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# Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels

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## Abstract

Asterids comprise 1/4–1/3 of all flowering plants and are classified in 10 orders and >100 families. The phylogeny of asterids is here explored with jackknife parsimony analysis of chloroplast DNA from 132 genera representing 103 families and all higher groups of asterids. Six different markers were used, three of the markers represent protein coding genes, *rbcL*, *ndhF*, and *matK*, and three other represent non-coding DNA; a region including *trnL* exons and the intron and intergenic spacers between *trnT* (UGU) to *trnF* (GAA); another region including *trnV* exons and intron, *trnM* and intergenic spacers between *trnV* (UAC) and *atpE*, and the *rps16* intron. The three non-coding markers proved almost equally useful as the three coding genes in phylogenetic reconstruction at the high level of orders and families in asterids, and in relation to the number of aligned positions the non-coding markers were even more effective. Basal interrelationships among Cornales, Ericales, Lamiales (new name replacing euasterids I), and campanulids (new name replacing euasterids II) are resolved with strong support. Family interrelationships are fully or almost fully resolved with medium to strong support in Cornales, Garryales, Gentianales, Solanales, Aquifoliales, Apiales, and Dipsacales. Within the three large orders Ericales, Lamiales, and Asterales, family interrelationships remain partly unclear. The analysis has contributed to reclassification of several families, e.g., Tetrameristaceae, Ebenaceae, Styracaceae, Montiniaceae, Orobanchaceae, and Scrophulariaceae (by inclusion of Pellicieraceae, Lissocarpaceae, Halesiaceae, Kaliphoraceae, Cyclocheilaceae, and Myoporaceae + Buddlejaceae, respectively), and to the placement of families that were unplaced in the APG-system, e.g., Sladeniaceae, Pentaphragmataceae, Plocospermataceae, Cardiopteridaceae, and Adoxaceae (in Ericales, Ericales, Lamiales, Aquifoliales, and Dipsacales, respectively), and Paracryphiaceae among campanulids. Several families of euasterids remain unclassified to order. © 2002 Elsevier Science (USA). All rights reserved.

**Keywords:** Asterids; Phylogeny; *rbcL*; *ndhF*; *matK*; *trnT-F*; *trnV-atpE*; *rps16*; coding DNA; non-coding DNA

## 1. Introduction

The asterids constitute one of the major clades of the flowering plants. They represent an evolutionary successful group with over 80,000 species or 1/4–1/3 of all flowering plants. Four of the 10 largest plant families belong to this group, Asteraceae (c. 22,750 species),

Rubiaceae (c. 10,200 species), Lamiaceae (c. 6700 species), and Apocynaceae s.l. (c. 4800 species). They are often herbaceous plants with bisexual, insect-pollinated flowers, stamens in one circle, and sympetalous corollas. Plants with such corollas, known as Sympetalae, have been recognised as a natural group since the 18th century (Jussieu, 1789). Takhtajan (1964, 1969) renamed the group as subclass Asteridae, although he later (Takhtajan, 1987, 1997) restricted his Asteridae to the core of the order Asterales (sensu APG, 1998). Cronquist (1981)

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maintained a more wide circumscription of the Asteridae, including the Asterales, Dipsacales, Gentianales, Lamiales, and Solanales as currently understood (APG, 1998). Dahlgren (1983), who stressed the importance of chemical characters for classification, placed Apiales (=Araliales) and Cornales close to Asterales and Dipsacales, respectively, and in his diagrams Ericales were surrounded by Cornales, Dipsacales, Gentianales, Lamiales, and Solanales. These placements were based on the occurrence of polyacetylenes and iridoids, which are common compounds in the Asteridae.

With molecular data, particularly from the *rbcL* gene of the chloroplast genome, it became evident that the “core” Asteridae (Asterales, Dipsacales, Gentianales, Lamiales, and Solanales) are nested in a larger monophyletic group, including not only Cornales, Ericales, and Apiales but also Garryales and Aquifoliales (Chase et al., 1993; Downie and Palmer, 1992; Olmstead et al., 1992, 1993). Later analyses including more taxa and/or based on more genes, in particular *ndhF*, *atpB*, and 18S rDNA, have corroborated the first molecular analyses and generated more detailed knowledge of the group (Backlund and Bremer, 1997; Hempel et al., 1995; Gustafsson et al., 1996; Morton et al., 1996; Plunkett et al., 1996; Savolainen et al., 1994; Soltis and Soltis, 1997; Soltis et al., 1997). The results from these studies are considered in the classification by the Angiosperm Phylogeny Group (APG, 1998), which is the starting point for the present study. Subsequent analyses are also considered here and in the forthcoming revision of the APG-system (Albach et al., 2001a; Backlund et al., 2000; Kårehed, 2001; Olmstead et al., 2000; Oxelman et al., 1999; APGII, in prep.). So far we know that all asterids form a strongly supported monophyletic group including 10 orders, viz. Cornales, Ericales, Garryales, Gentianales, Lamiales, Solanales, Aquifoliales, Apiales, Asterales, and Dipsacales. The last eight of these constitute the euasterids, which form two major subgroups, known as asterids I and II (Chase et al., 1993) or euasterids I (Garryales, Gentianales, Lamiales, and Solanales) and II (Apiales, Aquifoliales, Asterales, and Dipsacales) (APG, 1998). Since these names are awkward and easily confused, we here take the opportunity to rename euasterids I as **lamiids** and euasterids II as **campanulids**. More global analyses of the flowering plants (Soltis et al., 2000) have corroborated the monophyly of asterids and euasterids, and partly also the monophyly of lamiids, campanulids, and the ten APG-orders (APG, 1998, APGII, in prep.).

