Tracing the Mode and Speed of Intrageneric Evolution

A phylogenetic case study on genus *Acer* L. (Aceraceae) and genus *Fagus* L. (Fagaceae) using fossil, morphological, and molecular data

Dissertation
zur Erlangung des Grades eines Doktors der Naturwissenschaften

der Geowissenschaftlichen Fakultät
der Eberhard-Karls-Universität Tübingen

vorgelegt von
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aus Trier

2005

Tag der mündlichen Prüfung: 11.11.2003

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Für meine Eltern

In Erinnerung an meinem Vater, der mir beibrachte, immer hinter die Dinge zu sehen. Daran, dass ich ihm in Wesen, Art und mangelnder Kopfbehaarung nachgeschlagen bin. Und für den leicht zynischen Humor, den er an meine kleine Schwester und mich weitergegeben hat.

Für meine Mutter, die immer für mich da ist. Und all die Butterbrote und Schulsachen, die sie mir nachgetragen mußte, weil mein Kopf immer woanders war. Auch wenn sie es lieber gesehen hätte, dass ich einen sicheren Beruf gewählt hätte und Lehrer geworden wäre.

Zusammenfassung:

Aus der Korrelation fossiler, morphologischer und molekulargenetischer Datensätze wird ein detaillierter Einblick in die intragenerische Evolution zweier Baumgattungen aufgezeigt. Die ausgewählten Gattungen *Acer* (dt. Ahorn) und *Fagus* (dt. Buche) unterscheiden sich dabei sowohl in ihrer morphologischen Vielfältigkeit (*Acer* mit ca. 120 Arten, *Fagus* mit ca. 10 Arten) als auch ihrer genetischen Variabilität, die exemplarisch am intern transkribierten Spacer der nukleären ribosomalen DNS untersucht wird (*Acer*: variabel mit deutlich divergierenden Genmustern, *Fagus*: einheitlich, mit relativ wenigen, uneindeutigen Basenmutationen). Durch die Einführung neu beschriebener Methoden läßt sich nicht nur eine "nackte" phylogenetische Hypothese für die beiden Modellgenera ableiten, sondern die Evolutionsgeschichte und Artdifferenzierung auf einer molekularen Ebene im Detail verfolgen. Die neu beschriebenen Methoden umfassen ein Protokoll zum Erstellen eines möglichst objektiven und zuverlässigen Alignments, die Kodierung vorhandener intraspezifischer genetischer Variabilität als phylogenetisches Signal, visuelle Ansätze zur Klassifizierung und phylogenetischen Auswertung sogenannter "Oligonukleotidmuster" und die Zusammenführung verschiedener Datensätze und Ergebnisse durch "Mapping". Unter besonderer Berücksichtigung der Fossilgeschichte kann somit sowohl die Art und Weise als auch die Geschwindigkeit evolutionärer Prozesse innerhalb der beiden Modellgattungen qualitativ ermittelt werden. Aus den gesammelten Daten und Methoden ergibt sich schließlich ein erstes Abbild der historischen und aktuell wirksamen Prozesse, die zu den rezenten systematischen Gruppen und der erfaßten Biodiversität geführt haben, wie sie sich aus der Anzahl der allgemein akzeptierten Arten, den gefundenen Verwandschaftsverhältnissen unterschiedlicher Hierarchie (intraspezifisch bis infragenerisch) und der Aussagekraft gefundener morphologischer und genetischer Merkmale ergeben. Ferner wird die Bedeutung der Ergebnisse für die Forschungsbereiche intragenerische Evolution und Taxonomie der Pflanzen diskutiert und eine Grundlage geliefert für eine zukünftige Überarbeitung und Neubewertung des Fossilberichts.

Summary

By combination, comparison, and cross-validation of fossil, morphological, and genetical data an effort is undertaken to reveal a deep insight into intrageneric evolution in the arborescent plant genera *Acer* (maple) and *Fagus* (beech). The discriminative levels of morphological intrageneric diversity and differentiation within these two model genera (genus *Acer*: ~ 120 species; genus *Fagus*: ~ 10 species) is correlated to the detectable genetical divergence and variability, as it is exemplary exhibited in the nucleotide composition of the internal transcribed spacers of the nuclear ribosomal DNA (*Acer*: variable, and well differentiated; *Fagus*: conserved and exhibiting a high level of ambiguity). The introduction of new methodologies allows further to infer not only a 'naked' phylogeny for the analysed genera, but to trace and reconstruct the according pathways of molecular evolution and morphological diversification through space and time. Newly introduced methodologies include a protocol for an optimised alignment, the coding of intraspecific nucleotide site variabilities as phylogenetic signals, visual approaches to recognise and evaluate oligonucleotide motives in length polymorphic regions, and mapping strategies to correlate different data sets and hypotheses. Thus, with special respect to evidence from the fossil record, the mode and speed of evolutionary processes on a intrageneric level is qualitatively deduced for the model genera *Acer* and *Fagus*. The assembled data and conclusions from the analyses are, hence, utilised to draw a first comprehensive image of the processes that might have been the root and trigger for the current systematic setting and biodiversity, as it is exhibited by the number of accepted species, the particular intertaxonomic relationships, and the significance of defined morphologic and molecular genetic characteristics. Finally, the general impact of the results for the subjects areas of 'low-level' evolution and plant taxonomy, and for a future re-evaluation of the fossil record, respectively, is outlined.

Acknowledgements

Matthias Schlee and Martin Langer and his colleagues are thanked for collecting leaf material from Europe and North America.

Martin Langer is further acknowledged for conducting the primordial studies on the ITS in *Acer* and *Fagus*.

The technical assistance of Karin Stögerer in the laboratory is gratefully acknowledged. Without her help, it would not have been possible to retrieve DNA or positive clones from several "Gordian knot"-like samples and extractions.

I am in particular grateful to Thomas Denk for valuable discussions and productive collaboration to reconstruct and trace the evolution of *Fagus*, as well as for providing material form original stands of *Acer* and *Fagus* from the Black Sea region and eastern Asia, and his taxonomical expertise on northern hemisphere trees.

Vera Hemleben and Volker Mosbrugger are thanked for giving me the possibility to conduct this interdisciplinary study, providing the financial support for my work, and supervising and accompanying the dissertation.

Part of the laboratory work dealing with the ITS of *Acer* and *Fagus* was initially financially supported by the German Science Foundation (SFB 275, Tübingen).

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1 Introduction

The observation, description, and understanding of morphological characters was originally the only data set available to infer evolutionary pathways. In the second half of the last century, the biochemical characterisation of proteins and other metabolites contributed new data sets for systematics and phylogenetics. With the invention of the 'polymerase chain reaction' (PCR) by K. B. Mullis in the late 1970's (introduced to the scientific community by MULLIS & FALOONA 1987), an enormous amount of data became accessible in a rather easy and fast way. Modern automated sequencers allow to read more and more base pairs with increasing effectiveness and speed. As a consequence, the number of known nucleotide sequences increases exponentially, as well as the number of papers which infer phylogenetic relationships on the basis of sequence data, while the impact of biochemistry, morphology and especially the fossil record for phylogenetical and systematical purposes diminishes. However, the more molecular data become accessible, the more contradictions arise from the analyses of different genes, different taxa, and different analytic methods. Mainly two paths are taken to solve this problem: either new or modified analytic methods and models are introduced, or even more data from more genes are assembled.

With the enormous amount of sequence data on the one hand and fast, computerised analytic methods on the other hand, molecular phylogenies are often referred to as to be completely unbiased. The general idea is, that a ± statistical evaluation of more and more data leads to a final hypotheses, which reflects the true phylogeny, i.e. the true phylogeny is only a matter of the amount and usability of data. For example, SOLTIS et al. (2002) recently reconstructed the phylogeny of the spermatophytes by analysing eight genes from three genomes – chloroplast DNA (cpDNA), mitochondrial DNA (mtDNA), and genomic DNA – comprising 15,772 bp per used taxon. To minimise analytical bias, the phylogeny was inferred with Neighbour-Joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) via Bayesian inference (BI). Due to the enormous computational capacities which are needed to analyse an alignment comprising such a large number of basepairs, the five major spermatophyte groups (comprising approx. 500,000 species) were represented by 19 accessions of 19 distinct species. However, as it will be shown in this study, the reduction in the number of used accessions may cause serious problems, at least, if one tries to reconstruct low-level phylogenetic relationships (chapters 3.2, 3.6 & 4.6).

Since the reconstruction of the 'deep' phylogenies, e.g. to trace the origin of angiosperms, is occupied by numerous working groups (e.g. LEITH & HANSON 2002; ZANIS et al. 2002; cf. KUZOFF & GASSER 2000 for a compilation), it is understandable that an increasing number of researchers concentrate on 'low-level' evolution, i.e. intrafamiliar and intrageneric phylogenetic relationships. In fact, there are several reasons why low-level evolution is an interesting field in evolutionary sciences. Especially in the case of nearly related plant taxa, it is often difficult to impose a sound phylogenetic hypothesis based on morphological characters. Fossil ancestors commonly combine primitive and derived characteristics, a phenomenon known as heterobathmy. Convergences[1] are often found beyond near related taxa. Furthermore, those characters which are used to distinguish species or subspecies of the same genus – such as pubescence of leaves or other plant organs – may be constant for a group of taxa, but vary in another due to a slightly different ecological setting and/or genetical programme. By hybridisation, which occurs frequently among plants, morphological particularities can be further altered or exchanged. In addition, the ecological and biological parameters that control the development of specific[2] morphological characters are in most cases only poorly understood. Thus, genetical data are used to get a more detailed insight into the systematic and phylogenetic relationships. In this context, the internal transcribed spacers (ITS1 and ITS2; → Fig. 1-1) of the nuclear ribosomal DNA (nrDNA) were and are commonly used molecular markers to infer low-level phylogenetic relationships (e.g. Baldwin 1995; Jobst et al. 1998). But again, with more data available from an considerable number of plant species it has become apparent, that the genetic divergence of the ITS varies extremely between different plant genera, although the overall length of the region comprising the ITS1, 5.8S rDNA, and ITS2 is more or less the same (approx. 700bp; Fig. 1-1), at least for angiosperms (HEMLEBEN et al. 1988). Species of some genera, e.g. *Acer* (CHO et al. 1997; ACKERLY & DONOGHUE 1998; SUH et al. 2000; TIAN et al. 2002; new data[3]) exhibit a remarkably variable ITS1 and ITS2, while others such as *Fagus* (STANFORD 1998; MANOS & STANFORD 2001; DENK et al. 2002; new data) basically are uniform. Furthermore, most recent studies on *Acer* (PFOSSER et al. 2002) and *Fagus* (DENK et al. 2002; chapter 3.2) report a considerable amount of "ambiguous sites" (within the ITS of *Acer*: PFOSSER et al. 2002), respectively intraspecific and intragenomic variability. In the case of *Fagus*, the detected

[1] I.e. shared derived characters, that are not homologous (of a common origin).

[2] characters that define a species

[3] The terminus "new data" refers to data assembled for and presented in this study.

intraspecific variability of the ITS even exceeds the interspecific variability realised in the genus (DENK et al. 2002; chapter 3.2).

In fact, the actual evolutionary history of nearly related plant taxa might have been complex. It is known, that rather distinct related plant species can hybridise. Furthermore, hybridisation between more distantly related taxa can give rise to polyploids, that may form new species. Indeed, several *Acer* spp. are reported to have a polyploid chromosome set (→ appendix). Thus, a horizontal gene flow is possible and probable, if nearly related taxa are considered. In addition, the nrDNA including the ITS is tandemly organised (Fig. 1-1; up to 20,000 copies – paralogs – per genome: HEMLEBEN et al. 1988). It is conceivable, that two or more genotypes coexist within the genome of an individual and the gene pool of a population or species, respectively, due to hybridisation and/or incomplete concerted evolution (e.g. FOREST & BRUNEAU 2000 for *Corylus*; VOLKOV et al., in press for *Solanum*). Hence, phylogenetic relationships may be disguised in biparentally inherited molecular markers like the ITS. To avoid such problems, other molecular markers from the cpDNA and mtDNA have been used (e.g. *trn*L-F intergenic spacer for *Acer*: PFOSSER et al. 2002, TIAN et al. 2002; *mat*K for *Fagus*: STANFORD 1998) which are suspected to be only inherited from one parental lineage for most higher plants, and, consequently, thought to be more reliable for phylogenetical studies. But the genetical intrageneric divergence, that was found for most angiosperms like the here analysed genera *Acer* and *Fagus*, is so low, that the resulting hypotheses are mainly based on one or two mutations within hundreds of base pairs.

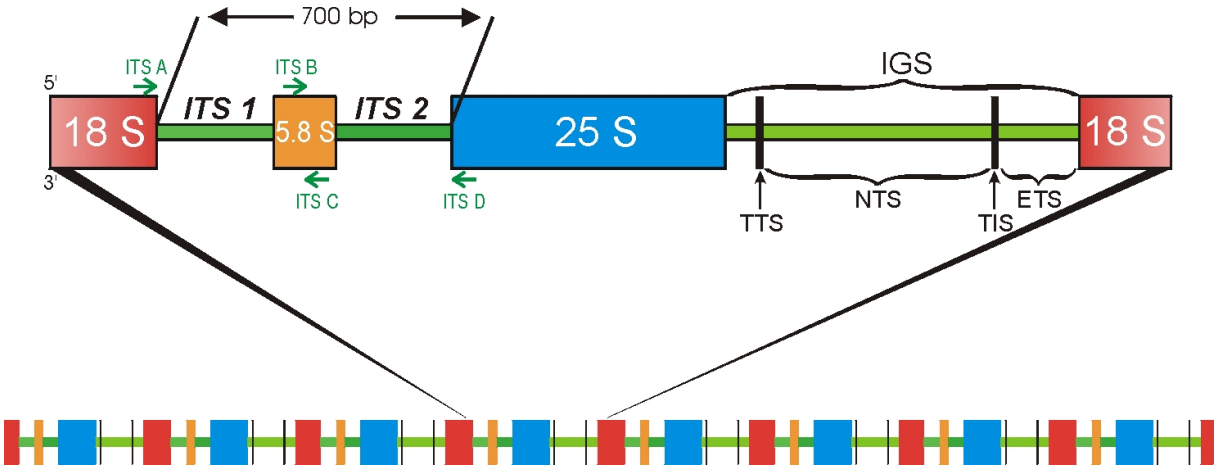


Figure 1-1: Scheme showing the general organisation of the repeatedly organised nrDNA.
Red, yellow, blue: regions coding for rRNA (i.e. rDNA). Green: intergenic spacers. **Abbr.:** 18S = 18S rDNA; 5.8S = 5.8S rDNA; 25S = 25S rDNA; ITS = internal transcribed spacer; IGS = 25S-18S intergenic spacer, comprising: TTS = transcription termination site, NTS = non-transcribed spacer, TIS = transcription initiation site, ETS = external transcribed spacer (based on HEMLEBEN et al. 1988).

As a consequence, the resulting phylogenies are often not convincing, since major divergence points lack crucial statistical support or an appropriate number of putatively synapomorphic mutations (in the case of MP-based analyses; cf. chapter 2.4.2). This is, in particular, the case in the here analysed genera *Acer* and *Fagus*, especially if the phylogenetic backbone of the analysed genus is considered. Like most phylogenetical studies dealing with the ITS and 'low-level' evolution in general, preceding studies of other authors suffer from three major problems (further discussed in chapters 3.2.3, 3.6, 4.3.1 & 4.6.2):

1. The phylogeny is inferred with distance methods and maximum parsimony, which is inappropriate for a 'base-per-base' analysis, at least for ITS data sets (chapter 2.4.2).
2. Directly sequenced PCR products are used. The ITS is a biparentally inherited molecular marker. By direct sequencing, there is a high probability to loose important information from an intraspecific level, which can be used for phylogenetical purposes (chapter 3.3). In addition, during any PCR wrong nucleotides may be incorporated in the product. Such 'artificial mutations' – herein also referred to as "misannealing" (→ special remark) – cannot be distinguished from naturally existing mutations. Hence, a divergence point based on a single mutation or the lack of a particular single mutation may be purely artificial.
3. Only one individual is taken as representative for a species (*Fagus*), respectively a single species for a group of taxa such as sections/series (*Acer*). A comparison between the here presented data with data of other authors (CHO et al. 1997; ACKERLY & DONOGHUE 1998; STANFORD 1998; SUH et al. 2000; MANOS & STANFORD 2001; TIAN et al. 2002) clearly demonstrates, that the genetic variability differs on an intra- and interspecific level within taxonomic units of different hierarchy and has to be evaluated for the studied genera, sections, series, and species, respectively.

In general, if a certain gene region is not accessible by standard analytic methods, this gene region is considered to be "useless" for phylogenetical purposes[4]. Nevertheless, any gene region, like the complete genome of an organism, is not a result of a purely statistical mutation pattern, but has to be in concordance with the actual evolutionary pathways, that lead to the origin of this organism. Like the morphology and ecology, the nucleotide composition of a certain taxon or group of taxa is a function of evolutionary processes

[4] PFOSSER et al. (2002, p.353) base their phylogeny on cpDNA and additional AFLP data, because the "...evaluation of ITS sequences in *Acer* did not always result in unambiguous data, which could be the result of incomplete concerted evolution and/or frequent hybridisation within a group of related taxa."

through space and time. Although the place and time of a single mutation are statistical phenomena, its contribution throughout the gene pool of the whole population and the fixation in the genome of the whole taxon must follow strictly the evolution and speciation history of the taxon. Thus, if the phylogeny can be inferred from the overall nucleotide composition, then the detailed nucleotide composition of any part of any gene or a particular gene region must mirror the evolutionary processes underlying the phylogeny.

Remark: The here presented data strongly indicate the occasional occurring of misannealing during the PCR. For both genera, mutations can be found at alignment sites (i.e. base positions), which are confined to a single of >100 cloned DNA sequences. Only in a few cases two accessions are identical. Such a unique mutation is either an artificial, by misannealing during PCR, or natural one, representing a genetic variability. However, the probability, that two or more cloned sequences from different PCR products show an identical artificial mutation at the same site, is minimal. Therefore, the assembling of a data base (here by a cloned DNA library) reflecting as well numerous taxa and/or populations as multiple PCR products retrieved from one extraction, allows to identify putative misannealings. Site variabilities, which are confined to a single population are per definition uninformative characters and, hence, are excluded in the new methodological approach proposed in chapter 3.3. Respectively, by analysing such a data base with ML-based methods, the impact of a misannealing for the phylogenetic reconstruction is further minimised: Instead of evaluating the potential mutations at a defined site (like it is done by MP), ML-based methods rely on a – here: general time reversible (GTR) – substitution model, in which general probabilities for point mutations are defined. The according probabilities are optimised on the base of the complete nucleotide data. Thus, the impact of misannealings on the substitution model diminishes in proportion to the number of used accessions.

Consequently, the major purpose of the following studies is not simply to infer intrageneric phylogenetic relationships, but to understand the composition of a selected gene region like the ITS on the basis of the putative evolution. To accomplish this task, most recently developed analytic methods to infer phylogenetic relationships on the basis of nuclear sequence data (i.e. Bayesian inference analysis: HUELSENBECK et al. 2001) are combined with newly introduced methodologies to trace and reconstruct pathways of the ITS evolution. On the basis of ITS data sets that comprehensively reflect the actual degree of intra- and interspecific variability, these methods allows to draw a concise image of the evolutionary pathways within these genera. Of course, if such a hypothesis is based solely on the data set used to infer the evolution, the analyses easily fall prey to circular arguments. Therefore, additional data have to be and are included. Fossils, as the only actual remnants of the evolutionary history, are the most valuable source for such a task. Up to now, reliable PCR

products from plant DNA cannot be retrieved from samples older than 5000 years (AUSTIN et al. 1997)[5]. Unlike animal DNA, which is rather well conserved and encapsulated in the marrow of bones, the hardest plant tissue, the wood, consists of death cells, while those cells, which contain the most DNA, are easily weathered and consumed during taphonomic processes. Hence, only morphology can be used to describe fossil taxa and order them into an evolutionary context. Out of the above mentioned reasons, the use of cladistic analyses merely based on morphological characters at an intrageneric level is difficult. To get a deeper insight into the pathways of molecular evolution and the underlying processes of speciation, the morphological data is herein analysed in correlation to and mapped against the molecular-based hypotheses. This study will show, that an intensive characterisation and reflection of the *recent* molecular setting – observed in the ITS of *Acer* and *Fagus* – can actually be correlated to equally sincere analysed morphological features and, in detail, to the putative history of fossil *and* recent taxa.

After introducing the general methodology (chapter 2) and recapitulating the basis for analyses about infrageneric relationships in *Acer* and *Fagus* (chapters 3.1, 4.1 & 4.2), phylogenetic hypotheses are computed based on numerous ITS accessions[6] reflecting the inter- and intraspecific variability within the analysed genera (chapters 3.2 to 3.4 & 4.3). These hypotheses are consecutively compared and mapped against morphological and fossil evidence, and further investigated to trace and reconstruct pathways of evolution (chapters 3.2, 3.5 & 4.4). A detailed investigation dealing with the taxonomical and systematical position of *F. sylvatica* has been already published (DENK et al. 2002; briefly summarised and emended by new evidence in chapter 3.2). The newly implemented methodology to infer phylogenetic relationships on the basis of intraspecific site variabilities (ISV analysis; chapters 3.3 & 3.4) is currently under review (G. Grimm, T. Denk, and V. Hemleben, submitted). The accumulated data and results are evaluated with respect to the usability for intrageneric phylogenetical studies in general – and in particular, for the here analysed genera – and further taxonomical and systematical purposes (chapters 3.6, 4.5 & 4.6). Finally, the major conclusions and implications for future studies are summarised (chapter 5).

[5] From 1990 to 1995, ancient plant DNA was reported from several sources aged up to 40 Ma, but the published sequences have to be considered as artefacts or contaminants.

[6] i.e. a nucleotide sequence of a certain gene region.

2 Material and Methods

2.1 Choice of analysed genera

The here presented analyses are focussed on two tree genera, i.e. *Acer* L. (maples) and *Fagus* L. (beeches), widely distributed throughout the northern hemisphere, that exhibit discriminative levels of morphological and genetical intrageneric differentiation. The genus *Acer* (fam. Aceraceae, order Sapindales) is morphologically (e.g. VAN GELDEREN et al. 1994 and literature cited herein) and genetically – considering the nucleotide composition of the ITS (CHO et al. 1997; ACKERLY & DONOGHUE 1998; SUH et al. 2000; TIAN et al. 2002; new data) – highly variable, does have a well-preserved fossil record dating back to at least 60 Ma (Paleocene; cf. WOLFE & TANAI 1987; PFR 2.2 database), and can be considered as a monophylum. Thus, the taxa assigned to this genus and to the family Aceraceae in general – including the sibling genus *Dipteronia* – are closely related and share a common history, which was not 'disturbed' by other genera[7]. *Fagus* (fam. Fagaceae, order Fagales) exhibits a lesser intrageneric differentiation from a morphological (e.g. SHEN 1992; DENK, in press) and genetical viewpoint (STANFORD 1998; MANOS & STANFORD 2001; DENK et al. 2002; new data). The fossil record is not as rich as in the case of *Acer*, but can also be traced back at least to the Middle Eocene (~ 45 Ma; PIGG & WEHR 2002). By comparison of molecular genetical evidence from the ITS it can be assumed, that *Fagus* is rather distinctly related to other genera of the Fagaceae and evolved – like *Acer* – independently from its origin on. Due to the lesser morphological intrageneric variability found, *Fagus* differs markedly from *Acer* in the number of distinguished and accepted species.

Besides the differing levels of morphological and genetical differentiation, *Acer* and *Fagus* are of comparable stratigraphic age and share a rather similar distribution history as arborescent elements within the northern hemispheric forests during the Tertiary and Quaternary (PFR 2.2 database; cf. TANAI 1983, WOLFE & TANAI 1987 for *Acer*; DENK 1999c, in press, MANOS & STANFORD 2001 for Fagaceae and *Fagus*, respectively). The analysis of these two genera allows to distinguish between two speciation strategies ("high" vs. "low speciation") and evaluate the impact of the speciation strategy onto the nucleotide composition of the ITS within these genera.

[7] For a most recent study on the relationship between *Dipteronia* and *Acer* and the origin of the Aceraceae refer to MCCLAIN & MANCHESTER (2002).

2.2 Sampling and taxonomical work

To trace evolutionary pathways within the here discussed genera – *Acer* and *Fagus* – intensive sampling is crucial. Samples comprise material from living specimen from original stands, as well as already herbarised samples collected by various researchers. Due to the limited number of taxa available from original stands, individuals cultivated in the botanical garden of the University of Tübingen (BGTue) as well the Morris Arboretum (MorArb) were sampled for means of comparison and completeness. The taxonomical identity of all specimen used for analysis has been confirmed using common identification keys and by comparison with herbarium specimen. Most of the material from the original stands was taxonomically revised by T. Denk, senior curator at the department of palaeobotany, Natural History Museum, Stockholm, Sweden. The collected material effectively covers the biogeographical range of the analysed taxa. Nevertheless, a stress in number of sampled individuals and populations lies in western Eurasia due to the major research interest of the involved collectors and researchers, respectively. Voucher information on all included taxa and specimen can be found in the appendix.

As far as certain morphologic characteristics and data from the fossil record of genus *Acer* are used in the study, the data are mainly taken from literature as it is cited. If possible, morphological features discussed and reported for *Acer* spp. were confirmed for all specimen used in the molecular analyses. Scans of Leaves, twigs, and/or fruits of most analysed *Acer* individuals can be electronically supplied upon request. The morphology of the genus *Fagus* has most recently undergone a major taxonomic, systematic, and historical re-investigation by T. Denk (person. comm.; but see also SHEN 1992, DENK 1999a, 1999b, 1999c; DENK et al. 2002; DENK, in press). The according studies were done in a direct context with the here presented molecular analyses. Thus, any further morphological investigations were not necessary for the here presented studies and conclusions.

2.3 Molecular genetical work

The total plant DNA has been extracted either from fresh leaf material or herbarised specimen following a modified CTAB-protocol (SAGHAI-MAROOF 1984). Up to 2 cm^2 of leaf tissue is put into a 1.5 ml Eppendorf cup and grounded in liquid nitrogen (N_2) to be pulverised with a micropistill. The pulverised leaf material is then re-suspended in 300 µl CTAB buffer[8]

[8] For ingredients of buffers and mediums refer to the appendix

and 5 µl of proteinase K is added. After vortexing, the suspension is incubated for 1 h at 37° C. Next, 300 µl of 1:24 mixture of isoamylalcohol-chloroform are added to separate the DNA from cellular metabolites and other organic remnants. The solution is intensively mixed and centrifuged shortly at 13,000 rpm. To precipitate the DNA, the upper phase is transferred into a 1.5 ml Eppendorf cup containing 300 µl (≙ 1 volume) of isopropanol (2-propanol). The cups are incubated for 15 min at room temperature and subsequently centrifuged for 15 min at 13,000 rpm. After centrifugation the alcohol is discarded and the remaining pellet is washed with 1 ml of 70% ethyl alcohol (ethanol) and again centrifuged at 13,000 rpm for 5 min. After complete removal of ethanol the pellet is dried and finally re-diluted in 50 µl of TE buffer. DNA solutions are stored at -20° C.

PCR reaction is done with Vent® polymerase (New England Biolabs, NEB) and plant specific primers ITS-A and ITS-D as described in DENK et al. (2002, introduced by JOBST et al. 1998) to amplify the ITS1, 5.8S rDNA, and ITS2. Up to 5 µl of DNA solution[9] are mixed with each 0.5 µl of 100mM primer solutions (ITS-A and ITS-D; ≙ final concentration of 2 mmol/l), Vent® polymerase (≙ 20 units), 100 mM $MgSO_4$, 4 µl dNTP mix (≙ 0.2 mmol/l dATP, dCTP, etc.), and 5 µl of 10×polymerase buffer (NEB). Finally, doubly distilled water is added to achieve a sample volume of 50 µl. PCR-cycler program follows specifications in DENK et al. (2002). The length of the PCR-products was checked on a 1% agarose gel and purified either with the Qiagen® purification kit, or - in cases with multiple bands - the Qiagen® gel purification kit.

If available, more than one PCR-product per sample are mixed and prepared for ligation. PCR-fragments are ligated into blunt-end cut pUC18 vectors[10] using a T4 ligase. Recombinant plasmids are then transformed into competent cells of *E. coli* strain DH5α via electroporation. Positive clones are detected by blue-white-screening and tested for appropriate inserts by PCR. Up to 10 positive colonies are cultivated overnight in microprep glass tubes containing 5 ml TY medium and 5 µl ampicillin solution (conc.: 100µg/l). 1 ml of the suspension is mixed 1:1 with glycerine and stored at –70°C for repeated plasmid isolation and data documentation. Cell cultures with transformed *E. coli* can be obtained upon request.

[9] Exact amount depends on the actual amount of DNA in the DNA solution. In particular, extractions from fresh leaf material collected in spring did contain increased amounts of DNA plus certain secondary metabolites. In these cases the PCR was done with a 1:10 or 1:100 dilution of the original extraction. Due to the unknown number of parameters that influence the result of the PCR, the exact volume is best evaluated by a trial-and-error procedure.

Plasmids are isolated with Roche® plasmid isolation kit and prepared for sequencing. Sequencing is done on a ABIPrism® automated sequencer at the CPMB[11], General Genetics, with standardised primers M13forward and M13reverse. Good runs provided up to 550 bp from the 5', respectively 3' end. A number of accessions were sequenced by a professional laboratory with primer M13forward reading the complete strand.

2.4 Phylogenetic analyses

The amount of DNA data assembled by PCR and cloning can only be analysed with computer-based methods. The choice of methods and the assembling of the underlying alignment do have a strong impact on the resulting phylogenetic hypothesis.

2.4.1 Aligning of nucleotide data

Sequences were edited with CHROMAS® V.1.45 (© C. McCarthy) and SeqMan II® (© DNAStar Inc) and, in the case of *Fagus*, submitted to gene bank. The gene bank accession numbers and voucher specifications for *Acer* and *Fagus* can be found in the appendix.

Complete sequence data were aligned with the Clustal method implemented in MegAlign® (© DNAStar Inc). In the case of *Acer*, the highly variable regions within ITS1 and ITS2 had to be manually realigned, as well as two regions within the ITS of *Fagus,* because of the occurrence of conspicuous length polymorphism. The recognition of similarity between complex patterns is a natural ability of the human brain. Up to now, such a task cannot be adequately performed by computer algorithms (→ Fig. 2-1). This is also illustrated by the strategies used by chess grandmasters to defeat chess computers with enormous computational capacities. Obviously, computational alignment algorithms can only be a help to find a start for an alignment, but a manual correction is essential, due to the enormous pattern recognition abilities of the human brain.

[10] Vector DNA is cut with the restriction enzyme *Sma* I by overnight incubation at 30°C.

[11] Centre of Plant Molecular Biology, Naturwissenschaftliche Institute (NWI), University of Tübingen

Figure 2-1: The chair paradigm.
Human observers, no matter of what age and intelligence, can readily recognise any of the chairs placed in this image. A task which cannot (yet) be accomplished by the most advanced computers and software packages for pattern recognition (Source: MPI/SZ).

To minimise potential subjective influence, the re-alignment followed a strict protocol:

1. Intragenomic (-population) differences, i.e. between two clones from one sample (locality), are minimised, e.g. in the number of gaps etc.
2. Interspecific differences (represented by number of nucleotides, occurrence of putative point mutations etc.) have to be conserved during the alignment.
3. Principally, the alignment of diverging molecular patterns follows the fixed character state optimisation as proposed by WHEELER (1999, 2001).[12]

In general, the alignment was done to principally avoid artificial grouping of taxa, if they did not show genetical similarities (see e.g. Fig. 4-13). The exact position of gaps was optimised for a ML analysis (cf. Figs. 4-13 & 4-31; chapter 4.6). The final alignment for the ITS nucleotide data of *Acer* and *Fagus* is documented in the appendix. The according NEXUS-files[13] can be supplied upon request.

[12] Due to the lack of reliable secondary structure models for the ITS of *Acer* and *Fagus*, a correlation to changes in the putative secondary structure (cf. DENDUANGBORIPANT & CRONK 2001) can not yet be taken into account.

[13] Files used for analyses are written in the NEXUS format which can be interpreted by numerous phylogenetic software packages.

2.4.2 Choice of methods

In my opinion, single nucleotides are *not* valid parsimony informative characters. Per definition, a parsimony informative character has to be independent from other characters and irreversible (FOREY et al. 1992, and literature cited therein). Due to the constraints on e.g. the secondary structure of the transcript, nucleotides of the rDNA may in fact be linked. Furthermore, since each nucleotide has only four possible characters states, i.e. "A" (= adenine), "C" (= cytosine), "G" (= guanosine), and "T" (= thymine), an irreversibility *a priori* can not be postulated for nucleotide data. All current and commonly used substitution models inferred to reconstruct the molecular evolution of a gene region imply, that transitions occur more often than transversions. Hence, it is more probable, that at a certain position (site) an "A" is changed into a "G" and back into an "A" again, than that the "A" is replaced by a "C" or "T". Such backmutations cannot be considered within a MP analyses. Therefore, maximum parsimony must not be used in nucleotide-based molecular analyses[14].

Although maximum parsimony is virtually useless for the 'base-per-base' analyses of nucleotides, it has proven its capability to analyse character matrices containing more complex characters, which, in addition, are to a convincing degree independent and irreversible. Cladistic analyses of well-defined morphological characters are commonly used to infer phylogeny and often present convincing hypotheses about the evolution of a certain group of taxa. Accordingly, maximum parsimony is used in this study to infer phylogenies based on (oligo)nucleotide motives (chapter 3.3). Such analyses are done analogously to cladistic analyses of morphological characters. As far as maximum parsimony is used, the analyses were performed with PAUP® 4.0 beta 10 (SWOFFORD 1998).

To infer the possible phylogeny based on the raw nucleotide data, maximum likelihood is up to now the most appropriate method (SANDERSON et al. 2000; STEEL & PENNY 2000; WHELAN et al. 2001). However, the computational capacities to find a topology which applies to the ML criterion, exceed the capability of personal computers. A statistical test ("Bootstrap", "Jackknife", etc.; for a compilation refer to WHELAN et al. 2001) of the computed topology is basically impossible. As a consequence, all here presented analyses were performed using the program MrBayes® 3.0 (HUELSENBECK & RONQUIST 2001). MrBayes® performs a Bayesian

[14] The uselessness of MP for nucleotide-based analyses has been proved by numerous theoretical and statistical studies. Still, some authors and journals protect and cultivate the usage of MP. Thus, the literature on this topic is vast. For a brief introduction into the discussion see STEEL & PENNY (2000).

inference (BI) analysis utilising Markov chains combined with a Monte-Carlo algorithm to estimate *a posteriori* probabilities of competing topologies under a given alignment (HUELSENBECK et al. 2001). This method allows to infer a ML phylogram and simultaneously test the according topology by statistical means (in principle: random sampling) with rather limited computational resources. The 5.8S rRNA gene was excluded during analysis. An artificial outgroup was not specified. Either the complete data set available for the ITS1 and ITS2, or a reduced set with only conserved data[15] was analysed. For each data set several runs were performed to verify the shown topology.

[15] "Conserved" is used here for directly alignable gene regions, i.e. gene regions *completely* lacking common length polymorphism (→ appended alignment). Within these regions, the homology of the nucleotides is directly given by the position, not the automated or manual alignment. Thus, such regions are generally free from subjective or methodological bias.

3 Microevolutionary Traits in Beeches (Genus *Fagus*, Fagaceae)

3.1 General introduction and current systematical knowledge

The beech tree (*Fagus* L.) is among the most abundant tree species in temperate forests of the Northern America, Europe and eastern Asia, especially if there is a strong anthropogenic impact on the natural vegetation (recent distribution of *Fagus* spp.: Fig. 3-1). From a morphological, ecological and genetical viewpoint, *Fagus* is a quite uniform and restricted genus (cf. SHEN 1992; PETERS 1997; MANOS & STANFORD 2001; DENK, in press; new data). Fossil and recent populations of *Fagus* are more or less identical in their gross morphology (T. Denk, person. comm.; see also DENK, in press). From the 8 to 13 species, which are recognised by various authors (→ Table 3-1), only in Japan and China populations can be found where different species of *Fagus* are associated with each other. In such a case, one representative of the subgenus *Fagus* (*sensu* SHEN 1992) is associated with exclusively one representative of the other subgenus *Engleriana* (*sensu* SHEN 1992). Natural hybrids are completely unknown. On the other hand, *Fagus* is clearly an invasive tree genus. If individuals of *Fagus* are found at a certain locality, they are always the dominant arborescent element at this locality (T. Denk, person. comm.; PETERS 1997), independent of the biogeographical setting. Furthermore, although probably extinct during the glacial period in Central Europe and most parts of North America, *Fagus* has become a dominating element of the forests in these regions throughout the last 10,000 years. The fossil history of *Fagus* can be traced back to the Middle Eocene of western North America and late Eocene of eastern Asia (FOTJANOVA 1982; PIGG & WEHR 2002). From this time on, *Fagus* was commonly distributed throughout the northern hemisphere, but apparently never did speciate in the same manner and intensity as other Fagaceae genera like *Castanea* and *Quercus*. Together with the invasive population strategy, this makes *Fagus* an excellent model plant to trace microevolutionary traits.

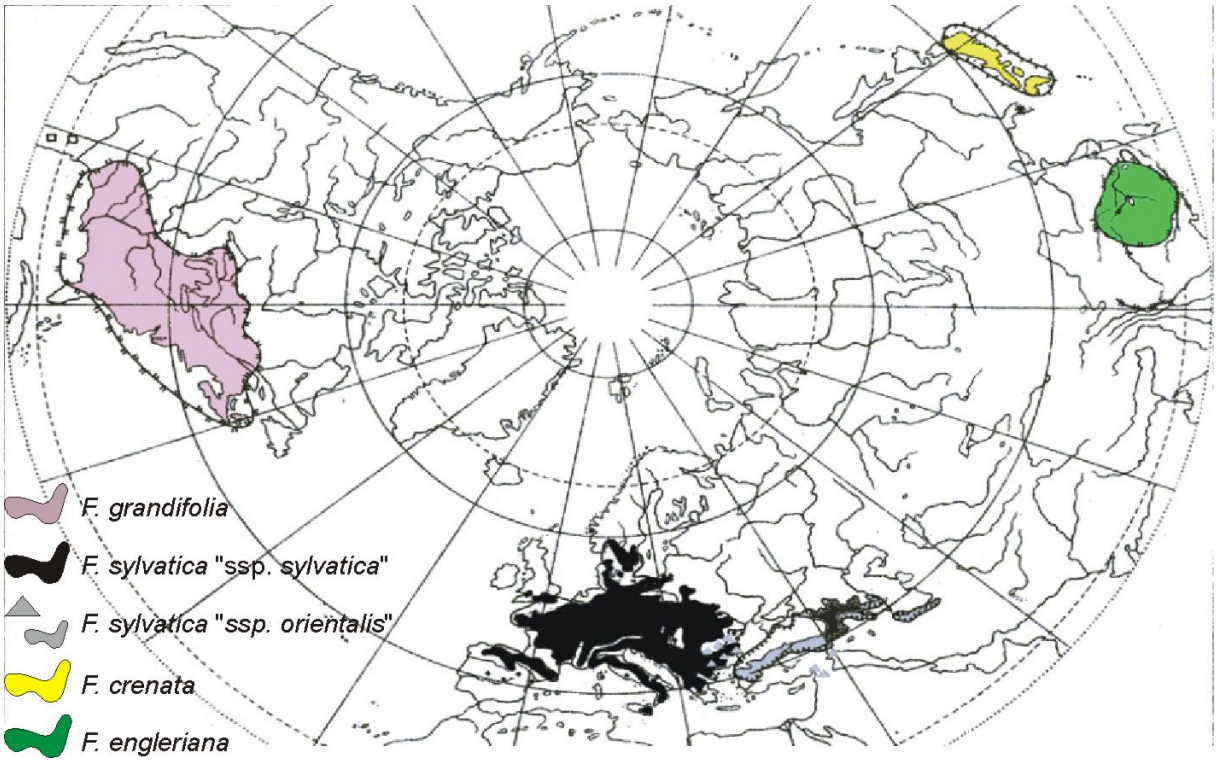


Figure 3-1: Distribution of major *Fagus* spp. throughout the northern hemisphere.
Not shown are the disjunct populations of *F. japonica* in Japan, *F. longipetiolata, F. lucida* in China, and *F. hayatae* in SE China and Taiwan. The species *F. okamotoi* (Japan) and *F. chienii*[16] (China), respectively, are solely available from herbarium specimen and possibly extinct at natural stands. Modified after MEUSEL et al. (1965).

The most recent monograph about *Fagus* by SHEN (1992) distinguishes – on the basis of morphological characters – two subgenera: *Engleriana* and *Fagus*, which are widely accepted (e.g. GRIN database[17]; cf. Table 3-1). Subgenus *Engleriana* comprises three species: *F. engleriana* SEEMEN (China mainland, Korean Ullung Is.), *F. japonica* MAXIM., and *F. okamotoi* SHEN[18] (latter two endemic to Japan). Subgenus *Fagus* comprises the remaining species (Table 3-1) distributed throughout western Eurasia, China, and eastern North America. STANFORD (1998) used molecular data (*mat*K, ITS) to clarify intrageneric affinities between species of *Fagus* and other genera, in respect to major trends affecting the biogeographic history of selected circumpacific genera. Her dendrograms strongly suffer from the earlier mentioned

[16] *F. chienii* is morphologically a close relative of *F. lucida* (DENK, in press). Unfortunately, T. Denk was not able to find living individuals of *F. chienii* while collecting material at original stands in China.

[17] URL: http://www.ars-grin.gov/npgs/tax/index.html

[18] *F. okamotoi* is reported from a few localities and is very similar to *F. japonica* (DENK, in press). KOIKE et al. (1998) comprehensively sampled numerous stands of *F. crenata* and *F. japonica* in Japan, but found no evidence of individuals of *F. okamotoi*.

problems often encountered in low-level molecular systematical studies (chapter 1). To reconstruct the phylogeographic history of the Fagaceae in the northern circumpacific area, MANOS & STANFORD (2001) assembled additional ITS and *mat*K data from several eastern Asian and North American populations of *Fagus*. Like in the preceding study (STANFORD 1998), the presented phylogram (MP) for *Fagus* did not sustain Shen's subgenera. In addition, accessions of different populations of *F. crenata* were found to be paraphyletic in relation to accessions of *F. grandifolia, F. sylvatica,* and *F. lucida*. Again, similar to STANFORD's (1998) study, only very few 'steps' (≙ point mutations) were used to reconstruct the phylogeny.

However, in contrary to the obviously low molecular genetical differentiation detected by STANFORD (1998) and MANOS & STANFORD (2001) in cpDNA and nrDNA markers, isoenzyme and RFLP[19] analyses were capable to reveal a complex genetical composition for species like *F. crenata* and *F. sylvatica* (e.g. DEMESURE et al. 1996, GÖMÖRY et al. 1999, COMPS et al. 2001 for *F. sylvatica*; KOIKE et al. 1998, OKAURA & HARADA 2002 for Japanese beeches; TOMARU et al. 1998, FUJII et al. 2002 for *F. crenata*), indicating a complex migratory and population history. In addition, DENK (1999a, 1999b) was able to demonstrate, that morphoclinal transitions characterise the populations of *F. sylvatica* in Europe and western Asia.

In general, the intrageneric systematical and taxonomical setting of the genus *Fagus* has been up to now only poorly understood and is in need for a comprehensive re-investigation.

[19] abbr. for restriction fragment length polymorphism

Table 3-1: Systematical concepts applied for genus *Fagus* (modified after DENK, in press**).**

species referred to in the original literature	KOLAKOWSKI 1960	ZETTER 1984	KVACEK & WALTHER 1991	SHEN 1992	
F. engleriana* Seemen	Gr. broch. spp.	*F. engleriana* group	Group 1	sg. *Engleriana*	
***F. japonica* Maxim.**					
***F. okamotoi* Shen**	not recognised				
***F. longipetiolata* Seemen**	Gr. crasp. spp.	*F. longipetiolata* group	Group 1	sg. *Fagus*	sect. *Longi-petiolata*
F. brevipetiolata Hu	not recognised				
F. bijiensis C.F.Wei & Y.T.Chang	not recognised				
F. tientaiensis T.N.Liou	not recognised				
***F. lucida* Rehder & Wilson**	Gr. crasp. spp.	*F. longipetiolata* group	Group 2		sect. *Lucida*
***F. chienii* Cheng**	not recognised				
***F. hayatae* Palibin†**	not recognised	*F. longipetiolata* group			
***F. crenata* Blume**	Gr. broch. spp.‡	*F. sylvatica* group	Group 3		sect. *Fagus*
***F. sylvatica* L.**					
F. orientalis Lipsky				not recognised	
F. moesiaca (Maly) Czecz.		not recognised		not recognised	
***F. grandifolia* Ehrh.**	Gr. crasp. spp.	*F. grandifolia* group	Group 4	sg. *Fagus*	sect. *Grandifolia*
F. mexicana Martinez	not recognised			not recognised	

bold printed: herein accepted species (see text)

Abbr.: Gr. broch. spp. = group of brochidodromous spp.; Gr. crasp. spp. = group of craspedodromous spp.

* syn. *F. multinervis* Nakai

† syn. *F. pashanica* C.C.Yang

‡ Kolakowski considered *F. moesiaca* and *F. orientalis* as very closely related

3.2 Western Eurasian beech populations: Defining Fagus sylvatica

The first approach, that has been undertaken in course of this study to understand the microevolutionary pathways within the genus *Fagus*, has already been published as DENK et al. (2002). In DENK et al. (2002), an effort was undertaken to clarify the taxonomic status of western Eurasian beech trees. The classical taxonomic system (e.g. Flora Europaea of 1967) divided these populations into two fully accepted species, i.e. *F. sylvatica* L. in Central Europe, the northern Mediterranean, and the Balkans, and *F. orientalis* LIPSKY, mainly distributed around the Black Sea, and a few other described species, which were only locally accepted. GREUTER & BURDET (1981) combined *F. sylvatica* and *F. orientalis* as subspecies under *F. sylvatica* and put all other names in synonymy, which was widely accepted, at least in European literature (cf. Flora Europaea, GRIN database). However, in the original publication, no convincing reason was given. [20]

DENK (1999a, 1999b) was able to show, that *F. sylvatica* ssp. *sylvatica* and ssp. *orientalis* cannot be separated by distinctive morphological characters. Instead, the transition from typical "*F. sylvatica*" into typical "*F. orientalis*" was found to be more or less dynamic. To contribute to this problem, numerous ITS accessions from various populations of *Fagus* studied by DENK (1999a, 1999b) were sequenced and analysed (DENK et al. 2002, Table 4, p. 219f). As a result, we were able to demonstrate, that *F. sylvatica* has to be understood as a complex species, that cannot (yet) be distinguished in clearly separated taxonomic entities like subspecies. Nevertheless, the molecular and the morphological data (DENK 1999a, 1999b; DENK et al. 2002) revealed a possibly starting or ongoing speciation process and showed that there is a significant difference within the variability found in Georgian (Transcaucasian) and other populations of *F. sylvatica*[21] (DENK et al. 2002, Table 6, p. 225ff, and Fig. 4, p. 228).

[20] for a detailed taxonomic history cf. DENK et al. (2002), Table 1, p.215.

[21] In the following, according to the data presented in DENK et al. (2002), *F. sylvatica* is used as the only valid taxon name for western Eurasian populations of *Fagus*. A further differentiation in subspecies is not appropriate.

3.2.1 Critical ITS data

By assembling ITS accessions of more *Fagus* populations, including new material collected in the north-western Mediterranean and northern Europe, it has become apparent, that some older accessions used in DENK et al. (2002) include potential sequence artefacts originating from the detection procedure, herein referred to as "misreadings". Most sequences representing Turkish and Iranian populations, as well as two Central European localities near Tübingen, were sequenced with a Pharmacia® ALF sequencer. This sequencer type has apparently problems with detecting and correctly reproducing certain nucleotide sequences (e.g. motives comprising AT- and CG-repeats, or multiple-"A", -"C", -"G", and –"T" motives). By comparison with clones from the same locality sequenced on the new ABI® sequencer, it is possible to detect these misreadings and exclude the according data from the analysis (→ Fig. 3-2)[22]. It has to be noted, that the protocol used for the ABI allows misannealings (cf. special remark, chapter 1) during the cycle sequencing PCR. However, in Tübingen no evidence of misannealings have been found up to now (T. Ertan[23], M. Schlee[24], A. Dressel[25], person. comm.; own observations). This may be due to the fact, that plasmid DNA is used for sequencing. The plasmid DNA provides a considerable amount of identical DNA strains. Consequently, occasional misannealings might have occurred, but are not detected during the reading process, because all other PCR replicates do contain the original sequence.

[22] Only in a few cases, the original clone cultures of ALF-sequenced accessions are still stored at the laboratory. Therefore, only accessions from newly assembled transformed cells could be sequenced in comparison.

[23] affiliated Institute of Earth Sciences (IFG), University of Tübingen

[24] affiliated with the Centre of Plant Molecular Biology (CPMB), University of Tübingen

[25] affiliated with the Centre of Plant Molecular Biology (CPMB), University of Tübingen

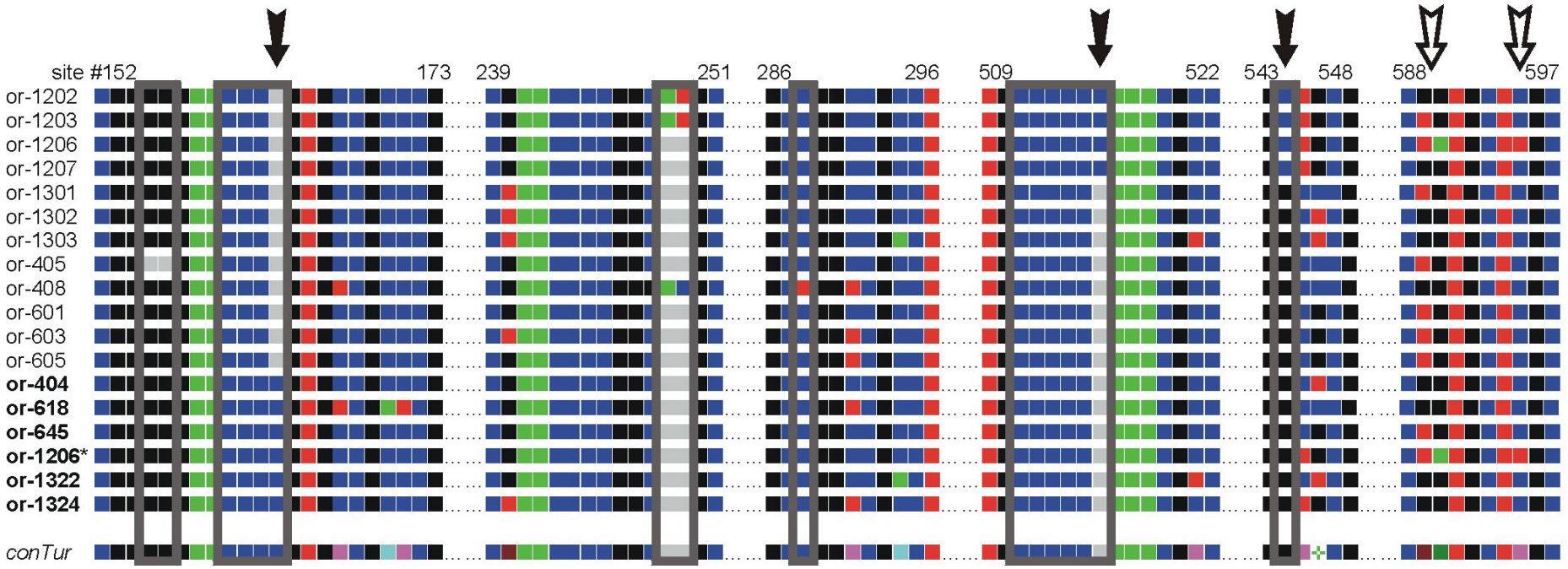


Figure 3-2: Parts of the ITS exhibiting putative sequence-artefacts (misreadings).

Aligned are accessions from clones representing populations of *F. sylvatica* from Turkey. Bold font: ABI-generated accessions; normal font: ALF-generated accessions. Site variabilities confined to ALF-generated accessions (grey outline; → appendix) are not recognised for computing the consensus sequence (accession *conTur*; cf. chapter 3.4.1) and during coding for ISV analysis (chapters 3.3 & 3.4.2). Black arrows indicate definite misreadings, white arrows sustained site variabilities within accessions representing the Çatalan gorge locality of *F. sylvatica* (clones or-12xx), detected by comparing the ALF-generated accession of clone or-1206 with the ABI-generated accession (**or-1206***). Standard colour code; site numbers refer to the complete alignment (→ appendix).

3.2.2 Emended phylogenetic hypothesis based on ITS data

The ML phylogram presented in DENK et al. (2002, Fig. 4, p. 228) was still computed via a heuristic search with PAUP and tested by bootstrapping using an according distance model. As already mentioned (chapters 1 & 2.4.2), the computational capacities to compute ML phylograms with 'classical' (pre-BI) algorithms are vast, and a statistical evaluation – e.g. by performing a bootstrap test – is virtually impossible. In addition, the bootstrap test provides primarily a statistical value for the consistency of the alignment, not – as in BI analyses – probabilities of the competing topologies[26]. In Figure 3-3 a majority consensus tree assembled via BI analyses is given, including the new data and taxa, respectively, from European and East Asian localities. ALF-generated accessions are only included from those localities, where the exact nucleotide composition could be confirmed by newly assembled ABI-generated sequences. The phylogram is in broad agreement with the topology shown in Figure 4, p. 228 in DENK et al. (2002). The subdivision into the two subgenera *sensu* SHEN (1992), i.e. *Engleriana* and *Fagus*, is well-sustained (100%, instead of a bootstrap value of 76). Also, *F. grandifolia* forms a distinct clade within subgenus *Fagus* (86%, incl. clone ho-1907 from Georgia, Transcaucasia, cf. DENK et al. 2002), while the remaining taxa – *F. crenata, F. hayatae* ssp. *pashanica* (not included in DENK et al. 2002), *F. longipetiolata, F. lucida, F. sylvatica* – cannot be resolved on a interspecific level by a 'base-per-base' analysis. However, some accessions of *F. hayatae* ssp. *pashanica* and *F. longipetiolata* are grouped together (prob. 100%). Like in the originally presented ML phylogram (DENK et al. 2002, Fig. 4, p. 228), accessions representing Georgian populations of *F. sylvatica* are distributed throughout the phylogram due to their conspicuous molecular genetical variability, indicated by black arrows in Figure 3-3. The inclusion of additional data from populations of *F. engleriana* and *F. japonica* demonstrates, that the original subdivision into two genetically distinguishable species (DENK et al. 2002, Fig. 4, p. 228) is a definite sampling error. As it will be shown in the following paragraphs, *F. engleriana* and *F. japonica* do have an identical gene pool and exhibit the same remarkable intraspecific/ -population variability.[27]

[26] for further details refer to HUELSENBECK et al. (2001), HUELSENBECK & RONQUIST (2001), and WHELAN et al. (2001).

[27] For further discussion, e.g. comparison with previous studies, taxonomic and systematic position of subspecies of *F. sylvatica*, etc., refer to DENK et al. (2002).

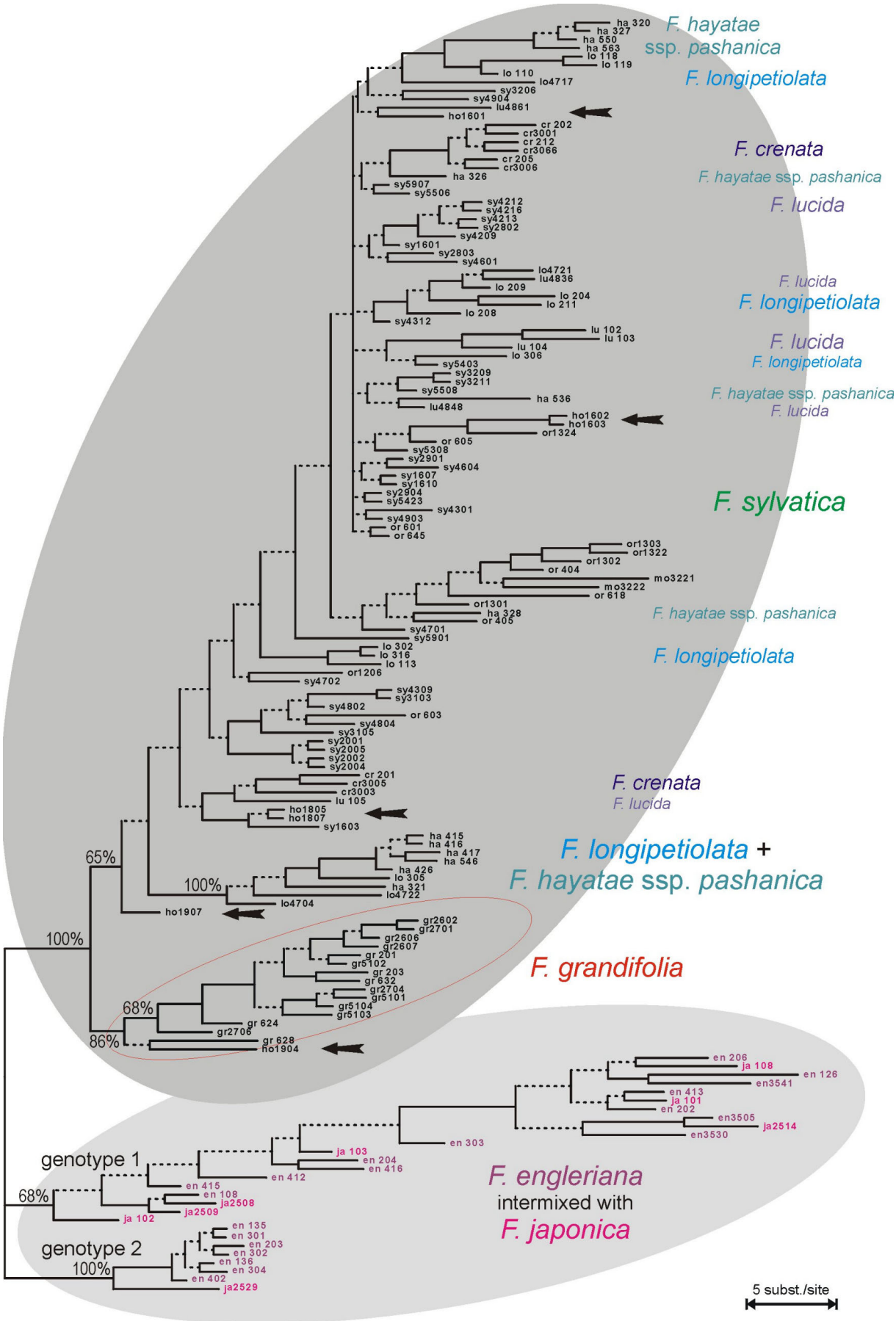

F. hayatae
ssp. pashanica
F. longipetiolata
F. crenata
F. hayatae ssp. pashanica
F. lucida
F. lucida
F. longipetiolata
F. lucida
F. longipetiolata
F. hayatae ssp. pashanica
F. lucida
F. sylvatica
F. hayatae ssp. pashanica
F. longipetiolata
F. crenata
F. lucida
F. longipetiolata +
F. hayatae ssp. pashanica
F. grandifolia
F. engleriana
intermixed with
F. japonica
genotype 1
genotype 2
65%
100%
100%
68%
86%
68%
100%
5 subst./site

Figure 3-3 (preceding page): ML phylogram of *Fagus* spp. including all assembled accessions. Shown is the all compatible consensus of 8033 trees (BI predefinitions: 1,000,000 generations, 5 chains, GTR+Γ+I, every 100th tree saved). The overall topology is similar with the topology in DENK et al. (2002). Subgenus *Engleriana* (light grey shadowed) is genetically separable from sg. *Fagus* (dark grey shadowed). Within sg. *Fagus* only *F. grandifolia* (red circled) is genetically specific. Note the miscellaneous position of Georgian clones (*F. sylvatica* ssp. *hohenackeriana sensu* SHEN), indicated by black arrows. Accession labels refer to clone numbers (see appendix), percentages at selected notes indicate *a posteriori* probabilities. Dotted lines: prob. <50%, solid lines: prob. >50%.

3.2.3 Impact of the assembled data for further studies

By sequencing the ITS of most species of *Fagus*[28] (STANFORD 1998; MANOS & STANFORD 2001; DENK et al. 2002; emended phylogram: Fig. 3-3) and analysing with various methods, the intrageneric relationships cannot be fully resolved. Besides ITS1 and ITS2, other molecular markers from cpDNA have been used (STANFORD 1998; MANOS & STANFORD 2001), but the detected infrageneric variability is too low to produce a reliable phylogeny for the genus. As demonstrated in the preceding chapter and DENK et al. (2002), the intraspecific variability found within the ITS1 and ITS2 in the *F. sylvatica*-complex not only exceeds the overall generic genetic divergence, but causes serious problems for

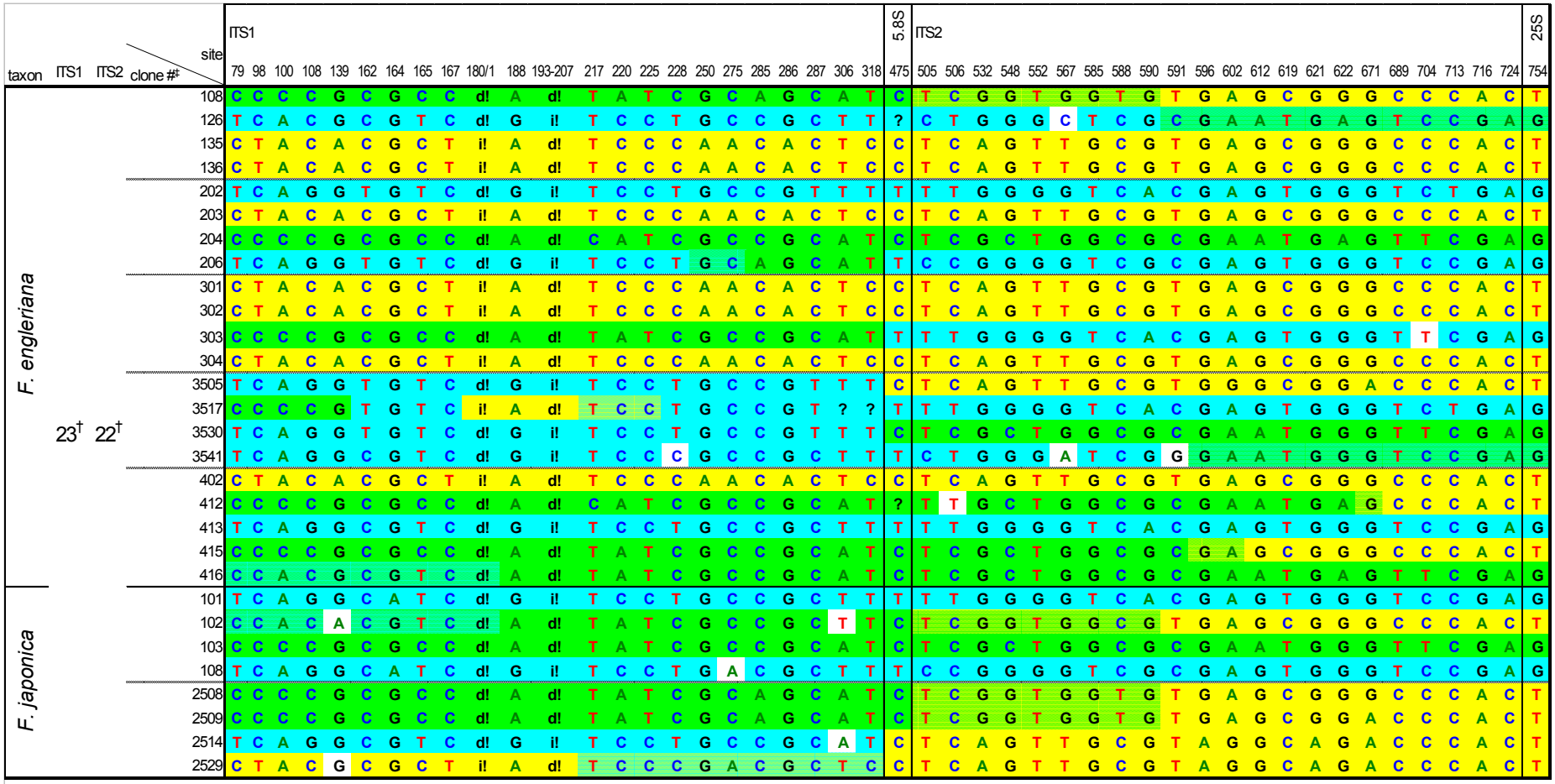
- the usage of directly sequenced PCR products as data source,
- the application of a one-accession-per-taxon strategy to resolve intrageneric relationships, and
- maximum parsimony as the analytic method.

Obviously, the assembling of a cloned DNA library comprehensively reflecting the intrageneric variability and the use of maximum likelihood to infer phylogenetic relationships (DENK et al. 2002; new data: Fig. 3-3) allow to better confirm recent systematic hypotheses obtained by morphological results, e.g. the subdivision in two subgenera as proposed by SHEN (1992), in contrast to earlier published studies (STANFORD 1998; MANOS & STANFORD 2001). In addition, the resolution limits of a classical 'base-per-base' analysis of the ITS, and hence, the reliability of the inferred phylogenetic hypotheses, can be evaluated and qualified *a priori* in the case of *Fagus*. The complete lacking of unambiguous sites, which are parsimony informative sites, is readily visible from the alignment of the assembled sequences (→ appendix). This is especially important, since some divergent points in the topology presented by MANOS & STANFORD (2001) are supported by appropriate bootstrap-values, but cannot be

[28] Accessions from the species *F. chienii* and *F. okamotoi* are missing (cf. footnotes 18 & 16).

sustained, if additional data like the here presented is included, hence, are due to a sampling error.

The most intriguing result of the here assembled data is the fact, that the genetical inter- and intraspecific variability alters remarkably within different 'species'. Moreover, the occurrence of certain site variabilities is obviously restricted to one species or two putative related species. In spite of the comparably low genetical interspecific variability – in comparison to e.g. *Acer* (chapter 4) – competing genotypes can be found within samples from one population (→ Fig. 3-4). In addition, identical genotypes are present in all, or most, populations analysed from a particular taxon, even if they are geographically separated (e.g. competing genotypes of sg. *Engleriana*, Fig. 3-4; consensus genotype found in populations of *F. sylvatica*, cf. appended alignment). As already stated in chapter 1, the actual nucleotide composition of any gene must mirror the phylogenetic history of the taxon and, furthermore, of the whole genus. Thus, the conspicuous intraspecific variability detected for morphologically distinguishable species of *Fagus* (i.e. *F. engleriana, F. crenata, F. hayatae, F. japonica, F. longipetiolata, F. lucida,* and *F. sylvatica*; SHEN 1992; DENK, in press) has to be and can be interpreted in an evolutionary context (following chapters).



† number of conspiciously variable sites, i.e. two or more accessions exhibit a nuclotide differing from the remaining clones of sg. *Engleriana*

‡ clone number, see appendix

Figure 3-4: Competing genotypes in *Fagus* sg. *Engleriana*.
The genotypic characteristics of *F. japonica* from Japan are similar to *F. engleriana* (China mainland and Ullung Is., Korea), especially in the ITS1. Equal background colours indicate an identical nucleotide composition (⇨ genotypes). White boxes differ at this site from the overall realised genotype. **Abbr.:** d! = deletion, i! = insertion (in relation to the consensus of all *Fagus* accessions).

3.3 Analyses of intraspecific nucleotide variabilities as phylogenetic characters – a new methodological approach

The characteristics of the ITS regions, i.e. conspicuous intraspecific variability in the ITS region, make *Fagus* a suitable model system to test the new approach proposed in this chapter, in the following referred to as "ISV analysis". Since substitution models for maximum likelihood contain only probabilities for point mutations, ambiguous data – here represented by intraspecific site variabilities, i.e. clones attained from one individual, population and/or species differ in their nucleotide composition at particular sites – are recognised as "missing" or "uncertain" and the phylogenetical information contained is lost. In an ISV analysis this information is preserved by the coding of site variabilities as characters for a matrix, that allows them to be treated in the same way as morphological characters. Such a matrix can be analysed either by maximum parsimony or maximum likelihood via Bayesian inference.

3.3.1 Data base

To maintain a first coarse statistical fundament, only populations and individuals, respectively, of *Fagus* were included in the analysis, from which at least four clones were sequenced (→ special remark). The nucleotide characteristics of ALF-generated accessions (Turkish localities) were included, if their nucleotide composition could be confirmed by according data from ABI-generated accessions of other clones (Fig. 3-2). Gene bank sequences of other authors (i.e. STANFORD 1998; MANOS & STANFORD 2001) are not included, because they do not provide substantial additional data and may lack crucial information due to the assembling procedure, i.e. direct sequencing of PCR products.

Remark: To provide statistical values (percentages) for the occurrence of co-existing genotypes, at least 10 (number of accessions) times 10 (number of PCR products per extraction) clones per individual and population would be appropriate. Of course, the work force and project money needed to assemble such an amount of data is immense and not fundable. However, from our own experience gained during the studies of intrageneric relationships, it can be assumed, that if two genotypes co-dominate within the ITS of an organism, and the first 2 clones are ± identical, the 3rd and 4th will exhibit the other genotypic composition.

3.3.2 Coding of ITS site variabilities as phylogenetic signals

For the MP analysis the nucleotide sites were combined and transferred into matrices of characters (see below, step 3). These characters comprise the phylogenetical information given in the alignment by the presence and absence of certain intraspecific variabilities. Each character contains up to nine possible states and covers either a single site or a number of sites, i.e. either an oligonucleotide motif or obviously linked sites. Characters in the data matrix meet the requirements for 'good' parsimonious informative characters (chapter 2.4.2), i.e. they are independent from each other as far as this can be achieved. For the ML analysis the characters are coded analogously (details see below).

Due to the overall low ITS variability the correlation of homologous nucleotide sites is easily accomplished by the alignment. In principle, each alignment site exhibiting differing nucleotides is recognised as a character (→ appendix). A site (nucleotide state) variability is defined as follows: clones of one locality – representing the intragenomic and, to a certain degree, the interpopulation variability – either show one or another nucleotide at this site. The composition of the ITS1 and ITS2 in *Fagus* requires not only the recognition of single site variabilities, but also the introduction of complex characters for oligonucleotide motives and linked sites, respectively. Thus, the coding follows a three step protocol:

1st step: 'Compression' of nucleotide data and definition of characters.

2nd step: Evaluation of interpopulation and intraspecific variability.

3rd step: Assembling and coding of character states.

Step 1: Character definition

The compression (transformation) of the nucleotide data is necessary primarily for oligonucleotide motives, such as characters including length polymorphism. Furthermore, several site variabilities at different positions within the analysed gene regions are obviously linked to each other. Thus, they are not strictly independent in a parsimonious sense and have to be treated as one character. One reason for such a linkage is the putative secondary structure of the transcript. Linked sites in the sense of complementary nucleotide pairs may e.g. be found within stem-loop structures and hairpins. In addition, mutations which provoke a shift in the secondary structure or the overall CG-content might be compensated by – not necessarily complementary – mutations in another region of the spacer (TORRES et al. 1990). In the matrices site variabilities are considered to be linked (dependent), when all clones with particular nucleotide types at one site show identical nucleotide types at the putatively linked site. To maintain a coherent layout, single site variabilities are equally transformed.

In general, the nucleotide state or oligonucleotide motif identical to the majority of all accessions included is labelled as A_0[29] (3rd column in Table 3-2). Nucleotide compositions derived through point mutations from the consensus are labelled as B_0, C_0, etc., those derived through insertions and deletions (indels) as X_1, X_2, where X = A,B,... if derived from A_0, B_0, etc (→ Fig. 3-5; 4th column in Table 3-2). The derivation of differing nucleotide compositions follows a maximum parsimony approach based on the fixed-character-state optimisation proposed by WHEELER (1999, 2001). Point mutations and indels are treated equally. Two basic types of site variabilities are recognised:

- **Single site variabilities**: The occurrence of a certain nucleotide state is labelled as A_0, B_0, C_0, etc.
- **Linked site variabilities** and **oligonucleotide motives**: The occurrence of the combination of certain nucleotides, i.e. a specific oligonucleotide motif, is labelled as A_0, B_0, C_0, etc. (A_1, A_2, etc. if indels occur).

Finally, nucleotide states and oligonucleotide motives confined to one locality, provenance or taxon are per definition parsimonious uninformative and neglected.

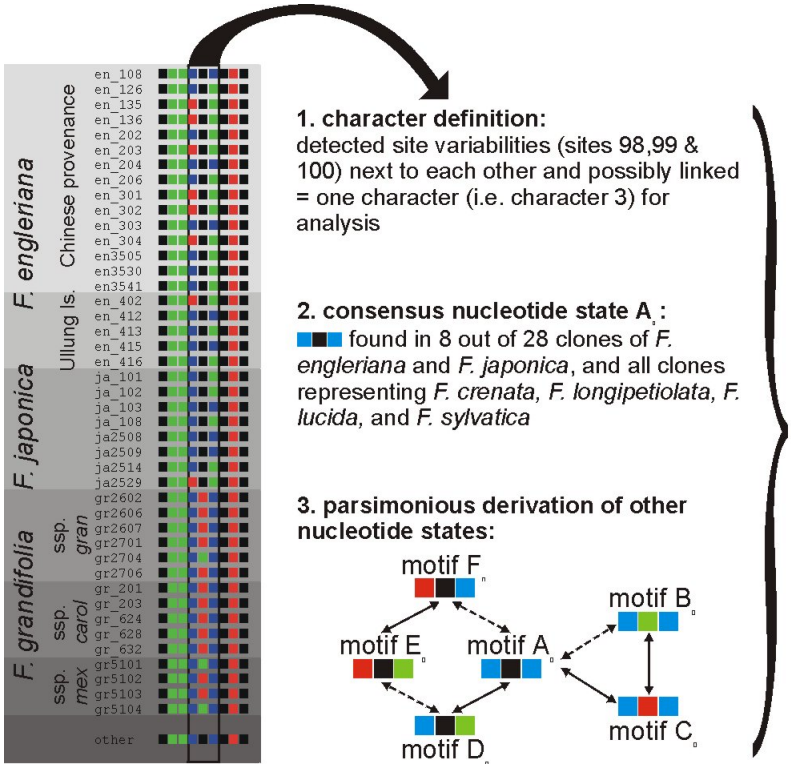


Table 3-2

		species		
		number of clones included in the analysis		
character	alignment sites	consensus (A_0-state)	other states	
1	78,79	CC	TC=B_0	TT=C_0
2	87	C	T=B_0	
3	98-100	CGC	CAC=B_0	
			CTC=C_0	
			CGA=D_0	TGA=E_0
			TGC=F_0	
4	108	C	G=B_0	
5	121	G	A=B_0 C=C_0	

Figure 3-5: Definition of characters and assembling of nucleotide states.

[29] This must not be confused with a hypothetical ancestral genetic composition/nucleotide state.

Table 3-2: Matrix of nucleotide states.

			species	*F. engleriana*				*F. grandifolia*			*F. hayatae*			*F. sylvatica*				
				China	Ullung Is., S.Korea	*F. japonica*	*F. crenata*	ssp. *grandifolia*	ssp. *caroliniana*	ssp. *mexicana*	ssp. *pashanica*	*F. longi-petiolata*	*F. lucida*	Georgia	Turkey	Hungary/ Slovenia	Germany	Italy/ Spain
			number of clones included in the analysis	16	5	8	9	7	5	4	13	16	8	7	10	8	9	16
character	alignment sites	consensus (A_0-state)	other states															
1	78,79	CC	**T**C=B_0 T**T**=C_0	$\{B_0C_0\}$	$\{B_0C_0\}$	$\{B_0C_0\}$	A_0	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	$\{A_0B_0\}$	$\{A_0B_0\}$	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	A_0	$\{A_0B_0\}$	$\{A_0B_0\}$
2	87	C	**T**=B_0	A_0	$\{A_0B_0\}$	A_0	A_0	A_0	A_0	A_0	A_0	$\{A_0B_0\}$	A_0	A_0	A_0	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0
3	98-100	CGC	C**A**C=B_0 C**T**C=C_0 CG**A**=D_0 T**G**C=F_0 T**GA**=E_0	$\{A_0D_0E_0\}$	$\{A_0D_0E_0\}$	$\{A_0D_0E_0\}$	A_0	$\{B_0C_0\}$	C_0	$\{B_0C_0\}$	A_0	A_0	A_0	$\{A_0C_0\}$	A_0	$\{A_0F_0\}$	A_0	A_0
4	108	C	**G**=B_0	$\{A_0B_0\}$	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0
5	121	G	**A**=B_0 **C**=C_0	A_0	A_0	A_0	$\{A_0B_0\}$	A_0	A_0	A_0	X_0	X_0	$\{A_0C_0\}$	$\{A_0B_0\}$	A_0	A_0	$\{A_0B_0\}$	$\{A_0B_0\}$
6	126-128	TTC	**C**TC=B_0 TT**T**=C_0	A_0	A_0	A_0	$\{A_0C_0\}$	A_0	A_0	A_0	A_0	A_0	$\{A_0C_0\}$	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	A_0	$\{A_0B_0\}$
7	135	T	**C**=B_0	A_0	A_0	A_0	A_0	B_0	$\{A_0B_0\}$	B_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0
8	139	G	**A**=B_0	$\{A_0B_0\}$	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	$\{A_0B_0\}$	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0
9	152-157	CGGGGG	C**A**GGGG=B_0 CGGG**xx**=$-_0$	$-_0$	$-_0$	$-_0$	A_0	$-_0$	$-_0$	$-_0$	$\{A_0-_0\}$	$\{A_0-_0\}$	A_0	$\{A_0-_0\}$	A_0	A_0	$\{A_0B_0\}$	X_0
10	159	A	**G**=B_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	$\{A_0B_0\}$	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	A_0	A_0	A_0	A_0
11	162-165	CCGT	**T**CGT=B_0 **A**CGT=C_0 C**T**GT=D_0 CCG**C**=E_0	$\{A_0B_0E_0\}$	$\{A_0E_0\}$	$\{A_0E_0\}$	A_0	A_0	$\{A_0C_0\}$	A_0	$\{A_0B_0D_0\}$	$\{A_0D_0\}$	A_0	$\{A_0B_0C_0\}$	A_0	A_0	A_0	A_0
12	167	C	**T**=B_0	$\{A_0B_0\}$	$\{A_0B_0\}$	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	A_0	A_0	A_0	A_0	A_0	A_0	$\{A_0B_0\}$	A_0	$\{A_0B_0\}$	A_0
13	171,172	CC	**T**C=B_0 **G**C=C_0 C**T**=D_0 C**A**=E_0	A_0	A_0	$\{A_0D_0E_0\}$	$\{A_0D_0\}$	A_0	A_0	$\{A_0C_0\}$	A_0	A_0	A_0	A_0	$\{A_0B_0\}$	A_0	$\{A_0D_0\}$	$\{A_0B_0D_0\}$
14	180-185	CACAAA	**xx**CAAA=$-_0$ C**G**CAAA=B_0 CACA**G**A=C_0	$\{A_0-_0\}$	$\{A_0-_0\}$	$\{A_0-_0\}$	$\{A_0B_0-_0\}$	$\{A_0C_0\}$	$\{A_0C_0\}$	$\{A_0C_0\}$	A_0	A_0	A_0	$\{A_0-_0\}$	A_0	A_0	A_0	A_0
15	187-207	short type	long type=B_0	$\{A_0B_0\}$	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0
16	212	G	**A**=B_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	A_0	A_0	A_0	A_0	A_0
17	216,217	GT	**A**T=B_0 G**C**=C_0	$\{A_0B_0C_0\}$	$\{A_0C_0\}$	A_0	A_0	A_0	A_0	A_0	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	A_0	A_0	A_0	A_0	A_0
18	220-226	CAACC	CAA**AA**CC=A_1 **T**AACC=B_0 **A**AACC=C_0 CAA**A**C=E_0 G**G**AA**G**C=F_0 AAA**T**C=D_0	$\{A_0D_0\}$	$\{A_0D_0\}$	$\{A_0D_0\}$	A_0	A_0	A_0	A_0	A_0	$\{A_0A_1\}$	$\{A_0C_0\}$	A_0	$\{A_0E_0\}$	$\{A_0B_0F_0\}$	$\{A_0F_0\}$	A_0
19	228	C	**T**=B_0 **G**=C_0	$\{A_0B_0\}$	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	A_0	A_0	A_0	A_0	$\{A_0C_0\}$	A_0	A_0	A_0	A_0	A_0	A_0
20	233,234	GT	**A**T=B_0 G**C**=C_0 G**A**=D_0	A_0	$\{A_0D_0\}$	A_0	$\{A_0B_0\}$	$\{A_0C_0\}$	A_0	A_0	A_0	A_0	$\{A_0C_0\}$	A_0	A_0	A_0	$\{A_0B_0\}$	A_0
21	240	G	**T**=B_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	A_0	A_0
22	249,250	CG	**T**G=B_0 C**A**=C_0	$\{A_0C_0\}$	$\{A_0C_0\}$	A_0	A_0	A_0	A_0	$\{A_0B_0\}$	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0
23	269	T	**C**=B_0	A_0	A_0	A_0	$\{A_0B_0\}$	$\{A_0B_0\}$	$\{A_0B_0\}$	B_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0
24	275	C	**A**=B_0	$\{A_0B_0\}$	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0
25	284-286	TCG	**C**CG=B_0 T**A**G=C_0 TC**A**=D_0	X_0/B_0 †	$\{A_0D_0\}$	$\{A_0C_0\}$	A_0	A_0	A_0	A_0	A_0	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	A_0	A_0	A_0	A_0
26	291,292	CC	**T**C=B_0 C**T**=C_0	A_0	A_0	$\{A_0C_0\}$	A_0	A_0	A_0	A_0	A_0	A_0	A_0	$\{A_0B_0\}$	$\{A_0B_0\}$	A_0	A_0	A_0
27	294	C	**A**=B_0	A_0	A_0	A_0	A_0	A_0	$\{A_0B_0\}$	A_0	A_0	A_0	A_0	A_0	$\{A_0B_0\}$	A_0	A_0	A_0
28	306,318	T...C	T...**T**=B_0 T...**A**=D_0 **A**...T=C_0	X_0/D_0	X_0/D_0	X_0/D_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0
29	310	C	**T**=B_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	$\{A_0B_0\}$
30	316	G	**A**=B_0	A_0	A_0	A_0	$\{A_0B_0\}$	A_0	A_0	A_0	A_0	$\{A_0B_0\}$	A_0	$\{A_0B_0\}$	A_0	A_0	A_0	A_0

Table 3-2 (cont.)

31	505,506	CC	**T**C=B_0 C**T**=C_0	T**T**=D_0	X_0‡	{B_0D_0}	X_0/C_0	{A_0B_0}	B_0	B_0	B_0	A_0	{A_0B_0}	{A_0B_0}	{A_0B_0}	{A_0B_0}	A_0	{A_0C_0}	X_0/D_0
32	512	C	**T**=B_0		A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	{A_0B_0}	{A_0B_0}	A_0	A_0	A_0	A_0	A_0
33	521-525	CCCC	CC**T**CC=A_1 CCC**T**=C_0		C_0	C_0	C_0	A_0	{A_0A_1}	{A_0A_1}	A_0	{A_0C_0}	{A_0C_0}	{A_0C_0}	A_0	A_0	A_0	A_0	A_0
34	527	G	**A**=B_0		A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	{A_0B_0}	A_0
35	531-533	CGC	**T**GC=B_0 C**A**C=C_0 CG**T**=D_0		{A_0C_0}	{A_0C_0}	{A_0C_0}	{A_0B_0}	A_0	A_0	A_0	{A_0B_0}	X_0/C_0	{A_0B_0}	A_0	A_0	A_0	A_0	A_0
36	534-538	gap	CTCCC insert=B_0		A_0	A_0	A_0	A_0	A_0	A_0	A_0	{A_0B_0}	A_0	A_0	A_0	A_0	A_0	A_0	A_0
37	544-548	GCGCG	**C**CGCG=B_0 G**T**GCG=C_0 GC**C**CG=D_0 GC**T**CG=E_0 GCG**T**G=F_0 GCGC**C**=G_0		{A_0G_0}	{A_0G_0}	{A_0G_0}	A_0	A_0	A_0	A_0	{$A_0C_0D_0F_0G_0$}	{$A_0B_0F_0$}	{$A_0B_0C_0D_0$}	A_0	{$A_0C_0D_0E_0$}	A_0	{A_0C_0}	{$A_0C_0E_0$}
38	552	T	**G**=B_0		{A_0B_0}	{A_0B_0}	{A_0B_0}	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0
39	555	C	**T**=B_0		{A_0B_0}	A_0	A_0	A_0	A_0	A_0	A_0	A_0	{A_0B_0}	A_0	A_0	{A_0B_0}	A_0	A_0	A_0
40	562-565	TGG	**C**GG=B_0 TG**A**=C_0	CGG**G**=B_1	A_0	A_0	A_0	A_0	{A_0B_0}	{A_0B_0}	A_0	X_0/B_0	{A_0B_0}	{A_0B_0}	A_0	A_0	A_0	{A_0C_0}	A_0
41	567	G	**A**=B_0 **T**=C_0 **C**=D_0		X_0	{A_0C_0}	A_0	A_0	A_0	A_0	A_0	A_0	{A_0B_0}	A_0	{A_0B_0}	{A_0B_0}	A_0	A_0	A_0
42	585	G	**T**=B_0		{A_0B_0}	{A_0B_0}	{A_0B_0}	A_0	A_0	A_0	A_0	{A_0B_0}	{A_0B_0}	A_0	A_0	A_0	A_0	A_0	A_0
43	588-591	CTGT	**T**GTG=B_0 C**G**GT=C_0 CT**A**T=G_0 CTG**C**=D_0 CTG**G**=F_0	CT**A**C=E_0	{$A_0B_0D_0E_0F_0$}	{$A_0D_0E_0$}	{$A_0B_0D_0E_0$}	A_0	A_0	A_0	A_0	A_0	{A_0D_0}	A_0	A_0	{$A_0C_0G_0$}	A_0	A_0	A_0
44	595,596	CG	**T**G=B_0 C**A**=C_0		A_0	A_0	{A_0C_0}	A_0	A_0	A_0	A_0	A_0	A_0	X_0	A_0	{A_0B_0}	A_0	A_0	A_0
45	602	A	**G**=B_0		{A_0B_0}	A_0	{A_0B_0}	A_0	A_0	A_0	A_0	{A_0B_0}	A_0	A_0	A_0	{A_0B_0}	A_0	A_0	A_0
46	612	G	**A**=B_0 **C**=C_0 **T**=D_0		{A_0B_0}	{A_0B_0}	{A_0B_0}	A_0	A_0	A_0	A_0	{A_0C_0}	{A_0C_0}	A_0	{A_0D_0}	A_0	{A_0D_0}	A_0	A_0
47	619	T	**C**=B_0		{A_0B_0}	{A_0B_0}	{A_0B_0}	{A_0B_0}	A_0	A_0	A_0	{A_0B_0}	{A_0B_0}	A_0	{A_0B_0}	A_0	A_0	A_0	{A_0B_0}
48	622	G	**A**=B_0 **T**=C_0		{A_0B_0}	{A_0B_0}	A_0	A_0	A_0	A_0	A_0	A_0	{A_0C_0}	A_0	A_0	A_0	A_0	A_0	A_0
49	626	C	**T**=B_0		A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	{A_0B_0}	A_0	A_0	A_0	A_0	A_0
50	653	T	**G**=B_0		B_0	B_0	B_0	A_0	B_0	B_0	B_0	{A_0B_0}	{A_0B_0}	A_0	{A_0B_0}	{A_0B_0}	A_0	A_0	A_0
51	671	G	**A**=B_0		{A_0B_0}	A_0	{A_0B_0}	A_0	A_0	A_0	A_0	A_0	{A_0B_0}	A_0	A_0	A_0	A_0	A_0	A_0
52	674	C	**T**=B_0		A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	{A_0B_0}	A_0	A_0	A_0	A_0
53	676	T	**C**=B_0		A_0	A_0	A_0	{A_0B_0}	A_0	A_0	A_0	{A_0B_0}	{A_0B_0}	{A_0B_0}	A_0	A_0	A_0	A_0	A_0
54	679	C	**T**=B_0		{A_0B_0}	A_0	{A_0B_0}	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0
55	686	C	**T**=B_0		A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	{A_0B_0}	A_0
56	689-691	CAA	CA**C**=B_0	**T**AC=C_0 C**G**C=D_0	{B_0C_0}	{B_0C_0}	{B_0C_0}	{A_0B_0}	B_0	B_0	B_0	{A_0C_0}	X_0/D_0	{A_0B_0}	{A_0B_0}	X_0/C_0	X_0/C_0	{A_0D_0}	X_0/C_0
57	702	G	**A**=B_0 **T**=C_0		A_0	A_0	A_0	X_0	A_0	A_0	A_0	A_0	{A_0B_0}	{A_0C_0}	A_0	A_0	A_0	A_0	A_0
58	704	C	**T**=B_0		{A_0B_0}	{A_0B_0}	{A_0B_0}	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0
59	709	C	**T**=B_0		A_0	{A_0B_0}	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	{A_0B_0}	A_0	A_0	A_0	A_0
60	713	C	**T**=B_0		{A_0B_0}	A_0	A_0	A_0	A_0	A_0	A_0	{A_0B_0}	{A_0B_0}	A_0	A_0	A_0	A_0	A_0	A_0
61	716...724	G...C	G...**A**=B_0 **A**...C=C_0		{B_0C_0}	{B_0C_0}	{B_0C_0}	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0	A_0
62	736	A	**G**=B_0		A_0	A_0	A_0	A_0	{A_0B_0}	{A_0B_0}	B_0	A_0	A_0	A_0	{A_0B_0}	A_0	A_0	A_0	A_0

† X_0/Y_0 = all (oligo)nucleotide states except for Y_0 realised

‡ X_0 = all (oligo)nucleotide states realised

Step 2: Evaluation of interpopulation and intraspecific variability

The nucleotide composition of all clones of one species or geographical origin – in the case of *F. engleriana, F. grandifolia,* and *F. sylvatica* – is summed up (→ Table 3-2, 5th and following columns). For example, taxon *A* is represented by two populations. If clones of population #1 exhibit either nucleotide state A_0 or B_0 and clones of population #2 A_0 or C_0, than the 'sum' for taxon *A* is "$\{A_0B_0C_0\}^{30}$", i.e. taxon *A* shows a site variability comprising the nucleotide states "A_0", "B_0" *and* "C_0". Due to the uncertain taxonomical status of certain subspecies/species of *Fagus* and different numbers of populations sampled per species, accessions of some species are summed up in slightly different ways: Accessions of *F. engleriana* are divided into Chinese and South Korean provenances, those of *F. grandifolia* into Mexican, south-eastern North American, and eastern North American provenances corresponding to three subspecies recognised for *F. grandifolia* (Flora of North America). *Fagus sylvatica* accessions are subdivided into geographical regions.

Step 3: Basic coding

The summed up variabilities (step 2) found for distinct taxa and defined geographical areas - in the case of *F. engleriana* and *F. sylvatica* - form the basis of the matrices. The coding is based on the occurrence and/or lack of certain variabilities. If some taxa show a nucleotide state A_0, others a state B_0, and the rest either A_0 or B_0, the matrices contain three character states: a = A_0, no variability; b = $\{A_0,B_0\}$, genetic variability is preserved in different populations and individuals, and c = B_0, no variability. Three character types are distinguished: (a) binary characters, (b) ordered characters, and (c) complex characters:

(a) **Binary characters** are defined by the occurrence of one type of site variability: a=A_0, no variability; b=$\{A_0,B_0\}$, conspicuous site variability. Binary characters are treated as unordered in both the MP and the ML analyses.

(b) **Ordered characters** comprise characters with two or more variabilities, as well as characters with two different nucleotide states like in the example given above (a = A_0, b = $\{A_0,B_0\}$, and c = B_0). An example for a simple ordered character with two variabilities and the underlying nucleotide composition is given in Figure 3-6 (a = $\{A_0B_0\}$, b = A_0, and c = $\{A_0C_0\}$). Ordered characters are defined as "ordered" for the analyses, i.e. it takes two steps from "a" to "c" for the given examples under maximum parsimony. Under the ML

method the direct transformation of "a" to "c" is prohibited by setting the character type to ordered.

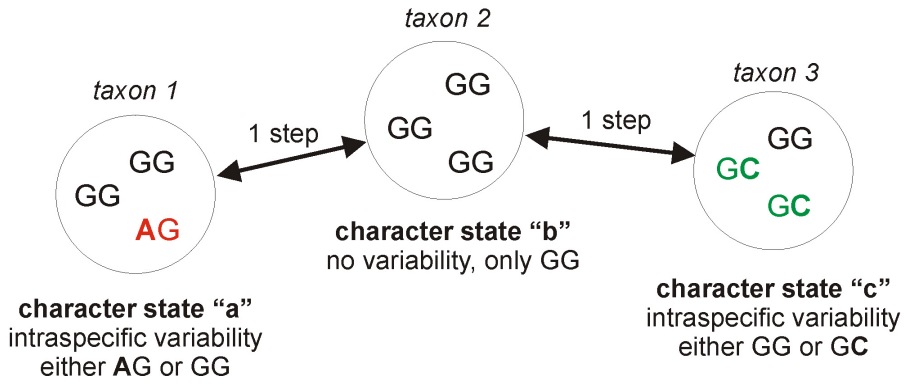


Figure 3-6: Example for a three-state ordered character.
Circles indicate the gene pool of taxon #1,#2 & #3 (≙ nucleotide states detected in clones from several populations or individuals).

(c) Characters comprising a number of possible variabilities and/or lack certain variabilities are coded as **complex characters**. Based on the steps required from one nucleotide type to another, a stepmatrix is defined, which codes the occurrence and loss of individual variabilities for this character. A very simple stepmatrix (3 competing site variabilities + lack of variability) is defined in the "div_var" stepmatrix (→ Fig. 3-7). Stepmatrices for all complex characters used in the analyses are provided in the appendix. Since maximum likelihood is a process-, not character-based analysing method, stepmatrices make no sense. Instead, characters coded as stepmatrices in the MP analyses are either treated as unordered characters, or divided into a group of binary and/or ordered characters. The resulting matrix for the basic coding is given in Table 3-3, for the complete code list (character number – character type – coding) refer to the appendix.

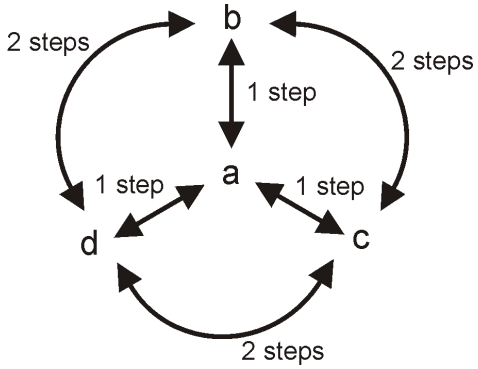


Figure 3-7 (right): DIV_VAR stepmatrix.
Example for a complex character with 4 possible character states. Character state "a": no intrataxonomic variability detected, character states "b", "c", and "d": different intrataxonomic variabilities found.

[30] The brackets are used according to the nomenclature for polymorphic states used in PAUP, MacClade, and other programs for phylogenetic analyses. {AB} equals A ***and*** B, (AB) equals A ***or*** B.

Table 3-3: Character matrix for basic coding.