Much has been learned from the published analyses, but many questions remain to be answered. Still there is no convincing support for the interrelationship among the three basal groups, i.e., Cornales, Ericales, and the euasterids. Different analyses, with low bootstrap or jackknife support values for the groupings, show contradictory results; *rbcL/atpB/18S* rDNA data (Soltis

et al., 2000) place Cornales as sister to Ericales while *ndhF* data alone (Olmstead et al., 2000) or *ndhF* together with *rbcL/atpB/18S* rDNA data (Albach et al., 2001a) show Cornales as sister to the rest of the asterids. Several studies indicate that lamiids and campanulids are sister taxa, although both groups have low to only medium support. Lamiids have jackknife or bootstrap values of 53/66% (Olmstead et al., 2000), 56% (Soltis et al., 2000), or 40% (Albach et al., 2001a). Campanulids have 68% (Olmstead et al., 2000), 88% (Soltis et al., 2000), or below 33% (Albach et al., 2001a). Despite all these studies based on many taxa and both three and four genes, the relationships among the orders within lamiids and campanulids, respectively, are in most parts unclear. The same applies to most family interrelationships within the orders.

As noted above, most molecular studies of higher-level (orders and families) phylogenetic interrelationships in asterids, and in flowering plants in general, are based on coding chloroplast DNA. In particular, the chloroplast genes *rbcL*, *ndhF*, and *atpB* have been used, but also nuclear 18S rDNA has been used. Non-coding chloroplast DNA have hitherto been utilised almost entirely for phylogenetic analyses at lower levels, and is generally taken to be phylogenetically uninformative at higher levels (e.g., Böhle et al., 1994; Gielly and Taberlet, 1994; Kelchner, 2000; Soltis and Soltis, 1998), since homoplasy from repeated mutations in saturated positions is assumed to swamp the phylogenetic signal. There are, however, analyses indicating that this latter assumption is erroneous, at least for silent mutations in third positions of coding chloroplast DNA (e.g., Källersjö et al., 1998; Sennblad and Bremer, 2000). Kelchner (2000) discussed the potential difficulties in using non-coding DNA, since it is highly structurally constrained and not randomly evolving. He gave several evolutionary mechanisms for non-coding sequence evolution (slipped-strand mispairing, stem-loop secondary structure, minute inversions, nucleotide substitutions, intramolecular recombination) which will influence the sequences and can cause problems with alignment. Kelchner argued that all matrices should be inspected prior to phylogenetic analyses and that the different mechanisms should be considered in the alignment.

As in this paper, non-coding DNA in chloroplasts is generally meant to include the non-coding single copy regions in the chloroplast DNA molecule. However, it should be noticed that from the conserved inverted repeat regions of the chloroplast DNA, it has been demonstrated that the very slowly evolving non-coding introns are informative for the basal angiosperms (Graham and Olmstead, 2000; Graham et al., 2000). Here we explore the phylogenetic utility of non-coding DNA (from the large single copy region) at the family/order level of asterid flowering plants, a level where this non-coding DNA generally is assumed to be useless.

The asterids are a biologically very diverse and species-rich group and their evolutionary success could be explained or traced with a resolved and well supported phylogeny as a basis for further research. The present study aims at presenting such a phylogeny and a refined classification of the asterids, based on both coding and non-coding DNA. The particular aims are to present: (1) supported phylogenetic interrelationships among the orders, families, and informal groups, (2) as far as possible re-circumscribe the asterid orders to include families presently unclassified to order, and (3) to test if non-coding DNA (e.g., *trnT-F*, *trnV-atpE*, *rps16*) is phylogenetically more or less informative and useful than the commonly used coding DNA (e.g., *rbcL*, *ndhF*, *matK*) at this higher taxonomic level and if such non-coding DNA, in combination with coding DNA, will increase support and resolution for the phylogeny of the asterids.

## 2. Materials and methods

### 2.1. Taxon sampling

The sampling strategy was to include one member of each of the 106 asterid families from the APG-system (APG, 1998). If easily available we chose a species from the type genus of the family. We obtained DNA representing 104 families and we failed to get material from two, Carlemanniaceae and Sphenostemonaceae. The genus *Hydrostachys* (Hydrostachyaceae in APG, 1998) caused many problems. All sequenced markers for this genus are considerably different from those of the other taxa. They were difficult to align and the analyses resulted in phylogenies with very long branches for *Hydrostachys*. Hence, with our data, the phylogenetic position of *Hydrostachys* could not be established with any degree of certainty. Published analyses (Albach et al., 2001b; Hempel et al., 1995; Olmstead et al., 2000) indicate that *Hydrostachys* is nested within or close to the family Hydrangeaceae and we therefore decided to exclude it from our analyses.

In addition to the representatives of the APG-families, we selected some further interesting taxa. From Lamiales, we included seven more genera since the number of families and their interrelationships within the order is very unclear. These genera, *Androya*, *Antirrhinum*, *Globularia*, *Peltanthera*, *Proboscidea*, *Sanango*, and *Selago*, have earlier been described as separate families or they have been transferred from other families. In five other cases, there have been indications that families are non-monophyletic and hence we have included additional genera, viz. *Pterostyrax* of Styraceae (Soltis et al., 2000), three genera of Icacinaceae (Kårehed, 2001; Savolainen et al., 2000a; Soltis et al., 2000), *Desfontainia* from Columelliaceae (Savolainen et al., 2000a), *Quintinia* of Escalloniaceae (Gustafsson et al., 1996) and *Schima* of

Theaceae (Morton et al., 1997). We also included *Maesa* of the family Maesaceae, described by Anderberg et al. (2000). From one bigeneric family, Montiniaceae, we also sequenced for the first time the genus *Grevea*. Furthermore, from the list of families with uncertain position in APG (1998), we included seven taxa, *Cardiopteris*, *Dipentodon*, *Kaliphora*, *Lissocarpa*, *Paracryphia*, *Pentaphylax*, and *Sladenia*. Because the monophyly of the asterids already has been convincingly demonstrated (Soltis et al., 2000), we have only selected two non-asterid outgroup taxa, *Paeonia* of the Paeoniaceae and *Vitis* of the Vitaceae. Both these genera assume basal positions in the core eudicots in general, where asterids constitute one of the major clades (APG, 1998). In the final analyses, we included in total 132 genera.