		ITS1																												
	character	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
F. engleriana	China	d	a	f	b	a	b	a	b	d	a	h	b	a	b	b	a	d	d	a	a	a	c	a	b	e	b	a	d	a	a
	Ullung	d	b	f	b	a	b	a	b	d	a	e	b	a	b	b	a	c	d	a	d	a	c	a	b	d	b	a	d	a	a
F. japonica		d	a	f	b	a	b	a	b	d	a	e	b	g	b	b	a	a	d	a	a	a	b	a	b	c	c	a	d	a	a
F. crenata		a	a	a	a	b	a	a	a	b	a	a	b	d	d	a	a	a	a	b	b	a	b	a	a	a	b	a	b	a	b
F. grandifolia	ssp. *grandifolia*	a	a	c	a	a	b	c	a	d	a	a	a	a	g	a	a	a	a	b	c	a	b	c	a	a	b	a	b	a	a
	ssp. *caroliniana*	b	a	c	a	a	b	b	a	d	a	c	a	a	f	a	a	a	a	b	a	a	b	b	a	a	b	b	b	a	a
	ssp. *mexicana*	a	a	d	a	a	b	c	a	d	a	a	a	c	f	a	a	a	a	b	a	a	a	c	a	a	b	a	b	a	a
F. hayatae	ssp. *pashanica*	b	a	a	a	e	b	a	a	c	b	g	a	a	a	a	b	b	a	b	a	a	b	a	a	a	b	a	b	a	a
F. longipetiolata		b	b	a	a	e	b	a	a	c	b	d	a	a	a	a	b	a	b	c	a	a	b	a	a	b	b	a	b	a	b
F. lucida		b	a	a	a	d	a	a	a	b	b	a	a	a	a	a	a	a	c	b	c	a	b	a	a	b	b	a	b	a	a
F. sylvatica	Georgien	b	a	b	a	b	c	a	a	c	a	f	a	a	b	a	a	a	a	b	a	b	b	a	a	a	a	a	b	a	b
	Turkey	a	a	a	a	a	c	a	a	b	a	a	b	b	a	a	a	a	e	b	a	b	b	a	a	a	a	b	b	a	a
	Hungary, Slovenia	a	b	g	a	a	b	a	a	b	a	a	a	a	a	a	a	a	g	b	a	a	b	a	a	a	b	a	b	a	a
	Germany	b	b	a	a	b	b	a	a	a	a	a	b	d	a	a	a	a	f	b	b	a	b	a	a	a	b	a	b	a	a
	Italy, Spain	b	a	a	a	b	b	a	a	e	a	a	a	f	a	a	a	a	a	b	a	a	b	a	a	a	b	a	b	b	a

		ITS2																													
	character	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62
F. engleriana	China	f	a	a	a	c	a	g	b	b	a	d	b	b	a	b	b	b	a	a	c	b	a	a	b	a	f	a	b	a	b	d	a
	Ullung	d	a	a	a	c	a	g	b	a	a	c	b	c	a	a	b	b	a	a	c	a	a	a	a	a	f	a	b	b	a	d	a
F. japonica		e	a	a	a	c	a	g	b	a	a	a	b	b	c	b	b	b	b	a	c	b	a	a	b	a	f	a	b	a	a	d	a
F. crenata		b	a	c	a	b	a	a	a	a	a	a	a	a	a	a	a	a	b	a	a	a	a	b	a	a	b	d	a	a	a	a	a
F. grandifolia	ssp. *grandifolia*	c	a	d	a	a	a	a	a	a	b	a	a	a	a	a	a	a	b	a	c	a	a	a	a	a	c	a	a	a	a	a	c
	ssp. *caroliniana*	c	a	d	a	a	a	a	a	a	b	a	a	a	a	a	a	a	b	a	c	a	a	a	a	a	c	a	a	a	a	a	b
	ssp. *mexicana*	c	a	c	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	a	c	a	a	a	a	a	c	a	a	a	a	a	c
F. hayatae	ssp. *pashanica*	a	a	b	a	b	b	f	a	a	d	a	b	a	a	b	c	b	b	a	b	a	a	b	a	a	d	a	a	a	b	a	a
F. longipetiolata		b	b	b	a	e	a	b	a	b	b	b	b	f	a	a	c	b	c	a	b	b	a	b	a	a	e	b	a	a	b	a	a
F. lucida		b	b	c	a	b	a	d	a	a	b	a	a	a	d	a	a	a	b	b	a	a	a	b	a	a	b	c	a	a	a	a	a
F. sylvatica	Georgien	b	a	c	a	a	a	a	a	a	a	b	a	a	a	a	d	b	b	a	b	a	b	a	a	a	b	a	a	b	a	a	b
	Turkey	b	a	c	a	d	a	e	a	b	a	b	a	e	b	b	a	a	b	a	b	a	a	a	a	a	h	a	a	a	a	a	a
	Hungary, Slovenia	a	a	c	a	a	a	a	a	a	a	a	a	a	a	a	d	a	b	a	a	a	a	a	a	a	h	a	a	a	a	a	a
	Germany	g	a	c	b	a	a	c	a	a	c	a	a	a	a	a	a	a	b	a	a	a	a	a	a	b	g	a	a	a	a	a	a
	Italy, Spain	h	a	c	a	a	a	i	a	a	a	a	a	a	a	a	a	b	b	a	a	a	a	a	a	a	h	a	a	a	a	a	a

3.3.3 Weighting

To take into account different kinds of characters (e.g., binary vs. complex characters, characters comprising one site variability vs. characters comprising several site variabilities), distinct weighting procedures were applied for MP analyses. Analyses have been performed with the following parameters (summed up in Table 3-4):

1. "Unweighted": no weighting applied, all characters of the same weight.
2. "Binary-doubled": ordered and complex characters = 1, binary characters = 2.
3. "Complex-penalised": complex characters =1, ordered and binary characters = 2.
4. "Levelled": characters weighted anti-proportional to number of character states.

The proposed weighting sets allow to distinguish between the impact of few-state binary and ordered characters (2 to 4 character states) on the phylogenetic hypotheses in comparison to many-state complex characters (up to 9 character states). Oligonucleotide motives often include site variabilities confined to a single taxon, hence, in a strict sense, are uninformative, but obviously derived from commonly distributed site variabilities, and consequently do influence the overall phylogenetic hypothesis. As it will be shown by the maximum parsimony reconstruction (MPR) in the following chapter (Fig. 3-14) the different classes of characters (binary, ordered, complex) contribute to a varying degree to the final phylogenetic reconstruction.

Since the application of weighting is from a methodically and theoretically point of view prohibited in ML analyses, the MP weighting was simulated by the duplication of the according characters and manipulating the character type (Table 3-4; for the appropriate character matrices refer to the NEXUS files provided in the appendix).

Table 3-4: Weighting sets for the MP analyses.

	binary doubled	complex penalised	levelled
binary characters	weight = 2	2	$N^{\dagger}/9$
ordered	1	2	$N^{\dagger}/9$
complex	1	1	$N^{\dagger}/9$
ML setting	binary duplicated	binary and complex duplicated	all set to 'unordered'

† N = number of character states (max. 9)

3.4 Reconstruction of intrageneric relationships inferred from intraspecific site variabilities within the ITS

The newly introduced methodological approach presented in the preceding chapter allows to infer phylogenetic relationships and admit a further insight in the pathways of molecular evolution in *Fagus*. In this chapter, the phylogenetic reconstruction based on the ISV analysis (chapter 3.4.2) is compared with a classical 'base-per-base' ML/BI analyses on the same data set and evidence from other data sources as provided by morphological investigations (DENK, in press) and the fossil record (DENK 1999c; T. Denk, person. comm.; T. Denk & G. Grimm, in prep.).

3.4.1 Classical 'base-per-base' analysis using Bayesian inference as control run

BI analyses were performed for means of comparison with the following parameters: 1,000,000 generations on five parallel Monte-Carlo-Markov-chains, each 100th tree saved. Numbers of possible substitutions sites was set to six, which were assumed to be gamma-distributed (≙ GTR+Γ+I substitution model; cf. FELSENSTEIN 2001; POSADA & CRANDALL 2001). Only accessions were included, which where used for the ISV analysis (cf. chapter 3.3.1). For means of direct comparison with the variability analysis (data summed up for species, respectively geographical provenances: cf. chapter 3.3.2), strict consensus sequences were constructed, which sum up the nucleotide data of all clones from one taxon, respectively geographical region in the case of *F. engleriana* and *F. sylvatica*, and analysed accordingly. For example, if five clones of taxon #1 exhibit an "A" at alignment site x, and one clone shows a "G", the appropriate base pair in the strict consensus sequence is "R" (for purine). In accordance with current ML models gaps are recognised as miscellaneous ("N", standard nucleotide code, → appendix). Statistics for the 'base-per-base' analysis including permuted likelihood parameters are provided in Table 3-5.

Table 3-5: Statistical parameters for the BI analysis.

				95% Cred. Interval	
	Parameter	Mean	Variance	Lower	Upper
substitution probabilities	r(G<->T)	1	0	1	1
	r(C<->T)	7.52	2.19	4.97	10.92
	r(C<->G)	0.6	0.03	0.34	1
	r(A<->T)	1.45	0.33	0.56	2.8
	r(A<->G)	6.74	2.41	4.2	10.25
	r(A<->C)	1.31	0.14	0.7	2.15
base frequencies	pi(A)	0.186	0	0.161	0.212
	pi(C)	0.333	0	0.301	0.366
	pi(G)	0.28	0	0.248	0.312
	pi(T)	0.201	0	0.176	0.227
shape parameter of gamma distribution		0.05	0	0.05	0.05
proportion of invariable sites		0.013	0	0.003	0.043

Based on a total of 7745 samples out of a total of 10001 samples recorded

An unrooted majority rule consensus tree representing the most probable phylogeny for *Fagus* on the basis of the ITS accessions used is given in Figure 3-8. The overall topology is equal to the ML phylograms presented in DENK et al. (2002, Fig. 4, p. 228) and Figure 3-3[31]. The accessions group into four distinct lineages. Very high *a posteriori* probabilities can be found for the common base of the subgenus *Engleriana* (lineage I, 100%) and accessions of *F. grandifolia* (lineage II, 89%). Two taxa represent the subgenus *Engleriana*: *F. engleriana* (China mainland and South Korea) and *F. japonica* (Japan). These taxa are genetically indistinct and share the presence of at least two genotypes, of which one can easily be distinguished by a prominent 13 bp long indel within the ITS1. Most accessions representing the Eurasian taxa of the subgenus *Fagus* are not resolved as distinct clades (lineage IV), with the exception of a number of accessions from clones of *F. hayatae* ssp. *pashanica* and *F. longipetiolata* (lineage III, 100%). Nevertheless, the distinction of lineage IV from the lineages I-III is supported by an *a posteriori* probability of 72%. Accessions of *F. crenata, F. hayatae* ssp. *pashanica*, *F. longipetiolata*, and *F. lucida* are distributed throughout the phylogenetical plateau representing most of the Eurasian accessions of the subgenus *Fagus*. However, these accessions never occur completely isolated but grouped with at least one more accession of the same taxon, but from another population. Also in lineage IV clones of *F. hayatae* ssp. *pashanica*, again, plot together with certain *F. longipetiolata* clones.

[31] For a detailed comparison between DENK et al. (2002), Fig. 4, and Fig. 3-3 refer to chapters 3.2.2 & 3.2.3.

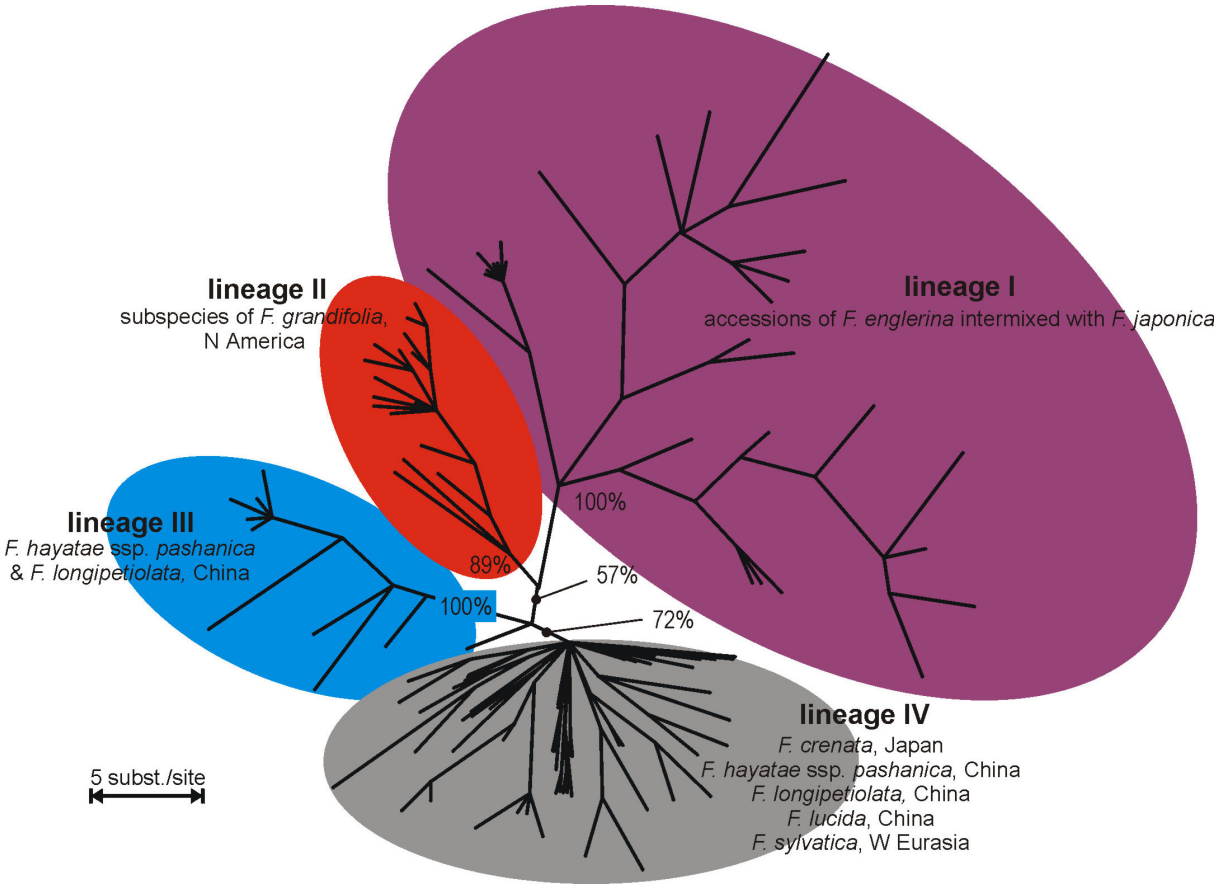


Figure 3-8: ML phylogram of *Fagus* based on the nucleotide data of ITS1 and ITS2.
Computed via BI. Consensus tree of 7745 saved topologies from 1,000,000 generations on 5 parallel chains. Note, that accessions representing *F. hayatae* ssp. *pashanica* and *F. longipetiolata* occur within two distinct genetic lineages. Lineage I correlates with sg. *Engleriana*, lineages II, III, IV include accessions of taxa assigned to sg. *Fagus*. Percentages at selected nodes indicate *a posteriori* probabilities of according topologies, branches found in less than 50% of the saved topologies are collapsed.

A similar topology is produced when the strict consensus sequences are used for the analysis (→ Fig. 3-9). Unambiguous sites are completely missing in the underlying alignment. Therefore, a 'base-per-base' MP analysis – like those conducted by STANFORD (1998) and MANOS & STANFORD (2001) with rather limited ITS data sets – is critical. However, ambiguity – actually representing intraspecific variability – of the subgenus *Engleriana* and *F. grandifolia* are not found in the consensus sequences of the Eurasian taxa of the subgenus *Fagus*. This is reflected by sufficient *a posteriori* probabilities segregating the lineages I (subgenus *Engleriana*, supported by 60%) and II (subspecies of *F. grandifolia*, 97%) from other *Fagus* spp. Within the lineage III+IV, the support values for putative divergence points are extremely low (<15%).

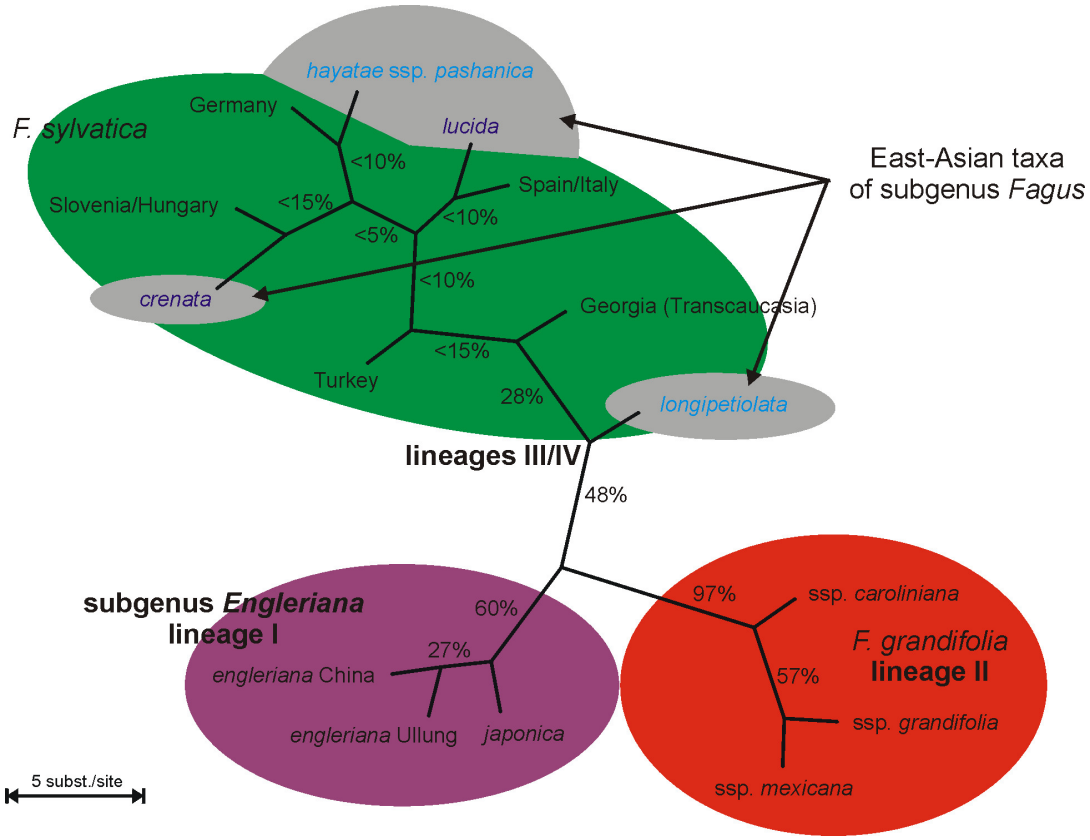


Figure 3-9: ML phylogram inferred from strict consensus sequences.
Computed via BI (1,000,000 topologies, 5 chains, every 100th topology saved). Percentages at nodes indicate *a posteriori* probabilities computed from 9988 topologies.

As indicated in chapter 3.2, apart from the distinction between the subgenera *Engleriana* and *Fagus*, only the systematical position of *F. grandifolia* is clearly determined by the 'base-per-base' analysis. The taxonomical position of *F. hayatae* ssp. *pashanica* and *F. longipetiolata,* with clones occurring in lineage III and IV, is obscure. Finally, the relationships of *F. crenata, F. lucida,* and *F. sylvatica* remain unresolved.

3.4.2 Phylogeny inferred by ISV analysis

With no weight set applied and standard ordered characters (cf. chapter 3.3.3), the branch-and-bound[32] analysis performed for the taxon matrix suggests *F. grandifolia* is intermediate between the subgenus *Engleriana* and the remaining species of the subgenus *Fagus* (in all 16 most parsimonious trees - MPT; → Fig. 3-10), which is in full accordance with the results of

[32] The "branch-and-bound" algorithm allows to actually find the most parsimonious tree (SWOFFORD 1998), as it is otherwise only achievable by computing and evaluating all possible trees. Unlike it is in the case of heuristic search algorithms, an additional statistical test (e.g. bootstrap), whether the computed topologies are actually the 'shortest' (most parsimonious), is not necessary.

the 'base-per-base' analyses. *Fagus hayatae* ssp. *pashanica* and *F. longipetiolata* are part of a clade comprising *F. crenata*, *F. lucida,* and *F. sylvatica* (all MPT). However, since variabilities are now treated as character changes, *F. hayatae* ssp. *pashanica* and *F. longipetiolata* are markedly derived and well-supported as sister taxa (all MPT). The most remarkable result is the placement of *F. crenata* and *F. lucida* as sister taxa in most MPT (12/16). Representatives of *F. sylvatica* come out as more or less derived, but do not form a distinct clade (cf. chapter 3.2).

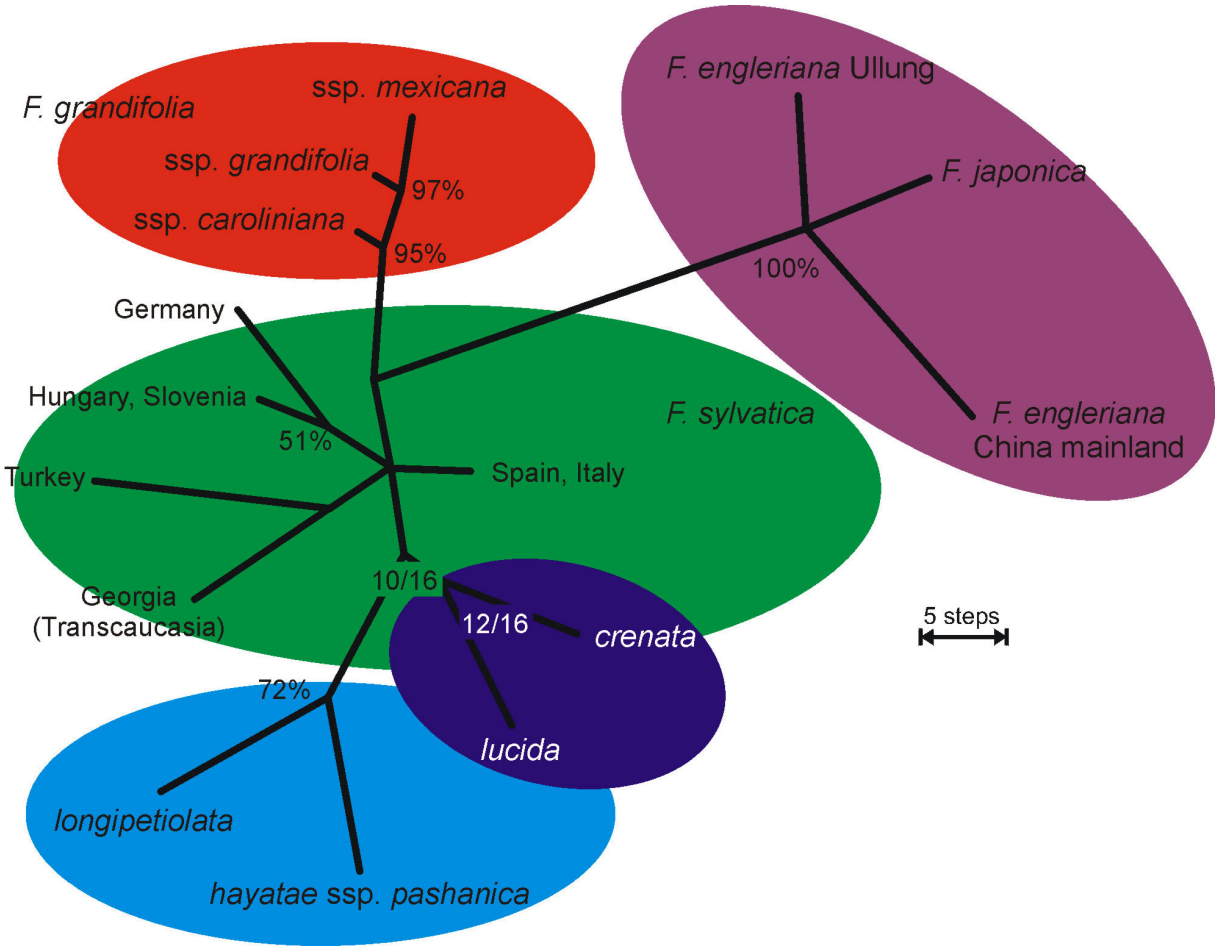


Figure 3-10: MP phylogram inferred from intraspecific variabilities with no weighting applied (weight set "unweighted").
The shown topology equals the topology of a majority rule consensus tree of 16 MPT computed via a branch-and-bound search. Branches occurring in less than half of the MPT are ignored. One step is equivalent to the loss or gain of a site variability (i.e. change of character state). Note, that *F. hayatae* ssp. *pashanica* and *F. longipetiolata*, as well as *F. crenata* and *F. lucida* are recognised as sister taxa. Numbers at nodes refer to the number of MPT, which show the according divergence point. Divergence points without numbers occur in all MPT. Percentages at branches indicate *a posteriori* probabilities (only >50% shown) computed from the analogously performed BI analysis.

Because of the miscellaneous composition of the characters a weighting system was applied to the data matrices. Figure 3-11 shows the consensus trees of all MP phylograms of the different runs performed with the above-described weighting sets "binary-doubled", "complex-penalised", and "levelled". Again, in all runs the subgenus *Engleriana* is most derived in comparison to the taxa of the subgenus *Fagus* (identical to Figs. 3-8 to 3-10). In addition, *Fagus hayatae* ssp. *pashanica* and *F. longipetiolata* consistently come out as sister taxa, and the subspecies of *F. grandifolia* form a monophyletic group. In contrast to the analyses without distinctive weighting, all MPT recognise *F. hayatae* ssp. *pashanica* + *F. longipetiolata* as sister clade to other representatives of the subgenus *Fagus*. *Fagus grandifolia* then appears as a distinct derived clade within or - in a few MPT - as a sister clade to the remaining taxa. Dependent on the applied weighting set, *F. crenata* and *F. lucida* are recognised as sister taxa in nearly all MPT ("binary-doubled" applied: 5 of 7 MPT, "complex-penalised": 17 of 19 MPT, "levelled": all 5 MPT). Primarily lacking any site variabilities, Central and eastern European populations of *F. sylvatica* are grouped together and placed as sister group to *F. crenata* + *F. lucida*. The exact position of Georgian + Turkish, and southern European *F. sylvatica* populations in relation to this group (Central and East European *F. sylvatica* and *F. crenata* + *F. lucida*) and *F. grandifolia*, respectively, varies according to the weighting set used.

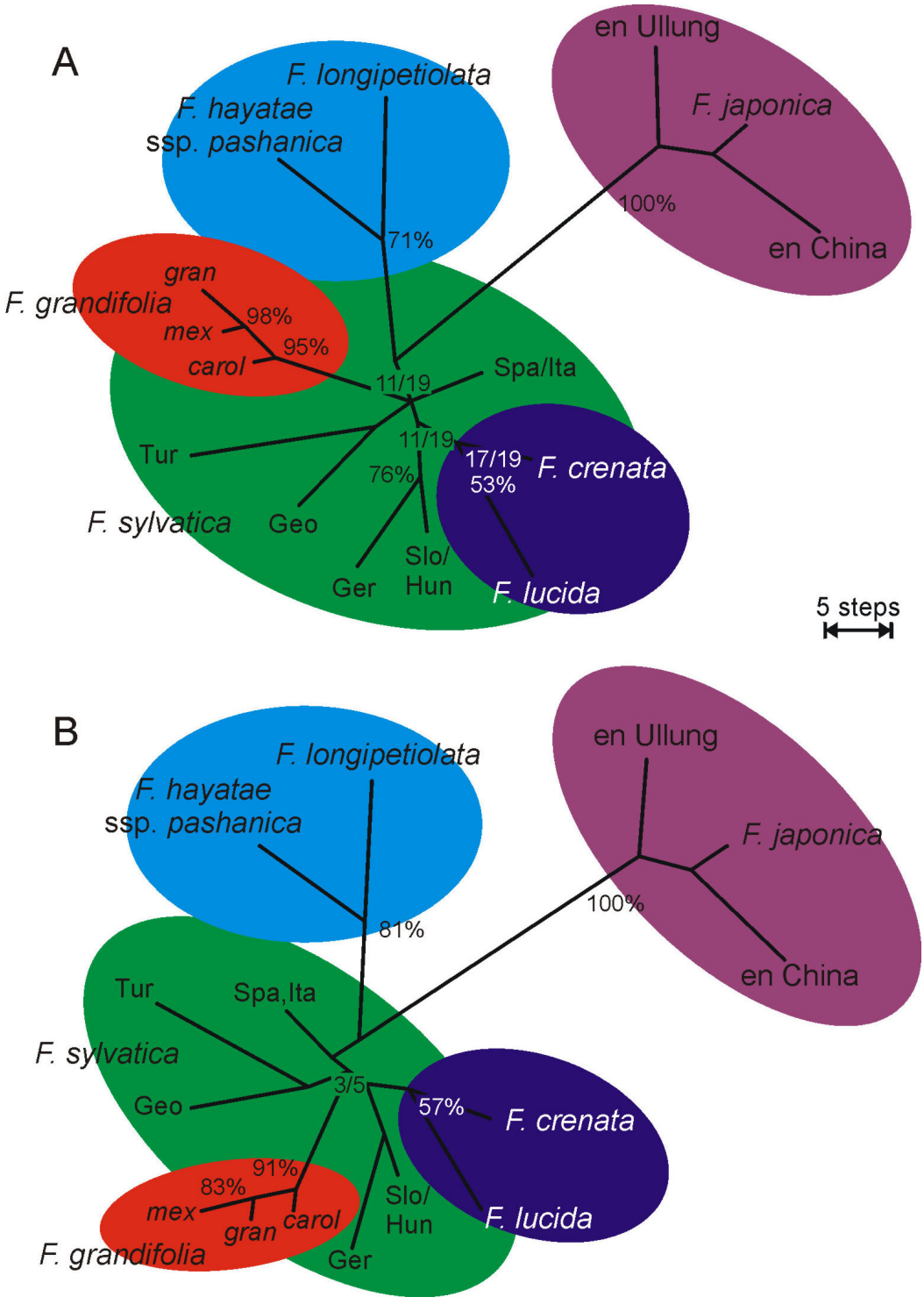


Figure 3-11: Impact by application of different weighting sets.
A: Weighting set "complex-penalised". The phylogram equals the majority rule consensus of 19 MPT. An identical topology is computed, if weighting set "binary-doubled" is used. **B:** 1 of 5 MPT computed with the "levelled" weighting set, exhibiting a topology identical to the majority rule consensus of all 5 MPT. **Abbr.:** en = *F. engleriana*; *F.* grandifolia: *carol* = spp. *caroliniana*, *gran* = ssp. *grandifolia*, *mex* = ssp. *mexicana*; *F. sylvatica*: Geo = Georgia (Transcaucasia), Ger = Germany, Hun = Hungary, Ita = Italy, Slo = Slovenia, Spa = Spain, Tur = Turkey.

3.4.3 Comparison of the results with preceding systematical studies

The results are in agreement with a most recent detailed morphological cladistic study (MP) undertaken by DENK (in press) and the preceding detailed analysis with emphasis on the *F. sylvatica* complex (DENK et al. 2002; chapter 3.2). The subgenus *Engleriana* is morphologically and genetically strongly derived in relation to the taxa of the subgenus *Fagus*. As it will be shown in detail in the following chapter, the derivation of subgenus *Engleriana* from subgenus *Fagus* – in contrary to a parallel evolution from a common, extinct ancestor – can actually be reconstructed on the basis of the occurring variabilities. *Fagus grandifolia* is either intermediate between the subgenus *Engleriana* and the remaining taxa of the subgenus *Fagus* (DENK et al. 2002; DENK, in press, 'base-per-base' BI analysis, MPT computed with "unweighted") or forms a distinct clade within the subgenus *Fagus* (MPT computed via "binary-doubled", "complex-penalised", and "levelled" weighting sets). In his monograph about *Fagus,* SHEN (1992) established the section *Lucida* comprising the series *Lucidae*, *Hayatae*, and *Crenatae*. While a closer relationship between *F. lucida* and *F. crenata* is supported by the present study, *F. hayatae* groups together with *F. longipetiolata* (cf. Figs. 3-10 & 3-11). A sister group relationship between *F. hayatae* and *F. longipetiolata* is also assumed by a recent morphological study (DENK, in press), whereas *F. lucida, F. crenata,* and *F. sylvatica* are unresolved. Comprehensive molecular analyses including ITS sequence data were also undertaken by STANFORD (1998) and MANOS & STANFORD (2001), but did not yield comprehensive results for the intrageneric phylogeny of *Fagus* (chapters 3.1 & 3.2.3). A comparison between 'cloned' ITS sequences from various genera[33], with gene bank accessions indicates that data from a genomic DNA library – like the data here presented – outperform data sets assembled by direct sequencing of PCR products. Accessions achieved from directly sequenced PCR products – like the data used by STANFORD (1998) and MANOS & STANFORD (2001) – do not contain the entire information about intraspecific down to intragenomic variability, at least for the biparental inherited genomic nrDNA.

[33] Besides the here presented accessions of *Acer* and *Fagus*, ITS data for comparison is available from *Nothofagus, Quercus, Tilia, Zelkova* and related "Ulmaceae" s.l. (own data) and *Lathyrus/Oxytropis* (M. Schlee, person. comm.). Furthermore, 25S rDNA data from several cycad taxa (GRIMM 1998) can be taken into account.

3.4.4 Phylogenetic implications and reliability of data

The analytic method presented in this paper allows to sufficiently resolve various interspecific relationships within *Fagus*, and the results are in agreement with data from other sources. For the model genus *Fagus* it becomes clear, that in the case of ITS sequence data the recognition and utilisation of genetical intraspecific variabilities as important phylogenetic signals result in phylogenetic trees, that exceed the resolution of 'base-per-base' analyses:

- A sibling relationship between *F. hayatae* ssp. *pashanica* and *F. longipetiolata* is assumed.
- Subgenus *Engleriana* (*F. engleriana, F. japonica*) is clearly derived from the taxa assigned to subgenus *Fagus*, and does not form a parallel evolutionary lineage. I.e. subgenus *Fagus* is, in a strict cladistic sense, paraphyletic in relation to the subgenus *Engleriana* (≙ definition of HENNIG 1950; cf. HENNIG & SCHLEE 1978).
- *F. sylvatica* is genetically closer to *F. crenata* and *F. lucida*, than to *F. grandifolia* and *F. hayatae* + *F. longipetiolata*. In addition, a sibling relationship between *F. crenata* and *F. lucida* is probable.

However, problems arise for the appropriate coding and weighting of site variabilities.

A detailed investigation of the data set (cf. competing genotypes: Fig. 3-4) together with a maximum parsimony reconstruction (MPR) of the used characters (Fig. 3-14; following chapter) indicate, that the independence of distinct characters is in fact difficult to evaluate. DENDUANGBORIPANT & CRONK (2001) used the putative secondary structure of a hypervariable region within the ITS2 of *Aeschynanthus* to optimise the according alignment. Obviously, the exact folding manner of the secondary structure plays an eminent role in mutation patterns. From the here presented alignment (→ appendix) it is clear, that certain mutations are always accompanied by mutations in another part of the spacer region (linked characters, cf. Figs. 3-4 & 3-12). Since these linked characters are not necessarily strictly complementary to each other, it cannot be decided, whether this is indeed due to a change or shift in the secondary structure (TORRES et al. 1990), occasional homoplasies[34], or the co-dominance of genotypes. A pilot study with the online DNA folding service by the Zuker group (SANTA LUCIA JR. 1998) did not result in a discrete trend. In addition, although characters are linked for a certain taxon or group of taxa, they are not necessarily linked for other taxa (→ Fig. 3-12). A possible explanation is, that linked mutations and an unlinked mutation pattern evolved convergently.

[34] i.e. a convergently evolved apomorphic character (↔ "homology")

Such an interpretation remains to a high degree hypothetical, because of the highly variable substitution model (GTR+Γ+I) that is assumed for the ITS1 and ITS2.

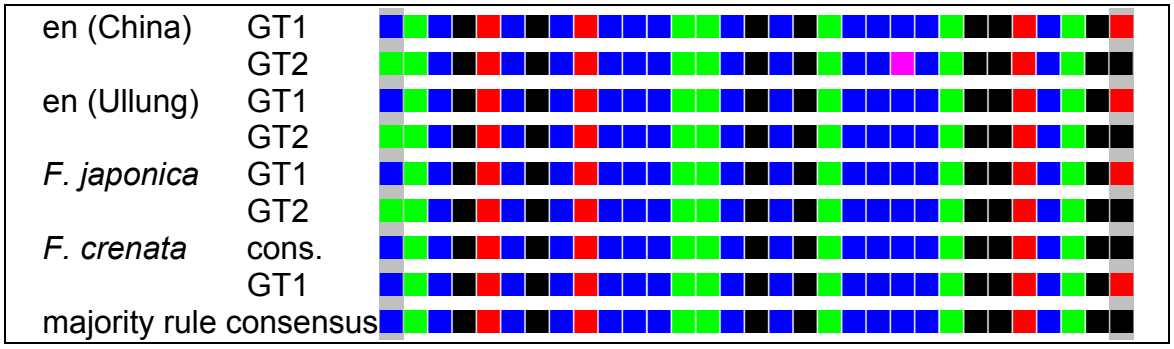


Figure 3-12: Putative linkage of nucleotides.
3' end of ITS2 and 5' end of 25S rDNA showing a conspicuous linkage between two site variabilities in *Fagus* spp. (grey background). Most *Fagus* spp. do not show a site variability at all, instead a "C" at the first position is always accompanied by a (complementary) "G" at the last position shown (i.e. majority rule consensus). All accessions of sg. *Engleriana* (a total of 28 clones, en = *F. engleriana*) can be assigned either to a genotype "GT1" ("C" linked with "T") or a genotype "GT2" ("A" linked with "G"), and both differ from the above mentioned consensus. However, accessions of *F. crenata* are either identical to the consensus or to GT1. Standard colour code.

The summation of the Bayesian parameters assembled during the analysis and a parallel likelihood ratio test (LRT; Modeltest 3.06; POSADA & CRANDALL 1998), propose a general and variable substitution model (like GTR+Γ+I[35]) for our data (Table 3-5). The probabilities for transitions exceed multiple times the probabilities for transversions. The 95% confidence intervals for the substitution probabilities span over a large range. A comparison with the alignment and the character matrices indicates that although transitions generally occur very often, they nevertheless can be very reliable and conservative phylogenetical signals at some sites. This observation can also be made in various differently conserved ITS data sets available from the gene bank, including the more variable data for *Acer* (chapters 4.2.3 & 4.3). To accommodate these findings with the data matrix, a very individual and variable weighting set combined with more complex stepmatrices simulating the probable mutation pathways have to be applied. Such weighting sets and character type definitions are more or less intuitive and lack strong statistical support. Therefore, new bioinformatic models with a strong statistical fundament ought to be developed and applied in future analyses.

[35] For an introduction and overview of currently used ML models see WHELAN et al. (2001)

3.5 Suppressed speciation or diversification on the run: hypothesising the history and future of beech trees

Table 3-6: Morphologically (SHEN 1992; DENK, in press) and genetically (new data) distinguishable *Fagus* spp.[36]

commonly accepted taxa*	morphology	molecular evidence
F. engleriana	sustained	share identical genotypes
F. japonica	sustained	
F. grandifolia ssp. *grandifolia*	species sustained, further subspecific differentiation questionable	species well sustained, subspecies ± identical‡
F. grandifolia ssp. *caroliniana*		
F. grandifolia ssp. *mexicana*		
F. hayatae	sustained	sustained
F. longipetiolata	sustained, 3 MT†	sustained
F. lucida	sustained	sustained
F. crenata	sustained	sustained
F. sylvatica spp. *moesica*	species sustained, several MT†; current subspecific differentiation not appropriate	
F. sylvatica spp. *orientalis*		
F. sylvatica spp. *sylvatica*		
F. sylvatica spp. *?taurica*		

* Flora Europea (print, online database); Flora of North America; Flora of China; GRIN database

† MT = morphotype *sensu* Denk (1999a, 1999b, in press, pers. comm.)

‡ ssp. *caroliniana* exhibits stronger resemblance with the consensus of subgenus *Fagus*

Taking into account morphological and molecular genetical evidence, it is clear that the species of *Fagus* represent a group of very closely related taxa. Morphologic (SHEN 1992; DENK, in press) and genetic characteristics (new data) allow to clearly separate a number of recent taxa (→ Table 3-6)[37], while others – like the western Eurasian *F. sylvatica* populations – show a gradual transition of different morphotypes or biogeographical races and only slight genetical differentiation, at least within the ITS (DENK 1999a, 1999b; DENK et al. 2002). On the other hand, morphologically distinguishable taxa – such as *F. engleriana* and *F. japonica* of the subgenus *Engleriana* (SHEN 1992; DENK, in press) – share the same, although highly variable, gene pool (see below; Table 3-6). Two scenarios can be applied to such a

[36] For a final taxonomic classification and verification more data from *F. crenata* and, in particular, *F. grandifolia* is needed.

[37] Since the possibility of natural and frequent hybridisation is unexplored in the case of *Fagus*, it cannot be determined if the morphologically, and to a certain degree genetically, distinguishable taxa are valid biological species. Possibly, only the subgeneric differentiation in *Engleriana* and *Fagus* defines valid "species" in a strict sense.

morphological and genetical setting: Either the speciation processes are suppressed (e.g. by frequent hybridisation events) or speciation is just about to happen. Whether the one case or the other is true cannot be inferred singly from the present molecular data and undertaken morphological analyses. However, the fossil record of *Fagus* allows a more particular insight (→ Fig. 3-13; DENK, in press; T. Denk, person. comm.).

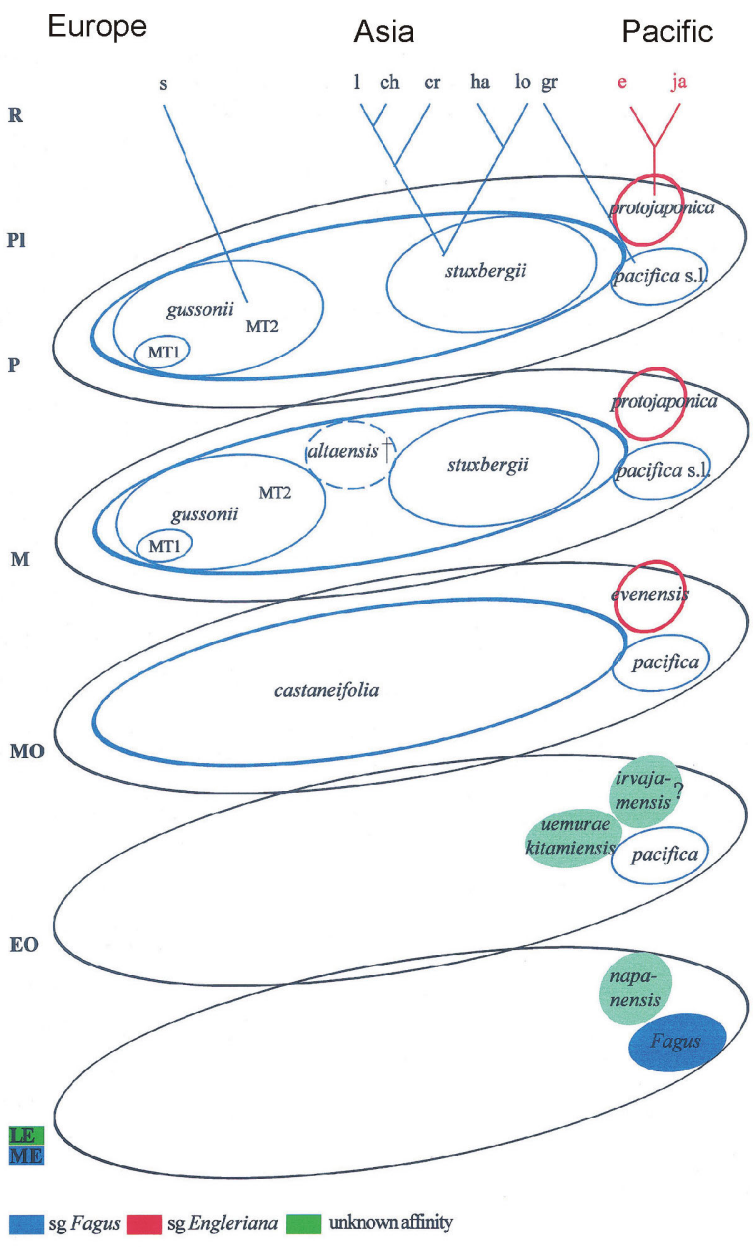


Figure 3-13 (left): Overview about the fossil history of *Fagus* with respect to the (recent) morphological differentiation.
The taxa are basically ordered from West (Europe) to East (circumpacific) within one time slice. Only 3 taxa are known from N America: the first fossils (*Fagus*), *F. pacifica*, and *F. grandifolia*. A weak morphological differentiation, although remarkable variability, is detected in Eurasian fossils belonging to sg. *Fagus*. **Abbr.:** ME = Middle Eocene, LE = late Eocene, EO = early Oligocene, MO = Middle Oligocene, M = Miocene, P = Pliocene, Pl = Pleistocene, R = recent; extant taxa: s = *F. sylvatica*, l = *F. lucida*, ch = *F. chienii*, cr = *F. crenata*, ha = *F. hayatae*, lo = *F. longipetiolata*, gr = *F. grandifolia*, e = *F. engleriana*, ja = *F. japonica* (incl. *F. okamotoi*). Courtesy of T. Denk.

The first fossils clearly belonging to *Fagus* are cupules and nuts from Middle Eocene sediments of western North America (PIGG & WEHR 2002). From Kamchatka leaves are reported from the latest Eocene (*F. napanensis*; FOTJANOVA 1982). By the early Oligocene, a number of morphologically distinguishable taxa occurred, which were subsequently replaced by the globally distributed mosaic taxon *F. castaneifolia*. During the Oligocene, the following two major divergences took place, which in detail can also be observed in the composition of the ITS (→ Figs. 3-14 & 3-15; chapter 3.4.2): (a) an initial West-East differentiation within the early beech populations and (b) the origin and separation of subgenus *Engleriana* from subgenus *Fagus*.

(a) *Fagus pacifica*, the presumed ancestor and putative progenitor of *F. grandifolia*, shows only few typical Asiatic morpho-elements (leaves), whereas cupules are strikingly similar to the modern *F. grandifolia*. In contrast, *F. castaneifolia* combines elements of *F. pacifica* + *F. grandifolia* and the later Eurasian taxa. In addition, the recent *F. grandifolia* is morphologically clearly distinguishable from other species of subgenus *Fagus*. Furthermore, the ITS of *F. grandifolia* exhibits a smaller overall intraspecific variability than *F. hayatae* + *F. longipetiolata*, but is most distinct in relation to the other taxa of the subgenus *Fagus*. This is exemplary illustrated by the gain of synapomorphic variabilities (red ⊕, Fig. 3-14) and the subsequent loss of the ancestral nucleotide state (black ⊖, Fig. 3-14) in ssp. *grandifolia* and ssp. *mexicana.*

(b) With *F. evenensis* in Kamchatka a possible ancestor and progenitor of the subgenus *Engleriana* is found. The subgenus *Engleriana* is morphologically as well as from a molecular genetical viewpoint clearly derived from the subgenus *Fagus* (DENK, in press; new data: Fig. 3-14). Molecular (Fig. 3-8) and morphological analyses (DENK, in press) propose an affinity between *F. grandifolia* and subgenus *Engleriana*. Genotypic characteristics that are shared between subgenus *Engleriana* and *F. grandifolia* are also represented in *F. longipetiolata* and/or *F. hayatae* (⇨ ancient polymorphism; cf. Fig. 3-14). Additionally, several genotypic characteristics are exclusively represented in *F. longipetiolata* or *F. hayatae* and populations of subgenus *Engleriana*. Thus, molecular evidence opposes a sibling relationship between *F. grandifolia* and subgenus *Engleriana* and a common origin of the according taxa. But, if a variable, but slightly differentiated ancestral gene pool[38] is assumed for the early *F. castaneifolia* + *F. pacifica*, it is conceivable that circumpacific populations share characteristics (either as

[38] e.g. in which a gradual transition of competing genotypes is realised

symplesiomorphies[39] or parallelisms[40]; → special remark), which are recently still realised in subgenus *Engleriana*, *F. grandifolia,* and, genetically, *F. hayatae* + *F. longipetiolata.* Furthermore, genetical similarities between *F. hayatae* + *F. longipetiolata* and subgenus *Engleriana* might be due to an ongoing gene flow (⇨ hybridisation) between the first populations of *F. evenensis* and ancestors of *F. hayatae* + *F. longipetiolata*.

But what happened to *F. castaneifolia*? By the Middle Miocene two Eurasian species of *Fagus* can be found: *F. gussonii* and *F. stuxbergii*. However, both taxa still combined morphologic characteristics of recent eastern Asian as well as western Eurasian taxa, and cannot be clearly distinguished. This is in accordance with the overall low genetical differentiation between the remaining subgenus *Fagus* taxa (Figs. 3-8, 3-9 & 3-14). The most interesting evidence in this context is the higher variability of *F. hayatae* ssp. *pashanica* (and *F. longipetiolata*) in comparison with *F. crenata, F. lucida,* and *F. sylvatica*, although the latter ones are morphologically more derived[41]. This implicates, that at least for these taxa, and probably the whole subgenus *Fagus*, an ancient polymorphism can be assumed[42](see also Figs. 3-14 & 3-15). In addition, a morphotype similar to the first typical *F. sylvatica* fossils can today only be found in Georgian populations, which, on the other hand, do show a remarkably higher genetic variability than Turkish, Iranian or Central European populations (Figs. 3-3 & 3-15). For example, otherwise *F. grandifolia*-specific genotypic characteristics can only be detected in relict populations of *F. sylvatica* in Georgia and *F. hayatae* + *F. longipetiolata* in China (Fig. 3-14; reconstruction of gene pools in Fig. 3-15), thus, sustaining an ancient polymorphism correlating to the mosaic patterns of *F. castaneifolia*. A transatlantic gene flow via hybridisation between North America and Europe in the Eocene, and than eastward into China, is not probable: *Fagus* fossils (pollen, macrofossils) are not reported from the Eocene of Europe, which was at that time segregated from the Asian floral province by the Turgai street. The genotypic characteristics and the fossil record of *F. sylvatica* clearly indicate an eastern origin of the European beech populations.

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[39] i.e. a shared ancestral and homologous state (opposite of synapomorphy)

[40] i.e. a convergent development due to a tendency or potential to be developed within a group of near relatives, e.g. because of an especial genetic programme.

[41] Whether the morphological characteristics of *F. lucida* are indeed derived, not rudimentary, has yet to be confirmed.

[42] To a lower degree, the relationship of *F. longipetiolata* and *F. hayatae* ssp. *pashanica* is characterised affirmatively. *Fagus longipetiolata* is morphologically clearly derived and markedly differentiated, although the detected genetic variability is not as high as in *F. hayatae* ssp. *pashanica*.

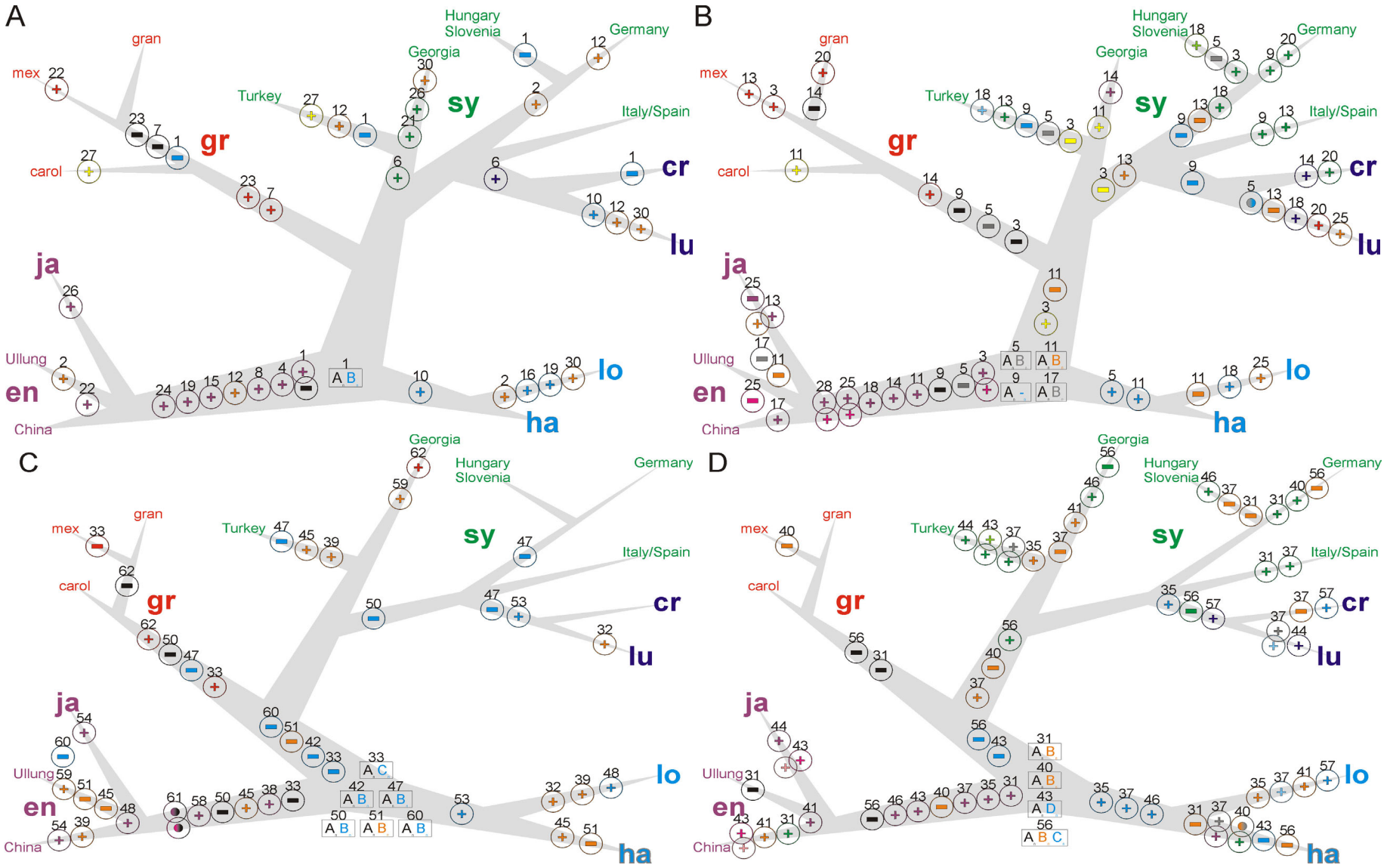

A
B
C
D
gran
mex
carol
Turkey
Hungary
Slovenia
Georgia
Germany
Italy/Spain
Ullung
China
gr
sy
cr
lu
ja
en
lo
ha

Figure 3-14 (preceding page): MPR of binary, ordered and complex characters for *Fagus*.
Letters in squares (e.g. "A_0B_0") indicate a putative ancient site variability for this character. "⊕" indicates the gain, "⊖" the loss of a particular intrataxonomic variability, respectively nucleotide state. Bi-coloured circles ("⊙") represent the replacement of one variability by another (≙ point mutation). Identical variabilities for a particular character are coloured equally. Black symbols refer to the consensus state (cf. Table 3-2). **A.** Binary and ordered characters, ITS1. **B**. Complex characters, ITS1. **C.** Binary and ordered characters, ITS2. **D**. Complex characters, ITS2. **Abbr.:** cr = *F. crenata*, en = *F. engleriana*, gr = *F. grandifolia*: carol = ssp. *caroliniana*, gran = ssp. *grandifolia*, mex = ssp. *mexicana*, ha = *F. hayatae* ssp. *pashanica*, ja = *F. japonica*, lo = *F. longipetiolata*, lu = *F. lucida*, sy = *F. sylvatica*; symbol numbers refer to character numbers (Table 3-3).

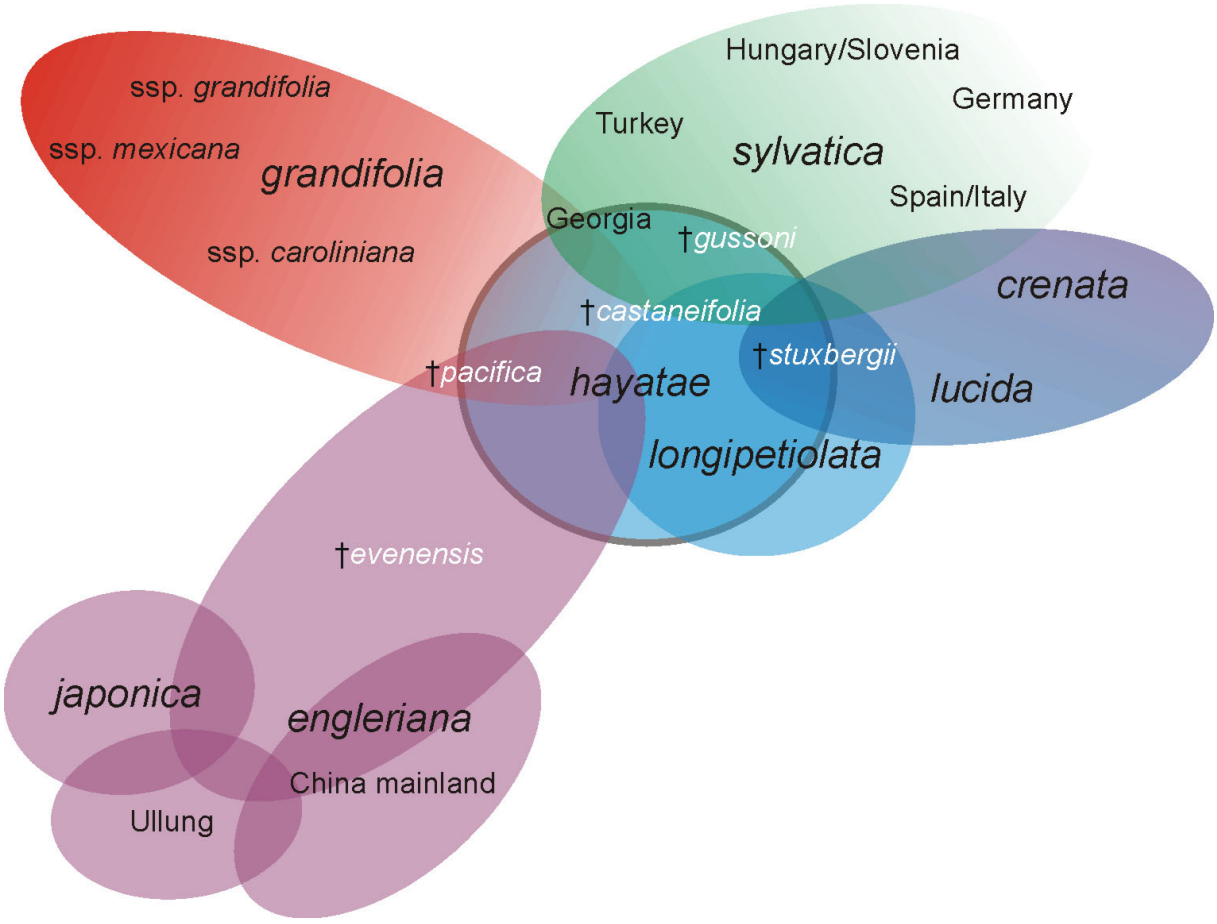


Figure 3-15: Ancient and recent gene pools of *Fagus*.
The reconstruction is based on the here presented analyses in respect of the fossil and biogeographic history of the genus (Fig. 3-13). Note, that any taxon shares genetic characteristics with at least one other taxon. *F. hayatae* (together with *F. longipetiolata*, light blue circles) covers genetical features that are shared by sg. *Fagus* (red, green, dark blue) and sg. *Engleriana* (purple). The intensifying or decay of colour represents the accumulation of unique (poss. true synapomorphic) genetic characteristics (*F. grandifolia*), or the loss of genetic variability *(F. sylvatica*). The bi-coloration (light ⇨ dark blue) of the *F. crenata* + *F. lucida* gene pool, refers to the genetical similarity of one *F. lucida* locality with *F. hayatae* + *F. longipetiolata* (cf. appended alignment). Extinct taxa ("†") are placed according to morphological and biogeographical evidence.

Remark: In phylogenetic systematics (cladistics) the termini "analogy", i.e. similar development of non-homologous structures, "homoiology", i.e. convergent modification on a homologous structure, and "parallelism" (definition cf. footnote 40) are used to describe derived characters that are not synapomorphies of a defined monophyletic group. Such a distinction is of minor or no importance for the phylogenetic reconstruction, because they all represent "convergences", i.e. non-homologous apomorphic characters (cf. HENNIG 1950; HENNIG & SCHLEE 1978; BECHLY 1998, and literature cited therein)[43]. Due to the unique composition of the here presented nrDNA data sets, the *potential* (cf. definition in footnote 40) to realise a point mutation at a distinct site or a defined oligonucleotide motif is obviously of strong phylogenetical significance (see chapters 3.4.4 & 4.4.1), hence, putative parallelisms have to be distinguished from analogously evolved motives and provide an equally important phylogenetical data source like synapomorphic motives. At least for the here analysed taxa *Acer* (chapter 4) and *Fagus* this supposedly applies to the realisation of certain morphological features (T. Denk, person. comm.; cf. data provided in DENK, in press; see also chapter 4.4.3). For morphological characters, the secluding verification of a parallelism can only be accomplished by developmental genetical or ontogenetical approaches.

The heightened genetic variability found within accessions of *F. engleriana* and *F. japonica* demonstrate that the fixation rate of *Fagus* is not in general slowed down. Instead, the intraspecific differences detected here can be compared with certain intrasectional differentiation found in *Acer* (chapter 4.2.3; appendix). On the other hand, although the genetic variability is increased in comparison to other *Fagus* spp., the consensus genotype of all *Fagus* spp. (nucleotide states found in subgenera *Engleriana* and *Fagus*) is still represented in the gene pool of *F. engleriana* and *F. japonica*. Therefore, it can be concluded, that at least at a historical and stratigraphical scale, speciation processes within the genus *Fagus* were suppressed during time and space. Neither the initial distribution and ecological manifestation of the genus in the circumpacific area (Oligocene), nor the westward expansion of *Fagus* into western Asia and Europe (Miocene) gave rise to clearly separated and distinct species. The nucleotide composition of the recent ITS in relation to the fossil record can only be explained by assuming an unhindered horizontal gene flow via frequent hybridisation events, also indicated by the lack of distinct morphologic characteristics within fossil populations of *Fagus*.

[43] However, in literature a "parallel evolution" is often distinguished from a "convergent evolution" to contribute to the fact, that the taxa in the case of a "parallel" evolution are closely related to each other, i.e. they can be *assigned to* the same monophyletic group, but do not *form* a monophylum. In this case, the terminus "parallelism" is used to describe "parallel evolved characters" *in contrast to* "convergences", i.e. characters originating from "convergent evolution" (see e.g. KÖNIGSMANN 1978).

It can be assumed that the molecular differentiation between disjunct populations of Asian (China, Transcaucasia, Turkey) *Fagus* populations will proceed. If no forestry efforts are undertaken, those population will probably loose genetic variability and give rise to genetically more differentiated species. Morphologically, *F. longipetiolata* is already clearly distinct from *F. hayatae*. In addition, several morphotypes can be distinguished within *F. longipetiolata*, which are putative predecessors of new subspecies and species. The same holds true for Georgian and Turkish populations of *F. sylvatica*, since they are conspicuously isolated from the European master population. The general tendency is here exhibited by the occurrence of distinguishable morphotypes (DENK 1999a, 1999b) together with the impoverished gene pool of many individual-rich populations of *F. sylvatica*, especially in Central and East Europe. By the introduction of *F. sylvatica* in North America, the gene pool of *F. grandifolia* is possibly enriched, although there is – up to now – no clear evidence for hybridisation between those two taxa[44]. Further population scale studies on this matter are clearly necessary.

From the assembled morphological, fossil, and molecular evidence it can be concluded, that the genus *Fagus* exhibits a peculiar evolutionary strategy, which is best described as "static frontier strategy". Populations of *Fagus* are obviously adapted to an especial ecological setting, i.e. defined macroclimate and microclimate constraints (cf. PETERS 1997; see e.g. altimeter migration of *Fagus* populations detectable in the fossil record of the Neogene of Georgia: SHATILOVA 1992). If these constraints are fulfilled, *Fagus* tends to quickly 'conquer' the appropriate area and niche, which is well-exhibited by the post-Pleistocene 'invasion' of Europe by populations of *F. sylvatica* (e.g. HUNTLEY & BIRKS 1983). The aggressive population strategy (high stemmed, densely crowned, ecto-mycorrhised trees forming nearly monotypic groves and/or forests; cf. e.g. PETERS 1997) is correlated to a low interspecific genetical differentiation and the perpetuation of a high intraspecific variability. Thus, populations of *Fagus* seldom speciate, but tend to retain genetic polymorphism, especially if growing in relict areas such as Georgia, and south-eastern China. Such a genetical peculiarity is also exhibited in case of the morphologically and genetically most derived subgenus *Engleriana*: Although new genotypic characteristics are fixed in the ITS and consecutively distributed over the gene pool (derived genetical features are commonly found in all populations, or only in Chinese populations, in Chinese and Korean populations, or in Korean

[44] Single clones from the south-eastern U.S. stands exhibit a intriguing resemblance to the genotype of *F. sylvatica*. Whether this can be a hint for hybridisation, has to be further analysed.

and Japanese populations; see Fig. 3-14), the putative ancestral and/or consensus nucleotide composition is always still realised. This concurs with observations of T. Denk (person. comm.) at natural stands in China, that indicate a stronger pioneer character for *F. engleriana* (shorter stemmed and branched and associated with other tree genera; cf. DENK, in press). Here, individuals of *F. engleriana* seem to 'infest' *Fagus*-free groves, and are subsequently followed by *F.* hayatae, *F. longipetiolata*, or *F. lucida*, until *Fagus* spp. are the dominating arborescent element within the grove.

3.6 Intraspecific variability in the ITS: chance or problem for the reconstruction of phylogeny?

Interpopulation and intragenomic variability are likely to disguise phylogenetic relationships when standard analytic methods are employed, especially if phylogenies are based on a rather variable gene region such as the ITS that is inherited by both parental lineages. The usage of Bayesian inference to model the putative phylogeny on the basis of single nucleotides seems to be more suitable than more classical methods such as distance methods and maximum parsimony. These methods are not sufficiently flexible to properly simulate the complex pattern of molecular evolution, at least for nuclear encoded rDNA spacer regions. However, the reduction of ML models to simple mutational categories (A→C, A→G, etc.) causes the loss of phylogenetical information provided by indels, and, even more important, by site variabilities. Gaps – resulting from indels – and site variabilities are treated as "missing" or "ambiguous" data by ML-based programs, like MrBayes. Thus, the phylogenetic hypothesis as shown in Figure 3-8 is not or only slightly altered by gaps and site variabilities, i.e. they have no negative impact. At the same time, important phylogenetical information provided by the presence of gaps and site variabilities is lost to a high degree, i.e. there is no positive impact (Fig. 3-9). The newly introduced ISV analysis (chapter 3.3) cannot generally replace statistical 'base-per-base' analytic methods such as Bayesian inference or other ML-based methods, but it does help to understand data sets, in which interpopulation and intragenomic variability are as high or almost as high as the overall interspecific variability. This is the case for the nucleotide composition of the ITS of the here used model system *Fagus.* A low overall genetical differentiation combined with a comparatively high intraspecific variability produces a data set, which cannot be completely resolved with 'base-per-base' analyses.

Studies dealing with subgeneric relationships in plants like *Fagus* may be strongly affected by recent and fossil hybridisation events and/or incomplete concerted evolution (e.g. VOLKOV

et al. 1999; FOREST & BRUNEAU 2001). Extant plant species possibly originated from complex reticulate evolution and have a complex biogeographic, migration, and speciation history. Thus, comprehensive assembling and making use of the genetic variability within closely related plant taxa are crucial for reconstructing a sound phylogeny based on molecular markers. Combined with data from other sources such as biogeography, ecology, morphology, and the fossil record, it should be possible to achieve a more probable and precise reconstruction of low-level evolution, and, eventually, a better understanding of the molecular differentiation in the course of speciation processes (Fig. 3-15).

4 Tracing the Phylogeny of Maples (Genus *Acer*, Aceraceae)

4.1 Introduction and compilation of preceding systematical and phylogenetical studies

The genus *Acer* L. is one of the most diverse and variable arborescent genera of the northern hemisphere. According to a recent monograph about 124 species are recognised (VAN GELDEREN et al. 1994), of which 27 comprise more than one subspecies. Numerous infrageneric sections and series (→ Table 4-1) have been proposed and partly accepted to describe the taxonomic and systematic relationships between individual taxa or groups of taxa. Comprehensive studies include phenomenological morphological (e.g. OGATA 1967; 27 sections, 15 series; DE JONG 1976, 14 sections, 20 series; MAY 1984, 4 subgenera divided into 17 sections and 29 series), biochemical (DELENDICK 1981, 21 sections, 3 series), and, most recent, molecular phylogenetical approaches (mainly ITS sequence data: CHO et al. 1997; ACKERLY & DONOGHUE 1998; SUH et al. 2000; TIAN et al. 2002; see PFOSSER et al. 2002 for cpDNA data). Aside the recognition of apparent near-relatives – e.g. species assigned to one series, section and/or morphologically very similar taxa – the interserial and -sectional and relationships could not be resolved and are up to now still a matter of debate. To solve the relationships, WOLFE & TANAI (1987) used fossils to trace not only the history of the different sections of *Acer*[45], but also to locate possible diversification centres of the genus and to follow its distribution throughout the northern hemisphere. A cladistic analysis carried out on the base of morphological characters of modern *Acer* brought some new insights on the intersectional relationships, which partly contrasted the original views about systematic relationships within *Acer*. However, their results were in agreement with the occurrence of corresponding macrofossils (leaves, samaras) and allowed to assign supersectional taxonomic groups. In addition, BOULTER et al. (1996) assembled megafossil data of *Acer* available from the PFR 2.2 database[46] to reconstruct the migratory and evolutionary history of the genus through space and time, including the extinct sections defined by WOLFE & TANAI (1987). The basic idea was to map the cladogram from WOLFE & TANAI (1987) against the

[45] defined based on an amended classification originally proposed by OGATA (1967)

[46] URL: http://ibs.uel.ac.uk/palaeo/pfr2/pfr.html

palaeobiogeographic record. As a preliminary result they stated, that it is impossible to divide the genus into morphological or geographical entities in pre-Neogene time (>35 Ma). Recent lineages seem to have aroused from this undifferentiated species-pool quasi-simultaneously. ACKERLY & DONOGHUE (1998) concentrated on canopy structure to understand the evolution and differentiation of *Acer*. They combined the results with a first molecular study using the ITS to test the hypothesis resulting from their morphometric analysis. The molecular phylogram (MP) presented by ACKERLY & DONOGHUE (1998) and a more recent analysis (MP and NJ) undertaken by SUH et al. (2000) enforced certain infrageneric relationships and questioned several others. Still, their dendrograms could not compete with the resolution of the cladogram of WOLFE & TANAI (1987), because ITS sequence data did not appear to have enough information to properly resolve the phylogenetic backbone of the whole genus. This was most recently underlined by a detailed analysis from PFOSSER et al. (2002) on endemic island *Acer* species. They concentrated on cpDNA data (*trn*L intron, *trn*L-F IGS), because a pilot study on ITS did not reveal enough unambiguous sites.

In the following it will be demonstrated, that the inability of preceding studies to comprehensively reconstruct the evolution of the genus, arise not from the used data source (morphology, ITS), but is related to two main problems, generally affecting low-level evolutionary reconstruction:

1. Convergent development of major morphologic characteristics within a group of nearly related taxa, e.g. the members of a genus. This, in general, contributes also to the use of maximum parsimony to evaluate molecular data (cf. chapter 2.4.2).
2. Sampling of too few species and populations, respectively, for genetical analyses in combination with unsuitable analytic models (cf. chapters 2.4.2, 3.2.3 & 3.6).

To infer a sound hypothesis about infrageneric evolution within the genus *Acer*, a brief recapitulation about current systematic models and morphologic characteristics together with a detailed characterisation of the ITS of the genus *Acer* is given. Next, by mapping these data against each other it is possible, not only to reconstruct the phylogeny of the genus, but also to infer and understand the pathways of morphological and molecular genetical infrageneric differentiation.

Table 4-1: Classification systems proposed for *Acer*.

current synopsis		PAX 1885ff		OGATA 1967			DELENDICK 1982		MAI 1984			WOLFE & TANAI 1987		
section	**series**	group	section	group	section	series	group	section	subgenus	section	series	group	section	series
Glabra	Arguta	Intrast./Extrast.	Ind./Spic.*	A	Arguta		V	Arguta	**Carpinifolia**	Arguta	Arguta	Macrantha	Arguta	
	Glabra	Perigyna	Glabra		Glabra			Glabra		Glabra			Glabra	
Wardiana			Macrantha		Macrantha	Macrantha		Macrantha		Macrantha	Wardiana		Macrantha	
Macrantha		Intrast./Perig.	Ind./Macr.*			Micrantha					Tegmentosa			
						Rufinervia					Rufinervia			
Ginnala		Extrastaminalia	Spicata		Trilobata		III	Ginnala		Ginnala			Trilobata	
Negundo	Cissifolia		Trifoliata		Cissifolia		V	Cissifolia	**Negundo**	Negundo			Cissifolia	
	Negundo	Adiscantha	Negundo		Negundo			Negundo					Negundo	
Parviflora	Distyla	Intrastaminalia	Indivisa		Distyla		I	Parviflora	**Carpinifolia**	Parviflora	Distyla	Spicata	Distyla	
	Parviflora	Perigyna	Macrantha		Parviflora					Arguta	Ukurunduensis		Parviflora	
	Caudata		Spicata		Spicata								Spicata	
Palmata	Penninervia	Extrastaminalia	Integrifolia		Palmata	Laevigata	II	Palmata		Palmata	Penninervia		Palmata	
	Palmata		Palmata			Palmata					Palmata			
	Sinensia		Spicata			Sinensia					Sinensia			
Rubra			Rubra		Rubra	Eriocarpa	III	Rubra	**Acer**	Rubra	Eriocarpa	Macrantha	Eriocarpa	
						Rubra					Rubra		Rubra	
Platanoidea		Perigyna	Campestria	B	Campestria		IV	Platanoidea	**Sterculiacea**	Platanoidea	Campestria	Platanoidea	Platanoidea	
			Platanoidea		Platanoidea						Platanoidea		Campestria	
Pubescentia			Campestria		Pubescentia			Pubescentia	**Carpinifolia**	Pubescentia			Pubescentia	
Acer	Acer			C	Syriaca			Acer	**Acer**	Acer	Acer	Macrophylla	Acer	Monspessulana
		Extrastaminalia	Spicata		Acer	Acer								Acer
	Monspessulana	Perigyna	Campestria		Goniocarpa	Monspessulana		Goniocarpa		Goniocarpa	Monspessulana			Monspessulana
						Opulifolium								Saccharodendron
	Saccharodendron	Extrastaminalia	Saccharina		Saccharina			Saccharina		Acer	Saccharodendron			
Pentaphylla	Trifida		Integrifolia	D	Integrifolia			Oblonga		Pentaphylla	Trifida		Integrifolia	
Trifoliata	Grisea		Trifoliata		Trifoliata	Grisea		Trifoliata	**Sterculiacea**	Trifoliata	Grisea			
	Mandshurica					Mandshurica							Acer	Monspessulana
Hyptiocarpa			Integrifolia	E	Decandra		III	Hyptiocarpa	**Acer**	Hyptiocarpa	Decandra	Platanoidea	Decandra	
					Laurina							Macrophylla	Laurina	
Lithocarpa	Lithocarpa	Perigyna	Lithocarpa		Lithocarpa		IV	Lithocarpa	**Sterculiacea**	Lithocarpa	Diabolica		Lithocarpa	
											Lithocarpa			
	Macrophylla	Extrastaminalia	Spicata		Macrophylla			Macrophylla		Trifoliata	Macrophylla		Macrophylla	
Indivisa		Intrastaminalia	Indivisa	F	Indivisa		V	Indivisa	**Carpinifolia**	Indivisa	Carpinifolia	Macrantha	Indivisa	
Pentaphylla	Pentaphylla	unknown to Pax		?	Pentaphylla		IV	Pentaphylla	**Acer**	Pentaphylla	Pentaphylla	Macrophylla	Acer	Monspessulana

Background colours indicate systematic and phylogenetic affinities. First two columns (bold font): current classification system (VAN GELDEREN et al. 1994).

* species of this taxonomic group either assigned partly to sects. *Indivisa* and *Spicata or* sects. *Indivisa* and *Macrantha* by Pax

4.2 *Morphological and genetical infrageneric composition of* Acer

The high number of species recognised for *Acer* is mirrored by a high morphological and genetical (ITS) variability. In addition, the overall variability is not constant in respect to the proposed systematic entities. Commonly accepted and presumably species-rich systematic entities like the sections *Acer* and *Macrantha* PAX may either be morphologically divers (sect. *Acer*) or rather homogenous (sect. *Macrantha*). As a consequence, the exact hierarchical positioning of taxonomic units – like subspecies, species, series, and sections – varies. This is especially true for the assignment of specific and subspecific ranks. For example, the most recent monograph on the genus *Acer* by VAN GELDEREN et al. (1994) places *A. ibericum* BIEBERST. as a subspecies of *A. monspessulanum* L., and *A. obtusatum* WALDST. & KIT. as a subspecies of *A. opalus* MILL., which is accepted in the GRIN database. In the Flora Europaea (print version; online database[47]) these taxa are all accepted as distinct species. Nevertheless, certain morphologically defined systematic entities exhibit an agreement on fundamentals with the nucleotide composition of the ITS within taxa assigned to these entities.

4.2.1 Current taxonomy and systematics

Due to the fact that various taxonomical sources (e.g. Flora Europaea, GRIN database, Flora of China etc.) disagree markedly in the classification of species and subspecies, the taxonomic nomenclature for herein used individuals follows mainly the most recent monograph about *Acer* by VAN GELDEREN et al. (1994) with exceptions given in Table 4-2. If a taxon comprising several subspecies is referred to with the species name only, the according data refers to the typical subspecies. Related ITS sequences are accessible from the gene bank, but have not been used in this analysis (see chapter 4.3.1).

Table 4-2: Taxonomic position of analysed *Acer* specimen other than classification proposed by VAN GELDEREN et al. (1994).

valid name according VAN GELDEREN et al. (1994)	here used taxon name	authority/reason
A. mono ssp. *mono* Maxim.	*A. pictum* ssp. *mono* H. Ohashi	GRIN database, OHASHI (1993)
A. monspessulanum ssp. *ibericum* Yalt.	*A. ibericum* Bieb. ex Willd.	Flora Europaea, cf. chapter 4.4
A. heldreichii ssp. *trautvetteri* A.E.Murray	*A. trautvetteri* Medv.	Flora Europaea

[47] URL: http://rbg-web2.rbge.org.uk/FE/fe.html

According to VAN GELDEREN et al. (1994) the genus *Acer* can be separated into 16 sections subdivided into 27 series (Table 4-1, first two columns). With the exception of section *Parviflora* KOIDZUMI, all sections are defined by the occurrence and combination of distinct morphological and biochemical features as reported and discussed by OGATA (1967), DE JONG (1976), and DELENDICK (1981). Section *Parviflora* comprises the species-poor series *Caudata* PAX, *Distyla* MURRAY, and *Parviflora* (latter two monospecific), which are combined to one section due to the lack of putatively derived floral and seed characteristics, respectively characteristical biochemical profiles.

Aside the grouping into series and sections, no further phylogenetic and systematic concept were applied. However, the morphological and biochemical data assembled in VAN GELDEREN et al. (1994) and literature cited therein allow to conclude diffuse intersectional relationships and infer the level of specialisation within certain sections:

The species-rich section *Acer* (3 series: *Acer*, *Monspessulana* POJÁRKOVA, *Saccharodendron* MURRAY) can be morphologically and biochemically linked to a number of species-poor and possibly related systematic entities, i.e. the series *Trifida* PAX of section *Pentaphylla* HU & CHENG and sections *Ginnala* NAKAI (monospecific, rather primitive), *Lithocarpa* PAX (2 series: *Lithocarpa, Macrophylla* POJÁRKOVA), and *Trifoliata* PAX (rather specialised, 2 series: *Grisea* POJÁRKOVA, *Mandshurica* POJÁRKOVA). A close relationship between sections *Lithocarpa* and *Trifoliata* is indicated by the biochemical composition. However, section *Lithocarpa* differs from the other sections of this group by having an unsure affinity to the bispecific section *Hyptiocarpa* FANG (highly specialised, tropical and subtropical SE Asia) and the primitive, but species-rich Eurasian section *Platanoidea* PAX[48]. Section *Platanoidea* is closely allied with the small Asian section *Pubescentia* OGATA, the latter one is supposed to be distantly related to section *Rubra* PAX, a predominant floral element of the North American forests[49]. Especially the floral characteristics and chromosome numbers of section *Rubra* are considered to be markedly derived, which makes it difficult to infer its systematical position in relation to other *Acer* sections. Beside section *Pubescentia*, the level of specialisation found within section *Rubra* indicates a distinct relatedness to either section *Hyptiocarpa* or section *Glabra* PAX[50] (2 series: *Arguta* REHDER, *Glabra*). Morphologically not related in any way to section *Ginnala*, the

[48] Taxa belonging to section *Platanoidea*, like *A. campestre*, *A. pictum*, and *A. platanoides* are beyond the most abundant *Acer* spp. in the temperate forests of Europe and Asia.

[49] I.e. *A. rubrum* and *A. saccharinum*. Several rare Japanese species are also assigned to this section.

[50] amended by MOMOTANI (1962)

biochemical profile of section *Rubra* and section *Ginnala* is indistinguishable (DELENDICK 1981). Section *Glabra* has a similar circumpacific distribution like section *Lithocarpa*, with a monospecific series (i.e. sers. *Glabra*, *Macrophylla*) in North America and a species-richer series in East Asia (sers. *Arguta, Lithocarpa*). However, biochemical and morphological evidence shows a strong relationship to the section *Parviflora*. The series of both sections (*Glabra, Parviflora*) are similar to section *Macrantha* PAX in lacking numerous putatively derived morphological features. The monotypic section *Wardiana* DE JONG is understood as an intermediate between sections *Macrantha* and *Parviflora*.

The three species-rich series distinguished within section *Palmata* PAX, i.e. the morphologically primitive series *Sinensia* POJÁRKOVA and the more derived series *Palmata* and *Penninervia* METCALF, are closely related, although their affinity to other sections is obscure. The biochemical bandwidth found in section *Palmata* exceeds that found elsewhere in the genus. VAN GELDEREN et al. (1994) therefore concluded that this section was separated from the remaining sections right since the origin of the genus.

With its pinnate leaves resembling the leaves of the sister genus *Dipteronia*, the highly specialised section *Negundo* MAXIMOWICZ (2 series: *Cissifolia* MOMOTANI, *Negundo*) is also considered to be a stratigraphically old *Acer* taxon, which is only remotely related to the remaining sections. The fruits of section *Negundo* resemble the fruits of the most specialised taxon found within the genus *Acer*, i.e. *A. carpinifolium* SIEB. & ZUCC. (section *Indivisa* PAX). Biochemically, *A. carpinifolium* is rather close to *Dipteronia*[51].

4.2.2 Morphology

Individuals of *Acer* are readily distinguished from other tree genera by a number of distinctive morphological features including leaf morphological, wood anatomical, floral and seed characteristics. The 'father of taxonomy', Carl von Linné (1707-1778) recognised the morphological peculiarity of *Acer* by establishing the genus. Hence, the morphology of *Acer* has been analysed and studied for more than two centuries. From the beginning of the last century, comprehensive and extensive morphological systematical investigations were undertaken by numerous researchers (e.g. PAX 1885, 1886; PÓJARKOVA 1933; OGATA 1967; WOLFE & TANAI 1987, including fossils) and intensively discussed and re-investigated (e.g. DE JONG 1976; MAI 1984; WOLFE & TANAI 1987). Therefore, I will give herein only a concise

[51] According to DELENDICK (1981), the biochemical profile of *Dipteronia sinensis* is closer to the 'typical' *Acer* profile than some *Acer* spp.

summary of the characters used to define the sections and series, which form the strong morphological fundament of the current systematic and taxonomic entities (cf. chapter 4.2.1; VAN GELDEREN et al. 1994) and are widely sustained by molecular evidence from the nrDNA (TIAN et al. 2002[52]; new data)[53] and cpDNA (PFOSSER et al. 2002; TIAN et al. 2002).

Fruit: The most prominent feature and securest synapomorphy of the genus *Acer* is the decisive morphology of the winged fruit, also known as samara (→ Fig. 4-1). The shape, size, pubescence and ornamentation of the nutlet (OGATA 1967; WOLFE & TANAI 1987) are of high taxonomical and systematical value to distinguish intrageneric taxonomic entities. Another important systematical character is the orientation of the nutlet's main axis in relation to the wing and the attachment scar. WOLFE & TANAI (1987) found that the variation in the nutlet angle and attachment angle (→ Fig. 4-2) is obviously limited to certain values for closely related taxa. Furthermore, the venation of the wing and nutlet surface can be used for the same purpose (WOLFE & TANAI 1987). REHDER (1905) was the first to recognise that the folding manner of the cotyledons is similar for related taxa and conspicuously conserved within the genus, why OGATA (1967) included this feature to define his sections.

Figure 4-1 (left): The samara of *Acer opalus* ssp. *obtusatum* (BGTue).
Note the markedly inflated nutlet together with the distinctive brown colour of the wing, which is typical for sect. *Acer* ser. *Monspessulana*. The brown colour can be seen directly after the development of the fruit. Enlarged 1½fold.

[52] Although TIAN et al. (2002) used a different taxonomical system, their data sustains the sectional division as proposed by VAN GELDEREN et al. (1994).

[53] In other studies (ITS) dealing with *Acer*, each taxonomic group is, in general, represented by only one 'typical' taxon.

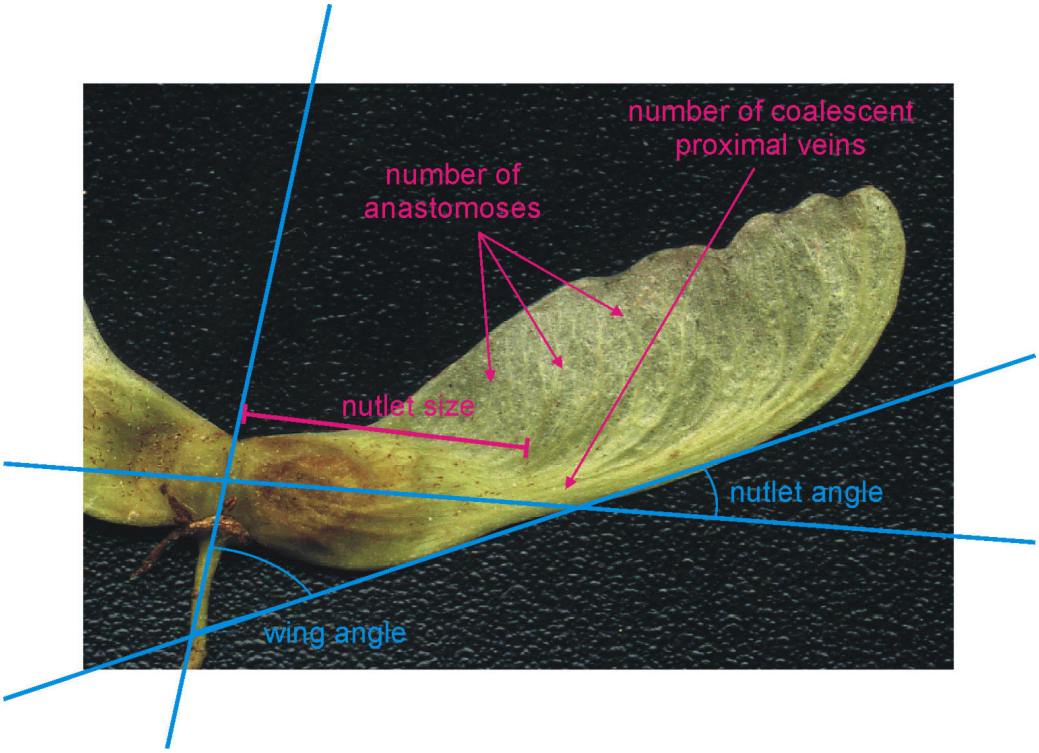


Figure 4-2: Biometric parameters of the samara used for taxonomical and systematical purposes (WOLFE & TANAI 1987).
Taxon shown: *A. davidii* ssp. *grosseri* (BGTue), sect. *Macrantha*.

Leaf: Typically, *Acer* spp. do have odd-lobed, perfectly actinodromous[54] leaves (→ Fig. 4-3). The number of lobes is strongly correlated to the number of basally originating primary veins. However, in some sections, the actinodromous leaf is replaced by pinnately organised or entire leaves. In general, the leaflets and entire leaves are pinnately veined, i.e. either craspedrodromous or eucamptodromous, but some taxa of sections *Ginnala, Macrantha, Platanoidea,* and series *Arguta* still show several equally dominant basal veins that are supposed to be homologous to the primary veins of the actinodromous leaves. This leaf type can be described as an imperfectly marginal actinodromous leaf. Sections like *Ginnala, Glabra, Palmata, Platanoidea* and the series *Monspessulana* and *Trifida* show transitions between entire and oligo-lobed (in general 3), respectively multi-lobed leaves (up to 11-lobed in ser. *Palmata*). Transitions within one individual from an unlobed to a lobed leaf, or *vice versa*, can be observed in sections *Ginnala, Glabra*, series *Trifida*, and cultivars of *A. negundo* (ser. *Negundo*; → Fig. 4-4). In contrary, palmatifoliolate or nearly palmatifoliolate

[54] The nomenclature for leaf characteristics such as venation etc. strictly follows HICKEY (1973). Otherwise used descriptive termini in the original literature were adjusted.

leaves are found in series *Pentaphylla* and Japanese cultivars of *A. palmatum* (ser. *Palmata*). The leaf margin of the lobed and unlobed leaves is either dentate, serrate, or entire. Within one series, respectively section, the exact serration type is somewhat variable. However, an exact analysis of the serration type is of taxonomical and contingent systematical value (OGATA 1967, WOLFE & TANAI 1987). The 2nd and higher order venation of *Acer* and the bracing of the lobal sinuses is more or less conserved within taxonomic entities and was intensively studied and described by WOLFE & TANAI (1987). Finally, the areolar venation pattern is of further taxonomical value.

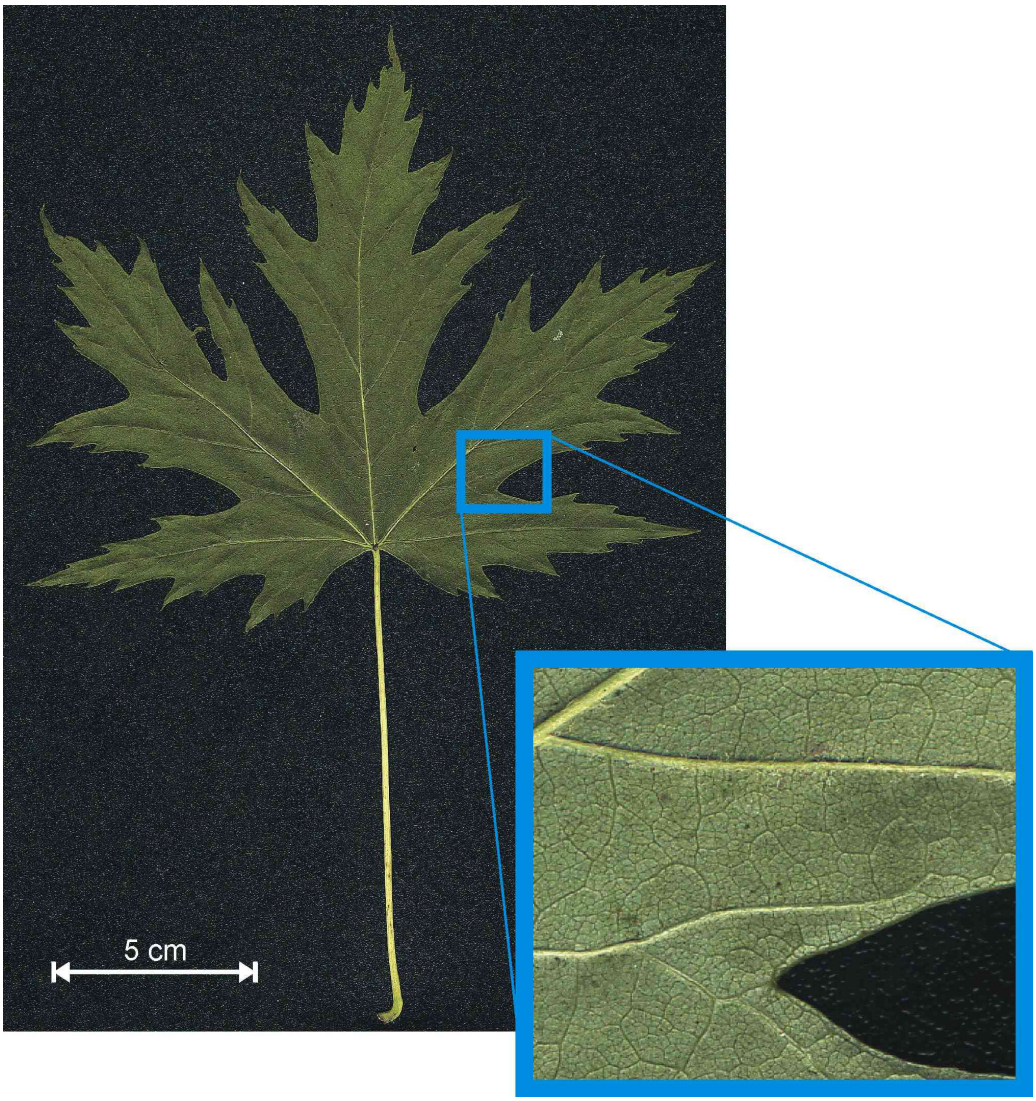


Figure 4-3: The actinodromous leaf of *A. saccharinum* 'Wieri' (sect. *Rubra*; BGTue).
A: Complete lamina with 5 well-developed lobes with acuminating apices. The leaf base is strongly cordate. **B:** Zoom on the lower leaf side exhibiting the distinctive bracing of the lobal sinuses by secondary and tertiary veins.

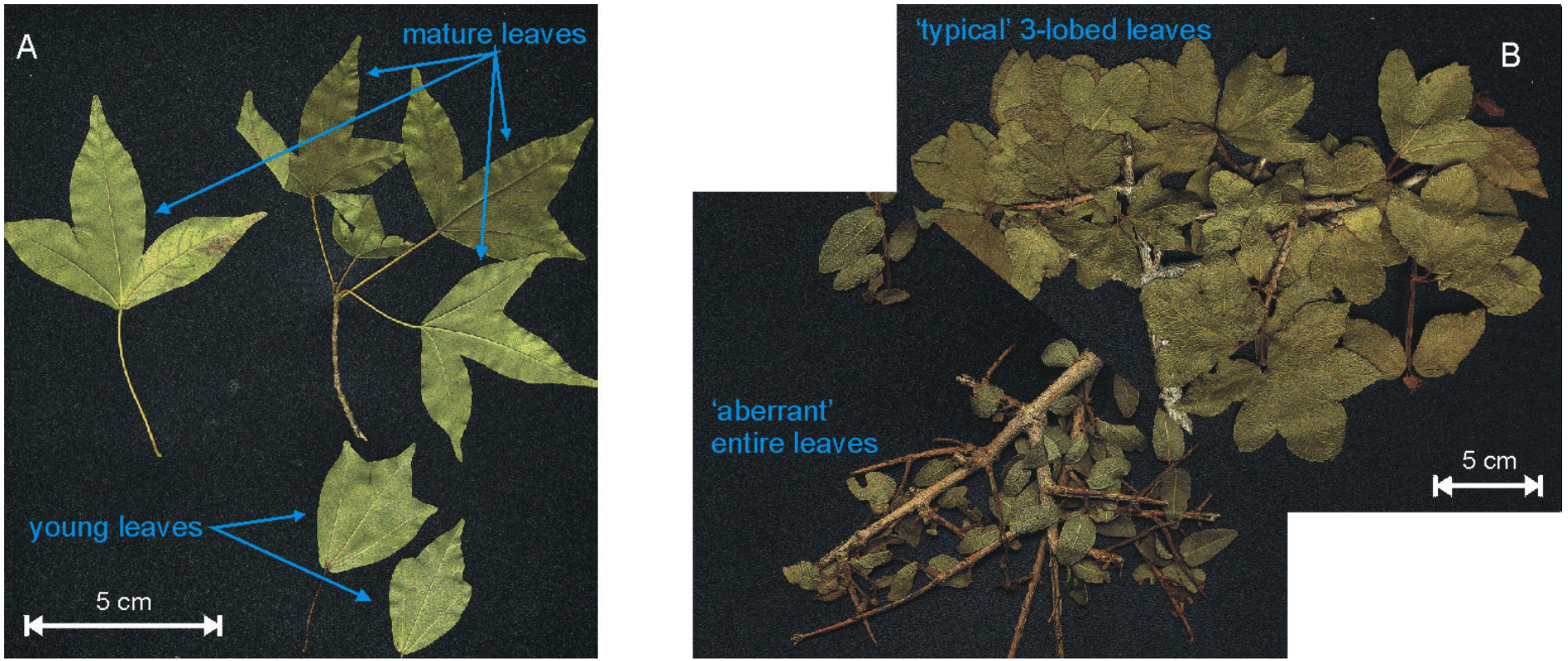


Figure 4-4: Leaf variability within individuals of *Acer*.
A: Young and mature leaves of *A. buergerianum* (sect. *Pentaphylla* ser. *Trifida*, BGTue). **B:** Shoots of *A. sempervirens* (sect. *Acer* ser. *Monspessulana*, Crete).

Wood: OGATA's (1967) classification of *Acer*, which is in wide agreement with the current systematics apart of the hierarchical position of the proposed taxonomic entities (cf. Fig. 4-1) and the placement of several, mostly western Eurasian species, puts a major stress on wood anatomy. In particular, the wood rays' shape, width, and height is rather conserved for the proposed sections. Also he was able to detect (by light microscopy) characteristical amounts of crystals and starch bearing fibres for taxa that he assigned to one section. Furthermore, he found that taxa belonging to sections *Lithocarpa* and *Platanoidea*[55] are exclusive in producing latex.

Flower & Inflorescence: The flowers of *Acer* are small and inconspicuous (→ Fig. 4-5). The perianth is often of greenish or yellowish colour, and up to 200 flowers are associated in raceme-like inflorescences, which emerge from terminal or lateral buds. The general flower formula is K5C5A8(G2), like it is found in the supposedly related Sapindaceae and the sister taxon *Dipteronia*. Derivations from the basic formula are found, but restricted to near-relatives or highly specialised taxa. Another systematical feature is the structure and position of the honey disc, also referred to as floral disc, which was used initially by PAX (1885) to define supersectional groups[56]. The inflorescences of *Acer* spp. exhibit an enormous morphological variability (DE JONG 1976; VAN GELDEREN et al. 1994) from elongated panicles to extremely reduced umbels (→ Fig. 4-6). Furthermore, the inflorescence type is strongly correlated to a number of other floral characteristics, such as the number of accompanying leaf pairs, whether the inflorescence originate from terminal or lateral buds, and the sexuality of the plant[57]. Individuals of *Acer* are either andromonoecious[58] (the putatively ancestral condition), androdioecious[59], or dioecious[60] in the case of section *Negundo*. The linkage between sexuality and floral elements was first recognised by OGATA (1967) and was intensively studied by DE JONG (1976). Table 4-3 sums up the condition of this character

[55] "Sections" according to current systematics. They comprise OGATA's (1967) sects. *Campestria* and *Platanoidea* (⇨ sect. *Platanoidea*), respectively *Lithocarpa* and *Macrophylla* (⇨ sers. *Lithocarpa, Macrophylla*; cf. Table 4-1)

[56] Although this floral element is of indisputable systematic value, the supersectional groups proposed by PAX (1885) are artificial, which was confirmed by later authors (OGATA 1967; WOLFE & TANAI 1987; DE JONG 1976; cf. Table 4-1)

[57] How far this is correlated to anemophily or zoophily is not known.

[58] i.e. male and mixed inflorescences found on the same individual

[59] i.e. either male or androgynous individuals

[60] i.e. males and females

complex for the currently proposed systematic groups. Andromonoecious taxa typically have a complex, multi-flowered inflorescence with ± developed cincinni – i.e. a panicle – emerging from terminal or terminal and lateral buds, and accompanied in general by two or three leaf pairs (type: T 2-3 P in Table 4-3). The shift via androdioecism to dioecism (only found in sect. *Negundo*) is always correlated with the reduction of the panicle into a less-flowered raceme or few-flowered umbel (in sect. *Rubra*), the decrease of accompanying leaf pairs (from 2-3 to 0-1), and/or that inflorescences emerge exclusively from lateral buds (⇨ type L 0-1 R/U). The coherence of these features is indicated by bold letters in Table 4-3. In addition, derivations from the 'typical' aceroid flower are predominately found in sections with androdioecism and dioecism, respectively.

Figure 4-5: 'Typical' *Acer* flower.
Shown is the greenish, pentamerous flower of *A. platanoides*. (Source: server of the Botanical Garden, Ruhr-Universität Bochum). Enlarged ~ 6fold.

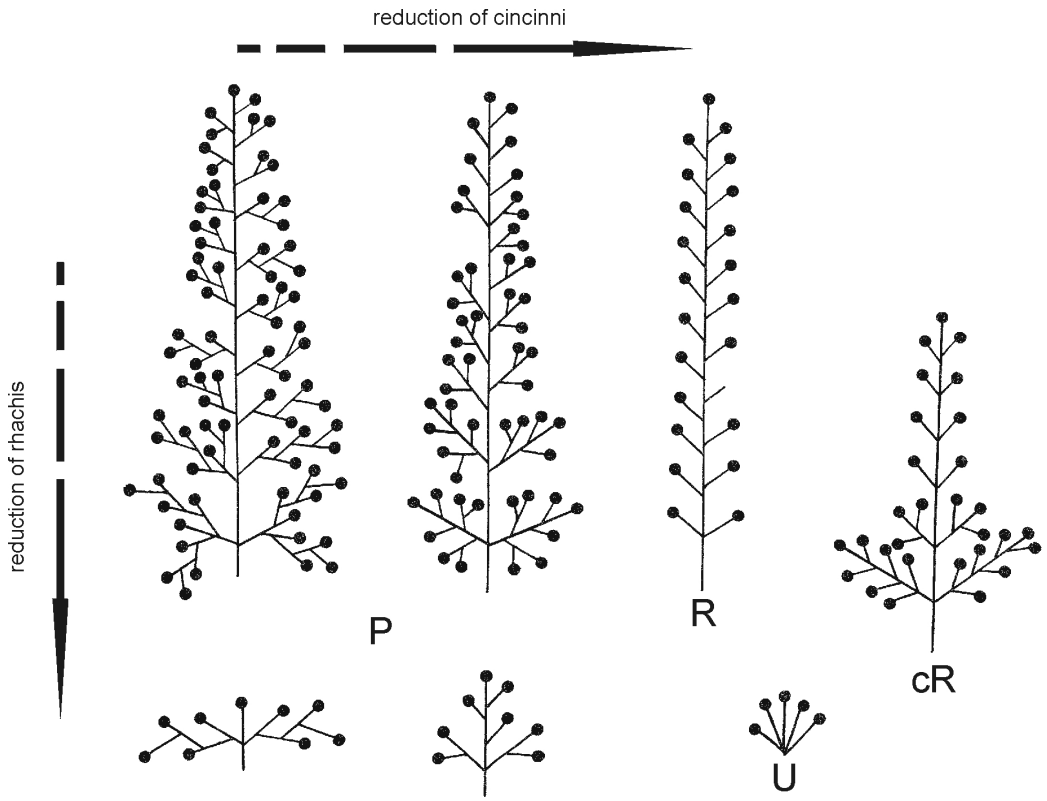


Figure 4-6: Inflorescence types of *Acer*.
General trends are the reduction of the rhachis length and number of cincinni. Note, that all imaginable transitions between two shown types are realised in genus *Acer* (cf. Table 4-3). **Abbr.:** P = panicle; R = raceme; U = umbel; cR = compound raceme. Basic schemes modified after VAN GELDEREN et al. 1994.

Table 4-3: Correlation between inflorescence type and sexuality in *Acer*

section	series	inflorescence type†	sexuality‡	derivations from the 'typical' aceroid flower
Acer		T 2-3 P	AM	
Indivisa		T **1 R**	**AD**	» 4-merous flower
Ginnala		T 2-3 P	AM	
Glabra	*Glabra*	T **1** P»**R**	AM»**AD**	
	Arguta	T **{0,1} R**	**AD**	4-merous flower
Hyptiocarpa		**L 0 {P,R}**	**AD**	up to 12 stamens
Macrantha		T **1 R**	**AD**	
Lithocarpa	*Lithocarpa*	**L 0 R**	**AD**	
	Macrophylla	T 2-3 P	AM	up to 12 stamens
Negundo	*Negundo*	**L (0,1)** ♂ **cR/**♀ **R**	**D**	honey disc reduced, » 4-merous flower
	Cissifolia	**L (0,1) R**	**D**	4-merous flower
Palmata		T 1-3 P	AM	
Parviflora		T (1,1-3,2-3) P	AM	
Pentaphylla		T (1,2-3) P	AM	
Platanoidea		T 2-3 P	AM	
Pubescentia		T 2-3 P	AM	
Rubra		**L 0 U**	**AD**	*A. saccharinum*: honey disc reduced
Trifoliata		T **1** ({P,**R**},**R**)	**AD**	up to 12 stamens
Wardiana		T ? P	AM	

† nomenclature follows OGATA (1967)
‡ AM = andromonoecious, AD = androdioecious, D = dioecious
"»" indicates a tendency within the species/individuals of this group

4.2.3 Nucleotide composition of the ITS

In the analyses 183 accessions are included representing 57 taxa of the major taxonomic groups of *Acer* (→ appendix). Three mono-, respectively bispecific sections (*Hyptiocarpa*, *Wardiana, Pubescentia*) and several series (*Glabra* of sect. *Glabra*, *Distyla/Parviflora* of sect. *Parviflora*, *Mandshurica* of sect. *Grisea*, *Penninervia* of sect. *Palmata*, *Pentaphylla* of sect. *Pentaphylla*) could not yet be taken into account because no material from original stands and/or botanical gardens was available. Accessions of 'typical' taxa of these sections and series can be taken from the gene bank, but have not been included in the analyses (cf. chapter 4.3.1). In addition, three accessions of the – bispecific – sister genus *Dipteronia* (*D. sinensis*) were assembled and used for comparison. The core parameters (number of nucleotides, CG-content, mean sequence divergence) for the amplified gene regions (ITS1, 5.8S rDNA, and ITS2) are given in the appendix. From the mere alignment, the taxonomical and systematical value of the ITS for *Acer* can be directly concluded:

1. **In general, the nucleotide composition of near-relatives (≙ assignation to a particular series and/or section) is strikingly similar.**

Clones from one sample, locality and/or taxon differ only slightly. Intraspecific length polymorphism is restricted to section *Macrantha* and series *Monspessulana.* With the exception of section *Macrantha*, all taxonomic groups exhibit a lower intraspecific, i.e. interpopulation and/or intragenomic, than interspecific variability. As a consequence, the nucleotide composition of the ITS is unique for most of the included taxa, allowing a taxonomical identification on the base of the sequence data. Especially the oligonucleotide motives in regions comprising length polymorphism (LP1 to LP4; cf. appended alignment) contain valuable taxonomical information. An example is given in Figure 4-7 for taxa of section *Platanoidea* and its putative sibling group section *Lithocarpa* (cf. chapter 4.3). Furthermore, taxa designated to morphologically well-described taxonomic groups, i.e. sections *Acer*, *Ginnala, Macrantha, Platanoidea, Palmata* (sers. *Palmata*, *Sinensia* combined), *Rubra*, and series *Caudata, Cissifolia*, *Grisea*, *Negundo*, share a convincing number of similar genetic patterns. Key features of these patterns are remarkably conserved even in variable gene regions (cf. chapter 4.5). Only the accessions of *A. campbelli* ssp. *campbelli* exhibit a genotype which is markedly different from the distinctive genotypic characteristics of the according section *Palmata*. In fact, a detailed 'base-per-base' investigation of the accessions reveals a striking similarity in several parts with the genotypic

composition of the ITS in section *Macrantha*, while other regions correlate with the condition found in the remaining taxa of section *Palmata* (→ special remark).

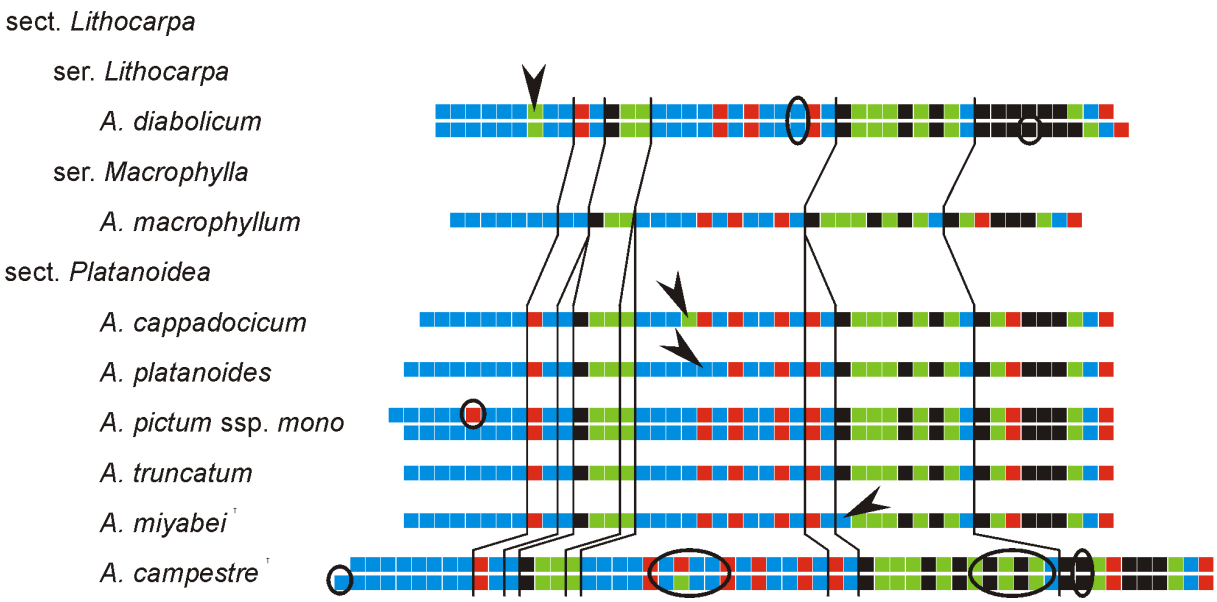


Figure 4-7: Oligonucleotide motives of clearly related *Acer* taxa (LP3).
Only *A. truncatum* shares an identical genotype with certain accessions of *A. pictum* ssp. *mono*. All other taxa are defined by specific molecular motives. Arrows indicate specific point mutations, specific insertions (up to 4bp in *A. campestre*) are encircled. Lines adumbrate presumably homologous gene regions.

2. **The accessions of *Dipteronia sinensis* are well distinguishable from all *Acer* spp. in a number of molecular genetical features (cf. chapters 4.3 & 4.4.1).**

In particular, several oligonucleotide motives contain nucleotide patterns which are not readily alignable to the general patterns detected within the genus *Acer*. Nevertheless, the overall genetic divergence in taxa-rich sections such as *Acer, Palmata,* and *Platanoidea* and their sister groups is comparatively high in relation to the genetical distance of *D. sinensis* to certain sections and taxa of *Acer* like section *Negundo*, series *Caudata*, and *A. caesium* (sect. *Acer* ser. *Acer*), especially if the conservative – readily alignable – regions are validated. Therefore, aside the arguments personated in chapter 2.4.2, maximum parsimony and distance methods should be avoided, if a phylogenetic hypothesis based on the assembled data set is computed (further discussed in chapter 4.6.2).

3. **Prominent, taxon-typical – sectional, serial, and specific – indels (≥ 5 bp) apart from the regions with common length polymorphism can be found within the sections *Indivisa*, *Ginnala, Rubra*, *Trifoliata,* and series *Trifida.***

Accessions of *A. griseum, A. maximowiczianum, A. triflorum* (ser. *Grisea*), *A. rubrum, A. saccharinum* (sect. *Rubra*), and *A. buergerianum* (ser. *Trifida*) lack numerous base pairs in the variable regions of ITS1, respectively ITS2[61]. Clones of *A. buergerianum* exhibit a prominent duplication of 6 bp and an additional insert of 7 bp in the 5' region of the ITS1. Insertions[62] in conservative regions are also exhibited in taxa of the subspecies of *A. tataricum* (sect. *Ginnala*) and *A. carpinifolium* (sect. *Indivisa*). Table 4-4 sums up significant indels found within the ITS of *Acer*. In addition, two neighbouring regions within the ITS1 (LP1, LP2), the 5' (LP3), and the 3' end (LP4; cf. Fig. 4-8) of the ITS2 comprise remarkable length polymorphism[63]. The polymorphic regions LP1 and LP2 are strongly correlated with the indels ID4 and ID5 (chapter 4.4.1). In the case of LP3, the length polymorphism is correlated with a strongly diverging molecular pattern comprising between 26 bp (*A. griseum*, ser. *Grisea*) and 57 bp (*A. campestre*, sect. *Acer*). Nevertheless, supposedly nearly related taxa – from a morphological and biochemical point of view – are remarkably uniform. This observation confers also to groups of taxa assignable to morphologically well-defined series and sections (Figs. 4-7 & 4-8; see above).

Table 4-4: List of conspicuous indels detected within ITS1 and ITS2 of *Acer*.

	length ITS1	ID1	ID2	ID3	ID4	ID5	length ITS2	ID6	ID7	ID8	ID9	ID10
sect. *Macrantha*	234-236bp	-	-	-	-	-	227-238bp	-	-	-	**I**or**D***	-
ser. *Arguta*	237/238bp	-	-	-	-	-	234bp	-	-	-	-	(+)
D. sinensis	233bp	-	-	-	-	-	238bp	-	-	-	**I***	-
sect. *Rubra*	221/222bp	-	-	-	-	+	233-235bp	-	-	-	-	-
sect. *Ginnala*	234/235bp	-	-	-	-	-	250/251bp	-	-	+	-	+
ser. *Grisea*	231-233bp	-	-	-	-	+	216-219bp	-	-	-	-	-
A.buergerianum, ser. *Trifida*	230bp	+	-	+	-	**+!**	236bp	-	-	-	-	-
A. carpinifolium, sect. *Indivisa*	220/227bp	-	±	-	-	**+!**	243bp	-	(+)	-	-	-
sect. *Acer*	233-242bp	-	-	-	±	-	233-242bp	-	+	-	-	-
A. ibericum	244/247bp	-	-	-	+/**+!**	-	244bp	+	+	-	-	-
arithmetic mean	234bp						236bp					

ID1...ID10 refer to indels ≥ 3 bp (→ alignment in the appendix).
Coloration refers to colours in Fig. 4-9ff.
* *Macrantha* genotype 1 exhibits + 6 bp (**I**), *D. sinensis* + 5 bp, in comparison to the consensus nucleotide composition, *Macrantha* genotype *2* lacks 6 bp (**D**).

[61] "Lack" in comparison with the majority rule consensus of all accessions.

[62] → footnote 35

[63] The sites comprised by LP1 to LP4 are denoted in the alignment (→appendix) at the according position.

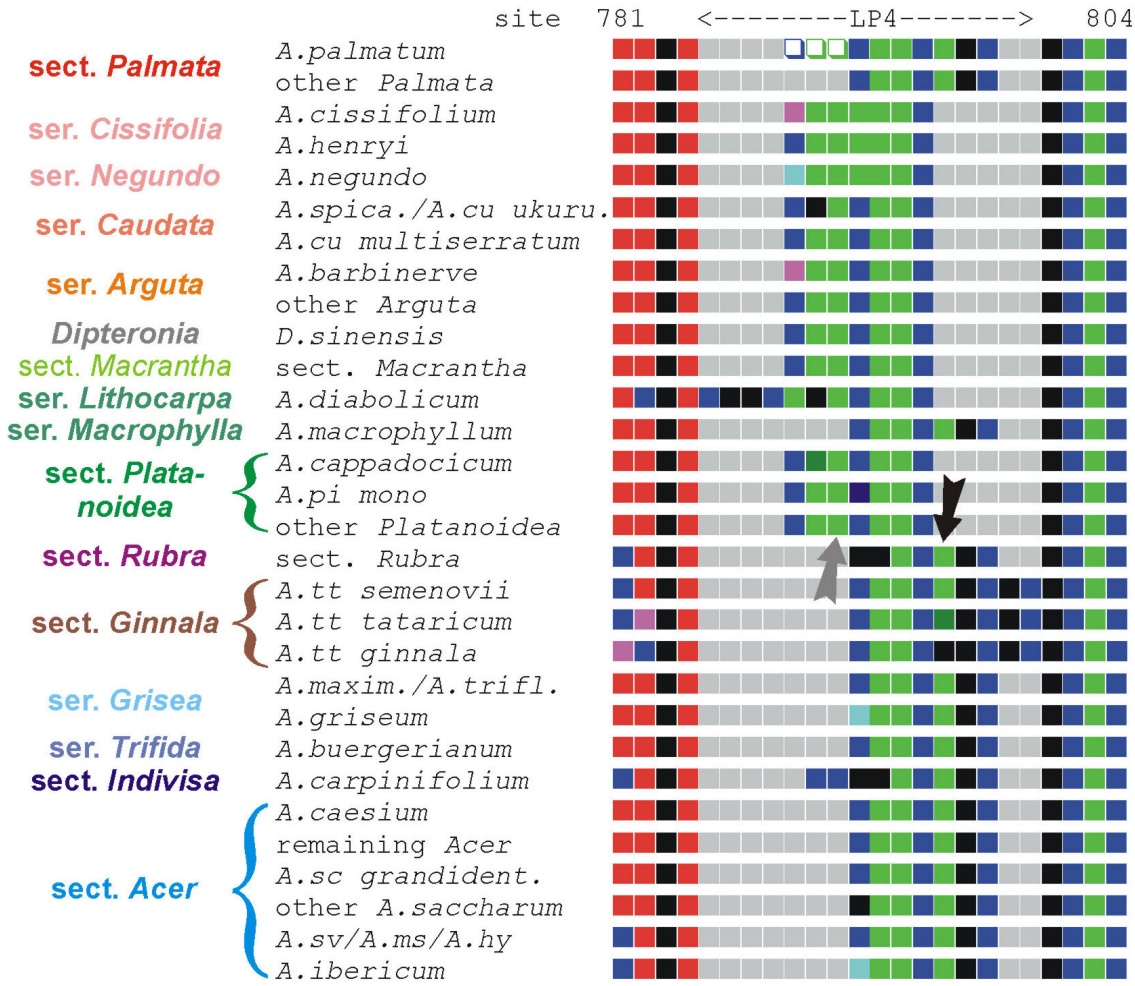


Figure 4-8: Nucleotide composition of LP4.
Main characteristics of this 7 to 11bp long motif are conserved within close relatives (following chapters) and currently accepted taxonomic groups (cf. Table 4-1). For example, a quintuple-"A" is only realised in sect. *Negundo* (sers. *Cissifolia, Negundo*). Subspecies of *A. tataricum* (tt, sect. *Ginnala*) are unique in sharing a 3' "GC" duplication. Also, varying levels of intra- and intertaxonomic variability are exhibited: A, in general, conservative nucleotide site (black arrow) exhibits a transition from "A" (ssp. *semenovii*) via "R" (ssp. *tataricum*) to "G" (ssp. *ginnala*) in the monospecific section *Ginnala*. All other accessions exhibit an "A" at this position (grey arrow indicates else possible homologous site; cf. Fig. 4-13, chapter 4.6.2). Note, that the motif of *D. sinensis* is identical to sect. *Macrantha* and taxa of sect. *Platanoidea*, ser. *Arguta,* and ser. *Caudata*. **Abbr.:** *cu* = caudatum ssp., *hy* = hyrcanum, *ms* = monspessulanum, *pi* = pictum ssp., *sc* = saccharum, *sv* = sempervirens; other taxon names abbreviated by the initial 5 letters. Identical accessions belonging to taxa assigned to the same taxonomic group are summed up. Standard colour code.

4. The detected intrasectional and –serial genetic variabilities vary remarkable and are not strictly linked to the number of taxa in the according taxonomic entities.

The three subspecies of *A. tataricum* (sect. *Ginnala*), the accessions of *A. diabolicum* (ser. *Lithocarpa*), and the taxa assigned to series *Caudata* (2 species, one represented by 2 subspecies) show a variability comparable to the taxa-rich section *Platanoidea*. On the other hand, the two species of section *Rubra* are remarkably uniform as well as taxa of each of the

two genotypes detected in taxa-rich section *Macrantha* (see also Fig. 4-28, chapter 4.5). In addition, the intrataxonomic variability also varies for near relatives. Taxa assigned to section *Acer* series *Monspessulana* are considered to be closely related. While the genetic variability of *A. ibericum* comprise intraspecific length polymorphism leading to distinct genotypes even within one population, accessions of *A. opalus* ssp. *opalus* and *A. opalus* ssp. *obtusatum* are practically identical at all sampled locations. The differing levels of inter- and intrataxonomic variability are exemplary illustrated in the genotypic characteristics of LP4 (→ Fig. 4-8).

No obvious 'pseudogenes' have been amplified and used in the analyses (cf. MAYOL & ROSELLO 2001). No accession shows deletions within the 18S, 5.8S, and 26S rRNA genes as far as they have been sequenced. The CG-contents of ITS1, ITS2, and 5.8S rDNA are within the range known for various tree species (55%-65%;→ appendix).[64]

Remark: DNA of *Acer campbelli* ssp. *campbelli* is available from a sampled leaf collected in the Morris arboretum in 1999 by Prof. Dr. M. Langer, professor for micropalaeontology, University of Bonn, Germany. Unfortunately, the sampled material is not optimally preserved and herbarised, hence a morphological taxonomical re-evaluation cannot be performed to exclude all possibility of doubt. In particular, leaves of *A. campbelli* ssp. *campbelli* are superficially similar to the leaves found in section *Macrantha*. However, none of the other samples assembled in the Morris arboretum was found to be a taxonomic misnomer or displayed a taxonomical problem. In addition, the accessions of *A. campbelli* ssp. *campbelli* are indeed intermediary between the 'typical' *Palmata*- and *Macrantha*-sequences (ITS1 ⇨ ± 'palmatoid', ITS2 ⇨ predominately 'macranthoid'). For these reasons, accessions of *A. campbelli* ssp. *campbelli* were included in the analyses to remain completeness (phylogram: Fig. 4-9; reconstruction of molecular evolution of oligonucleotide motives: Figs. 4-14 to 4-16), but their impact must not be overestimated. Also it cannot be completely ruled out, that the individual in the botanical garden is of hybrid origin.

[64] It has to be noted, that the current definition of 'pseudogenes' as e.g. used by MAYOL & ROSELLO (2001) may be inappropriate for rDNA genes in general (cf. studies dealing with nuclear dominance in hybrids, e.g. VOLKOV et al. 2001, in press).

4.3 Phylogeny of Acer *inferred from ITS sequence data*

Figure 4-9 shows the all compatible consensus tree representing a base of 9237 trees comprising data of all analysed taxa and inferred via Bayesian inference (parameters for all BI analyses: → Table 4-5). Morphologically well-defined taxonomic groups (OGATA 1967, emended by VAN GELDEREN et al. 1994) are sustained by high statistical support. In all topologies (100%) the genus *Acer* is a monophyletic[65] group with *D. sinensis* as its sister taxon, representing a natural, not artificial, outgroup. Topologies sustaining the sections *Ginnala*, *Macrantha*, *Platanoidea,* and *Rubra*, and the series *Arguta, Cissifolia, Grisea*, *Indivisa, Lithocarpa, Macrophylla*, *Negundo*, and *Trifida* (latter five represented by one taxon[66]) have a probability of 100%. The monophyly of series *Caudata* is sustained by 93%. Not or weakly sustained as monophyletic groups are sections (a) *Acer* and (b) *Palmata*:

(a) The monophyly of western Eurasian taxa of section *Acer* (including *A. carpinifolium* of sect. *Indivisa*) is sustained by an inappropriate *a posteriori* probability of 54%, due to the undetermined placement of *A. carpinifolium* (monospecific sect. *Indivisa*). Furthermore, the systematical position of the single Asian taxon *A. caesium* of section *Acer* in relation to remaining taxa of section *Acer* and *A. carpinifolium* is obscure.

(b) Due to its aberrant nucleotide composition, *A. campbelli* ssp. *campbelli* (cf. special remark, chapter 4.2.3) is placed as sister taxon to sect. *Macrantha* (93% prob.). However, the remaining taxa of section *Palmata* are definitely monophyletic (100%).

Thus, the inclusion of accessions of *A. carpinifolium* and *A. campbelli* ssp. *campbelli* distorts the overall phylogenetic hypotheses and is of concern in respect to the monophyly of sections *Acer* and *Palmata*.

Figure 4-9 (following page): ML phylogram (BI) of genus *Acer*.
Current sections and series are in general well-supported (>90%). In addition, accessions of the same species are grouped together, exceptions can only be found within sects. *Macrantha* and *Palmata*. The black arrow denotes the most probable root (see text). Topology shown is based on the all compatible consensus of 9237 trees, branches with ≤50% support are printed as dashed lines. Percentages at branches indicate probabilities of according divergence points. **Abbr.:** sspp.: several subspecies.

[65] "Monophyletic" is used only to refer to a group of taxa which have a putative common origin (≙ HENNIG 1950; cf. HENNIG & SCHLEE 1978). The termini "polyphyletic" and "paraphyletic" are used accordingly. In molecular systematical studies these termini are widely associated with MP, a method contrasting the process-based ML, in its character-based attitude.

[66] Sect. *Indivisa*, sers. *Macrophyllum, Negundo* are monospecific, ser. *Lithocarpa* comprises 2, ser. *Trifida* up to 13 species, mostly endemic to parts of China (according to VAN GELDEREN et al. 1994).

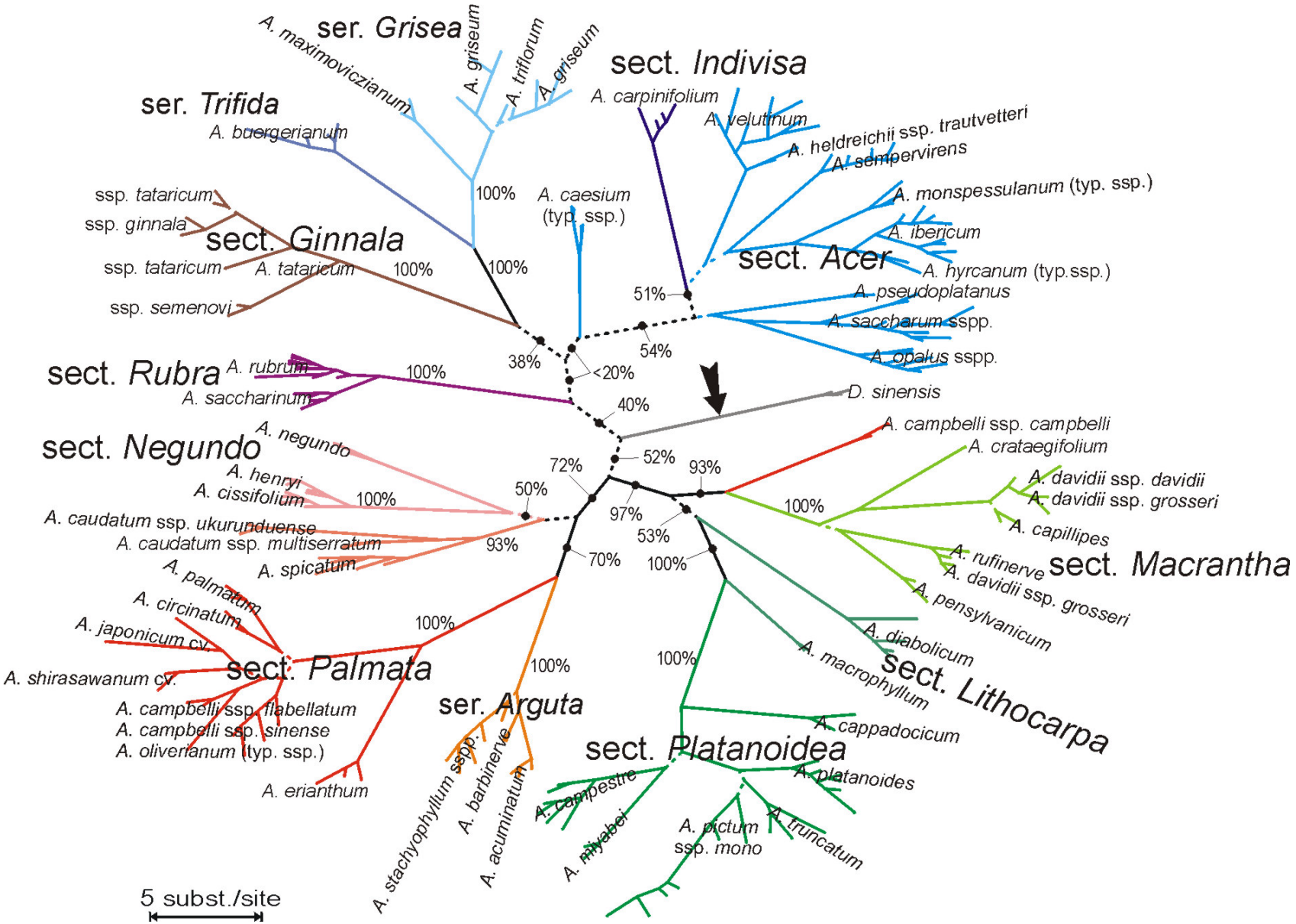

ser. Grisea
A. maximoviczianum
A. griseum
A. triflorum
A. griseum
sect. Indivisa
A. carpinifolium
A. velutinum
A. heldreichii ssp. trautvetteri
A. sempervirens
ser. Trifida
A. buergerianum
100%
A. caesium (typ. ssp.)
A. monspessulanum (typ. ssp.)
A. ibericum
ssp. tataricum
ssp. ginnala
sect. Ginnala
ssp. tataricum
A. tataricum
100%
100%
sect. Acer
A. hyrcanum (typ.ssp.)
51%
A. pseudoplatanus
A. saccharum sspp.
54%
A. opalus sspp.
38%
<20%
sect. Rubra
A. rubrum
100%
A. saccharinum
D. sinensis
40%
A. negundo
sect. Negundo
A. campbelli ssp. campbelli
A. crataegifolium
52%
72%
93%
A. henryi
A. cissifolium
100%
50%
A. davidii ssp. davidii
A. davidii ssp. grosseri
A. caudatum ssp. ukurunduense
97%
53%
100%
A. capillipes
A. caudatum ssp. multiserratum
93%
A. rufinerve
sect. Macrantha
A. davidii ssp. grosseri
A. spicatum
70%
100%
A. pensylvanicum
A. palmatum
A. circinatum
A. diabolicum
100%
100%
A. macrophyllum
A. japonicum cv.
sect. Palmata
sect. Lithocarpa
A. shirasawanum cv.
100%
ser. Arguta
A. cappadocicum
A. campbelli ssp. flabellatum
A. campbelli ssp. sinense
A. oliverianum (typ. ssp.)
sect. Platanoidea
A. platanoides
A. stachyophyllum sspp.
A. barbinerve
A. acuminatum
A. campestre
A. miyabei
A. pictum ssp. mono
A. truncatum
A. erianthum
5 subst./site

Table 4-5: Parameters for BI analyses performed for *Acer.*

	Mean			Variance			95% Cred. Interval					
							lower limit	upper limit	lower limit	upper limit	lower limit	upper limit
Parameter	Fig. 4.6	Fig. 4.7	Fig. 4.8	Fig. 4.6	Fig. 4.7	Fig. 4.8	Fig. 4.6		Fig. 4.7		Fig. 4.8	
r(G<->T)	set to 1						set to 1					
r(C<->T)	9,6	10,0	12,1	2,6	3,73	7,77	6,9	12,9	7,0	15,1	7,1	18,1
r(C<->G)	1,2	1,2	1,2	0,1	0,08	0,13	0,8	1,8	0,7	1,8	0,6	2,0
r(A<->T)	1,2	1,4	2,2	0,1	0,14	0,55	0,7	2,0	0,8	2,2	1,0	3,9
r(A<->G)	7,4	7,7	11,2	1,7	2,41	7,73	5,1	10,2	5,1	11,8	6,6	17,0
r(A<->C)	2,0	2,0	3,0	0,2	0,22	0,78	1,2	2,9	1,3	3,1	1,5	5,0
pi(A)	20%	21%	15%	± none	± none	± none	18%	23%	18%	23%	12%	17%
pi(C)	32%	31%	33%	± none	± none	± none	29%	35%	29%	34%	30%	37%
pi(G)	28%	27%	28%	± none	± none	± none	25%	31%	25%	30%	24%	32%
pi(T)	20%	21%	24%	± none	± none	± none	18%	22%	18%	23%	21%	27%
alpha parameter	0,050	0,050	0,050	± none	± none	± none	0,050	0,051	0,050	0,050	0,050	0,050
prop. of invariant sites	0,005	0,005	0,005	± none	± none	± none	0,000	0,017	0,000	0,016	0,000	0,018

Fig. 4.6: all data included, based upon 9237 samples
Fig. 4.7: complete ITS1 and ITS2; *A. campbelli* ssp. *campbelli* , *A. carpinifolium* excluded; 9220 samples
Fig. 4.8: only conservative sites; *A. campb.* ssp. *campb./A. carpinif.* excluded; 7996 samples

To draw a more concise image and further investigate these problems, a more detailed analysing of the data is necessary. The exclusion of *A. campbelli* ssp. *campbelli* and *A. carpinifolium* from the analysis (→ Fig. 4-10) allows to distinguish three major lineages: a ***Palmata*-clade** (97% prob.; comprising sect. *Palmata*, sers. *Arguta, Caudata, Cissifolia, Negundo*), a ***Platanoidea*-clade** (100% prob., sects. *Lithocarpa, Macrantha, Platanoidea*), both monophyletic, and a paraphyletic[67] "***Acer*"-group** (sects. *Acer*, *Ginnala, Rubra*, sers. *Grisea, Trifida*). A sibling relationship between the *Palmata*- and *Platanoidea*-clade is sustained by 54%. The terminal taxonomic groups (≙ series and sections as proposed by VAN GELDEREN et al. 1994) are optimally sustained (≥ 99%). In addition, further interserial and –sectional relationships are recognised:

- The phylogenetic backbone of the *Platanoidea*-clade is satisfyingly resolved. Section *Macrantha* is the sister group to a clade comprising series *Lithocarpa, Macrophylla*, and section *Platanoidea* (55%). *Acer macrophyllum* (ser. *Macrophylla*) is the direct sister taxon of section *Platanoidea* (100%).
- Within the *Palmata*-clade, series *Arguta* is the most probable sister group of section *Palmata* (64%). A probability of 55% sustains a section *Negundo* (≙ VAN GELDEREN et al. 1994) comprising the series *Cissifolia* and *Negundo*. Whether series *Caudata* is the sister group of *Arguta* + *Palmata* or *Cissifolia* + *Negundo* is not resolved in detail.
- Despite the apparent monophyly of series *Grisea* and *Trifida* (100% prob.), the exact phylogenetic position of sections *Acer*, *Ginnala, Rubra* in relation to *Grisea* + *Trifida* cannot be determined. A closer relationship between west Eurasian taxa of section *Acer* and the Asian taxon *A. caesium* (sect. *Acer* ser. *Acer*) is suggested by an *a posteriori* probability of 55%.

If the variable regions[68] are excluded during analysis (→ Fig. 4-11), the originally paraphyletic "*Acer*"-group (Figs. 4-9, 4-10) comes out to be a monophyletic ***Acer*-clade** (99% prob.). The *Palmata*-clade is broken up, but series *Arguta* is still the sister group of section *Palmata*. The monophyly of the *Platanoidea*-clade is sustained by a mere 51%, but section *Macrantha* forms a monophylum with *A. diabolicum* and *A. macrophyllum* (77% prob.; ⇨ sect. *Lithocarpa*). In Table 4-6 the systematic and phylogenetic implications of the BI analyses based on the new data are summed up.

[67] in relation to the *Palmata*- and *Platanoidea*-clades and with respect to the fact that the genus *Acer* is a monophylum

[68] I.e. all ITS regions that comprise common length polymorphism (→ appended alignment).

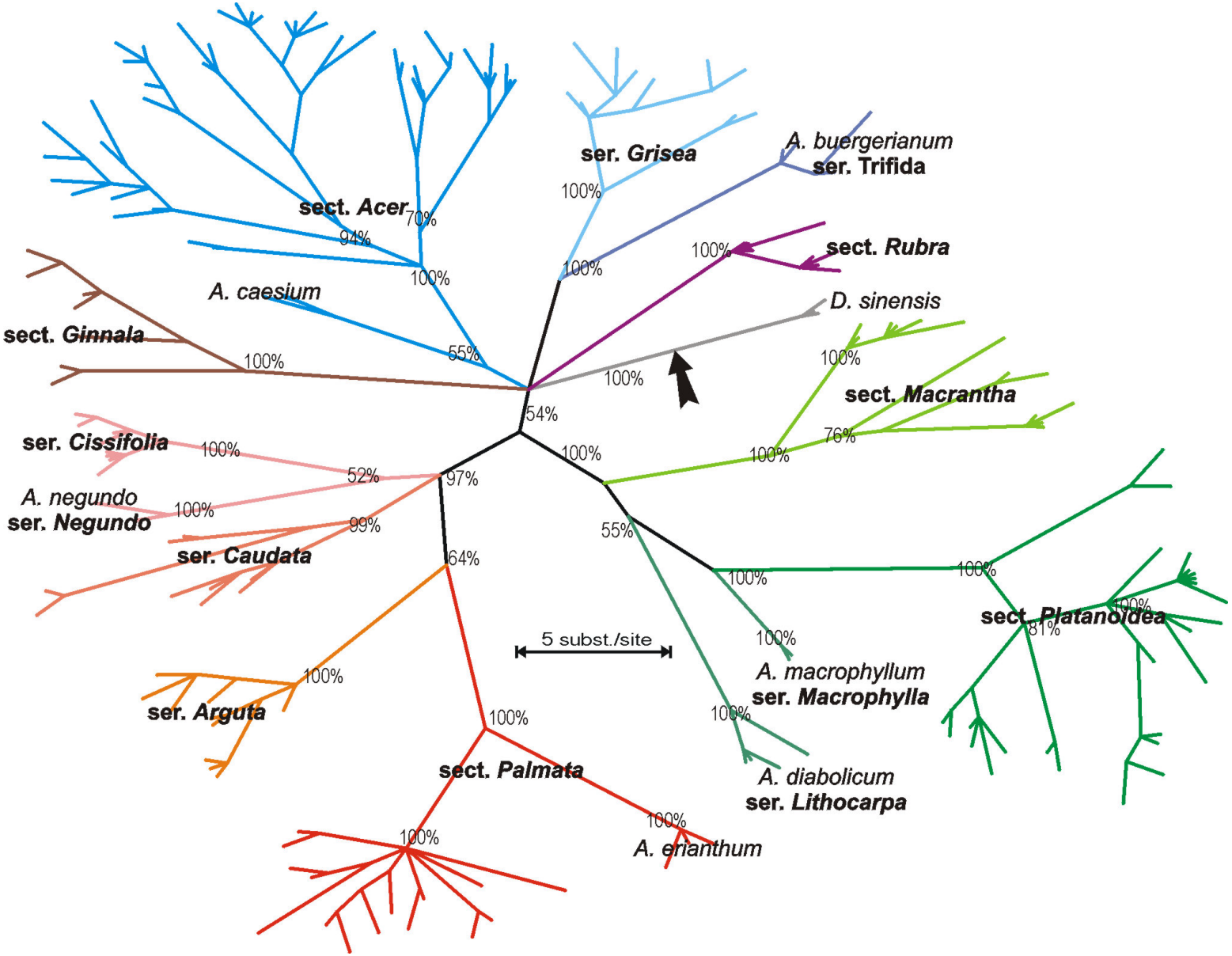

sect. Acer
ser. Grisea
A. buergerianum
ser. Trifida
sect. Rubra
D. sinensis
A. caesium
sect. Ginnala
sect. Macrantha
ser. Cissifolia
A. negundo
ser. Negundo
ser. Caudata
ser. Arguta
sect. Palmata
A. macrophyllum
ser. Macrophylla
A. diabolicum
ser. Lithocarpa
A. erianthum
sect. Platanoidea
5 subst./site
94%
70%
100%
100%
100%
100%
55%
100%
100%
54%
100%
100%
76%
100%
52%
97%
100%
99%
64%
55%
100%
100%
100%
81%
100%
100%
100%
100%
100%
100%

Figure 4-10 (preceding page): Phylogram (ML via BI) computed without accessions of *A. carpinifolium* and *A. campbelli* ssp. *campbelli*.
The *Palmata*- and *Platanoidea*-clades are well-sustained: 97% (instead of 72%, Fig. 4-9), respectively 100% (97%). *Acer caesium* is associated with the remaining taxa of sect. *Acer*, and the monophyly of the western Eurasian taxa of sect. *Acer* is now supported by 100% (54%, incl. *A. carpinifolium* of sect. *Indivisa*). The shown topology confers with a majority rule consensus of 9220 saved trees, branches <50% are collapsed. Labelling as in Fig. 4-9.

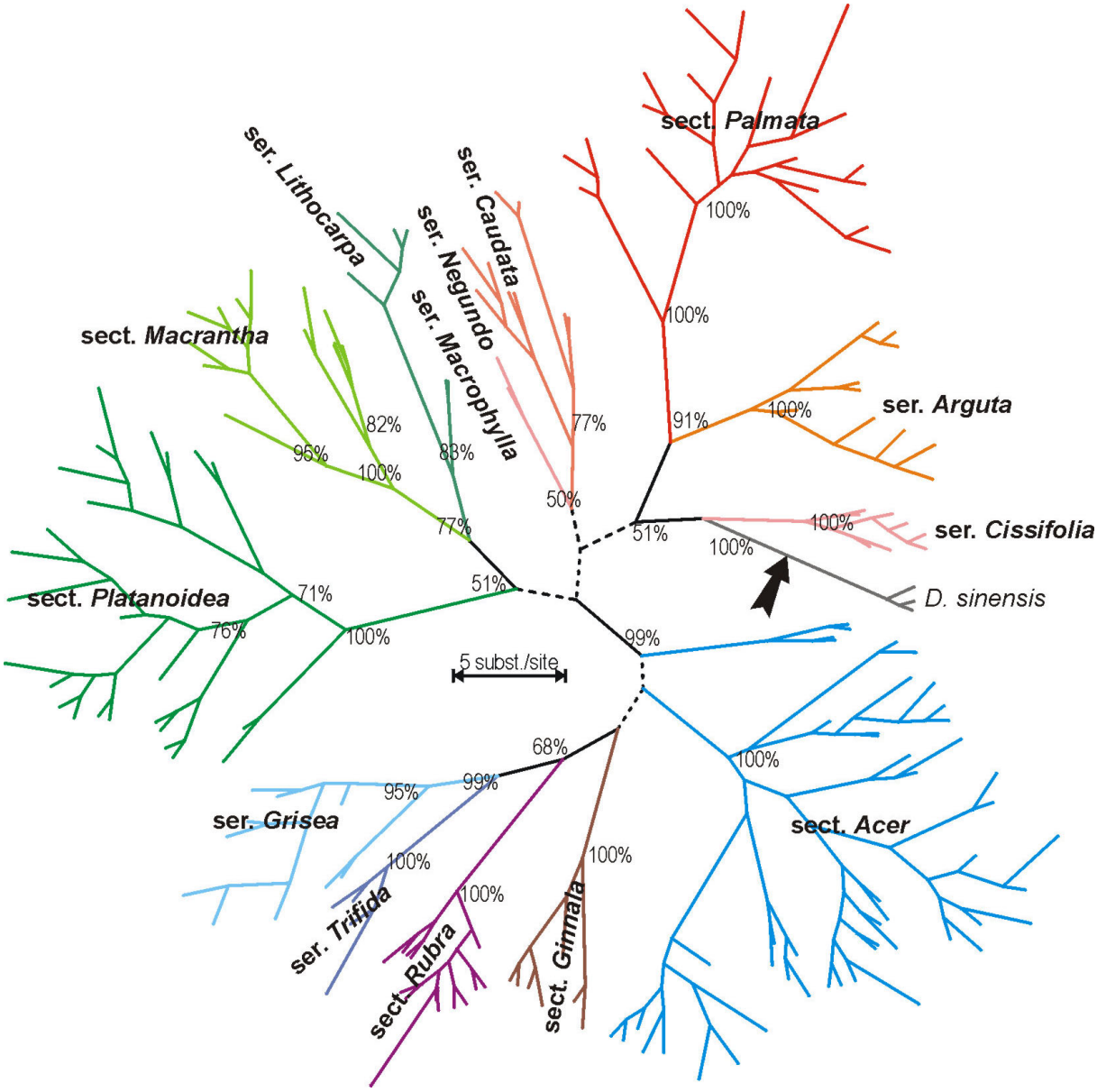


Figure 4-11: BI analysis based on the readily aligned ('conserved') ITS regions.
Taxa of the "*Acer*"-group are recognised as relatives: ⇨ *Acer*-clade (99% prob.). Within the *Palmata*- and *Platanoidea*-clades the support of the terminal relationships increases, while that of the basal divergences decreases. All compatible consensus tree, graphics/labelling as in Fig. 4-9.

Table 4-6: Sustain and phylogenetic relationships of currently accepted taxonomic entities inferred from Figs. 4-9, 4-10 & 4-11.

current synopsis			taxa analysed (incl. subspecies)	representing ... species	clade *sensu* new data	probability according new data: all data included (→ Fig. 4-9)	probability according new data: without *A. cb* ssp. *campbelli*, *A. carpinifolium* (→ Fig. 4-10)	probability according new data: only conservative regions used (→ Fig. 4-11)
section	series							
Parviflora	Caudata		3	2	*Palmata*-clade	93%	99%	77%
Negundo	Negundo			1		✓ 50%	✓ 52%	✓ 50%
	Cissifolia			2		✓	✓	✓
Glabra	Arguta		4	3		✓ 72%	✓ 97%	✓ n.r.
Palmata	Palmata			4		✓	✓	✓
	Sinensia	remaining taxa	5	3		✗ 100% 70%	✗ ✓ 64%	✗ ✓ 91%
		A. cb ssp. *campbelli**				✗	✗	✗
Macrantha			6	5	*Platanoidea*-clade*	93%	not included	
Lithocarpa	Lithocarpa			1		✓	✓	✓
	Macrophylla			1		✓ 97%	✓ 100%	✓ 77% 51%
Platanoidea				6		✓ 100% 53%	✓ 100% 55%	✓ 83%
Indivisa				1		✓	✓	✓
Acer	Saccharodendron		3	1	*Acer*-clade	✓	not included	
	Monspessulana		6	5		✓ 54% ✗	✓ ✗ 100% 55%	✓ ✗ ✓ ✗
	Acer	remaining taxa		4		✗ 40%	✗ n.r.	✗ 99%
		A. caesium				✓	✓	✓
Ginnala			3	1		✓	✓	✓
Rubra				2		✓	✓	✓
Trifoliata	Grisea			3		✓ 100%	✓ 100%	95% 99% 68%
Pentaphylla	Trifida			1		✓	✓	✓

✓ = series/section *sensu* VAN GELDEREN et al. 1994 sustained with 100%; ✗ = not sustained

n.r. = clade *sensu* new data not resolved as monophylum

* position of *A. campbelli* ssp. *campbelli* → special remark

In general, the following first results have to be pointed out for discussion:

- The genus *Acer* is monophyletic. *Dipteronia sinensis* is distinct from all other included taxa. As consequence, although no artificial outgroup is specified during analysis, all randomly computed topologies (via BI) put the root next to *D. sinensis*.
- The ITS region *is* capable of resolving intersectional and interserial relationships. Unlike former studies (discussed below) most divergence points are well-sustained by appropriate probabilities. Furthermore, the analyses strongly sustain the presence of three genetically distinguishable lineages, i.e. the *Acer-, Palmata-,* and *Platanoidea*-clades. This is due to an enhanced alignment (→ special remark; cf. chapter 2.4.1) and a more appropriate analytic method (ML via BI instead of MP and NJ; cf. chapters 4.3.1 & 4.6.2). The point of origin and initial diversification event of theses lineages cannot be resolved, which is exhibited by the underdetermined placement of *D. sinensis*[69].
- Due to the miscellaneous intersectional and -serial genetic divergence, a selective use of the data allows to focus on different hierarchies. Apparently, a constant molecular clock is not enforced. For example, a reduction to regions without length polymorphism resolve better the relationships within the *Acer*-clade, although the support of the *Palmata*- and *Platanoidea*-clades diminishes. *Vice versa*, the inclusion of the variable regions allow to resolve the position within the *Palmata*- and, in particular, the *Platanoidea*-clade. The overall topology of both analyses is identical.

Remark: Former authors studying the ITS of *Acer* (CHO et al. 1997; SUH et al. 2000; TIAN et al. 2002[70]) did not publish their alignments. In addition, they referred only shortly to the used alignment algorithm and software, respectively, and did not clearly specify where and how a manual re-alignment was undertaken. Therefore, it cannot be directly deduced, whether the here presented alignment is more appropriate or not. However, an alignment procedure as it is described in chapter 2.4.1, in which a major impact lies on the recognition of mutational patterns in regions comprising length polymorphism, is only possible with an appropriate database. Such a database, that reflects a comprehensive level of intra- and interspecific variability, can only be sufficiently provided by data from a cloned DNA library (here presented data). Since former authors relied on data from directly sequenced PCR products of one individual per taxon, and mainly one taxon per section and series, a comparable alignment could not have been generated (for further discussion see chapters 4.3.1 & 4.6).

[69] Either at the polyphyletic plateau of the *Acer*-clade (complete data) or as sister group to ser. *Cissifolia* and the remaining *Acer* sections (variable data excluded).

[70] The alignment of ACKERLY & DONOGHUE (1998) is accessible via the TreeBase databank (cf. following chapter).

4.3.1 Comparison with previous DNA studies

Only very few divergence points of the MP phylogram (ITS; → Fig. 4-12**A**) presented in ACKERLY & DONOGHUE (1998) are supported by high bootstrap values. SUH et al. (2000), including the data from CHO et al. (1997), were not able to resolve the phylogenetic backbone of the genus on the base of ITS sequence data, either (→ Fig. 4-12**B**). To reconstruct the origin of two endemic island species of *Acer* from the Korean Ullung island, PFOSSER et al. (2002) used sequence data from the chloroplast *trn*L intron, respectively the *trn*L-F IGS, and AFLP data. A MP phylogram (→ Fig. 4-12**C**) comprising mainly Asian taxa sustained the sectional division of VAN GELDEREN et al. (1994; Table 4-1) with bootstrap and jackknife values >50%, except for taxa assigned to the sections *Lithocarpa* (in a strict sense ⇨ ser. *Lithocarpa*: *A. macrophyllum* was not included in the study)*, Ginnala*, *Glabra* (in a strict sense ⇨ ser. *Arguta*: *A. glabrum* not included), and *Parviflora* (*A. distylum* of monotypic ser. *Distyla*; *A. caudatum* ssp. *ukurunduense* as only representative ser. *Caudata*; *A. nipponicum* of monotypic ser. *Parviflora* not included). Except for the obscure placement of accessions of *A. stachyophyllum*, the recognition of distinct series and sections, respectively, is in agreement with the analyses presented herein. One of two accessions assigned to *A. stachyophyllum*, a representative of series *Arguta,* is placed within section *Ginnala* (i.e. *A. stachyophyllum* ssp. *betulifolium*) and the other as sister taxon of *A. distylum* (i.e. *A. stachyophyllum* ssp. *stachyophyllum*). According to the here presented data these taxa belong to series *Arguta*. As consequence, series *Arguta* as well as section *Ginnala* are monophyletic and sustained with 100%. Again, the phylogenetic backbone could – similar to previous studies based on ITS data – not be resolved by PFOSSER et al. (2002), bootstrap and jackknife values were generally below 50%, and according clades comprised only very few character changes. The same holds true for the consensus tree presented by TIAN et al. (2002) based on MP analyses of ITS and *trn*L-F IGS data, respectively, including further ITS data available from the gene bank. Neither by analysing the ITS and *trn*L-F IGS data separately (by MP), nor by combining the two data sets (MP; → Fig. 4-12**D**) a topology was produced, which resolved appropriately sustained phylogenetic relationships. Simply the systematical affinity between closely related taxa was affirmed, that can be assigned to one of the currently accepted series and sections[71] (e.g. nine accessions, ITS, assignable to sect. *Palmata*[72]), with

[71] The sectional division used by TIAN et al. (2002) differs from the otherwise used systematic synopsis introduced by VAN GELDEREN et al. (1994). Deviations are indicated as footnotes.

[72] sects. *Microcarpa* and *Palmata* according to TIAN et al. (2002)

the exception of taxa assigned to section *Acer* (included were *A. caesium* ssp. *giraldii*, *A. trautvetteri* of ser. *Acer*; *A. monspessulanum* ssp. ?*monspessulanum, A. opalus* ssp. ?*opalus* of ser. *Monspessulana*; *A. saccharum* ssp. ?*saccharum*[73] of ser. *Saccharodendron*[74]).

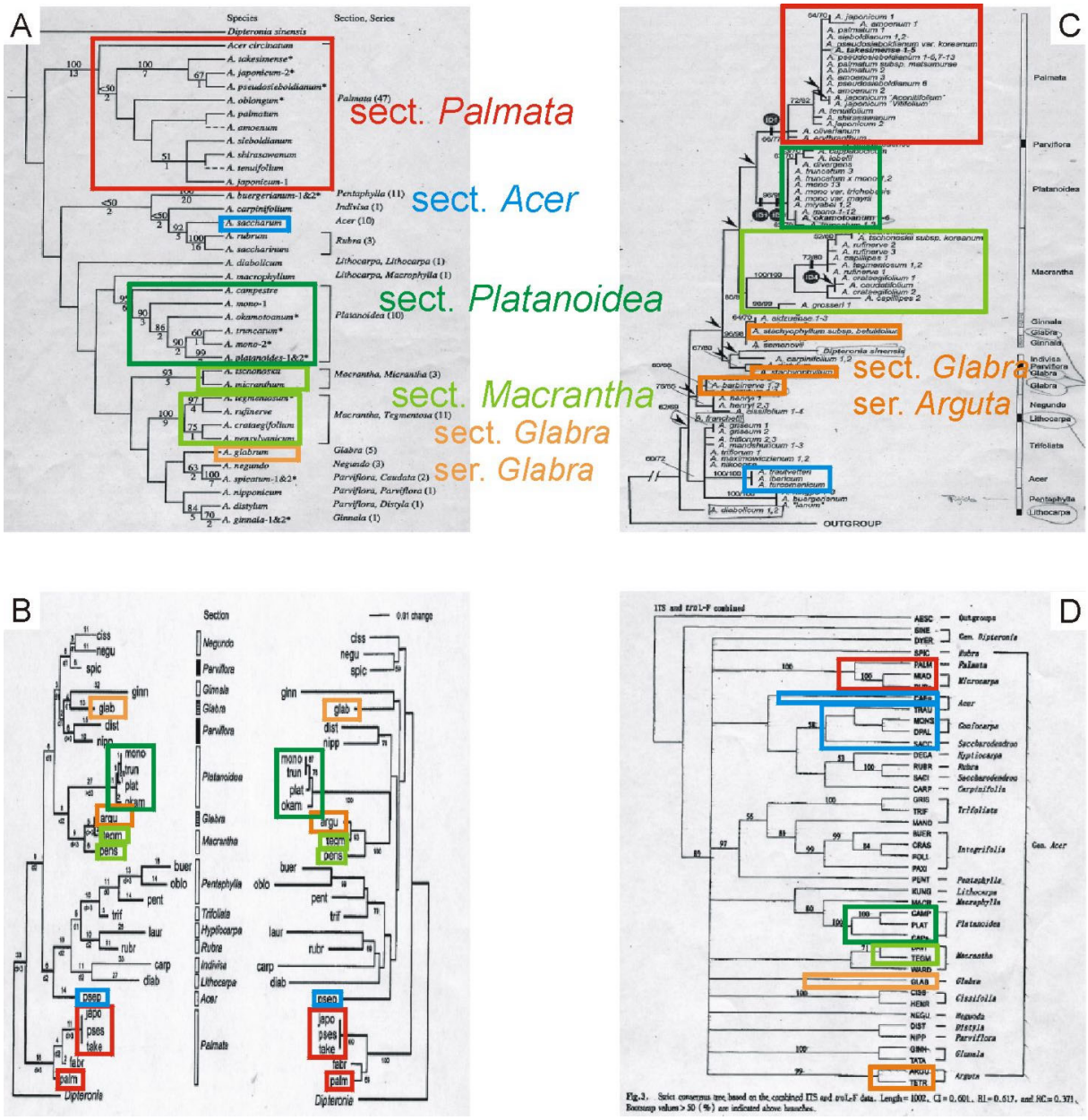


Figure 4-12: Dendrograms of previous molecular phylogenetical studies dealing with *Acer*. **A.** ACKERLY & DONOGHUE (1998; ITS; MP) **B.** SUH et al. 2000 (incl. data of CHO et al. 1997; ITS; MP & NJ;). **C.** PFOSSER et al. (2002; cpDNA; MP) **D.** TIAN et al. (2002; ITS + *trn*L-F IGS; consensus of MP & NJ). The position of important taxonomic groups is indicated by accordingly coloured circles.

[73] Accessions are presumably representing the typical subspecies.

[74] *A. saccharinum* (sect. *Rubra*) was wrongly assigned to sect. *Acer* ser. *Saccharodendron* by TIAN et al. (2002).

In spite of the difference in the general topology, i.e. phylogenetic backbone fully resolved by new data and not resolved by former studies, there are a number of similarities between former studies and our study considering the relatedness of two taxonomic entities[75]:

Position of taxa belonging to the *Palmata*-clade *sensu* new data

- In all former studies series *Caudata* (sect. *Parviflora*) plots together (± sustained) with series *Cissifolia* and *Negundo* (sect. *Cissifolia*), which is in agreement with our data. In TIAN et al. (2002) *A. spicatum* (ser. *Caudata*[76]) comes out to be somehow related (bootstrap <50) to series *Arguta, Cissifolia,* and *Negundo* in the strict consensus tree based on MP analysis of solely the ITS data (not reported in Fig. 4-12).
- Section *Palmata* forms a rather distinct group to other *Acer* spp., which can be directly concluded from the nucleotide composition of the ITS. A major difference is the placement of series *Arguta* as sibling of the above mentioned series and sections. SUH et al. (2000) placed *A. glabrum* (sect. *Glabra* ser. *Glabra*) next to "*A. ginnala*" (sect. *Ginnala*, syn. to *A. tataricum* ssp. *ginnala*), and *A. argutum* (sect. *Glabra* ser. *Arguta*) next to *A. tegmentosum* and *A. pensylvanicum* (sect. *Macrantha*). No representative of series *Arguta* was included by ACKERLY & DONOGHUE (1998), where *A. glabrum* comes next to *A. negundo* (ser. *Negundo*) and *A. spicatum* (ser. *Caudata*), although this placement is only weakly sustained. The cpDNA data of PFOSSER et al. (2002) confirm the distinctness of section *Palmata* and recognise *A. ukurunduense* (syn. to *A. caudatum* ssp. *ukurunduense*, ser. *Caudata*) as sibling taxon. However, their placement of taxa representing the other above mentioned sections and series is obscure. As stated above, although the ITS data of TIAN et al. (2002) indicate a relationships between series *Arguta, Caudata, Cissifolia* and *Negundo*, they were not able to determine the position of section *Palmata*.
- The phylograms presented in Figures 4-9, 4-10, and 4-11 group the four taxonomic entities *Arguta, Caudata, Cissifolia,* and *Negundo* as relatives to *Palmata*, a grouping, that is sustained by sufficient probabilities. Such a phylogenetic and systematic setting is not represented in any of the former studies, nor is a convincing alternative phylogeny produced.

[75] Only the studies of PFOSSER et al. (2002) and – to a lesser degree – of TIAN et al. (2002) provide sufficiently more than one taxon per taxonomic group.

[76] wrongly assigned as member of sect. *Rubra* by TIAN et al. (2002).

Position of taxa assigned to the *Platanoidea*-clade *sensu* new data

- In all studies, except for ACKERLY & DONOGHUE (1998), section *Macrantha* comes out to be somewhat related (statistical support <50%) to section *Platanoidea*, which is clearly well supported by the new data.
- Concentrating on East Asian taxa, neither SUH et al. (2000) nor PFOSSER et al. (2002) included *A. macrophyllum* in their studies. ACKERLY & DONOGHUE (1998) did so and found a weak support for the position of the north American *A. macrophyllum* (ser. *Macrophylla*) as sister taxon to the Eurasian section *Platanoidea*, which was confirmed by TIAN et al. (2002; bootstrap value of 80 in the combined analyses, <50 in the analyses based on ITS only, not resolved by *trn*L-F data). Such a placing is sustained by a 100% probability in our study.
- The appropriate phylogenetical position of *A. diabolicum* (sect. *Lithocarpa* ser. *Lithocarpa*) is also doubtful in former studies. Recognised as a basal sister taxon to a weakly sustained clade consisting of sections *Ginnala, Glabra, Macrantha, Platanoidea,* and *Parviflora* by ACKERLY & DONOGHUE (1998), SUH et al. (2000) placed it next to sections *Indivisa, Hyptiocarpa,* and *Rubra,* although also weakly supported. PFOSSER et al. (2002) – not using *D. sinensis* as a fixed outgroup taxon – found *A. diabolicum* to be rather distinct to other *Acer* accessions. Accordingly, it was placed as sister taxon to all other *Acer* spp. including *D. sinensis*. As well the ITS-based as the combined cladogram (Fig. 4-12**D**) of TIAN et al. (2002), who used two Sapindales taxa as outgroup, place series *Lithocarpa* (represented by an ITS accession of *A. diabolicum* and ITS and *trn*L-F accessions of *A. kungshanense*, respectively) next to series *Macrophylla* + section *Platanoidea* (bootstrap values <50). The genetical peculiarity of *A. diabolicum* can be confirmed by the newly assembled sequence data, in particular by the nucleotide composition of gene regions comprising length polymorphism. However, with the application of another analytic method – maximum likelihood via Bayesian inference instead of maximum parsimony – it is possible to assign this taxon to a well-sustained clade comprising *A. macrophyllum* (sect. *Lithocarpa* ser. *Macrophylla*) and sections *Macrantha* and *Platanoidea* (cf. Figs. 4-9, 4-10 & 4-11).

Position of taxa assigned to the *Acer*-clade *sensu* new data

Most interesting is the relation between taxa of the sections *Acer, Ginnala, Indivisa, Trifoliata, Pentaphylla,* and *Rubra* in the analyses. ACKERLY & DONOGHUE (1998), SUH et al. (2000), and PFOSSER et al. (2002) included merely a single, respectively three representatives

of section *Acer* (*A. saccharum*; *A. pseudoplatanus; A. heldreichii/ A. ibericum/ A. turcomanium*) in their studies. On the other hand, SUH et al. (2000) and PFOSSER et al. (2002) included three taxa each from section *Pentaphylla* (in PFOSSER et al. 2002, only taxa of ser. *Trifida*) and *A. laurinum* (SUH et al. 2000, sect. *Hyptiocarpa*), the only tropical *Acer* species. In TIAN et al. (2002) all series and sections are represented by one to two taxa (sect. *Acer*, incl. sers. *Acer, Monspessulana,* and *Saccharodendron*: 6 ITS, 5 *trn*L-F; sect. *Ginnala*: 2/2; sect. *Indivisa*[77]: 1/1; sect. *Trifoliata,* incl. sers. *Grisea* and *Mandshurica*: 3/3; sect. *Pentaphylla*, incl. sers. *Pentaphylla* and *Trifida*[78]: 6/5; sect. *Rubra*: 2/2; sect. *Hyptiocarpa*: 2[79]/1). Therefore, a direct comparison between their and the here presented analyses (numerous accessions representing all series of sect. *Acer*, but only *A. buergerianum* of sect. *Pentaphylla* ser. *Trifida* included, sect. *Hyptiocarpa* lacking) is difficult. Nevertheless, some former aspects of the internal relationship within this group are affirmed:

- ACKERLY & DONOGHUE (1998) found *A.* rubrum and *A. saccharinum* (sect. *Rubra*) to be closely related to *A. saccharum* (sect. *Acer*), with *A. carpinifolium* and *A. buergerianum* as sister taxa (but <50 bootstrap support). Such a position is partly sustained by TIAN et al. (2002), who found sections *Hyptiocarpa* and *Rubra* somewhat related to section *Acer* and section *Indivisa* (combined data set; <50 bootstrap support).
- Section *Trifoliata* is placed next to section *Pentaphylla* in SUH et al. (2000), next is section *Rubra*, and finally sections *Acer* and *Indivisa*. A sibling relationship between sections *Trifoliata* and *Pentaphylla* (including taxa of sers. *Pentaphylla* and *Trifida*) is well-supported by a bootstrap value of 97 in TIAN et al. (2002; combined data only).This placement was partly confirmed by PFOSSER et al. (2002), who report a rather sustained clade (bootstrap & jackknife values >60) comprising *Acer, Pentaphylla* and *Trifoliata*, but the mainly north American section *Rubra* was not included.
- The placement of section *Ginnala* is completely undetermined in each of the former studies. PFOSSER et al.'s (2002) positioning of *A. carpinifolium* (sect. *Indivisa*) and taxa assigned to section *Ginnala* disagrees with ACKERLY & DONOGHUE (1998), SUH et al. (2000), and our study. TIAN et al. (2002) could not determine more precisely the position of *A. tataricum* ssp. *tataricum* and *A. tataricum* ssp. *ginnala*, either.

[77] referred to as sect. *Carpinifolia*

[78] referred to as sect. *Integrifolia*

[79] *A. laurinum* (ITS) and *A. decandrum* (ITS, *trn*L-F), the latter name is actually a synonym of *A. laurinum* (VAN GELDEREN et al. 1994)

Quality of used accessions

The accessions (ITS) submitted to the gene bank by ACKERLY & DONOGHUE (1998) include a high degree of ambiguous base pairs, while those of CHO et al. (1997), SUH et al. (2000), and TIAN et al. (2002) are free from ambiguous sites. A close nucleotide-per-nucleotide comparison with the herein presented new data exhibits that the ambiguous base pairs of ACKERLY & DONOGHUE (1998) cannot be brought in accord with the intrataxonomic variability detected for new accessions of the same taxon, while sequences of CHO et al. (1997) and SUH et al. (2000), are differing from the newly assembled especially in the variable regions, where several key features (see above) are only partially represented. PFOSSER et al. (2002) – with the collaboration of Suh – state, that one of the sequences in SUH et al. (2000) is obviously wrongly labelled. On the other hand, several other accessions of SUH et al. (2000) are ± identical to the new data, but do not contain any additional information. A comparison of the sequences assembled by TIAN et al. (2002) shows an agreement in fundamentals, but again, represent only a limited scope of the actual variability represented in our data, especially in regard of the important western Eurasian representatives of section *Acer*. In addition, the quality seems to gradually 'fade' to the 3' end of the ITS in several accessions, exhibited by the lack of one nucleotide in multiple-A, -C, -G, -T motives (→ appendix). As a consequence, only some sequences of TIAN et al. (2002) can be used conditionally for means of comparison, while those of CHO et al. (1997) and SUH et al. (2000) representing not yet included taxa are difficult to handle. The sequences of ACKERLY & DONOGHUE (1998) are obviously not properly edited, if at all.

Summarising the discussion, two major points have to be emphasised to evaluate the significance of phylogenetic hypotheses based on sequence data for the genus *Acer*:

1. **Genetical affinity between two groups (i.e. mainly *one* taxon per group in former studies) shows some coarse similarities, although the according divergent points are generally supported to a higher degree in this study. The phylogenetic backbone is not resolved in former studies, but can be sufficiently resolved by the new data.**

This becomes especially apparent by the recognition of the *Acer-, Palmata-,* and *Platanoidea*-clades. One reason is the use of a more appropriate analytic software and method (i.e. maximum likelihood instead of distance methods and maximum parsimony), another the methodological dependence of former ITS studies on *Dipteronia* as a fixed outgroup. All studies indicate somehow the distinctness of the monophyletic sections *Palmata* and *Platanoidea* to other *Acer* spp. However, the precise placement varies extremely. In ACKERLY

& DONOGHUE (1998) and SUH et al. (2000) section *Palmata* is placed as an outgroup to all other *Acer* ssp., while section *Platanoidea* is either a sister group to sections *Ginnala, Macrantha, Parviflora,* and *A. glabrum* (ACKERLY & DONOGHUE 1998) or *Macrantha* and *A. glabrum* (SUH et al. 2000). In PFOSSER et al. (2002; cpDNA data) section *Palmata* is placed as a distinct 'crown group', forming a monophylum comprising *A. caudatum* ssp. *ukurunduense* of series *Caudata* (jackknife and bootstrap values: 66/77). Next is section *Platanoidea*. In our study, both sections, *Palmata* and *Platanoidea*, form discrete monophyla at the end of phylogenetic lineages.[80] Thus, the different position in former studies can possibly be attributed to long-branch attraction, a common problem of maximum parsimony (SANDERSON et al. 2000). *Dipteronia sinensis* is nearly equidistant to all major *Acer* lineages, in terms of character change (MP) as well as genetic distance. Dendrograms produced with maximum parsimony and distance methods without a fixation of *Dipteronia* as an outgroup, always place *Dipteronia* within *Acer* (PFOSSER et al. 2002; TIAN et al. 2002; here presented data, cladogram not shown). The reason for the recognition of genus *Acer* as a monophylum in our analysis is due to the tree-building method of the BI analyses. By producing a random tree from a random root on, topologies which place accessions of *D. sinensis* apart from accessions of *Acer* do always have a higher likelihood than an alternative placing, due to the composition of the underlying data.

2. **The use of cloned DNA sequences in the analysis, retaining not only the intertaxonomic, but also interpopulation and intragenomic variability, allows to produce a much more reliable alignment, but also, in combination with the Bayesian inference, a finer resolved phylogenetic hypothesis.**

The reason for this lies in the nucleotide composition of the ITS. The parameters from the Bayesian analysis (Table 4-5) show, that transitions occur up to ten times more than transversions. In addition, the probability for a specific point mutation follows a complex and general evolution model (GTR+Γ+I), which is to a high degree variable (Table 4-5). Such an underlying molecular evolution can only be reconstructed with process-based, mathematical-statistical methods like maximum likelihood. Genetic variability, respectively sequence divergence, which allows to directly infer phylogenetic relationships, is commonly accompanied by numerous indels in the variable regions. These indels pose a serious problem to the alignment process. Alignment algorithms (like the Clustal algorithm) are not able to

[80] The phylogenetic position sects. *Palmata* and *Platanoidea* in relation to other *Acer* spp. must be considered as undetermined in the study of TIAN et al. (2002)

make a proper alignment in the highly variable regions, because they lack the ability to recognise oligonucleotide motives. Such patterns are easily recognised by the human brain (cf. Fig. 2-1), but this poses the direct criticism of subjective bias (→ Fig. 4-13). On the other hand, the total neglect of regions with length polymorphism is no alternative, neither the pure ignorance of gaps (chapters 3.6 & 4.6.2). As it will be shown, the information contained in regions comprising length polymorphism is of a high taxonomical (chapters 4.2.3 & 4.5) and systematical (chapter 4.4.1) significance. Since taxonomic units are the result of evolutionary processes through space and time, a phylogenetic hypothesis about *Acer* must incorporate, recognise, and interpret these data (chapter 4.4).

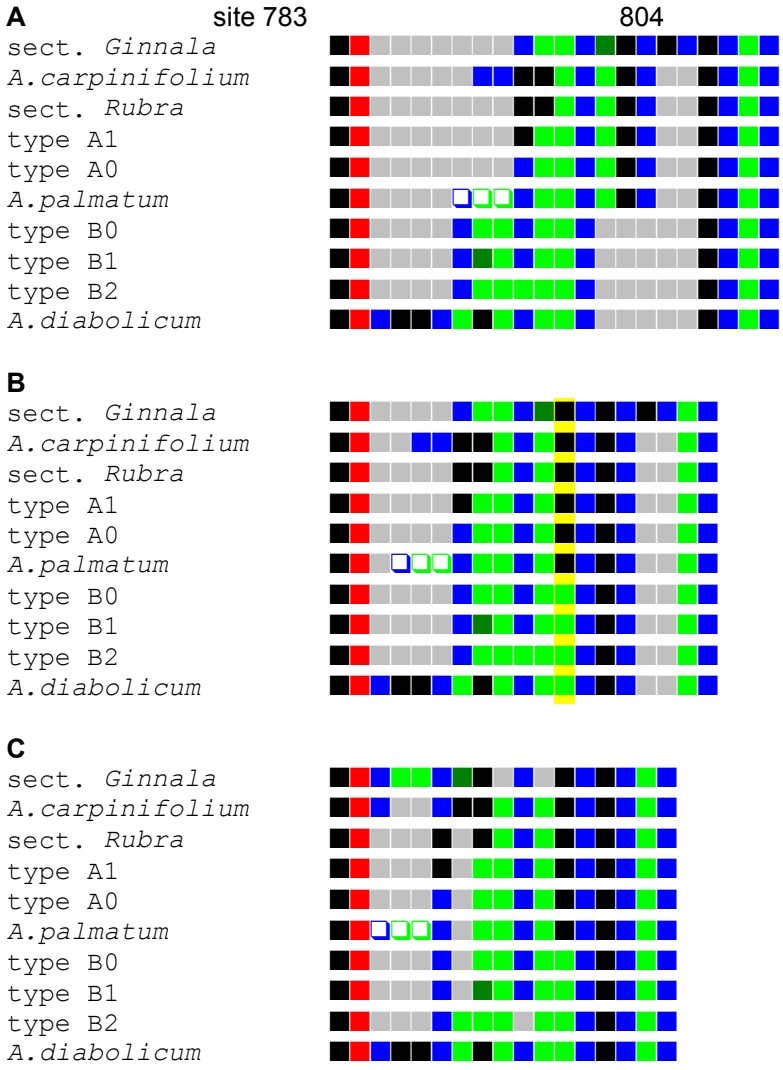


Figure 4-13: Competing alignments at the 3' end of ITS2 (LP 4).
Types A0, A1, B0, B1, and B2 are realised in distinct groups of taxa (cf. Fig. 4-16). **A.** Used alignment. **B.** Alternative alignment segregating two main lineages, distinguished by an A or G (yellow column). **C.** Alignment in which the number of gaps has been minimised (≈ computer-generated alignment). Standard colour code.

4.3.2 Comparison with morphological and biochemical studies

The herein presented molecular-based phylogenetic hypothesis both contrasts and confines aspects of phylogenetic concepts based on various morphological and biochemical (DELENDICK 1981) data sources.

The placement of *D. sinensis* as an outgroup to the genus *Acer* is widely accepted in literature. Morphological features of *Dipteronia* (like the pinnately arranged opposing leaves on vegetative shoots, the ultrastructure of the fruit etc.) are similar to certain *Acer* spp., although not identical. Most recently, MCCLAIN & MANCHESTER (2001), described and re-analysed fossils assigned to *Dipteronia* to clarify the origin of this taxon and its relationship to archetypal *Acer*. DELENDICK (1981) found a number of biochemical similarities between *Acer* and *Dipteronia*, and pointed out that *Dipteronia* is closer to a 'typical' *Acer* pattern than some *Acer* species (DELENDICK 1981). Similarly, in molecular analyses using parsimony and distance methods *Dipteronia* is commonly nested within the genus *Acer*, if it is not pre-defined as an outgroup (see above). Obviously, certain *Acer* clades – series and sections of the *Acer*-clade, section *Platanoidea*, and series *Lithocarpa* – have undergone a much more intense evolution than others – sections/series assignable to the *Palmata*-clade, the species-rich, but rather uniform[81], section *Macrantha*, and the monotypic series *Macrophylla* – and the sister genus *Dipteronia*. This is also represented by the number of extant species assigned to both taxa (2 for *Dipteronia*, 124 for *Acer*, cf. VAN GELDEREN et al. 1994).

OGATA (1967) arranged all *Acer* spp. into numerous sections, out of which he combined six "groups", mainly on the basis of leaf, inflorescence, and seed morphology and wood anatomy (cf. Tab. 4-1). Each "group" contained one or more sections, which he supposed to be ± related. His "group A" comprised primarily sections, which he thought to be primitive, including the more derived sections *Arguta* and *Palmata*. "Group B" and "C" represented the widely distributed sections *Campestria* and *Platanoidea* (+ *Pubescentia*) and various sections nowadays combined to one section, namely *Acer*. "Group D", "E" and "F" comprise sections with very few taxa. MAI (1984; cf. Tab. 4-1) reassigned certain taxa with emphasis on carpomorphologic characteristics, and proposed a merely serial rank for a number of Ogata's sections. He invented four subgenera, which do not map with Ogata's groups. Neither Ogata's groups nor Mai's subgenera seemed to be convincing, as far as they lack a cladistical or statistical fundament, an observation which is confirmed by the molecular data (→ Table 4-7). Nevertheless, the monophyly of Ogata's sections, which show an agreement in fundamentals

with the sections and series in VAN GELDEREN et al. (1994), the Flora Europaea, GRIN database etc., is definitely confirmed by our data as far as they have been analysed.

According to the cladistic analysis of WOLFE & TANAI (1987, based on the sections proposed by OGATA 1967), *Acer* can be divided into five "groups": *Spicata*, *Macrantha*, *Orba* (†), *Macrophylla*, *Platanoidea* (see Tables 4-1, 4-7), here ordered by their stratigraphical appearance. On the base of fossils it is argued that the "*Spicata*-group", or an extinct form related to the "*Spicata*-group", gave rise to the "*Macrantha*-group", and so on. Wolfe and Tanai stated, that the taxa-rich sections *Acer* including sect. *Trifoliata*, which they assumed to be polyphyletic), *Platanoidea*, and *Palmata* are rather advanced within the genus, an observation that is sustained by molecular data. A common origin of sections *Acer* and *Platanoidea* (i.e. a monophylum comprising "*Macrophylla*-" and "*Platanoidea*-groups") is sustained by a number of putative synapomorphies in the cladistic analysis of WOLFE & TANAI (1987), but is not supported in the same manner by the molecular analyses, where three major diverging genetic lineages are present. The distinctness to section *Palmata* and its relatives, is represented both in the morphological (⇨ "*Spicata*-group") and molecular genetical analyses. Going into detail it becomes apparent, that the placement of less diverse series, respectively sections, is not in agreement with WOLFE & TANAI (1987), but it is with non-cladistical approaches (VAN GELDEREN et al. 1994; cf. "affinities" reported in chapter 4.2.1). Series and sections which are combined in Wolfe and Tanai's "*Macrantha*-group" are genetically most distinct. Sections *Ginnala* and *Rubra* are genetically sister lineages of section *Acer*, *Macrantha* of *Platanoidea,* and *Negundo* and *Glabra* (only taxa of ser. *Arguta* analysed) of *Palmata*. This is reasonable according to the weak characters that define this group (mostly symplesiomorphies and occasional convergences), in contrast to the well-defined 'crown groups'. According to VAN GELDEREN et al. (1994; cf. chapter 4.2.1) there are some affinities between sections *Ginnala, Pentaphylla, Trifoliata* (represented by ser. *Grisea* in our study), and section *Acer*, which is confirmed by our molecular analyses. This holds true for the placement of section *Lithocarpa* as polyphyletic sister group (i.e. *A. macrophyllum* representing ser. *Macrophylla* and *A. diabolicum* representing ser. *Lithocarpa*) of section *Platanoidea*. Finally, the "synapomorphies" introduced by WOLFE & TANAI (1987) defining the genus' 'crown-clade' comprising sections *Acer, Hyptiocarpa, Lithocarpa,* and *Platanoidea* (⇨ "*Macrophylla*-" + "*Platanoidea*-group") can be considered as symplesiomorphies, hence

[81] from a morphological *and* genetical point of view

examples for the primitiveness of the sections *Acer* and *Platanoidea* (cf. chapter 4.4.3) [82]. All characters used by WOLFE & TANAI (1987) are defined by a putatively ancestral and one or several derived states. The ancestry is deduced by outgroup comparison with *Dipteronia* and according characters states represented in the remaining taxa of the order Sapindales. Furthermore, the direction (ancestral – derived) is also affected by the opinion, that the "*A. arcticum*" complex evolved in parallel to the genus *Acer* and *is not* an early representative of the genus. Furthermore, it has to be noted, that Wolfe and Tanai used a number of characters that are apparently linked to each other (cf. Table 4-3), and, thus, are not independent, why OGATA (1967), who used the same characters for primarily systematical purposes, argued that similarities in these characters are obviously coincidental, and not of phylogenetic origin (→ special remark). Wolfe and Tanai themselves acknowledged this fact, stating that (WOLFE & TANAI 1987, p. 19) "...clearly most advanced characters of *Acer* have been subject to parallel development."

Remark: The cladistic analysis performed by WOLFE & TANAI (1987) could only be a preliminary attempt to infer the phylogeny of the genus *Acer*. Besides the dependency of a number of character states, several systematically and taxonomically important leaf characters (such as the bracing of lobal sinuses) are confined to actinodromous leaves. Hence, they would have to be coded as unknown for taxa with unlobed leaves. Furthermore, a number of characters states vary markedly within taxa assigned to one section *sensu* OGATA (1967). Since Wolfe and Tanai did not published the original data matrix, it cannot be concluded, if their coding ("unknown", "polymorphic") was appropriate.

Beside the sustained division in sections and series, VAN GELDEREN et al. (1994) also presented a phylogenetic scheme based mainly on the studies of POJÁRKOVA (1933) and DELENDICK (1981), but was intended not to "... present a definite genealogical tree for the genus, but rather is an effort to represent in visual form the concept of possible evolution ... by the three authors ..." (VAN GELDEREN et al. 1994, p. 81/82). Therefore, the topology should not be overestimated. However, the grouping of some sections and series match partly with the analysis of the ITS data presented here. For example, section *Lithocarpa* is grouped with section *Platanoidea*, not with *Acer* (≙ WOLFE & TANAI 1987), which is sustained with high *a*

[82] WOLFE & TANAI (1987) found Ogata's section *Decandra* (from *A. decandrum*) to be related to *Platanoidea*, while Ogata's section *Laurina* (*A. laurinum*) was related to *Macrophylla* + *Lithocarpa* and *Acer*. In VAN GELDEREN (1994) and the GRIN database, *A. decandrum* is put as a synonym for *A. laurinum*, which is the only left valid species name for the *Acer* populations distributed throughout the tropical latitudes of SE Asia.

posteriori probabilities. Series *Caudata* is related to section *Palmata*, next is a group comprising sections *Glabra, Negundo* (≙ *Palmata*-clade introduced in chapter 4.3), *Indivisa* (genetically related to sect. *Acer*), and *Macrantha* (⇨ *Platanoidea*-clade). Also, section *Ginnala* is associated with section *Rubra.*

In conclusion, although molecular data from the ITS clearly affirms the morphologically and biochemically well-sustained series and sections, the deduced phylogenetic hypotheses – based on the assignment of supersectional groups – differ markedly. The comparison between the here introduced molecular-based phylogeny with former and current systematic models based on morphological and biochemical evidence (Tables 4-1 & 4-7) sustains the assumption of OGATA (1967) and others that morphological peculiarities of near-relatives are predominately convergences (following chapter). Furthermore, it becomes clear, that major similarities between the sections *Acer* and *Platanoidea* are either ancestral, namely symplesiomorphies of the genus, or analogies. In addition, the specialisation of sections comprising few or a single taxon – as it is stated by VAN GELDEREN et al. (1994; cf. chapter 4.2.1) and literature cited therein – is visualised and in accordance with a particular genetical distinctiveness of the according taxonomic entities.

Table 4-7: Former phylogenetical concepts applied for *Acer* in comparison to molecularly sustained superserial and -sectional groupings and clades.

phylogenetical and systematical implications based on BI analyses of the ITS of *Acer**				POJÁRKOVA 1933		OGATA 1967†	DELENDICK 1981†	MAI 1984		WOLFE & TANAI 1987	
				next relative (common progenitor)	further allied with			section	subgenus	section	group
ser. Cissifolia	sect. *Negundo*	*Arguta-Palmata-group*	*Palmata*-clade	*Negundo*	*Arguta, Rubra*	group A	group V	*Negundo*	*Negundo*	*Cissifolia*	*Macrantha*
ser. Negundo				*Cissifolia*						*Negundo*	
ser. Caudata				*Sinensia*	*Palm.,Macr.,Arguta, Ginn.+Trifida,(Acer)*		group I	*Arguta*	*Carpinifolia*	*Spicata*	*Spicata*
ser. *Arguta*				none	*Caud.+Sin.,Rubra*		group V			*Arguta*	*Macrantha*
"ser. *Palmata*"§	sect. *Palmata*			none	*Caud.+Sin.*			*Palmata*		*Palmata*	*Spicata*
"ser. *Sinensia*"§				*Caudata*	*Palm.,Macr.,Arguta, Ginn.+Trifida,(Acer)*		group II				
sect. *Macrantha*			*Platanoidea*-clade	none	*Ind.,Caud.+Sin.*		group V	*Macrantha*		*Macrantha*	*Macrantha*
ser. *Lithocarpa*		*Lithocarpa-Platanoidea-subclade*		*Macr.,(Acer)*‡	*Acer, Grisea*	group E	group IV	*Lithocarpa*	*Sterculiacea*	*Lithocarpa*	*Macrophylla*
ser. *Macrophylla*	*Macroph.-Platan.*-group			*Lith.,(Acer)*				***Trifoliata***		*Macrophylla*	
sect. *Platanoidea*				isolated		group B		*Platanoidea*		*Platan. + Campestria*	*Platanoidea*
A. caesium	sect. *Acer*	*Acer-subclade*	*Acer*-clade	(*Macr.+Lith.*)	*Macr.+Lith., Grisea*	group C		*Acer*	*Acer*	*Acer*	*Macrophylla*
"out-of-E Asia" taxa of sect. *Acer*								*Acer + Goniocarpa*			
sect. *Indivisa*				none	*Macrantha*	group F	group V	*Indivisa*	*Carpinifolia*	*Indivisa*	*Macrantha*
sect. *Ginnala*				*Trifida*	(*Acer*),*Caud.+Sin.*	group A	group III	*Ginnala*		*Trilobata*	
sect. *Rubra*		RTG-subclade		none	*Arguta, Negundo*			*Rubra*	*Acer*	*Eriocarpa + Rubra*	
ser. *Trifida*	*Grisea-Trifida*-group			*Ginnala*	(Acer),*Caud.+Sin.*	group D	group IV	*Pentaphylla*		*Integrifolia*	*Macrophylla*
ser. *Grisea*				none	*Acer, Macr.+Lith.*			***Trifoliata***	*Sterculiacea*		

* sectional and serial rank VAN GELDEREN et al. 1994

† current "sections" and "series" recognised as "sections" by OGATA (1967) and DELENDICK (1981); cf. Table 4-1

‡ "()" indicate partial relationships, i.e. only of or to some taxa

§ serial subdivision of sect. *Palmata* not sustained by molcelar data

4.4 *Reconstruction of the putative evolution of* Acer

It is obvious, that in the case of *Acer* with his numerous convergences including possible parallelisms, heterobathmies, and the lack of intrageneric synapomorphies, morphology cannot resolve the intrageneric phylogenetic relationships. In addition, although the use of ITS data allows to understand certain relationships of extant *Acer* spp. and infer a sound phylogeny for these taxa by distinguishing three major lineages (cf. Table 4-6), the sustain at important divergence nodes is not fully convincing, especially if one tries to focus on the very early diversification points. In addition, the selective use of data (complete ITS used, only conserved regions used) and the reliability of the resulting hypothesis ("*Acer*-group" vs. *Acer*-clade) has to be further verified. To install a sound hypothesis on the evolution of *Acer*, a detailed reconstruction of the molecular evolution of the ITS and its consecutive mapping against the fossil record is therefore absolutely necessary.

In general, for phylogenetical and systematical purposes, the computed molecular phylogenies are "as-it-is" compared with other data and then either taken as the true phylogeny (or the best possible approximation), or the used gene region(s) is (are) considered to be unsuitable to resolve the phylogeny. As already demonstrated for *Fagus*, molecular evolution and, in general, infrageneric evolutionary pathways can only be comprehensively understood, if all aspects of the molecular phylogeny and the underlying molecular data are in detail questioned, re-investigated and then further verified by other data from e.g. morphology and the fossil record.

4.4.1 General molecular evolutionary trends within the ITS

Since ancient DNA is only accessible for a rather short geological time (≤ 15,000 a; MAROTA & ROLLO 2002), the exact pathway of molecular evolution cannot be exactly determined. By the sequencing of DNA it cannot be concluded how often mutations occurred and during which time period they manifested in the genome of an organism, population, or species. Here, a method is introduced, which allows to get a 'feeling' about the time and speed of the molecular evolution processes, that finally led to the actual composition of the ITS of *Acer*. In addition, a deeper insight into the differentiation processes can be achieved that allows a qualitative re-evaluation of the statistically computed, molecular-based phylogenies. The most promising method to accomplish these tasks is to reconstruct the putative evolution of selected parts of the ITS, namely the length polymorphic regions, on the basis of pattern recognition and parsimonious derivation. As already introduced, the ITS comprises four

length polymorphic regions (LP1 to LP4) exhibiting discriminative levels of inter- and intrataxonomic variability (chapter 4.2.3; appended alignment). In addition, the detected oligonucleotide motives vary considerably in length and nucleotide composition. As it will be demonstrated, mutations in these regions are obviously following particular molecular evolutionary pathways and can be considered to be complex molecular characters (cf. chapters 3.3 & 3.6). Thus, a parsimonious derivation is – analogously to complex morphological characters – possible. From the reconstruction, an image of the evolutionary parameters of different taxonomic units can be deduced and used to improve and validate the molecular phylogeny and, consequently, for comparison with the data available from the fossil record.

The oligonucleotide motives realised in LP1 (→ Fig. 4-14**A**/**C**) and LP2 (→ Fig. 4-14**B**/**C**) within the ITS1 are distinctively correlated to the ML/BI-deduced phylogeny. Figures 4-14**A** and 4-14**B** demonstrate that *parsimonious* derivations of *complex* molecular characteristics, here: two 8 to 12 bp long oligonucleotide motives, are in full accordance with *likelihood* analyses computed without the according regions. This is not amazing, since complex oligonucleotide motives are the result of the same evolutionary history as the nucleotide composition of the complete ITS. Due to their complexity, oligonucleotide motives have the capability to be – in contrast to single nucleotides – valid parsimony informative characters. Furthermore, the mutational patterns exhibited in regions like the here reconstructed LP1 and LP2 are not 'free', i.e. purely coincidental, but confined to (a) particular genetical constraints (secondary structure of the transcript, etc.) and (b) evolutionary pathways (speciation, hybridisation, etc.). Examples are for:

(a) the multiple-"G" at the 5' end of LP1, which is generally limited to 6 or 7 "G" and susceptible to point mutations only at the first position. This susceptibility is utilised in distinct phylogenetic lineages (sects. *Macrantha* & *Platanoidea*; sers. *Arguta, Caudata* & *A. erianthum* of sect. *Palmata*; intraspecific variability in sects. *Ginnala* & *Rubra*). Point mutations other than a transition from "G" to "A" (and presumably *vice versa*) are obviously not possible, or cannot be fixed within the genome.

(b) the LP1 and LP2 pathway reconstructed for section *Acer*[83]. An initial "A" was fixed within the LP2 during the formation of a clade including *A. sempervirens, A. monspessulanum, A. hyrcanum* and *A. ibericum*. In course of evolution, the basic pattern

[83] The shown topology (detail) equals the topology in Fig. 4-10, not Fig. 4-11, since the variable regions are of importance to resolve lowest-level (intra- and interspecific) relationships.