### 2.2. Sequencing

We used six different DNA sequence regions from the chloroplast genome. Three represent coding genes, *rbcL*, *ndhF*, and *matK*. Three others represent non-coding DNA: (1) a region including *trnL* exons and the intron and intergenic spacers between *trnT* (UGU) to *trnF* (GAA), here abbreviated *trnL*, (2) a region including *trnV* exons and intron, *trnM* and intergenic spacers between *trnV* (UAC) and *atpE*, here abbreviated *trnV*, and (3) the *rps16* intron, here abbreviated *rps16*. All new sequences are listed in Appendix A.

Most of the sequencing (or 538 of the in all 547 new sequences) was done in the Evolutionary Biology Centre labs in Uppsala according to the following procedure. PCR reactions were performed using Taq polymerase. Amplified products were cleaned with Qiaquick PCR purification kit (Qiagen). Sequencing reactions were performed using two different protocols, either with BigDye™ terminator cycle sequencing kit (Applied Biosystems) and analysed on an ABI 377 (Applied Biosystems) or with DYEnamic™ ET termination cycle sequencing premix kit (Amersham Pharmacia Biotech), on a MegaBACE 1000 capillary machine (Amersham Pharmacia Biotech). Protocols followed that provided by the manufacturer. All PCR and sequencing primers are listed in Appendix B. Ten new *rbcL* sequences, 31 new *ndhF* sequences, 129 new *matK* sequences, 128 new *trnL* sequences, 124 new *trnV* sequences, and 125 new *rps16* sequences were produced for this study. One *rbcL* sequence, a pseudogene from *Orobanche*, was excluded due to difficulties in alignment. A limited number of taxa were not possible to sequence for some of the markers (cf. Appendix A) due to failure to amplify the targeted region.

### 2.3. Data matrices

Six separate matrices were produced for the six markers. In all data sets one or a few sequences were

missing. The coding genes were aligned manually by using the reading frames of the corresponding amino acid sequences. The non-coding DNA sequences were first aligned by Clustal W (Thomson et al., 1994) followed by manual corrections. We did not follow Kelchner's (2000) prealignment procedure but used a standard pragmatic alignment. Presumably homologous indel events (gaps), were coded as additional presence/absence characters. In some taxa where alignment left doubts about the homology of indels, their presence/absence was coded with a question mark. In the non-coding markers some regions, particularly poly-*N*-sequences (stretches of the same nucleotide) of different length (probably due to slipped-strand mispairing) could not be aligned, and were excluded from further phylogenetic analyses.

Each separate matrix was parsimony-jackknifed (see below) to get a preliminary phylogenetic tree from each DNA marker. If a taxon appeared in different jackknife-supported positions in the different trees, it was taken as an indication that the sequences could be erroneous and such taxa were re-sequenced, in a few cases also from a new DNA preparation. A few *rbcL* sequences from EMBL/GenBank were omitted, because they turned out to be clearly erroneous following the results of our preliminary analyses.

After the preliminary analyses, three data sets were constructed. To investigate the phylogenetic utility of coding and non-coding DNA for the taxonomic level of this study we merged the data from the coding genes (*rbcL*, *ndhF*, and *matK*) into one matrix, for short called the coding matrix or analysis, and we did the same for the non-coding markers (*trnL*, *trnV*, and *rps16*), for short called the non-coding matrix or analysis. To obtain the most comprehensive data set and the most well supported phylogeny for the asterids we merged all data into a combined matrix and analysis.

#### 2.4. Phylogenetic analyses

Each data matrix was analysed using PAUP\* 4.0 (beta version 4.0b8; Swofford, 1998) and parsimony analyses with a heuristic search strategy with 100 replicates of RANDOM stepwise additions of sequences and TBR branch swapping. Only informative characters were analysed. Support values for the nodes were obtained by jackknife analysis (Farris et al., 1996) with 1000 replicates with 5 RANDOM stepwise additions of sequences, and 37% of the characters deleted in each replicate, MULTREES off, and only one tree saved at each replicate. All jackknife values  $\geq 50\%$  in the strict consensus trees were summarised as a measure of total jackknife support for the whole tree. Total jackknife support in relation to the number of aligned characters was calculated as the quotient between these two numbers. The number of nodes with  $\geq 67\%$  jackknife sup-

port, i.e., a medium to high support, and the number of nodes with  $\geq 95\%$  jackknife support, i.e., a high or strong support, were also calculated.

### 3. Results

Table 1 includes number of parsimony-informative characters, number of equally parsimonious trees, tree lengths, consistency and retention indices, total jackknife support, and other data from the three analyses, namely, the coding analysis, the non-coding analysis, and the combined analysis, respectively. There are no great differences in the data from the coding versus the non-coding analyses. The coding matrix comprises 5717 aligned positions of which 1878 are constant, 898 autapomorphic (singletons), and 2941 parsimony-informative. The 2941 parsimony-informative characters include 18 indel characters, none in *rbcL*, three in *ndhF*, and 15 in the *matK* gene. The non-coding matrix comprises 4197 aligned positions of which 1458 are constant, 750 autapomorphic (singletons), and 1989 parsimony-informative. The 1989 parsimony-informative characters include 50 indel characters, 14 in *trnL*, 20 in *trnV*, and 16 in the *rps16* sequence.