(terminal "CTC", realised in the most basal sibling taxon *A. sempervirens* and all remaining taxa of sect. *Acer*) is lost and replaced by a strongly C-dominated and elongated motif. In the case of *A. ibericum* + *A. hyrcanum*, this motif is consecutively linked to a duplication of 3 bp within LP1 (structural compensation), respectively a second "CT" insertion, realised as intraspecific variability within populations of *A. ibericum* (≙ ID4; Table 4-4). Thus, the speciation history within the *A. sempervirens* + (*A. monspessulanum* + (*A. ibericum* + *A. hyrcanum*)) clade can be in detail traced by the mutational patterns and nucleotide composition of LP1 and LP2.

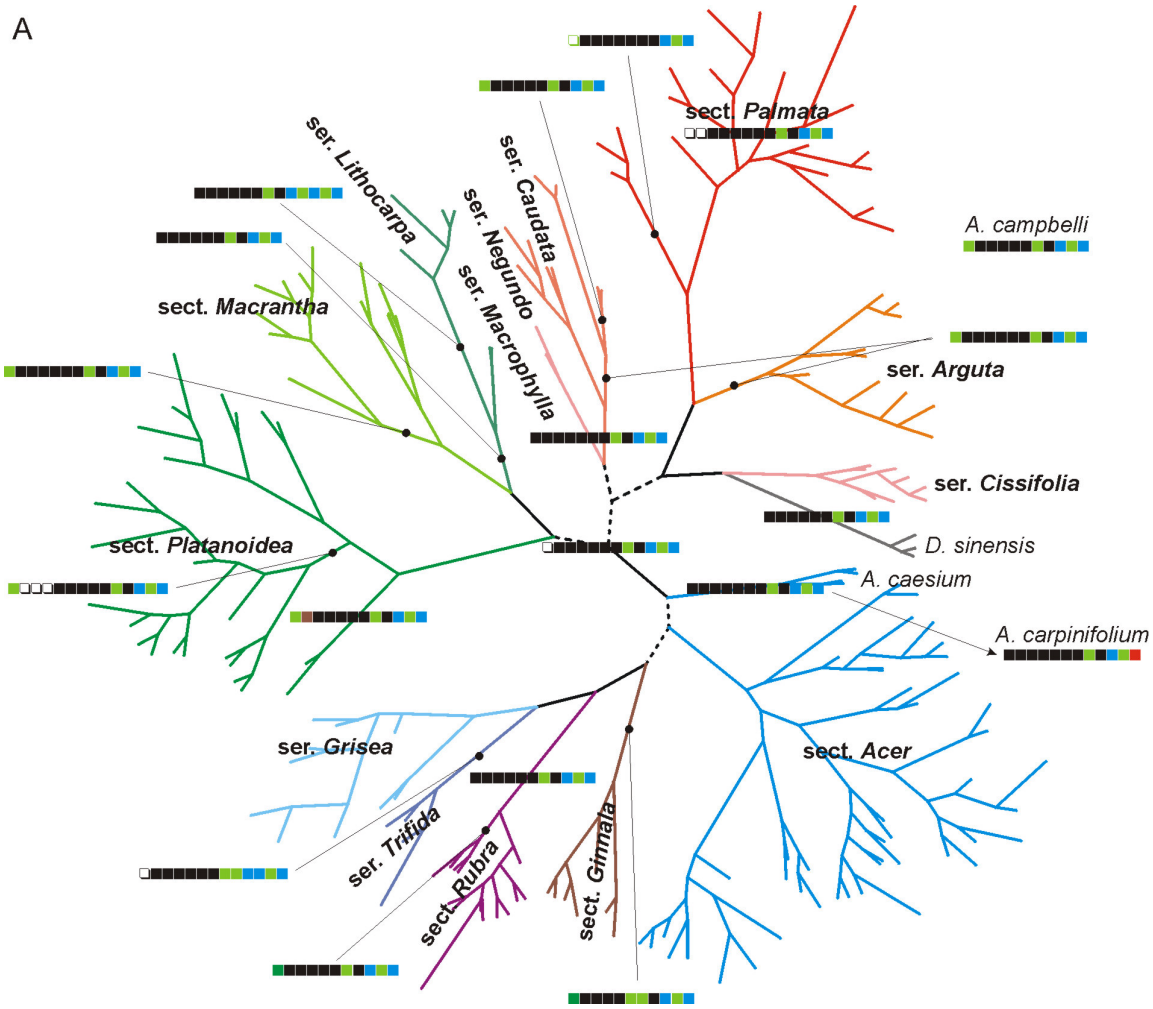
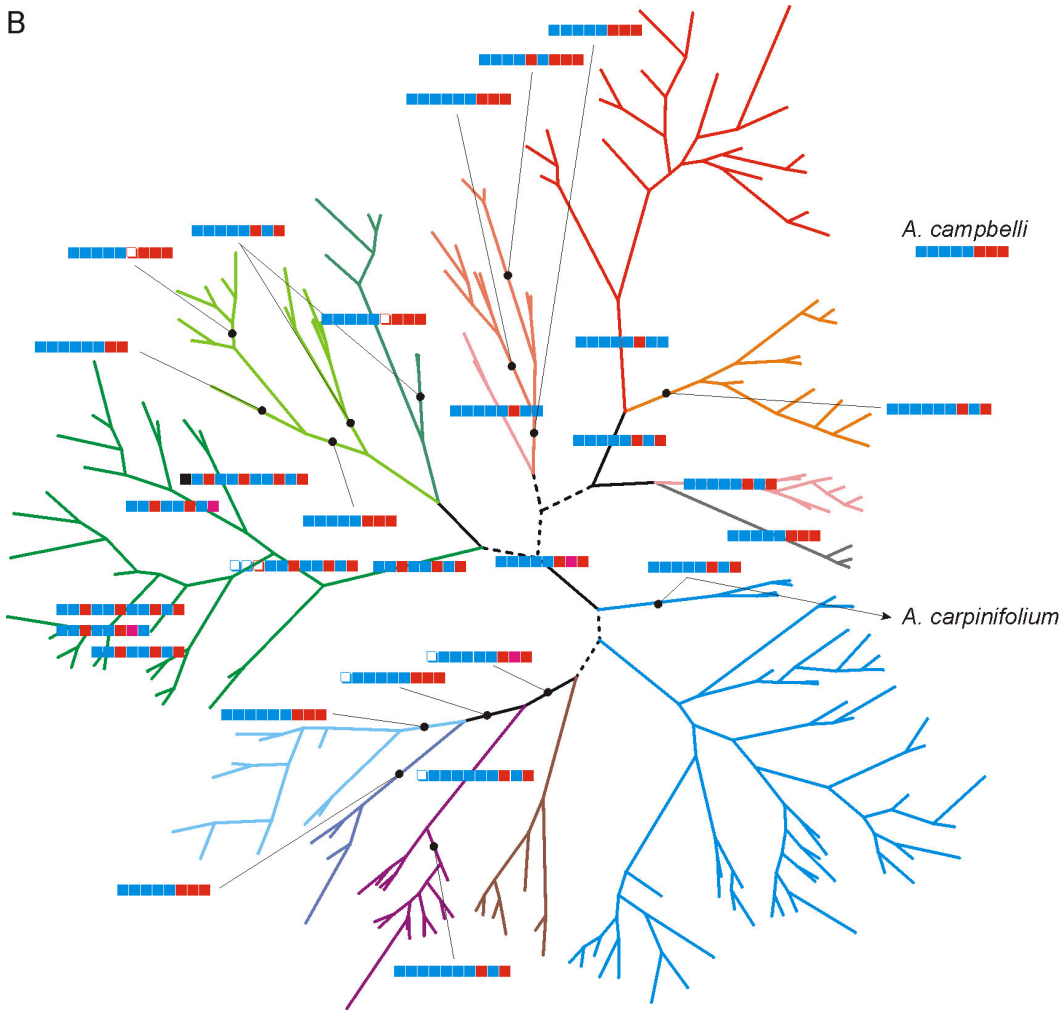
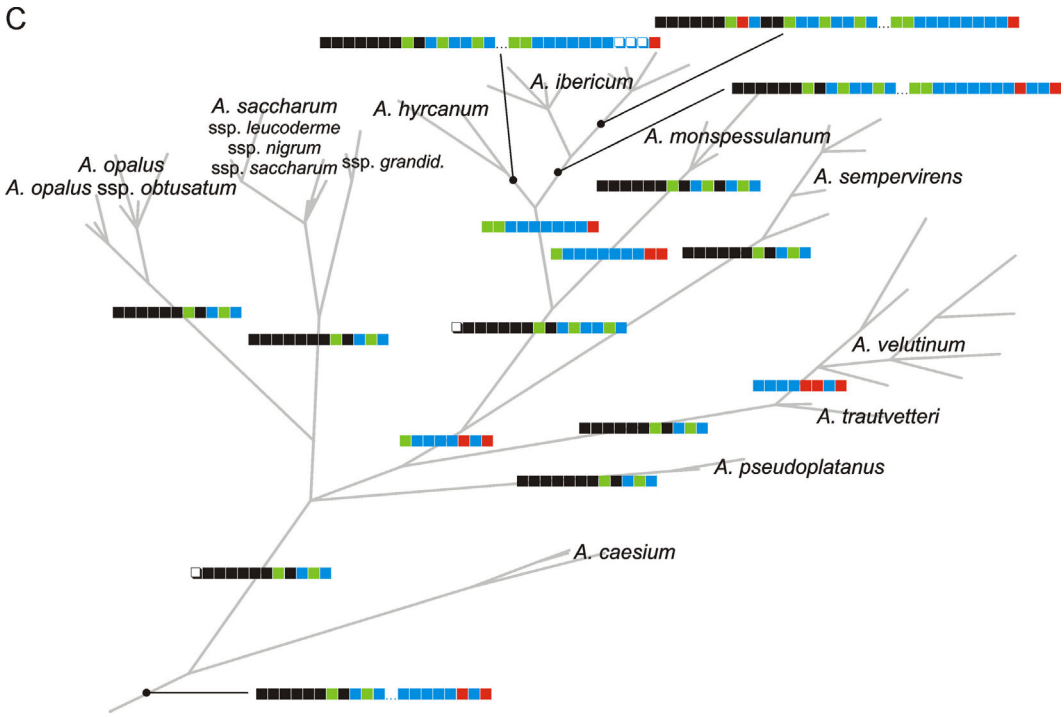


Figure 4-14 (above, following page): Putative evolution of polymorphic regions within the ITS1 comprising LP1 (incl. ID4), and LP2.
A. LP1. The motif "sex-/septuple-G-AGCAC" is ± conserved throughout the genus. Each shift can be accomplished by one mutational event **B.** LP2. Although more variable (cf. Figs. 4-7 & 4-31), the mutational patterns detected within this CT-dominated oligonucleotide motif can be reconstructed analogously to LP1. **C.** LP1 & LP2 of sect. *Acer*. The differentiation is markedly increased (cf. Figs. A. & B), indicating an old phylogenetic lineage (discussed further in the text). Topology shown in Fig. A & B ≙ Fig. 4-11; topology in Fig. C ≙ Fig. 4-10, cf. text); sustained taxonomic entities (cf. Table 4-6) coloured equally as in Figs. 4-9 to 4-11; standard colour code applied for nucleotide motives.


B
A. campbelli
A. carpinifolium


C
A. ibericum
A. saccharum
ssp. leucoderme
ssp. nigrum
ssp. saccharum
A. hyrcanum
ssp. grandid.
A. monspessulanum
A. opalus
A. opalus ssp. obtusatum
A. sempervirens
A. velutinum
A. trautvetteri
A. pseudoplatanus
A. caesium

The strongly diverging upstream CT-dominated part of LP3 (→ Fig. 4-15**A**) at the 5' end of the ITS2 is mainly of taxonomical and systematical value, i.e. in recognising unique molecular motives and patterns and, hence, sustaining current taxonomic units on a specific to sectional level (Table 4-8; cf. Fig. 4-7). A parsimonious reconstruction of this part is difficult (for details see below). The GA-dominated downstream part of LP3 is, in contrast, comparatively conservative in length and nucleotide composition (except for sect. *Acer*, see below). Of particular phylogenetical importance are here the discriminative levels of molecular differentiation within series and/or sections, exemplary illustrated in the species-rich sections. Sections *Macrantha, Palmata* and *Platanoidea* are more conservative in length and general composition of the LP3, while section *Acer* (similar to the condition detected within LP1 and LP2 in the ITS1) is more variable and allows to deduce precise pathways of molecular evolution (Fig. 4-15**C**). In general, the molecular evolution within the LP3 follows two major trends: increasing length, by the number of basepairs (in particular realised in sects. *Acer*, *Indivisa, Ginnala, Palmata, Platanoidea* and series *Lithocarpa*), and increasing CG-content, especially in the GA-dominated downstream part (sects. *Indivisa, Ginnala, Macrantha, Palmata, Rubra,* sers. *Caudata, Cissifolia, Negundo, Lithocarpa*).

Aside the general trends, the exact nucleotide composition and parsimonious derivation of LP3 allows to further qualify several inter- and intraserial relationships and phylogenetic and evolutionary implications of the ML/BI-analyses as introduced in Table 4-6:

- *Dipteronia sinensis* exhibits a short oligonucleotide motif, which is *similar*, but not *identical*, to the putative ancestral (→ special remark) or common sense motif. The detected derivations from the common sense are completely unique, no *Acer* sp. exhibits similar mutational patterns.
- The LP3 motif of section *Macrantha* has independently evolved within the *Platanoidea*-clade from the putative ancestral *Acer* motif (esp. Fig. 4-15**B**), while the motives of sections *Lithocarpa* and *Platanoidea* exhibit similar trends, that are not represented in any other *Acer* lineage (Fig. 4-15**A**; cf. Fig. 4-7; see above). Also, the derivedness of *A. diabolicum* (sect. *Lithocarpa* ser. *Lithocarpa*) and taxa assigned to section *Platanoidea* in comparison to *A. macrophyllum* (sect. *Lithocarpa* ser. *Macrophylla*) is well exhibited.
- The less-derived LP3 motives detected within series *Caudata* and *Negundo* are of identical length and similar general composition, although the taxa of series *Caudata* exhibit a remarkable inter- (between *A. caudatum*, *A. spicatum*) and intraspecific (between ssp. *ukurunduense* and ssp. *multiserratum* of *A. caudatum*) differentiation.

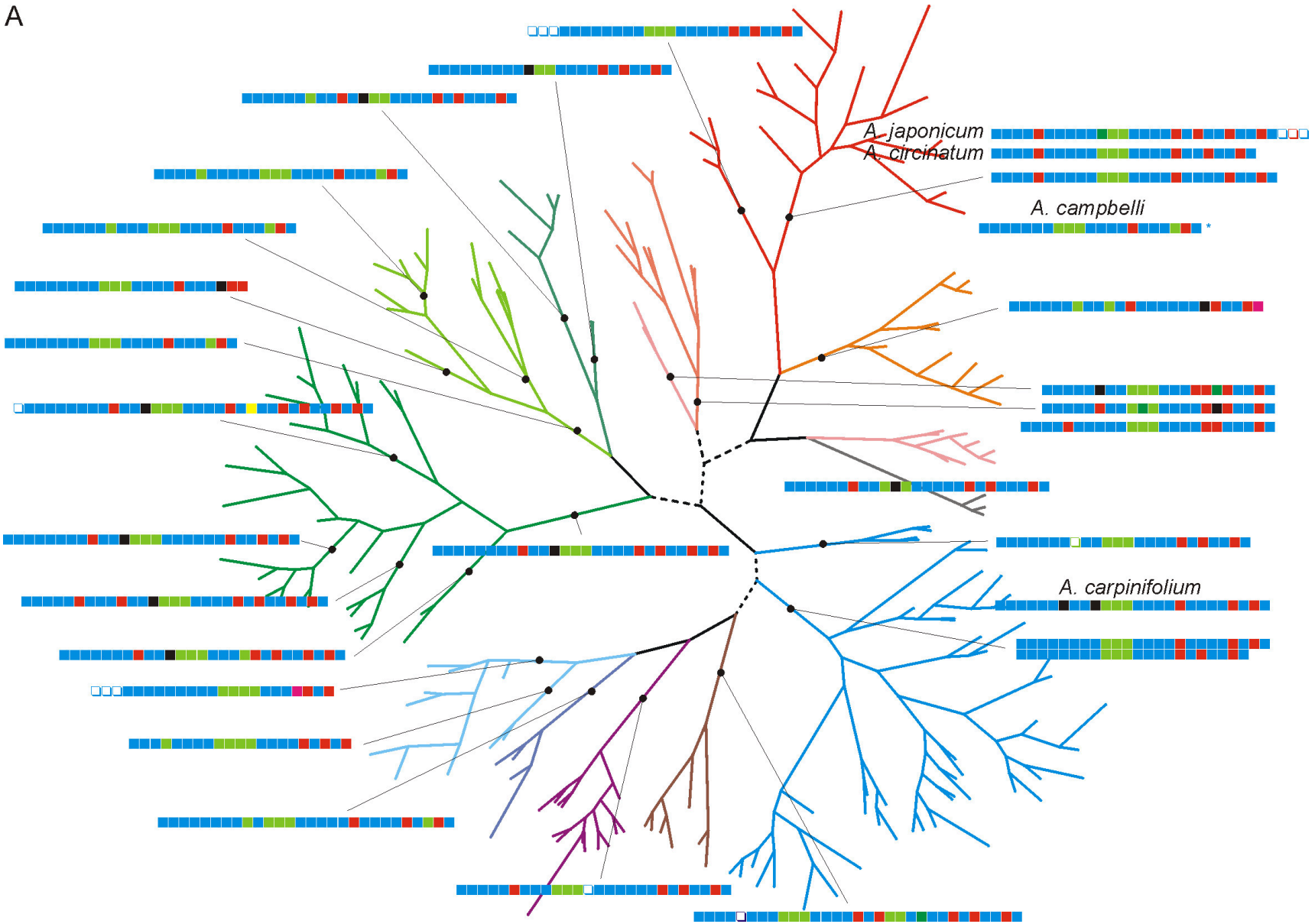
Table 4-8: Key motives of LP3 for taxonomical and systematical purposes within the *Platanoidea*-clade.

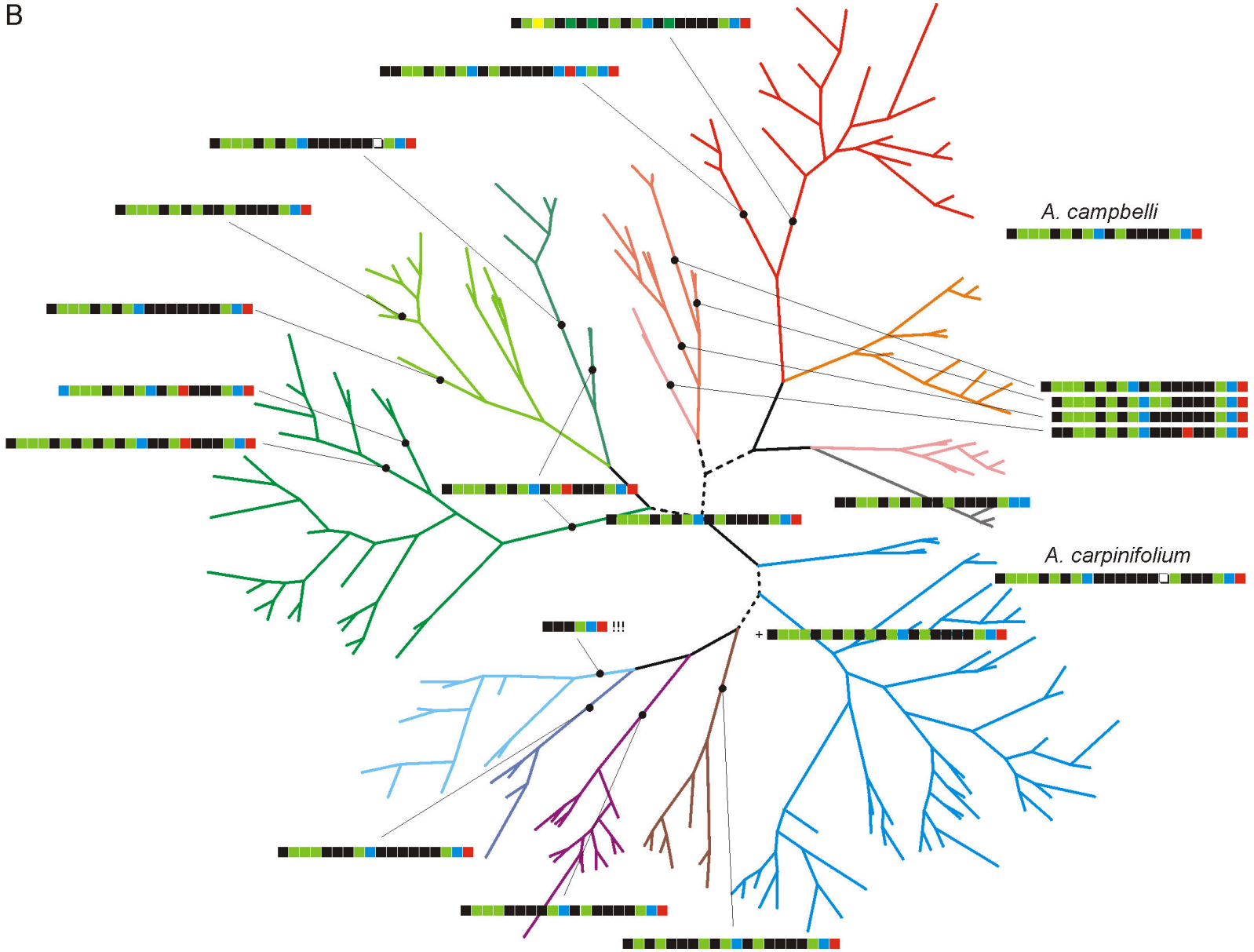
ancestral genotype of genus *Acer* (according reconstruction in Fig. 4-15)			not reconstructed						
***Platanoidea* -clade**		*A. crataegifolium*	6C	CCAAA	4C-T	**CCCGTT**	GAAAGAGAC	GA-4G / 7G	ACT
	sect. *Macrantha*	*A. capillipes*	6C-**AC***				GAAAGAGA**G**		
		A.davidii spp. davidii	**3C-A-4C**			**CCCATC**	GAAAGAGAC	GA-4G	
		A.davidii spp. *grosseri*							
		A. rufinerve	6C						
		A. pensylvanicum							
Lithocarpa-Platanoidea- subclade	sect. *Lithocarpa* ser. *Lithocarpa*	*A. diabolicum*	6C-**ACC**	CT**GAA**		TCT**CCC**TC		6/7G	
	sect. *Lithocarpa* ser. *Macrophylla*	*A. macrophyllum*	7C	CC**GAA**		TCTCCTC		GA**T**GGG	
	sect. *Platanoidea*	*A. cappadocium*		**T**CC**GAA**A	3C-**A**T				
		A. miyabei	8C		4C-T		**C**AAAGAGAC		
		A. campestre				**CWCC**TCTCCTC	GAAA(**GAGA**)$_2$C	GGA**T**GGG	
		A. platanoides				**C**CTCCTC	GAAAGAGAC	GA**T**GGG	
		A. pictum ssp. *mono*				TCTCCTC			
		A. truncatum							

TC

* prominent nucleotide patterns to indentify a taxonomic unit (species, series, section, subclade) are indicated by bold font

- The initial "4C-T-5C-AAA" detected in accessions of the presumed sibling series of series *Negundo*, series *Cissifolia* (cf. Tables 4-1; 4-6) correlates strongly to a – presumably not derived – pattern found within series *Arguta* + section *Palmata*. The remaining part of the motif is nearly identical to series *Negundo* (1 bp difference).
- The upstream part of the LP3 motif of series *Arguta* is strongly derived (e.g. lacks central "AAA" pattern), while the downstream part is identical to series *Cissifolia*, and the ancestral motif.
- The motives realised within section *Palmata* can be easily derived by mainly duplications from the ancestral motif of the *Palmata*-clade. The tendency for higher CG-amounts is especially represented in the upstream part by a putative "CCCCT"-duplication and in the downstream part by the co-occurrence of a – more ancestral – "GAGAGA" pattern (*A. japonicum, A. palmatum*) and a – putative derived – "GGGAGA" (*A. circinatum*) or "GAGGGA" pattern (remaining taxa, clones of *A. palmatum*, *A. japonicum*). The LP3 motif exhibited by *A. erianthum* is markedly similar to the motives of the remaining series and sections of the *Palmata*-clade.
- The putative reconstruction of the evolution of LP3 within section *Acer* exhibits – similar to LP1 and LP2 in the ITS1 – a linked mutational phenomenon (Fig. 4-15**C**): Although the general elongation tendency is followed, accessions of section *Acer* can be divided into two major groups, distinguishable by conspicuous indels. Here a duplication of 2 bp in the CT-dominated upstream part is accompanied (compensated) by a duplication of 4 bp in the downstream GA-dominated part. The fixed mutational activity is comparable with the mean *inter*serial and –sectional differentiation detected in the genus (≙ LP1, LP2).
- Identically to the situation in LP1 and LP2, the genotype of *A. caesium* is most similar to the putative ancestral genotype (⇨ basic type in Fig. 4-15**C**). In particular, the downstream part is identical with the downstream part of series *Arguta* and *Cissifolia* of the *Palmata*-clade and exhibits only a 1 bp difference to the downstream part shared by series *Macrophylla* and most accessions of section *Platanoidea*.
- The markedly derived motives detected within the remaining taxonomic entities assignable to the *Acer*-clade, i.e. series *Grisea* + *Trifida* and sections *Indivisa, Ginnala,* and *Rubra* are most likely independently evolved from the ancestral motif (↔ condition detected in LP1 and LP2). Most remarkable is the reduction of the GA-dominated part in series *Grisea* to 6 bp ("GGGACT"), while the CT-dominated upstream part is ± unreduced.


A
A. japonicum
A. circinatum
A. campbelli
A. carpinifolium


B
A. campbelli
A. carpinifolium
!!!
+

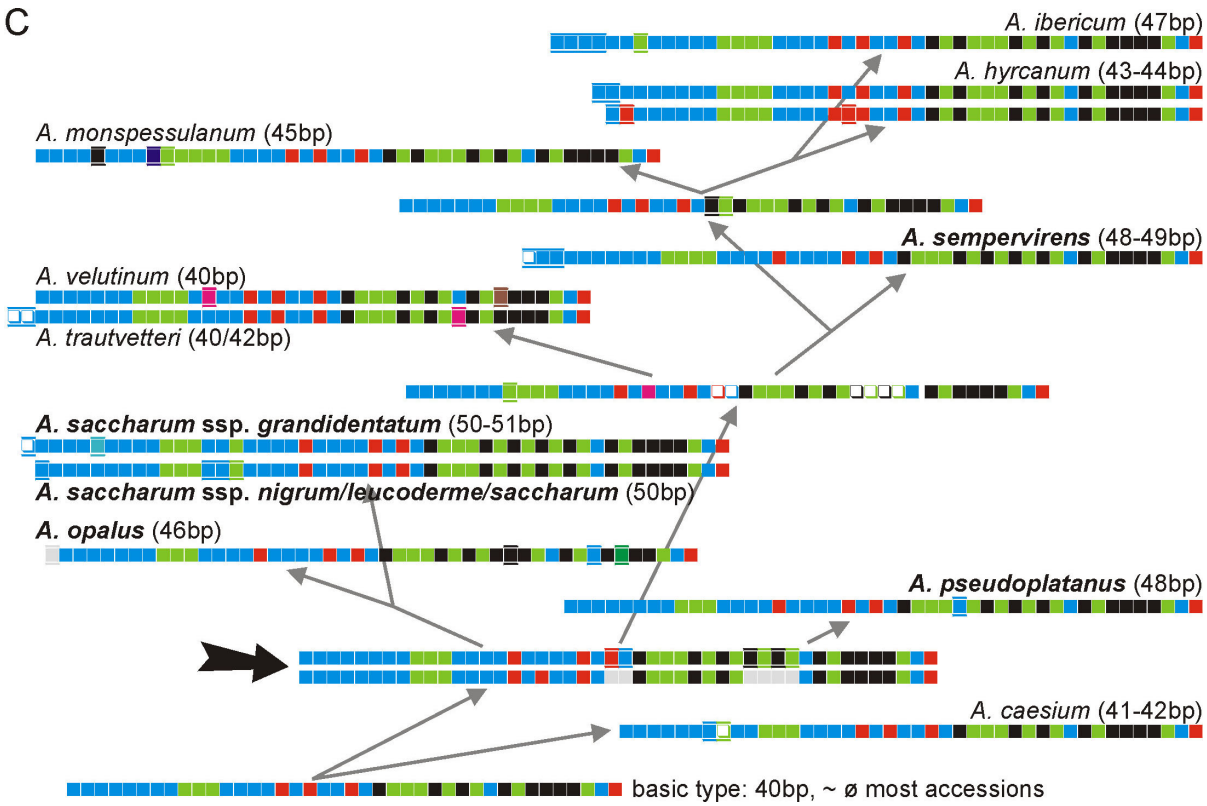


Figure 4-15 (preceding pages, this page): Nucleotide composition and putative evolution of the up to 57 bp long LP3, the *Acer* equivalent of the hypervariable arm of the ITS2 referred to in DENDUANGBORIPANT & CRONK (2001).
A. CT-dominated upstream part (5'), exhibiting a strongly diverging molecular pattern. The complete motif or prominent features are conserved within current sections and series (→ text). **B.** GA-dominated downstream part (3'), exhibiting an – in general – lower variability, but distinctive derivation of molecular patterns (→ text). In ser. *Grisea* this part is reduced to 6 bp (exclamation marks). **C.** LP3-genotypes in sect. *Acer*. A general tendency towards longer motives is exhibited. Note the co-occurrence of a linked insertion pattern (upstream "TC" duplication correlated to a downstream "GAGA" duplication) in different genetic clades/taxa (bold printed names), which is either a genetic parallelism or the remnant of a polymorphic ancestor (black arrow). All types of mutations from one type to another are above- and underlined. Standard colour code.

In comparison to the LP1 to LP3, the LP4 exhibits a conservative pattern of molecular evolution (→ Fig. 4-16). Due to the high conservation of the exact nucleotide composition within taxonomic units (cf. Fig. 4-8), the correlation and reconstruction allows to establish hypotheses about the general mode ('mutation-per-mutation') of molecular evolution (→ Fig. 4-17). The basic type is either "CAACAAC", predominately realised in the *Palmata*- and *Platanoidea*-clades and *D. sinensis*, or "CAACAGC", typical for *Acer*-clade and series *Macrophylla* and section *Palmata* (cf. Fig. 4-8). Whether this is due to an ancestral polymorphism (as shown in Fig. 4-16) or convergent (back)mutations cannot be clarified. It is imaginable, that structural constraints promote or selectively allow the transition at the 6th

position of the 7 bp long motif. Similar to the situation mentioned for the initial pattern of LP1, other point mutations are not realised and fixed, respectively. In addition, as reported for LP1 to LP3, the motives of *A. diabolicum* (ser. *Lithocarpa*; *Platanoidea*-clade), *A. carpinifolium* (monotypic sect. *Indivisa*), and the sections *Ginnala* and *Rubra* (latter three members of the *Acer*-clade) are strongly derived and show a tendency to increase the CG-content (see above; cf. chapters 4.2.3 & 4.3; Figs. 4-9 to 4-11, 4-14 & 4-15). But, the terminal part of the according basic motif ("CAAC" for *Platanoidea*-clade; "CAGC" for *Acer*-clade) is always represented. Of further phylogenetical importance are:

- the "(C/Y)AAA**A**AC" motif detected exclusively in *A. negundo* (ser. *Negundo*) and *A. cissifolium* + *A. henryi* (ser. *Cissifolia*), sustaining a section *Negundo* (cf. Table 4-6).
- the "CAACAGC"-based motives detected within the *Acer*-clade. The basic motif is realised or only slightly derived in section *Acer* (including *A. caesium*; cf. Table 4-8) and series *Grisea* + *Trifida* (cf. situation in LP3) and prominently derived (2 bp insertion; several point mutations) in sections *Indivisa* and *Ginnala*. The derived motives follow ± divergent evolutionary pathways.

Aside the phylogenetically important point mutations, unique site variabilities occur also in particular clades (further illustrated in Fig. 4-17):

- An initial "C" (basic type) *or* "T" (⇨ "Y") can be found exclusively in clones of *A. barbinerve* (ser. *Arguta*) and *A. cissifolium* (ser. *Cissifolia*) of the *Palmata*-clade.
- An initial "C" *or* "A" (⇨ "M") can be only found in *A. griseum* (ser. *Grisea*) and *A. ibericum* (sect. *Acer*) of the *Acer*-clade.

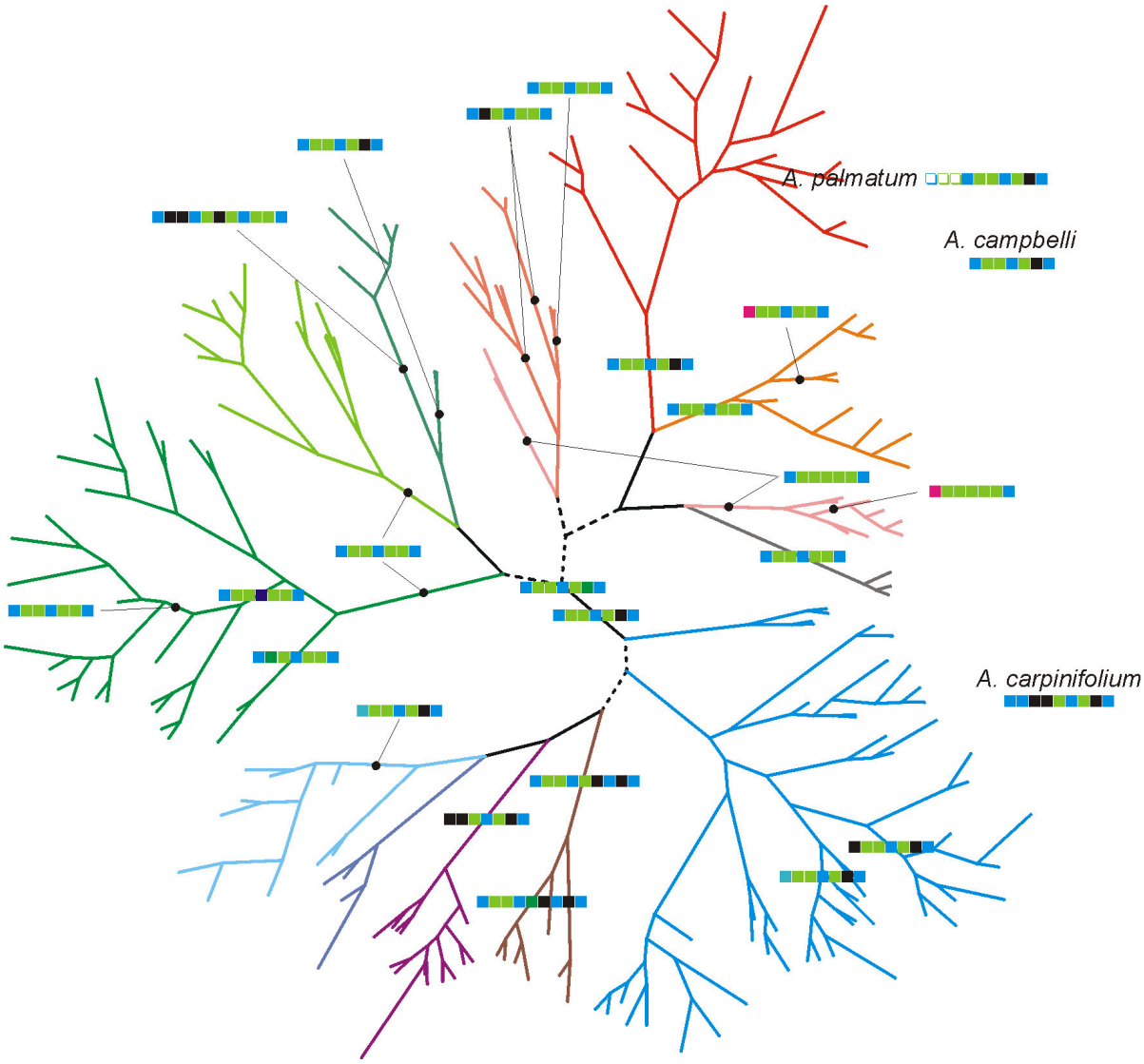


Figure 4-16: Putative evolution of LP4 (cf. Fig. 4-13).
The *Acer*-clade is defined by a basic "CAACAGC"-motif (A0-type in Fig. 4-13), which else can be found in ser. *Macrophylla* and sect. *Palmata* (incl. *A. campbelli* ssp. *campbelli*, ↔ sect. *Macrantha*). The *Palmata*- and *Platanoidea*-clade exhibit predominately a "CAACAAC"-based motif (B0-type, Fig. 4-13). In general, the motives are conserved within a section or series. Standard colour code.

It is obvious, that certain mutations can occur convergently and independently on very distinct genetic lineages. Nevertheless, the occurrence of particular mutation or site variabilities can be confined to clearly related taxa, such as sibling species. Although intraspecific variability is commonly detected in the analysed *Acer* taxa, its impact is negligible for a phylogenetic reconstruction, unlike the condition found in the ITS of *Fagus* (chapters 3.4 & 3.5). On a serial or sectional level, the co-occurrence of site variability in genetically and morphologically ± related series and sections, respectively, even if a rather conservative and simple ≥7 bp long motif is analysed (Figs. 4-8 & 4-16), is either a striking coincidental analogy or a definitive molecular genetical parallelism and, consequently, of

strong phylogenetical impact. Such a mutation may indeed reflect definitive structural constraints or evolutionary events. Furthermore, such a parallelism may in fact also be a chronological phenomenon. Due to repeated events of mutation and backmutation, and with regard to the possibility of incomplete concerted evolution, it can be assumed that an actual synapomorphic genotypic characteristic is realised in a number, but not yet, anymore, and/or again in all taxa of the monophylum. To illustrate this hypothesis, the shared site variability of *A. cissifolium* (sect. *Negundo* ser. *Cissifolium*; see above; cf. 4-16) and *A. barbinerve* of series *Arguta* is discussed in the following. All other *Acer* spp. never exhibit a "Y", respectively a "T", at this position (cf. Fig. 4-8). The supposedly sibling series of series *Cissifolia* – series *Negundo* – exhibits a similar motif as series *Cissifolia*, but a different variability at the 1st site ("M" ≙ "A" *or* "C"). It has to be noted, that accessions of *A. negundo* are sampled from only one representative[84]. Possibly, by assembling more data from original stands of the various subspecies recognised for this taxon (cf. VAN GELDEREN et al. 1994) a similar variability may be detected. From the molecular analyses, is it clear that section *Negundo* (comprising *A. negundo* of monotypic ser. *Negundo*, and *A. cissifolium, A. henryi* of bispecific ser. *Cissifolium*) and series *Caudata* (here represented by ssp. *multiserratum* and spp. *ukurunduense* of *A. caudatum* and *A. spicatum*) are definitely related to series *Arguta* + section *Palmata* (⇨ *Palmata*-clade; Figs. 4-10 & 4-11) and, hence, are monophyletic, i.e. they share a common ancestor. The exact position of series *Caudata, Cissifolia*, and *Negundo* in relation to *Arguta* + *Palmata* (⇨ *Arguta-Palmata*-group) is debatable (cf. Figs. 4-9 to 4-11). If one assumes the following hypothetical relationship (cf. following chapter), namely (*Negundo* + *Cissifolia*)(*Caudata* (*Arguta* + *Palmata*)) and frequent (back)mutations at the 1st site of the oligonucleotide motif, the molecular genetic history and future evolution of LP4 can be hypothesised as shown in Figure 4-17. That the variability "C"↔"T" (1st position) is never found in series *Caudata* and *Palmata* may/might be due to an incompatibility between mutations at differing positions in the motif (2nd bp in the case of ser. *Caudata*; 6th bp in the case of sect. *Palmata*; cf. situation in *Acer*- and *Platanoidea*-clades).

[84] More ITS accessions representing cultivars of *A. negundo* have been assembled and exhibit conspicuous intragenomic variability, but are not included in the current study, due to the unknown propagation history of the sampled individuals.

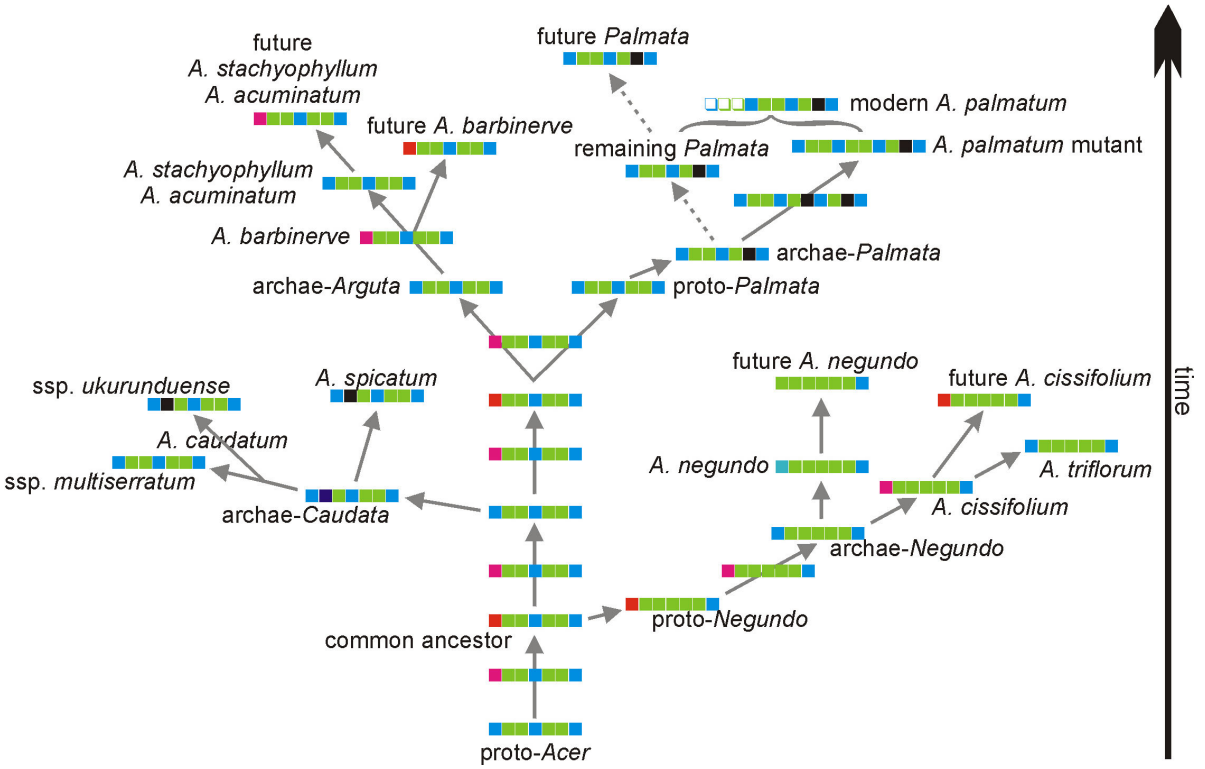


Figure 4-17: Notional evolutionary history and future of LP4 within the *Palmata*-clade.
The here presented scheme – based on here presented ITS data – illustrates imaginable pathways of molecular evolution that might have lead – hypothetically – to the recent molecular genetical condition. For further details see text. Standard colour code.

Figures 4-14, 4-15, and 4-16 demonstrate, that the evolution of conservative and – on a generic level differently – variable regions within the ITS is decisively correlated. It is possible, to plot a MP-derived evolution of an oligonucleotide motif on a ML phylogram, that is computed without any region comprising length polymorphism, i.e. totally free from subjective bias introduced during the alignment process. Furthermore, a conspicuous linkage between the molecular evolution of LP1, ID4, and LP2, respectively the CT-dominated 5' and GA-dominated 3' strand of LP3 is apparent. Taxa and taxonomic groups – like series and sections – that accumulate putatively derived mutations within the conservative regions often comprise prominent indel motives in the variable regions. Furthermore, the insertion of several bp in one part is correlated with an equivalent shift in the other part. The most probable constraint for such a linkage is the secondary structure of the transcript (DENDUANGBORIPANT & CRONK 2001) and the balancing of nucleotide content and amount in certain genome regions (TORRES et al. 1990). The oligonucleotide motives are more or less conserved for taxonomic entities – like series and sections – and even within the supersectional clades. The reconstruction and plotting of section-specific oligonucleotide motives can also be correlated with morphological specialisation in taxa-poor sections.

Presumably highly specialised taxa like sections *Ginnala, Indivisa,* and *Rubra* exhibit remarkably derived oligonucleotide patterns. A reason for this could be either an old stratigraphic age and early isolation or an accelerated evolution rate within the specialised taxon during the niching process, e.g. by passing an evolutionary bottleneck. In this context, the role of section *Acer* has to be further analysed. The series and taxa included in section *Acer* exhibit an evolutionary plurality, which is comparable to the degree of the overall mutation activity[85] in the genus. The putative molecular evolution of oligonucleotide motives within this section can be analogously reconstructed like the overall mutation patterns (Figs. 4-14C & 4-15C). Thus, a comprehensive and extensive assembling of sequence data from this section is apparently crucial for molecular analyses. This might have been another reason, why preceding molecular studies (CHO et al. 1997; ACKERLY & DONOGHUE 1998; SUH et al. 2000; PFOSSER et al. 2002; TIAN et al. 2002) were not able to resolve the phylogenetic backbone of the genus at all.

Remark: The ancestry or derivedness of particular molecular patterns are of course hypothetical. In the case of the here analysed length polymorphic regions, the putative ancestry or derivedness are readily deduced from Figs. 4-14, 4-15 & 4-16, because of three observations:

1. All BI analyses and the overall nucleotide composition of the ITS recognise *D. sinensis* as outtaxon. However, the exact nucleotide composition of LP1 to LP4 of *D. sinensis* is on the one hand (nearly) identical to a common sense nucleotide composition of all *Acer* spp. (in the case of LP1, LP2, and LP4; length of LP3) but, on the other hand, if, then uniquely derived (e.g. initial "multiple-C-AGA" instead of the basic aceroid "multiple-C-multiple-A"; unique LP3 terminal motif).
2. The actual oligonucleotide motives in the "basal" lineages are similar to a common sense pattern and *D. sinensis*, respectively, and stronger derived on terminal branches. Furthermore, the putatively ancestral motives may be still represented in terminal branches, but in these cases, the more basal sister lineages never show markedly derived motives.
3. Derived motives in the different clades (*Acer*-, *Palmata*-, *Platanoidea*-clade) are often divergent to each other, i.e. the easiest way to get from one motif to another is via the common sense pattern, respectively the hypothetical ancestral motif. Within a clade, the evolution obviously follows often a definable direction and/or general trend.

Since the underlying topology is computed under neglect of the according regions, it is at least improbable that such a comprehensive and always fitting pattern is due to convergent evolution and completely coincidental mutational events.

[85] It has to be noted, that the recent genetical variability only mirrors the actual mutation rate. Beside cellular constraints, the actual fixation rate is strongly affected by population dynamics, hybridisation, and randomly occurring evolutionary events.

4.4.2 Emended molecular phylogeny

The reconstruction of the putative pathways in the evolution of oligonucleotide motives (preceding chapter) allows to get an impression about the actual mutation events that occurred in the course of evolution. In the following, unresolved or miscellaneous divergence points (Table 4-6) from the phylogenetic hypothesis computed via BI analyses (Figs. 4-9, 4-10 & 4-11) will be further evaluated on the basis of the putative evolution and recent composition of oligonucleotide motives (Figs. 4-14, 4-15 & 4-16). In particular, phylogenetically informative mutations are favoured against phylogenetically uninformative mutations as it can be deduced from the reconstructions in the preceding chapter. The conclusions thus obtained are consecutively used to fully clarify the phylogenetic relationships within the genus *Acer* and anticipate a synoptical molecular phylogeny.

Position of section *Indivisa* in relation to the taxa of section *Acer*

Acer carpinifolium, the only representative of section *Indivisa*, is genetically clearly distinct from other *Acer* spp. (→ appended alignment) and placed within a polyphyletic section *Acer* during the BI analysis (Fig. 4-9). The genetical peculiarity of this taxon is further illustrated by several indels (Table 4-4). Nevertheless, a presumed relationship between *A. carpinifolium* and section *Acer* can be affirmed by the genotypic characteristics of the length polymorphic regions LP1 to LP4. Although the detected oligonucleotide motives in these regions are strongly derived, they share the most similarities with the genotypic characteristics realised within the section *Acer*, in particular in respect to *A. caesium*. Thus, although strongly derived from the typical *Acer* pattern, the closest extant relative of *A. carpinifolium* is found beyond the taxa assigned to section *Acer* (⇨ *Acer*-subclade; Table 4-7).

Exact position of section and series within the *Acer*-clade

The best resolution via BI analyses is achieved, if only conserved regions are included in the analysis: According to Figure 4-11 section *Acer* (incl. *A. caesium*) is basal to section *Ginnala*, next comes section *Rubra*, and series *Grisea* + *Trifida* (⇨ RGT-subclade, Table 4-7). A sibling relationship between series *Grisea* and *Trifida* (⇨ *Grisea*-*Trifida*-group, Table 4-7) is obvious and well-sustained by the BI analyses (prob. >95%; Figs. 4-9 to 4-11), and the nucleotide composition of LP1 to LP4 (Figs. 4-14 to 4-16). As already mentioned, the level of interspecific genetic divergence within section *Acer* is comparable to the overall exhibited interserial and –sectional differentiation. In correlation with the putative primitiveness of accessions of *A. caesium* (especially with respect to the genotypic characteristics of LP1 to

LP4), a basal position of section *Acer* is most appropriate. Accessions of the remaining taxonomic entities (sects. *Ginnala, Rubra*, sers. *Grisea, Trifida*) are apparently derived from a genotype similar to the recent nucleotide composition of *A. caesium*. The assumption, that sections *Ginnala, Rubra*, respectively the *Grisea-Trifida*-group, evolved independently from ancestors which were close relatives of *A. caesium* (hence, extinct members of sect. *Acer*) is well exhibited in the genotypic characteristics of LP1 to LP4. However, the genotypic characteristics are less derived in series *Grisea* than in the remaining entities. In addition, series *Grisea* exhibits similar evolutionary trends like those detected within section *Acer* (LP2, LP4).

Mutational patterns that would indicate a common origin of two entities are lacking. A RGT-subclade, like it is proposed by the BI/ML analyses based on the conserved regions concurs with the occurrence of a remarkable deletion detected within the ITS1 (ID5, located in between LP1 and LP2; Table 4-4). But, a similar deletion is also found in accessions of *A. carpinifolium* (sect. *Indivisa*). In conclusion, the sections *Indivisa, Ginnala, Rubra,* and the series *Grisea* + *Trifida* are thought to represent independent descendants of a 'stem-section' *Acer* including *A. caesium*, whereas sections *Ginnala* and *Rubra* are most distinct to the common ancestor.

Monophyly or paraphyly of section *Lithocarpa* in relation to sections *Macrantha* and *Platanoidea*

In conclusion, the LP1 to LP4, with special respect to LP3, sustain a *Lithocarpa-Platanoidea* subclade (Table 4-6; cf. Fig. 4-7) in contrast to the less probable subclade comprising the sections *Lithocarpa* and *Macrantha* (topology shown in Figs. 4-14, 4-15 & 4-16). According to the BI analyses of the complete data set (Figs. 4-9 & 4-10) section *Lithocarpa* – comprising the genetically distinct taxon *A. diabolicum* as representative of series *Lithocarpa* and the ± genetically primitive taxon *A. macrophyllum* (monotypic ser. *Macrophylla*) – is paraphyletic in relation to section *Platanoidea* (⇨ *Macrophylla-Platanoidea*-group; *Lithocarpa-Platanoidea*-subclade; Table 4-7; higher probabilities at divergence points). The presumed primitiveness and strong ancestral character of *A. macrophyllum* (analogous to the position of *A. caesium* within the *Acer*-clade) and distinctive derivation of *A. diabolicum* is further sustained by the BI analyses of the conserved regions (compare branch lengths in Fig. 4-11) and the reconstruction of the putative evolution of LP1 to LP4 (preceding chapter; Figs. 4-14 to 4-16). Although the BI analysis of the conserved regions proposes a monophyletic section *Lithocarpa* next to section *Macrantha*, the

composition of LP1 to LP4 strongly indicates a closer relationship between sections *Lithocarpa* and *Platanoidea* than between sections *Lithocarpa* and *Macrantha*. This is especially apparent in the composition of LP3. Here, as well the genotypic characteristics of section *Platanoidea* as well as those of *A. diabolicum* (ser. *Lithocarpa*) are derivable by rather few mutational events from the state found in *A. macrophyllum* (ser. *Macrophylla*). If the occurrence of indels (in particular ID 10; Table 4-4) is further taken into account, the positioning according the BI analyses based on the complete data appears to be more appropriate, i.e. a *Lithocarpa-Platanoidea* subclade with section *Macrantha* as sibling group. To stress the similarities between *A. diabolicum* and *A. macrophyllum* and the distinctiveness of between *A. diabolicum* and section *Platanoidea* (which is strikingly apparent form the molecular patterns) a section *Lithocarpa* is retained at first instance, including the 'ancestral' series *Macrophylla* and 'derived' series *Lithocarpa* (cf. Fig. 4-27).

Monophyletic or polyphyletic section *Negundo*

The reconstruction of the evolution of length polymorphic regions indicate a monophyletic origin of the series *Cissifolium* and *Negundo*, which is only moderately sustained in the BI analyses (complete data only; Figs. 4-9 & 4-10) and strongly demanded by morphological evidence (cf. chapters 4.2.2 & 4.4.3; WOLFE & TANAI 1987; VAN GELDEREN et al. 1994). Therefore, a monophyletic section *Negundo* can be postulated from the available data.

Exact position of series *Caudata* in relation to *Cissifolia* + *Negundo* and *Arguta* + *Palmata*

Except for a position within the *Palmata*-clade, the BI/ML analyses do not further clarify the position of the series *Caudata*. Most interestingly, LP1 motives detected within taxa assigned to series *Caudata* may be identical to series *Arguta* or series *Negundo.* Similar observation can be done if the LP2 and LP3 motives are considered. The LP4 motif is either identical to the putative ancestral motif or uniquely derived by a single mutation. Morphologically, series *Caudata* is special in basically lacking characters, that are considered to be derived (⇨ sect. *Parviflora* proposed by VAN GELDEREN et al. 1994). In combination with the data from the length polymorphic regions, the most probable scenario is that series *Caudata* represents a stratigraphically very old lineage (cf. chapter 4.3.2; analysis of WOLFE &

TANAI 1987) and, hence, needs to be positioned basally to both, section *Negundo* and the *Arguta-Palmata*-group.[86]

The emended molecular phylogeny is illustrated in Figure 4-18. It has to be noted, that the here proposed phylogeny must not necessarily mirror the actual evolution of the genus *Acer*, but is considered to reflect the *molecularly inferred* intrageneric relationships of *extant* taxonomic entities. Although the identification of molecular trends allows to construct a theory about ancestral and derived molecular motives, a rooting of the molecular tree is always most debatable, especially if all assumed divergent points are considered.

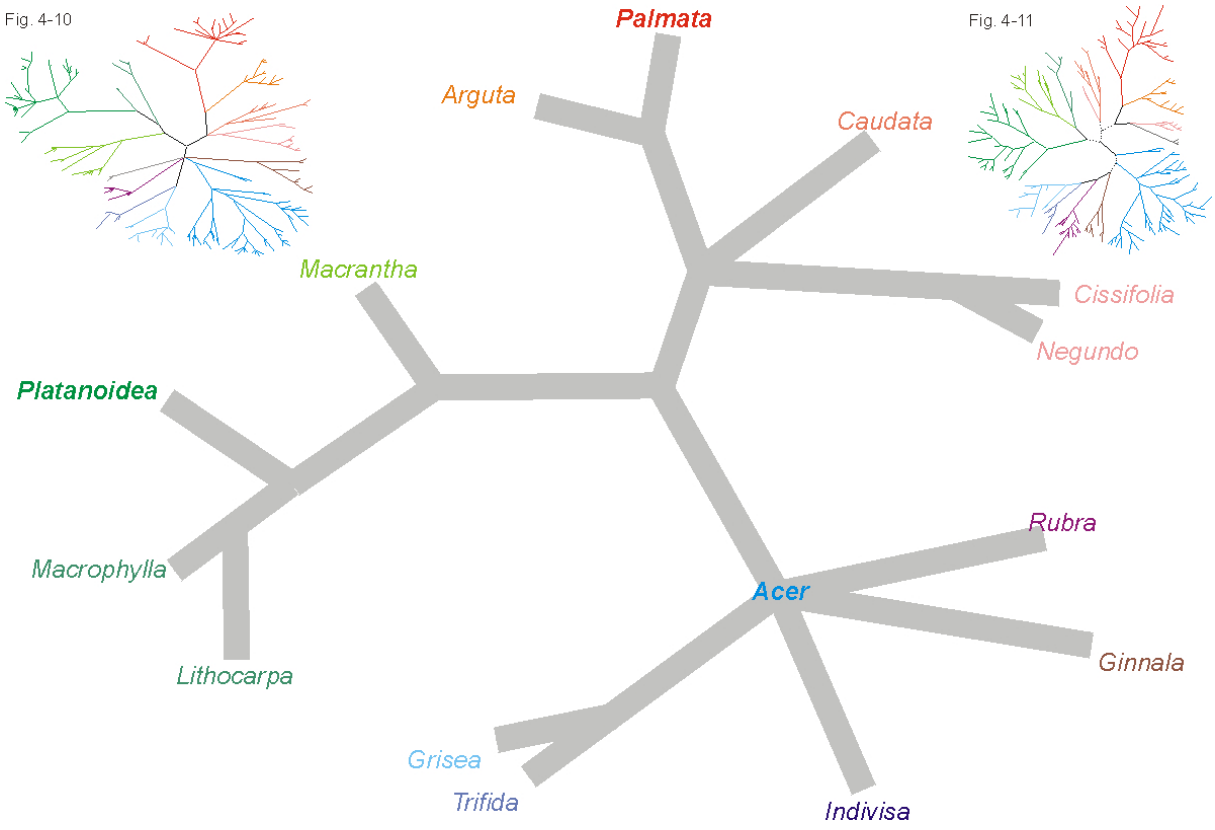


Figure 4-18: Emended molecular phylogeny of genus *Acer* inferred from ITS data.
The shown cladogram indicates phylogenetic affinities between the molecularly, biochemically and morphologically sustained taxonomic entities based on the results of the BI analyses (chapter 4.3) and pattern recognition (chapter 4.4.1). Colours refer to coloration used in preceding figures. Top left: phylogram based on BI analysis of complete data (cf. Fig. 4-10), top right: phylogram based on the conserved regions only (cf. Fig. 4-11) for comparison.

[86] This does *not* implement a direct sibling relationship between section *Negundo* and the *Arguta-Palmata*-group.

4.4.3 Mapping against morphology and the fossil record

As shown in preceding chapters, molecular data clearly sustains the morphologically well-defined taxonomic entities like series and sections. Accordingly, the combination of certain morphological features is typical for – and obviously conserved within – the so defined taxonomic entities (cf. OGATA 1967; also exhibited in Figs. 4-19 to 4-23, see below) allowing to assign a number of fossil morphospecies to recent taxonomic groups and dating back the occurrence of these sections (WOLFE & TANAI 1987, additional data included from WALTHER 1972, for C Europe, and TANAI 1983, for E Asia, complete list → appendix)[87]. However, to deduce a comprehensive evolutionary hypotheses, i.e. not only a 'naked' phylogeny of the genus, but a reconstruction of the actual evolutionary pathways (e.g. development of certain characteristics in course of speciation processes, identification of tendencies and possibilities to colonise new niches, etc.) further information is needed. Thus, one needs to include fossils, which cannot be clearly assigned to recent taxonomic entities, and a more particular insight into the morphological trends realised within the different clades. In addition, since the molecular-based clades are supposed to be monophyletic, recent taxonomic units are derived from common ancestors, which have to be further defined for palaeobotanical and evolutionary purposes.

In Figures 4-19 to 4-23 the variability and derivation of systematically relevant morphological features (see chapter 4.2.2) are mapped against the background of the ITS-based phylogeny (conservative and variable regions included; Fig. 4-10) to comprehensively correlate morphologic characteristics to the molecular-based clades. Apparently, important morphological inventions, e.g. the adaptation of the floral elements in course of the development of (andro)dioecism, are persistently homoplastic, i.e. convergently developed. Ecologically specialised taxa (cf. chapter 4.2) accumulate molecular genetical (preceding chapter) and morphological peculiarities. For example, series *Grisea* (representative of the molecularly sustained *Acer*-clade including sects. *Acer, Indivisa, Ginnala, Rubra*, and ser. *Trifida*) exhibits prominent indels within the ITS (cf. Table 4-4; Fig. 4-15), floral characteristics linked to an androdioecious tendency (sects. *Acer, Ginnala*, ser. *Trifida* andromonoecious, specialised sects. *Indivisa, Rubra* androdioecious), a slightly derived

[87] Other assemblages of fossil data (like PFR 2.2; BOULTER et al. 1996) are difficult to handle due to the miscellaneous attribution to intrageneric systematic groups. E.g., numerous fossil taxa in PFR 2.2 are assigned by the original inventors to section *Campestria* as defined by Pax, but belong to ser. *Monspessulana* (sect. *Acer*) and are not related in any kind to *A. campestre* etc. of sect. *Platanoidea*.

flower formula[88] with outside the disc inserted stamens (standard flower formula and inside inserted stamens realised in sects. *Acer, Ginnala,* and ser. *Trifida*; sects. *Indivisa, Rubra* with outside inserted stamens, but tendency to 4-merous flower), and trifoliate leaves (in general 3- to 5-lobed leaves found within the *Acer*-clades, unlobed leaves restricted to 2 species: *A. carpinifolium*, sect. *Indivisa*, and individuals of *A. sempervirens*, sect. *Acer*).

In addition, several morphologic characteristics do predominately or exclusively occur in genetically distinguishable lineages. Such a "predominant feature" realised in a group of near-relatives is either a parallelism originating from a homologous genetic programme or an indication, that a genetic programme to evolve a particular derived character is not available. Examples are:

- The always 3- to 5-lobed actinodromous leaves of the *Platanoidea*-clade (Fig. 4-19). Derived pinnately organised (including trifoliate leaves) or entire leaves are restricted to species-poor, rather specialised taxonomic entities of the *Acer*- and *Palmata*-clades.
- The laticiferous tissues (Fig. 4-20) of taxa assigned to sections *Lithocarpa* and *Platanoidea* (OGATA 1967), which are genetically clearly related (⇨ *Lithocarpa-Platanoidea*-subclade; cf. Fig. 4-18)[89].
- The perfectly smooth fusiform wood rays (Fig. 4-20) of sections *Acer* and series *Trifida*.
- The dioecism restricted to series *Cissifolia* and *Negundo* (⇨ sect. *Negundo*; VAN GELDEREN et al. 1994; cf. Fig. 4-21), which are of a possibly monophyletic origin based on the molecular evidence, in particular if the nucleotide composition of the oligonucleotide motives is considered (Fig. 4-18).
- The ± distinct nutlet venation found in all representatives of the *Palmata*-clade, which can else be found in section *Ginnala*, a molecular distinct and rather specialised section belonging to the *Acer*-clade, and series *Lithocarpa,* a molecular markedly distinct representative of the *Platanoidea*-clade (Fig. 4-23).

[88] standard flower formula of genus *Acer*: K5C5A8(G2)

[89] Thus, laticiferous tissues represent the only putative morphological synapomorphy on a intrageneric, but superserial/-sectional level, here sustaining the monophyly of sers. *Lithocarpa* + *Macrophylla* and section *Platanoidea* (⇨ *Lithocarpa-Platanoidea*-subclade).

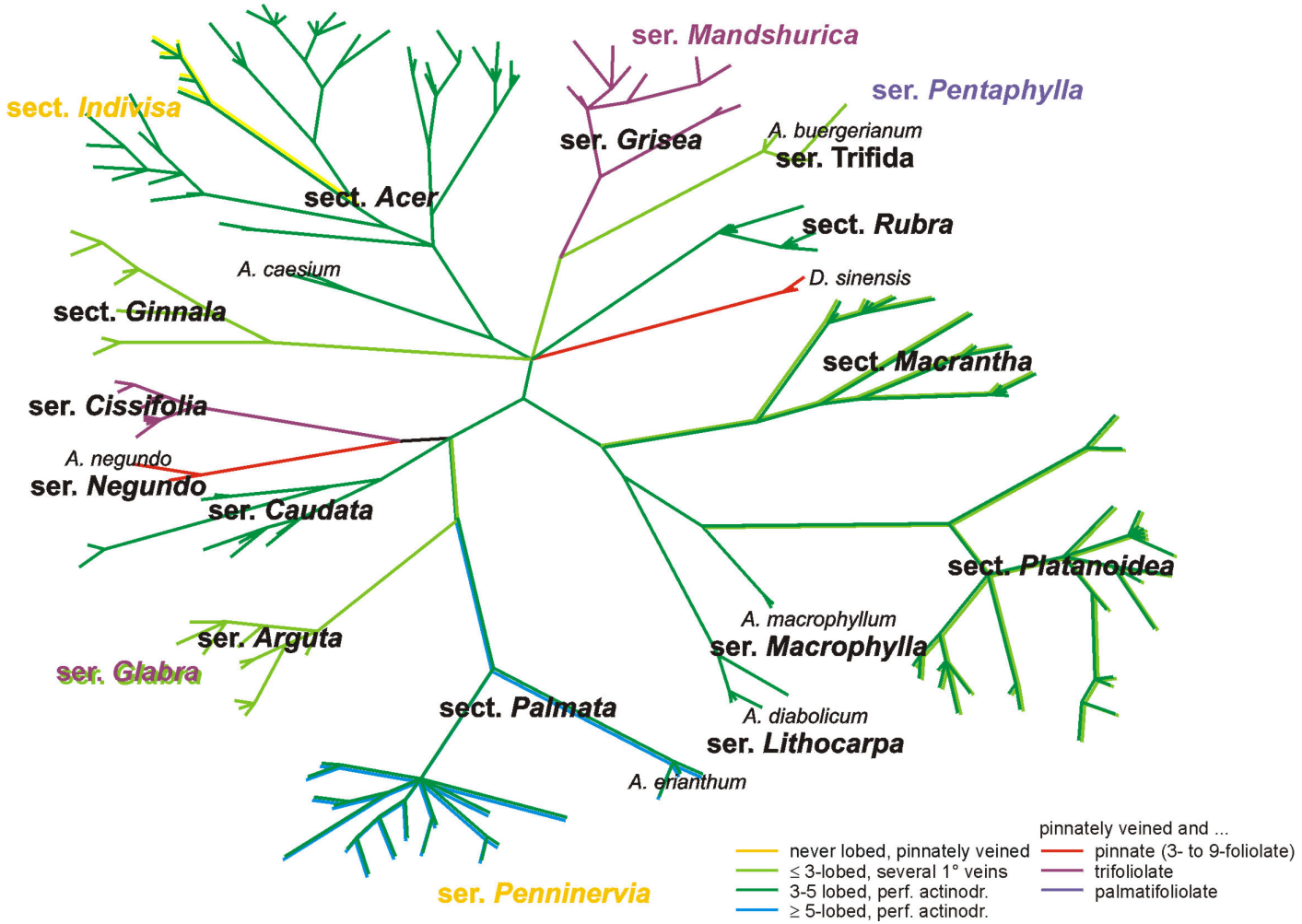


Figure 4-19: Leaf morphology in comparison to the molecularly derived phylogeny.
Apparently the pinnate and trifoliate leaves of sects. *Negundo* and *Trifoliata* are convergently developed. The condition found in sibling series of analysed groups, i.e. sers. *Arguta, Grisea, Palmata* + *Sinensia* (⇨ sect. *Palmata*), and *Trifida*, which were not included in the molecular analyses, i.e. sers. *Glabra, Mandshurica, Penninervia,* and *Pentaphylla*, is given as reference.

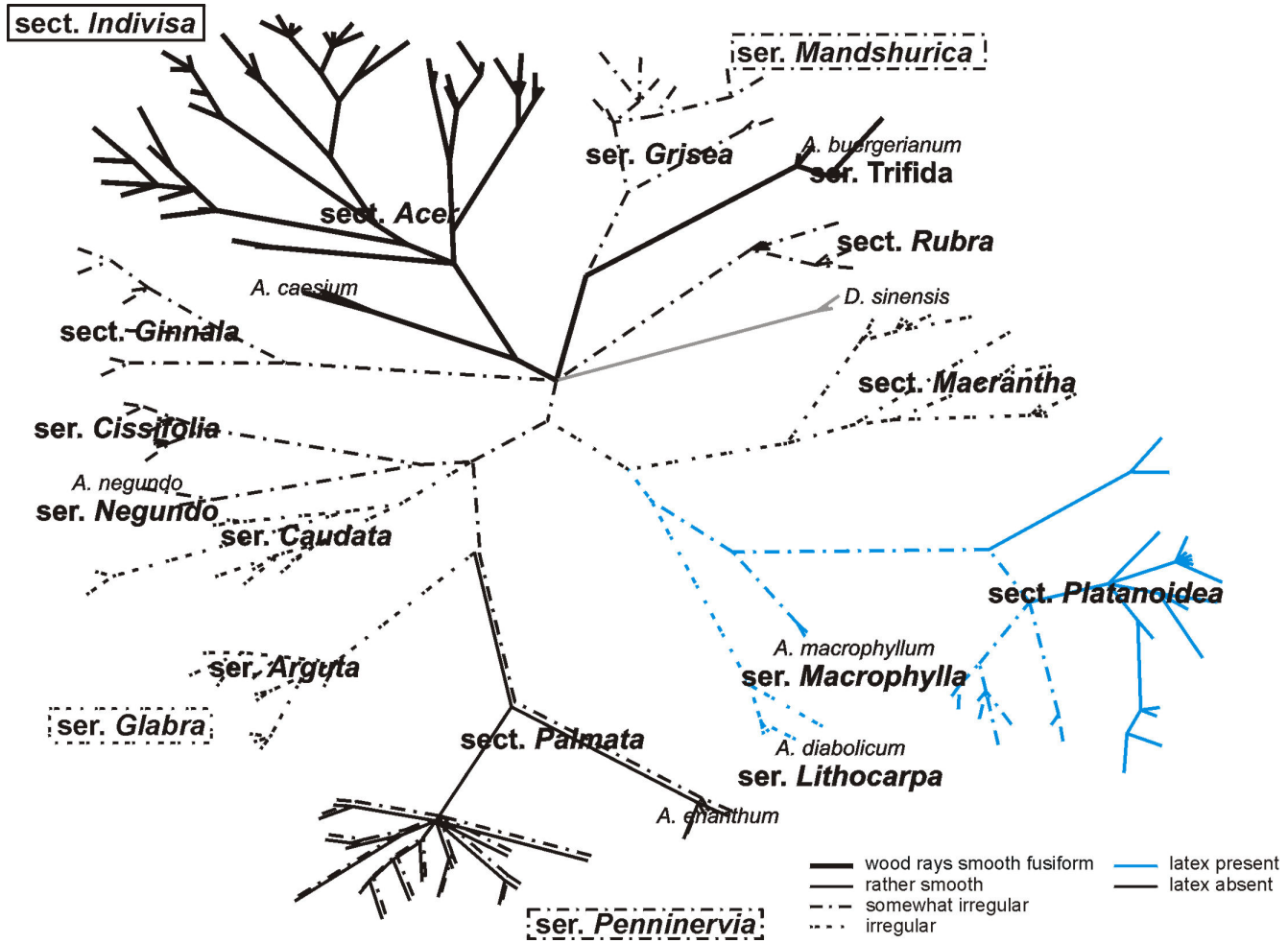


Figure 4-20: Wood anatomical characters analysed by OGATA (1967; *A. pentaphyllum*, ser. *Pentaphylla* not analysed) mapped on the molecular phylogram.
Most remarkable the exclusive occurrence of laticiferous bearing tissues in sects. *Lithocarpa* and *Platanoidea*, which form a possibly monophyletic group from a molecular genetical point of view (Fig. 4-10).

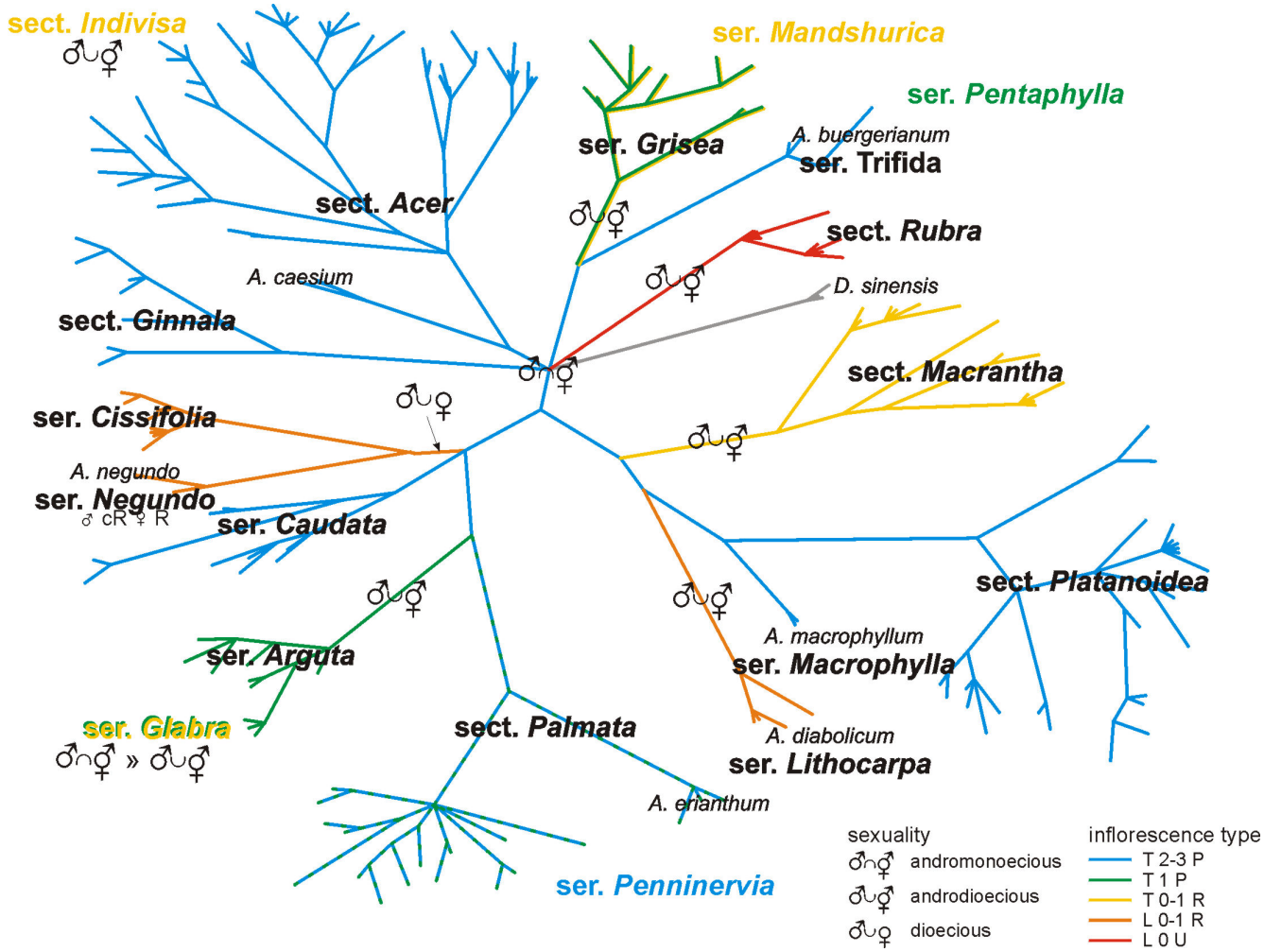


Figure 4-21: Morphological advances related to the inflorescence type in course with a shift in the predominant sexuality (cf. Tab. 4-3).
Obviously a tendency from andromonoecism to dioecism exists in all 3 molecularly sustained clades, but is confined to ± taxa-poor, ± specialised series and sections.

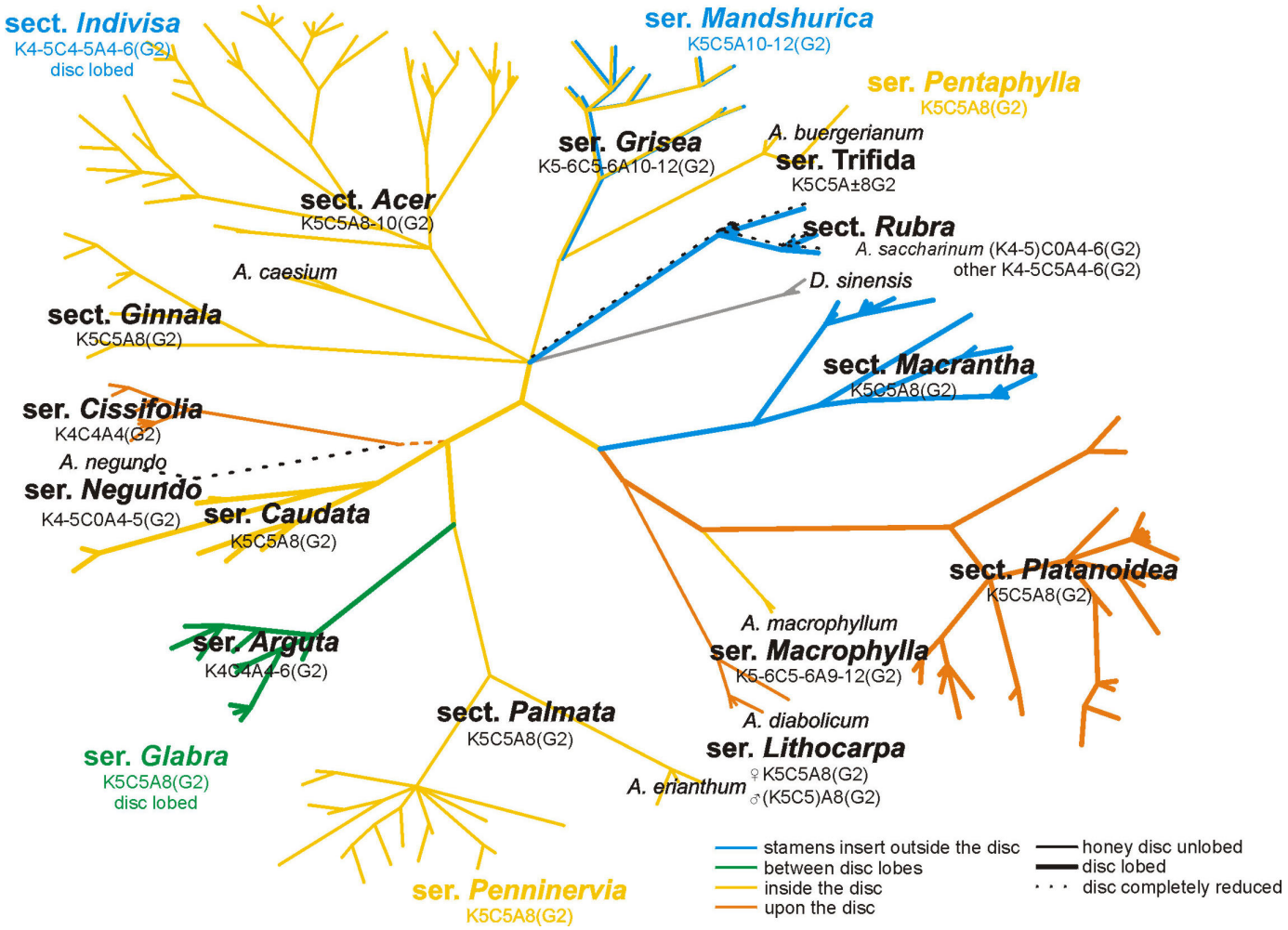


Figure 4-22: Derivations in flower anatomy and morphology.
Aside the relatedness of series, no further phylogenetical trends are apparent, neither concerning derivations in the flower formula, typically K5C5A8(G2), nor characteristics of the honey disc.

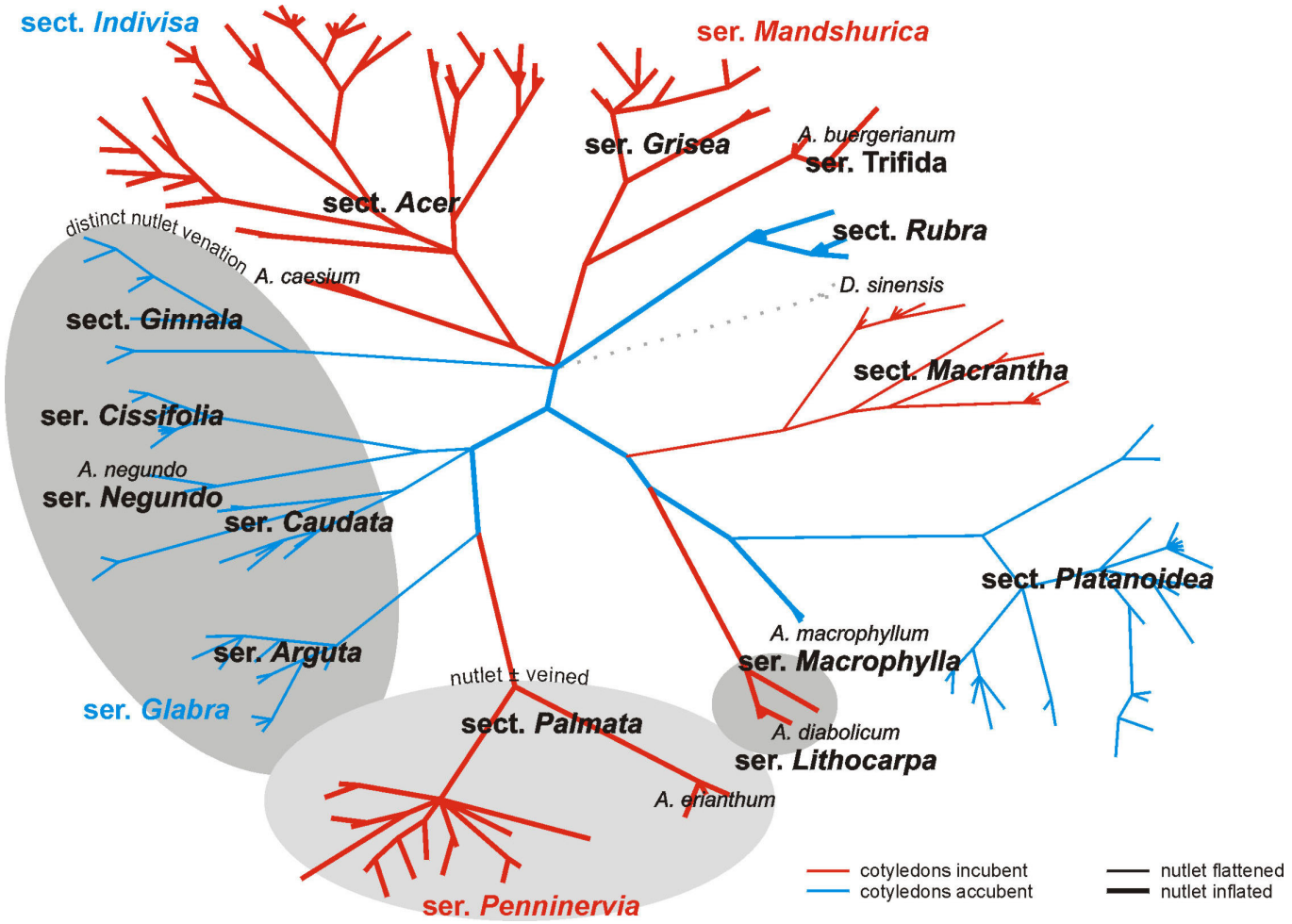


Figure 4-23: Seed characteristics of the molecular-based *Acer* clades.
A ± distinct nutlet venation is predominately found within the *Palmata*-clade. The folding manner of the cotyledons and the flattening of the nutlet do not follow certain tendencies within molecularly related groups of taxa.

As shown above, by plotting morphological features onto a molecular-based phylogram a tool is given, which allows to identify possible progenitors of two or more series and/or sections and distinguish putative morphological analogies against morphological parallelisms within the major clades. In addition, tendencies and probabilities to evolve certain morphologic characteristics during niching and speciation processes can be qualitatively evaluated. In Figure 4-24 several morphological features that can be traced in the fossil record are mapped onto the cladogram which combines the results from the 'classic' molecular analyses (ML via BI, cf. Figs. 4-9, 4-10 & 4-11), filtered by the evidence from the reconstruction of the evolution of oligonucleotide motives (⇨ Fig. 4-18) to identify general morphological trends within the recognised intrageneric clades and phylogenetic groups. The plotted morphological features include characters dealing with the gross morphology of leaves (such as number of lobes, serration type, etc.) and samaras (cf. Fig. 4-2) and particular leaf and/or wing venation patterns (e.g. type of lobal bracing ≙ definitions of WOLFE & TANAI 1987). The assembled data represent mainly the condition detected at the material, which was actually used for the genetic analyses (cf. special remark, chapter 4.3.2), verified with additional information as reported in literature (OGATA 1967; WOLFE & TANAI 1987; VAN GELDEREN et al. 1994), and classified according to the character definitions of WOLFE & TANAI (1987)[90].

Figure 4-24 (following page): Cladogram summing up general morphological trends within the major *Acer* lineages.
The here shown topology is based on the BI analyses (chapter 4.3; Figs. 4-10 & 4-11) filtered by evidence from the genotypic characteristics of the oligonucleotide patterns (preceding chapter). Only morphological features of recent taxonomic units are used, which are of relevance for the fossil record (cf. characters analysed by WOLFE & TANAI 1987). **Abbr.:** L = leaf characteristics; S = samara characteristics; "»" indicates a general tendency.

[90] Since WOLFE & TANAI (1987) did not publish the character state matrix, which they used for the cladistic analyses, the data had to be newly assembled (cf. special remark, chapter 4.3.2).

Fig. 4-10

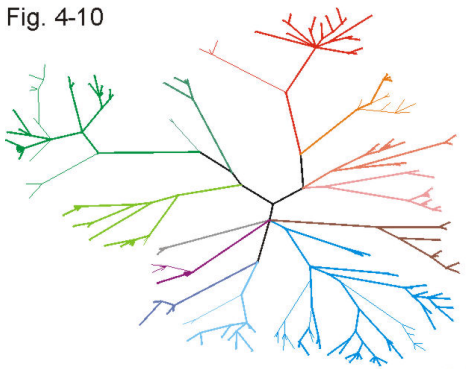
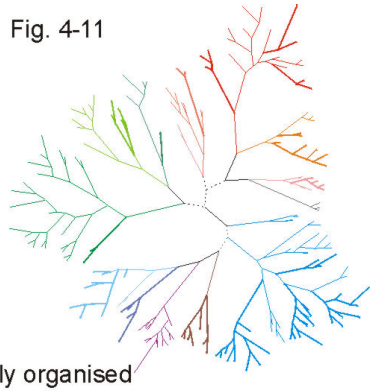


Fig. 4-11



Palmata

L: lobal braces type C
(markedly) inflated nutlets
attachment angles >50° realised

Arguta

nutlet angle always >20°

Caudata

L: » 3-lobed to unlobed
» truncate base

L: predom. acuminating apices
craspedodromous 2° veins, bifurcations occur
veinlets may branch > 2-times
S: nutlet flattened (except *Palmata*)
wing venation with only few anastomoses

L: pinnately organised
entire
few or none teeth
no subsidary teeth
S: wing apical

Cissifolia

Negundo

Macrantha

lobal braces ± var.

L: never derived types/organisation
apices variable, base broadly rounded
» camptodromous 2° veins
S: » assym. inflated/flattened nutlets
attachment angles >50° realised

archetypical *Acer*
L: 5-lobed, perf. actinodromous
veinlets branch 1- to 2-times, many teeth
S: wing extending distally
wing vein anastomoses common

Platanoidea

L: » cordate
lobal braces type A (F)
veinlets unbranched or absent
few or none teeth, no subsidary teeth
lobations present

Macrophylla

L: base cordate
lobal braces predom. type A/B
S: nutlet angle always >20°
attachment angles <40° realised

Rubra

Acer

lobal braces type D known

L: » acute (rounded) apices
» fewer teeth, other than D1-4 type
no subsidary teeth, lobations present
veinlets typ. unbranched or absent
S: predom. markedly inflated nutlets
wing may be apical and with sulcus

Ginnala

teeth other than D1-4 type

Lithocarpa

Grisea

L: » pinnately organised, entire

flattened nutlets

Trifida

Indivisa

In a next step, the extinct sections defined by WOLFE & TANAI (1987) are classified on the basis of the combination of the plotted morphological features (→ Table 4-9) and included in the cladogram (→ Fig. 4-25). Due to the different preservation quality of the fossils and the sometimes diffuse and varying differences between the major *Acer* lineages (cf. Fig. 4-24), such a classification can – on the base of the currently available data – only be done qualitatively by a phenetical comparison, not in a parametrical form, e.g. by a cladistic analysis based on morphological characters. Here, the phenetical assignment to a phylogenetic lineage is mainly done by a falsifying approach: If the fossil taxon exhibits a morphologic characteristic, which is never realised in according phylogenetic lineage, it cannot be part of or related to this lineage (cf. chapter 5).

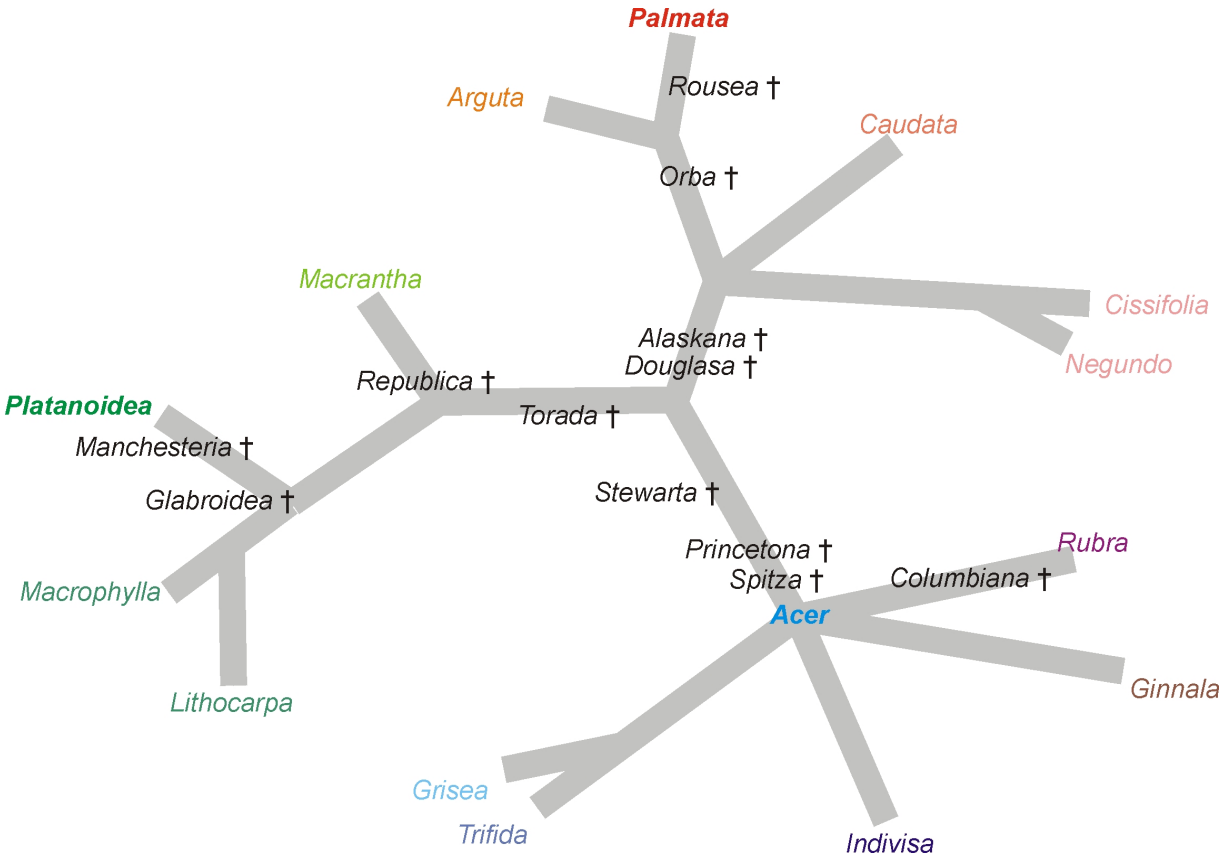


Figure 4-25: Cladogram including extinct sections of *Acer*.
The positioning is done based on the co-occurrence or lack of several morphological features which are supposed to be ± typical for the identified *Acer* clades (cf. Fig. 4-24, Table 4-9).

Table 4-9: Morphologic characteristics of extinct *Acer* sections as described by WOLFE & TANAI (1987).

section	*Douglasa*	*Alaskana*	*Columbiana*	*Glabroidea*	*Manchesteria*	*Orba*	*Stewarta*	*Torada*	*Princetona*	*Republica*	*Rousea*	*Spitza*
leaf type	leaves actinodromous								unknown	unknown	unknown	unknown
number of lobes	5	(2-)3	3(5)	3(5)	3	3	5	3				
number of primary veins	5	3	3-5	3-5	3	3	5	3				
leaf base: c = cordate, r = rounded	±c	±c	?	±r	r	var.	?	±c				
leaf apex: r = rounded, a = acute, a! = acuminate	a»a!	?	?	(a,a!)	?	a	?	a!				
secondary venation	predom. craspedodromous											
tertiary venation type	R-{RA}	R-R	A-A	var.	A-{AR}	R-{AR}	R-R	A-R				
highest order	5°	5°	4°	4°»5°*	?	4°	?4°†	5°				
areoles (4° or 5°): p = irregularly polygonal, q = quadrangular	p	p	q	q	q	p	p†	p				
veinlets: - = none, s = unbranched, b_i = bifurcating i-times	$b_{>2}$	b_{1-2}	(-,b_1)	(-,s)	s	$b_{>2}$	b_{1-2}†	b2				
lobal bracing (type according to WOLFE & TANAI 1987, p.58)	B	E	(C,E)	(A,B)	C	B»C	A	A				
teeth': + = several, ++ = numerous	++	++	var.	+	+	+	+	+				
dentation type (after HICKEY 1973)	D1	D1		D1»A1	A1	D1	D1	D1/3				
number of subsidary teeth	1-2	1		=1	0	=1	0	0				
lobations: - = none, + = present, +! =strongly developed	-	-	+!	+	-	-	-	-				
nutlet outline: a = asymmetrical, c = circular, e = elliptic, t = triangular; i = inflated)	unknown	unknown	e	(e,t)	(a,e)	t	c	±a	e	e	e»c	c
nutlet shape: f = flattened, i = inflated, i! = markedly inflated			±i	±i	f	i!	i!	±i	i!	±i	i	i!
nutlet surface: s = smooth, f = flanged, r = ridged			s	f	±s	f!	±f	±f	f	f»r	±f	f
nutlet angle				var.	<30°		20°	10-20°	20°	10°	0-5°	30°
attachment angle			25-30°	var.	>40°	40-50°	40°	20-30° 45°	30°	50°	80-90°	40°
wing extending apically = a, distally = d			d	d	d	d	(a,±d)	a	a	d	?	a
sulcus present = +, absent = -			-	-	-	-	+	-	-	-	-	-
wing venation with many anastomoses			?	-	-	+	-	-	+	±	-	+

* gradual transitions from 4° to 5° venation

† diagnosis for sect. *Stewarta* differs from the type species *A.stewarti*: 5° forming areoles, veinlets branch more than twice

The assembling of selected morphological evidence (see above; Figs. 4-19 to 4-22; Fig. 4-24), recognition and classification of fossil *Acer* (WOLFE & TANAI 1987; Table 4-9; Fig. 4-25) and detailed comparison with the nucleotide composition (chapters 4.2.3 & 4.4.1) and phylogenetic hypotheses (chapter 4.3; Figs. 4-9, 4-10 & 4-11) now allows to reconstruct the evolution and fossil history of *Acer* in a first approximation (→ Fig. 4-26).

By the beginning of the Tertiary (**Paleocene**), genus *Acer* was already distinguished from the sister genus *Dipteronia* (WOLFE & TANAI 1987; MCCLAIN & MANCHESTER 2001). Fossil remains of these first *Acer* are sparse and not well preserved (TANAI 1983; WOLFE & TANAI 1987; VAN GELDEREN et al. 1994; BOULTER et al. 1996, and literature cited herein; PFR 2.2 database). The first common macrofossils of *Acer* (actinodromous leaves) from this time are predominately attributed to the "*A. arcticum*"-complex, a diffuse morphospecies which comprises numerous early Tertiary actinodromous leaves[91] distributed globally on the northern hemisphere. *Acer arcticum* s.s., i.e. early tertiary leaf fossils that look like recent actinodromous *Acer* leaves, were included by TANAI (1983) within section *Spicata* (cf. OGATA 1967; ≙ here used series *Caudata*)[92]. Beside *A. arcticum*, fossils are reported from the Paleocene of North America by WOLFE & TANAI (1987) as *A. alaskense* (⇨ sect. *Alaskana* WOLFE & TANAI) with a similar affinity (cf. footnote 92).

During the **Lower Eocene** a first diversification event in the pacific area must be assumed which gave rise to the three major lineages: the *Acer*-clade, the *Platanoidea*-clade, and the *Palmata*-clade. Such a diversification event is well-illustrated for the North American provenance (fossil sections invented by WOLFE & TANAI 1987; assigned to the molecular-based lineages by the evolutionary reconstruction shown above): The *Acer*-clade is here represented by the extinct sections *Stewarta, Princetona* + *Spitza,* and *Columbiana* (poss. related to sect. *Acer*, the latter one with affinities to section *Rubra*), the *Palmata*-clade by the extinct sections *Alaskana, Douglasa, Orba,* and *Rousea*, and the *Platanoidea*-clade by the extinct sections *Glabroidea* (related to sect. *Platanoidea*), *Republica* (a progenitor of sect.

[91] The taxonomical status of numerous fossils labelled *A. arcticum* is obscure, since the name has been used as a taxon for numerous late Cretaceous and early Tertiary lobed leaves, including e.g. fossils of *Liquidambar* and *Platanus* (T. Denk, person. comm.; cf. e.g. revision of *Acer* fossils by TANAI 1983 and WOLFE & TANAI 1987).

[92] The leaves of the recent *A. spicatum*, the type species of section *Spicata* (syn. ser. *Caudata*), do completely lack derived or unique leaf characteristics. Here included leaf fossils assigned by WALTHER (1972) and TANAI (1983) to section "*Spicata*" (→ appendix) could also be representatives/descendants of the archetypical, not yet differentiated, *Acer* as deduced in Fig. 4-24.

Macrantha), *Torada* (uncertain affinity). In eastern Asia, besides fossils assigned to *A. arcticum* (possibly assignable to the *Palmata*-clade; cf. footnote 92), a first representative of section *Platanoidea* is found, namely *A. kushiroanum* (TANAI 1983; sect. *Campestria sensu* classification of OGATA 1967).

During the **Middle** and **Upper Eocene** a second diversification event is exhibited by the occurrence of fossils in the northern circumpacific area, which can be assigned to recent taxonomic groups (TANAI 1983, WOLFE & TANAI 1987). By the end of the Eocene, the *Platanoidea*-clade is fully differentiated into the recent taxonomic entities: section *Platanoidea* (including fossils assigned to the extinct sections *Glabroidea* and *Manchesteria*) with representatives in Eastern Asia and western North America, and the sections *Lithocarpa* (including both representatives of sers. *Macrophylla* and *Lithocarpa*) and *Macrantha* in North America. From the recent taxonomic entities of the *Acer*-clade only section *Rubra* is documented by fossils in East Asia and North America, while sections *Acer* and *Ginnala* (differentiated at the end of the Eocene) are only reported from North America. The recent sections of the *Palmata*-clade are only weakly documented during Late Eocene. However, with *A. ivanofense* a representative of series *Arguta* is found in North America (WOLFE & TANAI 1987), as well as two relatives – *A. sinuofluviatilis, A. macginitiei* (WOLFE & TANAI 1987) – of *A. negundo* (series *Negundo*).

After closure of the Turgai-street, during **Oligocene** the sections *Acer, Rubra, Platanoidea*, and possibly also series *Caudata* undergone a westward expansion and gained ground in western Asia (TANAI 1983) and Europe (WALTER 1972).

Back in eastern Asia, during the **Late Oligocene** and **Lower Miocene**, a special development took place in populations of section *Acer* and gave rise to the sibling series *Trifida* (poss. Late Oligocene; TANAI 1983) and *Grisea* (Miocene; TANAI 1983) and the highly specialised section *Indivisa* (*A. subcarpinifolium*, reported for the Miocene and Pliocene of E Asia; TANAI 1983). At the same time, the series *Cissifolia* was separated from series *Negundo* (both forming a specialised lineage within the *Palmata*-clade) and the first representatives of the Asian section *Palmata* are recorded.

With the beginning of the **Upper Miocene** all taxonomic units of the genus *Acer* analysed and included in this study were fully differentiated.

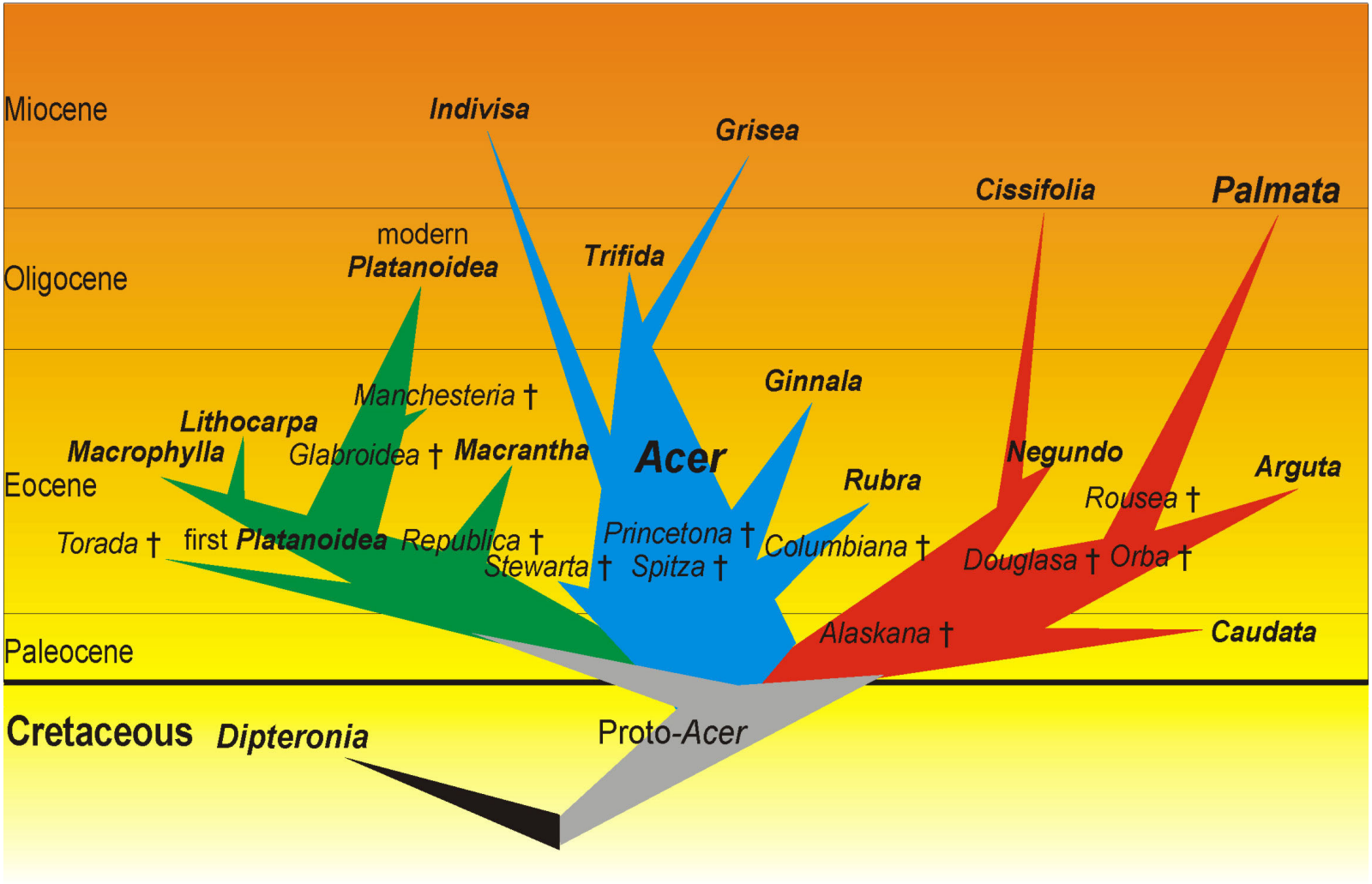


Figure 4-26: Putative evolution of *Acer* since the end of the Cretaceous based on the assembled molecular data, and mapped against the fossil record.
The position of names indicates the first recorded appearance of the according taxonomic group. Blue: *Acer*-clade, green: *Platanoidea*-clade, red: *Palmata*-clade.

From the series and sections that were not included in the molecular analyses (sects. *Pubescentia, Hyptiocarpa, Wardiana*, sers. *Distyla, Glabra*, *Mandshurica, Parviflora, Pentaphylla*), only the series *Distyla* and *Glabra* are documented by fossils. Series *Distyla* is reported by one taxon from the Middle and Upper Eocene of East Asia (*A. protodistylum*; TANAI 1983), and series *Glabra* by *A. traini* from the Lower and Middle Miocene of western North America (WOLFE & TANAI 1987). While the systematical position of the monotypic series *Distyla* is obscure, *A. glabrum* is from a morphological (e.g. current systematics: VAN GELDEREN et al. 1994; cf. Figs. 4-19 to 4-23) and genetical point of view (by comparison between ITS gene bank accessions of *A. glabrum* with new data assembled for ser. *Arguta*) a close relative of the Asian series *Arguta*, respectively a North American sister lineage. It has to be further evaluated, if the fossils of series *Arguta* reported from North America can be predecessors of *A. glabrum*, series *Glabra.*

4.4.4 Deduction of evolutionary rates

From the reconstruction of the evolution in respect to the fossil history from the genus, relative molecular evolution rates in each group for defined parts of the ITS1, respectively ITS2, can be directly deduced (→ Fig. 4-27, branch length ~ evolution rate). Absolute mutation and fixation rates cannot be deduced from the mapping of fossil and molecular data, due to undetectable 'silent' mutations. A number of point mutations are statistically deleted in the gene pool of a certain taxon, while others are still present in recent taxa (cf. theory of neutral evolution, KIMURA 1983). Also, the amount of backmutations cannot be evaluated. The composition of the different ITS regions clearly demonstrates, that certain regions and single nucleotide sites are susceptible to mutations (preceding chapter; see below, Fig. 4-28). As a consequence, if at a defined site a certain nucleotide is realised, it is conceivable that the recent condition will be changed in another thousand or million years, depending on the overall mutation rate, number of paralogs (gene copies) per genome, and the size of the population. Hence, the detected difference at this site may be only a function of the isolation time (and population size) of the taxon, not of the taxon's phylogenetic relationships. Whether the initial mutation is realised or not, is just coincidence (cf. chapter 4.4.1; Fig. 4-17).

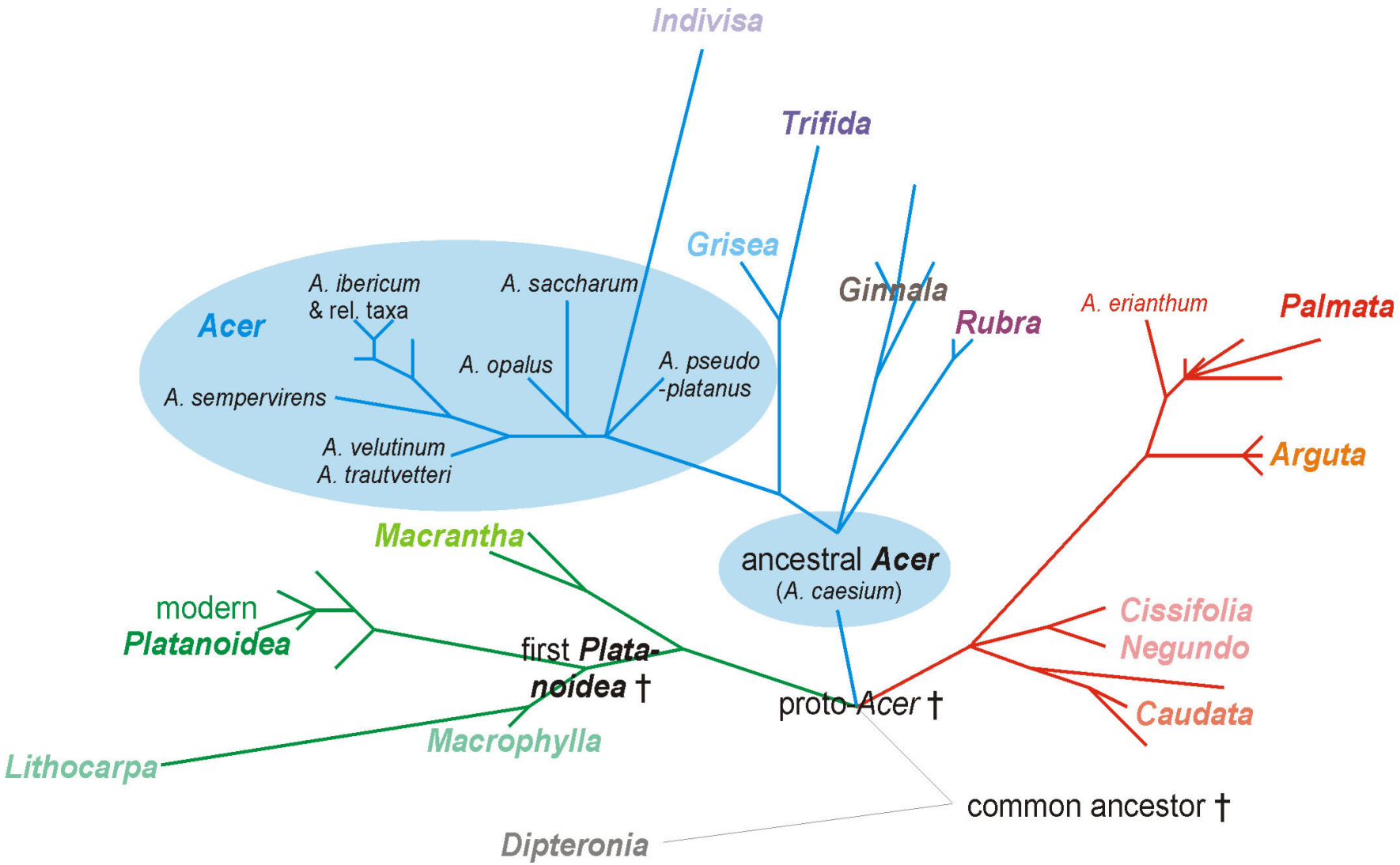


Figure 4-27: Tempo of molecular evolution in *Acer*.
Branch lengths indicate qualitatively the fixation rate (≙ rate of molecular evolution) within distinct lineages of *Acer*.

According to the fact, that *D. sinensis* still – after at least 65 Ma of parallel evolution – comprise genotypic characteristics remarkably similar to the consensual nucleotide composition of genus *Acer*, it can be assumed that the fixation rates (evolution speed) in genus *Acer* is comparably high in relation to extant *Dipteronia*, which is indicated in Figure 4-27 by a equidistant position of *Dipteronia* and the predecessors of the recent *Acer* groups to the common ancestor of the Aceraceae.

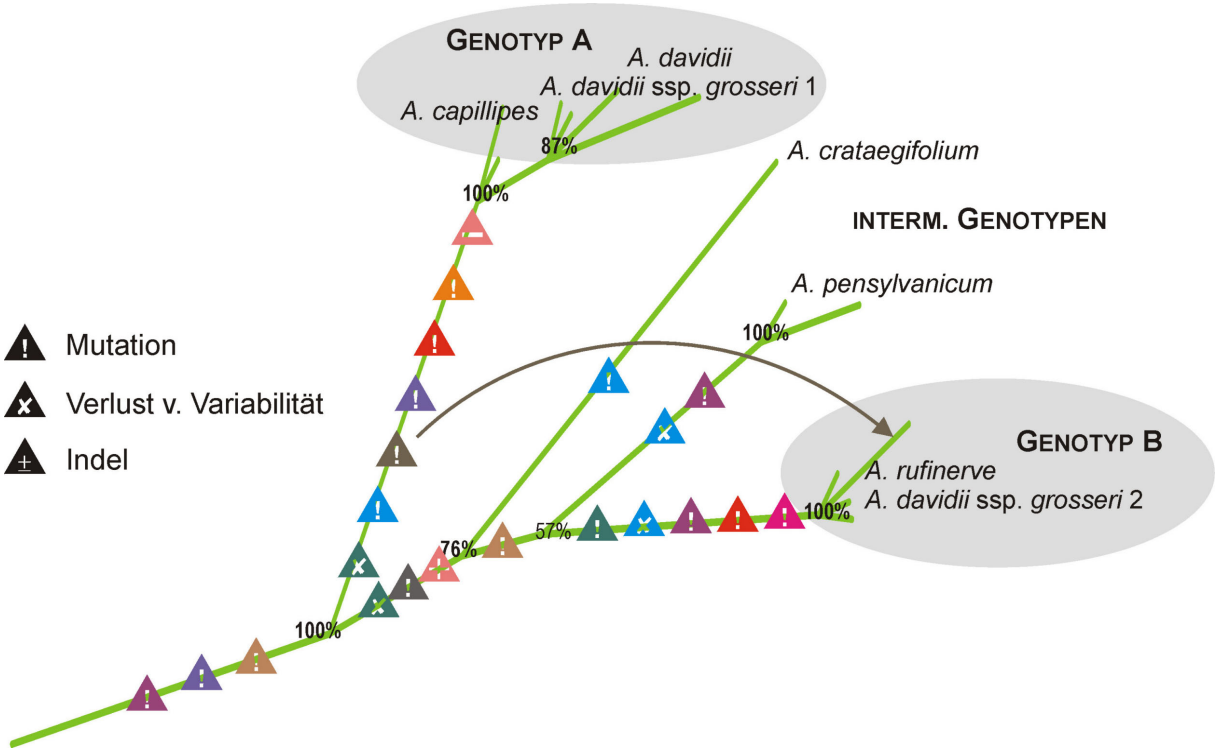


Figure 4-28: Molecular genetical differentiation within sect. *Macrantha*.
The assumed ancestral genotype equals the consensus of the *Platanoidea*-clade (≙ Figs. 4-14, 4-15 & 4-16). The accessions differ in merely 11 nucleotide sites or oligonucleotide motives. Note that changes occur predominately at the same position within the ITS (equally coloured triangles).

The presented molecular data clearly indicates that section *Macrantha* (known since Middle/Upper Eocene) accumulated numerous mutational peculiarities, although the molecular intrasectional differentiation is minimal (→ Fig. 4-28). Series *Macrophylla* is considered to be most similar to the common ancestor of the remaining taxonomic entities of the *Platanoidea*-clade. Since the fossil record of this series can be traced back to the Middle Eocene, the rate of molecular evolution was comparatively low, especially if compared to the slightly younger series *Lithocarpa*, known since the Upper Eocene, which is one of the (genetically) most derived taxonomic entities within genus *Acer*. Recent taxa assigned to section *Platanoidea* share numerous obviously derived genotypic characteristics, but, on the

other hand, the section comprises also the oldest known fossils assigned to the *Platanoidea*-clade. Hence, it must be assumed, that those oldest representatives of section *Platanoidea* are extinct and, accordingly, their genotypic characteristics are lost.

The molecularly detected plurality between extant members of section *Acer* is illustrated by long intrasectional branches. Since the fossil record of section *Acer* can be traced back to the Lower Eocene of North America and Asia, the molecular differentiation found within this genus could also be a result of the high stratigraphic age of the section. However, the primitiveness of the eastern Asian *A. caesium* (cf. chapters 4.4.1 & 4.4.2) indicates that the recent, mostly western Eurasian, representatives of this sections are markedly derived from their Eocene ancestors. Similar to section *Platanoidea*, recent taxa of section *Acer* – including the North American *A. saccharum* – share numerous, presumably derived genotypic characteristics. While the sections *Ginnala* and *Rubra* are only slightly younger than section *Acer* (Eocene), sections *Indivisa* and series *Grisea* + *Trifida* are relatively modern descendants of the *Acer*-clade. Thus, the genetical derivedness of series *Ginnala* and *Rubra* can be primarily assigned to the high stratigraphic age, while the derivedness of sections *Indivisa* and series *Grisea* + *Trifida* is apparently due to an increasing evolution rate. Such an increased evolution rate concurs with the specialised character of these taxonomic entities (cf. chapter 4.2.2).

Since accessions of series *Caudata, Cissifolia,* and *Negundo* exhibit more similarities to the more "primitive" taxa *A. caesium, A. macrophyllum* and *D. sinensis* within the length polymorphic regions than series *Arguta* and *Palmata*, it can be deduced that the first diversification events within the *Palmata*-clade were only accompanied by rather few fixed mutations. But, on the other hand, the intrasectional, respectively intraserial, molecular differentiation (i.e. between *A. negundo*, ser. *Negundo* and *A. cissifolium* and *A. henryi*, ser. *Cissifolia* of section *Negundo* and between species and subspecies of ser. *Caudata*) found within this group is remarkable, and can be partly attributed to the high stratigraphic age (?Paleocene, Eocene) of these series. The evolution rate within the *Palmata*-*Arguta*-group is obviously higher, especially in respect to the section *Palmata* (known from the Miocene on). Of special interest is here the parallel evolved taxon *A. erianthum* (cf. Fig. 4-9, 4-10 & 4-11), where genetical affinities with the series *Caudata, Cissifolia,* and *Negundo* are combined with typical derived characteristics of section *Palmata*. The position of *A. erianthum* in relation to the remaining taxa of section *Palmata* is apparently analogous to the position of *A. macrophyllum* within the *Lithocarpa*-*Platanoidea*-subclade and *A. caesium* within section *Acer* and the *Acer*-clade, respectively.

4.5 Implications for the taxonomy and systematics of Acer

Although not all described *Acer* taxa are analysed, the now available data allow to draw a comprehensive image of the evolutionary pathways, especially by the combination with other data sources aside from molecular data. Obviously, the value of ITS sequence data for taxonomical and systematical purposes is yet unexplored. Since cpDNA is inherited only from one parent, it is less difficult to produce a molecular phylogeny, due to the homogeneity of the according data set. However, the underlying speciation processes – possibly occurring or occurred hybridisation, reticulate evolution, the gradual or punctual origin of new subspecies, respectively species – might have been complex and, thus, require gene regions, which preserved at least some of this complex history.

For *Acer* the following systematical results and implications for future taxonomical and phylogenetical studies on the genus have to be pointed out:

- **The here presented data propose, that three main genetic lineages – leading to the sections *Acer*, *Platanoidea*, and *Palmata* – can be distinguished for the ITS of the genus *Acer*.**

Each 'crown section' (W Eurasian & N American taxa of sect. *Acer,* sects. *Platanoidea* and *Palmata*) is accompanied by a more ancestral sister group, respectively taxon (ser. *Macrophylla* ⇨ *A. cappadocicum/Platanoidea*, ser. *Arguta* ⇨ *A. erianthum/Palmata*, *A. caesium* ⇨ remaining taxa of sect. *Acer*), and the corresponding divergence points are sustained by appropriate *a posteriori* probabilities and the occurrence of unique oligonucleotide motives, which can be defined as molecular synapomorphies (cf. Figs. 4-14, 4-15 & 4-16). These sister groups exhibit less genetic divergence and morphological variability, especially in the case of leaf characteristics, also reflected by the overall number of taxa. In conclusion, the crown sections *Acer*, *Platanoidea*, and *Palmata* exhibit a general trend from primitive to derived genetical features. As a consequence, for a comprehensive analyses it is necessary to assemble as much data as possible for these taxa-rich sections, from as much taxa and populations per taxon as possible.

- **The relationship of sections *Ginnala, Trifoliata*, *Rubra*, and *Pentaphylla* to section *Acer* (including *A. caesium*) has to be classified more precisely.**

Here, an old phylogenetic lineage represented by a single extant Asian taxon (*A. caesium* of sect. *Acer*) gave rise not only to a group of morphologically and genetically related but well differentiated taxa (remaining species assigned to sect. *Acer*), but also to a number of distinctively specialised series and sections (chapter 4.3). The next step will be to assemble

new data from the remaining taxa of series *Trifida* (sect. *Pentaphylla*), section *Trifoliata* series *Mandshurica*, and *A. pentaphyllum* (representative of the monotypic ser. *Pentaphylla* of sect. *Pentaphylla*)[93]. Furthermore, a detailed study on the genotypic setting of populations of *A. rubrum* and *A. saccharinum*, respectively their natural occurring hybrid *A.* x *freemanii*, may reveal interesting relationships concerning the possibility and processes of hybridisation and speciation, since these taxa are all polyploid. In comparison, population-scale studies on the diploid and tetraploid representatives of section *Acer* have to been undertaken.

↳ **Morphologically well defined taxonomic groups are mostly genetically sustained.**

With the exception of *A. caesium* and *A. campbelli* spp. *campbelli* (cf. special remark, chapter 4.2.3), all sequenced taxa plot within the sections and series they are assigned to from a morphological and biochemical point of view (≙ sections of OGATA 1967 and systematical synopsis proposed by VAN GELDEREN et al. 1994, but infrasectional division of sects. *Acer* and *Palmata* not confined: → Fig. 4-29). This is not surprising, if one considers the numerous morphological features, respectively their combination, that are preserved in different groups of *Acer* spp. and were studied in detail and thus recognised by various authors (e.g. POJÁRKOVA 1933; OGATA 1967; DE JONG 1976; MAY 1984; WOLFE & TANAI 1987).

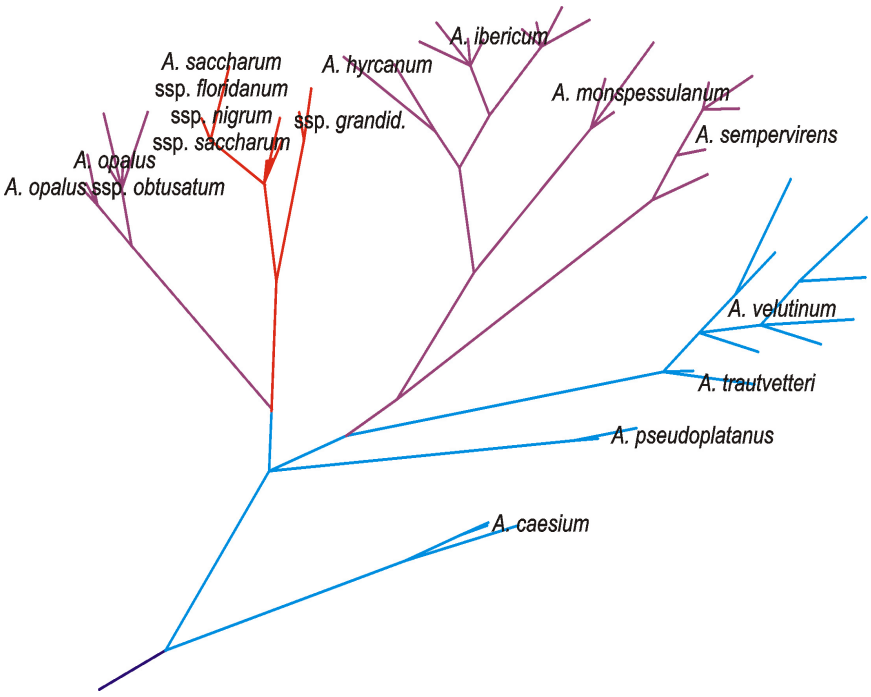


Figure 4-29 (left): Part of the ML phylogram (Fig. 4-10) focussing on the serial relationships within sect. *Acer*. Clearly, the currently used series are not sustained. Furthermore, the inclusion of *A. ibericum* as subspecies of *A. monspessulanum* is doubtful (see text). Blue: ser. *Acer*, pink: ser. *Monspessulana*, red: ser. *Saccharodendron*. Percentages at nodes indicate probabilities; complete data from ITS1 & ITS2; *A. carpinifolium* (sect. *Indivisa*) not included in the analysis.

[93] Aside the here analysed taxa, gene bank accessions are available from *A. mandshuricum* (sect. *Grisea* ser. *Mandshurica*), *A. oblongum* (sect. *Pentaphylla* ser. *Trifida*), and *A. pentaphyllum* (sect. *Pentaphylla* ser. *Pentaphylla*), but not included in the study due to problems mentioned in the preceding chapter.

↳ **Small taxonomic entities – on a species and/or subspecies level – can be readily recognised by ITS sequence data.**

Taxonomic entities that exhibit a high morphological variability *and* differentiation do commonly comprise more "species" than morphologically uniform identities. In the case of genus *Acer*, morphological variability is in general correlated to the variability and genetical differentiation detected within the ITS. As a rule, morphological distinctness is comparable with genetical distinctness. This possibly applies also to other tree genera. As shown in chapter 3, *Fagus* exhibits a low overall genetical diversity and differentiation within the ITS in comparison to other tree genera. The morphological variability, and so the number of taxa assigned to this genus, is accordingly low. Other *Fagaceae* like *Quercus*, which show a higher morphological diversity, exhibit also a higher genetical ITS diversity (e.g. SAMUEL et al. 1998; MANOS et al. 1999; MUIR et al. 2001; MANOS & STANFORD 2001). This is amazingly enough, since the ITS is a non-coding miscellaneous rDNA region not linked to a special ontogenetical process leading to the formation of a morphological character.

In the context of taxonomical hierarchy, a still unsolved problem is (a) the application of sectional and serial ranks for morphologically and molecularly well-defined taxonomic entities and (b) the assignment of morphologically distinguishable taxa to a subspecific or specific level:

(a) The series *Cissifolia* and *Negundo* form a section *Negundo* in current systematics. A sister relationship between these two series can be sustained by the nucleotide composition of the oligonucleotide motives and morphological particularities, but is only moderately to weakly sustained by the BI analyses. On the other hand, series *Grisea* is from a genetical point of view (BI analyses, oligonucleotide motives) definitely closely related to series *Trifida*. A comparison of the here presented data with gene bank accessions of series *Mandshurica* (sers. *Grisea* and *Mandshurica* ⇨ sect. *Trifoliata*) and series *Pentaphylla* (sers. *Trifida* + sers. *Pentaphylla* ⇨ sect. *Pentaphylla*) indicate a very close relationship between series *Grisea* and *Mandshurica* and a more distant relationship between series *Trifida* and *Pentaphylla* (→ appendix). From a purely genetical point of view, the interserial and –sectional genetical difference would be best illustrated by assigning a sectional rank to series *Cissifolia* and *Negundo* and retaining the serial rank for series *Grisea* – including taxa assigned to series *Mandshurica* – and series *Trifida,* but combine these series to a section which may include also a series *Pentaphylla* or series *Pentaphylla* must be assigned a sectional rank. From an evolutionary point of view a discussion about

a serial or sectional rank is superfluous, because it is of no important concern whether one looks at sister sections or sister series, if it is clear that they have a common origin.

(b) Lowest-level hierarchical problems do pose a direct criticism on the possibility to count biodiversity by reporting number of species. The mean genetic divergence for taxa assigned to one species in VAN GELDEREN et al. (1994) and various online databanks (GRIN database, Flora Europaea, etc.) varies substantially (→ appendix), which concurs also to the historical assignment of the according taxa to specific and subspecific ranks. Some taxa of *Acer* do have a remarkable nomenclatural history, well documented in VAN GELDEREN et al. (1994). The general tendency seems to be, to combine described species as subspecies of one species, as it was already done for former species of section *Ginnala* (⇨ *A. tataricum*) and series *Saccharodendron* (⇨ *A. saccharum*). That such a reduction in numbers of species, and hence, amount of biodiversity, is questionable and may cause further taxonomical problems is well exhibited in the case of the mainly Transcaucasian taxon *A. ibericum* (ser. *Monspessulana*, reported from S Caucasus, NE Turkey, and NW Iran). *Acer ibericum* was assigned as a subspecies of *A. monspessulanum* by YALTIRIK (1967) together with various other oriental *Acer* spp. This placing is approved by VAN GELDEREN et al. (1994) and the GRIN database. According to the identification key of the Flora Europaea, *A. monspessulanum* is a close relative of *A. opalus,* named the "*opalus*-group". Both taxa – *A. monspessulanum* and *A. opalus* – frequently hybridise in nature, especially on the Balkans (VAN GELDEREN et al. 1994, T. Denk, person. comm.). MURRAY (1970) combined all taxa related to *A. hyrcanum* (Asia Minor) and *A. opalus* as subspecies of *A. opalus*. According to molecular data, *A. ibericum* is markedly derived within series *Monspessulana* and a close relative of *A. hyrcanum*[94], while *A. opalus* is rather distinct to the above mentioned taxa (Fig. 4-29). Put in a taxonomical context, if *A. ibericum* is a subspecies of *A. monspessulanum*, than *A. hyrcanum* has to be assigned subspecific rank not of *A. opalus* but of *A. monspessulanum*. Furthermore, from a strict reproduction biological viewpoint, *A. opalus* and *A. monspessulanum* form a complex species (because of the frequent natural hybridisation), providing a morphologic and genetic divergence far exceeding those of other species of section *Acer*, like *A. saccharum*, *A. sempervirens*, and *A. pseudoplatanus.* In addition, the number of species recognised for series *Monspessulana*, and hence, the biodiversity importance of this series, would have to be reduced from nine (accord. FLORA EUROPAEA), respectively five (accord. VAN GELDEREN

[94] ITS sequences assembled from the typical subspecies, i.e. *A. hyrcanum* ssp. *hyrcanum*.

et al. 1994) to two species: *A. monspessulanum* and *A. sempervirens* compared to one species (*A. saccharum*) in North America, and four species (*A. caesium, A. pseudoplatanus, A. velutinum,* and *A. heldreichii* + *A. trautvetteri*[95] representing series *Acer* in Eurasia. Since the taxa assigned to series *Monspessulana* provide the dominating or even only *Acer* element in the warm-temperate and subtropical regions of western Eurasia, such a reduction clearly does not reflect the ecological, evolutionary and biodiversity importance of this group.

In the context of phylogeny and evolution the question remains, to which proportion the morphological evolution is due to irreversible mutational events related to the underlying genetic programme of the individual organism or direct adaptations to definite ecological parameters within the limits of possible morphological variation. The morphological variability in correlation to genetical differentiation as it is exhibited to different degrees in the phylogenetic lineages within the genus *Acer*, will allow – in combination with further biogeographical, ecological, ontogenetical, and development genetical studies – to trace in detail the circumstances and core parameters of low-level evolution and differentiation. Thus, in contrast to the "static frontier strategy" exhibited in *Fagus*, genus *Acer* can be used as a model to analyse the presumed basal mechanisms of evolution as proposed by the synthetic evolution theory and, hence, the origin of species. The general task for *Acer* has to be to understand, why some *Acer* sections and series did speciate more than others, how they were able to do so, and what did prevent speciation processes or constitute inter- and intraspecific variability. For example in the case of section *Acer*, why does section *Acer* comprises so many species, how could *A. caesium* survive in Southwest China and the Himalayan and *A. saccharum* in eastern North America, while the remaining species dispersed into different habitats throughout western Eurasia (→ Table 4-10 & Fig. 4-30), and what is the reason for the peculiar morphological variability found in leaf morphology of the evergreen taxon *A. sempervirens*, respectively the ITS variability detected for populations of *A. ibericum*[96] (see below).

[95] Both subspecies of *A. heldreichii* according to VAN GELDEREN et al. (1994). *Acer heldreichii* not included in the current study.

[96] In addition, *A. ibericum* exhibits a remarkable leaf dimorphism (T. Denk, person. comm.)

Table 4-10: Habitats of taxa assigned to section *Acer*.

species	habitat	climate*	hardiness zone*
caesium	subtropical montaneous forests	Cw	V
heldreichii	mixed temperate forests	Cf	V-VII
hyrcanum	(montaneous) bushland	Df,Ds	IV-V
ibericum	mixed temperate forests	Cf	V-VII
monspessulanum	mediterranean semi-deciduous forests	Cs,(Cf)	V-VI
opalus	montaneous mixed forests	Cf,(Cs)	IV-V
pseudoplatanus	montaneous mixed forests; sub-alpine habitats	Cf	IV
saccharum	mixed & evergreen temperate forests; subtropical montaneous & mixed forests	Cf,Df	III-VII
sempervirens	mediterranean semi-deciduous forests	Cs	VI-VII
trautvetteri	montaneous mixed forests	Cf,Cs	V
velutinum	temperate forests and bushland	Cf	V

* Koeppen classification

† after VAN GELDEREN et al. 1994

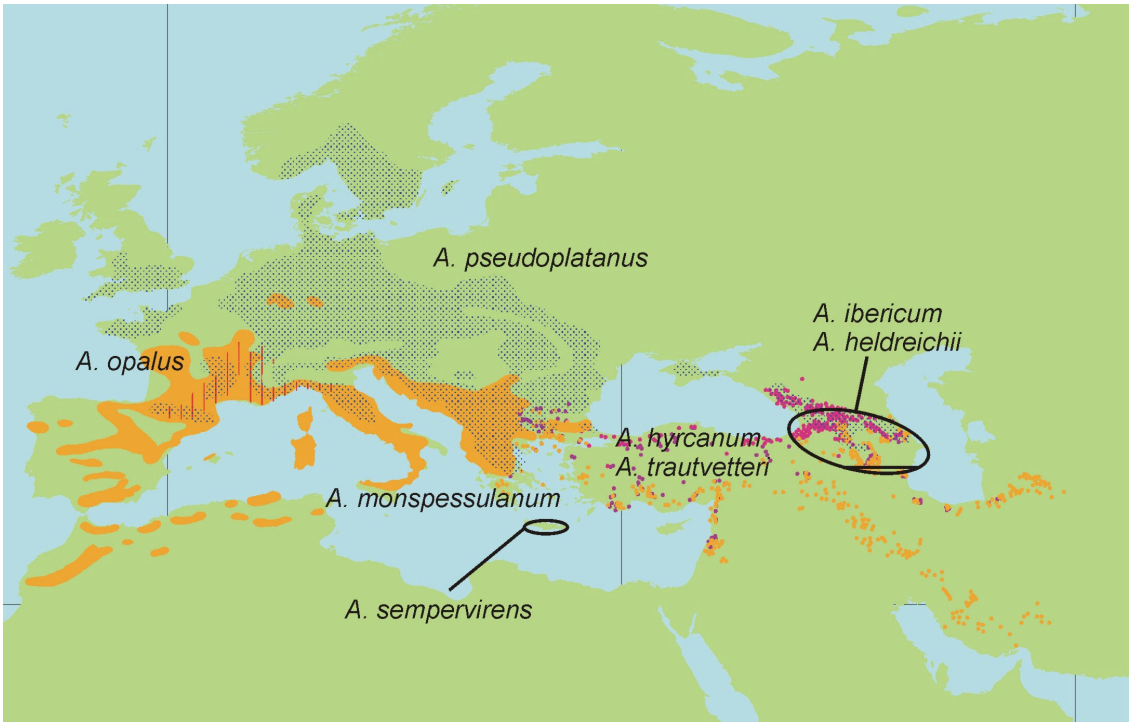


Figure 4-30: Biogeographical distribution of section *Acer* in western Eurasia.
Distribution of *Acer* spp. emended after MEUSEL et al. (1978), that are assigned to section *Acer*. Blue sparkled: *A. pseudoplatanus*; pink dots: *A. hyrcanum* & *A. trautvetteri*; red lined: *A. opalus* ssp. *opalus*; orange: subspecies of *A. monspessulanum*, in southern Europe/Balkans commonly associated/hybridised with *A. opalus* ssp. *hispanicum,* respectively *A. opalus* ssp. *obtusatum* (not shown). Original distribution maps digitised (GIS) at the Institute for Earth Science, University of Tübingen (courtesy of C. Traiser).

4.6 Implications for infrageneric studies on Acer *and other tree genera*

From the analyses performed for *Acer*, general aspects for intrageneric phylogenetic reconstructions can be deduced concerning the usability of morphology as data source, the necessity of incorporating fossils, the quality and quantity of the molecular data needed, and the appropriate analytic model and methodological approach to infer a infrageneric phylogeny.

4.6.1 Morphological data as evidence to infer low-level evolution

The current study confirms the recent systematical grouping of *Acer* spp. into taxonomic entities like sections and series, respectively, that comprise morphologically and genetically near-related taxa. In particular, the morphologically well-defined sections *Acer* (infrasectional subdivision not confined), *Indivisa*, *Ginnala*. *Lithocarpa* (incl. sers. *Macrophylla* and *Lithocarpa*), *Negundo* (incl. sers. *Cissifolia, Negundo*), *Palmata* (infrasectional subdivision not confined), *Platanoidea*, and *Rubra*, and series *Arguta*, *Grisea*, and *Trifida* can be recognised in the nucleotide composition of the ITS and are sustained as monophyletic groups by the phylogenetic reconstruction. Due to the high morphological variability of the genus, the designation of certain taxa to infrageneric taxonomic groups like sections and/or series can be and was done with a high precision (preceding chapter; see also chapters 4.2.1, 4.2.2 & 4.3.2). But, the application of a molecular phylogenetic hypothesis based on ITS sequence data reveals, that the morphological features of *Acer* spp. are obviously to an extremely high degree the result of convergent evolution, i.e. far the most morphological characters have been developed in parallel (parallelisms) or analogously (analogies, homoiologies; Figs. 4-19 to 4-23; cf. special remark, chapter 3.5). In addition, heterobathmies are common: Derived morphological features may be found on genetically 'primitive' (possibly ancestral) taxa or putatively old lineages, while taxa exhibiting a sum of presumably ancestral organic features (5-lobed leaf, small samaras, high number of flowers, etc.; cf. Figs. 4-19 to 4-23) may be genetically rather distinct. As a consequence, although the *combination of morphological characters* allows to define taxonomic entities, the phylogeny and evolutionary history cannot be reconstructed by cladistic (MP) analyses based on the *individually realised character states* within distinguishable taxonomic units (as attempted by WOLFE & TANAI 1987). This is no surprise, since all taxa forming a genus are ± closely related and share presumably a widely identical genetic programme. The occurrence or lack of a particular morphological

feature in two or more taxonomic units can be just coincidence or primarily due to ecological adaptations, because in principle all *Acer* spp. have the appropriate genetic programme to develop such a feature.

The molecularly reconstructed phylogeny – based on ITS sequence data – is virtually independent from the morphological characters, which are used to define the currently accepted sections and series. Hence, by mapping the morphological features onto a ITS-based topology, it is possible to reckon morphological analogies and distinguish them from putative parallelisms and general tendencies realised within different intrageneric lineages (cf. chapter 4.4.3). This allows to deal with three common problems of low-level evolution, which also may affect higher level evolution:

1. **The morphologic characteristics of fossils can be evaluated in a new light.**

   It is possible to decide, whether a particular morphological feature exhibited by a fossil taxon is of phylogenetical relevance (homologies, parallelisms, general trends within one lineage) or not. Furthermore, the direction – ancestral or derived – can be rendered more precisely and independently from character states realised in extinct and extant taxa. WOLFE & TANAI's (1987) re-evaluation of the fossil record showed, that some fossil taxa – especially from the Paleocene and Eocene – cannot be assigned to any of the recent sections and series, because they exhibit unique combinations of morphological characters. By identifying general trends within the intrageneric lineages, their placement can be clarified (cf. chapter 4.4.3; Fig. 4-25)[97].

2. **Future distinction between primarily ecological adaptation and genetical mutation, respectively molecular evolution (cf. chapters 4.4.3 & 4.5) is possible.**

   Some morphological features are obviously variable to a certain degree within an individual, population, and/or taxon – leaf size, number of lobes, etc. – while others are developed apparently due to a particular genetic programme[98]. In addition, the realisable range of a particular character may be limited in one lineage and ± free in another. Hence, the

[97] For a precise placing of the extant fossil sections, all reported *Acer* fossils would have to be re-evaluated on the base of the new results concerning the evolution of *Acer*, including fossils assigned to *A. arcticum.*

[98] Which character, in the case of *Acer*, is developed due to a particular genetical program has yet to be analysed by development genetical and ontogenetical approaches.

according lineage has or lacks the ability to disperse into a new niche[99] or survive, if its current niche is ecologically altered.

3. **The more precise evaluation of fossils then allows to decide, whether the genetic distance of a taxon to its sibling taxa or the common origin is due to a higher stratigraphic age or an accelerated evolution rate (≙ fixation rate in case of the nucleotide composition) as it shown in Figure 4-27.**

The internal (e.g. molecular mechanisms) and external factors (ecological adaptation and climate constraints) that lead to a higher or lower fixation rate are only poorly understood and studied mainly on the basis of theoretical-mathematical models (cf. literature on population genetics). The herein shown data for the ITS of *Acer* – and *Fagus*, respectively (chapter 3) – implicates, that the fixation rate is indeed variable. It is conceivable, that such a variability does have an impact on the possibility and speed of speciation and might be affected by the individual evolutionary pathway of the genus, the corresponding intrageneric group, and/or the particular species.

As already demonstrated for *Fagus* (chapter 3) the combination of molecular and morphological data is imminent to trace the actual evolutionary pathways which gave rise to the recent setting. A stable general phylogeny, which allows to deduce systematical relationships, might be reconstructed without morphological evidence on the basis of molecular data in the case of *Acer*, but a stand-alone molecular-based phylogenetic hypothesis does not provide enough information about the speed and mode of evolution. The task of molecular genetical studies should be to contribute to the comprehensive understanding of evolution, not to merely reconstruct phylogenies and sustain systematic relationships.

4.6.2 Quality and quantity of molecular data and best analytic model

The amount of data and the sampling method involving the intensive accumulation of reliable inter- and intraspecific variability by cloning allows to distinguish between putative ancestral and derived oligonucleotide molecular motives and genetic lineages. An initial hypotheses about the evolutionary pathways of the ITS region can be put up, but clearly more data are needed. The application of maximum likelihood via Bayesian inference is for a molecular data set like the one presented here the most suitable. The recent composition of the ITS region is the sum of a complex evolutionary history. Up to now, possible evolutionary

[99] To accomplish such a task, more ecological, (micro)climatical, and morphological data for *Acer* is needed.

constraints by external factors are not known[100]. But, apparently the mutational patterns are not purely coincidental but follow certain pathways. The reconstruction of these pathways is difficult. The implementation of a randomising factor during the analysis (cf. HUELSENBECK & RONQUIST 2001) together with the permanent permutation of the evolutionary model seem to be a promising approximation of the actual circumstances.

However, oligonucleotide patterns and the resulting secondary structure play obviously an important and still unsolved role during the molecular evolution, posing serious concerns about the optimal alignment (Figs. 4-13 & 4-31) and the general neglect of length polymorphic regions in numerous studies or the recognition of gaps as phylogenetic signals (as supposed by e.g. KELCHNER 2000 and SIMMONS & OCHOTORENA 2000). Apparently, the information encrypted on a nucleotide level within the genome, at least for maternally and paternally inherited molecular marker, is only used in a preliminary way in current molecular systematics. New methods and models have to be developed and applied, including intuitive and visual approaches, as it is shown here. Nevertheless, especially the taxonomic implications show, that the here assembled data and performed analyses can be only a first step to understand the evolution of *Acer*. I am convinced by my ongoing studies, that the phylogeny presented here is still a working hypothesis, like all phylogenies based on molecular studies, although it is well sustained.

The weakness of fixed alignments is further demonstrated: The reconstruction of the hypervariable regions (Figs. 4-14 & 4-15) shows that oligonucleotide motives apparently evolve as one character complex. An identification of homologous sites from the pure nucleotide data *before* the analysis is at least difficult, if not impossible (cf. Figs. 4-7, 4-8 & 4-13). This is exemplary illustrated in the nucleotide composition of LP2 realised within section *Platanoidea* (cf. Fig. 4-14): several accessions of *A. campestre* and all accessions of *A. cappadocicum* and *A. platanoides* exhibit a 8 bp long motif "CCTCCTCT" (⇨ ancestral motif "P_0" of sect. *Platanoidea*; → Fig. 4-31), which is only slightly derived from the elsewhere in realised motives "5C-TCT" (sects. *Indivisa, Macrantha*, sers. *Arguta, Cissifolia, Macrophylla, Negundo, A. caesium*) or "5C-TTT" (sect. *Macrantha*, sers. *Caudata, Trifida*, *D. sinensis*). The remaining clones of *A. campestre* exhibit a "CCTCCTC**C**" motif (⇨ motif P_1), which can be derived by a single transition from "T" to "C" at the last bp from motif P_0. Accessions of *A. pictum* ssp. *mono* are distinguished into two lineages: one exhibits motif P_1

[100] E.g., the processing of the rDNA, which probably has an impact on the evolution of this region, is only studied in detail for the yeast genome.

and the other a motif P_2 ("CCT**CCT**CCTCT") derivable by a duplication of the initial three bp ("CCT") of motif P_0. Accessions of *A. miyabei* exhibit a similar motif P_2', where the initial "C" is replaced by a "G". Motif P_1 can also be derived from motif P_2 by a deletion of the last three bp. According to the BI/ML analyses (Figs. 4-9, 4-10 & 4-11) *A. cappadocicum* is basal to the remaining taxa of section *Platanoidea*. Furthermore, the Chinese *A. miyabei* is a sister species of the European *A. campestre* while the other European representative *A. platanoides* is more closely related to the remaining Asian taxa (*A. pictum* spp. *mono*, A. *truncatum* ⇨ *A. pictum*-group)[101]. In conclusion, the alignment #2 in Figure 4-31 is more appropriate than the alignment #1. Thus, motif P_1 is actually derived from P_2 by deletion of three bp. If future data from additional Asian *Platanoidea* taxa and populations reposition *A. miyabei* within the *A. pictum*-group and indicate a more basal position for *A. campestre*, a re-alignment would become necessary in which the P_1 motif represents in fact two non-homologous motives: One derived within the *A. pictum*-group by deletion from motif P_2 and the other (*A. campestre*) by a transition at the last bp from motif P_0.

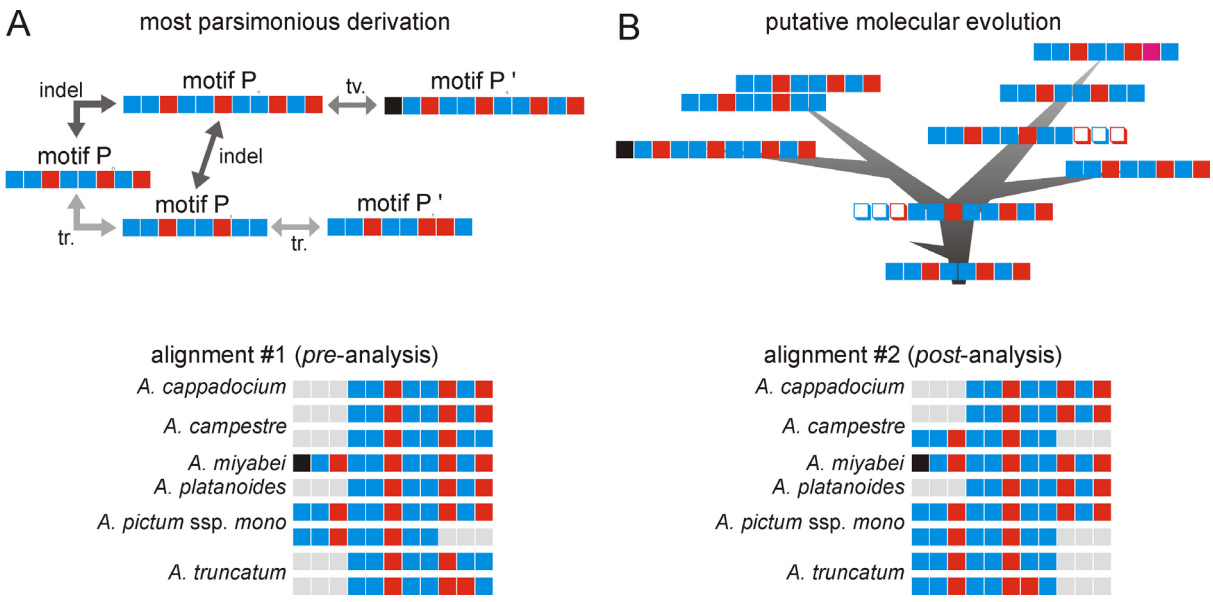


Figure 4-31: *Pre*- and *post*-analysis optimal alignment of LP2 in respect to section *Platanoidea*. **A.** Most parsimonious derivation of detected oligonucleotide motives and according alignment (cf. chapter 2.4.1). **B.** Putative evolution of LP2 as inferred from the BI analyses and overall genotypic characteristics and ML-optimised alignment. Refer to text for further details; standard colour code.

[101] Morphological evidence (e.g. OGATA 1967) clearly indicate a closer relationship between *A. campestre* and *A. miyabei* (⇨ sect. *Campestria sensu* Ogata) than between *A. miyabei* and the remaining *Platanoidea* taxa (*A. pictum* ssp. *mono, A. platanoides,* and *A. truncatum* ⇨ sect. *Platanoidea sensu* Ogata)

In consequence, the alignment has to be adopted to the analytic methods (ML via BI), to not produce artificial homologies, which then influence the analyses. In the case of section *Platanoidea*, the used alignment for LP2 (alignment #2, Fig. 4-31) is actually analytically neutral in respect to the intrasectional phylogenetic relationships.

A spite the general problem to find an optimal alignment for regions comprising common length polymorphism, the phylogenetical and systematical information contained in these oligonucleotide motives cannot be ignored, since they are highly complex characters. The exact composition of such a characters is a function of the actual evolutionary pathway of the analysed taxon. A detailed investigation of the putative evolution of such motives, as it is presented in chapter 4.4.2, reveals a deep insight into the intrageneric phylogeny. Hence, a valuable tool is given to further increase the resolution of the molecular-based hypothesis (chapter 4.4.2) aside the statistical and computerised evaluation of the data. In extreme cases – as it is indicated in Figures 4-14, 4-15, and 4-16 in comparison to the phylogenetic hypotheses based on the conservative regions – the phylogenetical information encrypted within an oligonucleotide pattern can compete with the phylogenetical information provided by one or several molecular markers (⇨ complete gene regions) such as the occurrence and development of certain morphological and biochemical features, respectively (e.g. quadrupedal organisation of terrestrial vertebrates, occurrence of chlorophyll A and B in green algae and higher plants).

5 Conclusions and Perspectives

By comprehensively analysing the ITS of two only distantly related angiosperm genera, i.e. *Acer* (order Sapindales, fam. Aceraceae) and *Fagus* (order Fagales, fam. Fagaceae), it is possible to resolve intrageneric relationships further than it was possible with morphological, biochemical (in the case of *Acer*), and earlier molecular genetical (nrDNA, cpDNA) evidence. The data provided by nucleotides help to establish an independent viewpoint on the relations between and within taxonomic entities of differing hierarchy. Certain taxonomic groupings, in particular on the specific level, can be questioned (e.g. subspecific division of *F. sylvatica*, taxonomic position of *A. ibericum*) or affirmed (e.g. relatedness of *F. engleriana* and *F. japonica* ⇨ subgenus *Engleriana*, placing of *A. obtusatum* WALDST. & KIT. as ssp. of *A. opalus* ⇨ *A. opalus* ssp. *obtusatum* GAMS). But, for a comprehensive phylogenetic hypothesis, the mere assembling of molecular data and their consecutive analysis with standard methods by the computer, is as disputable as the usage of stand-alone morphological and biochemical data. The biparental inherited ITS does have a complex evolutionary history, which can result in an low interspecific, but comparable high intraspecific, variability (*Fagus*) or strongly diverging molecular patterns (*Acer*). Reticulate evolution, which has to be assumed for most angiosperms because of frequent hybridisation events, may contribute to this. Competing genotypes within one individual or population have been documented for all plant genera, as far as data were assembled via cloning (e.g. GREBENSTEIN et al. 1998 for grasses; FOREST & BRUNEAU 2002 for *Corylus*; VOLKOV et al., in press, for *Solanum*; M. Schlee, person. comm., for *Lathyrus*). Such complex patterns cannot yet be solely investigated by computer. As well the underlying alignment as the resulting phylogenetic hypothesis need a detailed re-investigation by the researcher. Aside from the construction of an alignment and the computation of a topology the researcher must *look* at the data, which she/he uses as basis for the computation. As an example, to deduce a morphological character, botanists look at a number of individuals from a taxon to establish a general feature or to assess the natural variability. Taxonomists gain a feeling for the reliability of morphological features as taxonomical characters by a mixture of expert knowledge and field experience. The same should be done with molecular data. The final task cannot be to assemble as much data as possible from different markers but to *understand* the recent composition of the according gene region. In the case of the here studied organisms, 'simpler' data sets retrieved from the only maternally inherited cpDNA did not contain enough variability to impose any

phylogenetic hypothesis, nor are any further gene regions known, which provide a more 'suitable' (for computers) differentiation. As it is demonstrated herein for *Acer* and *Fagus*, the complex pattern of evolution can be inferred, if the decent understanding of the molecular data is combined with even more detailed and extensive morphological studies and, finally, the inclusion of and mapping against the fossil record.

Genus *Fagus*

For *Fagus*, the data assembled are concise. The proposed phylogeny inferred by the newly introduced ISV analyses (chapters 3.4.2 & 3.3) can be put into perfect accordance with the fossil record (chapters 3.4.3 & 3.5; T. Denk, G. Grimm, in prep.) and allows a detailed insight into the evolutionary pathways of the genus. The fundament is laid to trace the Tertiary and Quaternary evolution of the genus, and in addition, a theory about ancient gene pools can be put up. Thus, first evidence is provided for fossil hybridisation events, suppressed speciation, and an ancient horizontal gene flow in *Fagus*. For completeness, further sampling of the widely distributed North American *F. grandifolia* and the Japanese *F. crenata*, and *F. japonica*, is necessary for a statistical comparison with the data assembled for *F. sylvatica* in western Eurasia and *F. longipetiolata, F. lucida,* and *F. hayatae* in China. In addition, for a more precise reconstruction of the differentiation, distribution, and migration of the genus throughout the northern hemisphere, another – more variable – gene region (e.g. 25S-18S IGS of the nrDNA; cf. Fig. 1-1) needs to be incorporated. Such data can also be used to confirm the conclusions made on the basis of site variabilities and to further trace and characterise putative ancient hybridisation or isolation events. In addition, it would be interesting, whether a more variable gene region exhibits also a greater genetic distance aside from the introduction of new site variabilities between the subgenus *Engleriana* and the remaining *Fagus* species.

Fagus exhibits a specialised evolutionary strategy. This strategy ("static frontier strategy") is exhibited in a low number of rather weakly differentiated – genetically and morphologically – species, that apparently retain a certain amount of genetical and morphological variability. This is correlated with an aggressive population strategy (chapter 3.5) and the limitation to a yet to define general ecological setting. For a deeper understanding in this matter, future *Fagus* studies should deal primarily with the question, if – and to which extent – the extant representatives of *Fagus* are clearly separated biological species. Open questions are for example, whether the alien *F. sylvatica* and the native *F. grandifolia* hybridise in North America, and whether hybrids between cultivated individuals of species of subgenus *Fagus*

can be found in botanical gardens[102]. In addition, studies dealing with the nucleolar dominance in putative hybrids – similar to studies performed e.g. on *Nicotiana* ssp. (VOLKOV ET AL. 1999) – are needed to trace the molecular genetical basis for such an evolutionary strategy and mode.

Genus *Acer*

For *Acer* a completely new phylogenetic concept – i.e. an evolutionary hypothesis distinguishing three main lineages: the *Acer-, Palmata-,* and *Platanoidea*-clade – is proposed, which, in addition, sustains the current systematic groupings – i.e. the recognition of near-related species – on a serial, respectively sectional, level. Thus, the monophyly of sections *Acer* (excluding *A. caesium*)[103], *Indivisa, Ginnala, Macrantha, Negundo*[104]*, Palmata, Platanoidea, Rubra* and series *Caudata, Grisea, Macrophylla, Lithocarpa, Trifida* (originally proposed by OGATA 1967; emended by VAN GELDEREN et al. 1994, and literature cited herein) is sustained. Still, a limited scope of all *Acer* taxa is represented by ITS accessions at the moment. Although the gene bank provides accessions of all series and sections that are not yet included in the analysis, the quality and composition of these accessions (cf. chapters 4.3.1 & 4.6.2) makes it difficult to use them in the analysis. Gene bank accessions of *A. laurinum* (sect. *Hyptiocarpa*) and *A. fabri* (sect. *Palmata* ser. *Penninervia*) differ remarkably from the here presented nucleotide patterns. Either new general genotypes are introduced by these taxa, or data published are not typical for these species[105]. The new data for section *Acer* clearly demonstrates, that the reduction of a taxa-rich series and/or section to a single representative – as it was done in former studies – vitiates any attempt to infer a first phylogenetic hypothesis. Therefore, to accommodate the presented hypotheses, clearly more data especially from Chinese taxa are needed. The phylogenetical information contended in solely the ITS – in respect to different taxonomic hierarchies – is sufficient to execute future phylogenetical and systematical studies without expanding on another gene region. In addition, tools are given

[102] In fact, first genetic and morphological evidence can be found for a putative hybrid between *F. crenata* and *F. sylvatica* cultivated in the arboretum of the Botanical Garden, University of Tübingen.

[103] cf. chapters 4.3, 4.4 & 4.5

[104] mainly sustained by the composition of oligonucleotide motives in length polymorphic regions LP1, LP2, LP3, and LP4.

[105] This is also true for the taxonomical identification (cf. chapter 4.3.1). E.g., for one gene bank taxon, it is difficult to find the appropriate synonym in literature, and consequently, the appropriate section, although the bibliographic history is comprehensively assembled in VAN GELDEREN et al. (1994).

for an extensive re-evaluation of the fossil record, especially if the fossil data of Europe and western Asia are considered. Such a re-evaluation can consequently be used to precise the stratigraphic and palaeobiographic history of the genus in the Tertiary of the northern hemisphere. Consequently, new historical aspects will allow a further insight in the molecular and morphological differentiation and evolution of genus *Acer*.

The distinctive grades of morphological and molecular differentiation and evolutionary rate, respectively, exhibited in the different phylogenetic lineages make the genus *Acer*, in contrast to the "static" genus *Fagus*, a future model plant to identify and trace larger-scale processes of ecological adaptation, niching, and speciation within a comparatively old monophyletic group (≥ 65 Ma) of arborescent angiosperms. Thus, the genus *Acer* holds as an example for the processes of evolution as originally described by Charles Darwin and emended by numerous researchers (⇨ "synthetic theory of evolution"). Future comprehensive and interdisciplinary analyses of genus *Acer* will further allow to define the causes and modes for and the amount of biodiversity, which is currently only weakly inferred by the counting of "species".

The here presented data and methodological approaches allow a particular insight in the processes of intrageneric evolution. Furthermore, it is clear that such detailed, and to a certain degree, intuitive analyses need the fundament of as much data as possible – by sampling numerous taxa, populations, and individuals – from as much sources – nucleotide sequences, morphology and ontogeny, biogeography, ecology, fossil record, and in future: developmental genetics – as possible. Only by the combination of different data sets a hypothesis can be put up, that is able to reconstruct low-level evolution. Finally, the general impact of such studies for the problems of the 'higher' phylogenies and conservation biology has yet to be explored.

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Appendix I: Abbrevations

Alphabetical list of abbreviations used in text and figures.

A.	*Acer*
ABI	Applied Biosystems®
AFLP	amplified fragment length polymorphism
ALF	automated light florescence
BGTue	botanical garden, University of Tübingen, Germany
BI	Bayesian inference
bp	base pair
carol	*F. grandifolia* ssp. *caroliniana*
CG-...	cytosine and guanosine, e.g. CG-content: amount of cytosine and guanosine in a certain region
CMBP	Centre of Plant Molecular Biology, NWI, University of Tübingen
cpDNA	chloroplast DNA
CT-...	cytosine and thymine (→ CG-...)
CTAB	buffer; → appendix
D.	*Dipteronia*
Div_Var	"divergent site variabilities" (stepmatrix)
DNA	deoxyribonucleic acid
EO	early Oligocene
ETS	external transcribed spacer
fam.	family (taxonomic unit)
F.	*Fagus*
GA-...	guanosine and adenine (→ CG-...)
Geo/GEO	Georgia (Transcaucasia)
Ger/GER	Germany
gran	*F. grandifolia* ssp. *grandifolia*
GRIN	Germplasm Resources Information Network database
GTR+Γ+I	general substitution model ("general time reversible", substitution rates gamma-distributed, and proportion of sites are invariant)
Hun/HUN	Hungary
ID1...ID11	ITS region comprising an indel (*Acer*)
IGS	25S-18S intergenic spacer
ISV	intraspecific site variability
Ita/ITA	Italy
ITS	internal transcribed spacer
LE	late Eocene
LP1...LP4	(hypervariable) length polymorphic ITS region (*Acer*)
LRT	likelihood ratio test
ML	maximum likelihood
MorArb	Morris Arboretum, ???, U.S.
MP	maximum parsimony
MPR	maximum parsimonious reconstruction
MPT	most parsimonious tree(s)
mtDNA	mitochondrial DNA
N	unknown nucleic base (in alignments)
NEB	New England Biolabs
NEXUS	data format used by common phylogenetic programs
NJ	Neighbour-Joining algorithm
(n)rDNA	(nuclear) ribosomal RNA gene
NTS	non-transcribed spacer
NWI	Naturwissenschaftliche Institute, University of Tübingen; Auf der Morgenstelle, D-72076 Tübingen
PAUP	Phylogenetic Analyses Using Parsimony, analysing software

PCR	polymerase chain reaction
PFR 2.2	Plant Fossil Record 2.2. database
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rRNA	ribosomal RNA
sect.	section (taxonomic unit)
sects.	sections
ser.	series (singular, taxonomic unit)
sers.	series (plural)
sg.	subgenus (taxonomic unit)
s.l.	*sensu latu*, in a broad sense
Slo/SLO	Slovenia
sp.	species (*singular*, taxonomic unit)
Spa/SPA	Spain
spp.	species (*plural*, taxonomic unit)
s.s.	*sensu strictu,* in a strict sense
ssp.	subspecies (taxonomic unit)
TE	buffer; → appendix
TIS	transcription initiation site
TTS	transcription termination site
Tur/TUR	Turkey
TY	medium; → appendix

Abbreviations used for nucleotides

Standard nucleotide code

A	adenine
B	"not A", i.e. C, G, or T
C	cytosine (nucleic base)
D	"not C", i.e. A, G, or T
G	guanosine
H	"not G", i.e. A, C, or T
K	either G or T
M	either A or C
N	miscellaneous/unknown nucleotide
R	purine (A or G)
S	strong bond, i.e. C or G
T	thymine
V	"not T", i.e. A, C, or G
W	weak bond, i.e. A or T
Y	pyrimidine (C or T)

Standard colour code for alignments/oligonucleotide motives (G. Grimm, unpublished)
single nucleotides:
■ = A, ■ = C, ■ = G, ■ = T, ■ = gap
(site) variability comprising 2 possible nucleotides:
■ = K, ■ = M, ■ = R, ■ = S, ■ = W, ■ = Y
(site) variability comprising >2 possible nucleotides:
✣ = B, ✣ = D, ✣ = H, ✣ = V
(site) variability including gaps/length polymorphism: ❑, e.g. ■■❑❑ = either GA or GAGA
nucleotide state unknown: ■ = N

Appendix II: Ingredients of buffers, mediums, etc.

ampicillin solution: final concentration of 100 g/l, dissolved in $H_2O_{bidest.}$

CTAB buffer:	6,05 g	Tris/HCl (≙ 100 mmol/l; pH = 8)
	40,9 g	NaCl (≙ 1,4 mol/l)
	2,29 g	EDTA (≙ 20 mmol/l)
	10 g	Cetyltrimethylammonium bromide (CTAB; ≙ 2% solution)
	1 ml	2-Merkaptoethanol

proteinase K solution: final concentration of 20 g/l, dissolved in $H_2O_{bidest.}$

IPTG (isopropyl-β-D-thiogalactopyranoside) solution: final concentration of 40 g/l; dissolved in $H_2O_{bidest.}$

100x TE buffer:	105.55 g	Tris (Trishydroxymethylaminomethane)
	18,61 g	EDTA
	500 ml	H_2O

pH adjusted to 8 with hydrochloric acid (HCl)

2TY medium:	16 g	Trypton® (proteine extract; Becton, Dickenson & Co.)
	10 g	yeast extract
	5 g	NaCl
	16 g	agar
		dissolved in 1 l $H_2O_{bidest.}$

for "blue-white" screening: each 1ml of X-Gal, IPTG, and ampicillin

X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) solution: final concentration of 40 g/l; dissolved in Dimethylformamide

Appendix III: Voucher information about used material

Voucher table for material and accessions of genus *Acer*

clones	species	subspecies cultivar	locality
ac 0, ac 1	acuminatum		MorArb
bn 0	barbinerve		BGTue, arboretum
bu 0	buergerianum		BGTue, arboretum
cm 0	caesium	caesium	MorArb
cm 1	caesium	caesium	Shennongjia Forest District, W Hubei, China
cb 0	campbellii	campbellii	MorArb
fl 1	campbellii	flabellatum	Shennongjia Forest District, E Daba Shan, W Hubei, China
si 0	campbellii	sinense	MorArb
ca 3	campestre		Passo della Cisa, Italy
ca15	campestre		E Austria
ca16	campestre		E Bulgaria
cp 0	capillipes		BGTue, arboretum
cd 0	cappadocicum	cappadocicum	BGTue, arboretum
cf 0	carpinifolium		BGTue, arboretum
mt 1	caudatum	multiserratum	Shennongjia Forest District, E Daba Shan, W Hubei, China
uk 0	caudatum	ukurunduense	MorArb
cc 0	circinatum		MorArb
cs 0	cissifolium		BGTue, arboretum
cs 1	cissifolium		MorArb
cr 0	crataegifolium		BGTue, arboretum
da 1	davidii	davidii	S of Longmenhe, Shinshan County, Ichang area, China
gs 0	davidii	grosseri	BGTue, arboretum; labelled as *A. grosseri*
gs 1	davidii	grosseri	BGTue, arboretum; labelled as *A. grosser var. hersii*
db 0	diabolicum		BGTue, arboretum
er 0	erianthum		MorArb
gr 0	griseum		BGTue, arboretum
gr 1	griseum		S of Longmenhe, Shinshan County, Ichang area, China
he 0, he 1	henryi		BGTue, arboretum; one tree (he 1) mislabelled as *A. franchetii*
ib 1	ibericum		N of Alpadera, Georgia (Transcaucasia)
ib 2	ibericum		Vashlovani, Georgia (Transcaucasia)
ib 3	ibericum		Norawank, Armenia (Transcaucasia)
ja 0	japonicum	'Aconitifolium'	BGTue
mp 0	macrophyllum		BGTue, arboretum
mx 0	maximowiczianum		MorArb
my 0	miyabei		MorArb
ms 0, ms 1	monspessulanum	monspessulanum	BGTue, arboretum, 2 trees
ms 4	monspessulanum	monspessulanum	W of San Lorenzo de El Escorial within UNESCO-parc "Bosque de la Herrería", prov. Madrid, Spain
ne 2	negundo	negundo	Albany Co., New York State, U.S.A.
ol 0	oliverianum	oliverianum	MorArb
ol 1	oliverianum	oliverianum	S of Longmenhe, Shinshan County, Ichang area, China
op 5	opalus	opalus	3km W of Caussols, NW of Grasse, dépt. Alpes-Maritimes, France
ot 1	opalus	obtusatum	BGTue, arboretum; originally from the vicinity of Naples, S Italy
pa 0	palmatum	palmatum	BGTue

clones	species	subspecies cultivar	locality
pe 1	pensylvanicum		Franklin Co., New York State, U.S.A.
mo 1	pictum	mono	Shennongjia Forest District, E Daba Shan, W Hubei, China
mo 2	pictum	mono	Shennongjia Forest District, E Daba Shan, W Hubei, China
mo 3	pictum	mono	S of Longmenhe, Shinshan County, Ichang area, China
pl 4	platanoides	platanoides	Königsfeld, Germany
pl12	platanoides	platanoides	Sisteron, dépt. Alpes-de-Haute-Provence, France
pl13	platanoides	platanoides	W Georgia (Transcaucasia)
pl15	platanoides	platanoides	Skeen, 10km W of Ljungby, central S Sweden
pp17, pp18	pseudoplatanus		BGTue, arboretum; naturally growing
pp22	pseudoplatanus		near Budapest, Hungary
ru 0	rubrum		BGTue, arboretum
ru 1	rubrum		Rensselaer, Conneticut, U.S.A.
ru 2	rubrum		Rensselaer, Conneticut, U.S.A.
rn 0	rufinerve		BGTue, arboretum
sa 1	saccharinum		BGTue, arboretum
sa 3	saccharinum		Rensselaer, Conneticut, U.S.A.
gd 0	saccharum	grandidentatum	MorArb
ni 1	saccharum	nigrum	Albany Co., New York State, U.S.A.
ni 2	saccharum	nigrum	Albany Co., New York State, U.S.A.
sc 1	saccharum	saccharum	Rensselaer, Conneticut, U.S.A.
fd 1	saccharum	floridanum	Florida Cavern S.P., Florida, U.S.A.
sv 1	sempervirens		N over Lake Kournes, Crete
sv 2	sempervirens		near Vrisses, along road to Ashifon, Crete
sv 3	sempervirens		Eligia gorge, Crete
sh 0	shirasawanum	'Aureum'	BGTue, arboretum
sp 0	spicatum		BGTue, arboretum
bf 0	stachyophyllum	betulifolium	BGTue, arboretum
st 0	stachyophyllum	stachyophyllum	MorArb
gi 0	tataricum	ginnala	BGTue, arboretum
se 0	tataricum	semenovii	BGTue, arboretum
tt 0	tataricum	tataricum	BGTue, arboretum
tv 1	trautvetteri		W Georgia (Transcaucasia)
tf 0	triflorum		MorArb
tr 0	truncatum		BGTue, arboretum
ve 1	velutinum		NW Iran
ve 2	velutinum		NW Iran
ve 3	velutinum		NW Iran

* **bold font**: material collected at original stand from presumably not cultivated (wild) individual(s); normal font: obviously cultivated specimen (botanical gardens, planted)

Material from all sampled populations has been herbarised (Tübingen; Museum of Natural History, Stockholm). Further information can be supplied upon request.

Table of used accessions' core parameters (CG-content, number of nucleotides) for *Acer*

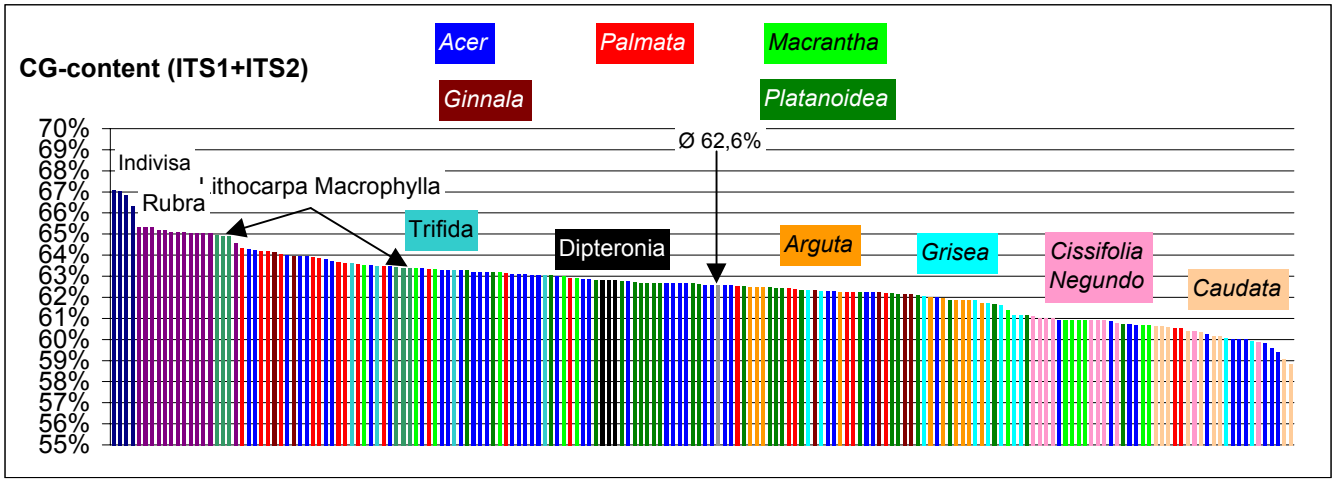
Clone numbers refer to voucher table. Last column: Difference between the CG-content of ITS1 and ITS2.

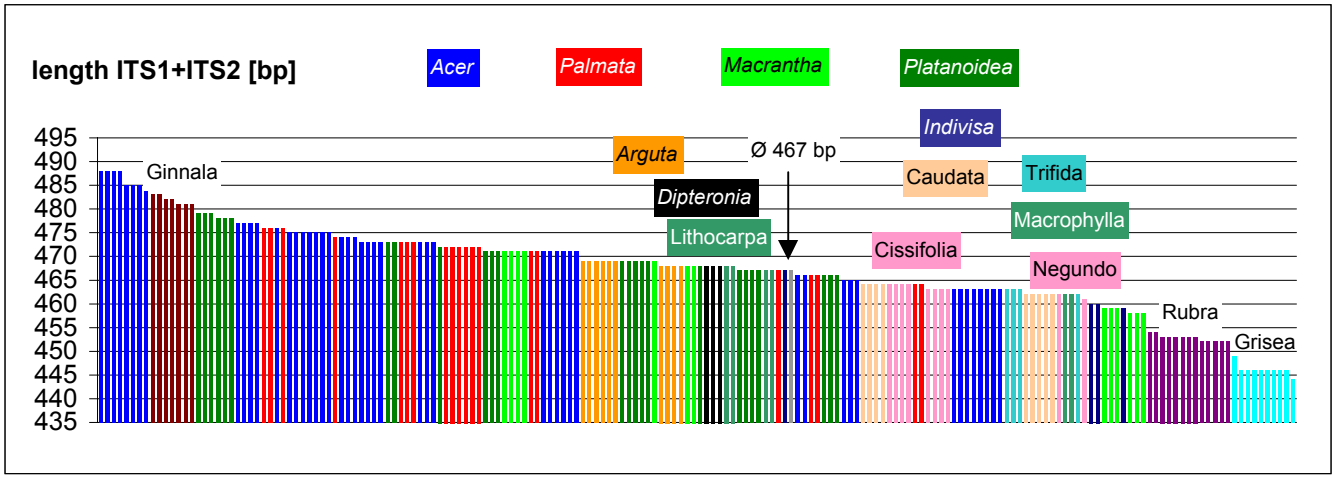
		ITS1						ITS2						
taxonomic entity	clone	CG-content	A	C	G	T	length [bp]	CG content	A	C	G	T	length [bp]	$\Delta_{CG\text{-}content}$ (ITS2-ITS1)
Dipteronia	Di1 6	**61,4%**	20%	34%	27%	18%	**233**	**64,3%**	18%	33%	31%	18%	**235**	**2,9%**
	Di1 19	**61,4%**	20%	34%	27%	18%	**233**	**64,3%**	18%	33%	31%	18%	**235**	**2,9%**
	Di1 45	**61,4%**	20%	34%	27%	18%	**233**	**64,3%**	17%	33%	31%	18%	**235**	**2,9%**
sect. Acer	cm 001	**62,8%**	18%	34%	29%	19%	**234**	**62,5%**	19%	33%	29%	19%	**232**	**-0,3%**
	cm 003	**62,8%**	18%	34%	29%	19%	**234**	**62,5%**	19%	33%	29%	19%	**232**	**-0,3%**
	cm 122	**62,4%**	18%	33%	29%	20%	**234**	**62,8%**	19%	33%	29%	19%	**231**	**0,4%**
	cm 145	**62,4%**	18%	33%	29%	20%	**234**	**62,8%**	19%	33%	29%	19%	**231**	**0,4%**
	fd 102	**65,5%**	18%	37%	29%	16%	**235**	**63,1%**	19%	33%	30%	18%	**241**	**-2,5%**
	fd 119	**65,0%**	19%	37%	28%	16%	**237**	**62,9%**	19%	33%	30%	18%	**240**	**-2,1%**
	gd 025	**65,0%**	18%	36%	29%	17%	**237**	**62,9%**	19%	33%	30%	18%	**240**	**-2,1%**
	gd 035	**64,6%**	18%	36%	29%	18%	**237**	**62,9%**	19%	33%	30%	18%	**240**	**-1,6%**
	hy 102	**64,9%**	19%	37%	28%	16%	**242**	**61,4%**	20%	32%	30%	19%	**233**	**-3,5%**
	hy 133	**64,0%**	19%	36%	28%	17%	**239**	**62,0%**	20%	32%	29%	18%	**234**	**-2,1%**
	ib 108	**63,5%**	20%	37%	27%	16%	**244**	**61,8%**	20%	32%	30%	18%	**241**	**-1,7%**
	ib 111	**63,6%**	20%	36%	27%	16%	**247**	**62,7%**	20%	33%	30%	18%	**241**	**-0,9%**
	ib 113	**64,0%**	20%	37%	27%	16%	**247**	**62,7%**	20%	33%	30%	18%	**241**	**-1,3%**
	ib 117	**64,0%**	20%	37%	27%	16%	**247**	**62,7%**	20%	33%	30%	18%	**241**	**-1,3%**
	ib 201	**63,9%**	20%	37%	27%	16%	**244**	**61,8%**	20%	32%	30%	18%	**241**	**-2,1%**
	ib 238	**63,9%**	20%	37%	27%	16%	**244**	**61,8%**	20%	32%	30%	18%	**241**	**-2,1%**
	ib 303	**63,6%**	20%	37%	27%	16%	**247**	**62,7%**	20%	33%	30%	18%	**241**	**-0,9%**
	ib 307	**63,9%**	20%	37%	27%	16%	**244**	**61,4%**	20%	32%	29%	19%	**241**	**-2,5%**
	ms 011	**63,4%**	19%	36%	28%	18%	**238**	**61,7%**	20%	31%	30%	18%	**235**	**-1,7%**
	ms 408	**63,4%**	19%	35%	28%	18%	**238**	**61,7%**	20%	32%	30%	18%	**235**	**-1,7%**
	ms 411	**63,9%**	19%	36%	28%	17%	**238**	**61,7%**	20%	32%	30%	18%	**235**	**-2,2%**
	ni 101	**65,5%**	18%	37%	29%	16%	**235**	**62,5%**	19%	33%	30%	18%	**240**	**-3,0%**
	ni 206	**65,1%**	18%	37%	29%	17%	**235**	**61,7%**	20%	32%	30%	19%	**240**	**-3,4%**
	ni 209	**65,5%**	18%	37%	29%	16%	**235**	**62,1%**	20%	33%	30%	18%	**240**	**-3,4%**
	op 404	**63,7%**	19%	35%	29%	18%	**234**	**63,3%**	19%	33%	30%	18%	**237**	**-0,4%**
	op 407	**63,2%**	19%	35%	28%	18%	**234**	**63,3%**	19%	33%	30%	18%	**237**	**0,0%**
	op 502	**63,7%**	18%	35%	29%	18%	**234**	**62,8%**	20%	33%	30%	18%	**239**	**-0,9%**
	op 509	**63,7%**	19%	35%	29%	18%	**234**	**62,8%**	20%	33%	30%	18%	**239**	**-0,9%**
	ot 103	**63,2%**	19%	35%	29%	18%	**234**	**62,9%**	19%	33%	30%	18%	**237**	**-0,4%**
	ot 104	**63,7%**	18%	35%	29%	18%	**234**	**62,8%**	20%	33%	30%	18%	**239**	**-0,9%**
	ot 105	**63,7%**	18%	35%	29%	18%	**234**	**62,4%**	20%	33%	29%	18%	**237**	**-1,2%**
	pp1702	**61,5%**	19%	33%	29%	20%	**234**	**60,3%**	20%	32%	29%	20%	**237**	**-1,2%**
	pp2209	**61,5%**	19%	33%	29%	20%	**234**	**59,9%**	20%	31%	29%	20%	**237**	**-1,6%**
	sc 110	**65,5%**	18%	37%	29%	16%	**235**	**62,9%**	19%	33%	30%	18%	**240**	**-2,6%**
	sc 140	**64,6%**	19%	36%	28%	16%	**237**	**62,5%**	19%	33%	30%	19%	**240**	**-2,1%**
	sv 102	**61,9%**	19%	34%	28%	19%	**236**	**62,8%**	21%	34%	28%	17%	**239**	**0,9%**
	sv 124	**62,3%**	19%	34%	28%	19%	**236**	**62,3%**	21%	34%	28%	17%	**239**	**0,1%**
	sv 201	**61,9%**	19%	34%	28%	19%	**236**	**62,2%**	21%	33%	29%	17%	**238**	**0,3%**
	sv 220	**61,9%**	19%	34%	28%	19%	**236**	**62,6%**	21%	34%	29%	17%	**238**	**0,7%**
	sv 309	**61,9%**	19%	34%	28%	19%	**236**	**62,6%**	21%	34%	29%	17%	**238**	**0,7%**
	tv 101	**60,9%**	18%	32%	29%	21%	**233**	**60,4%**	20%	31%	29%	20%	**230**	**-0,5%**
	tv 103	**60,5%**	18%	32%	28%	21%	**233**	**61,2%**	19%	32%	29%	19%	**232**	**0,7%**
	ve 112	**59,7%**	18%	31%	28%	22%	**233**	**60,0%**	20%	32%	28%	20%	**230**	**0,3%**
	ve 144	**59,7%**	19%	31%	28%	21%	**233**	**60,4%**	20%	32%	29%	20%	**230**	**0,8%**
	ve 202	**60,1%**	18%	32%	28%	21%	**233**	**60,0%**	20%	31%	29%	20%	**230**	**-0,1%**
	ve 207	**58,8%**	19%	31%	28%	22%	**233**	**60,0%**	20%	31%	29%	20%	**230**	**1,2%**
	ve 240	**60,1%**	18%	32%	28%	22%	**233**	**60,0%**	20%	31%	29%	20%	**230**	**-0,1%**
	ve 306	**59,7%**	18%	31%	28%	22%	**233**	**59,6%**	20%	32%	28%	20%	**230**	**-0,1%**
	ve 308	**59,7%**	18%	31%	28%	22%	**233**	**60,9%**	20%	32%	29%	20%	**230**	**1,2%**

		ITS1						ITS2						
taxonomic entity	clone	CG-content	A	C	G	T	length [bp]	CG content	A	C	G	T	length [bp]	$\Delta_{CG\text{-}content}$ (ITS2-ITS1)
ser. *Arguta*	ac 002	**61,3%**	20%	33%	28%	19%	**238**	**63,6%**	19%	34%	29%	17%	**231**	**2,3%**
	ac 101	**61,3%**	20%	33%	28%	19%	**238**	**63,6%**	19%	34%	29%	17%	**231**	**2,3%**
	ac 102	**61,3%**	20%	33%	28%	19%	**238**	**63,6%**	19%	34%	29%	17%	**231**	**2,3%**
	bf 002	**60,9%**	20%	34%	27%	19%	**238**	**62,8%**	19%	33%	29%	18%	**231**	**1,8%**
	bf 005	**60,9%**	20%	34%	27%	19%	**238**	**62,8%**	19%	34%	29%	18%	**231**	**1,8%**
	bn 001	**61,2%**	20%	33%	28%	19%	**237**	**62,3%**	19%	32%	30%	18%	**231**	**1,1%**
	bn 002	**61,2%**	20%	33%	28%	19%	**237**	**62,6%**	19%	33%	30%	18%	**231**	**1,4%**
	bn 003	**61,2%**	20%	33%	28%	19%	**237**	**62,9%**	19%	33%	30%	18%	**231**	**1,7%**
	st 001	**61,3%**	20%	34%	28%	18%	**238**	**63,2%**	19%	34%	29%	18%	**231**	**1,9%**
	st 002	**61,2%**	20%	33%	28%	19%	**237**	**62,8%**	19%	33%	29%	18%	**231**	**1,6%**
ser. *Caudata*	mt 105	**58,7%**	19%	32%	27%	22%	**237**	**59,0%**	20%	30%	30%	21%	**227**	**0,4%**
	mt 106	**59,1%**	19%	32%	27%	22%	**237**	**59,0%**	20%	30%	30%	21%	**227**	**0,0%**
	sp 001	**60,0%**	19%	31%	29%	21%	**235**	**61,2%**	19%	30%	31%	20%	**227**	**1,2%**
	sp 002	**60,4%**	19%	32%	29%	21%	**235**	**60,4%**	19%	30%	30%	21%	**227**	**-0,1%**
	sp 003	**59,6%**	20%	32%	28%	21%	**235**	**60,8%**	19%	30%	30%	21%	**227**	**1,2%**
	sp 005	**60,0%**	19%	31%	29%	21%	**235**	**61,2%**	19%	31%	30%	20%	**227**	**1,2%**
	sp 008	**60,0%**	18%	31%	29%	22%	**235**	**60,4%**	19%	30%	30%	21%	**227**	**0,4%**
	uk 004	**59,7%**	19%	33%	27%	21%	**236**	**61,0%**	19%	29%	32%	20%	**228**	**1,2%**
	uk 020	**60,2%**	19%	33%	28%	21%	**236**	**61,0%**	19%	29%	32%	20%	**228**	**0,8%**
sect. *Ginnala*	gi 021	**61,5%**	18%	34%	28%	20%	**234**	**62,8%**	21%	33%	30%	17%	**247**	**1,2%**
	gi 022	**61,5%**	18%	34%	28%	20%	**234**	**62,8%**	21%	34%	29%	16%	**247**	**1,2%**
	se 021	**63,4%**	18%	36%	28%	18%	**235**	**64,5%**	20%	34%	30%	16%	**248**	**1,1%**
	se 025	**63,4%**	18%	36%	28%	18%	**235**	**64,9%**	20%	35%	30%	15%	**248**	**1,5%**
	tt 005	**61,5%**	19%	34%	27%	20%	**234**	**63,2%**	21%	34%	30%	16%	**247**	**1,6%**
	tt 008	**62,0%**	19%	34%	28%	19%	**234**	**62,9%**	21%	33%	29%	17%	**248**	**0,9%**
	tt 014	**61,7%**	19%	34%	27%	20%	**235**	**62,8%**	21%	34%	29%	16%	**247**	**1,1%**
sect. *Indivisa*	cf 001	**65,0%**	19%	37%	28%	16%	**220**	**69,2%**	16%	35%	34%	15%	**240**	**4,2%**
	cf 002	**64,3%**	19%	37%	28%	17%	**227**	**68,3%**	17%	35%	33%	15%	**240**	**4,0%**
	cf 003	**65,0%**	19%	37%	28%	16%	**220**	**69,0%**	16%	36%	33%	15%	**239**	**4,0%**
	cf 004	**65,0%**	19%	37%	28%	16%	**220**	**68,8%**	16%	35%	34%	15%	**240**	**3,8%**
ser. *Lithocarpa*	db 021	**61,8%**	19%	34%	27%	19%	**233**	**65,1%**	18%	34%	31%	17%	**235**	**3,3%**
	db 025	**64,7%**	19%	37%	28%	17%	**232**	**65,1%**	18%	34%	31%	17%	**235**	**0,5%**
	db 034	**64,7%**	19%	37%	28%	17%	**232**	**65,1%**	18%	34%	31%	17%	**235**	**0,5%**
	db 038	**64,7%**	19%	37%	28%	17%	**232**	**65,3%**	18%	34%	31%	17%	**236**	**0,6%**
ser. *Macrophylla*	mp 001	**64,4%**	18%	36%	29%	18%	**233**	**62,4%**	18%	32%	30%	20%	**229**	**-1,9%**
	mp 002	**64,4%**	18%	36%	29%	18%	**233**	**62,4%**	18%	32%	30%	20%	**229**	**-1,9%**
sect. *Macrantha*	cp 021	**62,1%**	18%	34%	28%	20%	**235**	**63,8%**	18%	34%	30%	18%	**224**	**1,7%**
	cp 023	**62,6%**	18%	34%	28%	19%	**235**	**63,8%**	18%	34%	30%	18%	**224**	**1,3%**
	cr 041	**61,3%**	18%	33%	28%	20%	**235**	**61,5%**	18%	31%	30%	21%	**234**	**0,3%**
	da 121	**62,8%**	18%	35%	28%	19%	**234**	**63,8%**	18%	34%	30%	18%	**224**	**1,0%**
	da 122	**63,0%**	18%	35%	28%	19%	**235**	**63,8%**	18%	34%	30%	18%	**224**	**0,9%**
	gs 011	**61,0%**	19%	33%	28%	20%	**236**	**60,9%**	20%	31%	29%	20%	**235**	**-0,2%**
	gs 015	**61,0%**	18%	33%	28%	21%	**236**	**60,9%**	20%	31%	29%	20%	**235**	**-0,2%**
	gs 101	**62,8%**	18%	35%	28%	19%	**234**	**64,3%**	18%	34%	30%	18%	**224**	**1,5%**
	gs 102	**62,4%**	18%	35%	27%	19%	**234**	**63,4%**	18%	34%	29%	19%	**224**	**1,0%**
	pe 107	**59,6%**	19%	32%	28%	22%	**235**	**61,8%**	19%	31%	30%	19%	**233**	**2,2%**
	pe 110	**59,6%**	19%	32%	28%	22%	**235**	**61,8%**	19%	31%	30%	19%	**233**	**2,2%**
	rn 005	**61,0%**	18%	33%	28%	21%	**236**	**60,9%**	20%	31%	29%	20%	**235**	**-0,2%**
	rn 024	**61,0%**	18%	33%	28%	21%	**236**	**60,9%**	20%	31%	29%	20%	**235**	**-0,2%**
ser. *Cissifolia*	cs 009	**60,9%**	19%	33%	28%	20%	**235**	**61,1%**	19%	31%	30%	20%	**229**	**0,3%**
	cs 023	**60,9%**	19%	33%	28%	20%	**235**	**61,1%**	19%	31%	30%	20%	**229**	**0,3%**
	cs 102	**60,9%**	19%	33%	28%	20%	**235**	**61,1%**	19%	31%	30%	20%	**229**	**0,3%**
	cs 103	**60,9%**	19%	33%	28%	20%	**235**	**60,7%**	19%	31%	30%	20%	**229**	**-0,2%**
	he 005	**61,5%**	19%	34%	28%	19%	**234**	**60,7%**	19%	31%	30%	20%	**229**	**-0,8%**
	he 111	**61,1%**	19%	33%	28%	20%	**234**	**60,7%**	19%	31%	30%	20%	**229**	**-0,4%**
	he 112	**61,1%**	19%	34%	27%	20%	**234**	**60,7%**	19%	31%	30%	20%	**229**	**-0,4%**
	he 136	**61,1%**	19%	33%	28%	20%	**234**	**60,7%**	19%	31%	30%	20%	**229**	**-0,4%**

		ITS1						ITS2						
taxonomic entity	clone	CG-content	A	C	G	T	length [bp]	CG content	A	C	G	T	length [bp]	$\Delta_{CG\text{-}content}$ (ITS2-ITS1)
ser. *Negundo*	ne 201	**60,4%**	19%	33%	28%	20%	**235**	**60,4%**	19%	30%	31%	21%	**227**	**-0,1%**
	ne 203	**60,3%**	19%	32%	28%	21%	**234**	**59,5%**	19%	30%	30%	22%	**227**	**-0,8%**
sect. *Palmata*	cb 014	**60,1%**	20%	32%	28%	20%	**233**	**61,0%**	18%	31%	30%	21%	**231**	**0,9%**
	cb 017	**60,9%**	19%	33%	28%	20%	**233**	**60,2%**	19%	30%	30%	21%	**231**	**-0,8%**
	cc 004	**63,8%**	19%	36%	28%	17%	**235**	**63,1%**	19%	33%	30%	18%	**236**	**-0,7%**
	cc 009	**64,7%**	19%	37%	28%	16%	**235**	**63,1%**	19%	33%	30%	18%	**236**	**-1,5%**
	er 002	**61,5%**	20%	34%	28%	18%	**234**	**62,9%**	19%	33%	30%	18%	**232**	**1,4%**
	er 003	**61,6%**	19%	34%	28%	19%	**232**	**63,4%**	19%	34%	30%	18%	**235**	**1,8%**
	er 005	**61,1%**	20%	33%	28%	19%	**234**	**63,4%**	19%	33%	31%	18%	**232**	**2,3%**
	fl 103	**63,1%**	19%	35%	28%	18%	**236**	**64,0%**	19%	34%	30%	17%	**236**	**0,8%**
	fl 114	**62,7%**	19%	34%	28%	18%	**236**	**64,0%**	19%	34%	30%	17%	**236**	**1,3%**
	ja 005	**64,3%**	19%	35%	29%	17%	**235**	**63,1%**	20%	33%	30%	17%	**241**	**-1,2%**
	ja 008	**64,6%**	19%	35%	29%	16%	**237**	**64,1%**	19%	34%	30%	17%	**237**	**-0,4%**
	ol 037	**63,1%**	19%	35%	28%	18%	**236**	**62,7%**	19%	33%	30%	18%	**236**	**-0,4%**
	ol 039	**64,0%**	19%	36%	28%	17%	**236**	**64,1%**	19%	34%	30%	17%	**237**	**0,2%**
	ol 111	**63,6%**	19%	35%	29%	17%	**236**	**64,1%**	19%	34%	30%	16%	**237**	**0,6%**
	pa 005	**63,6%**	19%	35%	28%	17%	**236**	**61,3%**	20%	32%	29%	18%	**240**	**-2,3%**
	pa 006	**63,6%**	19%	35%	28%	17%	**236**	**60,8%**	21%	32%	29%	18%	**240**	**-2,7%**
	sh 001	**64,7%**	19%	37%	28%	16%	**235**	**63,7%**	19%	33%	30%	18%	**237**	**-1,0%**
	sh 002	**64,7%**	19%	37%	28%	16%	**235**	**63,7%**	19%	33%	30%	17%	**237**	**-1,0%**
	si 003	**63,6%**	19%	35%	28%	17%	**236**	**62,7%**	19%	33%	30%	18%	**236**	**-0,8%**
	si 006	**63,6%**	19%	35%	28%	17%	**236**	**63,7%**	19%	34%	30%	17%	**237**	**0,2%**
sect. *Platanoidea*	ca 301	**64,5%**	17%	34%	30%	18%	**235**	**60,5%**	18%	31%	30%	22%	**243**	**-4,0%**
	ca 306	**64,3%**	17%	34%	30%	18%	**235**	**61,1%**	18%	32%	30%	21%	**244**	**-3,2%**
	ca1407	**63,4%**	17%	34%	30%	19%	**235**	**61,1%**	18%	32%	30%	21%	**244**	**-2,3%**
	ca1507	**63,7%**	18%	34%	29%	19%	**234**	**61,1%**	18%	32%	30%	21%	**244**	**-2,6%**
	ca1616	**64,0%**	17%	34%	30%	19%	**236**	**60,9%**	18%	31%	30%	21%	**243**	**-3,1%**
	ca1633	**63,2%**	18%	33%	30%	19%	**234**	**61,1%**	18%	31%	30%	21%	**244**	**-2,2%**
	cd 001	**61,5%**	19%	32%	29%	20%	**234**	**60,8%**	17%	31%	30%	22%	**232**	**-0,8%**
	cd 018	**61,1%**	19%	32%	29%	20%	**234**	**60,3%**	18%	30%	30%	22%	**232**	**-0,8%**
	mo 103	**63,0%**	18%	34%	29%	19%	**238**	**62,6%**	17%	32%	31%	20%	**235**	**-0,5%**
	mo 106	**63,0%**	18%	34%	29%	19%	**238**	**62,4%**	17%	32%	31%	21%	**234**	**-0,6%**
	mo 202	**62,7%**	18%	34%	29%	19%	**233**	**61,5%**	17%	31%	31%	21%	**234**	**-1,1%**
	mo 203	**62,7%**	18%	34%	29%	19%	**233**	**62,2%**	17%	32%	30%	21%	**233**	**-0,4%**
	mo 303	**63,4%**	18%	34%	29%	19%	**238**	**63,0%**	17%	32%	31%	20%	**235**	**-0,5%**
	mo 324	**63,4%**	18%	34%	29%	19%	**238**	**63,1%**	17%	32%	31%	20%	**233**	**-0,4%**
	my 005	**64,3%**	17%	35%	29%	18%	**238**	**61,4%**	18%	32%	30%	21%	**233**	**-2,9%**
	my 009	**64,3%**	17%	35%	29%	18%	**238**	**61,8%**	18%	32%	30%	21%	**233**	**-2,5%**
	pl 403	**62,7%**	18%	33%	30%	19%	**236**	**62,7%**	17%	32%	31%	20%	**233**	**-0,1%**
	pl1108	**62,7%**	18%	33%	30%	19%	**236**	**62,7%**	17%	32%	31%	20%	**233**	**-0,1%**
	pl1203	**62,6%**	18%	33%	29%	20%	**235**	**62,7%**	17%	32%	31%	20%	**233**	**0,1%**
	pl1306	**62,7%**	18%	33%	30%	19%	**236**	**62,7%**	17%	32%	31%	20%	**233**	**-0,1%**
	pl1501	**62,7%**	18%	33%	30%	19%	**236**	**62,2%**	18%	32%	30%	20%	**233**	**-0,5%**
	pl1503	**62,7%**	18%	33%	30%	19%	**236**	**62,7%**	17%	32%	31%	20%	**233**	**-0,1%**
	tr 002	**62,4%**	18%	33%	29%	20%	**234**	**60,9%**	18%	31%	30%	21%	**233**	**-1,4%**
	tr 024	**62,4%**	18%	33%	29%	20%	**234**	**61,4%**	17%	31%	30%	21%	**233**	**-1,0%**
	tr 027	**63,0%**	18%	33%	30%	19%	**234**	**61,4%**	18%	31%	30%	21%	**233**	**-1,6%**
sect. *Rubra*	ru 007	**64,9%**	18%	37%	27%	17%	**222**	**65,2%**	17%	34%	31%	17%	**230**	**0,4%**
	ru 008	**64,9%**	18%	37%	27%	17%	**222**	**65,2%**	17%	34%	31%	17%	**230**	**0,4%**
	ru 025	**64,9%**	18%	37%	27%	17%	**222**	**65,4%**	17%	34%	31%	17%	**231**	**0,5%**
	ru 028	**64,9%**	18%	37%	27%	17%	**222**	**65,5%**	17%	34%	31%	17%	**232**	**0,7%**
	ru 029	**65,5%**	19%	38%	28%	16%	**222**	**65,2%**	17%	34%	31%	17%	**231**	**-0,2%**
	ru 103	**64,4%**	18%	37%	27%	17%	**222**	**64,8%**	17%	34%	31%	18%	**230**	**0,4%**
	ru 107	**64,9%**	18%	37%	27%	17%	**222**	**65,4%**	17%	34%	31%	17%	**231**	**0,5%**
	ru 209	**64,9%**	18%	37%	27%	17%	**222**	**65,2%**	17%	34%	31%	17%	**230**	**0,4%**
	ru 210	**64,9%**	18%	37%	27%	17%	**222**	**65,2%**	17%	34%	31%	17%	**230**	**0,4%**

		ITS1						ITS2						
taxonomic entity	clone	CG-content	A	C	G	T	length [bp]	CG content	A	C	G	T	length [bp]	$\Delta_{CG\text{-}content}$ (ITS2-ITS1)
sect. *Rubra* (cont.)	sa 105	**64,0%**	19%	36%	27%	17%	**222**	**66,4%**	17%	35%	31%	16%	**232**	**2,4%**
	sa 109	**64,4%**	19%	37%	27%	17%	**222**	**66,2%**	17%	35%	31%	16%	**231**	**1,8%**
	sa 306	**64,3%**	19%	37%	28%	17%	**221**	**66,4%**	17%	35%	31%	16%	**232**	**2,1%**
	sa 309	**63,8%**	19%	37%	27%	17%	**221**	**66,4%**	17%	35%	31%	16%	**232**	**2,6%**
ser. *Trifida*	bu 004	**62,0%**	21%	36%	26%	17%	**229**	**65,2%**	17%	33%	32%	18%	**233**	**3,2%**
	bu 015	**61,7%**	20%	35%	27%	18%	**230**	**64,8%**	17%	34%	31%	18%	**233**	**3,1%**
	bu 040	**61,3%**	20%	35%	27%	18%	**230**	**64,8%**	17%	34%	31%	18%	**233**	**3,5%**
	bu 045	**61,7%**	20%	35%	27%	18%	**230**	**65,2%**	17%	34%	31%	18%	**233**	**3,5%**
ser. *Grisea*	gr 001	**61,8%**	20%	35%	27%	18%	**233**	**60,6%**	18%	32%	29%	22%	**213**	**-1,2%**
	gr 002	**61,8%**	20%	35%	27%	18%	**233**	**60,6%**	18%	32%	29%	21%	**213**	**-1,2%**
	gr 007	**61,8%**	20%	35%	27%	18%	**233**	**61,5%**	18%	33%	29%	21%	**213**	**-0,3%**
	gr 015	**61,9%**	20%	35%	27%	18%	**231**	**61,5%**	18%	33%	29%	20%	**213**	**-0,4%**
	gr 118	**62,7%**	19%	35%	27%	18%	**233**	**61,5%**	18%	33%	29%	21%	**213**	**-1,2%**
	gr 128	**62,7%**	19%	35%	27%	18%	**233**	**61,0%**	18%	32%	29%	21%	**213**	**-1,6%**
	mx 001	**58,5%**	22%	33%	26%	20%	**232**	**61,7%**	18%	34%	28%	20%	**214**	**3,2%**
	mx 007	**58,2%**	22%	32%	26%	19%	**232**	**61,7%**	18%	34%	28%	20%	**214**	**3,5%**
	tf 003	**62,2%**	19%	35%	27%	18%	**233**	**62,5%**	17%	34%	28%	20%	**216**	**0,3%**
	tf 004	**62,7%**	19%	35%	27%	18%	**233**	**62,0%**	17%	33%	29%	21%	**213**	**-0,7%**
	Ø	**62,5%**	19%	34%	28%	19%		**62,6%**	19%	33%	30%	19%		**0,1%**





Levels of sequence diversity within taxonomic entities of *Acer*

Within group averages calculated via Kimura 2-parameter substitution model, gamma-distributed (MEGA 2.1®)

		N (species)*	mean intraspecific diversity	mean interspecific diversity	overall diversity		level of intrataxonomic diversification†
	Dipteronia	1	0.001	n/c	0.001		n/c
***Acer*-clade**	sect. *Acer*	10	0.08	0.101	0.109	0.511	±
	sect. *Ginnala*	1	0.050	n/c	0.050		n/c
	ser. *Grisea*	3	0.006	0.019	0.026		+
	sect. *Indivisa*	1	0.005	n/c	0.005		n/c
	sect. *Rubra*	2	0.002	0.004	0.006		+
	ser. *Trifida*	1	0.008	n/c	0.008		n/c
***Palmata*-clade**	ser. *Arguta*	3	0.004	0.010	0.015	0.313	+
	ser. *Caudata*	2	0.049	0.021	0.070		±
	ser. *Cissifolia*	2	0.002	0.003	0.005		±
	ser. *Negundo*	1	0.010	n/c	0.010		n/c
	sect. *Palmata*	7	0.011	0.067	0.078		+
***Platanoidea*-clade**	ser. *Lithocarpa*	1	0.013	n/c	0.013	0.518	n/c
	ser. *Macrophylla*	1	0.000	n/c	0.000		n/c
	sect. *Macrantha*	5	0.014‡	0.041‡	0.06		±
	sect. *Platanoidea*	6	0.009	0.042	0.051		+

* according to herein used taxonomic classification (VAN GELDEREN et al. 1994; emended in chapter 4.1, Table 4-2)

† optimal intrasectional/-serial diversifivication is indicated by: high overall diversity > level of interspecific diversity > level of intraspecific diversity

‡ in the case of taxa of sect. *Macrantha* the comparably low intraspecific diversity and the high interspecific diversity is due two the occurrence of two distinct genotypes. Species, which can be assigned to the same genotype are basically identical (not differentiated on a molecular level).