The strict consensus tree from the combined analysis with jackknife values for the nodes is shown in Figs. 1A–C. One of the trees is shown with branch lengths in Figs. 2A–C. There are some differences in the topology of the trees from the three different analyses. Most of these differences are within clades of few taxa and are not in conflict with family or order classification. Of the 130 possible nodes (the number of taxa minus two), 36 nodes show contradictions between the three analyses. Most of these cases concern clades with low to medium support (<95%). In two cases the support is high in two of the analyses for a particular node not occurring in the third analysis. There is 100% support for *Eucommia* in Garryales in the combined analysis and the coding analysis but less than 50% support in the non-coding analysis. In the combined analysis and in the coding analysis there is high support for *Sphenoclea* and *Hydrolea* as sister taxa, 90% and 99%, respectively, but less than 50% support in the non-coding data. In these cases, one non-coding marker each is missing in our data (in *Eucommia trnV* and in *Hydrolea rps16*). In two other cases, the different analyses support different phylogenies between Apocynaceae/Gelsemiaceae/Gentianaceae/Loganiaceae, and between *Buddleja/Scrophularia/Selago*; see Section 4).

All except 17 of the 129 ingroup taxa are placed in well supported clades representing orders of the APG (1998). Solanales are supported with 90% and the other 10 orders with 100% jackknife support. Six of the eight possible nodes representing interrelationships among the 10 orders are supported (>50%) by the jackknife analysis (cf. Fig. 1). Seven of the ingroup taxa represented

Table 1  
Data from matrices, analyses, and trees

	Aligned characters	Parsimony-informative characters	Indels	Percent informative of aligned	Number of trees	Tree length	Consistency index	Retention index	Total jackknife support	Total jackknife support index	Number of nodes with support $\geq 67\%$	Number of nodes with support $\geq 95\%$
	na	nc	ni	nc/na	np	steps	CI	RI	TJ	TJ/na	nn-67	nn-95
6 Markers	9914	4930	68	49.7	24	42203	0.294	0.503	9831	0.99	91	64
3 Coding	5717	2941	18	51.4	24	26146	0.274	0.501	8550	1.67	79	61
3 Non-coding	4197	1989	50	47.4	7452	15954	0.329	0.511	8009	1.91	78	41

families of uncertain position (APG, 1998) and six are nested within the asterids. One genus, *Dipentodon* of Dipentodontaceae, unclassified in APG (1998), is more closely related to the outgroup taxa and apparently does not belong in the asterids. Table 2 summarizes classification of the asterids and the changes introduced.

#### 4. Discussion

We here show that Cornales are sister to the other asterids, i.e., Ericales and euasterids, and that the latter two are sister taxa. Earlier studies have not resolved convincingly the relationships among the basal branches, the support values have been low, and the results have been contradicting. The *rbcLlatpB/18S* rDNA data (Soltis et al., 2000) place Cornales as sister to Ericales while *ndhF* data alone (Olmstead et al., 2000) or *ndhF* together with *rbcLlatpB/18S* rDNA data place Cornales as sister to the rest of the asterids (Albach et al., 2001a), as in this study. From the **Cornales** we have included four families and of these Cornaceae together with Grubbiaceae are the sister group to Hydrangeaceae and Loasaceae. We did not sample *Curtisia* in our study since it has been included in Cornaceae (APG, 1998; Xiang et al., 1993, 1998) but recent analysis indicates that *Curtisia* is more closely related to Grubbiaceae and it is thus re-instated as a family in APGII (in prep.).

**Ericales** comprise many families but except for the balsaminoid and the primuloid groups discussed below, family interrelationships have hitherto been largely unknown or uncertain. Here we identify a number of jackknife-supported family groups, which have also been found in analysis of chloroplast and mitochondrial genes in combination (Anderberg et al., 2002). At the base Ericales are split in two strongly supported clades, a resolution hitherto not demonstrated with any degree of support. The smaller balsaminoid group has been identified in several earlier analyses (e.g., Källersjö et al., 1998) but the strongly supported monophyly of the rest of the order is new (and also found in Anderberg et al., 2002). The balsaminoid group is totally resolved and the relationships among its three families are strongly supported. Marcgraviaceae are sister to the rest, and Balsaminaceae and Tetrameristaceae are sister groups. *Pelliciera* was formerly in a family of its own (APG, 1998), but *Pentamerista*, the sister genus of *Tetramerista*, shares many morphological similarities with *Pelliciera* (Cronquist, 1981), including unusual glandular pits on the inner surface of the sepals, and it seems unnecessary to maintain two separate families for only three genera. Hence we merge *Pelliciera*, *Pentamerista* (not included in the analyses), and *Tetramerista* in a single family Tetrameristaceae (also in APGII, in prep.). The other basal clade of the Ericales comprises most of the families, still with partly unresolved interrelationships as