Numerical diversity maxima are indicated with gray background colours.

Table of intra- and interspecific distances within *Acer*

Pairwise distances calculated with gamma-distributed Kimura 2-parameter model (MEGA 2.1®), gaps deleted pairwise

		sect. *Acer*											ser. *Arguta*					sect. *Macrantha*						ser. *Cissifolia*			sect. *Palmata*										ser. *Caudata*		sect. *Platanoidea*						sect. *Rubra*			ser. *Grisea*		
species	*Dipteronia sinensis*	*A. caesium*	*A. saccharum*	*A. hyrcanum*	*A. ibericum*	*A. monspessulanum*	*A. opalus*	*A. pseudoplatanus*	*A. sempervirens*	*A. trautvetteri*	*A. velutinum*	*A. tataricum* (sect. *Ginnala*)	*A. acuminatum*	*A. barbinerve*	*A. stachyophyllum*	*A. carpinifolium* (sect. *Indivisa*)	*A. diabolicum* (ser. *Lithocarpa*)	*A. macrophyllum* (ser. *Macrophylla*)	*A. capillipes*	*A. crataegifolium*	*A. davidii*	*A. pensylvanicum*	*A. rufinerve*	*A. cissifolium*	*A. henryi*	*A. negundo* (ser. *Negundo*)	*A. campbelli* ssp. *campbelli*	*A. circinatum*	*A. erianthum*	*other A. campbelli* spp.	*A. japonicum*	*A. oliverianum*	*A. palmatum*	*A. shirasawanum*	*A. spicatum*	*A. caudatum*	*A. campestre*	*A. cappadocicum*	*A. pictum*	*A. miyabei*	*A. platanoides*	*A. truncatum*	*A. rubrum*	*A. saccharinum*	*A. buergerianum* (ser. *Trifida*)	*A. griseum*	*A. maximowiczianum*	*A. triflorum*
Dipteronia sinensis	**0,00**	1,58	1,87	1,64	1,47	1,56	1,92	2,13	2,6	1,58	2,11	2,92	1,25	1,13	1,29	**6,11**	**5,73**	1,75	3,14	2,18	2,8	1,57	2,02	1,2	1,28	1,77	1,79	1,48	2,23	1,93	2,27	1,81	1,95	1,48	1,53	1,32	3,58	2,63	4,81	3,18	4,16	4,45	2,06	2,24	3,39	2,46	3,07	1,93
A. caesium	1,58	**0,01**	0,16	0,2	0,19	0,23	0,16	0,19	0,29	0,17	0,22	0,44	0,37	0,38	0,48	1,31	0,92	0,18	0,25	0,39	0,25	0,29	0,22	0,27	0,29	0,29	0,32	0,37	0,46	0,41	0,36	0,49	0,38	0,36	0,2	0,33	0,54	0,58	0,72	0,42	0,55	0,64	0,49	0,47	0,43	0,31	0,27	0,23
A. saccharum	1,87	0,16	**0,02**	0,08	0,08	0,09	0,06	0,09	0,14	0,1	0,14	0,94	0,74	1,14	0,97	1,03	0,94	0,41	0,47	0,68	0,52	0,54	0,53	0,57	0,63	0,63	0,7	1,04	1,77	1,11	0,98	1,08	0,83	0,8	0,5	0,65	0,95	1,01	1,5	1,05	1,19	1,7	0,63	0,59	0,59	0,4	0,36	0,32
A. hyrcanum	1,64	0,2	0,08	**0,01**	0,01	0,04	0,07	0,09	0,12	0,12	0,15	0,61	0,59	0,9	0,79	1,16	1,01	0,44	0,49	0,85	0,61	0,77	0,67	0,52	0,56	0,57	0,64	0,96	1,17	1,2	1,07	1,25	1	0,86	0,41	0,6	0,7	0,66	1,08	0,95	0,96	1,36	0,45	0,43	0,64	0,33	0,26	0,27
A. ibericum	1,47	0,19	0,08	0,01	**0,01**	0,03	0,08	0,1	0,11	0,12	0,13	0,56	0,51	0,74	0,69	1,04	1,02	0,41	0,47	0,7	0,51	0,64	0,5	0,5	0,55	0,53	0,61	0,87	1,18	1,1	1	1,14	1,02	0,79	0,42	0,54	0,71	0,61	1,14	0,96	0,97	1,2	0,41	0,39	0,58	0,29	0,21	0,24
A. monspessulanum	1,56	0,23	0,09	0,04	0,03	**0,01**	0,11	0,13	0,12	0,13	0,15	0,68	0,64	0,93	0,86	1,15	0,74	0,5	0,63	0,76	0,65	0,56	0,57	0,7	0,77	0,7	0,69	1,09	1,48	1,38	1,22	1,44	1,27	0,98	0,45	0,53	0,85	0,8	1,4	1,08	1,22	1,34	0,56	0,53	0,54	0,31	0,27	0,25
A. opalus	1,92	0,16	0,06	0,07	0,08	0,11	**0,01**	0,09	0,12	0,1	0,13	0,83	0,57	0,71	0,71	1,06	0,87	0,46	0,48	0,72	0,51	0,56	0,52	0,49	0,54	0,53	0,59	0,99	1,58	1,07	0,94	1,04	0,8	0,76	0,42	0,49	1	0,79	1,75	0,91	1,28	1,72	0,61	0,58	0,46	0,35	0,28	0,28
A. pseudoplatanus	2,13	0,19	0,09	0,09	0,1	0,13	0,09	**0,00**	0,14	0,13	0,17	1,2	0,87	1,09	0,87	1,94	1,32	0,41	0,37	0,55	0,41	0,45	0,42	0,44	0,49	0,39	0,5	0,98	1,68	1,05	0,93	1,06	0,79	0,76	0,37	0,52	0,74	0,78	1,74	0,89	1,14	1,54	0,9	0,84	0,64	0,51	0,36	0,41
A. sempervirens	2,6	0,29	0,14	0,12	0,11	0,12	0,12	0,14	**0,00**	0,14	0,18	0,82	0,57	0,76	0,87	1,65	0,89	0,37	0,53	0,85	0,6	0,56	0,63	0,7	0,76	0,88	0,9	0,77	1,84	0,93	0,86	0,92	0,92	0,73	0,72	0,71	1,09	1,04	2,22	1,32	1,7	2,24	0,8	0,75	0,63	0,43	0,39	0,35
A. trautvetteri	1,58	0,17	0,1	0,12	0,12	0,13	0,1	0,13	0,14	**0,01**	0,01	0,71	0,57	0,72	0,73	1,4	1,5	0,45	0,53	0,79	0,56	0,44	0,54	0,39	0,43	0,53	0,61	0,99	1,19	1,06	0,94	1,16	0,83	0,98	0,34	0,4	1,01	0,84	1,62	0,86	1,23	1,3	0,72	0,68	0,65	0,36	0,34	0,29
A. velutinum	2,11	0,22	0,14	0,15	0,13	0,15	0,13	0,17	0,18	0,01	**0,01**	0,89	0,74	0,92	0,94	1,78	1,85	0,54	0,69	1,01	0,68	0,58	0,55	0,5	0,55	0,65	0,76	1,28	1,52	1,38	1,2	1,49	1,08	1,26	0,43	0,5	1,23	0,99	1,93	1,04	1,46	1,52	0,94	0,88	0,83	0,49	0,36	0,39
A. tataricum	2,92	0,44	0,94	0,61	0,56	0,68	0,83	1,2	0,82	0,71	0,89	**0,05**	1,18	1,54	1,87	3,5	4	0,76	0,64	1,24	0,75	0,82	0,94	0,95	1,04	1,1	1,03	1,61	2,27	2,6	2,31	2,5	2,64	2,5	0,85	0,93	2,13	1,95	3,09	1,9	2,46	2,67	1,11	1,21	1,19	0,72	0,65	0,61
A. acuminatum	1,25	0,37	0,74	0,59	0,51	0,64	0,57	0,87	0,57	0,57	0,74	1,18	**0,00**	0,01	0,02	**7,93**	1,76	0,29	0,61	0,66	0,6	0,49	0,56	0,23	0,19	0,25	0,27	0,31	0,38	0,38	0,45	0,39	0,52	0,37	0,23	0,24	1,61	1,25	2,14	1,33	1,27	1,96	1,33	1,25	1,86	1,08	0,72	1,02
A. barbinerve	1,13	0,38	1,14	0,9	0,74	0,93	0,71	1,09	0,76	0,72	0,92	1,54	0,01	**0,01**	0,02	**5,94**	1,84	0,31	0,67	0,73	0,65	0,54	0,59	0,17	0,13	0,26	0,27	0,23	0,38	0,29	0,33	0,3	0,39	0,28	0,24	0,26	1,36	1,26	1,74	1,09	1,15	1,53	1,12	1,05	1,39	0,86	0,47	0,76
A. stachyophyllum	1,29	0,48	0,97	0,79	0,69	0,86	0,71	0,87	0,87	0,73	0,94	1,87	0,02	0,02	**0,01**	**5,74**	2,73	0,46	0,99	0,98	0,96	0,78	0,89	0,24	0,18	0,32	0,34	0,32	0,47	0,43	0,5	0,47	0,53	0,38	0,3	0,35	1,42	1,58	2,26	1,38	1,49	2,07	1,67	1,56	2,16	1,41	0,85	1,24
A. carpinifolium	**6,11**	1,31	1,03	1,16	1,04	1,15	1,06	1,94	1,65	1,4	1,78	3,5	**7,93**	**5,94**	**5,74**	**0,01**	3,54	3,08	1,35	3,23	2,54	2,89	4,71	2,4	2,68	3,48	**7,2**	**5,12**	**6,99**	**5,15**	3,95	4,47	4,84	2,7	3,77	**6,02**	4,44	4,84	4,55	3,1	**5,72**	**7,16**	1,26	1,22	2,5	2,24	1,75	1,72
A. diabolicum	**5,73**	0,92	0,94	1,01	1,02	0,74	0,87	1,32	0,89	1,5	1,85	4	1,76	1,84	2,73	3,54	**0,01**	0,4	1,01	1,11	1,08	1	1,12	1,98	2,06	2,19	0,88	1,38	2,7	1,55	1,27	1,42	1,61	1,21	0,88	1,32	1,31	1,52	2,19	1,39	1,69	2,14	2,28	2,14	1,51	1,23	1,55	0,97
A. macrophyllum	1,75	0,18	0,41	0,44	0,41	0,5	0,46	0,41	0,37	0,45	0,54	0,76	0,29	0,31	0,46	3,08	0,4	**0,00**	0,2	0,26	0,21	0,24	0,2	0,37	0,4	0,5	0,22	0,33	0,6	0,46	0,39	0,49	0,58	0,49	0,32	0,38	0,25	0,21	0,32	0,18	0,22	0,28	0,61	0,58	0,63	0,44	0,43	0,35
A. capillipes	3,14	0,25	0,47	0,49	0,47	0,63	0,48	0,37	0,53	0,53	0,69	0,64	0,61	0,67	0,99	1,35	1,01	0,2	**0,00**	0,08	0,03	0,09	0,08	0,25	0,3	0,46	0,24	1,25	1,7	1,01	1,06	1,13	1,12	1,11	0,51	0,61	0,59	0,63	0,49	0,46	0,38	0,54	0,81	0,77	0,96	0,66	0,46	0,57
A. crataegifolium	2,18	0,39	0,68	0,85	0,7	0,76	0,72	0,55	0,85	0,79	1,01	1,24	0,66	0,73	0,98	3,23	1,11	0,26	0,08	**n/c**	0,09	0,07	0,09	0,57	0,61	0,75	0,18	1,36	1,3	1,24	1,23	1,32	1,42	1,21	0,46	0,67	0,83	0,76	0,93	0,99	0,62	0,71	1,36	1,28	1,5	1,09	0,9	0,86
A. davidii	2,8	0,25	0,52	0,61	0,51	0,65	0,51	0,41	0,6	0,56	0,68	0,75	0,6	0,65	0,96	2,54	1,08	0,21	0,03	0,09	**0,05**	0,08	0,06	0,32	0,35	0,53	0,22	1,17	1,75	0,99	1,02	1,12	1,11	1,04	0,55	0,56	0,73	0,66	0,65	0,61	0,47	0,58	1,09	1,03	0,95	0,7	0,49	0,61
A. pensylvanicum	1,57	0,29	0,54	0,77	0,64	0,56	0,56	0,45	0,56	0,44	0,58	0,82	0,49	0,54	0,78	2,89	1	0,24	0,09	0,07	0,08	**0,01**	0,04	0,38	0,37	0,49	0,14	1,22	1,18	1,12	1,11	1,18	1,28	1,09	0,42	0,36	1,01	0,84	0,95	1	0,69	0,72	1,23	1,16	1,15	1,03	0,68	0,92
A. rufinerve	2,02	0,22	0,53	0,67	0,5	0,57	0,52	0,42	0,63	0,54	0,55	0,94	0,56	0,59	0,89	4,71	1,12	0,2	0,08	0,09	0,06	0,04	**0,00**	0,39	0,38	0,57	0,15	1,01	1,37	0,93	0,92	1,07	1,06	0,9	0,48	0,41	0,89	0,7	0,86	0,91	0,57	0,66	1,41	1,33	0,89	0,63	0,46	0,57
A. cissifolium	1,2	0,27	0,57	0,52	0,5	0,7	0,49	0,44	0,7	0,39	0,5	0,95	0,23	0,17	0,24	2,4	1,98	0,37	0,25	0,57	0,32	0,38	0,39	**0,00**	0,01	0,17	0,22	0,3	0,52	0,27	0,37	0,3	0,28	0,27	0,16	0,23	0,62	0,58	0,76	0,57	0,65	0,68	0,64	0,77	0,91	0,8	0,53	0,75
A. henryi	1,28	0,29	0,63	0,56	0,55	0,77	0,54	0,49	0,76	0,43	0,55	1,04	0,19	0,13	0,18	2,68	2,06	0,4	0,3	0,61	0,35	0,37	0,38	0,01	**0,00**	0,18	0,23	0,33	0,57	0,3	0,38	0,32	0,3	0,29	0,18	0,23	0,63	0,53	0,77	0,57	0,66	0,63	0,7	0,85	0,91	0,89	0,52	0,84
A. negundo	1,77	0,29	0,63	0,57	0,53	0,7	0,53	0,39	0,88	0,53	0,65	1,1	0,25	0,26	0,32	3,48	2,19	0,5	0,46	0,75	0,53	0,49	0,57	0,17	0,18	**0,01**	0,26	0,66	0,58	0,66	0,8	0,63	0,66	0,65	0,15	0,19	0,64	0,86	1,14	0,83	0,78	1,01	1,12	1,06	0,79	0,56	0,39	0,54
A. campbelli ssp. *campbelli*	1,79	0,32	0,7	0,64	0,61	0,69	0,59	0,5	0,9	0,61	0,76	1,03	0,27	0,27	0,34	7,2	0,88	0,22	0,24	0,18	0,22	0,14	0,15	0,22	0,23	0,26	**0,01**	0,53	0,51	0,52	0,5	0,55	0,51	0,52	0,17	0,18	0,51	0,48	0,67	0,56	0,55	0,62	1,25	1,39	1,05	0,84	0,52	0,75
A. circinatum	1,48	0,37	1,04	0,96	0,87	1,09	0,99	0,98	0,77	0,99	1,28	1,61	0,31	0,23	0,32	**5,12**	1,38	0,33	1,25	1,36	1,17	1,22	1,01	0,3	0,33	0,66	0,53	**0,01**	0,16	0,02	0,03	0,03	0,03	0,03	0,58	0,71	0,95	1,01	1,42	0,85	1,47	1,56	1,13	1,22	1,12	0,89	1,04	0,7
A. erianthum	2,23	0,46	1,77	1,17	1,18	1,48	1,58	1,68	1,84	1,19	1,52	2,27	0,38	0,38	0,47	**6,99**	2,7	0,6	1,7	1,3	1,75	1,18	1,37	0,52	0,57	0,58	0,51	0,16	**0,01**	0,19	0,22	0,2	0,25	0,22	0,57	0,86	2,6	1,37	2,37	1,76	2,35	2,79	1,91	2,22	2,69	1,92	1,77	1,51
other A. campbelli spp.	1,93	0,41	1,11	1,2	1,1	1,38	1,07	1,05	0,93	1,06	1,38	2,6	0,38	0,29	0,43	**5,15**	1,55	0,46	1,01	1,24	0,99	1,12	0,93	0,27	0,3	0,66	0,52	0,02	0,19	**0,01**	0,03	0,02	0,03	0,02	0,58	0,82	1,2	1,38	1,58	1,07	1,69	2	1,42	1,53	1,65	1,26	1,09	0,99
A. japonicum	2,27	0,36	0,98	1,07	1	1,22	0,94	0,93	0,86	0,94	1,2	2,31	0,45	0,33	0,5	3,95	1,27	0,39	1,06	1,23	1,02	1,11	0,92	0,37	0,38	0,8	0,5	0,03	0,22	0,03	**0,03**	0,04	0,04	0,03	0,64	0,94	1,09	1,2	1,7	0,99	1,65	1,94	1,44	1,56	1,41	1,21	1,07	0,97
A. oliverianum	1,81	0,49	1,08	1,25	1,14	1,44	1,04	1,06	0,92	1,16	1,49	2,5	0,39	0,3	0,47	4,47	1,42	0,49	1,13	1,32	1,12	1,18	1,07	0,3	0,32	0,63	0,55	0,03	0,2	0,02	0,04	**0,02**	0,04	0,03	0,58	0,84	1,25	1,48	1,83	1,15	1,96	2,32	1,33	1,44	1,64	1,29	1,08	1
A. palmatum	1,95	0,38	0,83	1	1,02	1,27	0,8	0,79	0,92	0,83	1,08	2,64	0,52	0,39	0,53	4,84	1,61	0,58	1,12	1,42	1,11	1,28	1,06	0,28	0,3	0,66	0,51	0,03	0,25	0,03	0,04	0,04	**0,00**	0,04	0,65	0,88	1,08	1,34	1,63	0,87	1,69	1,99	1,43	1,55	1,5	1,14	1,1	0,9
A. shirasawanum	1,48	0,36	0,8	0,86	0,79	0,98	0,76	0,76	0,73	0,98	1,26	2,5	0,37	0,28	0,38	2,7	1,21	0,49	1,11	1,21	1,04	1,09	0,9	0,27	0,29	0,65	0,52	0,03	0,22	0,02	0,03	0,03	0,04	**0,00**	0,58	0,77	1,07	0,93	1,55	0,95	1,67	1,77	1,1	1,19	1,61	1,19	1,01	0,94
A. spicatum	1,53	0,2	0,5	0,41	0,42	0,45	0,42	0,37	0,72	0,34	0,43	0,85	0,23	0,24	0,3	3,77	0,88	0,32	0,51	0,46	0,55	0,42	0,48	0,16	0,18	0,15	0,17	0,58	0,57	0,58	0,64	0,58	0,65	0,58	**0,01**	0,09	0,6	0,69	1,23	0,81	0,76	0,88	1,33	1,26	0,82	0,64	0,56	0,57
A. caudatum	1,32	0,33	0,65	0,6	0,54	0,53	0,49	0,52	0,71	0,4	0,5	0,93	0,24	0,26	0,35	**6,02**	1,32	0,38	0,61	0,67	0,56	0,36	0,41	0,23	0,23	0,19	0,18	0,71	0,86	0,82	0,94	0,84	0,88	0,77	0,09	**0,09**	1,07	0,9	1,56	1	1,03	1,06	1,12	1,15	0,94	0,57	0,51	0,51
A. campestre	3,58	0,54	0,95	0,7	0,71	0,85	1	0,74	1,09	1,01	1,23	2,13	1,61	1,36	1,42	4,44	1,31	0,25	0,59	0,83	0,73	1,01	0,89	0,62	0,63	0,64	0,51	0,95	2,6	1,2	1,09	1,25	1,08	1,07	0,6	1,07	**0,01**	0,1	0,07	0,05	0,05	0,06	0,81	0,77	0,75	0,84	0,64	0,74
A. cappadocicum	2,63	0,58	1,01	0,66	0,61	0,8	0,79	0,78	1,04	0,84	0,99	1,95	1,25	1,26	1,58	4,84	1,52	0,21	0,63	0,76	0,66	0,84	0,7	0,58	0,53	0,86	0,48	1,01	1,37	1,38	1,2	1,48	1,34	0,93	0,69	0,9	0,1	**0,01**	0,1	0,11	0,07	0,1	0,75	0,74	1,53	1,44	1,1	1,27
A. pictum	4,81	0,72	1,5	1,08	1,14	1,4	1,75	1,74	2,22	1,62	1,93	3,09	2,14	1,74	2,26	4,55	2,19	0,32	0,49	0,93	0,65	0,95	0,86	0,76	0,77	1,14	0,67	1,42	2,37	1,58	1,7	1,83	1,63	1,55	1,23	1,56	0,07	0,1	**0,02**	0,09	0,03	0,04	0,96	0,91	1,52	1,31	0,94	1,17
A. miyabei	3,18	0,42	1,05	0,95	0,96	1,08	0,91	0,89	1,32	0,86	1,04	1,9	1,33	1,09	1,38	3,1	1,39	0,18	0,46	0,99	0,61	1	0,91	0,57	0,57	0,83	0,56	0,85	1,76	1,07	0,99	1,15	0,87	0,95	0,81	1	0,05	0,11	0,09	**0,00**	0,06	0,08	0,64	0,62	0,82	0,87	0,62	0,71
A. platanoides	4,16	0,55	1,19	0,96	0,97	1,22	1,28	1,14	1,7	1,23	1,46	2,46	1,27	1,15	1,49	**5,72**	1,69	0,22	0,38	0,62	0,47	0,69	0,57	0,65	0,66	0,78	0,55	1,47	2,35	1,69	1,65	1,96	1,69	1,67	0,76	1,03	0,05	0,07	0,03	0,06	**0,00**	0,02	0,92	0,87	1,26	1,21	0,93	1,08
A. truncatum	4,45	0,64	1,7	1,36	1,2	1,34	1,72	1,54	2,24	1,3	1,52	2,67	1,96	1,53	2,07	**7,16**	2,14	0,28	0,54	0,71	0,58	0,72	0,66	0,68	0,63	1,01	0,62	1,56	2,79	2	1,94	2,32	1,99	1,77	0,88	1,06	0,06	0,1	0,04	0,08	0,02	**0,01**	1,16	1,12	1,44	1,48	0,83	1,32
A. rubrum	2,06	0,49	0,63	0,45	0,41	0,56	0,61	0,9	0,8	0,72	0,94	1,11	1,33	1,12	1,67	1,26	2,28	0,61	0,81	1,36	1,09	1,23	1,41	0,64	0,7	1,12	1,25	1,13	1,91	1,42	1,44	1,33	1,43	1,1	1,33	1,12	0,81	0,75	0,96	0,64	0,92	1,16	**0,00**	0,01	0,65	0,51	0,44	0,41
A. saccharinum	2,24	0,47	0,59	0,43	0,39	0,53	0,58	0,84	0,75	0,68	0,88	1,21	1,25	1,05	1,56	1,22	2,14	0,58	0,77	1,28	1,03	1,16	1,33	0,77	0,85	1,06	1,39	1,22	2,22	1,53	1,56	1,44	1,55	1,19	1,26	1,15	0,77	0,74	0,91	0,62	0,87	1,12	0,01	**0,00**	0,62	0,49	0,42	0,39
A. buergerianum	3,39	0,43	0,59	0,64	0,58	0,54	0,46	0,64	0,63	0,65	0,83	1,19	1,86	1,39	2,16	2,5	1,51	0,63	0,96	1,5	0,95	1,15	0,89	0,91	0,91	0,79	1,05	1,12	2,69	1,65	1,41	1,64	1,5	1,61	0,82	0,94	0,75	1,53	1,52	0,82	1,26	1,44	0,65	0,62	**0,01**	0,17	0,14	0,14
A. griseum	2,46	0,31	0,4	0,33	0,29	0,31	0,35	0,51	0,43	0,36	0,49	0,72	1,08	0,86	1,41	2,24	1,23	0,44	0,66	1,09	0,7	1,03	0,63	0,8	0,89	0,56	0,84	0,89	1,92	1,26	1,21	1,29	1,14	1,19	0,64	0,57	0,84	1,44	1,31	0,87	1,21	1,48	0,51	0,49	0,17	**0,01**	0,05	0,01
A. maximowiczianum	3,07	0,27	0,36	0,26	0,21	0,27	0,28	0,36	0,39	0,34	0,36	0,65	0,72	0,47	0,85	1,75	1,55	0,43	0,46	0,9	0,49	0,68	0,46	0,53	0,52	0,39	0,52	1,04	1,77	1,09	1,07	1,08	1,1	1,01	0,56	0,51	0,64	1,1	0,94	0,62	0,93	0,83	0,44	0,42	0,17	0,05	**0,00**	0,04
A. triflorum	1,93	0,23	0,32	0,27	0,24	0,25	0,28	0,41	0,35	0,29	0,39	0,61	1,02	0,76	1,24	1,72	0,97	0,35	0,57	0,86	0,61	0,92	0,57	0,75	0,84	0,54	0,75	0,7	1,51	0,99	0,97	1	0,9	0,94	0,57	0,51	0,74	1,27	1,17	0,71	1,08	1,32	0,41	0,39	0,14	0,01	0,04	**0,00**

bold font: mean intraspecific distance

light gray: K2P distance between 1 and 2,5

medium gray, white font: K2P distance between 2,5 and 5

dark gray, bold white font: K2P distance > 5

Voucher table for material and accessions of genus *Fagus*

clone*	taxon	location
cr 2	*crenata*	C Honshu, Japan
cr30	*crenata*	BGTue, arboretum
en 1	*engleriana*	Longmenhe, W Hubei, China (locality 3, 300km from locality 1 and 2)
en 2	*engleriana*	Longmenhe, W Hubei, China (locality 4, 10 km from loc. 3)
en 3	*engleriana*	Shennongjia, Hubei, China (locality 5, 50km from locs. 3 and 4)
en 4	*engleriana*	Seo-Myun, western part of Ullung Do, S Korea
en35	*engleriana*	BGTue, arboretum
gr 2	*grandifolia* ssp. *caroliniana*	Piedmont Park, Atlanta, S Georgia, U.S.A.
gr 6	*grandifolia* ssp. *caroliniana*	Florida Caverns S.P., Florida, U.S.A.
gr26	*grandifolia* ssp. *grandifolia*	Fulton Co., New York State, U.S.A.
gr27	*grandifolia* ssp. *grandifolia*	Renssalaer Co., New York State, U.S.A.
gr51	*grandifolia* ssp. *mexicana*	Zacualtipán, Hidalgo, Mexiko
ha 3	*hayatae ssp. pashanica*	Longmenhe, W Hubei, China (locality 3)
ha 4	*hayatae ssp. pashanica*	Longmenhe, W Hubei, China (locality 4)
ha 5	*hayatae ssp. pashanica*	Shennongjia, Hubei, China (locality 5)
ja 1	*japonica*	C Honshu, Japan
ja25	*japonica*	Mt. Fuji, Honshu, Japan
lo 1, lo 2, lo 3	*longipetiolata*	Longmenhe, W Hubei, China (locality 3); different morphotypes
lo47	*longipetiolata*	Long Xi Shan, Fujian, China
lu 1	*lucida*	SW Hubei, near border to Sechuan (localities 1 & 2), China
lu48	*lucida*	Guizhou, China
ho16	*sylvatica*	Mt. Mtiralla, SE of Batumi, Georgia (Transcaucasia)
ho18, ho 19	*sylvatica*	Tezami, 40km N of Tiflis, CE Georgia
mo32	*sylvatica*	Loveč, Bulgaria
or 4	*sylvatica*	Alapli, NW Turkey
or 6	*sylvatica*	Bahceköy, 20km W of Istanbul , Turkey
or12, or13	*sylvatica*	Çatalan, near Erbae, N Turkey
sy16	*sylvatica*	Picos de Europa, Asturia, N Spain
sy20	*sylvatica*	János-hegy, near Buda (Budapest), Hungary
sy28, sy29	*sylvatica*	Baiderschwang, Allgäu, SW Germany
sy31, sy32	*sylvatica*	Masserberg, Thuringia, C Germany
sy43	*sylvatica*	Podčetrtek, road to Olimje, Slovenia
sy46	*sylvatica*	Passo della Cisa, prov. Parma, Emilia Romagna, Italy
sy47	*sylvatica*	near Monte Baldo, Lago di Garda, prov. Verona, N Italy
sy48	*sylvatica*	below Monte Sirente, SE of Róvere, prov. L'Aquila, C Italy
sy49	*sylvatica*	NE of Rocca di Mezzo, prov. L'Aquila, C Italy
sy54	*sylvatica*	San Juan de la Peña, prov. Huesca, Aragon, Spain
sy55	*sylvatica*	Túnel de Vielha, S of Vall d'Aran, prov. Lleida/Lérida, Catalonia, Spain
sy59	*sylvatica*	Black Isle, N Iverness, N of Ethie, Scotland mainland
sy42	*sylvatica* var. *atropunicea*	BGTue, arboretum
sy53	*sylvatica* var. *laciniata*	Bourges, dépt. Cher, France; cultivated

* **bold font**: material collected at original stand from presumably not cultivated (wild) individual(s); normal font: obviously cultivated specimen (botanical gardens, planted)

Material from all sampled populations has been herbarised (Tübingen; Museum of Natural History, Stockholm). Further information can be supplied upon request.

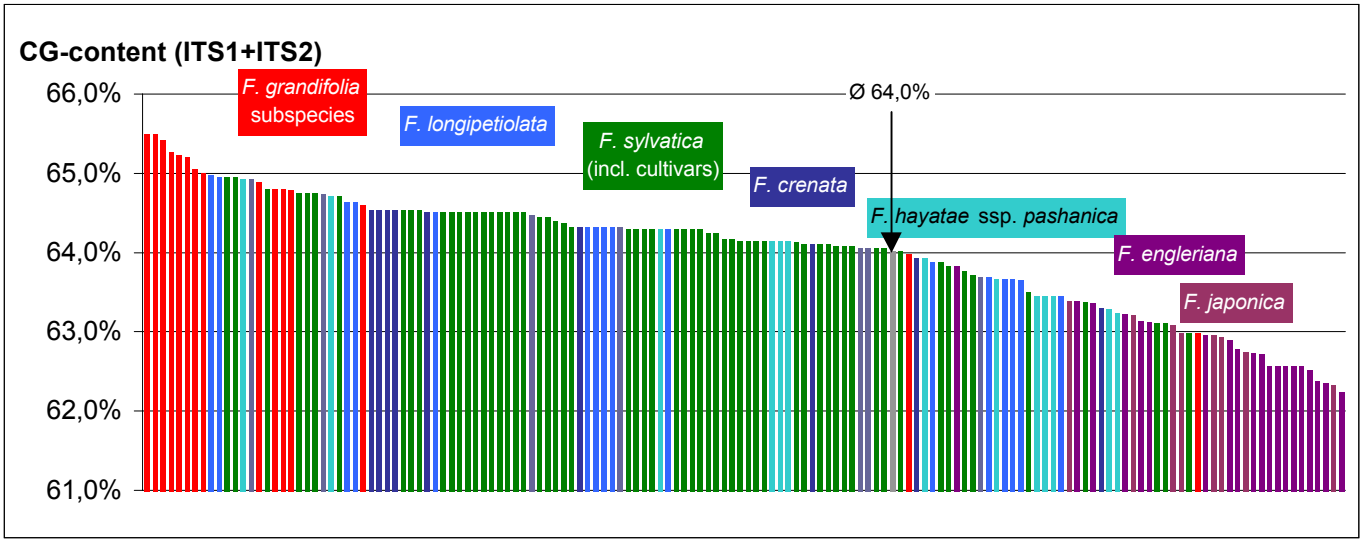
Table of used accessions' core parameters (CG-content, number of nucleotides) for *Fagus*

Clone numbers refer to voucher table. Last column: Difference between the CG-content of ITS1 and ITS2.

		ITS1						ITS2						
taxon/origin	clone	CG-content	A	C	G	T	length [bp]	CG content	A	C	G	T	length [bp]	$\Delta_{CG\text{-content}}$ (ITS2-ITS1)
F. sylvatica var. *atropunicea*	sy4209	**63,8%**	22%	35%	29%	14%	**257**	**64,3%**	17%	34%	30%	19%	**230**	0,5%
	sy4212	**63,8%**	22%	35%	29%	14%	**257**	**64,8%**	16%	34%	30%	19%	**230**	1,0%
	sy4213	**63,8%**	22%	35%	29%	14%	**257**	**64,8%**	16%	34%	30%	19%	**230**	1,0%
	sy4216	**63,8%**	22%	35%	29%	14%	**257**	**64,8%**	16%	34%	30%	19%	**230**	1,0%
... var. *laciniata*	sy5308	**63,4%**	22%	34%	29%	15%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,8%
F. engleriana China mainland	en 108	**61,7%**	23%	33%	28%	15%	**253**	**64,8%**	16%	34%	31%	20%	**230**	3,1%
	en 126	**62,3%**	22%	32%	30%	16%	**268**	**62,2%**	17%	32%	30%	21%	**230**	-0,1%
	en 135	**60,8%**	24%	33%	27%	15%	**255**	**64,3%**	16%	34%	30%	20%	**230**	3,6%
	en 136	**60,8%**	24%	33%	27%	15%	**255**	**64,3%**	16%	34%	30%	20%	**230**	3,6%
	en 202	**61,9%**	21%	31%	31%	17%	**268**	**63,5%**	16%	33%	31%	20%	**230**	1,5%
	en 203	**60,8%**	24%	33%	27%	15%	**255**	**63,9%**	17%	34%	30%	20%	**230**	3,1%
	en 204	**62,8%**	23%	34%	29%	15%	**253**	**62,6%**	17%	33%	30%	20%	**230**	-0,2%
	en 206	**61,9%**	22%	31%	31%	16%	**268**	**64,8%**	16%	33%	31%	20%	**230**	2,8%
	en 301	**60,8%**	24%	33%	27%	15%	**255**	**64,3%**	16%	34%	30%	20%	**230**	3,6%
	en 302	**60,8%**	24%	33%	27%	15%	**255**	**64,3%**	16%	34%	30%	20%	**230**	3,6%
	en 303	**62,5%**	23%	34%	29%	15%	**253**	**63,5%**	16%	33%	31%	20%	**230**	1,0%
	en 304	**60,8%**	24%	33%	27%	15%	**255**	**64,8%**	16%	35%	30%	19%	**230**	4,0%
	en3505	**61,9%**	21%	31%	31%	17%	**268**	**64,3%**	16%	34%	30%	20%	**230**	2,4%
	en3530	**61,6%**	22%	31%	31%	17%	**268**	**63,5%**	16%	33%	30%	20%	**230**	1,9%
	en3541	**62,3%**	22%	32%	30%	16%	**268**	**63,9%**	17%	33%	31%	20%	**230**	1,6%
F. engleriana Ullung Is.	en 402	**60,8%**	24%	33%	27%	15%	**255**	**64,3%**	16%	34%	30%	20%	**230**	3,6%
	en 412	**62,8%**	23%	34%	29%	14%	**253**	**63,9%**	17%	34%	30%	20%	**230**	1,1%
	en 413	**62,3%**	21%	31%	31%	16%	**268**	**63,5%**	16%	33%	31%	20%	**230**	1,2%
	en 415	**62,5%**	23%	34%	29%	15%	**253**	**65,2%**	16%	35%	30%	19%	**230**	2,8%
	en 416	**61,3%**	23%	32%	29%	16%	**253**	**63,5%**	17%	33%	30%	20%	**230**	2,2%
F. japonica	ja 101	**61,9%**	22%	31%	31%	16%	**268**	**63,9%**	16%	33%	31%	20%	**230**	2,0%
	ja 102	**60,7%**	23%	32%	29%	16%	**252**	**64,8%**	16%	34%	31%	20%	**230**	4,1%
	ja 103	**62,5%**	23%	34%	29%	15%	**253**	**63,5%**	16%	33%	30%	20%	**230**	1,0%
	ja 108	**61,6%**	22%	31%	31%	16%	**268**	**65,2%**	16%	34%	31%	19%	**230**	3,7%
	ja2508	**62,1%**	23%	33%	29%	15%	**253**	**63,9%**	17%	33%	30%	20%	**230**	1,9%
	ja2509	**62,1%**	23%	33%	29%	15%	**253**	**64,3%**	16%	34%	30%	20%	**230**	2,3%
	ja2514	**62,7%**	22%	32%	31%	16%	**268**	**63,5%**	17%	34%	29%	20%	**230**	0,8%
	ja2529	**61,2%**	23%	33%	29%	16%	**255**	**63,5%**	17%	34%	29%	20%	**230**	2,3%
F. crenata	cr 201	**62,3%**	22%	33%	29%	16%	**257**	**64,3%**	15%	34%	30%	20%	**230**	2,1%
	cr 202	**63,4%**	22%	34%	29%	15%	**257**	**65,2%**	16%	35%	30%	19%	**230**	1,8%
	cr 205	**63,4%**	22%	35%	29%	14%	**257**	**65,7%**	16%	35%	31%	19%	**230**	2,2%
	cr 212	**63,4%**	22%	35%	29%	14%	**257**	**65,7%**	16%	35%	31%	19%	**230**	2,2%
	cr3001	**63,8%**	22%	35%	29%	14%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,4%
	cr3003	**63,4%**	21%	34%	29%	15%	**257**	**64,8%**	15%	34%	30%	20%	**230**	1,4%
	cr3005	**62,6%**	23%	34%	28%	15%	**257**	**65,2%**	15%	35%	30%	20%	**230**	2,6%
	cr3006	**63,4%**	22%	34%	29%	15%	**257**	**65,7%**	16%	35%	31%	19%	**230**	2,2%
	cr3066	**63,4%**	22%	34%	29%	15%	**257**	**65,7%**	16%	35%	31%	19%	**230**	2,2%
F. grandifolia ssp. *grandifolia*	gr2602	**64,3%**	22%	36%	29%	14%	**255**	**66,7%**	15%	35%	32%	19%	**231**	2,4%
	gr2606	**64,3%**	22%	36%	29%	14%	**255**	**66,2%**	15%	35%	32%	19%	**231**	1,9%
	gr2607	**64,3%**	22%	36%	29%	14%	**255**	**65,8%**	15%	34%	32%	19%	**231**	1,5%
	gr2701	**64,3%**	22%	36%	29%	14%	**255**	**66,7%**	15%	35%	32%	19%	**231**	2,4%
	gr2704	**63,9%**	22%	36%	28%	14%	**255**	**66,5%**	14%	34%	32%	19%	**230**	2,6%
	gr2706	**62,7%**	22%	35%	28%	15%	**255**	**65,2%**	16%	34%	31%	19%	**230**	2,5%
F. grandifolia ssp. *caroliniana*	gr 201	**64,3%**	22%	36%	29%	14%	**255**	**66,5%**	15%	35%	32%	19%	**230**	2,2%
	gr 203	**63,9%**	22%	35%	29%	14%	**255**	**65,7%**	15%	34%	32%	19%	**230**	1,7%
	gr 624	**63,5%**	22%	35%	29%	15%	**255**	**65,7%**	15%	34%	31%	19%	**230**	2,1%
	gr 628	**61,2%**	23%	33%	28%	16%	**255**	**64,8%**	15%	33%	31%	20%	**230**	3,6%
	gr 632	**63,5%**	22%	35%	28%	14%	**255**	**66,2%**	15%	35%	32%	19%	**231**	2,7%
F. grandifolia ssp. *mexicana*	gr5101	**63,5%**	22%	35%	28%	14%	**255**	**66,1%**	15%	34%	32%	19%	**230**	2,6%
	gr5102	**64,3%**	22%	36%	29%	14%	**255**	**66,1%**	15%	34%	32%	19%	**230**	1,8%
	gr5103	**63,5%**	22%	35%	29%	15%	**255**	**66,1%**	15%	34%	32%	19%	**230**	2,6%
	gr5104	**63,9%**	22%	36%	28%	14%	**255**	**66,1%**	15%	34%	32%	19%	**230**	2,2%

		ITS1						ITS2						
taxonomic entity	clone	CG-content	A	C	G	T	length [bp]	CG content	A	C	G	T	length [bp]	$\Delta_{CG\text{-}content}$ (ITS2-ITS1)
F. hayatae ssp. *pashanica*	ha 320	**62,7%**	22%	35%	28%	15%	**255**	**65,1%**	15%	36%	29%	20%	**235**	2,4%
	ha 321	**63,8%**	22%	35%	29%	14%	**257**	**64,8%**	15%	35%	30%	20%	**230**	1,0%
	ha 326	**64,2%**	22%	35%	29%	14%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,0%
	ha 327	**62,7%**	22%	35%	28%	15%	**255**	**65,5%**	15%	36%	29%	19%	**235**	2,8%
	ha 328	**64,2%**	21%	35%	29%	14%	**257**	**65,7%**	16%	35%	30%	19%	**230**	1,5%
	ha 415	**63,4%**	22%	34%	29%	15%	**257**	**63,5%**	15%	33%	30%	21%	**230**	0,1%
	ha 416	**63,4%**	22%	34%	29%	15%	**257**	**63,5%**	15%	33%	30%	21%	**230**	0,1%
	ha 417	**63,4%**	22%	34%	29%	15%	**257**	**63,0%**	16%	33%	30%	21%	**230**	-0,4%
	ha 426	**63,4%**	22%	34%	29%	15%	**257**	**63,5%**	15%	33%	30%	21%	**230**	0,1%
	ha 536	**61,5%**	23%	34%	28%	15%	**257**	**65,1%**	16%	34%	31%	19%	**232**	3,6%
	ha 546	**63,4%**	22%	34%	29%	15%	**257**	**63,9%**	15%	33%	31%	21%	**230**	0,5%
	ha 550	**62,7%**	22%	35%	28%	15%	**255**	**65,5%**	15%	36%	29%	19%	**235**	2,8%
	ha 563	**62,7%**	23%	35%	28%	15%	**255**	**65,5%**	15%	36%	29%	19%	**235**	2,8%
F. longipetiolata	lo 110	**63,4%**	22%	34%	29%	15%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,8%
	lo 113	**63,4%**	22%	34%	29%	15%	**257**	**66,5%**	15%	36%	31%	18%	**230**	3,1%
	lo 118	**63,4%**	22%	34%	29%	15%	**257**	**64,3%**	16%	34%	30%	20%	**230**	0,9%
	lo 119	**63,4%**	22%	34%	29%	15%	**257**	**63,9%**	16%	34%	30%	20%	**230**	0,5%
	lo 204	**62,2%**	23%	34%	29%	15%	**259**	**65,2%**	16%	35%	30%	19%	**230**	3,1%
	lo 208	**63,8%**	21%	35%	29%	15%	**257**	**65,2%**	16%	35%	30%	19%	**230**	1,4%
	lo 209	**63,8%**	22%	35%	29%	14%	**257**	**66,1%**	15%	35%	31%	19%	**230**	2,3%
	lo 211	**63,8%**	22%	34%	30%	14%	**257**	**64,8%**	16%	35%	30%	19%	**230**	1,0%
	lo 302	**62,7%**	22%	35%	28%	15%	**255**	**66,5%**	15%	36%	31%	18%	**230**	3,8%
	lo 305	**63,4%**	22%	34%	29%	15%	**257**	**63,9%**	15%	34%	30%	21%	**230**	0,5%
	lo 306	**63,4%**	22%	34%	29%	15%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,8%
	lo 316	**62,7%**	22%	35%	28%	15%	**255**	**66,5%**	15%	36%	31%	18%	**230**	3,8%
	lo4704	**63,4%**	22%	35%	29%	14%	**257**	**65,2%**	15%	34%	31%	20%	**230**	1,8%
	lo4717	**63,8%**	22%	35%	29%	14%	**257**	**63,5%**	16%	33%	30%	20%	**230**	-0,3%
	lo4721	**63,4%**	22%	34%	29%	14%	**257**	**65,2%**	16%	35%	30%	19%	**230**	1,8%
	lo4722	**63,4%**	22%	35%	29%	15%	**257**	**63,5%**	15%	33%	30%	21%	**230**	0,1%
F. lucida	lu 102	**63,0%**	22%	34%	29%	15%	**257**	**64,3%**	16%	33%	31%	20%	**230**	1,3%
	lu 103	**63,4%**	22%	35%	29%	14%	**257**	**65,2%**	16%	35%	30%	19%	**230**	1,8%
	lu 104	**64,6%**	21%	35%	29%	14%	**257**	**64,3%**	16%	34%	30%	20%	**230**	-0,2%
	lu 105	**64,2%**	21%	35%	30%	14%	**257**	**63,9%**	15%	33%	30%	21%	**230**	-0,3%
	lu4836	**64,2%**	21%	35%	29%	14%	**257**	**65,7%**	16%	35%	30%	19%	**230**	1,5%
	lu4848	**63,8%**	22%	35%	29%	14%	**257**	**65,7%**	16%	35%	31%	19%	**230**	1,8%
	lu4861	**64,2%**	22%	35%	29%	14%	**257**	**63,9%**	17%	34%	30%	20%	**230**	-0,3%
F. sylvatica Georgia (Transcaucasus)	ho1601	**63,5%**	22%	35%	29%	14%	**255**	**65,2%**	16%	35%	30%	19%	**230**	1,7%
	ho1602	**61,9%**	23%	34%	28%	16%	**257**	**64,3%**	17%	34%	30%	19%	**230**	2,5%
	ho1603	**61,9%**	23%	34%	28%	16%	**257**	**64,3%**	17%	34%	30%	19%	**230**	2,5%
	ho1805	**63,4%**	22%	35%	29%	14%	**257**	**65,7%**	15%	35%	31%	19%	**230**	2,2%
	ho1807	**63,4%**	22%	35%	29%	14%	**257**	**65,7%**	15%	35%	31%	19%	**230**	2,2%
	ho1904	**62,1%**	22%	34%	28%	16%	**253**	**63,9%**	15%	33%	30%	21%	**230**	1,9%
	ho1907	**63,4%**	22%	34%	29%	15%	**257**	**66,1%**	15%	34%	32%	19%	**230**	2,7%
F. sylvatica Turkey	or1206	**64,2%**	22%	35%	29%	14%	**257**	**63,9%**	16%	33%	30%	20%	**230**	-0,3%
	or1301	**63,3%**	22%	34%	29%	15%	**256**	**65,2%**	16%	34%	31%	19%	**230**	1,9%
	or1302	**63,7%**	22%	35%	29%	14%	**256**	**65,2%**	16%	34%	31%	19%	**230**	1,5%
	or1303	**62,9%**	22%	34%	29%	15%	**256**	**64,8%**	16%	34%	31%	20%	**230**	1,9%
	or1322	**63,8%**	22%	35%	29%	14%	**257**	**64,8%**	16%	34%	31%	20%	**230**	1,0%
	or1324	**62,6%**	22%	34%	29%	15%	**257**	**64,8%**	16%	34%	31%	19%	**230**	2,1%
	or 404	**64,2%**	21%	35%	30%	14%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,0%
	or 405	**63,0%**	22%	35%	28%	15%	**254**	**65,2%**	16%	35%	30%	19%	**230**	2,2%
	or 601	**63,7%**	22%	34%	29%	14%	**256**	**65,2%**	16%	34%	31%	19%	**230**	1,5%
	or 603	**62,9%**	22%	34%	29%	15%	**256**	**65,2%**	15%	34%	31%	20%	**230**	2,3%
	or 605	**63,3%**	22%	34%	29%	15%	**256**	**65,2%**	16%	34%	31%	19%	**230**	1,9%
	or 618	**62,3%**	22%	33%	29%	16%	**257**	**66,5%**	15%	35%	32%	18%	**230**	4,3%
	or 645	**63,8%**	22%	35%	29%	14%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,4%

		ITS1						ITS2						
taxonomic entity	clone	CG-content	A	C	G	T	length [bp]	CG content	A	C	G	T	length [bp]	$\Delta_{CG\text{-}content}$ (ITS2-ITS1)
F. sylvatica Bulgaria	mo3221	**61,1%**	22%	33%	28%	17%	**257**	**65,7%**	16%	35%	30%	19%	**230**	4,6%
	mo3222	**63,2%**	23%	35%	28%	14%	**253**	**64,8%**	16%	34%	30%	19%	**230**	1,5%
F. sylvatica Hungary/Slovenia	sy2001	**62,6%**	22%	33%	29%	16%	**257**	**65,7%**	15%	35%	31%	19%	**230**	3,0%
	sy2002	**62,6%**	22%	33%	29%	16%	**257**	**65,7%**	15%	35%	31%	19%	**230**	3,0%
	sy2004	**62,6%**	22%	33%	29%	16%	**257**	**65,7%**	15%	35%	31%	19%	**230**	3,0%
	sy2005	**62,6%**	22%	33%	29%	16%	**257**	**65,7%**	15%	35%	31%	19%	**230**	3,0%
	sy4301	**63,4%**	22%	34%	30%	15%	**257**	**64,8%**	16%	34%	30%	20%	**230**	1,4%
	sy4309	**63,4%**	22%	33%	30%	15%	**257**	**66,1%**	15%	35%	31%	19%	**230**	2,7%
	sy4312	**63,8%**	22%	35%	29%	14%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,4%
F. sylvatica Germany	sy2802	**63,8%**	22%	35%	29%	14%	**257**	**64,8%**	16%	34%	30%	19%	**230**	1,0%
	sy2803	**63,4%**	22%	35%	29%	14%	**257**	**64,8%**	16%	34%	30%	19%	**230**	1,4%
	sy2901	**63,8%**	22%	35%	29%	14%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,4%
	sy2904	**63,8%**	22%	35%	29%	14%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,4%
	sy3103	**63,4%**	22%	33%	30%	15%	**257**	**66,1%**	15%	35%	31%	19%	**230**	2,7%
	sy3105	**62,3%**	23%	34%	28%	15%	**257**	**66,1%**	15%	35%	31%	19%	**230**	3,8%
	sy3206	**62,6%**	22%	34%	29%	15%	**257**	**64,3%**	16%	33%	31%	20%	**230**	1,7%
	sy3209	**63,8%**	22%	35%	29%	14%	**257**	**64,3%**	16%	34%	30%	20%	**230**	0,5%
	sy3211	**63,8%**	22%	35%	29%	14%	**257**	**64,3%**	16%	34%	30%	20%	**230**	0,5%
F. sylvatica Scotland	sy5901	**63,4%**	22%	34%	29%	14%	**257**	**65,7%**	16%	35%	31%	19%	**230**	2,2%
	sy5907	**63,8%**	22%	35%	29%	14%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,4%
F. sylvatica Spain	sy1601	**63,8%**	22%	35%	29%	14%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,4%
	sy1603	**61,9%**	23%	34%	28%	15%	**257**	**65,7%**	15%	35%	31%	19%	**230**	3,8%
	sy1607	**63,8%**	22%	35%	29%	14%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,4%
	sy1610	**63,8%**	22%	35%	29%	14%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,4%
	sy5403	**63,8%**	22%	35%	29%	14%	**257**	**64,8%**	16%	34%	31%	20%	**230**	1,0%
	sy5423	**63,8%**	22%	35%	29%	14%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,4%
	sy5506	**63,1%**	22%	35%	29%	15%	**255**	**65,2%**	16%	34%	31%	19%	**230**	2,1%
	sy5508	**63,8%**	22%	35%	29%	14%	**257**	**64,8%**	16%	34%	31%	20%	**230**	1,0%
F. sylvatica Italy	sy4601	**63,4%**	22%	34%	29%	15%	**257**	**64,3%**	17%	34%	30%	19%	**230**	0,9%
	sy4604	**63,0%**	22%	34%	29%	15%	**257**	**65,2%**	16%	34%	31%	19%	**230**	2,2%
	sy4802	**63,8%**	22%	35%	29%	14%	**257**	**66,1%**	15%	35%	31%	19%	**230**	2,3%
	sy4804	**63,5%**	22%	35%	29%	15%	**257**	**66,1%**	15%	35%	31%	19%	**230**	2,6%
	sy4903	**63,8%**	22%	35%	29%	14%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,4%
	sy4904	**63,8%**	22%	35%	29%	14%	**257**	**64,8%**	16%	34%	31%	20%	**230**	1,0%
	sy4701	**63,8%**	22%	35%	29%	14%	**257**	**65,2%**	16%	34%	31%	19%	**230**	1,4%
	sy4702	**63,8%**	22%	35%	29%	14%	**257**	**66,1%**	15%	35%	31%	19%	**230**	2,3%
	Ø	**63,1%**	22%	34%	29%	15%		**65,0%**	16%	34%	31%	19%		1,9%



Mean genetic distances between species and populations of *Fagus* originating from different geographic regions

Pairwise ditances calculated with MEGA 2.1® using a gamma-distributed Kimura 2-parameter model (gaps pairwise deleted)

		sg. *Engleriana*			sg. *Fagus*															
	F. sylvatica cv.	*F. engleriana* China	*F. engleriana* Ullung Is.	*F. japonica*	*F. crenata*	*F. grandifolia ssp. grandifolia*	*F. grandifolia ssp. caroliniana*	*F. grandifolia ssp. mexicana*	*F. hayatae ssp. pashanica*	*F. longipetiolata*	*F lucida*	*F sylvatica* Georgia	*F. sylvatica* Turkey	*F. sylvatica* Bulgaria	*F. sylvatica* Hungary	*F. sylvatica* Slovenia	*F. sylvatica* Germany	*F. sylvatica* Scotland	*F. sylvatica* Spain	*F. sylvatica* Italy
F. sylvatica cv.	**0.003**	0.090	0.080	0.083	0.017	0.031	0.031	0.026	0.027	0.021	0.019	0.018	0.014	0.035	0.013	0.009	0.009	0.012	0.007	0.008
F. engleriana China	0.090	**0.105**	0.088	0.093	0.112	0.119	0.114	0.109	0.112	0.105	0.114	0.097	0.110	0.156	0.096	0.095	0.097	0.098	0.084	0.092
F. engleriana Ullung Is.	0.080	0.088	**0.079**	0.078	0.098	0.101	0.097	0.092	0.101	0.094	0.097	0.086	0.098	0.139	0.088	0.081	0.085	0.092	0.074	0.081
F. japonica	0.083	0.093	0.078	**0.086**	0.101	0.107	0.103	0.098	0.106	0.097	0.101	0.090	0.101	0.145	0.091	0.085	0.089	0.094	0.076	0.084
F. crenata	0.017	0.112	0.098	0.101	**0.016**	0.045	0.044	0.039	0.042	0.034	0.031	0.026	0.027	0.045	0.025	0.021	0.021	0.025	0.017	0.020
F. grandifolia ssp. grandifolia	0.031	0.119	0.101	0.107	0.045	**0.012**	0.017	0.010	0.058	0.049	0.045	0.039	0.043	0.076	0.037	0.035	0.036	0.038	0.029	0.033
F. grandifolia ssp. caroliniana	0.031	0.114	0.097	0.103	0.044	0.017	**0.021**	0.015	0.056	0.049	0.045	0.038	0.042	0.074	0.036	0.034	0.036	0.037	0.029	0.033
F. grandifolia ssp. mexicana	0.026	0.109	0.092	0.098	0.039	0.010	0.015	**0.006**	0.051	0.045	0.040	0.034	0.037	0.066	0.031	0.029	0.031	0.033	0.025	0.028
F. hayatae ssp. pashanica	0.027	0.112	0.101	0.106	0.042	0.058	0.056	0.051	**0.035**	0.040	0.044	0.041	0.038	0.069	0.035	0.031	0.032	0.035	0.027	0.030
F. longipetiolata	0.021	0.105	0.094	0.097	0.034	0.049	0.049	0.045	0.040	**0.034**	0.034	0.033	0.031	0.058	0.029	0.024	0.025	0.028	0.021	0.023
F lucida	0.019	0.114	0.097	0.101	0.031	0.045	0.045	0.040	0.044	0.034	**0.028**	0.032	0.029	0.051	0.029	0.024	0.024	0.028	0.019	0.023
F sylvatica Georgia	0.018	0.097	0.086	0.090	0.026	0.039	0.038	0.034	0.041	0.033	0.032	**0.025**	0.026	0.049	0.025	0.022	0.022	0.022	0.017	0.021
F. sylvatica Turkey	0.014	0.110	0.098	0.101	0.027	0.043	0.042	0.037	0.038	0.031	0.029	0.026	**0.020**	0.042	0.023	0.018	0.018	0.020	0.015	0.017
F. sylvatica Bulgaria	0.035	0.156	0.139	0.145	0.045	0.076	0.074	0.066	0.069	0.058	0.051	0.049	0.042	**0.063**	0.049	0.042	0.040	0.042	0.034	0.039
F. sylvatica Hungary	0.013	0.096	0.088	0.091	0.025	0.037	0.036	0.031	0.035	0.029	0.029	0.025	0.023	0.049	**0.000**	0.015	0.016	0.018	0.014	0.014
F. sylvatica Slovenia	0.009	0.095	0.081	0.085	0.021	0.035	0.034	0.029	0.031	0.024	0.024	0.022	0.018	0.042	0.015	**0.013**	0.012	0.016	0.010	0.011
F. sylvatica Germany	0.009	0.097	0.085	0.089	0.021	0.036	0.036	0.031	0.032	0.025	0.024	0.022	0.018	0.040	0.016	0.012	**0.013**	0.016	0.010	0.011
F. sylvatica Scotland	0.012	0.098	0.092	0.094	0.025	0.038	0.037	0.033	0.035	0.028	0.028	0.022	0.020	0.042	0.018	0.016	0.016	**0.018**	0.013	0.014
F. sylvatica Spain	0.007	0.084	0.074	0.076	0.017	0.029	0.029	0.025	0.027	0.021	0.019	0.017	0.015	0.034	0.014	0.010	0.010	0.013	**0.007**	0.009
F. sylvatica Italy	0.008	0.092	0.081	0.084	0.020	0.033	0.033	0.028	0.030	0.023	0.023	0.021	0.017	0.039	0.014	0.011	0.011	0.014	0.009	**0.010**

bold font: mean intraspecific/intrapopulation distance

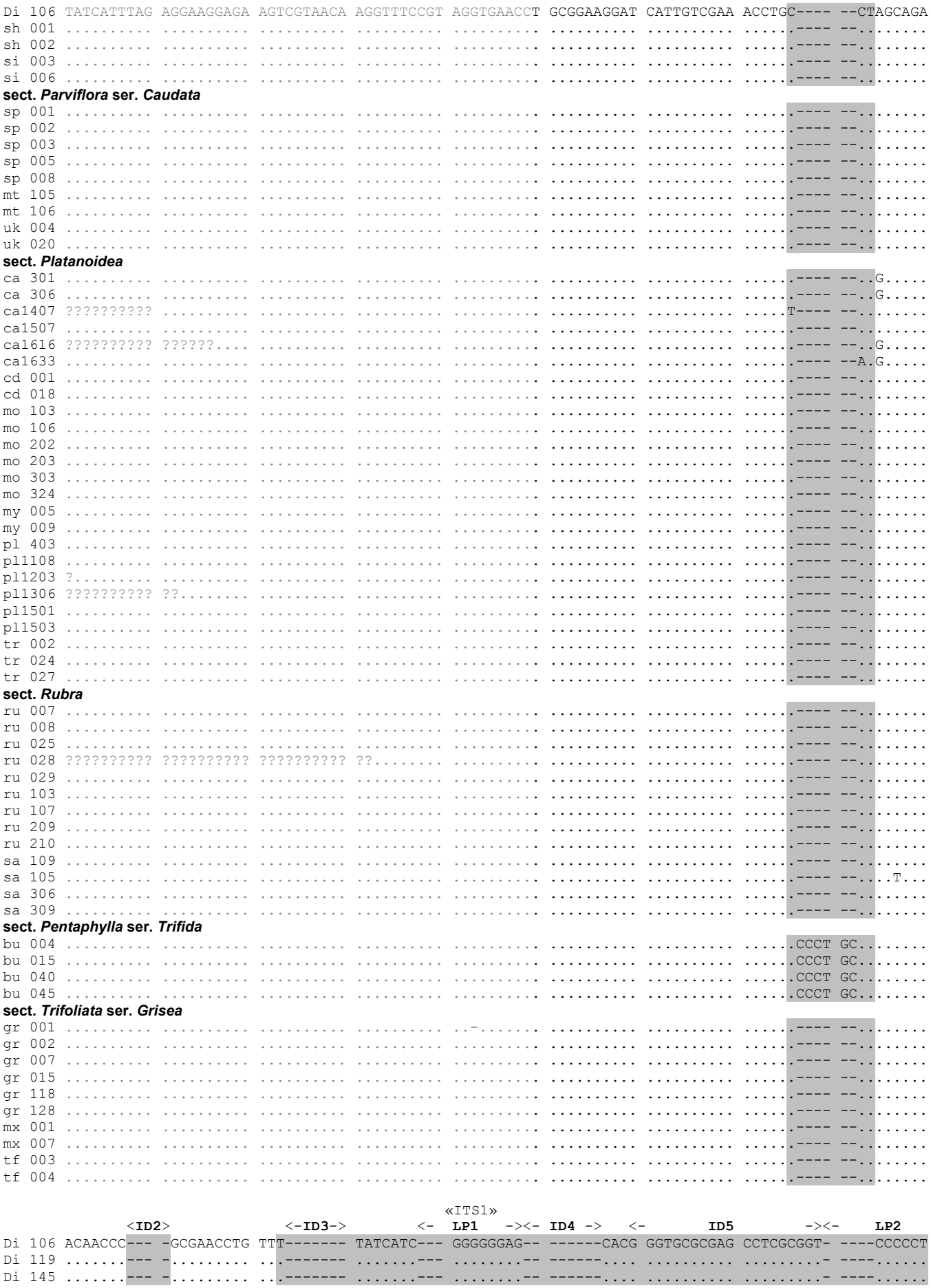
Appendix IV: Alignments

Alignment of *Acer* accessions (new data only) comprising the 3' end of the 18S rDNA, the ITS1, the 5.8S rDNA, the ITS2, and the 5' end of the 26S rDNA

Standard nucleotide code, "." indicates identity with reference sequence (clone Di 106), "?" uncertain data (not available or poor quality). Grey font: rRNA gene data, not used for analyses. Grey background: 'variable' regions within the ITS1 and ITS2 (excluded from the analysis based on conserved regions only; → Fig. 4-11). Accession labels refer to column #1 in the voucher table. Accessions are ordered alphabetically and grouped into series/sections as proposed by current systematics.

```
                                            3' end 18S rDNA-><-5' end ITS1
                                                                                       <-ID1->
Dipteronia sinensis
Di 106 TATCATTTAG AGGAAGGAGA AGTCGTAACA AGGTTTCCGT AGGTGAACCT GCGGAAGGAT CATTGTCGAA ACCTGC---- --CTAGCAGA
Di 119 .......... .......... .......... .......... .......... .......... .......... ......---- --........
Di 145 .......... .......... .......... .......... .......... .......... .......... ......---- --........
sect. Acer
cm 001 .......... .......... .......... .......... .......... .......... .......... ......---- --........
cm 003 .......... .......... .......... .......... .......... .......... .......... ......---- --........
cm 122 .......... .......... .......... .......... .......... .......... .......... ......---- --........
cm 145 .......... .......... .......... .......... .......... .......... .......... ......---- --........
fd 102 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
fd 119 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
gd 025 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
gd 035 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
hy 102 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
hy 133 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
ib 108 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
ib 111 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
ib 113 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
ib 117 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
ib 201 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
ib 238 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
ib 303 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
ib 307 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
ms 011 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
ms 408 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
ms 411 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
ni 101 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
ni 206 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
ni 209 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
op 404 .......... .......... .......... .......... .......... .......... .......... ......---- --G.......
op 407 .......... .......... .......... .......... .......... .......... .......... ......---- --A.......
op 502 .......... .......... .......... .......... .......... .......... .......... ......---- --G.......
op 509 .......... .......... .......... .......... .......... .......... .......... ......---- --G.......
ot 103 .......... .......... .......... .......... .......... .......... .......... ....A.---- --G.......
ot 104 .......... .......... .......... .......... .......... .......... .......... ......---- --G.......
ot 105 .......... .......... .......... .......... .......... .......... .......... ......---- --G.......
pp1702 ?????????? ?????????? ?????????? ???????... .......... .......... .......... ......---- --........
pp2209 ??........ .......... .......... .......... .......... .......... .......... ......---- --........
sc 110 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
sc 140 .......... .......... .......... .......... .......... .......... .......... ......---- --.C......
sv 102 .......... .......... .......... .......... .......... .......... .......... ......---- --........
sv 124 ..A....... .......... .......... .......... .......... .......... .......... ......---- --........
sv 201 .......... .......... .......... .......... .......... .......... .......... ......---- --........
sv 220 .......... .......... .......... .......... .......... .......... .......... ......---- --........
sv 309 .......... .......... .......... .......... .......... .......... .......... ......---- --........
tv 101 .......... .......... .......... .......... .......... .......... .......... ......---- --........
tv 103 .......... .......... .......... .......... .......... .......... .......... ......---- --........
ve 112 .......... .......... .......... .......... .......... .......... .......... ......---- --........
ve 144 .......... .......... .......... .......... .......... .......... .......... ......---- --........
ve 202 .......... .......... .......... .......... .......... .......... .......... ......---- --........
ve 207 .......... .......... .......... .......... .......... .......... .......... ......---- --........
ve 240 .......... .......... .......... .......... .......... .......... .......... ......---- --........
ve 306 ?????????? ?????????? ?????????? ?????????? ?????????? ?......... .......... ......---- --........
ve 308 .......... .......... .......... .......... .......... .......... .......... ......---- --........
```

```
Di 106 TATCATTTAG AGGAAGGAGA AGTCGTAACA AGGTTTCCGT AGGTGAACCT GCGGAAGGAT CATTGTCGAA ACCTGC---- --CTAGCAGA
```
sect. *Ginnala*
```
gi 021 .......... .......... .......... .......... .......... .......... .......... .....C---- --.C......
gi 022 .......... .......... .......... .......... .......... .......... .......... .....C---- --.C......
se 021 .......... .......... .......... .......... .......... .......... .......... .....C---- --.C......
se 025 .......... .......... .......... .......... .......... .......... .......... .....C---- --.C......
tt 005 .......... .......... .......... .......... .......... .......... .......... .....C---- --.C......
tt 008 .......... .......... .......... .......... .......... .......... .......... .....C---- --.C......
tt 014 .......... .......... .......... .......... .......... .......... .......... .....C---- --.C......
```
sect. *Glabra* ser. *Arguta*
```
ac 002 .......... .......... .......... .......... .......... .......... .......... .....T---- --.C......
ac 101 .......... .......... .......... .......... .......... .......... .......... .....T---- --.C......
ac 102 .......... .......... .......... .......... .......... .......... .......... .....T---- --.C......
bn 001 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
bn 002 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
bn 003 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
bf 002 .......... .......... .......... .......... .......... .......... .......... .....T---- --.C......
bf 005 .......... .......... .......... .......... .......... .......... .......... .....T---- --.C......
st 001 .......... .......... .......... .......... .......... .......... .......... .....T---- --.C......
st 002 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
```
sect. *Indivisa*
```
cf 001 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
cf 002 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
cf 003 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
cf 004 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
```
sect. *Lithocarpa* ser. *Lithocarpa*
```
db 021 .......... .......... .......... .......... .......... .......... .........G .....C---- --........
db 025 .......... .......... .......... .......... .......... .......... .........G .....C---- --........
db 034 .......... .......... .......... .......... .......... .......... .........G .....C---- --........
db 038 ???....... .......... .......... .......... .......... .......... .........G .....C---- --........
```
sect. *Lithocarpa* ser. *Macrophylla*
```
mp 001 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
mp 002 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
```
sect. *Macrantha*
```
cp 021 ?????????? ?......... .......... .......... .......... .......... .......... .....C---- --........
cp 023 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
cr 041 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
da 121 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
da 122 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
gs 011 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
gs 015 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
gs 101 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
gs 102 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
pe 107 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
pe 110 .......... .......... .......... .......... .......... .......... .......... .....T---- --........
rn 005 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
rn 024 ????????. .......... .......... .......... .......... .......... .......... .....C---- --........
```
sect. *Negundo* ser. *Cissifolia*
```
cs 009 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
cs 023 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
cs 102 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
cs 103 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
he 005 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
he 111 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
he 112 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
he 136 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
```
sect. *Negundo* ser. *Negundo*
```
ne 201 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
ne 203 .......... .......... .......... .......... .......... ..T....... .......... .....C---- --........
```
sect. *Palmata*
```
cb 014 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
cb 017 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
cc 004 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
cc 009 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
er 002 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
er 005 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
er 003 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
fl 103 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
fl 114 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
ja 005 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
ja 008 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
ol 037 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
ol 039 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
ol 111 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
pa 005 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
pa 006 .......... .......... .......... .......... .......... .......... .......... .....C---- --........
```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sh 001
sh 002
si 003
si 006
sect. Parviflora ser. Caudata
sp 001
sp 002
sp 003
sp 005
sp 008
mt 105
mt 106
uk 004
uk 020
sect. Platanoidea
ca 301
ca 306
ca1407 ??????????
ca1507
ca1616 ?????????? ??????....
ca1633
cd 001
cd 018
mo 103
mo 106
mo 202
mo 203
mo 303
mo 324
my 005
my 009
pl 403
pl1108
pl1203 ?.....
pl1306 ?????????? ??.....
pl1501
pl1503
tr 002
tr 024
tr 027
sect. Rubra
ru 007
ru 008
ru 025
ru 028 ?????????? ?????????? ?????????? ??.....
ru 029
ru 103
ru 107
ru 209
ru 210
sa 109
sa 105
sa 306
sa 309
sect. Pentaphylla ser. Trifida
bu 004
bu 015
bu 040
bu 045
sect. Trifoliata ser. Grisea
gr 001
gr 002
gr 007
gr 015
gr 118
gr 128
mx 001
mx 007
tf 003
tf 004
«ITS1»
<ID2>
<-ID3->
<- LP1 -><- ID4 ->
<- ID5 -><- LP2
Di 106 ACAACCC--- -GCGAACCTG TTT------- TATCATC--- GGGGGGAG-- ------CACG GGTGCGCGAG CCTCGCGGT- ----CCCCCT
Di 119 ....--- -.......... ...------- ....--- ....-- ------.... ....- ----......
Di 145 ....--- -.......... ...------- ....--- ....-- ------.... ....- ----.....

```
Di 106 ACAACCC--- -GCGAACCTG TTT------- TATCATC--- GGGGGGAG-- ------CACG GGTGCGCGAG CCTCGCGGT- ----CCCCCT
cm 001 ..G....--- -.......C. ...------- C......--G ........-- ------.... .......... .....T...- ----......
cm 003 ..G....--- -.......C. ...------- C......--G ........-- ------.... .......... .....T...- ----......
cm 122 ..G....--- -.T.....C. ...------- C......--G ........-- ------.... .......... .....T...- ----......
cm 145 ..G....--- -.T.....C. ...------- C......--G ........-- ------.... .......... .....T...- ----......
fd 102 ..G....--- -.......C. ...T------ ........--G ........-- ------.... ..C....A.. ..C......- ----......
fd 119 ..G....--- -.......C. ...T------ ........--G ........-- ------.... ..C....A.. ..C......- ----......
gd 025 ..G....--- -.T.....C. ...T------ ........--G ........-- ------.... ..C....A.. ..C..T...- --CC...T.C
gd 035 ..G....--- -.T.....C. ...T------ ........--G ........-- ------.... ..C....A.. ..C..T...- --CC...T.C
hy 102 ..G....--- -.......C. ...------- ..C....--G ........-- ---CAC.... .......A.. .....T...A ACCC.....C
hy 133 ..G....--- -.......C. ...------- ..C....--G ........-- ---CAC.... .......A.. .....T...A ACCC..---C
ib 108 ..G....--- -.......C. ...------- ..C....--- ........-- ---CAC.... .......A.A .....T...A ACCC....TC
ib 111 ..G....--- -.......C. ...------- ..C....--- .......TCG GACCAC.... .......A.. .....T...A ACCC...--C
ib 113 ..G....--- -.......C. ...------- ..C....--- .......TCG GACCAC.... .......A.. .....T...A ACCC...--C
ib 117 ..G....--- -.......C. ...------- ..C....--- .......TCG GACCAC.... .......A.. .....T...A ACCC...--C
ib 201 ..G....--- -.......C. ...------- ..C....--- ........-- ---CAC.... .......A.. .....T...A ACCC....TC
ib 238 ..G....--- -.......C. ...------- ..C....--- ........-- ---CAC.... .......A.. .....T...A ACCC....TC
ib 303 ..G....--- -.......C. ...------- ..C....--- .......TCG GACCAC.... .......A.. .....T...A ACCC...--C
ib 307 ..G....--- -.......C. ...------- ..C....--- ........-- ---CAC.... .......A.. .....T...A ACCC....TC
ms 011 ..G....--- -.......C. ...------- ..C....--- ........-- ---CAG.... .......A.. .....T...- ACCC...--C
ms 408 ..G....--- -.......C. ...------- ..C....--- ........-- ---CAG.... .......A.. .....T...- ACCC...--C
ms 411 ..G....--- -.......C. ...------- ..C....--- ........-- ---CAG.... .......A.. .....T...- ACCC...--C
ni 101 ..G....--- -.......C. ...T------ ........--G ........-- ------.... ..C....A.. ..C......- ----......
ni 206 ..G....--- -.......C. ...T------ ........--G ........-- ------.... ..C....A.. ..C......- ----......
ni 209 ..G....--- -.......C. ...T------ ........--G ........-- ------.... ..C....A.. ..C......- ----......
op 404 ..G....--- -.......C. ...------- .......--- ........-- ------.... .......A.. A.C..T...- ---C...T..
op 407 ..G....--- -.......C. ...------- .......--- ........-- ------.... .......A.. A.C..T...- ---C...T..
op 502 ..G....--- -.......C. ...------- .......--- ........-- ------.... .......A.. A.C..T...- ---C...T..
op 509 ..G....--- -.......C. ...------- .......--- ........-- ------.... .......A.. A.C..T...- ---C...T..
ot 103 ..G....--- -.......C. ...------- .......--- ........-- ------.... .......A.. A.C..T...- ---C...T..
ot 104 ..G....--- -.......C. ...------- .......--- ........-- ------.... .......A.. A.C..T...- ---C...T..
ot 105 ..G....--- -.......C. ...------- .......--- ........-- ------.... .......A.. A.C..T...- ---C...T..
pp1702 .TG....--- -.......C. ...------- ........--G ........-- ------.... .......A.. .....T...- ----......
pp2209 .TG....--- -.......C. ...------- ........--G ........-- ------.... .......A.. .....T...- ----......
sc 110 ..G....--- -.......C. ...T------ ........--G ........-- ------.... ..C....A.. ..C......- ----......
sc 140 ..G....--- -.......C. ...T------ ........--G ........-- ------.... ..C....A.. ..C......- ----......
sv 102 ..G....--- -.......C. ...------- .....C.--- ........-- ------.... .......A.. .T...T.C.- ----A.....
sv 124 ..G....--- -.......C. ...------- .....C.--- ........-- ------.... .......A.. .T...T.C.- ----A.....
sv 201 ..G....--- -.......C. ...------- .....C.--- ........-- ------.... .......A.. .T...T.C.- ----A.....
sv 220 ..G....--- -.......C. ...------- .....C.--- ........-- ------.... .......A.. .T...T.C.- ----A.....
sv 309 ..G....--- -.......C. ...------- .....C.--- ........-- ------.... .......A.. .T...T.C.- ----A.....
tv 101 ..G....--- -......... ...------- .......--- ........-- ------.... ......TA.. ..C..T...- ----......
tv 103 ..G....--- -......... ...------- .......--- ........-- ------.... ......TA.. ..C..T...- ----......
ve 112 ..G....--- -......... ...------- .......--- ........-- ------.... ......TA.. ..C..T...- ----....T.
ve 144 ..G....--- -......... ...------- .......--- ........-- ------.... ......TA.. ..C..T...- ----....T.
ve 202 ..G....--- -......... ...------- .......--- ........-- ------.... ......TA.. ..C..T...- ----....T.
ve 207 .TG....--- -......... ...------- .......--- ........-- ------.... ......TA.. ..C..T...- ----....T.
ve 240 ..G....--- -......... ...------- .......--- ........-- ------.... ......TA.. ..C..T...- ----....T.
ve 306 ..G....--- -......... ...------- .......--- ........-- ------..T. ......TA.. ..C..T...- ----....T.
ve 308 ..G....--- -......... ...------- ......T--- ........-- ------.... ......TA.. ..C..T...- ----....T.
gi 021 ..G....--- -......TC. ...------- .....C.--- .....A..-- ------.... .......... .....T.T.- ----......
gi 022 ..G....--- -......TC. ...------- .....C.--- .....A..-- ------.... .......... .....T.T.- ----......
se 021 ..G....--- -......TC. ...------- .....C.--- A....A..-- ------.... .......... ..C..T.T.- ----......
se 025 ..G....--- -......TC. ...------- .....C.--- A....A..-- ------.... .......... ..C..T.T.- ----......
tt 005 ..G....--- -......TC. ...------- .....C.--- A....A..-- ------.... .......... .....T.T.- ----......
tt 008 ..G....--- -......TC. ...------- .....C.--- A....A..-- ------.... .......... .....T.T.- ----......
tt 014 ..G....--- -......TC. ...------- .....C.--- A....A..-- ------.... .......... .....T.T.- ----......
ac 002 ..G....--- -......T.. ...------- ........--A ........-- ------.... .......... .....T..G- ---C......
ac 101 ..G....--- -......T.. ...------- ........--A ........-- ------.... .......... .....T..G- ---C......
ac 102 ..G....--- -......T.. ...------- ........--A ........-- ------.... .......... .....T..G- ---C......
bn 001 ..G....--- -......T.. ...------- ........--A ........-- ------.... .......... .....T..C- ---C......
bn 002 ..G....--- -......T.. ...------- ........--A ........-- ------.... .......... .....T..C- ---C......
bn 003 ..G....--- -......T.. ...------- ........--A ........-- ------.... .......... .....T..C- ---C......
bf 002 ..G....--- -......T.. ...------- ........--A ........-- ------.... .......... .....T..C- ---C......
bf 005 ..G....--- -......T.. ...------- ........--A ........-- ------.... .....A.... .....T..C- ---C......
st 001 ..G....--- -......A.. ...------- ........--A ........-- ------.... .......... .....T..C- ---C......
st 002 ..G....--- -......T.. ...------- ........--A ........-- ------.... .......... .....T..C- ---C......
cf 001 ..G....--- -.......C. ...------- ..C....CCG ........-- -------.T. CA.------- ---------- ----......
cf 002 ..G....GCT C.......C. ...------- ..C....CCG ........-- -------.T. CA.------- ---------- ----......
cf 003 ..G....--- -.......C. ...------- ..C....CCG ........-- -------.T. CA.------- ---------- ----......
cf 004 ..G....--- -.......C. ...------- ..C....CCG ........-- -------.T. CA.------- ---------- ----......
db 021 ..G....--- -.......C. ...------- .......--- ........-- ----CA.... ..AC.T.C-- ---..T...- ---C....T.
db 025 ..G....--- -.......C. ...------- ..C....--- ........-- ----CA.... ..AC.T.C-- ---..T...- ----......
db 034 ..G....--- -.......C. ...------- ..C....--- ........-- ----CA.... ..AC.T.C-- ---..T...- ----......
db 038 ..G....--- -.......C. ...------- ..C....--- ........-- ----CA.... ..AC.T.C-- ---..T...- ----......
mp 001 ..G....--- -......... ...------- .....C.--- ........-- ------.... .......... .....T...- ----......
mp 002 ..G....--- -......... ...------- .....C.--- ........-- ------.... .......... .....T...- ----......
```

```
Di 106 ACAACCC--- -GCGAACCTG TTT------- TATCATC--- GGGGGGAG-- ------CACG GGTGCGCGAG CCTCGCGGT- ----CCCCCT
cp 021 ..G.....--- -......T.. ..CT------ ..C....--G ........-- ------.... .-........ .....T...- ----......
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gs 101 ..G.....--- -......T.. ..CT------ ..C....--- ........-- ------.... .-........ .....T...- ----......
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pe 107 ..G.....--- -......T.. ..CT------ .......--A ........-- ------.... .-........ .....T...- ----......
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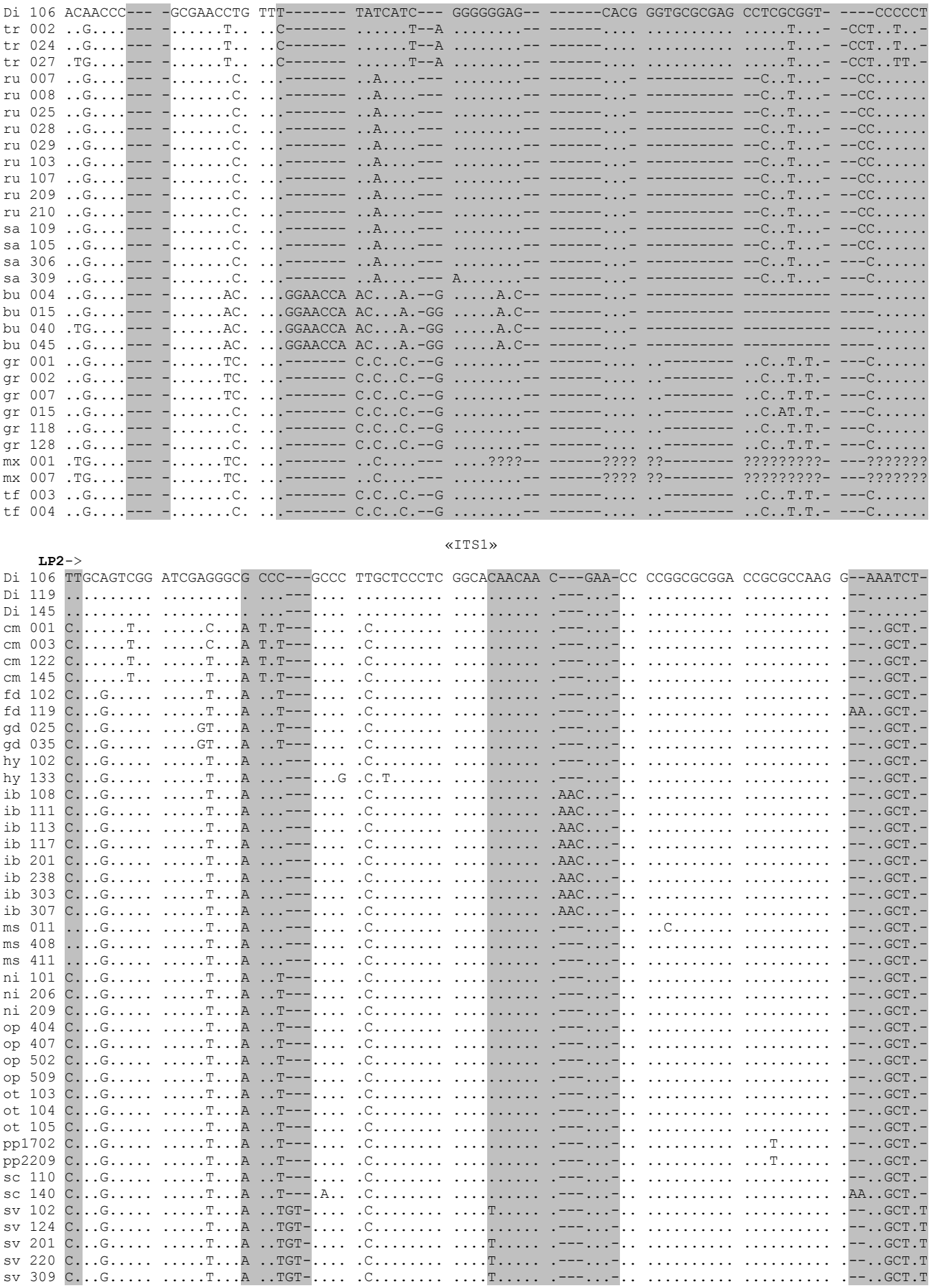
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tr 027 .TG....--- -......T.. ..C------- ......T--A ....-- ------.... ....T...- -CCT..TT.-
ru 007 ..G....--- -.......C. ...------- ..A...--- ....-- ------...- ---------- --C..T...- --CC......
ru 008 ..G....--- -.......C. ...------- ..A...--- ....-- ------...- ---------- --C..T...- --CC......
ru 025 ..G....--- -.......C. ...------- ..A...--- ....-- ------...- ---------- --C..T...- --CC......
ru 028 ..G....--- -.......C. ...------- ..A...--- ....-- ------...- ---------- --C..T...- --CC......
ru 029 ..G....--- -.......C. ...------- ..A...--- ....-- ------...- ---------- --C..T...- --CC......
ru 103 ..G....--- -.......C. ...------- ..A...--- ....-- ------...- ---------- --C..T...- --CC......
ru 107 ..G....--- -.......C. ...------- ..A...--- ....-- ------...- ---------- --C..T...- --CC......
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sa 109 ..G....--- -.......C. ...------- ..A...--- ....-- ------...- ---------- --C..T...- --CC......
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sa 309 ..G....--- -.......C. ...------- ..A...--- A.......-- ------...- ---------- --C..T...- ---C......
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gr 007 ..G....--- -......TC. ...------- C.C..C.--G ....-- ------.... ..-------- ..C..T.T.- ---C......
gr 015 ..G....--- -.......C. ...------- C.C..C.--G ....-- ------.... ..-------- ..C.AT.T.- ---C......
gr 118 ..G....--- -.......C. ...------- C.C..C.--G ....-- ------.... ..-------- ..C..T.T.- ---C......
gr 128 ..G....--- -.......C. ...------- C.C..C.--G ....-- ------.... ..-------- ..C..T.T.- ---C......
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fd 119 C..G..... .....T...A ..T---.... .C........ ....---...-.. .... .AA..GCT.-
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ib 108 C..G..... .....T...A ...---.... .C........ .... .AAC...-.. ....--..GCT.-
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ib 113 C..G..... .....T...A ...---.... .C........ .... .AAC...-.. ....--..GCT.-
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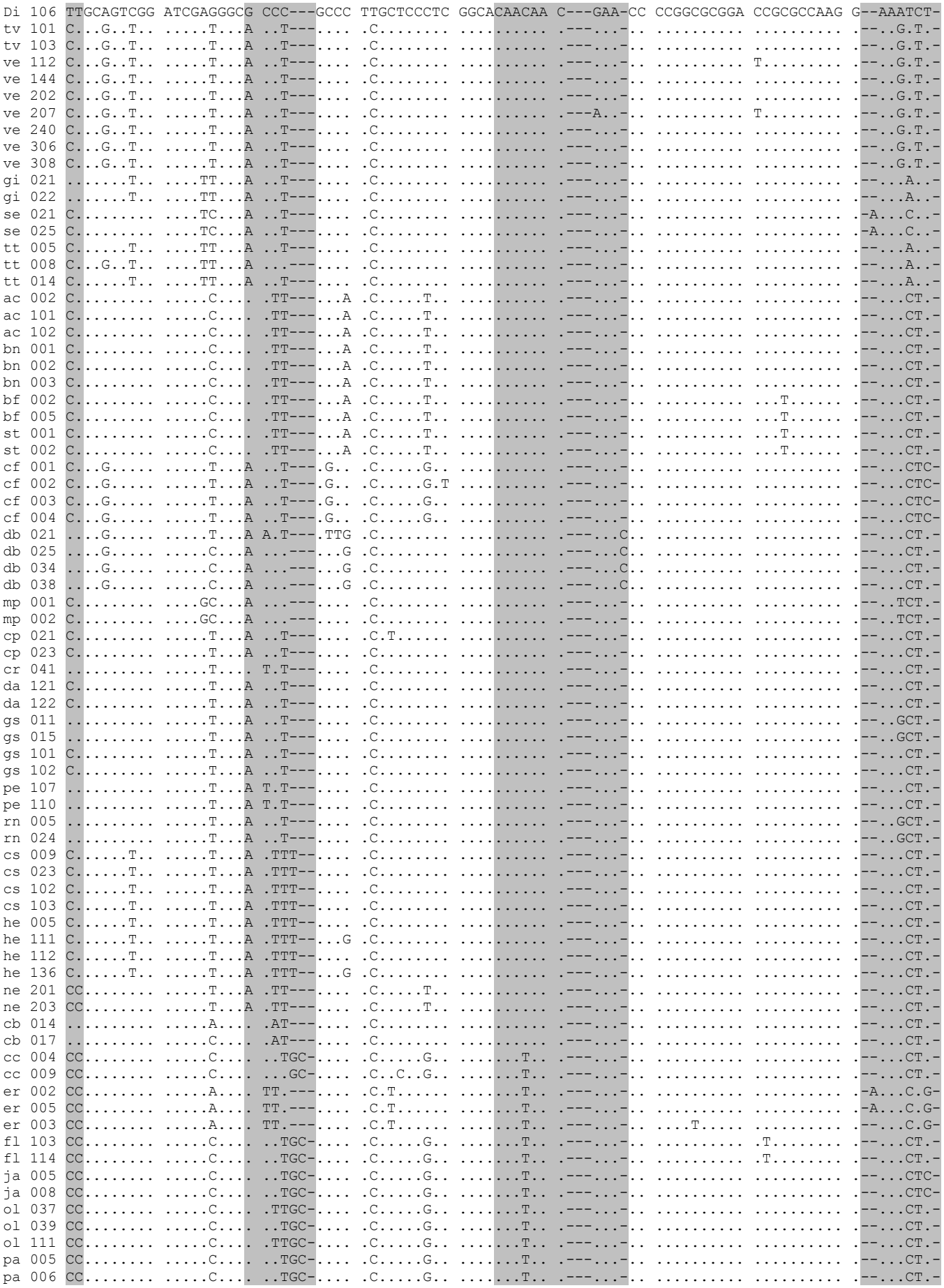
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db 025 ....G..... .....C...A ...---...G .C..... ..... .---...C.. ....--...CT.-
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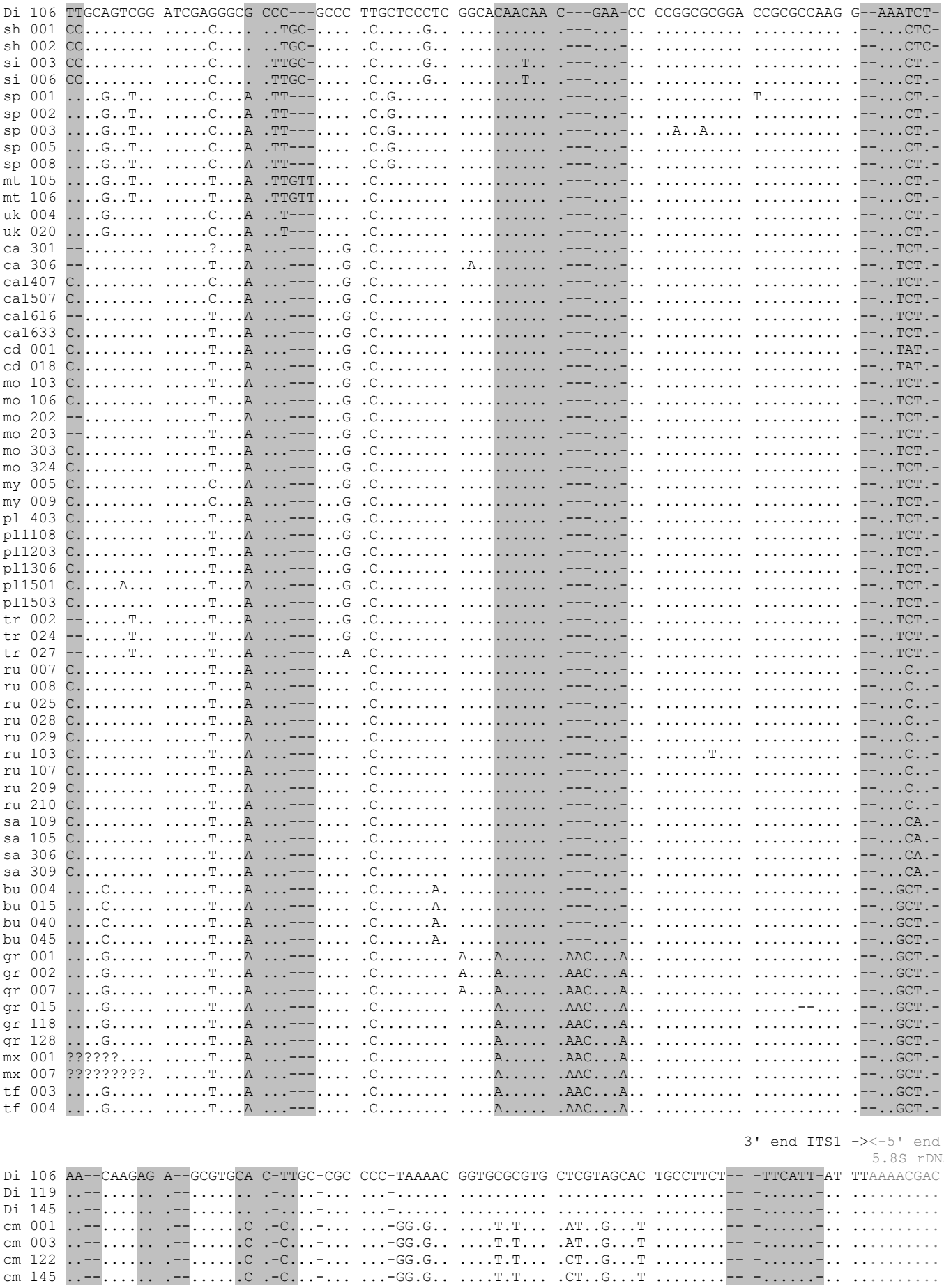
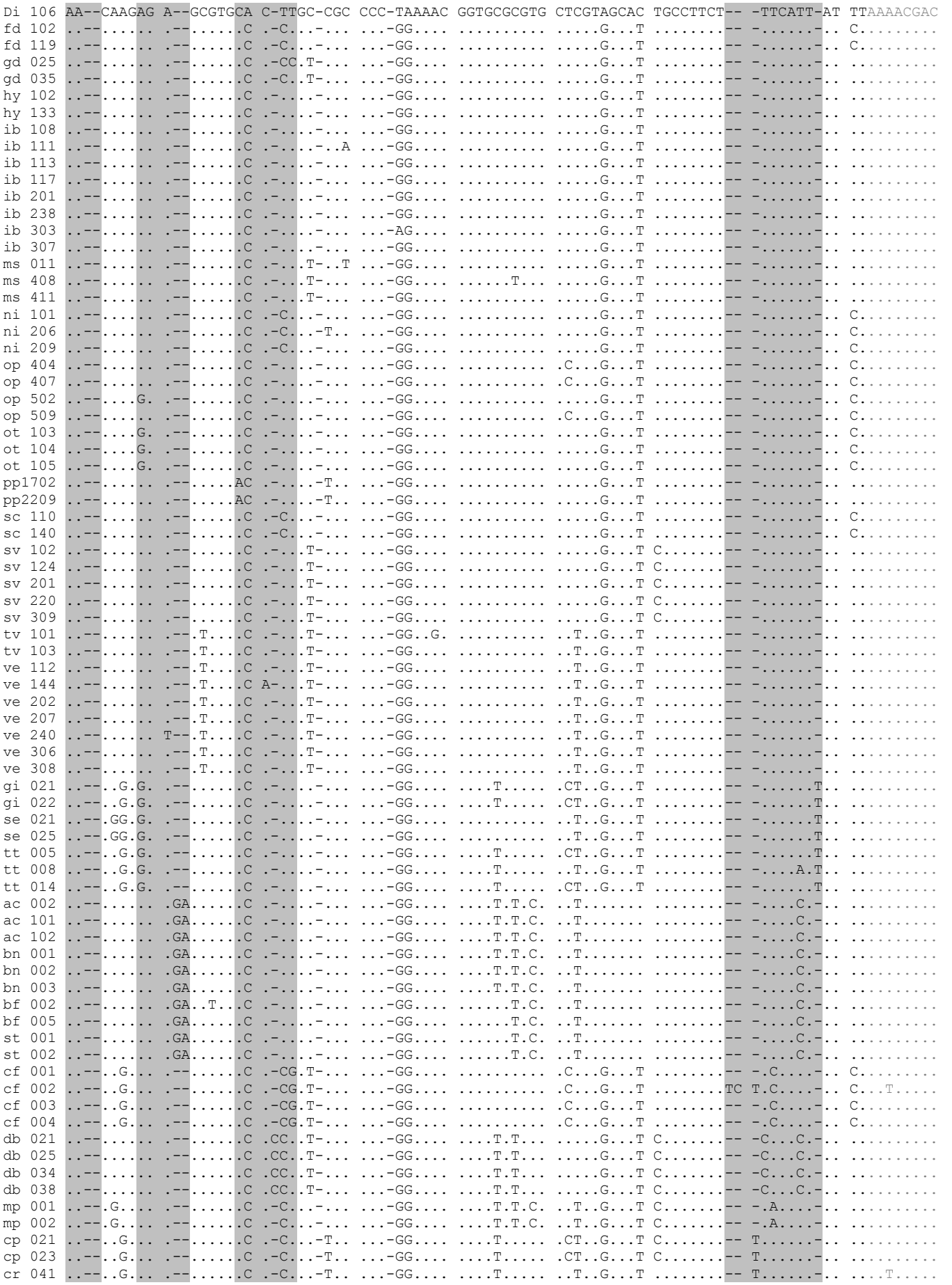
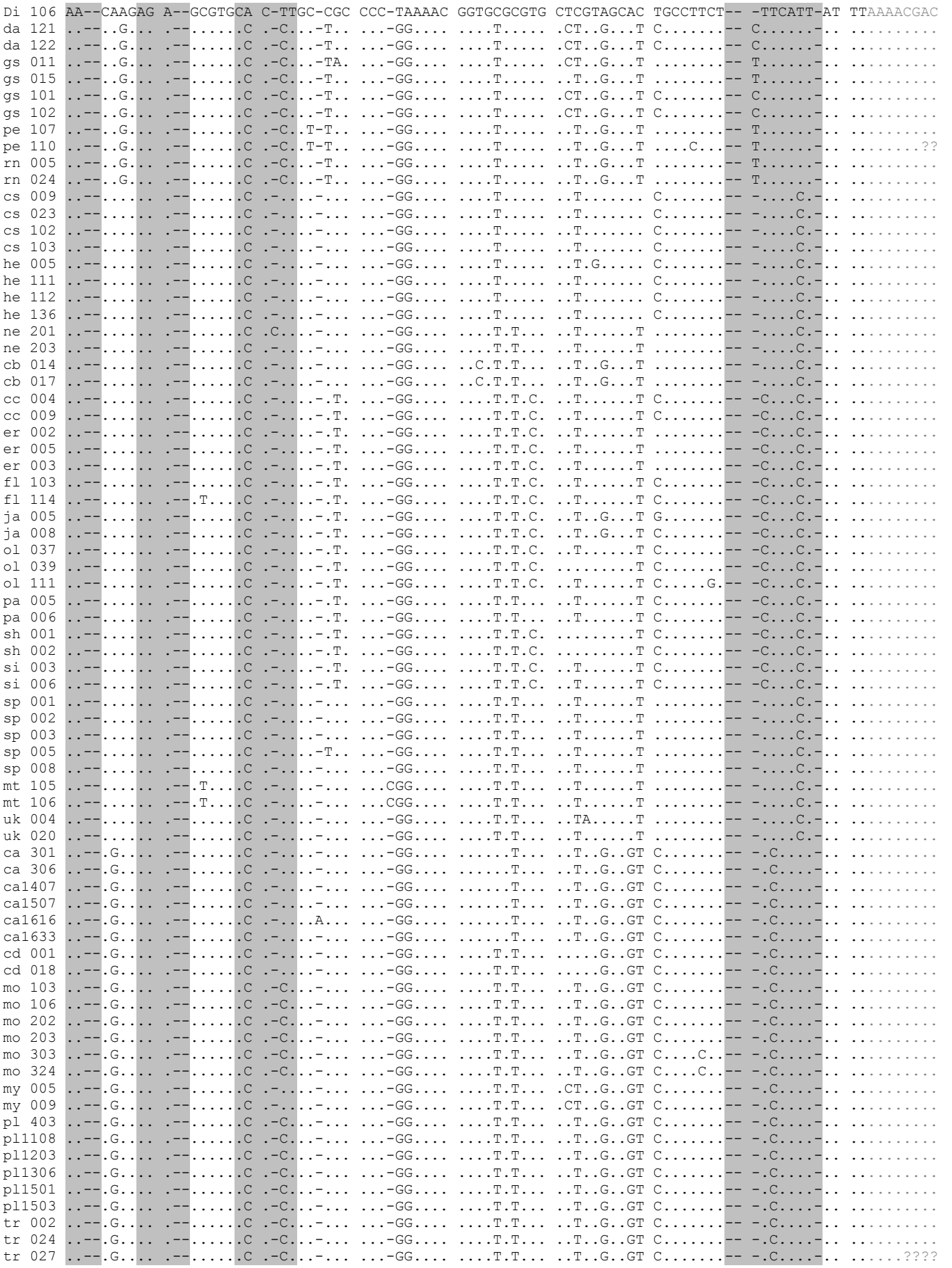
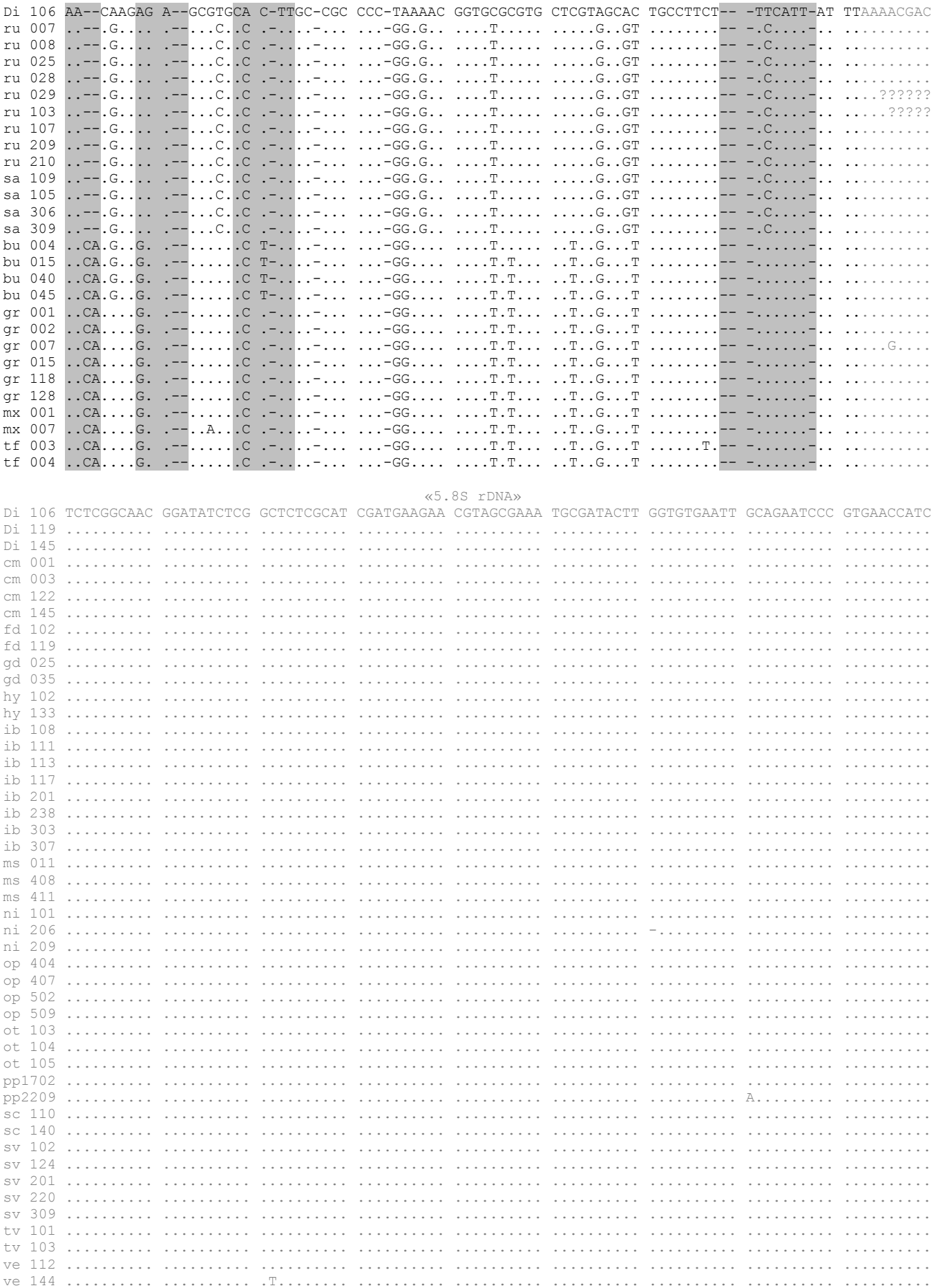

3' end ITS1 -><-5' end
5.8S rDNA






«5.8S rDNA»

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Di 106 TCTCGGCAAC GGATATCTCG GCTCTCGCAT CGATGAAGAA CGTAGCGAAA TGCGATACTT GGTGTGAATT GCAGAATCCC GTGAACCATC
ve 202 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ve 207 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ve 240 .......... .......... .....A.... .......... .......... .......... .......... .......... ..........
ve 306 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ve 308 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gi 021 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gi 022 .......... .......... .......... .......... .......... .......... .......... .......... ..........
se 021 .......... .......... .......... .......... .......... .......... .......... .......... ..........
se 025 .......... .......... .......... .......... .......... .......... T......... .......... ..........
tt 005 .......... .......... .......... .......... .......... .......... .......... .......... ..........
tt 008 .......... .......... .......... .......... .......... .......... .......... .......... ..........
tt 014 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ac 002 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ac 101 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ac 102 .......... .......... .......... .......... .......... .......... .......... .......... ..........
bn 001 .......... .......... .......... .......... .......... .......... .......... .......... ..........
bn 002 .......... .......... .......... .......... .......... .......... .......... .......... ..........
bn 003 .......... .......... .......... .......... .......... .......... .......... .......... ..........
bf 002 ......T... .......... .......... .......... .......... .......... .......... .......... ..........
bf 005 .......... .......... .......... .......... .......... .......... .......... .......... ..........
st 001 .......... .......... .......... .......... .......... .......... .......... ........A. ..........
st 002 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cf 001 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cf 002 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cf 003 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cf 004 .......... .......... .......... .......... .......... .......... .......... .......... ..........
db 021 .......... .......... .......... .......... .......... .......... .......??? ?????????? ??????????
db 025 .......... .......... .......... .......... .......... .......... .......... .......... ..........
db 034 .......... .......... .......... .......... .......... .......... .......... .......... ..........
db 038 .......... .......... .......... .......... .......... .......... .......... .......... ..........
mp 001 .......... .......... .......... .......... .......... .......... .T........ .......... ..........
mp 002 .......... .......... .......... .......... .......... .......... .T........ .......... ..........
cp 021 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cp 023 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr 041 .......... .......... .......... .......... .......... .......... .......... .......... ..........
da 121 .......... .......... .......... .......... .......... .......... .......... .......... ..........
da 122 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gs 011 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gs 015 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gs 101 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gs 102 .......... .......... .......... .......... .......... .......... .......... .......... ..........
pe 107 .......... .......... .......... .......... .......... .......... .......... .......... ..........
pe 110 ?????????? ?????????? ?????????? ???....... .......... .......... .......... .......... ..........
rn 005 .......... .......... .......... .......... .......... .......... .......... .......... ..........
rn 024 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cs 009 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cs 023 .......... .......... ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
cs 102 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cs 103 .......... .......... .......... .......... .......... .......... .......... .......... ..........
he 005 .......... .......... .......... .......... .......... .......... .......... .......... ..........
he 111 .......... .......... .......... .......... .......... .......... .......... .......... ..........
he 112 .......... .......... .......... .......... .......... .......... .......... .......... ..........
he 136 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ne 201 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ne 203 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cb 014 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cb 017 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cc 004 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cc 009 .......... .......... .......... .......... .......... .......... .......... .......... ..........
er 002 .......... .......... .......... .......... .......... .......... .......... .......... ..........
er 005 .......... .......... .......... .......... .......... .......... .......... .......... ..........
er 003 .......... .......... .......... .......... .......... .......... .......... .......... ..........
fl 103 .......... .......... .......... .......... .......... .......... .......... .......... ..........
fl 114 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ja 005 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ja 008 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ol 037 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ol 039 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ol 111 .......... .......... .......... .......... .......... .......... .......... .......... ..........
pa 005 .......... .......... .......... .......... .......... .......... .......... .......... ..........
pa 006 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sh 001 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sh 002 .......... .......... .......... .......... .......... .......... .......... .......... ..........
si 003 .......... .......... .......... .......... .......... .......... .......... .......... ..........
si 006 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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3' end 5.8S rDNA->
<-5' end ITS2
<- upstream LP3

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hy 102 .......... .......... .......... .......... .......... .......... .......... ...----.T. ...C.A.A.-
hy 133 .......... .......... .......... .......... .......... .......... .......... ...---C... ...C.A.A.-
ib 108 .......... .......... .......... .......... .......... .......... .......... ...CCCC..A ...C.A.A.-
ib 111 .......... .......... .......... .......... .......... .......... .......... ...CCCC..A ...C.A.A.-
ib 113 .......... .......... .......... .......... .......... .......... .......... ...CCCC..A ...C.A.A.-
ib 117 .......... .......... .......... .......... .......... .......... .......... ...CCCC..A ...C.A.A.-
ib 201 .......... .......... .......... .......... .......... .......... .......... ...CCCC..A ...C.A.A.-
ib 238 .......... .......... .......... .......... .......... .......... .......... ...CCCC..A ...C.A.A.-
ib 303 .......... .......... .......... .......... .......... .......... .......... ...CCCC..A ...C.A.A.-
ib 307 .......... .......... .......... .......... .......... .......... .......... ...CCCC..A ...C.A.A.-
ms 011 .......... .......... .A........ .......... .......... .......... .......... ...--CC..G ...GAA.A.-
ms 408 .......... .......... .......... .......... .......... .......... .......... ...--CC..G ...CAA.A.-
ms 411 .......... .......... .......... .......... .......... .......... .......... ...--CC..G ...CAA.A.-
ni 101 .......... .......... .......... .......... .......... .......... .......... ...----... ...C...A.-
ni 206 .......... .......... .......... .......... .......... .......... .......... ...----... .A.C...A.-
ni 209 .......... .......... .......... .......... .......... .......... .......... ...----... .A.C...A.-
op 404 .......... .......... .......... .......... .......... .......... .......... ...------. ...C...A.-
op 407 .......... .......... .......... .......... .......... .......... .......... ...------. ...C...A.-
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pp1702 .......... .......... .......... .......... .......... .......... .......... ...-----.. ...C...A.-
pp2209 .......... .......... .......... .......... .......... .......... .......... ...-----.. ...C...A.-
sc 110 .......... .......... .......... .......... .......... .......... .......... ...----... ...C...A.-
sc 140 .......... .......... .......... .......... .......... .......... .......... ...----... ...C...A.-
sv 102 .......... .......... .......... .......... .......... .......... .......... ...--CC... ...C.A.A.-
sv 124 .......... .......... .......... .......... .......... .......... .......... ...--CC... ...C.A.A.-
sv 201 .......... .......... .......... .......... .......... .......... .......... ...---C... ...C.A.A.-
sv 220 .......... .......... .......... .......... .......... .......... .......... ...---C... ...C.A.A.-
sv 309 .......... .......... .......... .......... .......... .......... .......... ...---C... ...C.A.A.-
tv 101 .......... .......... .......... .......... .......... .......... .......... ...-----.. ...C.A.A.-
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ve 144 .......... .......... .......... .......... .......... .......... .......... ...-----.. ...C.A.A.-
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ve 207 .......... .......... .......... .......... .......... .......... .......... ...-----.. ...C.A.A.-
ve 240 .......... .......... .......... .......... .......... .......... .......... ...-----.. ...C.A.A.-
ve 306 .......... .......... .......... .......... .......... .......... .......... ...-----.. ...C.A.A.-
ve 308 .......... .......... .......... .......... .......... .......... .......... ...-----.. ...C.A.A.-
gi 021 .......... .......... .......... .......... .......... .......... .......... ...-----.. ..-C...A.C
gi 022 .......... .......... .......... .......... .......... .......... .......... ...-----.. ..-C...A.C
se 021 .......... .......... .......... .......... .......... .......... .......... ...-----.. ..GC...A.C
se 025 .......... .......... .......... .......... .......... .......... .......... ...-----.. ..GC...A.C
tt 005 .......... .......... .......... .......... .......... .......... .......... ...-----.. ..-C...A.C
tt 008 .......... .......... .......... .......... .......... .......... .......... ...-----.. ...C...A.C
tt 014 .......... .......... .......... .......... .......... .......... .......... ...-----.. ...-...A.C
ac 002 .......... .......... .......... .......... .......... .......... .......... ...----... ...A...---
ac 101 .......... .......... .......... .......... .......... .......... .......... ...----... ...A...---
ac 102 .......... .......... .......... .......... .......... .......... .......... ...----... ...A...---
bn 001 .......... .......... .......... .......... .......... .......... .......... ...----..? ??.A...---
bn 002 .......... ?????????? ?????????. .......... .......... .......... .......... ...----..? ???A...---
bn 003 ........?? ?????????? ??????.... .......... .......... .......... .......... ...----..? ?..A...---
bf 002 .......... .......... .......... .......... .......... .......... .......... ...----... ...A...---
bf 005 .......... .......... .......... .......... .......... .......... .......... ...----... ...A...---
st 001 .......... .......... .......... .......... .......... .......... .......... ...----... ...A...---
st 002 .......... .......... .......... .......... .......... .......... .......... ...----... ...A...---
cf 001 .......... .......... .......... .......... .......... .......... .......... ...---C... ..GC.G.A.-
cf 002 .......... .......... .......... .......... .......... .......... .......... ...---C... ..GC.G.A.-
cf 003 .......... .......... .......... .......... .......... .......... .......... ...---C... ..GC.G.A.-
cf 004 .......... .......... .......... .......... .......... .......... .......... ...---C... ..GC.G.A.-
db 021 ?????????? ?????????? ?????????? ?????????? .......... .......... .......... ...--CC... .A.CT.GA.-
db 025 .......... .......... .......... .......... .......... .......... .......... ...--CC... .A.CT.GA.-
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cp 023 .......... .......... .......... .......... .......... .......... .......... ...---C... ..AC...A.-
cr 041 .......... .......... .......... .......... .......... .......... .......... ...-----.. ...C...A.-
da 121 .......... .......... .......... .......... .......... .......... .......... ...---C... ..AC...A.-
da 122 .......... .......... .......... .......... .......... .......... .......... ...---C... ..AC...A.-
gs 011 .......... .......... .......... .......... .......... .......... .......... ...---C... A..C...A.-
gs 015 .......... .......... .......... .......... .......... .......... .......... ...---C... A..C...A.-
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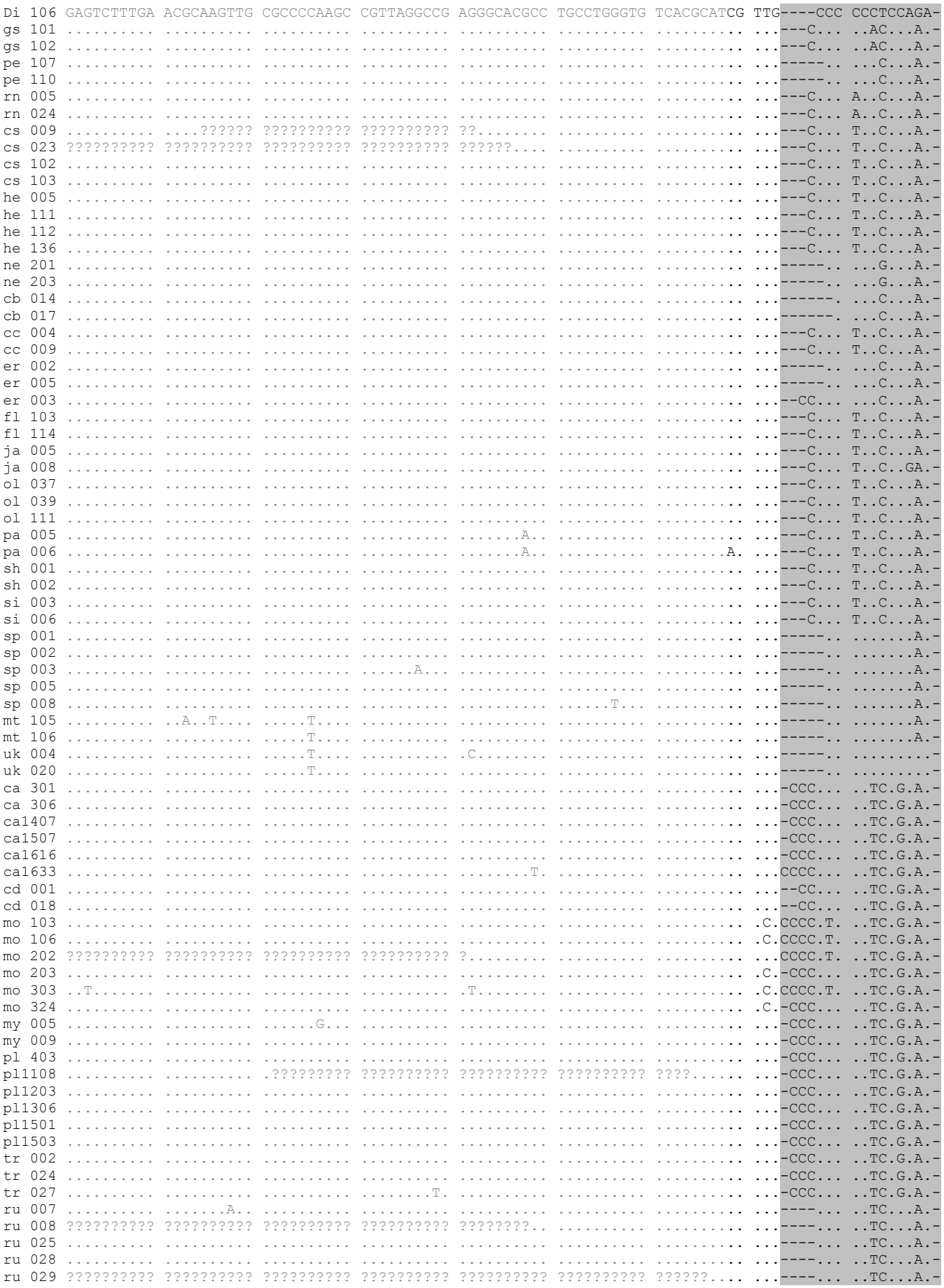
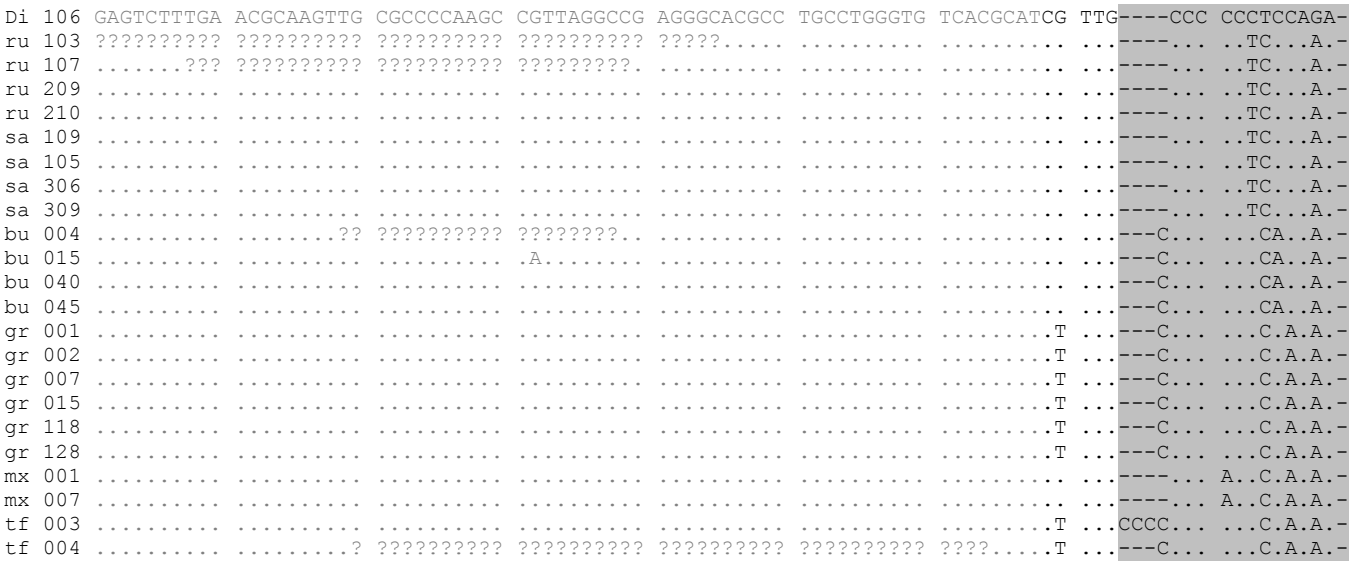
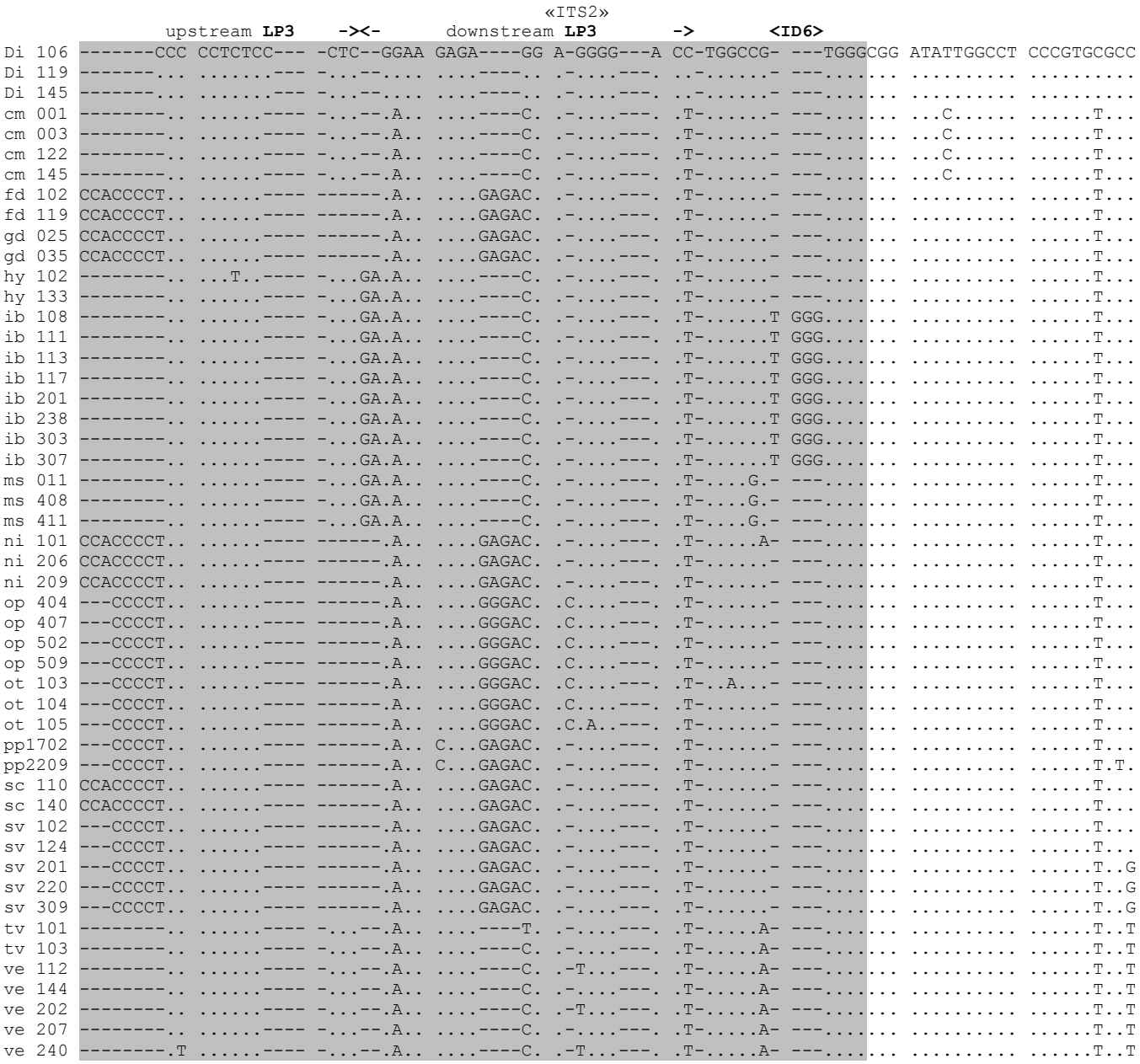
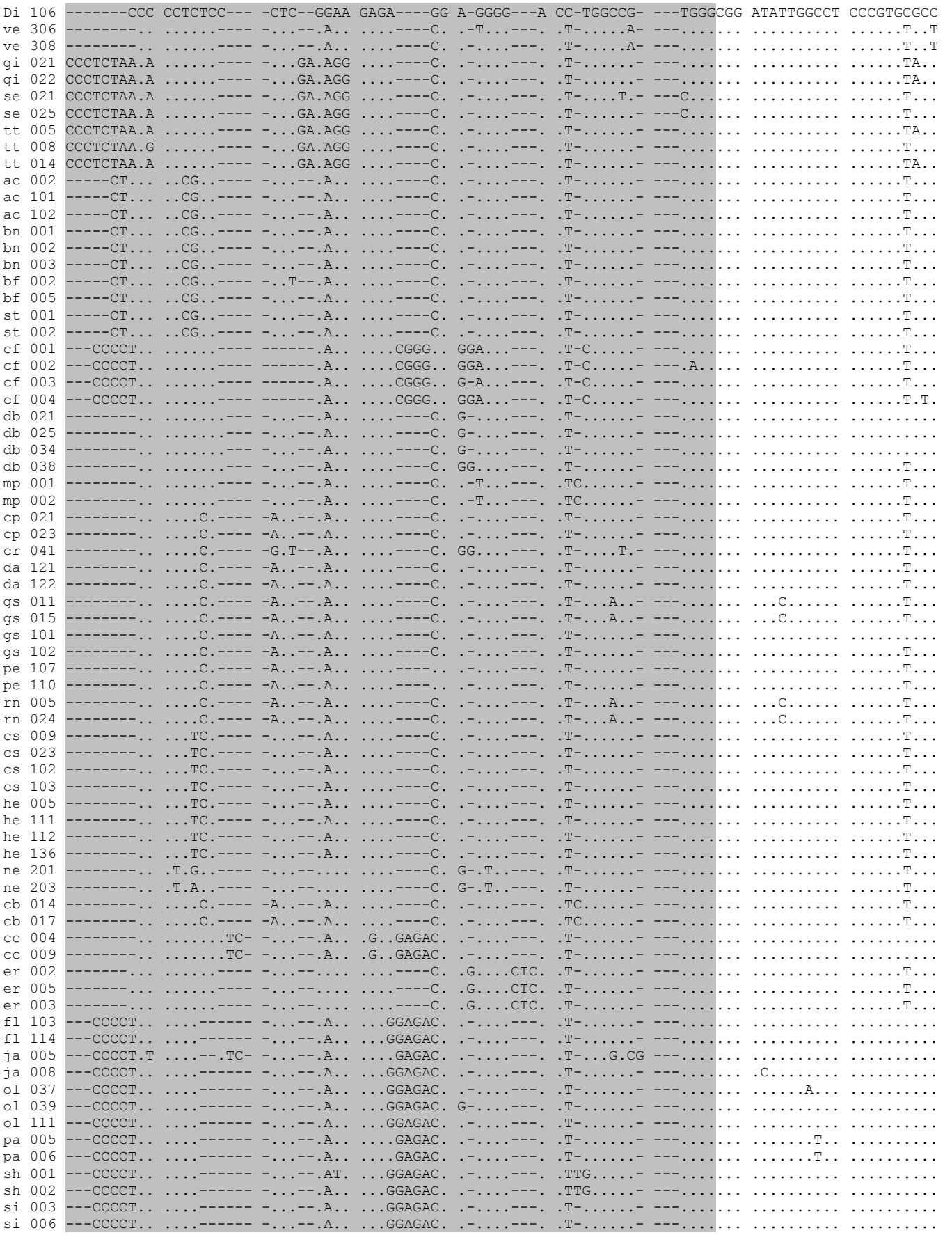
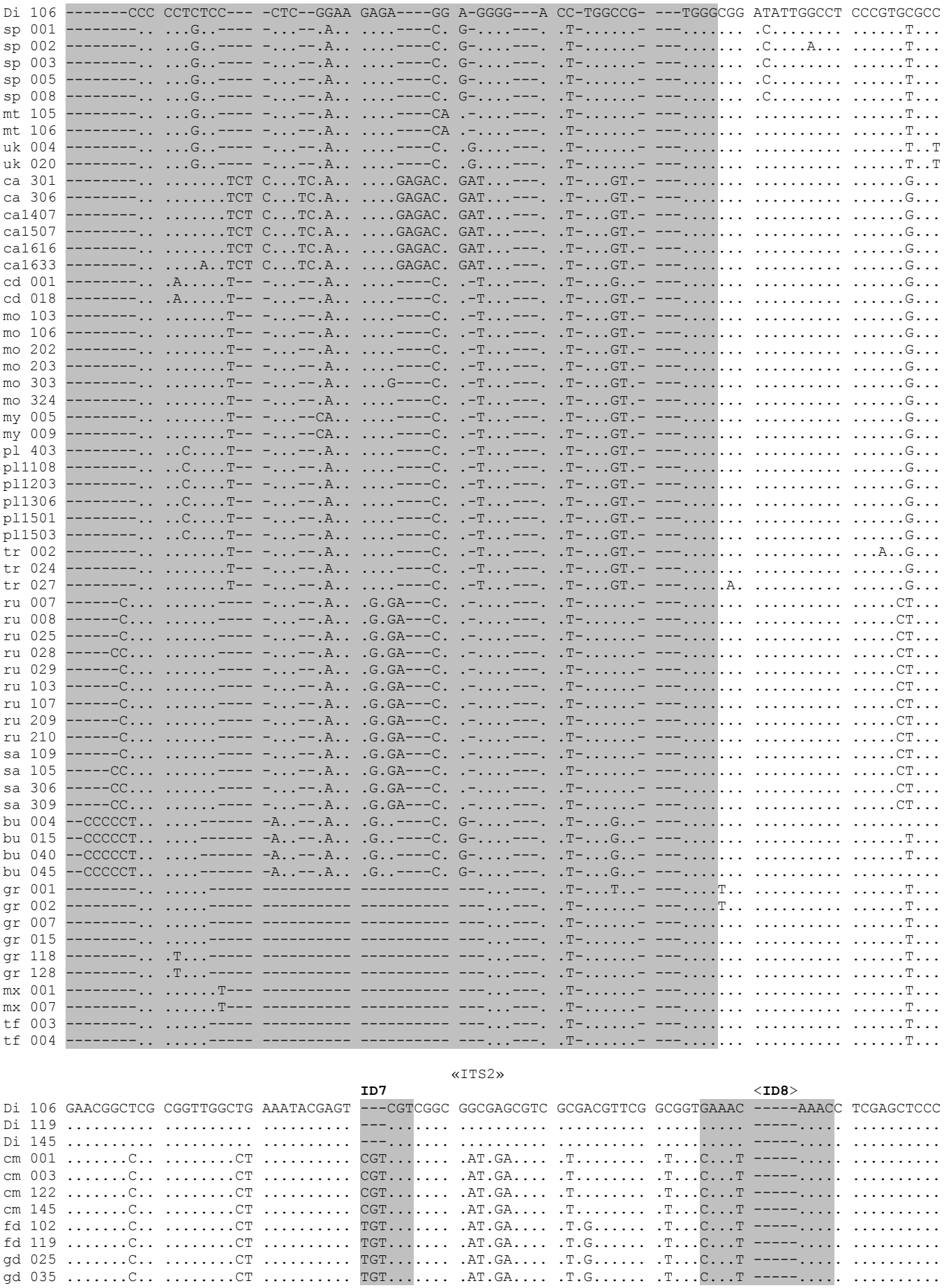





«ITS2»
upstream LP3
->
<-
downstream LP3
->
<ID6>




«ITS2»
ID7
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hy 102 .......C.. ........CT .....A.... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
hy 133 .......C.. ........CT .....A.... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ib 108 .......C.. ........CT .....A.... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ib 111 .......C.. ........CT .....A.... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ib 113 .......C.. ........CT .....A.... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ib 117 .......C.. ........CT .....A.... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ib 201 .......C.. ........CT .....A.... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ib 238 .......C.. ........CT .....A.... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ib 303 .......C.. ........CT .....A.... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ib 307 .......C.. ........CT .....A.... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ms 011 .......C.. ........CT .......... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ms 408 .......C.. ........CT .......... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ms 411 .......C.. ........CT .......... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ni 101 .......C.. ........CT .......... TGT..... .AT.GA.... .T.G...... .T...C...T -----.... ..........
ni 206 .......C.. ........CT .......... TGT..... .AT.GA.... .T.G...... .T...C...T -----.... ..........
ni 209 .......C.. ........CT .......... TGT..... .AT.GA.... .T.G...... .T...C...T -----.... ..........
op 404 .......C.. ........CT .......... TGT..C.... .AT.GA.... .T........ .T...C...T -----.... ..........
op 407 .......C.. ........CT .......... TGT..C.... .AT.GA.... .T........ .T...C...T -----.... ..........
op 502 .......C.. ........CT .......... TGT..C.... .AT.GA.... .T........ .T...C...T ---AA.... ..........
op 509 .......C.. ........CT .......... TGT..C.... .AT.GA.... .T........ .T...C...T ---AA.... ..........
ot 103 .......C.. ........CT .......... TGT..C.... .AT.GA.... .T........ .T...C...T -----.... ..........
ot 104 .......C.. ........CT .......... TGT..C.... .AT.GA.... .T........ .T...C...T ---AA.... ..........
ot 105 .......C.. ........CT .......... TAT..C.... .AT.GA.... .T........ .T...C...T -----.... ..........
pp1702 .......CT. ........CT .......... TGT..... .ATAGA.... .T........ .T...C...T ----A.... ..........
pp2209 .......CT. ........CT .......... TGT..... .ATAGA.... .T........ .T...C...T ----A.... ..........
sc 110 .......C.. ........CT .......... TGT..... .AT.GA.... .T.G...... .T...C...T -----.... ..........
sc 140 .......C.. .......TCT .......... TGT..... .AT.GA.... .T.G...... .T...C...T -----.... ..........
sv 102 .......C.. ........CT .......... TGT.AC..A. .A..GA.... .T........ .T...C...T -----.... ..........
sv 124 .......CT. ........CT .......... TGT.AC..A. .A..GA.... .T........ .T...C...T -----.... ..........
sv 201 .......C.. ........CT .......... TGT.AC..A. .A..GA.... .T........ .T...C...T -----.... ..........
sv 220 .......C.. ........CT .......... TGT.AC..A. .A..GA.... .T........ .T...C...T -----.... ..........
sv 309 .......C.. ........CT .......... TGT.AC..A. .A..GA.... .T........ .T...C...T -----.... ..........
tv 101 .......C.. ........CT .......... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
tv 103 .......C.. ........CT .......... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ve 112 .......C.. ........CT .......... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ve 144 .......C.. ........CT .......... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ve 202 .......C.. ........CT .......... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ve 207 .......C.. ........CT .......... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ve 240 .......C.. ........CT .......... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ve 306 .......C.A ........CT .......... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
ve 308 .......C.. ........CT .......... TGT..... .AT.GA.... .T........ .T...C...T -----.... ..........
gi 021 .......C.. ........CT .......... ---.A..... .A..GA.... .T........ .T..... GAAAT.... ..........
gi 022 .......C.. ........CT .......... ---.A..... .A..GA.... .T........ .T..... GAAAT.... ..........
se 021 .......C.. ........CT .......... ---..... .A..GA..C. .T........ .T...C.... GAAAT.... ..........
se 025 .......C.. ........CT .......... ---..... .A..GA..C. .T........ .T...C.... GAAAT.... ..........
tt 005 .......C.. ........CT .......... ---.A..... .A..GA.... .T........ .T..... GAAAT.... ..........
tt 008 .......C.. ........CT .......... ---.A..... .A..GA.... .T........ .T...C.... GAAAT.... ........A.
tt 014 .......C.. ........CT .......... ---.A..... .A..GA.... .T........ .T..... GAAAT.... ..........
ac 002 .......... ........CT .......... ---..C..A. .A..GA.... .T........ .T...C...T -----.... ..........
ac 101 .......... ........CT .......... ---..C..A. .A..GA.... .T........ .T...C...T -----.... ..........
ac 102 .......... ........CT .......... ---..C..A. .A..GA.... .T........ .T...C...T -----.... ..........
bn 001 .......... ........CT .......... ---..C..A. .AT.GA.... .T........ .T...C...T -----.... ..........
bn 002 .......... ........CT .......... ---..C..A. .A..GAT... .T........ .T...C...T -----.... ..........
bn 003 .......... ........CT .......... ---..C..A. .A..GAT... .T........ .T...C...T -----.... ..........
bf 002 .......... ........CT .......... ---..C..A. .AT.GA.... .T........ .T...C...T -----.... ..........
bf 005 .......... ........CT .......... ---..C..A. .AT.GA.... .T........ .T...C...T -----.... ..........
st 001 .......... ........CT .......... ---..C..A. .AT.GA.... .T........ .T...C...T -----.... ..........
st 002 .......... ........CT .......... ---..C..A. .AT.GA.... .T........ .T...C...T -----.... ..........
cf 001 .G.....C.. ........CT .......... CT-..... ..T.GA.... .......... .....C.... -----.... ..........
cf 002 .G.....C.. ........CT .......... CT-..... ..T.GA.... .......... .....C.... -----.... ..........
cf 003 .G.....C.. ........CT .......... CT-..... ..T.GA.... .......... .....C.... -----.... ..........
cf 004 .G.....C.. ........CT .......... CT-..... ..T.GA.... .......... .....C.... -----.... ..........
db 021 .C........ ........CT .......... ---..A.... .A..GA.... ......A... .T...CG..T -----.... .....G..T.
db 025 .C........ ........CT .......... ---..A.... .A..GA.... ......A... .T...CG..T -----.... .....G..T.
db 034 .C........ ........CT .......... ---..A.... .A..GA.... ......A... .T...CG..T -----.... .....G..T.
db 038 .C........ ........CT .......... ---..A.... .A..GA.... ......A... .T...CG..T -----.... .....G..T.
mp 001 .......... ........CT .......... ---..... .A..GA.... .T........ .T...CG..T -----.... .........T.
mp 002 .......... ........CT .......... ---..... .A..GA.... .T........ .T...CG..T -----.... .........T.
cp 021 .......C.. ........CT .......... ---..... .A..GA.... .T........ .T...CG..T -----.... .........T.
cp 023 .......C.. ........CT .......... ---..... .A..GA.... .T........ .T...CG..T -----.... .........T.
cr 041 .......... ........CT .....T.... ---..... .A..TA.... .T........ .T...CG..T -----.... .........T.
da 121 .......C.. ........CT .......... ---..... .A..GA.... .T........ .T...CG..T -----.... .........T.
da 122 .......C.. ........CT .......... ---..... .A..GA.... .T........ .T...CG..T -----.... .........T.
gs 011 .......... ........CT .......... ---..... .A..GA.... .T........ .T...CG..T -----.... .........T.
gs 015 .......... ........CT .......... ---..... .A..GA.... .T........ .T...CG..T -----.... .........T.
```

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Di 106 GAACGGCTCG CGGTTGGCTG AAATACGAGT ---CGTCGGC GGCGAGCGTC GCGACGTTCG GCGGTGAAAC -----AAACC TCGAGCTCCC
gs 101 .......C.. ........CT .......... ---..... .A..GA.... .T........ .T...CG..T -----.... .........T.
gs 102 .......C.. ........CT .......... ---..... .A..GA.... .T........ .TT..CG..T -----.... .........T.
pe 107 .......... ........CT ...A...... ---..... .A..GA.... .T....G... .T...CG..T -----.... ..........
pe 110 .......... ........CT ...A...... ---..... .A..GA.... .T....G... .T...CG..T -----.... ..........
rn 005 .......... ........CT .......... ---..... .A..GA.... .T........ .T...CG..T -----.... .........T.
rn 024 .......... ........CT .......... ---..... .A..GA.... .T........ .T...CG..T -----.... .........T.
cs 009 ...T...... ........CT .......... ---..... ..T.GA.... .T........ .T...C...T -----.... ..........
cs 023 ...T...... ........CT .......... ---..... ..T.GA.... .T........ .T...C...T -----.... ..........
cs 102 ...T...... ........CT .......... ---..... ..T.GA.... .T........ .T...C...T -----.... ..........
cs 103 ...T...... ........CT .......... ---..... ..T.GA.... .T........ .T...C...T -----.... ..........
he 005 ...T...... ........CT .......... ---..... ..T.GA.... .T........ .T...C...T -----.... ..........
he 111 ...T...... ........CT .......... ---..... ..T.GA.... .T........ .T...C...T -----.... ..........
he 112 ...T...... ........CT .......... ---..... ..T.GA.... .T........ .T...C...T -----.... ..........
he 136 ...T...... ........CT .......... ---..... ..T.GA.... .T........ .T...C...T -----.... ..........
ne 201 .......CT. ........CT .....T.... ---..... .AT.GA..C. .T........ .T...C...T -----.... ..........
ne 203 .......CT. ........CT .......... ---..... .AT.GA..C. .T........ .T...C...T -----.... .T........
cb 014 ...T...... ........CT ...C.T.... ---..... .AT.GA.... .T........ .T...CG..T -----.... .....G..T.
cb 017 ...T...... ........CT ...C.T.... ---..... .AT.GA.... .T........ .T...CG..T -----.... .....G..T.
cc 004 ...G...... ........CT .......... ---....A. ..T.GA.... .T........ .T...C.... -----.... ..........
cc 009 ...G...... ........CT .......... ---....A. ..T.GA.... .T........ .T...C.... -----.... ..........
er 002 .G.A...... ........CT .......... ---....A. ..T.GA.... .T........ .T...C...T -----.... A.........
er 005 .G.A...... ........CT .......... ---....A. ..T.GA.... .T........ .T...C...T -----.... A..G......
er 003 .G.A...... ........CT .......... ---....A. ..T.GA.... .T........ .T...C...T -----.... A.........
fl 103 ...G...... ........CT .......... ---....A. ....GA.... .T........ .T...C.... -----.... ..........
fl 114 ...G...... ........CT .......... ---....A. ....GA.... .T........ .T...C.... -----.... ..........
ja 005 ...G...... ........CT .......... ---....A. ....GA.... .T........ .T...C.... -----.... ..........
ja 008 ...G...... ........CT .......... ---....A. ..T.GA.... .T........ .T...C.... -----.... ..........
ol 037 ...G...... ........CT .......... ---....A. ..T.GA.... .T........ .T...C.... -----.... ..........
ol 039 ...G...... ........CT .......... ---....A. ...TGA.... .T........ .T...C.... -----.... ..........
ol 111 ...G...... ........CT .......... ---....A. ....GA.... .T........ .T...C.... -----.... ..........
pa 005 ...G...... ........CT .......... ---....A. ..T.GA.... .T........ .T...C.... -----.... ..........
pa 006 ...G...... ........CT .......... ---....A. ..T.GA.... .T........ .T...C.... -----.... ..........
sh 001 ...G...... ........CT .......... ---....A. ..T.GA.... .T........ .T...C.... -----.... ..........
sh 002 ...G...... ........CT .......... ---....A. ..T.GA.... .T........ .T...C.... -----.... ..........
si 003 ...G...... ........CT .......... ---....A. ..T.GA.... .T........ .T...C.... -----.... ..........
si 006 ...G...... ........CT .......... ---....A. ..T.GA.... .T........ .T...C.... -----.... ..........
sp 001 .......... ........CT .....T.... ---..... .AT.GA.... .T........ .T.....T -----.... ..........
sp 002 .......... ........CT .....T.... ---..... .AT.GA.... .T........ .T...C...T -----.... .......T..
sp 003 .......... ........CT .....T.... ---..... .AT.GA.... .T........ .T...C...T -----.... .......T..
sp 005 .......... ........CT .....T.... ---..... .AT.GA.... .T........ .T...C...T -----.... ..........
sp 008 .......... ........CT .....T.... ---..... .AT.GA.... .T......A. .T...C...T -----.... .......T..
mt 105 ...G...... ........CT .....T.... ---..... .AT.GA.... .T........ .T...C...T -----.... ..........
mt 106 ...G...... ........CT .....T.... ---..... .AT.GA.... .T........ .T...C...T -----.... ..........
uk 004 ...G...... ........CT .......... ---..... .A..GA..C. .T........ .T...C...T -----.... ...GATAA..
uk 020 ...G...... ........CT .......... ---..... .A..GA..C. .T........ .T...C...T -----.... ...GATAA..
ca 301 .......... ........CT ....TT.... ---..... .AT.GA..C. .T........ .T...CG..T -----.... .........T.
ca 306 .......... ........CT ....TT.... ---..... .AT.GA..C. .T........ .T...CG..T -----.... .........T.
ca1407 .......... ........CT ....TT.... ---..... .AT.GA..C. .T........ .T...CG..T -----.... .........T.
ca1507 .......... ........CT ....TT.... ---..... .AT.GA..C. .T........ .T...CG..T -----.... .........T.
ca1616 .......... ........CT ....TT.... ---..... .AT.GA..C. .T........ .T...CG..T -----.... .........T.
ca1633 ......G... ........CT ....TT.... ---..... .AT.GA..C. .T........ .T...CG..T -----.... .........T.
cd 001 .......... .T......CT ....CT.... ---..... ..T.GA.... .T........ .T...CG..T -----..G. .........T.
cd 018 .......... ........CT ....CT.... ---..... ..T.GA.... .T........ .T...CG..T -----..G. .........T.
mo 103 .......... ........CT ....TT.... ---..... ..T.GA..C. .T........ .T...CG..T -----.... .........T.
mo 106 .......... ........CT ....TT.... ---..... ..T.GA..C. .T........ .T...CG..T -----.... .........T.
mo 202 .......... ........CT ....TT.... ---..... .AT.G...C. .T........ .T...CG..T -----.... .........T.
mo 203 .......... ........CT ....TT.... ---..... .AT.G...C. .T........ .T...CGG.T -----.... .........T.
mo 303 .......... ........CT ....TT.... ---..... ..T.GA..C. .T........ .T...CG..T -----.... .........T.
mo 324 .......... ........CT ....TT.... ---..... ..T.GA..C. .T........ .T...CG..T -----.... .........T.
my 005 .......... ........CT ....T..... ---..... .AT.GA..C. .T........ .T...CG..T -----.... .........T.
my 009 .......... ........CT ....T..... ---..... .AT.GA..C. .T........ .T...CG..T -----.... .........T.
pl 403 .......... ........CT ....CT.... ---..... .AT.G...C. .T........ .T...CG..T -----.... .........T.
pl1108 .......... ........CT ....CT.... ---..... .AT.G...C. .T........ .T...CG..T -----.... .........T.
pl1203 .......... ........CT ....CT.... ---..... .AT.G...C. .T........ .T...CG..T -----.... .........T.
pl1306 .......... ........CT ....CT.... ---..... .AT.G...C. .T........ .T...CG..T -----.... .........T.
pl1501 .......... ........CT ....CT.... ---..... .AT.G...C. .T........ .T...CG..T -----.... .........T.
pl1503 .......... ........CT ....CT.... ---..... .AT.G...C. .T........ .T...CG..T -----.... .........T.
tr 002 .......... ........CT ....TT.... ---..... .AT.G.T.C. .T........ .T...CG..T -----.... .........T.
tr 024 .......... ........CT ....TT.... ---..... .AT.G...CT .T........ .T...CG..T -----.... .........T.
tr 027 .......... ........CT ....TT.... ---..... .AT.G...C. .T........ .T...CG..T -----.... .........T.
ru 007 ...T...... ........CT .......... ---..... .A..GA..C. .T........ .T...C...G -----.... ..........
ru 008 ...T...... ........CT .......... ---..... .A..GA..C. .T........ .T...C...G -----.... ..........
ru 025 ...T...... ........CT .......... ---..... .A..GA..C. .T........ .T...C...G -----.... ..........
ru 028 ...T...... ........CT .......... ---..... .A..GA..C. .T........ .T...C...G -----.... ..........
ru 029 ...T...... ........CT .......... ---..... .A..GA..C. .T........ .T...C...G -----.... ..........
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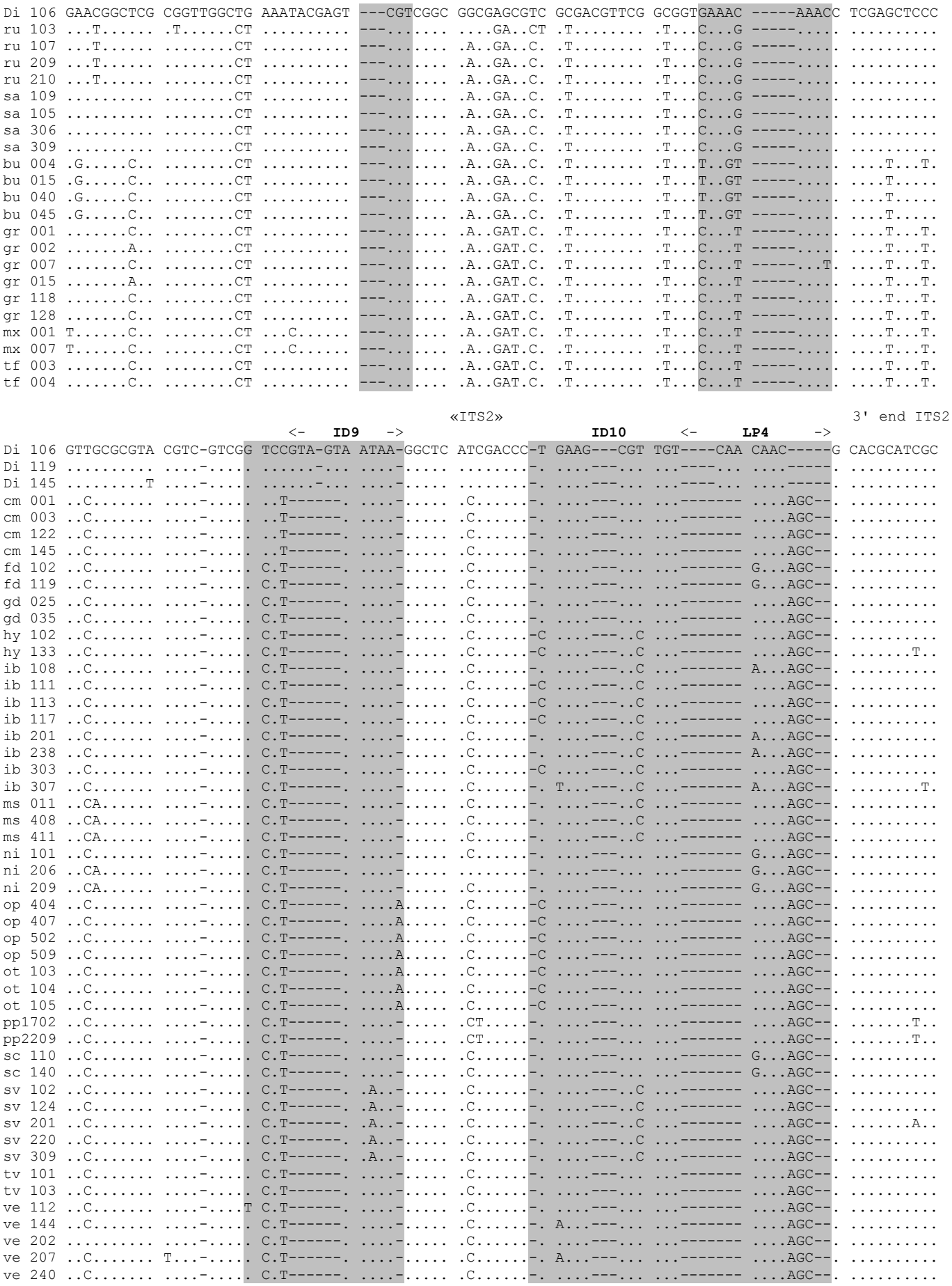
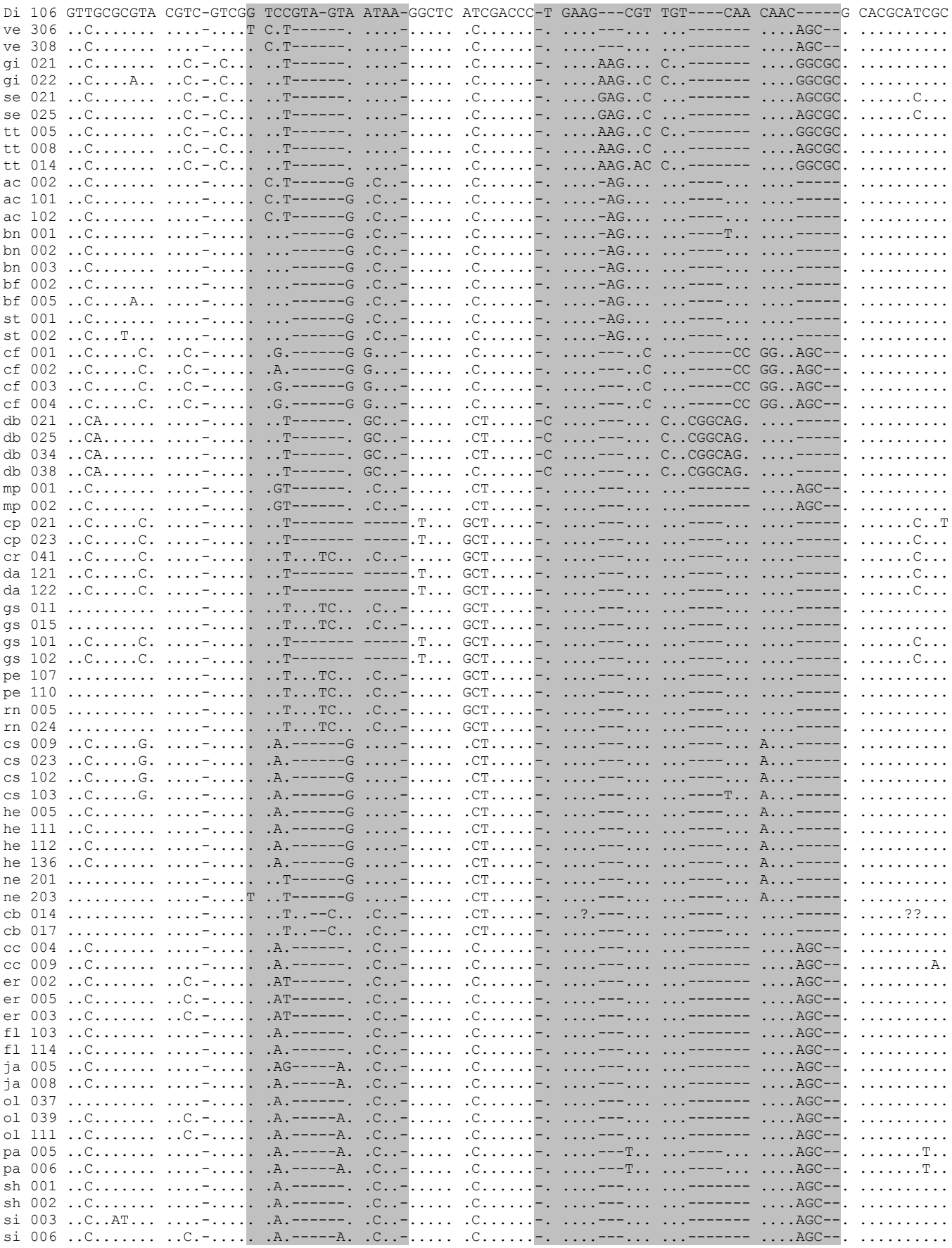

«ITS2»
3' end ITS2
<- ID9 ->
ID10
<- LP4 ->
Di 106 GAACGGCTCG CGGTTGGCTG AAATACGAGT ---CGTCGGC GGCGAGCGTC GCGACGTTCG GCGGTGAAAC -----AAACC TCGAGCTCCC
ru 103 ...T..... .T.....CT ..... ---..... ....GA..CT .T..... .T...C...G -----.... .....
ru 107 ...T..... .....CT ..... ---..... .A..GA..C. .T..... .T...C...G -----.... .....
ru 209 ...T..... .....CT ..... ---..... .A..GA..C. .T..... .T...C...G -----.... .....
ru 210 ...T..... .....CT ..... ---..... .A..GA..C. .T..... .T...C...G -----.... .....
sa 109 ....CT ..... ---..... .A..GA..C. .T..... .T...C...G -----.... .....
sa 105 ....CT ..... ---..... .A..GA..C. .T..... .T...C...G -----.... .....
sa 306 ....CT ..... ---..... .A..GA..C. .T..... .T...C...G -----.... .....
sa 309 ....CT ..... ---..... .A..GA..C. .T..... .T...C...G -----.... .....
bu 004 .G.....C.. .....CT ..... ---..... .A..GA..C. .T..... .T...T..GT -----.... ....T...T.
bu 015 .G.....C.. .....CT ..... ---..... .A..GA..C. .T..... .T...T..GT -----.... ....T.....
bu 040 .G.....C.. .....CT ..... ---..... .A..GA..C. .T..... .T...T..GT -----.... ....T.....
bu 045 .G.....C.. .....CT ..... ---..... .A..GA..C. .T..... .T...T..GT -----.... ....T.....
gr 001 .....C.. .....CT ..... ---..... .A..GAT.C. .T..... .T...C...T -----.... ....T...T.
gr 002 .....A.. .....CT ..... ---..... .A..GAT.C. .T..... .T...C...T -----.... ....T...T.
gr 007 .....C.. .....CT ..... ---..... .A..GAT.C. .T..... .T...C...T -----...T. ....T...T.
gr 015 .....A.. .....CT ..... ---..... .A..GAT.C. .T..... .T...C...T -----.... ....T...T.
gr 118 .....C.. .....CT ..... ---..... .A..GAT.C. .T..... .T...C...T -----.... ....T...T.
gr 128 .....C.. .....CT ..... ---..... .A..GAT.C. .T..... .T...C...T -----.... ....T...T.
mx 001 T.....C.. .....CT ...C..... ---..... .A..GAT.C. .T..... .T...C...T -----.... ....T...T.
mx 007 T.....C.. .....CT ...C..... ---..... .A..GAT.C. .T..... .T...C...T -----.... ....T...T.
tf 003 .....C.. .....CT ..... ---..... .A..GAT.C. .T..... .T...C...T -----.... ....T...T.
tf 004 .....C.. .....CT ..... ---..... .A..GAT.C. .T..... .T...C...T -----.... ....T...T.
Di 106 GTTGCGCGTA CGTC-GTCGG TCCGTA-GTA ATAA-GGCTC ATCGACCC-T GAAG---CGT TGT----CAA CAAC-----G CACGCATCGC
Di 119 ....-.... ......-... ....-..... .....-. ....---... ...----... ....-----. .....
Di 145 .....T .....-.... ......-... ....-..... .....-. ....---... ...----... ....-----. .....
cm 001 ..C..... ....-.... ..T------. ....-..... .C.....-. ....---... ...------- ....AGC--. .....
cm 003 ..C..... ....-.... ..T------. ....-..... .C.....-. ....---... ...------- ....AGC--. .....
cm 122 ..C..... ....-.... ..T------. ....-..... .C.....-. ....---... ...------- ....AGC--. .....
cm 145 ..C..... ....-.... ..T------. ....-..... .C.....-. ....---... ...------- ....AGC--. .....
fd 102 ..C..... ....-.... C.T------. ....-..... .C.....-. ....---... ...------- G...AGC--. .....
fd 119 ..C..... ....-.... C.T------. ....-..... .C.....-. ....---... ...------- G...AGC--. .....
gd 025 ..C..... ....-.... C.T------. ....-..... .C.....-. ....---... ...------- ....AGC--. .....
gd 035 ..C..... ....-.... C.T------. ....-..... .C.....-. ....---... ...------- ....AGC--. .....
hy 102 ..C..... ....-.... C.T------. ....-..... .C.....-C ....---..C ...------- ....AGC--. .....
hy 133 ..C..... ....-.... C.T------. ....-..... .C.....-C ....---..C ...------- ....AGC--. ....T..
ib 108 ..C..... ....-.... C.T------. ....-..... .C.....-. ....---..C ...------- A...AGC--. .....
ib 111 ..C..... ....-.... C.T------. ....-..... .C.....-C ....---..C ...------- ....AGC--. .....
ib 113 ..C..... ....-.... C.T------. ....-..... .C.....-C ....---..C ...------- ....AGC--. .....
ib 117 ..C..... ....-.... C.T------. ....-..... .C.....-C ....---..C ...------- ....AGC--. .....
ib 201 ..C..... ....-.... C.T------. ....-..... .C.....-. ....---..C ...------- A...AGC--. .....
ib 238 ..C..... ....-.... C.T------. ....-..... .C.....-. ....---..C ...------- A...AGC--. .....
ib 303 ..C..... ....-.... C.T------. ....-..... .C.....-C ....---..C ...------- ....AGC--. .....
ib 307 ..C..... ....-.... C.T------. ....-..... .C.....-. T...---..C ...------- A...AGC--. .....T.
ms 011 ..CA..... ....-.... C.T------. ....-..... .C.....-. ....---..C ...------- ....AGC--. .....
ms 408 ..CA..... ....-.... C.T------. ....-..... .C.....-. ....---..C ...------- ....AGC--. .....
ms 411 ..CA..... ....-.... C.T------. ....-..... .C.....-. ....---..C ...------- ....AGC--. .....
ni 101 ..C..... ....-.... C.T------. ....-..... .C.....-. ....---... ...------- G...AGC--. .....
ni 206 ..CA..... ....-.... C.T------. ....-..... .....-. ....---... ...------- G...AGC--. .....
ni 209 ..CA..... ....-.... C.T------. ....-..... .C.....-. ....---... ...------- G...AGC--. .....
op 404 ..C..... ....-.... C.T------. ....A..... .C.....-C ....---... ...------- ....AGC--. .....
op 407 ..C..... ....-.... C.T------. ....A..... .C.....-C ....---... ...------- ....AGC--. .....
op 502 ..C..... ....-.... C.T------. ....A..... .C.....-C ....---... ...------- ....AGC--. .....
op 509 ..C..... ....-.... C.T------. ....A..... .C.....-C ....---... ...------- ....AGC--. .....
ot 103 ..C..... ....-.... C.T------. ....A..... .C.....-C ....---... ...------- ....AGC--. .....
ot 104 ..C..... ....-.... C.T------. ....A..... .C.....-C ....---... ...------- ....AGC--. .....
ot 105 ..C..... ....-.... C.T------. ....A..... .C.....-C ....---... ...------- ....AGC--. .....
pp1702 ..C..... ....-.... C.T------. ....-..... .CT.....-. ....---... ...------- ....AGC--. ....T..
pp2209 ..C..... ....-.... C.T------. ....-..... .CT.....-. ....---... ...------- ....AGC--. ....T..
sc 110 ..C..... ....-.... C.T------. ....-..... .C.....-. ....---... ...------- G...AGC--. .....
sc 140 ..C..... ....-.... C.T------. ....-..... .C.....-. ....---... ...------- G...AGC--. .....
sv 102 ..C..... ....-.... C.T------. .A..-..... .C.....-. ....---..C ...------- ....AGC--. .....
sv 124 ..C..... ....-.... C.T------. .A..-..... .C.....-. ....---..C ...------- ....AGC--. .....
sv 201 ..C..... ....-.... C.T------. .A..-..... .C.....-. ....---..C ...------- ....AGC--. ....A..
sv 220 ..C..... ....-.... C.T------. .A..-..... .C.....-. ....---..C ...------- ....AGC--. .....
sv 309 ..C..... ....-.... C.T------. .A..-..... .C.....-. ....---..C ...------- ....AGC--. .....
tv 101 ..C..... ....-.... C.T------. ....-..... .C.....-. ....---... ...------- ....AGC--. .....
tv 103 ..C..... ....-.... C.T------. ....-..... .C.....-. ....---... ...------- ....AGC--. .....
ve 112 ..C..... ....-....T C.T------. ....-..... .C.....-. ....---... ...------- ....AGC--. .....
ve 144 ..C..... ....-.... C.T------. ....-..... .C.....-. A...---... ...------- ....AGC--. .....
ve 202 ....-.... C.T------. ....-..... .C.....-. ....---... ...------- ....AGC--. .....
ve 207 ..C..... T...-.... C.T------. ....-..... .C.....-. A...---... ...------- ....AGC--. .....
ve 240 ..C..... ....-.... C.T------. ....-..... .C.....-. ....---... ...------- ....AGC--. .....


Di 106 GTTGCGCGTA CGTC-GTCGG TCCGTA-GTA ATAA-GGCTC ATCGACCC-T GAAG---CGT TGT----CAA CAAC-----G CACGCATCGC
ve 306 ..C..... ....-....T C.T------. ....-..... .C.....-. ....---... ...------- ....AGC--. ....
ve 308 ..C..... ....-..... C.T------. ....-..... .C.....-. ....---... ...------- ....AGC--. ....
gi 021 ..C..... ..C.-.C.. ..T------. ....-..... .C.....-. ....AAG... C..------- ....GGCGC. ....
gi 022 ..C....A.. ..C.-.C.. ..T------. ....-..... .C.....-. ....AAG..C C..------- ....GGCGC. ....
se 021 ..C..... ..C.-.C.. ..T------. ....-..... .C.....-. ....GAG..C ...------- ....AGCGC. ......C...
se 025 ..C..... ..C.-.C.. ..T------. ....-..... .C.....-. ....GAG..C ...------- ....AGCGC. ......C...
tt 005 ..C..... ..C.-.C.. ..T------. ....-..... .C.....-. ....AAG..C C..------- ....GGCGC. ....
tt 008 ..C..... ..C.-.C.. ..T------. ....-..... .C.....-. ....AAG..C ...------- ....AGCGC. ....
tt 014 ..C..... ..C.-.C.. ..T------. ....-..... .C.....-. ....AAG.AC C..------- ....GGCGC. ....
ac 002 ..C..... ....-.... C.T------G .C..-..... .C.....-. ....-AG... ...----... ....-----. ....
ac 101 ..C..... ....-.... C.T------G .C..-..... .C.....-. ....-AG... ...----... ....-----. ....
ac 102 ..C..... ....-.... C.T------G .C..-..... .C.....-. ....-AG... ...----... ....-----. ....
bn 001 ..C..... ....-.... ...------G .C..-..... .C.....-. ....-AG... ...----T.. ....-----. ....
bn 002 ..C..... ....-.... ...------G .C..-..... .C.....-. ....-AG... ...----... ....-----. ....
bn 003 ..C..... ....-.... ...------G .C..-..... .C.....-. ....-AG... ...----... ....-----. ....
bf 002 ..C..... ....-.... ...------G .C..-..... .C.....-. ....-AG... ...----... ....-----. ....
bf 005 ..C....A.. ....-.... ...------G .C..-..... .C.....-. ....-AG... ...----... ....-----. ....
st 001 ..C..... ....-.... ...------G .C..-..... .C.....-. ....-AG... ...----... ....-----. ....
st 002 ..C...T... ....-.... ...------G .C..-..... .C.....-. ....-AG... ...----... ....-----. ....
cf 001 ..C.....C. ..C.-.... .G.------G G...-..... .C.....-. ....---..C ...-----CC GG..AGC--. ....
cf 002 ..C.....C. ..C.-.... .A.------G G...-..... .C.....-. ....---..C ...-----CC GG..AGC--. ....
cf 003 ..C.....C. ..C.-.... .G.------G G...-..... .C.....-. ....---..C ...-----CC GG..AGC--. ....
cf 004 ..C.....C. ..C.-.... .G.------G G...-..... .C.....-. ....---..C ...-----CC GG..AGC--. ....
db 021 ..CA..... ....-.... ..T------. GC..-..... .CT.....-C ....---... C..CGGCAG. ....-----. ....
db 025 ..CA..... ....-.... ..T------. GC..-..... .CT.....-C ....---... C..CGGCAG. ....-----. ....
db 034 ..CA..... ....-.... ..T------. GC..-..... .CT.....-C ....---... C..CGGCAG. ....-----. ....
db 038 ..CA..... ....-.... ..T------. GC..-..... .C.....-C ....---... C..CGGCAG. ....-----. ....
mp 001 ..C..... ....-.... .GT------. .C..-..... .CT.....-. ....---... ...------- ....AGC--. ....
mp 002 ..C..... ....-.... .GT------. .C..-..... .CT.....-. ....---... ...------- ....AGC--. ....
cp 021 ..C.....C. ....-.... ..T------- -----.T... GCT.....-. ....---... ....----... ....-----. ......C..T
cp 023 ..C.....C. ....-.... ..T------- -----.T... GCT.....-. ....---... ....----... ....-----. ......C...
cr 041 ..C.....C. ....-.... ..T...TC.. .C..-..... GCT.....-. ....---... ....----... ....-----. ......C...
da 121 ..C.....C. ....-.... ..T------- -----.T... GCT.....-. ....---... ....----... ....-----. ......C...
da 122 ..C.....C. ....-.... ..T------- -----.T... GCT.....-. ....---... ....----... ....-----. ......C...
gs 011 ....-.... ..T...TC.. .C..-..... GCT.....-. ....---... ....----... ....-----. ....
gs 015 ....-.... ..T...TC.. .C..-..... GCT.....-. ....---... ....----... ....-----. ....
gs 101 ..C.....C. ....-.... ..T------- -----.T... GCT.....-. ....---... ....----... ....-----. ......C...
gs 102 ..C.....C. ....-.... ..T------- -----.T... GCT.....-. ....---... ....----... ....-----. ......C...
pe 107 ....-.... ..T...TC.. .C..-..... GCT.....-. ....---... ....----... ....-----. ....
pe 110 ....-.... ..T...TC.. .C..-..... GCT.....-. ....---... ....----... ....-----. ....
rn 005 ....-.... ..T...TC.. .C..-..... GCT.....-. ....---... ....----... ....-----. ....
rn 024 ....-.... ..T...TC.. .C..-..... GCT.....-. ....---... ....----... ....-----. ....
cs 009 ..C.....G. ....-.... .A.------G ....-..... .CT.....-. ....---... ...----... A...-----. ....
cs 023 ..C.....G. ....-.... .A.------G ....-..... .CT.....-. ....---... ...----... A...-----. ....
cs 102 ..C.....G. ....-.... .A.------G ....-..... .CT.....-. ....---... ...----... A...-----. ....
cs 103 ..C.....G. ....-.... .A.------G ....-..... .CT.....-. ....---... ....----T.. A...-----. ....
he 005 ..C..... ....-.... .A.------G ....-..... .CT.....-. ....---... ...----... A...-----. ....
he 111 ..C..... ....-.... .A.------G ....-..... .CT.....-. ....---... ...----... A...-----. ....
he 112 ..C..... ....-.... .A.------G ....-..... .CT.....-. ....---... ...----... A...-----. ....
he 136 ..C..... ....-.... .A.------G ....-..... .CT.....-. ....---... ...----... A...-----. ....
ne 201 ....-.... ..T------G ....-..... .CT.....-. ....---... ...----... A...-----. ....
ne 203 ....-....T ..T------G ....-..... .CT.....-. ....---... ...----... A...-----. ....
cb 014 ....-.... ..T..--C.. .C..-..... .CT.....-. ..?.---... ...----... ....-----. .....??...
cb 017 ....-.... ..T..--C.. .C..-..... .CT.....-. ....---... ...----... ....-----. ....
cc 004 ..C..... ....-.... .A.------. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....
cc 009 ..C..... ....-.... .A.------. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....A.
er 002 ..C..... ..C.-.... .AT------. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....
er 005 ..C..... ..C.-.... .AT------. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....
er 003 ..C..... ..C.-.... .AT------. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....
fl 103 ..C..... ....-.... .A.------. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....
fl 114 ..C..... ....-.... .A.------. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....
ja 005 ..C..... ....-.... .AG-----A. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....
ja 008 ..C..... ....-.... .A.-----A. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....
ol 037 ....-.... .A.------. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....
ol 039 ..C..... ..C.-.... .A.-----A. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....
ol 111 ..C..... ..C.-.... .A.-----A. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....
pa 005 ..C..... ....-.... .A.-----A. .C..-..... .C.....-. ....---T.. ...----... ....AGC--. ....T..
pa 006 ..C..... ....-.... .A.-----A. .C..-..... .C.....-. ....---T.. ...----... ....AGC--. ....T..
sh 001 ..C..... ....-.... .A.------. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....
sh 002 ..C..... ....-.... .A.------. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....
si 003 ..C..AT... ....-.... .A.------. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....
si 006 ..C..... ..C.-.... .A.-----A. .C..-..... .C.....-. ....---... ...------- ....AGC--. ....

```
Di 106 GTTGCGCGTA CGTC-GTCGG TCCGTA-GTA ATAA-GGCTC ATCGACCC-T GAAG---CGT TGT----CAA CAAC-----G CACGCATCGC
sp 001 ..C....... ....-.... ..T------. ....-..... .CT.....-. ....---... ...----.G. ....-----. ..........
sp 002 ..C....... ....-.... ..T------. ....-..... .CT.....-. ....---... ...----.G. ....-----. ..........
sp 003 ..C....... ....-.... ..T------. ....-..... .CT.....-. ....---... ...----.G. ....-----. ..........
sp 005 ..C....... ....-.... ..T------. ....-..... .CT.....-. ....---... ...----.G. ....-----. ..........
sp 008 ..C....... ....-.... ..T------. ....-..... .CT.....-. ....---... ...----.G. ....-----. ..........
mt 105 .......... ....-.... ..T------. ....-..... .CT.....-. ....---... ...----... ....-----. ..........
mt 106 .......... ....-.... ..T------. ....-..... .CT.....-. ....---... ...----... ....-----. ..........
uk 004 .......... ....-.... ..T------G ....-..... .CT.....-. ....---... ...----.G. ....-----. ..........
uk 020 .......... ....-.... ..T------G ....-..... .CT.....-. ....---... ...----.G. ....-----. ..........
ca 301 ..C......C .T..-.... .AT------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
ca 306 ..C......C .T..C.... .A.------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
ca1407 ..C......C .T..C.... .A.------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
ca1507 ..C......C .T..C.... .A.------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
ca1616 ..C......C .T..-.... .A.------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
ca1633 ..C......C .T..-.... .A.------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
cd 001 ..C..T...C ....-.... .AT------. ....-..... .CT.....-. ....---... ...----.G. ....-----. ..........
cd 018 ..C..T...C ....-.... .AT------. ....-..... .CT.....-. ....---... ...----... ....-----. ..........
mo 103 ..C......C ....-.... .A.------. G...-..... .C......C. ....---... ...----... G...-----. ..........
mo 106 ..C......C ....-.... .A.------. G...-..... .C.....-. ....---... ...----... G...-----. ..........
mo 202 ..C......C ....-.... .A.------. G...-..... .CT.....-. ....---... ...----... G...-----. ..........
mo 203 A.C......C ....-.... .A.------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
mo 303 ..C......C ....-.... .A.------. G...-..... .C......C. ....---... ...----... G...-----. ..........
mo 324 ..C......C ....-.... .A.------. GC..-..... .C.....-. ....---... ...----... G...-----. ..........
my 005 ..C......C ....-.... .A.------. G...-..... .CT..A..-. ....---... ...----... ....-----. ..........
my 009 ..C......C ....-.... .A.------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
pl 403 ..C......C ....-.... .AG------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
pl1108 ..C......C ....-.... .AG------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
pl1203 ..C......C ....-.... .AG------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
pl1306 ..C......C ....-.... .AG------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
pl1501 ..C......C .A..-.... .AG------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
pl1503 ..C......C ....-.... .AG------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
tr 002 ..C......C ....-.... .A.------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
tr 024 ..C......C ....-.... .A.------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
tr 027 ..C......C ....-.... .A.------. G...-..... .CT.....-. ....---... ...----... ....-----. ..........
ru 007 ..C....... ....-.... ...------G ....-..... .C......-. ...----..C ...------- GG..AGC--. ......C...
ru 008 ..C....... ....-.... ...------G ....-..... .C......-. ...----..C ...------- GG..AGC--. ......C...
ru 025 ..C....... ....-.... ...------G ....-..... .C......-. ....---..C ...------- GG..AGC--. ......C...
ru 028 ..C....... ....-.... ...------G ....-..... .C......-. ....---..C ...------- GG..AGC--. ......C...
ru 029 ..C....... ....-.... ...------G ....-..... .C......-. ....---..C ...------- GG..AGC--. ......C...
ru 103 ..C....... ....-.... ...------G ....-..... .C......-. ...----..C ...------- GG..AGC--. ......C...
ru 107 ..C....... ....-.... ...------G ....-..... .C......-. ....---..C ...------- GG..AGC--. ......C...
ru 209 ..C....... ....-.... ...------G ....-..... .C......-. ...----..C ...------- GG..AGC--. ......C...
ru 210 ..C....... ....-.... ...------G ....-..... .C......-. ...----..C ...------- GG..AGC--. ......C...
sa 109 ..C....... ....-.... ...------G ....-..... .C......-. ....---..C ..C------- GG..AGC--. ......C...
sa 105 ..C....... ....-.... ...------G ....-..... .C......-. ....---..C ..C------- GG..AGC--. ......C...
sa 306 ..C....... ....-.... ...------G ....-..... .C......-. ....---..C ..C------- GG..AGC--. ......C...
sa 309 ..C....... ....-.... ...------G ....-..... .C......-. ....---..C ..C------- GG..AGC--. ......C...
bu 004 ..C.G..... ....-.... ...------G G...-..... .C......-. ....---... ...------- ....AGC--. ..........
bu 015 ..C....... ....-.... ...------G ....-..... .C......-. ....---... ...------- ....AGC--. ..........
bu 040 ..C....... ....-.... ...------G ....-..... .C......-. ....---... ...------- ....AGC--. ..........
bu 045 ..C....... ....-.... ...------G ....-..... .C......-. ....---... ...------- ....AGC--. ..........
gr 001 ..C....... ....-.... ...------G ....-..... ........-. ....---... ...------- A...AGC--. ..........
gr 002 ..C....... ....-.... ...------G ....-..... ........-. ....---... ...------- A...AGC--. ..........
gr 007 ..C....... ....-.... A..------G ....-..... ........-. ....---... ...------- ....AGC--. ..........
gr 015 ..C....... ....-.... A..------G ....-..... ........-. ....---... ...------- ....AGC--. ..........
gr 118 ..C....... ....-.... A..------G ....-..... ........-. ....---... ...------- ....AGC--. ..........
gr 128 ..C....... ....-.... ...------G ....-..... ........-. ....---... ...------- A...AGC--. ..........
mx 001 ..C....... ....-.... ...------G ....-..... .C......-. A...---... ...------- ....AGC--. ..........
mx 007 ..C....... ....-.... ...------G ....-..... .C......-. A...---... ...------- ....AGC--. ..........
tf 003 ..C....... ....-.... ...------G ....-..... ........-. ....---... ...------- ....AGC--. ..........
tf 004 ..C....... ....-.... ...------G ....-..... ........-. ....---... ...------- ....AGC--. ..........

       5' end 26S rDNA
Di 106 GACCCCAGGT CAGGCGGGAT TACCCGCTGA GTT
Di 119 .......... .......... .......... ...
Di 145 .......... .......... .......... ...
cm 001 .......... .......... .......... ...
cm 003 .......... .......... ........?? ???
cm 122 .......... .......... .......... ...
cm 145 .......... .......... .......... ...
fd 102 .......... .......... .......... ...
fd 119 .......... .......... .......... ...
gd 025 .......... .......... .......... ...
gd 035 .......... .......... .......... ...
hy 102 .......... .......... .......... ...
hy 133 .......... .......... .......... ...
```