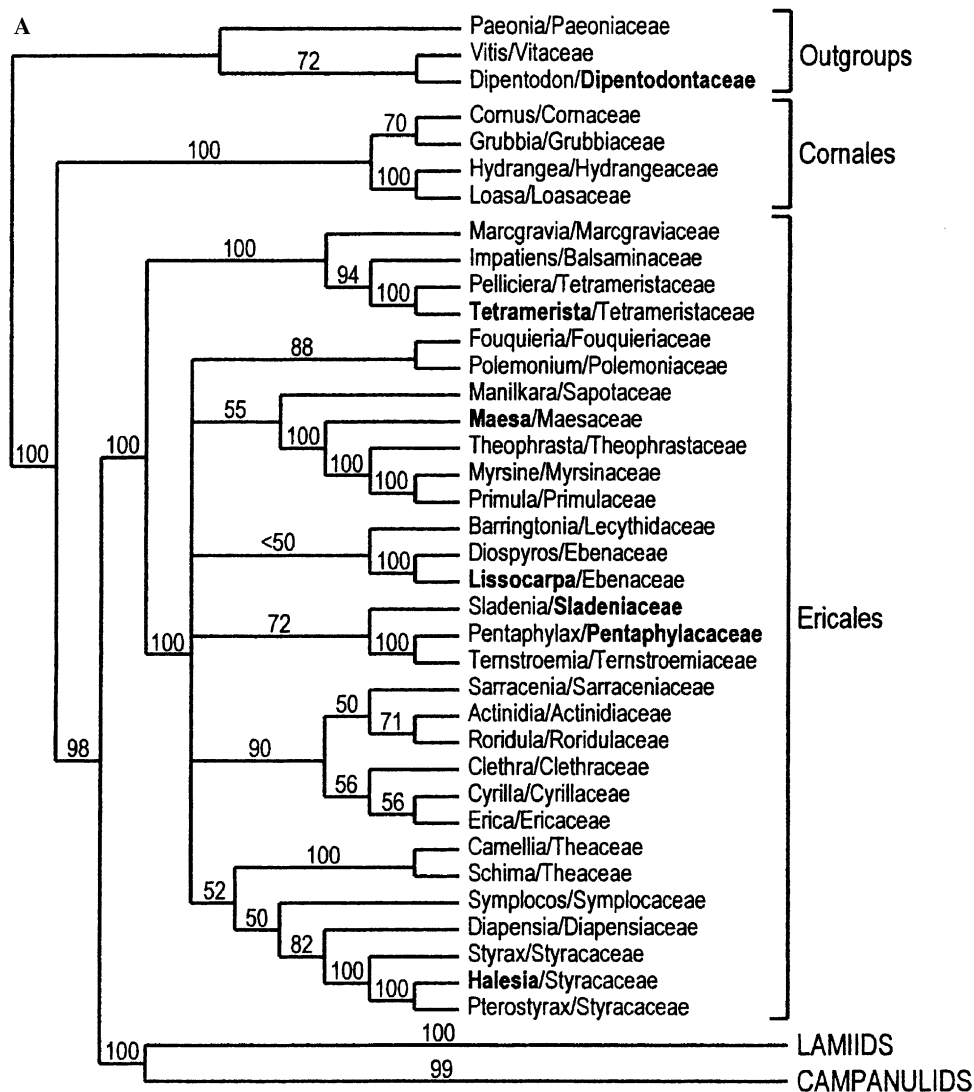


Fig. 1. Strict consensus tree from the combined analysis of all 6 markers (coding and non-coding) with jackknife values for the nodes. (A) Outgroups, Cornales, and Ericales. (B) Lamiids. (C) Campanulids. Genera in bold have a new family placement and families in bold a new position compared to APG (1998).

indicated by the polytomy in Fig. 1A. However, there are a number of well supported groups of families. The primuloid group of families (Primulales of Cronquist, 1981) includes Maesaceae, Theophrastaceae, Myrsinaceae, and Primulaceae (Anderberg et al., 2000; Källersjö et al., 2000). Another group of families supported here is the ericoid group which contains six families with only weakly supported and uncertain interrelationships, namely, Sarraceniaceae, Actinidiaceae, Roridulaceae, Clethraceae, Cyrillaceae, and Ericaceae (Fig. 1A). The enigmatic Fouquieriaceae are here supported (88%) as sister to Polemoniaceae. The position of Fouquieriaceae was much debated before molecular data was available, e.g., close to Ericaceae (Dahlgren, 1980, 1983) or Violaceae (Cronquist, 1981; Takhtajan, 1987). In one of the first molecular analyses including Fouquieriaceae (Downie and Palmer, 1992) they were found to be sister

taxon to Polemoniaceae, but in that study no other Ericales were included. Later studies (Johnson et al., 1996; Johnson et al., 1999) including also Ericales taxa showed the same relationship to Polemoniaceae but with very low support (<50%).

The genus *Lissocarpa* was before molecular investigations placed close to Ebenaceae (Cronquist, 1981), a position confirmed by this study, as well as by Anderberg et al. (2002). The genus has recently been unplaced, as Lissocarpaceae with uncertain position by APG (1998), or misplaced in Rutaceae (Savolainen et al., 2000a). It is now included in Ebenaceae (APGII, in prep.). Earlier classifications included *Ternstroemia* in Theaceae but this placement is not supported here, nor in other molecular investigations (Anderberg et al., 2002; Savolainen et al., 2000b; Soltis et al., 2000). Instead *Ternstroemia* forms a clade together with two genera of

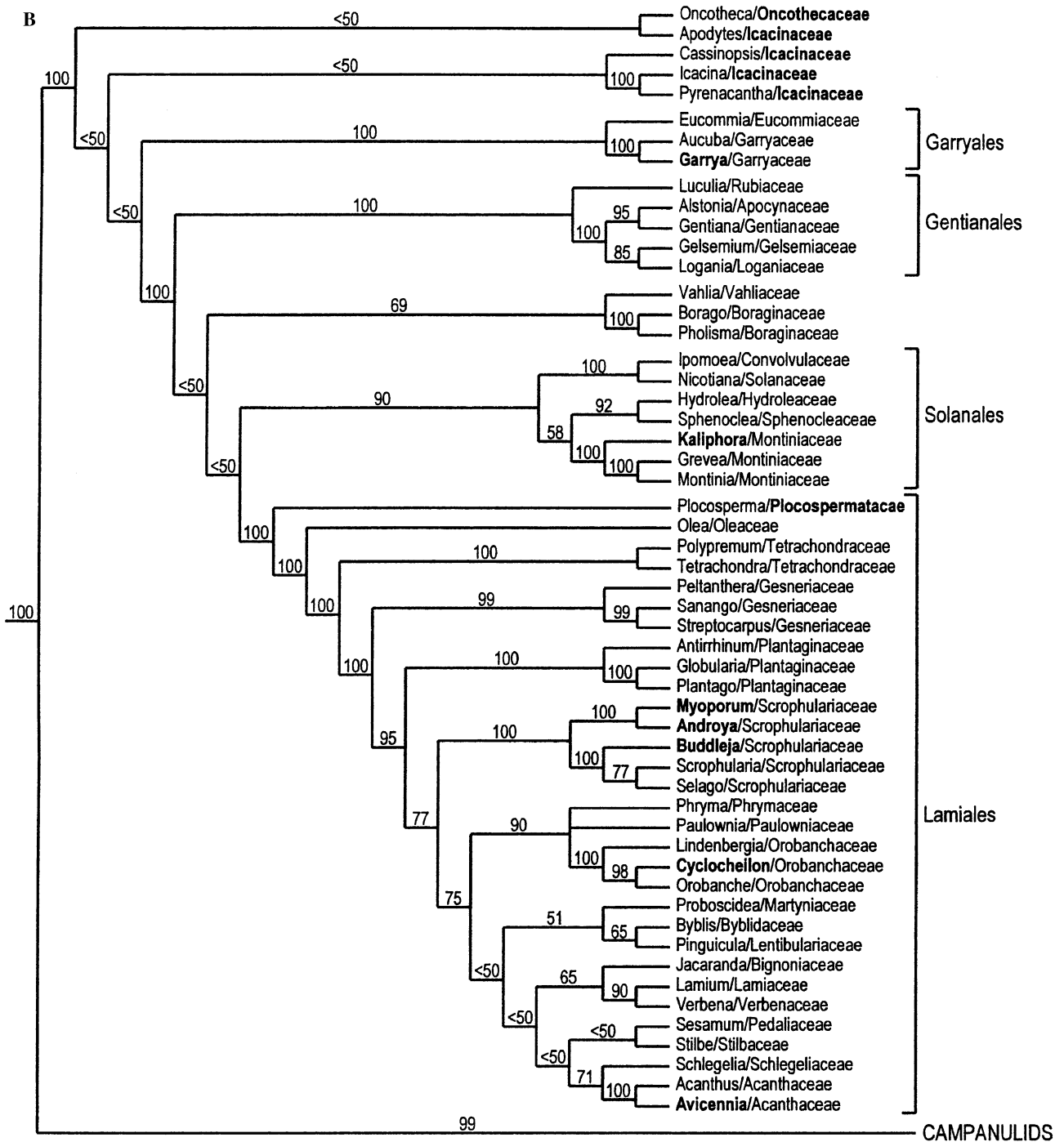


Fig. 1. (continued)

uncertain position and listed as unplaced families by APG (1998), namely, *Sladenia* and *Pentaphylax*. Savolainen et al. (2000a) investigated both *Sladenia* and *Pentaphylax*. The former was, without support, placed in Ternstroemiaceae, whereas *Pentaphylax* appeared in a totally different position, in Cardiopteridaceae of the campanulids. The sequence that they used may be erroneous, since there is another sequence of *Pentaphylax* in GenBank (AF320785 submitted by S.Q. Tang and

S.H. Shi) showing the same Ericales relationship as our sequence. The relationship between *Sladenia*, *Pentaphylax*, and *Ternstroemia* was also found by Anderberg et al. (2002). The exact position of Theaceae within Ericales is still unclear, although the family is here with low support close to Symplocaceae, Diapensiaceae, and Styrcaceae, the latter including *Halesia* of the former Halesiaceae (Soltis et al., 2000; APGII, in prep.). There is a close relationship between Diapensiaceae and