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Di 106 GACCCCAGGT CAGGCGGGAT TACCCGCTGA GTT
ib 108 .......... .......... .......... ...
ib 111 .......... .......... .......... ...
ib 113 .......... .......... .......... ...
ib 117 .......... .......... .......... ...
ib 201 .......... .......... .......... ...
ib 238 .......... .......... .......... ...
ib 303 .......... .......... .......... ...
ib 307 .......... .......... .......... ...
ms 011 .......... .......... .......... ...
ms 408 .......... .......... .......... ...
ms 411 .......... .......... .......... ...
ni 101 .......... .......... .......... ...
ni 206 .......... .......... .......... ...
ni 209 .......... .......... .......... ...
op 404 .......... .......... .......... ...
op 407 .......... .......... .......... ...
op 502 .......... .......... .......... ...
op 509 .......... .......... .......... ...
ot 103 .......... .......... .......... ...
ot 104 .......... .......... .......... ...
ot 105 .......... .......... .......... ...
pp1702 .......T.. .......... .......... ...
pp2209 .......... .......... .......... ...
sc 110 .......... .......... .......... ...
sc 140 .......... .......... .......... ...
sv 102 .......... .......... .......... ...
sv 124 .......... .......... .......... ...
sv 201 .......... .......... .......... ...
sv 220 .......... .......... .......... ...
sv 309 .......... .......... .......??? ???
tv 101 .......... .......... .......... ...
tv 103 .......... .......... .......... ...
ve 112 .......... .......... .......... ...
ve 144 .......... .......... .......... ...
ve 202 .......... .......... .......... ...
ve 207 .......... .......... .......... ...
ve 240 .......... .......... .......... ...
ve 306 .......... .......... .......... ...
ve 308 .......... .......... .......... ...
gi 021 .......... .......... .......... ...
gi 022 .......... .......... .......... ...
se 021 .......... .......... .......... ...
se 025 .......... .......... .......... ...
tt 005 .......... .......... .......... ...
tt 008 .......... .......... .......... ...
tt 014 .......... .......... .......... ...
ac 002 .......... .......... .......... ...
ac 101 .......... .......... .......... ...
ac 102 .......... .......... .......... ...
bn 001 .......... .......... .......... ...
bn 002 .......... .......... .......... ...
bn 003 .......... .......... .......... ...
bf 002 .......... .......... .......... ...
bf 005 .......... .......... .......... ...
st 001 .......... .......... .......... ...
st 002 .......... .......... .......... ...
cf 001 .......... .......... C......... ...
cf 002 .......... .......... C......... ...
cf 003 .......... .......... C......... ...
cf 004 .........C .......... C......... ...
db 021 .......... .......... .......... ...
db 025 .......... .......... .......... ...
db 034 .......... .......... .......... ...
db 038 .......... .......... .......... ...
mp 001 .......... .......... .......... ...
mp 002 .......... .......... .......... ...
cp 021 .......... .......... .......... ...
cp 023 .......... .......... .......... ...
cr 041 .......... .......... .......... ...
da 121 .......... .......... .......... ...
da 122 .......... .......... .......... ...
gs 011 .......... .......... .......... ...
gs 015 .......... .......... .......... ...
gs 101 .......... .......... .......... ...
gs 102 .......... .......... .......... ...
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Di 106 GACCCCAGGT CAGGCGGGAT TACCCGCTGA GTT
pe 107 .......... .......... C......... ...
pe 110 .......... .......... C...T..... ...
rn 005 .......... .......... .......... ...
rn 024 .......... .......... .......... ...
cs 009 .......... .......... .......... ...
cs 023 .......... .......... .......... ...
cs 102 .......... .......... .......... ...
cs 103 .......... .......... .......... ...
he 005 .......... .......... .......... ...
he 111 .......... .......... .......... ...
he 112 .......... .......... .......... ...
he 136 .......... .......... .......... ...
ne 201 .......... .......... .......... ...
ne 203 .......... .......... .......... ...
cb 014 ......???? ?????????? ?????????? ???
cb 017 .......... .......... .......... ...
cc 004 .......... .......... .......... ...
cc 009 .......... .......... .......... ...
er 002 .......... .......... .......... ...
er 005 .......... .......... .......... ...
er 003 .......... .......... .......... ...
fl 103 .......... .......... .......... ...
fl 114 .......... .......... .......... ...
ja 005 .......... .......... .......... ...
ja 008 .......... .......... .?........ ...
ol 037 .......... .......... .......... ...
ol 039 .......... .......... ......T... ...
ol 111 .......... .......... .......... ...
pa 005 .......... .......... .......... ...
pa 006 .......... .......... .......... ...
sh 001 .......... .......... .......... ...
sh 002 .......... .......... .......... ...
si 003 .......... .......... ...??????? ???
si 006 .......... .......... .......... ...
sp 001 .......... .......... .......... ...
sp 002 .......... .......... .......... ...
sp 003 .......... .......... .......... ...
sp 005 .......... .......... .......... ...
sp 008 .......... .......... .......... ...
mt 105 .......... .......... .......... ...
mt 106 .......... .......... .......... ...
uk 004 .......... .......... .......... ...
uk 020 .......... .......... .......... ...
ca 301 .......... .......... .......... ...
ca 306 .......... .......... .......... ...
ca1407 .......... .......... ......T... ...
ca1507 .......... .......... .......... ...
ca1616 .......... .......... .......... ...
ca1633 .......... .......... .......... ...
cd 001 .......... .......... .......... ...
cd 018 .......... .......... .......... ...
mo 103 .......... .......... .......... ...
mo 106 .......... .......... .......... ...
mo 202 .......... .......... .......... ...
mo 203 .......... .......... .......... ...
mo 303 .......... .......... .......... ...
mo 324 .......... .......... .......... ...
my 005 .......... .......... .......... ...
my 009 .......... .......... .......... ...
pl 403 .......... .......... .......... ...
pl1108 .......... .......... .......... ...
pl1203 .......... .......... .......... ...
pl1306 .......... .......... .......... ...
pl1501 .......... .......... .......... .??
pl1503 .......... .......... .......... ...
tr 002 .......... .......... .......... ...
tr 024 .......... .....A.... .......... ...
tr 027 .......... .......... .......... ...
ru 007 .......... .......... .......... ...
ru 008 .......... .......... .......... ...
ru 025 .......... .......... .......... ...
ru 028 .......... .......... .......... ...
ru 029 .......... .......... .......... ...
ru 103 .......... .......... .......... ...
ru 107 .......... .......... .......... ...
```

```
Di 106 GACCCCAGGT CAGGCGGGAT TACCCGCTGA GTT
ru 209 .......... .......... .......... ...
ru 210 .......... .......... .......... ...
sa 109 .......... .......... .......... ...
sa 105 .......... .......... .......... ...
sa 306 .......... .......... .......... ...
sa 309 .......... .......... .......... ...
bu 004 .......... .......... .......... ...
bu 015 .......... .......... .......... ...
bu 040 .......... .......... .......... ...
bu 045 .......... .......... .......... ...
gr 001 .......... .......... .......... ...
gr 002 .......... .......... ......T... ...
gr 007 .......... .......... .......... ...
gr 015 .......... .......... .......... ...
gr 118 .......... .......... .......... ...
gr 128 .......... .......... .......... ...
mx 001 .......... .......... .......... ...
mx 007 .......... .......... .......... ...
tf 003 .......... .......... .......... ...
tf 004 .......... .......... .......... ...
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Alignment of *Fagus* accessions (own data only) comprising the 3' end of the 18S rDNA, the ITS1, the 5.8S rDNA, the ITS2, and the 5' end of the 26S rDNA

Standard nucleotide code, "." indicates identity with reference sequence (clone sy4209), "?" uncertain data (not available or poor quality). Grey font: rRNA gene data, not used for analyses. Accession labels refer to column #1 in the voucher table. Accessions are grouped according species and geographic origin.

```
                                                                            3' end 18S rDNA-><- 5' end ITS1
site #          1 1111111112 2222222223 3333333334 4444444445 5555555556 6666666667 7777777778 8888888889
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
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cultivars of *F. sylvatica*

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sy4209 TATCATTTAG AGGAAGGAGA AGTCGTAACA AGGTTTCCGT AGGTGAACCT GCGGAAGGAT CATTGTCGAA ACCTGCCCCA GCAGAACGAC
sy4212 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4213 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4216 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5308 .......... .......... .......... .......... .......... .......... .......... .......... ......T...
```

***F. engleriana* China mainland**

```
en_108 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
en_126 .........A .......... .......... .......... .......... .......... .......... .......TT. ..........
en_135 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
en_136 .......... .......... C......... .......... .......... .......... .......... .......T.. ..........
en_202 .......... .......... .......... .......... .......... .......... .......... .......TT. ..........
en_203 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
en_204 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
en_206 .......... .......... .......... .......... .......... .......... .......... .......TT. ..........
en_301 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
en_302 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
en_303 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
en_304 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
en3505 .......... .......... .......... .......... .......... .......... .......... .......TT. ..........
en3530 .......... .......... .......... .......... .......... .......... .......... .......TT. ..........
en3541 .......... .......... .......... .......... .......... .......... .......... .......TT. ..........
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***F. engleriana* Ullung Is., Korea**

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en_402 ????...... .......... .......... .......... .......... .......... .......... .......T.. ..........
en_412 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
en_413 .......... .......... .......... .......... .......... .......... .......... .......TT. ......T...
en_415 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
en_416 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
```

F. japonica

```
ja_101 ?????????? ?????????? ??????.... .......... .......... .......... .......... .......TT. ..........
ja_102 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
ja_103 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
ja_108 .......... .......... .......... .......... .......... .......... .......... .......TT. ..........
ja2508 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
ja2509 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
ja2514 .......... .......... .......... .......... .......... .......... .......... .......TT. ..........
ja2529 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
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sy4209 TATCATTTAG AGGAAGGAGA AGTCGTAACA AGGTTTCCGT AGGTGAACCT GCGGAAGGAT CATTGTCGAA ACCTGCCCCA GCAGAACGAC
```

F. crenata

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cr_201 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr_202 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr_205 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr_212 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr3001 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr3003 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr3005 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr3006 .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr3066 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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F. grandifolia* ssp. *grandifolia

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gr2602 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr2606 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr2607 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr2701 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr2704 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr2706 ???????... .......... .......... .......... .......... .......... .......... .......T.. ..........
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F. grandifolia* ssp. *caroliniana

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gr_201 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr_203 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr_624 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr_628 .......... .......... .......... .......T.. .......... .......... .......... .......T.. ..........
gr_632 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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F. grandifolia* ssp. *mexicana

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gr5101 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr5102 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr5103 .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr5104 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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F. hayatae* ssp. *pashanica

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ha_320 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
ha_321 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_326 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_327 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
ha_328 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_415 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_416 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_417 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_426 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_536 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
ha_546 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_550 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
ha_563 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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F. longipetiolata

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lo_110 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
lo_113 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
lo_118 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
lo_119 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
lo_204 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
lo_208 .......... .......... .......... .......... .......... .......... .......... .......... ......T...
lo_209 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_211 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_302 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
lo_305 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_306 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_316 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
lo4704 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo4717 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo4721 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo4722 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
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F. lucida

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lu_102 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
lu_103 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
lu_104 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu_105 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu4836 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu4848 .......... .......... .......... .......... ..........T.......... .......... .......... ..........
lu4861 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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***F. sylvatica* Georgia (Transcaucasus)**

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ho1601 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ho1602 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
ho1603 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
ho1805 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ho1807 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ho1904 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
ho1907 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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sy4209 TATCATTTAG AGGAAGGAGA AGTCGTAACA AGGTTTCCGT AGGTGAACCT GCGGAAGGAT CATTGTCGAA ACCTGCCCCA GCAGAACGAC
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***F. sylvatica* Turkey**
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or1206 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or1301 ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????..... .......... ..........
or1302 ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????..... .......... ..........
or1303 ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????..... .......... ..........
or1322 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or1324 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_404 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_405 ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????..... .......... ..........
or_601 ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????..... .......... ..........
or_603 ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????..... .......... ..........
or_605 ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????..... .......... ..........
or_618 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_645 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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***F. sylvatica* Bulgaria** (≙ *F. moesica sensu* Czeczkott)
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mo3221 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
mo3222 .......... .......... .......... .......... .......... .......... .......... ....A..... ..........
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***F. sylvatica* Hungary**
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sy2001 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2002 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2004 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2005 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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***F. sylvatica* Slovenia**
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sy4301 .........C .......... .......... .......... ...C...... .......... .......... .......... ..........
sy4309 .......... .......... .......... .......... .......... .......... .......... .......... ......T...
sy4312 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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***F. sylvatica* Germany**
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sy2802 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2803 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2901 .......... .........A. .......... .......... .......... .......... .......... .......... ..........
sy2904 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy3103 .......... .......... .......... .......... .......... .......... .......... .......... ......T...
sy3105 .......... .......... .......... .......... .......... .......... .......... .......T.. ..........
sy3206 .......... .......... .......... .......... .......... .......... .......... .......... .......A..
sy3209 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy3211 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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***F. sylvatica* Scotland**
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sy5901 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5907 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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***F. sylvatica* N Spain**
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sy1601 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy1603 ?????????? ?????????? ??????.... .......... .......... .......... .......... .......T.. ..........
sy1607 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy1610 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5403 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5423 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5506 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5508 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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***F. sylvatica* N Italy**
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sy4601 .......... .......... .......... .......... .......... .......... .......... ..........T.........
sy4604 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4802 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4804 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4903 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4904 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4701 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4702 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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                                                                «ITS1»
             1 1111111111 1111111111 1111111111 1111111111 1111111111 1111111111 1111111111 1111111111
      9999999990 0000000001 1111111112 2222222223 3333333334 4444444445 5555555556 6666666667 7777777778
      1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
sy4209 CCGAGAACGC GTGATAACCA CACGGGGCGA GGGGCTTCGC GGCCTTTCGT CCCCAAACGG TCGGGGGAAC CCCGTGCCGC CCGTCTGGCC
sy4212 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4213 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4216 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5308 .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_108 .......... .......... .......... .......... .......... .......... .....--... ....C..... .........-
en_126 .........A .......... .......... ......A... .......... .......... .....--... .......... .........-
en_135 .......T.A .......... .......... .......... ........A. .......... .....--... ....C.T... ..........
en_136 .......T.A .......... .......... .......... ........A. .......... .....--... ....C.T... ..........
en_202 .........A .......G.. .......... .......... .......... .......... .....--... .T........ .........-
en_203 .......T.A .......... .......... .......... ........A. .......... .....--... ....C.T... ..........
en_204 .......... .......... .......... .......... .......... .......... .....--... ....C..... .........-
en_206 .........A .......G.. .......... .......... .......... .......... .....--... .T........ .........-
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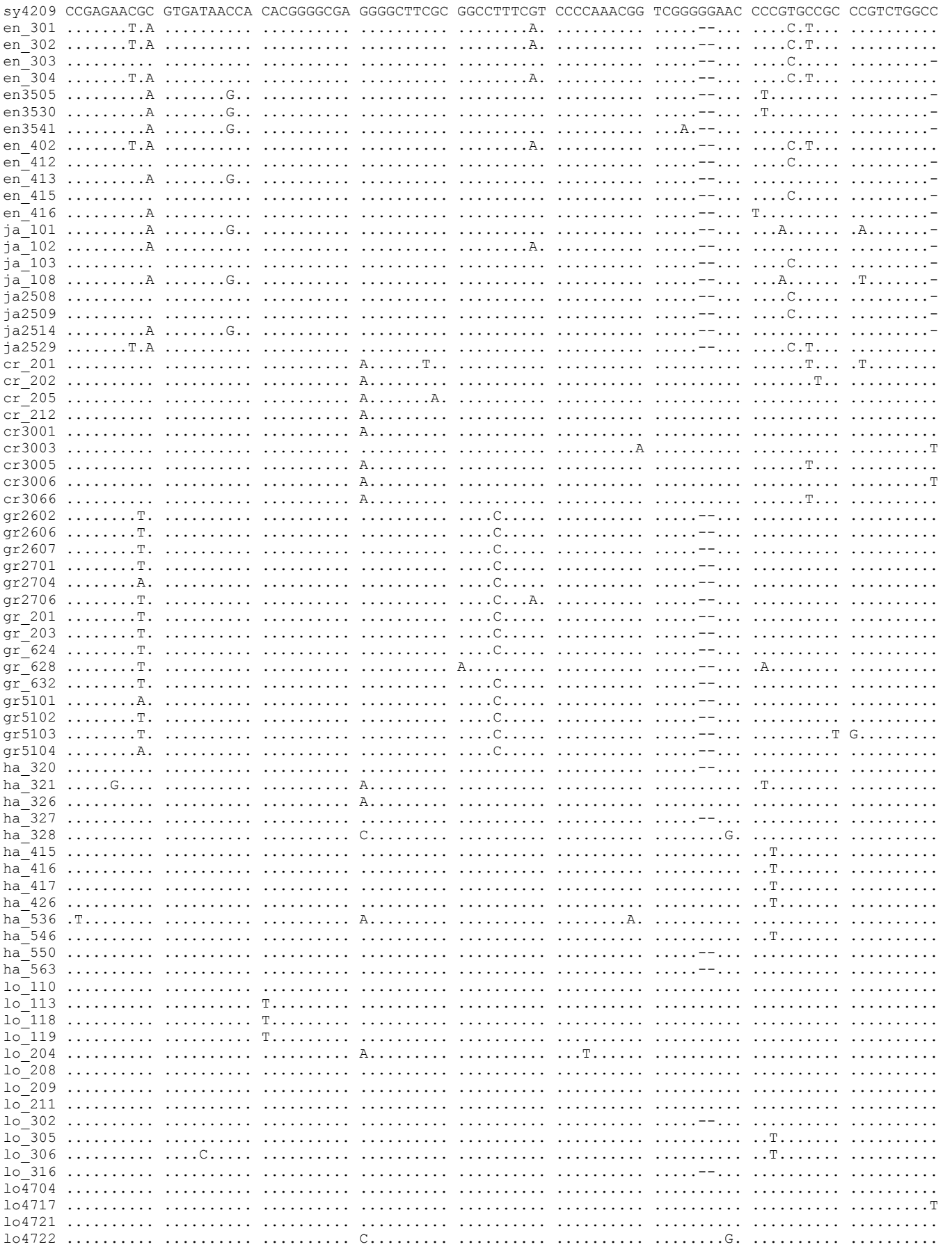
sy4209 CCGAGAACGC GTGATAACCA CACGGGGCGA GGGGCTTCGC GGCCTTTCGT CCCCAAACGG TCGGGGGAAC CCCGTGCCGC CCGTCTGGCC
en_301 ....T.A ....A. ....--... ....C.T... ....
en_302 ....T.A ....A. ....--... ....C.T... ....
en_303 ....--... ....C..... ....-
en_304 ....T.A ....A. ....--... ....C.T... ....
en3505 ....A ....G.. ....--... .T........ ....-
en3530 ....A ....G.. ....--... .T........ ....-
en3541 ....A ....G.. ....A.--... ....-
en_402 ....T.A ....A. ....--... ....C.T... ....
en_412 ....--... ....C..... ....-
en_413 ....A ....G.. ....--... ....-
en_415 ....--... ....C..... ....-
en_416 ....A ....--... T......... ....-
ja_101 ....A ....G.. ....--... ...A...... .A.......-
ja_102 ....A ....A. ....--... ....-
ja_103 ....--... ....C..... ....-
ja_108 ....A ....G.. ....--... ...A...... .T.......-
ja2508 ....--... ....C..... ....-
ja2509 ....--... ....C..... ....-
ja2514 ....A ....G.. ....--... ....-
ja2529 ....T.A ....--... ....C.T... ....
cr_201 .... A......T.. ....T... .T........
cr_202 .... A......... ....T.. ....
cr_205 .... A.......A. ....
cr_212 .... A......... ....
cr3001 .... A......... ....
cr3003 ....A ....T
cr3005 .... A......... ....T... ....
cr3006 .... A......... ....T
cr3066 .... A......... ....T... ....
gr2602 ....T. ....C..... ....--... ....
gr2606 ....T. ....C..... ....--... ....
gr2607 ....T. ....C..... ....--... ....
gr2701 ....T. ....C..... ....--... ....
gr2704 ....A. ....C..... ....--... ....
gr2706 ....T. ....C...A. ....--... ....
gr_201 ....T. ....C..... ....--... ....
gr_203 ....T. ....C..... ....--... ....
gr_624 ....T. ....C..... ....--... ....
gr_628 ....T. .... A......... ....--... .A........ ....
gr_632 ....T. ....C..... ....--... ....
gr5101 ....A. ....C..... ....--... ....
gr5102 ....T. ....C..... ....--... ....
gr5103 ....T. ....C..... ....--... ....T G.........
gr5104 ....A. ....C..... ....--... ....
ha_320 ....--... ....
ha_321 .....G.... .... A......... .... .T........ ....
ha_326 .... A......... ....
ha_327 ....--... ....
ha_328 .... C......... ....G. ....
ha_415 ....T....... ....
ha_416 ....T....... ....
ha_417 ....T....... ....
ha_426 ....T....... ....
ha_536 .T........ .... A......... ....A. ....
ha_546 ....T....... ....
ha_550 ....--... ....
ha_563 ....--... ....
lo_110 ....
lo_113 .... T......... ....
lo_118 .... T......... ....
lo_119 .... T......... ....
lo_204 .... A......... ....T...... ....
lo_208 ....
lo_209 ....
lo_211 ....
lo_302 ....--... ....
lo_305 ....T....... ....
lo_306 ....C..... ....T....... ....
lo_316 ....--... ....
lo4704 ....
lo4717 ....T
lo4721 ....
lo4722 .... C......... ....G. ....

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sy4209 CCGAGAACGC GTGATAACCA CACGGGGCGA GGGGCTTCGC GGCCTTTCGT CCCCAAACGG TCGGGGGAAC CCCGTGCCGC CCGTCTGGCC
lu_102 .......... .......... .......... .......T.. .......... .......... .......... .......... ..........
lu_103 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu_104 .......... .......... .......... .......... .......... .......... ........G. .......... ..........
lu_105 .......... .......... .......... .......... .......... .......... ........G. .......... ..........
lu4836 .......... .......... .......... C......... .......... .......... ........G. .......... ..........
lu4848 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu4861 .......... .......... .......... .......... .......... .......... ........G. .......... ..........
ho1601 .......... .......... .......... .....C.... .......... .......... .....--... .A........ ..........
ho1602 .......... .......... .......... A......... .......... .......... .......... .......... ..........
ho1603 .......... .......... .......... A......... .......... .......... .......... .......... ..........
ho1805 .......... .......... .......... A......... .......... .......... .......... .......... ..........
ho1807 .......... .......... .......... A......... .......... .......... .......... .......... ..........
ho1904 ........TT .......... .......... .......... .......... .......... .....--... .T........ .........-
ho1907 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or1206 .......... .......... .......... .....C.... .......... .......... .......... .......... ..........
or1301 .......... .......... .......... .......... .......... .......... .......... ..-....... ..........
or1302 .......... .......... .......... .....C.... .......... .......... .......... ..-....... ..........
or1303 .......... .......... .......... .......... .......... .......... .......... ..-....... ..........
or1322 .......... .......... .......... .....C.... .......... .......... .......... .......... ..........
or1324 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_404 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_405 .......... .......... ......C... A......... .......... .......... .....--... ..-....... ..........
or_601 .......... .......... .......... .......... .......... .......... .......... ..-....... ..........
or_603 .......... .......... .......... .......... .......... .......... .......... ..-....... ..........
or_605 .......... .......... .......... .......... .......... .......... .......... ..-....... ..........
or_618 .......... .......... .......... .......... .......... .......... .......... ......T..A T.........
or_645 .......... .......... .......... .......... .......... .......... .......... .......... ..........
mo3221 .......... .......... .T........ A.......T. .......... .......... .......... ......T... ..........
mo3222 .......... .......... .......... .......... .......... .......... .....--... .......... .........-
sy2001 .......T.. .......... .......T.. .......... .......... .......... .......... .......... ..........
sy2002 .......T.. .......... .......T.. .......... .......... .......... .......... .......... ..........
sy2004 .......T.. .......... .......T.. .......... .......... .......... .......... .......... ..........
sy2005 .......T.. .......... .......T.. .......... .......... .......... .......... .......... ..........
sy4301 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4309 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4312 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2802 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2803 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2901 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2904 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy3103 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy3105 .......... .......... .......... A......... .......... .......... ..A....... .......... .T........
sy3206 .......... .......... .......... .......... .......... .......... .......... ......T... ..........
sy3209 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy3211 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5901 .......... .......... .......... .......... .......... .......... .......... .T........ ..........
sy5907 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy1601 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy1603 .......... .......... .......... A......... .......... .......... ..A....... .......... .T........
sy1607 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy1610 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5403 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5423 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5506 .......... .......... .......... .......... .......... .......... .T...--... .......... ..........
sy5508 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4601 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4604 .......... .......... .......... .......... .......... .......... .......... .......... T.........
sy4802 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4804 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4903 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4904 .T........ .......... .......... .....C.... .......... .......... .......... .......... ..........
sy4701 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4702 .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                                                «ITS2»
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       8888888889 9999999990 0000000001 1111111112 2222222223 3333333334 4444444445 5555555556 6666666667
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
sy4209 ACAAACCATT CG-------- -------AAG CGACCGTGGC AA--CCTCGT CCGTAAACCG AACCCCGGCG CGGAATGTGC CAAGGAACTG
sy4212 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
sy4213 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
sy4216 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
sy5308 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
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sy4209 ACAAACCATT CG-------- -------AAG CGACCGTGGC AA--CCTCGT CCGTAAACCG AACCCCGGCG CGGAATGTGC CAAGGAACTG
en_108 -......... ..-------- -------... .........A ..--T..... .......... .......... .......... .........A
en_126 -......G.. ..CGGGATGG CCGGACG... .....A.... ..--...T.. .......... .......... .......... .........T
en_135 .......... ..-------- -------... .......... ..--...... .......... .........A .......... ..........
en_136 .......... ..-------- -------... .......... ..--...... .......... .........A .......... ..........
en_202 -......G.. ..CGGGATGG TCGGACG... .......... ..--...T.. .......... .......... .......... ..........
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en_204 -......... ..-------- -------... ......C..A ..--T..... .......... .......... .......... ..........
en_206 -......G.. ..CGGGATGG TCGGACG... .......... ..--...T.. .......... .......... .......... ..........
en_301 .......... ..-------- -------... .......... ..--...... .......... .........A .......... ..........
en_302 .......... ..-------- -------... .......... ..--...... .......... .........A .......... ..........
en_303 -......... ..-------- -------... .........A ..--T..... .......... .......... .......... ..........
en_304 .......... ..-------- -------... .......... ..--...... .......... .........A .......... ..........
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en3541 -......G.. ..CGGGATGG TCAGACG... .......... ..--...... .......... .......... .......... ..........
en_402 .......... ..-------- -------... .......... ..--...... .......... .........A .......... ..........
en_412 -......... ..-------- -------... ......C..A ..--T..... ...A...... .......... .......... ..........
en_413 -......G.. ..CGGGATGG TCGGACG... .......... ..--...T.. .......... .......... .......... ..........
en_415 -......... ..-------- -------... .........A ..--T..... .......... .......... .......... ..........
en_416 -......... ..-------- -------... .........A ..--T..... .......... .......... .......... ..........
ja_101 -......G.. ..CGGGATGG TCGGACG... .......... ..--...T.. .......... .......... .......... ..........
ja_102 --........ ..-------- -------... .........A ..--T..... .......... .......... .......... ..........
ja_103 -......... ..-------- -------... .........A ..--T..... .......... .......... .......... ..........
ja_108 -......G.. ..CGGGATGG TCGGACG... .......... ..--...T.. .......... .......... .......... ......G...
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ja2509 -......... ..-------- -------... .........A ..--T..... .......... .......... .......... ..........
ja2514 -......G.. ..CGGGATGG TCGGACG... .......... ..--...T.. .......... .......... .......... ..........
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cr_205 G......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
cr_212 G......... ..-------- -------... .......... ..--...... ..A....... .......... .......... ..........
cr3001 G......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
cr3003 G......... ..-------- -------... .......... ..--...... ..........T. .......... .......... ..........
cr3005 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
cr3006 G......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
cr3066 G......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
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gr2606 ...G...... ..-------- -------... .......... ..--...... .......... .......... .......... .........C.
gr2607 ...G...... ..-------- -------... .......... ..--...... .......... .......... .......... .........C.
gr2701 ...G...... ..-------- -------... .......... ..--...... .......... .......... .......... .........C.
gr2704 .......... ..-------- -------... .......... ..--...... .......... .......... .......... .........C.
gr2706 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
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gr_203 ...G...... ..-------- -------... .......... ..--...... .......... .......... .......... .........C.
gr_624 ...G...... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
gr_628 .......... ..-------- -------... T......... ..--...... .......... .......... .......... ..........
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gr5101 .......... ..-------- -------... .......... ..--...... .......... .........T. .......... .........C.
gr5102 ...G...... ..-------- -------... .......... ..--...... .......... .......... .......... .........C.
gr5103 .......... ..-------- -------... .......... ..--...... .......... .......... .......... .........C.
gr5104 .......... ..-------- -------... .......... ..--...... .......... .......... .......... .........C.
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lo_119 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
lo_204 .......... ..-------- -------... .......... ..AA...... .......... .......... .......... ..........
lo_208 .......... ..-------- -------... .......... ..--...... ....C..... .......... .......... ..........
lo_209 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
lo_211 .......... ..-------- -------... .......... ..--...G.. .......... .......... .......... ..........
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sy4209 ACAAACCATT CG-------- -------AAG CGACCGTGGC AA--CCTCGT CCGTAAACCG AACCCCGGCG CGGAATGTGC CAAGGAACTG
lo_302 .......... ..-------- -------... .A........ ..--...... .......... .......... .......... ..........
lo_305 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
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ho1603 .......... ..-------- -------... .......... ..--...... .........T .......... .......... ..........
ho1805 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
ho1807 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
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ho1907 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
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or1303 .......... ..-------- -------... .......... ..--...... .........T .......... .......... ..........
or1322 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
or1324 .......... ..-------- -------... .......... ..--A..... .........T .......... .......... ..........
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or_405 .......... ..-------- -------... .........G ..--...... .......... .......... .......... ..........
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or_603 .......... ..-------- -------... .......... ..--...... .........T .......... .......... ..........
or_605 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
or_618 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
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sy2002 ......T... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
sy2004 ......T... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
sy2005 ......T... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
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sy2904 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
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sy3105 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
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sy3209 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
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sy1607 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
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sy4903 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
sy4904 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
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sy4702 .......... ..-------- -------... .......... ..--...... .......... .......... .......... ..........
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                                                                                 3' end ITS1 -><- 5' end 5.8S rDNA
       2222222222 2222222222 2222222223 3333333333 3333333333 3333333333 3333333333 3333333333 3333333333
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       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
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sy4212 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4213 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4216 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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ja2509 .......... ....A..... .......... .....A.... .......T.. .......... .......... .......... ..........
ja2514 .......... .......... .......... .....A.... .......T.. .......... .......... .......... ..........
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ha_536 .......... .......... .......... .......... .......... .........A .......... .......... ..........
ha_546 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_550 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_563 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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sy4209 AAACCAAAGA GCGTCGCCGG CCGCCTCGGA CACGATGTGC GTGCCGGCGT CGACGTCTTG TATTTATCCA AAACGACTCT CGGCAACGGA
lo_110 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_113 .......... ...C...... .......... .......... .......... .......... .......... .......... ..........
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lo4717 .......... ...C...... .......... .......... .......... .......... .......... .......... ..........
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lo4722 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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lu_103 ........C. ...C...... .......... .......... .......... .......... .......... .......... ..........
lu_104 ........C. ...C...... .......... .......... .......... .......... .......... .......... ..........
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lu4836 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu4848 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu4861 .......... .......... .......... .......... ..A....... .......... .......... .......... ..........
ho1601 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ho1602 .......... .......... T......... .......... .....A.... .......... .......... .......... ..........
ho1603 .......... .......... T......... .......... .....A.... .......... .......... .......... ..........
ho1805 .......... .......... .......... .......... .......... .......... .......... .......... .......???
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ho1904 .......... .......... .......... .......... .......... .......... .......... .......... .........?
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or1206 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or1301 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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or_601 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_603 .......... .......... T......... .......... .......... .......... .......... .......... ..........
or_605 .......... .......... T......... .......... .......... .......... .......... .......... ..........
or_618 .......... .......... T......... .......... .......... .......... .......... .......... ..........
or_645 .......... .......... .......... .......... .......... .......... .......... .......... ..........
mo3221 .......... .......... .......... .........T ....T..... .......... .......... .......... ......T...
mo3222 .......... .......... .......... .......... .......A.. .....C.... .......... .......... ..........
sy2001 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2002 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2004 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2005 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4301 .......... .......... .......... .......... .......... ......T... .......... .......... ..........
sy4309 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4312 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2802 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2803 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2901 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2904 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4209 AAACCAAAGA GCGTCGCCGG CCGCCTCGGA CACGATGTGC GTGCCGGCGT CGACGTCTTG TATTTATCCA AAACGACTCT CGGCAACGGA
sy3103 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy3105 .......... .......... .......... .......... .......... .......... .......... .......A.. ..A.......
sy3206 .......... .......... .......... .......... .......... T......... .......... .......... ..........
sy3209 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy3211 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5901 .......... .......... .......... .......... .......... .....A.... .......... .......... ..........
sy5907 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy1601 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy1603 .......... .......... .......... .......... A......... .......... .......... .......... ..........
sy1607 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy1610 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5403 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5423 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5506 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5508 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4601 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4604 .......... .......... .......... .........T .......... .......... .......... .......... ......T...
sy4802 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4804 .......... ......T... .......... .......... ......??.. .......... .......... .......... ..........
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sy4209 AAACCAAAGA GCGTCGCCGG CCGCCTCGGA CACGATGTGC GTGCCGGCGT CGACGTCTTG TATTTATCCA AAACGACTCT CGGCAACGGA
sy4903 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4904 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4701 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4702 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                                                «5.8S rDNA»
       3333333333 3333333333 3333333333 3333333334 4444444444 4444444444 4444444444 4444444444 4444444444
       6666666667 7777777778 8888888889 9999999990 0000000001 1111111112 2222222223 3333333334 4444444445
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
sy4209 TATCTCGGCT CTCGCATCGA TGAAGAACGT AGCGAAATGC GATACTTGGT GTGAATTGCA GAATCCCGTG AATCATCGAG TCTTTGAACG
sy4212 .........?? ?????????? ?????????? ?????????? ???????... .......... .......... .......... ..........
sy4213 .......... .......??? ?????????? ?????????? ?????..... .......... .......... .......... ..........
sy4216 .....????? ?????????? ?????????? ?????????? ??????.... .......... .......... .......... ..........
sy5308 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_108 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_126 .......... .......... ........A. .......... .......... .......... .......... .......... .......... ..........
en_135 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_136 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_202 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_203 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_204 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_206 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_301 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_302 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_303 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_304 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en3505 ......???? ?????????? ?????????? ??????.... .......... .......... .......... .......... ..........
en3530 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en3541 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_402 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_412 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_413 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_415 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_416 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ja_101 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ja_102 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ja_103 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ja_108 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ja2508 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ja2509 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ja2514 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ja2529 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr_201 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr_202 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr_205 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr_212 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr3001 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr3003 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr3005 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr3006 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
cr3066 .......... .......... .......... .......??? ?????????? ???....... .......... .......... .......... ..........
gr2602 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr2606 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr2607 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr2701 .......... .......... ........?? ?????????? .......... .......... .......... .......... ..........
gr2704 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr2706 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr_201 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr_203 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr_624 .......... .......... .......... .......... .......... .......... .......... .....T.... .......... ..........
gr_628 .......... .......... .......... .......... .......... .......... .......... .....T.... .......... ..........
gr_632 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr5101 .....T.... ..T....... .......... .......... .......... .......... .......... .......... .......... ..........
gr5102 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr5103 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
gr5104 .....T.... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_320 .......... .......... .......... ...A...... .......... .......... .......... .......... .......... ..........
ha_321 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_326 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_327 ?????????? ?????????? ????????.. ...A...... .......... .......... .......... .......... .......... ..........
ha_328 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_415 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_416 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_417 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_426 .......... .......... .......... .......... .......... .......... .......... .......... .......... ..........
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sy4209 TATCTCGGCT CTCGCATCGA TGAAGAACGT AGCGAAATGC GATACTTGGT GTGAATTGCA GAATCCCGTG AATCATCGAG TCTTTGAACG
ha_536 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_546 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_550 .......... .......... .......... ...A...... .......... .......... .......... .......... ..........
ha_563 .......... .......... .......... ...A...... .......... .......... .......... .......... ..........
lo_110 .......... .......... .......... .......... .......... .......... .......... .......... .........T.
lo_113 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_118 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_119 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_204 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_208 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_209 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_211 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_302 .......... .......... .......... ...A...... .......... .......... .......... .......... ..........
lo_305 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_306 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_316 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo4704 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo4717 .......... .......... .......... .......... .......... .......... A......... .......... ..........
lo4721 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo4722 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu_102 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu_103 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu_104 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu_105 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu4836 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu4848 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu4861 .......... .......... .......... .......... ...??????? ????????? .......... .......... ..........
ho1601 .......... .......... .......... .......... ...??????? ????????? .......... .......... ..........
ho1602 .......... .......... .......... .????????? ?????????? ?????????? ?????????? ???????... ..........
ho1603 .......... .......... .......... ...??????? ?????????? ?????????? .......... .......... ..........
ho1805 ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ???????... .......... ..........
ho1807 .......... ......???? ?????????? ?????????? .......... .......... .......... .......... ..........
ho1904 ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????????
ho1907 .......... .....????? ?????????? ?????????? ?????????? ???????... .......... .......... .........T.
or1206 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or1301 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or1302 .......... .......... .......... .......... ......G... .......... .......... .......... ..........
or1303 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or1322 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or1324 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_404 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_405 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_601 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_603 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_605 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_618 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_645 .......... .......... .......... .......... .......... .......... .......... .......... ..........
mo3221 .......... .......... .......... ......???? ?????????? ?????????? ???????... .......... ..........
mo3222 .......... .......... .......... .......... ??????.... .......... .......... .......... ..........
sy2001 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2002 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2004 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2005 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4301 ..???????? ?????????? ?????????? ?????????? ?????????? ?????????? ??????.... .......... ..........
sy4309 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4312 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2802 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2803 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2901 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2904 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy3103 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy3105 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy3206 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy3209 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy3211 .......... .......... .......... ......???? ?????????? ?????????? ?......... .......... ..........
sy5901 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5907 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy1601 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy1603 .......... .......... .......... ......???? ?????????? ????..... .......... .......... ..........
sy1607 .......... .......??? ?????????? ????????.. .......... .......... .......... .......... ..........
sy1610 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5403 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5423 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5506 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5508 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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sy4209 TATCTCGGCT CTCGCATCGA TGAAGAACGT AGCGAAATGC GATACTTGGT GTGAATTGCA GAATCCCGTG AATCATCGAG TCTTTGAACG
sy4601 .......... .......... .......... .......... .......... ........T. .......... .......... ..........
sy4604 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4802 .......A.. .......... .......... .......... .......... .......... .......... .......... ..........
sy4804 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4903 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4904 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4701 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4702 .......... .......... .......... .......... .......... .......... .......... .......... ..........

                                                  3' end 5.8S rDNA ->&lt;- 5' end ITS2
       4444444444 4444444444 4444444444 4444444444 4444444445 5555555555 5555555555 5555555555 5555555555
       5555555556 6666666667 7777777778 8888888889 9999999990 0000000001 1111111112 2222222223 3333333334
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
sy4209 CAAGTTGCGC CCGACGCCAT TCGGCCGAGG GCACGTCTGC CTGGGTGTCA CGCACCGTTG CCCCAAAACG CC-CCCACCT CGC-----AA
sy4212 .......... .......... .......... .......... .......... .......... ....C..... ..-....... ...-----..
sy4213 .......... .......... .......... .......... .......... .......... ....C..... ..-....... ...-----..
sy4216 .......... .......... .......... .......... .......... .......... ....C..... ..-....... ...-----..
sy5308 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
en_108 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... ...-----..
en_126 .......... ........?? ???????... .......... .......... .....T.... ....C...T. ..-.T.G... ...-----..
en_135 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... .A.-----..
en_136 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... .A.-----..
en_202 .......... .......... ....T..... .......... .......... ....TT.... ....C..... ..-.T.G... ...-----..
en_203 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... .A.-----..
en_204 .......... .......... .......... .......... .......... ....T..... ....T..... ..-.T.G... ...-----..
en_206 .......... .......... ....T..... .......... .......... .......... ....C..... ..-.T.G... ...-----..
en_301 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... .A.-----..
en_302 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... .A.-----..
en_303 .......... .......... ....T..... .......... .......... ....TT.... ....C..... ..-.T.G... ...-----..
en_304 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... .A.-----..
en3505 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... .A.-----..
en3530 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... ...-----..
en3541 .......... .......... ....T..... ...T...... .......... .....T.... ....C..... ..-.T.G... ...-----..
en_402 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... .A.-----..
en_412 .????????? ?????????? ?????????? ?????????? ???....... ....TT.... ....C..... ..-.T.G... ...-----..
en_413 .......... .......... ....T..... .......... .......... ....TT.... ....C..... ..-.T.G... ...-----..
en_415 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... ...-----..
en_416 .......... .......... .....T.... .......... .......... ....T..... ....C..... ..-.T.G... ...-----..
ja_101 .......... .......... ....T..... .......... .......... ....TT.... ....C..... ..-.T.G... ...-----..
ja_102 .......... .......... .......... .......... .......... ....T..... ..T.C..... ..-.T.G... ...-----..
ja_103 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... ...-----..
ja_108 .......... .......... ....T..... .......... .......... .......... ....C..... ..-.T.G... ...-----..
ja2508 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... ...-----..
ja2509 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... ...-----..
ja2514 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... .A.-----..
ja2529 .......... .......... .......... .......... .......... ....T..... ....C..... ..-.T.G... .A.-----..
cr_201 .......... .......... .......... .......... .......... ....T..... ....C..... ..-...G... T..-----..
cr_202 .......... .......... .......... .......... .......... T......... ....C..... ..-...G... ...-----..
cr_205 .......... .......... .......... .......... .......... T......... ....C..... ..-...G... ...-----..
cr_212 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
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gr2701 .......... .......... .......... .......... .......... ....T..... ....C..... ..T...G... ...-----..
gr2704 .......... .......... .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
gr2706 .......... .......... .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
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gr_203 .......... .......... .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
gr_624 .......... ........C. .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
gr_628 .......... ........C. .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
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gr5101 .......... .......... .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
gr5102 .......... .......... .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
gr5103 .......... .......... .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
gr5104 .......... .......... .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
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ha_321 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
ha_326 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
ha_327 .......... ..A....... .......... .......... .......... .......... ....C..... ..-...G... ...CTCCC..
ha_328 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
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sy4209 CAAGTTGCGC CCGACGCCAT TCGGCCGAGG GCACGTCTGC CTGGGTGTCA CGCACCGTTG CCCCAAAACG CC-CCCACCT CGC-----AA
ha_415 .......... .......... .......... .......... .......... .......... ....C..... ..-.T.G... T..-----..
ha_416 .......... .......... .......... .......... .......... .......... ....C..... ..-.T.G... T..-----..
ha_417 .......... .......... .......... .......... .......... .......... ....C..... ..-.T.G... T..-----..
ha_426 .......... .......... .......... .......... .......... .......... ....C..... ..-.T.G... T..-----..
ha_536 .......... .......... .......... .......... .......... .......... ....C..... ..C...G... ...-----..
ha_546 .......... .......... .......... .......... .......... .......... ....C..... ..-.T.G... T..-----..
ha_550 .......... ..A..... .......... .......... .......... .......... ....C..... ..-...G... ...CTCCC..
ha_563 .......... ..A..... .......... .......... .......... .......... ....C..... ..-...G... ...CTCCC..
lo_110 .......... .........C .......... .......... .......... .......... ....C..... ..-...GT.. ...-----..
lo_113 .......... .........C .......... .......... .......... .......... ....C..... ..-...G... ...-----..
lo_118 .......... .......... .......... .......... .......... .......... .T..C..... ..-...GT.. ...-----..
lo_119 .......... .......... .......... .......... .......... .......... .T..C..... ..-...GT.. ...-----..
lo_204 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
lo_208 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
lo_209 .......... .......... .......... .......... .....C.... .......... ....C..... ..-...G... ...-----..
lo_211 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
lo_302 .......... ..A..... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
lo_305 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
lo_306 .......... .......... .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
lo_316 .......... .........C .......... .......... .......... .......... ....C..... ..-...G... ...-----..
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lo4717 .......... .......... .......... .......... .......... .......... ....C..... ..-.T.G... ..T-----..
lo4721 .......... .......... .....G.... .......... .......... .......... ....C..... ..-...G... T..-----..
lo4722 .......... .......... .......... .......... .......... .......... ....C..... ..-.T.G... ..T-----..
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lu_104 .......... .......... .......... .......... .......... ....T..... ....C..... ..-...G... T..-----..
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lu4836 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
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ho1602 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
ho1603 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
ho1805 .......... .T........ .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
ho1807 .......... .T........ .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
ho1904 ?????????? ?????????? ?????????? ?????????? ????????.. ....T..... ....C..... ..-...G... ...-----..
ho1907 .......... .......... .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
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or1301 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
or1302 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
or1303 .......... .......... .......... .......... .......... .......... ....C..... T.-...G... ...-----..
or1322 .......... .......... .......... .......... .......... .......... ....C..... T.-...G... ...-----..
or1324 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
or_404 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
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or_601 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
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or_605 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
or_618 .......... .......... .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
or_645 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
mo3221 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
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sy2001 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy2002 .......... .......... ........?? ?????????? ?.......... .......... ....C..... ..-...G... ...-----..
sy2004 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy2005 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy4301 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy4309 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy4312 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy2802 ......A... .......... .......... .......... .......... .......... ....C..... ..-........ ...-----..
sy2803 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy2901 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy2904 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy3103 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy3105 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy3206 .......... .......... .......... .......... .......... .....T.... ....C..... ..-...G... ...-----..
sy3209 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy3211 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy5901 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy5907 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy1601 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy1603 .......... .T........ .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
sy1607 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy1610 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
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sy4209 CAAGTTGCGC CCGACGCCAT TCGGCCGAGG GCACGTCTGC CTGGGTGTCA CGCACCGTTG CCCCAAAACG CC-CCCACCT CGC-----AA
sy5403 .......... .......... .......... .......... .......... ....T..... ....C..... ..-...G... ...-----..
sy5423 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy5506 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy5508 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy4601 .......... .......... .......... .......... .......... .A........ ....C..... ..-...G... ...-----..
sy4604 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy4802 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy4804 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy4903 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..
sy4904 .......... .......... .......... .......... .......... .....T.... ....C..... ..-...G... ...-----..
sy4701 .......... .......... ...A...... .......... .......... .......... ....C..... ..-...G..G ...-----..
sy4702 .......... .......... .......... .......... .......... .......... ....C..... ..-...G... ...-----..

                                                    «ITS2»
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       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
sy4209 GGGGCGCGGG ATCTCGTTTG GTGG-CGGAA GTTGGCCTCC CGTGGGCCTG TGCTCGCGGT TAGCCTAAAA AGGAGTCCTC GGCGACGAGC
sy4212 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy4213 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy4216 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy5308 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
en_108 .......... .......... ....-..... .......... .......T.. .......... .......... ........C. ..........
en_126 .......... .G..T..... ....-.C... .......... ....T..... C......... .......... .A........ .A........
en_135 .......... .......... ....-.T... .......... .......... .......... .......... ........C. ..........
en_136 .......... .......... ....-.T... .......... .......... .......... .......... ........C. ..........
en_202 .......... .G........ ....-..... .......... ....T....A C......... .......... .......... ..........
en_203 .......... .......... ....-.TA.. .......... .......... .......... .......... ........C. ..........
en_204 .......C.. .......... ....-..... .......... .......... C......... .......... .A........ .A........
en_206 .......... .G........ ....-..... .......... ....T..... C......... .......... .......... ..........
en_301 .......... .......... ....-.T... .......... .......... .......... .......... ........C. ..........
en_302 .......... .......... ....-.T... .......... .......... .......... .......... ........C. ..........
en_303 .......... .G........ ....-..... .......... ....T....A C......... .......... .......... ..........
en_304 .......... .......... ....-.T... .......... .......... .......... .......... ........C. ..........
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en3530 .......C.. .......... ....-..... .......... .......... C......... .......... .A........ ..........
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en_402 .......... .......... ....-.T... .......... .......... .......... .......... ........C. ..........
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en_413 .......... .G........ ....-..... .......... ....T....A C......... .......... .......... ..........
en_415 .......C.. .......... ....-..... .......... .......... C......... .......... ........C. ..........
en_416 .......C.. .......... ....-..... .......... .......... C......... .......... .A........ .A........
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ja_102 .......... .......... ....-..... .......... .......... .......... .......... ........C. ..........
ja_103 .......C.. .......... ....-..... .......... .......... C......... .......... .A........ ..........
ja_108 .......... .G........ ....-..... .......... ....T..... C......... .......... .......... ..........
ja2508 .......... .......... ....-..... .......... .......T.. .......... .......... ........C. ..........
ja2509 .......... .......... ....-..... .......... .......T.. .......... .......... ........C. ..........
ja2514 .......... .......... ....-.T... .......... .......... .....A.... .G........ ........C. A........
ja2529 .......... .......... ....-.T... .......... .......... .....A.... .G........ ........C. A........
cr_201 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
cr_202 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
cr_205 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
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cr3001 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
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cr3005 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
cr3006 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
cr3066 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
gr2602 .......... .......C.. .C..-..... .......... .......... .......... .......... .......... ..........
gr2606 .......... .......... .C..-..... .......... .......... .......... .......... .......... ..........
gr2607 .......... .......... .C..-..... .......... .......... ..T....... .......... .......... ..........
gr2701 .......... .......C.. .C..-..... .......... .......... .......... .......... .......... ..........
gr2704 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
gr2706 .......... .......... ....-..... .......... .......... .......... .......... ..A....... ..........
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gr_203 ......A... .......... ....-..... .......... .......... .......... .......... .......... ..........
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gr_628 .......... .......... ....-..... .......... T......... .......... ...T...... .......... ..........
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gr5103 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
gr5104 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
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sy4209 GGGGCGCGGG ATCTCGTTTG GTGG-CGGAA GTTGGCCTCC CGTGGGCCTG TGCTCGCGGT TAGCCTAAAA AGGAGTCCTC GGCGACGAGC
ha_320 .......C.. .......... ....-..... .......... .......... .......... .......... .CT.....C. ..........
ha_321 .....C.... .......... ....-..... .......... ....T..... .......... .......... .C........ ..........
ha_326 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
ha_327 .......C.. .......... ....-..... .......... .......... .......... .......... .C......C. ..........
ha_328 .....C.... .......... ....-..... .......... .......... .......... .......... .......... ..........
ha_415 ......T... .......... ....-..... .......... ....T..... .......... .......... .C........ ..........
ha_416 ......T... .......... ....-..... .......... ....T..... .......... .......... .C........ ..........
ha_417 ......T... .......... ...A-..... .......... ....T..... .......... .......... .C........ ..........
ha_426 ......T... .......... ....-..... .......... ....T..... .......... .......... .C........ ..........
ha_536 ....T..... .......... .C..G..... .......... .......... .......... .......... .......... ..........
ha_546 ......T... .......... ....-..... .......... ....T..... .......... .G........ .C........ ..........
ha_550 .......C.. .......... ....-..... .......... .......... .......... .......... .C......C. ..........
ha_563 .......C.. .......... ....-..... .......... .......... .......... .......... .C......C. ..........
lo_110 .......... .......... ....-..... .......... .......... .......... .......... ........C. ..........
lo_113 .......... .......... ....-..... .......... .......... C......... .......... .......... ..........
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lo_204 ...C...... .......... ....-.A... .......... .......... .......... .......... .......... ..........
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lo_209 ...C...... .......... .C..-..... .......... .......... .......... .......... .......... ..........
lo_211 ...C.A.... .......... ....-.A... .......... .......... .......... .......... .......... ..........
lo_302 .......... .......... ....-..... .......... .......... C......... .......... .......... ..........
lo_305 .......... .......... ....-..... .......... ....T..... .......... ........T. .C........ .T........
lo_306 .......... .......... .C..-..... .......... .......... .......... .......... .......... ..........
lo_316 .......... .......... ....-..... .......... .......... C......... .......... .......... ..........
lo4704 .......... .......... ....-..... .......... ....T..... .......... .......... .C........ ..........
lo4717 ......T... .......... ....-..... .......... .......... .......... .......... ........C. ......A...
lo4721 ...C...... .......... .C..-..... .......... .......... .......... .......... .......... ..........
lo4722 ......T... ....T..... ....-..... .......... ....T..... .......... .......... .C........ ..........
lu_102 .....C.... .......... ....-..... .......... .......... .......... .......... .......... ..........
lu_103 ...C...... .......... ....-..... .......... .......... .......... .......... .......... ..........
lu_104 .....C.... .......... ....-..... .......... .......... .......... .......... .......... ..........
lu_105 ....T..... .......... ....-..... .......... .......... ....T..... .......... C......... ..........
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lu4848 .......... .......... .C..-..... .......... .......... .......... .......... .......... ..........
lu4861 .......... .......... ....-..... .......... .......... .....A.... .......... .......... ...A.T....
ho1601 .......... .......... ....-..C.. .......... .......... .......... .......... .......... ..........
ho1602 .......... .......... ....-.A... .......... .......... .......A.. .......... .......... ..........
ho1603 .......... .......... ....-.A... .......... .......... .......A.. .......... .......... ..........
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ho1807 .......... .......... ....-..... .......... .......... .......... .......... ........C. ..........
ho1904 .......... .......... ....-..... .......... .......... .......... .......... .T........ ..........
ho1907 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
or1206 ....T..... ....T..... ....-..... .......... .........A ....T..... .......... .......... ..........
or1301 .....C.... .......... ....-..... .......... .......... .......... .......... .......... ..........
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or1303 .....T.... .......... ....-..... .......... ........G. .......... .......... .......... ..........
or1322 .....T.... .......... ....-..... .......... ........G. .......... .......... .......... ..........
or1324 ....T..... .......... ....-.A... .......... ........G. .......... .......... .......... ..........
or_404 .....T.... .......... ....-..... .......... ........G. .......... .......... .......... ..........
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or_603 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
or_605 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
or_618 .....C.... .......... ....-.A... .......... ........G. .......... .G........ .......... ..........
or_645 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
mo3221 .C...C.... .......... ....-..... .......... ........G. .......... .......... .......... .....T....
mo3222 .....C.... .......... ....-..... .......... ........G. .......... .......... .......... ..........
sy2001 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy2002 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy2004 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy2005 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy4301 .......... .......... ....-..... .......... .......... .......... .......... .T........ ..........
sy4309 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy4312 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy2802 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy2803 .......... .......... ...A-..... .......... .......... .......... .......... .......... ..........
sy2901 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy2904 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy3103 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy3105 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy3206 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy3209 ....T..... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy3211 ....T..... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy5901 .......... .......... ....-.A... .......... ........G. .......... .......... .....C.... ..........
sy5907 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
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sy4209 GGGGCGCGGG ATCTCGTTTG GTGG-CGGAA GTTGGCCTCC CGTGGGCCTG TGCTCGCGGT TAGCCTAAAA AGGAGTCCTC GGCGACGAGC
sy1601 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy1603 .......... .......... ....-..... .......... .......... .......... .......... ........C. ..........
sy1607 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy1610 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy5403 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy5423 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy5506 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy5508 ....T..... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy4601 .......... .......... ....-..... .......... .......... .......... .......... .......... ........A.
sy4604 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy4802 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy4804 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy4903 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy4904 .......... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy4701 .....T.... .......... ....-..... .......... .......... .......... .......... .......... ..........
sy4702 .......... .......... ....-..... .......... .......... ..G....... .......... ........C. ..........

                                                                «ITS2»
       6666666666 6666666666 6666666666 6666666666 6666666666 6666666666 6666666667 7777777777 7777777777
       3333333334 4444444445 5555555556 6666666667 7777777778 8888888889 9999999990 0000000001 1111111112
       1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890 1234567890
sy4209 GCCACGACAA TCGGTGGTTG ATTAGACCTC GGTCCCCGTC GTGCGTGTCT GGTCGCCACA AGGTGTGACT CGTCGACCCT AACGCGTCGT
sy4212 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4213 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4216 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5308 .......... .......... .......... .......... .......... .......... .......... .......... ..........
en_108 .......... .......... ..G....... .......... .......... .......... C......... .......... .....A....
en_126 .......... .......... ..G....... .......... .......... ........T. C......... .......... ....T..T..
en_135 .......... .......... ..G....... .......... .......... .......... C......... .......... .....A....
en_136 .......... .......... ..G....... .......... .......... .......... C......... .......... .....A....
en_202 .......... .......... ..G....... .......... .......... ........T. C......... .......... ..T.......
en_203 .......... .......... ..G....... .......... .......... .......... C......... .......... .....A....
en_204 .......... .......... ..G....... A......... .......... ........T. C......... ...T...... ..........
en_206 .......... .......... ..G....... .......... .......... ........T. C......... .......... ..........
en_301 .......... .......... ..G....... .......... .......... .......... C......... .......... .....A....
en_302 .......... .......... ..G....... .......... .......... .......... C......... .......... .....A....
en_303 .......... .......... ..G....... .......... .......... ........T. C......... ...T...... ..........
en_304 .......... .......... ..G....... .......... .......... .......... C....C.... .......... .....A....
en3505 .......... .......... ..G....... .......... A......... .......... C......... .......... .....A....
en3530 .......... .......... ..G....... .......... ........T. ........T. C......... ...T...... ..........
en3541 .......... .......... ..G....... .......... .......... ........T. C......... .......... ..........
en_402 .......... .......... ..G....... .......... .......... .......... C......... .......... .....A....
en_412 .......... .......... ..G....... .......... .......... .......... C......... .......... .....A....
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en_415 .......... .......... ..G......T .......... .......... .......... C......... .......... .....A....
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ja_101 .......... .......... ..G....... .......... .......... ........T. C......... .......... ..........
ja_102 .......... .......... ..G....... .......... .......... .......... C......... .......... .....A....
ja_103 .......... .......... ..G....... .......... ........T. ........T. C......... ...T...... ..........
ja_108 .......... .......... ..G....... .......... .......... ........T. C......... .......... ..........
ja2508 .......... .......... ..G...A.. .......... .......... ....A..... C......... .......... .....A....
ja2509 .......... .......... ..G....... .......... A......... .......... C......... .......... .....A....
ja2514 .......... .......... ..G....... .......... A......... .......... C......... .......... .....A....
ja2529 .......... .......... ..G....... .......... A......... .......... C......... .......... .....A....
cr_201 .......... .......... .......... .......... .......... .......... C.........T........ ..........
cr_202 .......... .......... .......... .......... ..A..C.... .......... .......... .......... ..........
cr_205 .......... .......... .......... .......... .....C.... .......... .......... .......... ..........
cr_212 .......... .......... .......... .......... .....C.... .......... .......... .......... ..........
cr3001 .......... .......... .......... .......... .....C.... .......... .......... .......... ....T.....
cr3003 .......... .......... .......... .......... .......... .......... C.........T........ ..........
cr3005 .......... .......... .......... .......... .....C.... .......... C.........T........ ..........
cr3006 .......... .......... .......... .......... .....C.... .......... .......... .......... ..........
cr3066 .......... .......... .......... .......... .....C.... .......... .......... .......... ..........
gr2602 .......... .......... ..G....... .......... .......... .......... C......... .......... ..........
gr2606 .......... .......... ..G....... .......... .......... .......... C......... .......... ..........
gr2607 .......... .......... ..G....... .......... .......... .......... C......... .......... ..........
gr2701 .......... .......... ..G....... .......... .......... .......... C......... .......... ..........
gr2704 .......... .......... ..G....... .......... .......... .......... C......G.. .......... ..........
gr2706 .......... .......... ..G....... .......... .......... .......... C......... .......... ..........
gr_201 .......... .......... ..G....... ..C....... .......... .......... C......... .......... ..........
gr_203 .......... .......... ..G....... .......... .......... .......... C......... .......... ..........
gr_624 .......... .......... ..G....... .......... .......... .......... C......... .......... ..........
gr_628 .......... .......... ..G....... .......... .......... .......... C......... .......... ..........
gr_632 .......... .......... ..G....... .......... .......... .......... C......... .......... ..........
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gr5101 .......... .......... ..G....... .......... .......... .......... C......... .......... ..........
gr5102 .......... .......... ..G....... .......... .......... .......... C......... .......... ..........
gr5103 .......... .......... ..G....... .......... .......... .......... C......... .......... ..........
gr5104 .......... .......... ..G....... .......... .......... .......... C......... .......... ..........
ha_320 .......... .......... .......... .......... .......... .......... .......... .......... ..T.......
ha_321 .......... .......... ..G....... .......... .......... ........T. C......... .......... ..T.......
ha_326 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ha_327 .......... .......... .......... .......... .......... .......... .......... .......... ..T.......
ha_328 .......... .......... .......... .......... .....C.... .......... .......... .......... ..........
ha_415 .......... .......... ..G....... .......... .......... ........T. C......... .......... ..T.......
ha_416 .......... .......... ..G....... .......... .......... ........T. C......... .......... ..T.......
ha_417 .......... .......... ..G....... .......... .......... ........T. C......... .......... ..T.......
ha_426 .......... .......... ..G....... .......... .......... ........T. C......... .......... ..T.......
ha_536 .......... .......... .......... .A........ .......... .......... .......... .......... ..........
ha_546 .......... .......... ..G....... .......... .......... ........T. C......... .......... ..T.......
ha_550 .......... .......... .......... .......... .......... .......... .......... .......... ..T.......
ha_563 .......... .......... .......... .......... .......... .......... .......... .......... ..T.......
lo_110 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_113 .......... .......... .......... .......... .....C.... .......... C......... .......... ..........
lo_118 .......... .......... .......... .......... .......... .......... .......... .......... ..........
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lo_204 .......... .......... .......... .......... .......... .......... .......... .......... G.........
lo_208 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_209 .......... .......... .......... .......... .......... .......... .......... .......... G.........
lo_211 .......... .......... .......... .......... .......... .......... .......... .......... G.........
lo_302 .......... .......... .......... .......... .....C.... .......... C......... .......... ..........
lo_305 .......... .......... ..G....... .......... A......... ........T. C......... .......... ..T.......
lo_306 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo_316 .......... .......... .......... .......... .....C.... .......... C......... .......... ..........
lo4704 .......... .......... ..G....... .......... .......... ........T. C......... .......... ..........
lo4717 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo4721 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lo4722 .......... .......... ..G....... .......... .......... ........T. C......... .......... ..........
lu_102 .......... .......... .......... .......... .......... .......... ........G. .......... ..........
lu_103 .......... .......... .......... .......... .....C.... .......... .......... .......... ..........
lu_104 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu_105 .......... .......... .......... .......... .......... .......... C......... .T........ ..........
lu4836 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu4848 .......... .......... .......... .......... .......... .......... .......... .......... ..........
lu4861 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ho1601 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ho1602 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ho1603 .......... .......... .......... .......... .......... .......... .......... .......... ..........
ho1805 .......... .......... .......... .......... .......... .......... C......... .......... ..........
ho1807 .......... .......... .......... .......... .......... .......... C......... .......... ..........
ho1904 .......... .......... .......... .......... ...T...... .......... C......... ........T. ..........
ho1907 .......... .......... ..G....... .......... .......... .......... C......... .......... ..........
or1206 .......... .......... .......... .......... .......... .......... C......... .......... ..........
or1301 .......... .......... .......... .......... .......... ........G. .......... .......... ..........
or1302 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or1303 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or1322 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or1324 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_404 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_405 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_601 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_603 .......... .......... ..A....... .......... .......... .........G C......... .......... ..........
or_605 .......... .......... .......... .......... .......... .......... .......... .......... ..........
or_618 ........C. .......... ..G....... .......... .......... .......... .......... .......... ..........
or_645 .......... .......... .......... .......... .......... .......... .......... .......... ..........
mo3221 .......... .......... .......... .......... .........C .......... .......... .......... ..........
mo3222 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2001 .......... .......... .......... .......... .......... .......... C......... .......... ..........
sy2002 .......... .......... .......... .......... .......... .......... C......... .......... ..........
sy2004 .......... .......... .......... .......... .......... .......... C......... .......... ..........
sy2005 .......... .......... .......... .......... .......... .......... C......... .......... ..........
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sy4309 .......... .......... .......... .......... .......... .........G C......... .......... ..........
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sy2901 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy2904 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy3103 .......... .......... .......... .......... .......... .........G C......... .......... ..........
sy3105 .......... .......... .......... .......... .......... .........G C......... .......... ..........
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sy4209 GCCACGACAA TCGGTGGTTG ATTAGACCTC GGTCCCCGTC GTGCGTGTCT GGTCGCCACA AGGTGTGACT CGTCGACCCT AACGCGTCGT
sy3206 .......... .......... .......... .......... .......... .....T.... .......... .......... ..........
sy3209 .......... .......... .......... .......... .......... A......... .......... .......... ..........
sy3211 .......... .......... .......... .......... .......... A......... .......... .......... ..........
sy5901 .......... .......... .......... .......... ...T...... .......... C......... .......... ..........
sy5907 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy1601 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy1603 .......... .......... .......... .......... .......... .......... C......... .......... ..........
sy1607 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy1610 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5403 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5423 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5506 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy5508 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4601 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4604 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4802 .......... .......... .......... .......... .......... .........G C......... .......... ..........
sy4804 .......... .......... .......... .......... .......... .........G C......... .......... ..........
sy4903 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4904 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4701 .......... .......... .......... .......... .......... .......... .......... .......... ..........
sy4702 .......... .......... .......... .......... .......... .......... C......... .......... ..........

          3' end ITS2 ->&lt;- 5' end 26S rDNA
       7777777777 7777777777 7777777777 7777777777 7777777777 777
       2222222223 3333333334 4444444445 5555555556 6666666667 777
       1234567890 1234567890 1234567890 1234567890 1234567890 123
sy4209 ACCCACGTCG CTCCCAACGC GACCCCAGGT CAGGCGGGAC TACCCGCTGA GTT
sy4212 .......... ..........  .......... .......... .......... ...
sy4213 .......... ..........  .......... .......... .......... ...
sy4216 .......... ..........  .......... .......... .......... ...
sy5308 .......... ..........  .......... .......... .......... ...
en_108 .......... ..........  .......... ...T...... .......... ...
en_126 ...A...... ..........  ....T..... .......... .......... ...
en_135 .......... ..........  .......... ...T...... .......... ...
en_136 .......... ..........  .......... ...T...... .......... ...
en_202 ...A...... ..........  .......... .......... .......... ...
en_203 .......... ..........  .......... ...T...... .......... ...
en_204 ...A...... ..........  .......... .......... .......... ...
en_206 ...A...... .......T..  .......... .......... .......... ...
en_301 .......... ..........  .......... ...T...... .......... ...
en_302 .......... ..........  .......... ...T...... .......... ...
en_303 ...A...... ..........  .......... .......... .......... ...
en_304 .......... ..........  .......... ...T...... .......... ...
en3505 .......... ..........  .......... ...T...... .......... ...
en3530 ...A...... ..........  .......... .......... .......... ...
en3541 ...A...... ..........  .......... .......... .......... ...
en_402 .......... ..........  .......... ...T...... .......... ...
en_412 .......... ..........  .......... ...T...... .......... ...
en_413 ...A...... ..........  .......... .......... .......... ...
en_415 .......... ..........  .......... ...T...... .......... ...
en_416 ...A...... ..........  .......... .......... .......... ...
ja_101 ...A...... ..........  .......... .......... .......... ...
ja_102 .......... ..........  .......... ...T...... .......... ...
ja_103 ...A...... ..........  .......... .......... .......... ...
ja_108 ...A...... ..........  .......... .......... .......... ...
ja2508 .......... ..........  .......... ...T...... .......... ...
ja2509 .......... ..........  .......... ...T...... .......... ...
ja2514 .......... ..........  .......... ...T...... .......... ...
ja2529 .......... ..........  .......... ...T...... .......... ...
cr_201 .......... ..........  .......... .......... .......... ...
cr_202 .......... ..........  .......... .......... .......... ...
cr_205 .......... ..........  .......... .......... .......... ...
cr_212 .......... ........T.  .......... .......... .......... ...
cr3001 .......... ..........  .......... .......... .......... ...
cr3003 .......... ..........  .......... .......... .......... ...
cr3005 .......... ..........  .......... .......... .......... ...
cr3006 .......... ..........  .......... .......... .......... ...
cr3066 .......... ..........  .......... .......... .......... ...
gr2602 .......... .....G....  .......... .......... .......... ...
gr2606 .......... .....G....  .......... .......... .......... ...
gr2607 .......... .....G....  .......... .......... .......... ...
gr2701 .......... .....G....  .......... .......... .......... ...
gr2704 .......... .....G....  .......... .......... .......... ...
gr2706 .......... ..........  .......... .......... .......... ...
gr_201 .......... .....G....  .......... .......... .......... ...
gr_203 .......... .....G....  .......... ......A... .......... ...
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sy4209 ACCCACGTCG CTCCCAACGC GACCCCAGGT CAGGCGGGAC TACCCGCTGA GTT
gr_624 .......... .......... .......... .......... .......... ...
gr_628 .......... .......... .......... .......... .......... ...
gr_632 .......... .....G.... .......... .......... .......... ...
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gr5103 .......... .....G.... .......... .......... .......... ...
gr5104 .......... .....G.... .......... .......... .......... ...
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ha_321 .......... .......... .......... .......... .......... ...
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ha_327 .......... .......... .......... .......... .......... ...
ha_328 .......... .......... .......... .......... .......... ...
ha_415 .......... .......... .......... .......... .......... ...
ha_416 .......... .......... .......... ....G..... .......... ...
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ha_426 .......... .......... .......... .......... .......... ...
ha_536 .......... .......... .......... .......... .......... ...
ha_546 .......... .......... .......... .......... .......... ...
ha_550 .......... .......... .......... .......... .......... ...
ha_563 .......... .......... .......... .......... .......... ...
lo_110 .......... .......... .......... .......... .......... ...
lo_113 .......... .......... .......... .......... .......... ...
lo_118 .......... .......... .......... .......... .......... ...
lo_119 .......... .......... .......... .......... .......... ...
lo_204 .......... .......... .......... .......... .......... ...
lo_208 .......... .......... .......... .......... .......... ...
lo_209 .......... .......... .......... .......... .......... ...
lo_211 .......... .......... .......... .......... .......... ...
lo_302 .......... .......... .......... .......... .......... ...
lo_305 .......... .......... .......??? ?????????? ?????????? ???
lo_306 .......... .......... .......... .......... .......... ...
lo_316 .......... .......... .......... .......... .......... ...
lo4704 .......... .......... .......... .......... .......... ...
lo4717 .......... .......T.. .......... .......... .......... ...
lo4721 .......... .......... .......... .......... .......... ...
lo4722 .......... .......... .......... .......... .......... ...
lu_102 .......... .......... .......... .......... .......... ...
lu_103 .......... .......... .......... .......... .......... ...
lu_104 .......... .......... .......... .......... .......... ...
lu_105 .......... .......... .......... .......... .......... ...
lu4836 .......... .......... .......... .......... .......... ...
lu4848 .......... .......... .......... .......... .......... ...
lu4861 .......... .......... .......... .......... .......... ...
ho1601 .......... .......... .......... .......... .......... ...
ho1602 .......... .......... .......... .......... .......... ...
ho1603 .......... .......... .......... .......... .......... ...
ho1805 .......... .......... .......... .......... .......... ...
ho1807 .......... .......... .......... .......... .......... ...
ho1904 .......... .......... .......... .......... .......... ...
ho1907 .......... .....G.... .......... .......... .......... ...
or1206 .......... .......... .......... .......... .......... ...
or1301 .......... ........?? ?????????? ?????????? ?????????? ???
or1302 .......... ........?? ?????????? ?????????? ?????????? ???
or1303 .......... ........?? ?????????? ?????????? ?????????? ???
or1322 .......... .......... .......... .......... .......... ...
or1324 .......... .......... .......... .......... .......... ...
or_404 .......... .......... .......... .......... .......... ...
or_405 .......... ........?? ?????????? ?????????? ?????????? ???
or_601 .......... ........?? ?????????? ?????????? ?????????? ???
or_603 .......... .......T?? ?????????? ?????????? ?????????? ???
or_605 .......... ........?? ?????????? ?????????? ?????????? ???
or_618 .......G.. .......... .......... .......... .......... ...
or_645 .......... .......... .......... .......... .......... ...
mo3221 .......... .......... .......... .......... .......... ...
mo3222 ......A... .......... .......... .......... .......... ...
sy2001 .......... .......... .......... .......... .......... ...
sy2002 .......... .......... .......... .......... .......... ...
sy2004 .......... .......... .......... .......... .......... ...
sy2005 .......... .......... .......... .......... .......... ...
sy4301 .......... .......... .......... .......... .......... ...
sy4309 .......... .......... .......... .......... .......... ...
sy4312 .......... .......... .......... .......... .......... ...
sy2802 .......... .......... .......... .......... .......... ...
sy2803 .......... .......... .......... .......... .......... ...
sy2901 .......... .......... .......??? ?????????? ?????????? ???
sy2904 .......... .......... .......... .......... .......... ...
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sy4209 ACCCACGTCG CTCCCAACGC GACCCCAGGT CAGGCGGGAC TACCCGCTGA GTT
sy3103 .......... .........  .......... .......... .......... ...
sy3105 .......... ........A. .......... .......... .......... ...
sy3206 .......... .........  .......... .......... .......... ...
sy3209 .......... .........  .......... .......... .......... ...
sy3211 .......... .........  .......... .......... .......... ...
sy5901 .......... .........  .......... .......... .......... ...
sy5907 .......... .........  .......... .......... .......... ...
sy1601 .......... .........  .......... .......... .......... ...
sy1603 .......... .........  .......... .......... .......... ...
sy1607 .......... .........  .......... .......... .......... ...
sy1610 .......... .........  .......... .......... .......... ...
sy5403 .......... .........  .......... .......... .......... ...
sy5423 .......... .........  .......... .......... .......... ...
sy5506 .......... .........  ........C. .......... .......... ...
sy5508 .......... .........  .......... T......... .......... ...
sy4601 .......... .........  .......... .......... .......... ...
sy4604 .......... .........  .......... .......... .......... ...
sy4802 .......... .........  .......... .......... .......... ...
sy4804 .......... .........  .......... .......... .......... ...
sy4903 .......... .........  .......... .......... .......... ...
sy4904 .......... .........  .......... .......... .......... ...
sy4701 .......... .........  .......... .......... .......... ...
sy4702 .......... .........  .......... .......... .......... ...
```

Appendix V: Additional information for ISV analyses

Stepmatrices used for character coding

Character set "complex" (see below) includes the following stepmatrices:

A) Stepmatrices with 4 character states:

		a	b	c	d
characters #17, #44 & #57	a	0	1	1	2
	b	1	0	2	1
	c	1	2	0	1
	d	2	1	1	0
character #40	a	0	1	1	3
	b	1	0	2	3
	c	1	2	0	2
	d	3	3	2	0
character #41	a	0	1	1	3
	b	1	0	2	2
	c	1	2	0	2
	d	3	2	2	0

B) Stepmatrices with 5 character states:

		a	b	c	d	e
character #5	a	0	1	2	1	2
	b	1	0	1	2	1
	c	2	1	0	3	2
	d	1	2	3	0	1
	e	2	1	2	1	0
character #9	a	0	1	2	3	1
	b	1	0	1	2	2
	c	2	1	0	1	1
	d	3	2	1	0	2
	e	1	2	1	2	0
character #25	a	0	1	1	1	2
	b	1	0	2	2	3
	c	1	2	0	2	1
	d	1	2	2	0	1
	e	2	3	1	1	0
character #35	a	0	1	1	1	2
	b	1	0	2	2	1
	c	1	2	0	2	3
	d	1	2	2	0	1
	e	2	1	3	1	0

C) Stepmatrices with 7 character states:

		a	b	c	d	e	f	g
character #3	a	0	1	2	3	2	2	1
	b	1	0	1	2	3	3	2
	c	2	1	0	1	4	4	3
	d	3	2	1	0	5	5	4
	e	2	3	4	5	0	1	2
	f	2	3	4	5	1	0	3
	g	1	2	3	4	2	3	0
character #13	a	0	1	1	1	1	2	2
	b	1	0	2	2	2	1	3
	c	1	2	0	2	2	3	3
	d	1	2	2	0	2	1	1
	e	1	2	2	2	0	3	1
	f	2	1	3	1	3	0	2
	g	2	3	3	1	1	2	0
character #14	a	0	1	2	1	2	1	2
	b	1	0	1	2	3	2	3
	c	2	1	0	3	4	3	4
	d	1	2	3	0	1	2	3
	e	2	3	4	1	0	3	4
	f	1	2	3	2	3	0	1
	g	2	3	4	3	4	1	0
character #18	a	0	1	1	2	1	2	3
	b	1	0	2	3	2	3	4
	c	1	2	0	2	2	3	4
	d	2	3	2	0	3	4	5
	e	1	2	2	3	0	3	4
	f	2	3	3	4	3	0	1
	g	3	4	4	5	4	1	0
character #43	a	0	3	2	1	2	1	2
	b	3	0	1	4	5	2	5
	c	2	1	0	3	4	1	4
	d	1	4	3	0	1	2	1
	e	2	5	4	1	0	3	2
	f	1	2	1	2	3	0	3
	g	2	5	4	1	2	3	0

D) Stepmatrices with 8 character states:

		a	b	c	d	e	f	g	h
character #11	a	0	1	1	1	1	2	2	2
	b	1	0	2	2	2	1	1	1
	c	1	2	0	2	2	1	3	3
	d	1	2	2	0	2	3	1	3
	e	1	2	2	2	0	3	3	1
	f	2	1	1	3	3	0	2	2
	g	2	1	3	1	3	2	0	2
	h	2	1	3	3	1	2	2	0
character #31	a	0	1	2	3	2	3	1	2
	b	1	0	1	2	1	2	2	1
	c	2	1	0	1	2	3	3	2
	d	3	2	1	0	1	2	4	3
	e	2	1	2	1	0	1	3	2
	f	3	2	3	2	1	0	2	1
	g	1	2	3	4	3	2	0	1
	h	2	1	2	3	2	1	1	0
character #56	a	0	1	2	2	2	3	2	2
	b	1	0	1	2	1	2	2	1
	c	2	1	0	3	2	1	3	2
	d	2	2	3	0	1	2	4	3
	e	2	1	2	1	0	1	3	2
	f	3	2	1	2	1	0	4	3
	g	2	2	3	4	3	4	0	1
	h	2	1	2	3	2	3	1	0

E) Stepmatrices with 9 character states :

		a	b	c	d	e	f	g	h	i
character #56	a	0	2	1	3	3	4	1	2	2
	b	2	0	3	3	5	3	3	4	4
	c	1	3	0	2	2	3	2	3	1
	d	3	3	2	0	2	3	4	3	3
	e	3	5	2	2	0	3	4	3	1
	f	4	3	3	3	3	0	3	4	4
	g	1	3	2	4	4	3	0	3	3
	h	2	4	3	3	3	4	3	0	4
	i	2	4	1	3	1	4	3	4	0

Complete list of character for ISV analysis

	character #	type	number of character states	coding
ITS1	1	ordered	3(4)*	A_0=a {A_0B_0}=b {B_0C_0}=d
	2	ordered	3	A_0=a {A_0B_0}=b B_0=c
	3	complex†	7	A_0=a {A_0C_0}=b C_0=c {B_0C_0}=d {A_0E_0}=e {$A_0D_0E_0$}=f {A_0F_0}=g
	4	binary	2	A_0=a {A_0B_0}=b
	5	complex	5	A_0=a {A_0B_0}=b B_0=c {A_0C_0}=d X_0=e‡
	6	ordered	3	{A_0C_0}=a A_0=b {A_0B_0}=c
	7	ordered	3	A_0=a {A_0B_0}=b B_0=c
	8	binary	2	A_0=a {A_0B_0}=b
	9	complex	5	{A_0B_0}=a A_0=b {A_{0-0}}=c $_{-0}$=d X_0=e
	10	binary	2	A_0=a {A_0B_0}=b
	11	complex	8	A_0=a {A_0B_0}=b {A_0C_0}=c {A_0D_0}=d {A_0E_0}=e {$A_0B_0C_0$}=f {$A_0B_0D_0$}=g {$A_0B_0E_0$}=h
	12	binary	2	A_0=a (A_0B_0)=b
	13	complex	7	A_0=a {A_0B_0}=b {A_0C_0}=c {A_0D_0}=d {A_0E_0}=e {$A_0B_0D_0$}=f {$A_0D_0E_0$}=g
	14	complex	7	A_0=a {A_{0-0}}=b $_{-0}$=c {A_0B_0}=d B_0=e {A_0C_0}=f C_0=g
	15	binary	2	A_0=a {A_0B_0}=b
	16	binary	2	A_0=a {A_0B_0}=b
	17	complex	4	A_0=a {A_0B_0}=b {A_0C_0}=c X_0=d
	18	complex	7	A_0=a {A_0A_1}=b {A_0C_0}=c {A_0D_0}=d {A_0E_0}=e {A_0F_0}=f {$A_0B_0F_0$}=g
	19	ordered	3	{A_0B_0}=a A_0=b {A_0C_0}=c
	20	complex	4	A_0=a {A_0B_0}=b {A_0C_0}=c {A_0D_0}=d
	21	binary	2	A_0=a {A_0B_0}=b
	22	ordered	3	{A_0B_0}=a A_0=b {A_0C_0}=c
	23	ordered	3	A_0=a {A_0B_0}=b B_0=c
	24	binary	2	A_0=a {A_0B_0}=b
	25	complex	5	A_0=a {A_0B_0}=b {A_0C_0}=c {A_0D_0}=d {$A_0C_0D_0$}=e
	26	ordered	3	{A_0B_0}=a A_0=b {A_0C_0}=c
	27	binary	2	A_0=a {A_0B_0}=b
	28	complex	4	{A_0D_0}=a A_0=b {A_0B_0}=c {$A_0B_0C_0$}=d
	29	binary	2	A_0=a {A_0B_0}=b
	30	binary	2	A_0=a {A_0B_0}=b
ITS2	31	complex	8	A_0=a {A_0B_0}=b B_0=c {B_0D_0}=d {$A_0B_0D_0$}=e X_0=f {A_0C_0}=g {$A_0B_0C_0$}=h
	32	binary	2	A_0=a {A_0B_0}=b
	33	ordered	4	C_0=a {A_0C_0}=b A_0=c {A_0A_1}=d
	34	ordered	3	A_0=a {A_0B_0}=b B_0=c
	35	complex	5	A_0=a {A_0B_0}=b {A_0C_0}=c {A_0D_0}=d {$A_0B_0D_0$}=e
	36	binary	2	A_0=a {A_0B_0}=b
	37	complex	9	A_0=a {$A_0B_0F_0$}=b {A_0C_0}=c {$A_0B_0C_0D_0$}=d {$A_0C_0D_0E_0$}=e {$A_0C_0D_0F_0G_0$}=f {A_0G_0}=g D_0=h {$A_0C_0E_0$}=i
	38	binary	2	A_0=a {A_0B_0}=b
	39	binary	2	A_0=a {A_0B_0}=b
	40	complex	4	A_0=a {A_0B_0}=b {A_0C_0}=c {$A_0B_1C_0$}=d
	41	complex	4	A_0=a {A_0B_0}=b {A_0C_0}=c X_0=d
	42	binary	2	A_0=a {A_0B_0}=b
	43	complex	7	A_0=a {$A_0B_0D_0E_0F_0$}=b {$A_0D_0E_0$}=c {A_0C_0}=d {$A_0C_0G_0$}=e {A_0D_0}=f C_0=g
	44	complex	4	A_0=a {A_0B_0}=b {A_0C_0}=c X_0=d
	45	binary	2	A_0=a {A_0B_0}=b
	46	complex	4	A_0=a {A_0B_0}=b {A_0C_0}=c {A_0D_0}=d
	47	binary	2	A_0=a {A_0B_0}=b
	48	ordered	3	{A_0B_0}=a A_0=b {A_0C_0}=c
	49	binary	2	A_0=a {A_0B_0}=b
	50	ordered	3	A_0=a {A_0B_0}=b B_0=c
	51	binary	2	A_0=a {A_0B_0}=b
	52	binary	2	A_0=a {A_0B_0}=b
	53	binary	2	A_0=a {A_0B_0}=b
	54	binary	2	A_0=a {A_0B_0}=b
	55	binary	2	A_0=a {A_0B_0}=b
	56	complex	8	A_0=a {A_0B_0}=b B_0=c {A_0C_0}=d {$A_0B_0C_0$}=e {B_0C_0}=f {A_0D_0}=g {$A_0B_0D_0$}=h
	57	complex	4	A_0=a {A_0B_0}=b {A_0C_0}=c X_0=d
	58	binary	2	A_0=a {A_0B_0}=b
	59	binary	2	A_0=a {A_0B_0}=b
	60	binary	2	A_0=a {A_0B_0}=b
	61	ordered	3(4)	A_0=a X_0=c {B_0C_0}=d
	62	ordered	3	A_0=a {A_0B_0}=b B_0=c

* in the case of ordered characters, single transitional characters states may be lacking

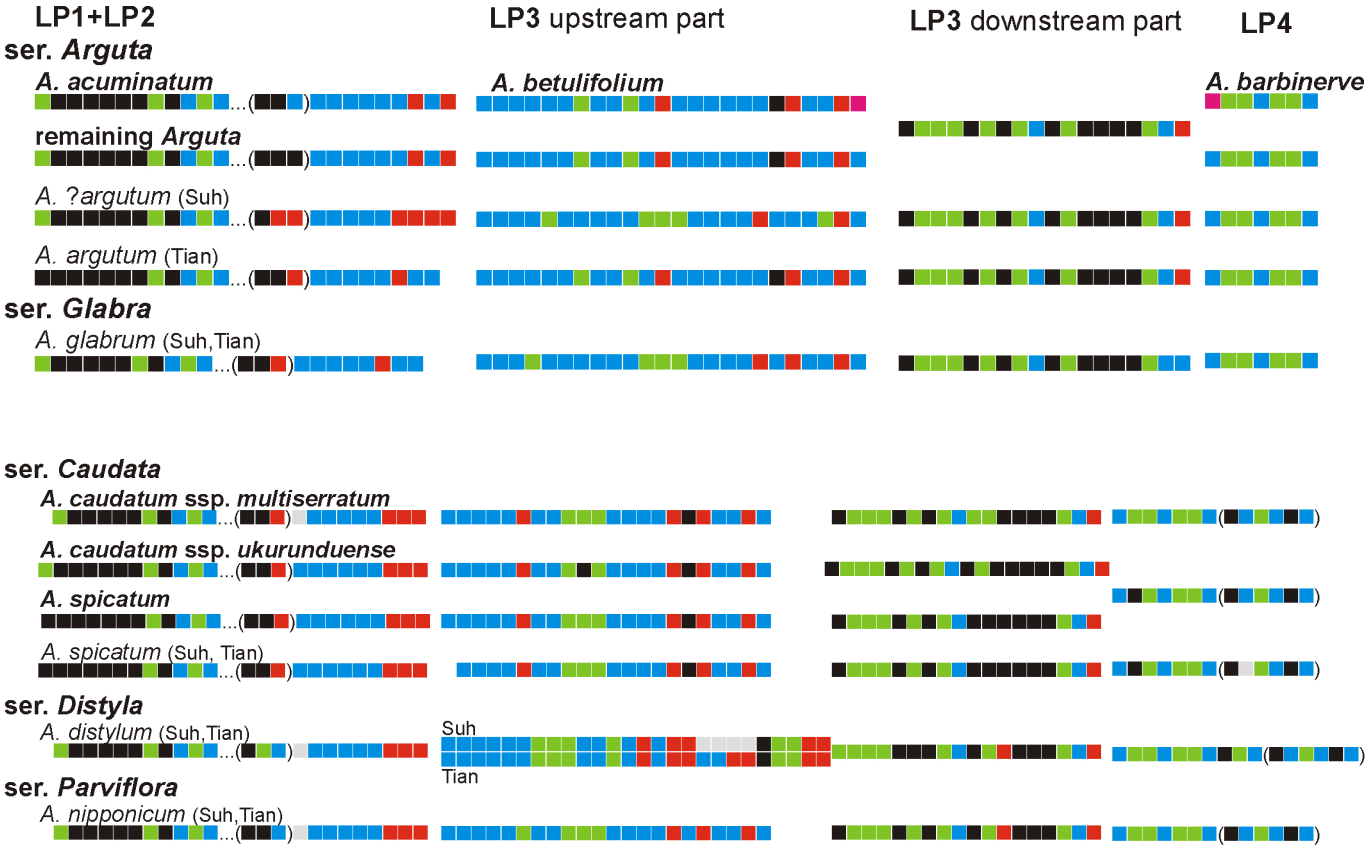
† see above for further details on complex characters

‡ X_0 = all (oligo)nucleotide states realised

Appendix VI: ITS accessions from the gene bank

Comparison between new (bold font) and gene bank accessions (SUH et al. 2000; TIAN et al. 2002; normal font) assigned to series *Arguta* and its presumed sister series *Glabra* (⇨ sect. *Glabra*), respectively series *Caudata, Distyla,* and *Parviflora* (⇨ sect. *Parviflora*). Standard colour code, "()" indicate flanking regions, which are not part of LP1 to LP4.

The motives of Suh's sequence of *A. argutum* are identical to the typical *Macrantha* pattern (not shown) and differ strikingly from the new data and the sequence of TIAN et al. (2002). This observation confers also to the remaining parts of the ITS1 and ITS2. Thus, the systematic position of *A. glabrum* with a LP1/LP2 motif identical to Tian's *A. argutum*, but exhibiting a macranthoid LP3 region, can not be validated.



The gene bank accessions of *A. spicatum* are widely identical to the new data (but note the lack of 1 bp at the 3' end of ITS2 in a highly conserved region, which is most probable a detection or editing artefact). Most distinct to the other accessions is *A. distylum*: its LP3 motif exhibits a nucleotide composition not reported in any other *Acer* taxon, while the elongated LP4 motif is identical to the 11 bp motif detected in newly assembled accessions of *A. palmatum* (sect. *Palmata*). Whether the indel within the upstream part of LP3 is a true polymorphism or detection artefact, cannot be decided.

labelled as	synonymous to*	assigned to†	source	comparison to newly assembled data
A. caesium ssp. *giraldii*		*Acer*	Tian et al. 2002	falls within the variability found for *A. caesium* ssp. *caesium*
A. trautvetteri		*Acer*	Tian et al. 2002	identical to new sequences
A. monspes-sulanum		*Monspess.*	Tian et al. 2002	exhibits typical *A. monspessulanum* sequence
A. opalus		*Monspess.*	Tian et al. 2002	exhibits typical *A. opalus* sequence
A. saccharum		*Saccharod.*	Tian et al. 2002	exhibits typical *A. saccharum* sequence, 2 obvious detection faults towards the 3' end of ITS2
A. ginnala	*A. tataricum* ssp. ~	*Ginnala*	Suh/Tian	ITS1 identical, ITS2 differs in composition of LP3 and LP4 (in the case of LP4 obvious detection faults)
A. glabrum		*Glabra*	Suh/Tian	affinities to ser. *Arguta*, but only few derived geotypic characteristics
A. argutum		*Arguta*	Tian et al. 2002	similar to other *Arguta* sequences
A. argutum		*Arguta*	Suh et al. 2000	sequence strikingly similar to *Macrantha*-genotype
A. laurinum		*Hyptiocarpa*	Suh et al. 2000	strongly derived, affinities to *A. diabolicum* and sect. *Macrantha;* Suh's and Tian's accessions differ from each other in the ITS2
A. decandrum	*A. laurinum*	*Hyptiocarpa*	Tian et al. 2002	
A. carpini-folium		*Indivisa*	Tian et al. 2002	important molecular characteristics present
A. carpini-folium		*Indivisa*	Suh et al. 2000	the same, but 3' part of ITS2 with conspicuous derivations
A. diabolicum		*Lithocarpa*	Suh et al. 2000	major molecular characteristics present
A. kung-shanense	*A. franchetii ssp.* ~	*Lithocarpa*	Tian et al. 2002	most similar to *A. diabolicum*
A. macro-phyllum		*Macrophylla*	Tian et al. 2002	widely identical to new sequences
A. davidii		*Macrantha*	Tian et al. 2002	widely identical to new sequences
A. tegmen-tosum		*Macrantha*	Cho/Suh/Tian	fits within detected genotypes of sect. *Macrantha*
A. cissifolium		*Cissifolia*	Tian et al. 2002	identical to new sequences
A. cissifolium		*Cissifolia*	Suh et al. 2000	identical to new sequences (1 bp missing in LP4)
A. henryi		*Cissifolia*	Tian et al. 2002	identical to new sequences
A. negundo		*Negundo*	Cho/Suh/Tian	exhibits typical *A. negundo* sequence
A. japonicum		*Palmata*	Cho/Suh	similar to *A. japonicum* 'Aconitifolium', but LP3 appears not well-sequenced
A. palmatum		*Palmata*	Suh/Tian	widely identical to new sequences
A. pseudosie-boldianum		*Palmata*	Cho et al. 1997	fits within section *Palmata*
A. takesi-mense	*A. pseudosie-boldianum* ssp. ~	*Palmata*	Cho/Suh	fits within section *Palmata*
A. crassum		*Penninervia*	Tian et al. 2002	differs markedly in the length polymorphic regions/indels, with affinities to sect. *Palmata* (especially *A. erianthum*) and sect. *Macrantha*
A. fabri		*Penninervia*	Suh et al. 2000	~ *A. crassum*, but differs even stronger from the elsewhere realised patterns in the variable regions
A. miao-shanicum		*Sinensia*	Tian et al. 2002	fits perfectly to section *Palmata*
A. pubinerve	*A. campbelli* ssp. *sinense*	*Sinensia*	Tian et al. 2002	wide agreement with *A. campbelli* ssp. *campbelli*

labelled as	synonymous to*	assigned to†	source	comparison to newly assembled data
A. spicatum		*Caudata*	Cho/Suh	widely identical to new sequences
A. distylum		*Distyla*	Suh/Tian	exhibits predominately ancestral motives; similar to ser. *Caudata*, some affinities to sect. *Platanoidea*
A. nipponi-cum		*Parviflora*	Suh/Tian	~ *A. distylum*, but no affinities to sect. *Platanoidea*
A. campestre		*Platanoidea*	Tian et al. 2002	falls within the detected intraspecific variability
A. cappado-cicum		*Platanoidea*	Tian et al. 2002	definite *Platanoidea*-sequence, nearly identical to *A. pictum* ssp. *mono*, specific features of *A. cappadocicum* are missing
A. mono	*A. pictum* ssp. ~	*Platanoidea*	Cho/Suh	falls mainly within the variability found for *A. pictum* ssp. *mono/A. truncatum*
A. okamoto-anum		*Platanoidea*	Cho/Suh	falls mainly within the variability found for *A. pictum* ssp. *mono/A. truncatum*
A. platanoides		*Platanoidea*	Cho/Tian	widely identical to new sequences
A. truncatum		*Platanoidea*	Cho et al. 1997	definite *Platanoidea*-sequence, more similar to *A. platanoides* than to *A. pictum* ssp. *mono/A. truncatum*
A. rubrum		*Rubra*	Tian et al. 2002	identical to new sequences
A. saccha-rinum		*Rubra*	Tian et al. 2002	identical to new sequences
A. penta-phyllum		*Pentaphylla*	Tian et al. 2002	most similar to ser. *Trifida*; LP1-/ID4-/ID5-region markedly derived
A. buerger-ianum		*Trifida*	Cho/Suh/Tian	all specific features of *A. buergerianum* are represented
A. paxii		*Trifida*	Tian et al. 2002	*A. buergerianum*-specific features are mainly represented; distinctively composited LP1-/ID4-/ID5-region
A. polio-phyllum	not recognised‡	?*Trifida*	Tian et al. 2002	most similar to *A. paxii* (ser. *Trifida*)
A. griseum		*Grisea*	Tian et al. 2002	widely identical to new sequences
A. triflorum		*Grisea*	Tian et al. 2002	identical to new sequences
A. mandshu-ricum		*Mandshurica*	Tian et al. 2002	similar to ser. *Grisea*, but LP1/ID4/ID5/LP2 distinct
A. wardii		*Wardiana*	Tian et al. 2002	ITS1 most similar to sect. *Platanoidea*, ITS2 miscellaneous; although distinct, derived motives are lacking

* taxonomic nomenclature according GRIN database, VAN GELDEREN et al. (1994)

† section/series according to current systematic synopsis (VAN GELDEREN et al. 1994)

‡ taxon not recognised as species name or synonym by OGATA (1967), VAN GELDEREN (1994), GRIN database etc.

Bildungsgang

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