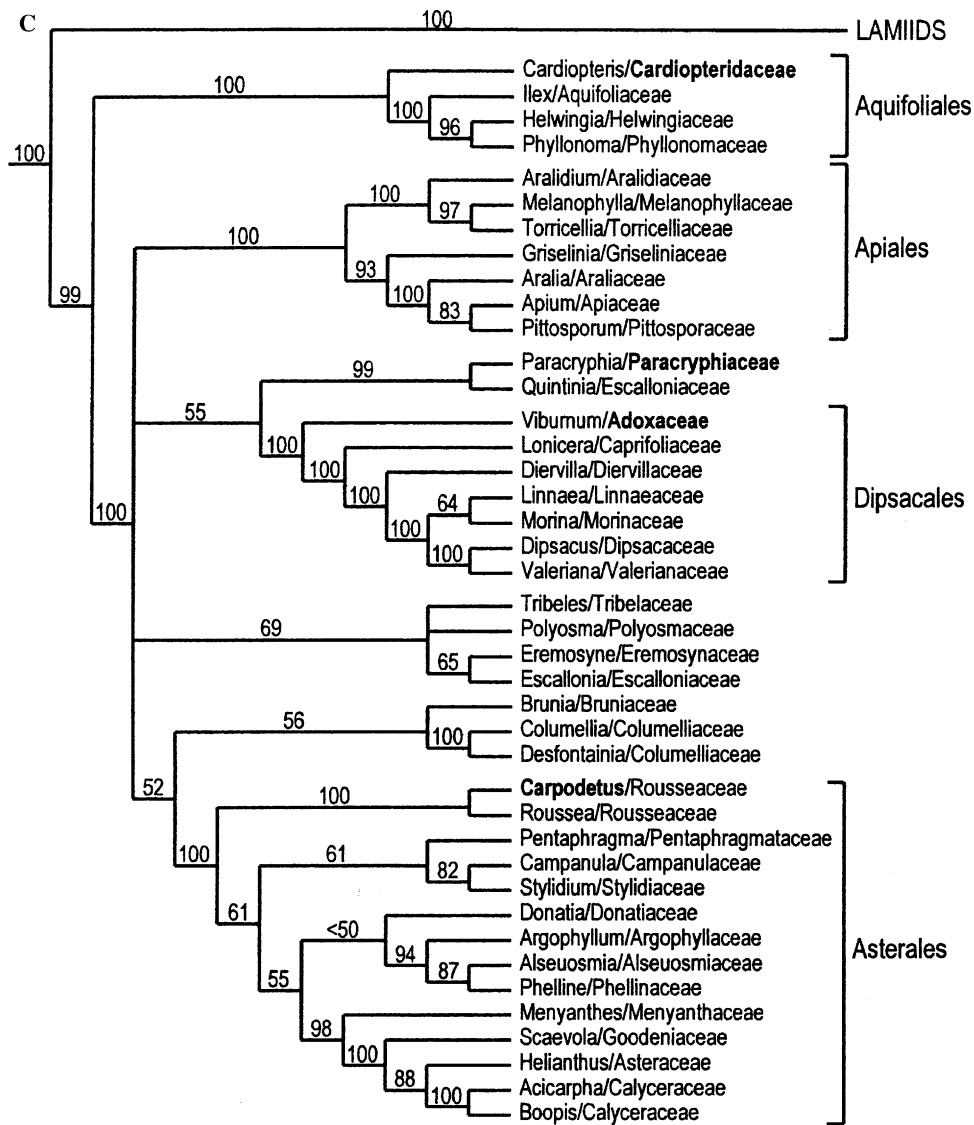


Fig. 1. (continued)

Styracaceae (also found in Anderberg et al., 2002), but relationships of Sapotaceae and Lecythidaceae are poorly supported and their positions are still unclear.

The sister group relationship between Ericales and the **euasterids** is here highly supported (100%) and so is also that between the two branches of the euasterids, lamiids and campanulids. The support for these two groups together is 100% and each group is supported as monophyletic by jackknife values of 100% and 99%, respectively. The monophyly of each group has been more or less accepted however hitherto without strong support, the published jackknife or bootstrap values have not exceeded 66% (lamiids, Olmstead et al., 2000) and 88% (campanulids, Soltis et al., 2000), respectively.

The basal relationships of the **lamiids** are still partly obscure. The problems involve taxa of the Icacinaceae and the **Garryales**. The latter order is strongly supported (100%) with two families only, Garryaceae (in-

cluding Aucubaceae following APGII, in prep.) and Eucommiaceae. The family Icacinaceae has in recent studies been demonstrated to be at least biphyletic (Savolainen et al., 2000a,b; Soltis et al., 2000) with one part related to the campanulids and with a core of genera around *Icacina* positioned at the base of the lamiids. In our limited sample of genera only *Pyrenacantha* and *Icacina* are supported (100%) as a group while the relationships to *Casssinopsis* and *Apodytes* are uncertain. In other studies (Kårehed, 2001; Soltis et al., 2000) Icacinaceae have been suggested to be included in Garryales, but such a relationship is not supported here. Another unplaced taxon at the base of the lamiids is *Oncotheca* (Oncothecaceae), which has been classified earlier in Theales (Cronquist, 1981) or in Garryales (APG, 1998). Information from the six markers used here is not enough to resolve basal relationships among the lamiids.



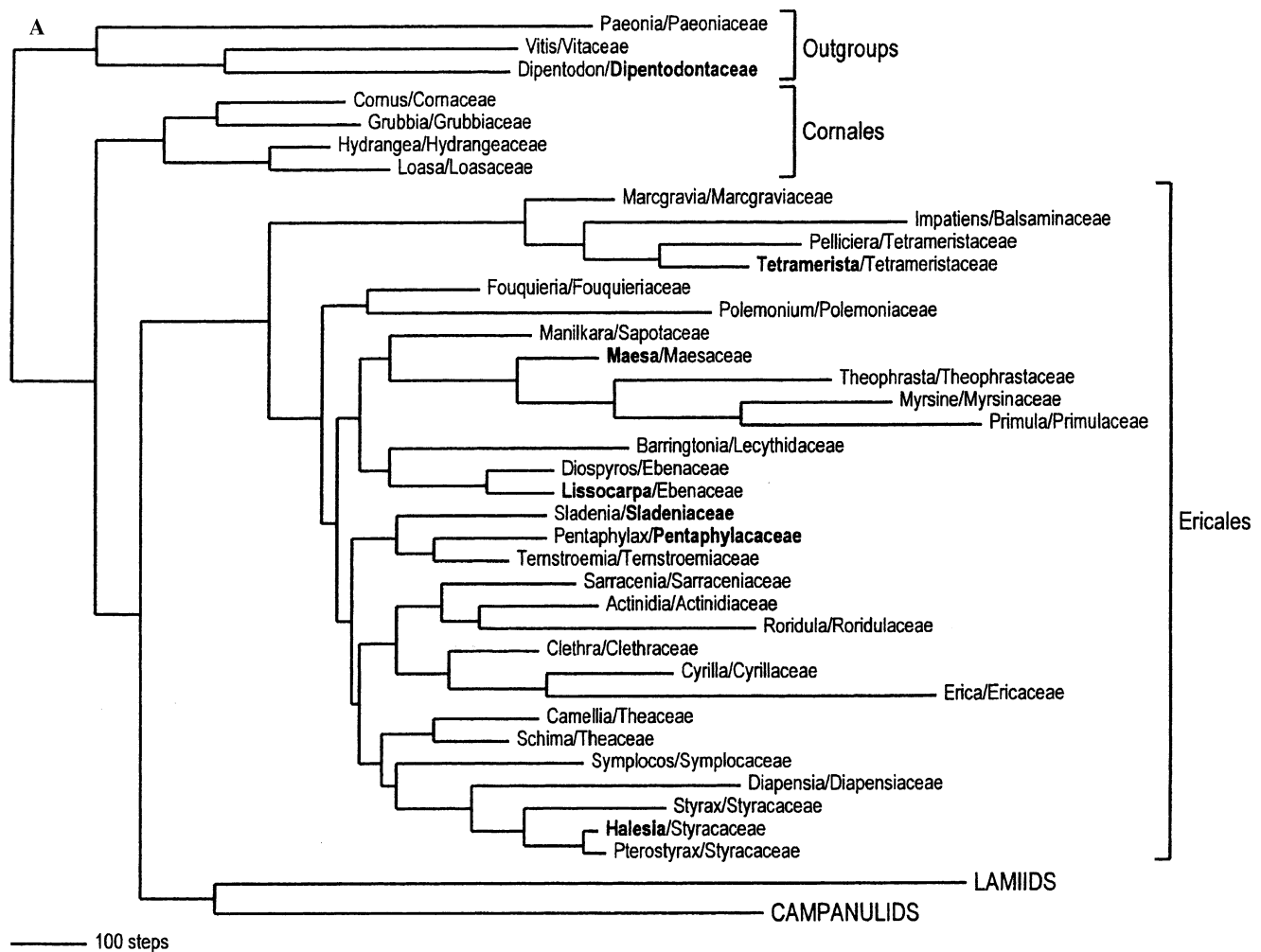


Fig. 2. One of the 24 trees from the combined analysis of all 6 markers (coding and non-coding) drawn proportional to branch lengths. (A) Outgroups, Cornales, and Ericales. (B) Lamiids. (C) Campanulids. Genera in bold have a new family placement and families in bold a new position compared to APG (1998).

Above these unresolved basal branches in the tree there is a strongly supported (100%) and taxon-rich group of lamiids, both in terms of species number and number of families. Here belong the three orders Gentianales, Lamiales, and Solanales, each strongly supported as monophyletic, and further Boraginaceae and Vahliaceae. Ever since the first molecular cladistic analyses of a comprehensive asterid set of taxa (Chase et al., 1993; Olmstead et al., 1993) it has been clear that these taxa are closely related, but their exact sister group relationships remain an open question. In investigations of *rbcL* and *atpB* data (Savolainen et al., 2000b) there is weak bootstrap support (66%) for a sister group relationship between Gentianales and Lamiales and between Solanales and Boraginaceae (60%), relationships shown also in trees from *ndhF* analysis (Olmstead et al., 2000), but it disappears with the addition of 18S rDNA data (Soltis et al., 2000). In the consensus tree from the 3-genes analyses of *rbcL/atpB/18S* rDNA (Soltis et al., 2000) there is a grade with Gentianales as sister to the

rest followed by Solanales, Boraginaceae, Vahliaceae, and Lamiales, however, without any jackknife support for these interrelationships. In the 4-genes analysis of *rbcL/atpB/ndhF/18S* rDNA (Albach et al., 2001a) there is still no jackknife support for the interrelationships of these taxa, and unfortunately the same holds also for our combined analysis.

**Gentianales** comprise five families and the five representatives show totally resolved and well supported interrelationships. However, the taxon sampling is small and our different 3-markers analyses yield partly different results compared to that of the combined analysis. The Rubiaceae, the second largest asterid family, are both here and in earlier molecular and morphological investigations shown to be the sister group to the rest of the order (Backlund et al., 2000; Bremer et al., 2001; Olmstead et al., 2000; Oxelman and Bremer, 2000; Soltis et al., 2000). Considering the other four families our combined analysis show supported relationships with Apocynaceae and Gentianaceae as sister groups, and



Fig. 2. (continued)

these two together as sister to the pair of Gelsemiaceae and Loganiaceae. Interrelationships among these four families are different in our 3-marker analyses (in the coding analysis there is a grade, part of which is only weakly supported, with Gelsemiaceae at the base followed by Apocynaceae, Gentianaceae, and Loganiaceae, while in the non-coding analysis Loganiaceae have shifted placed with Apocynaceae compared to the result of the coding analysis). A study with more taxa but only two genes did not resolve the interrelationships (Backlund et al., 2000).

We here include representatives of all five **Solanales** families and for the first time show that they are supported as a monophyletic group (90%). In Savolainen et al.'s (2000a) analysis all five families are included but

they do not constitute a clade. In other analyses only four of the families have been included. *Sphenoclea* of Sphenocleaceae and *Kaliphora* of the former Kaliphoraceae were not included by Olmstead et al. (2000), Soltis et al. (2000) or Albach et al. (2001a,b). The close relationship between Solanaceae and Convolvulaceae has been long known and here receives 100% jackknife support. In the earlier analyses of four families Montiniaceae were sister to Hydroleaceae (Albach et al., 2001a; Olmstead et al., 2000; Soltis et al., 2000). Disregarding *Sphenoclea* and *Kaliphora* that were absent from these earlier analyses this relationship is congruent with our results but here we also show that Hydroleaceae are closer to Sphenocleaceae. In our tree, the two families together form the sister to the strongly supported family

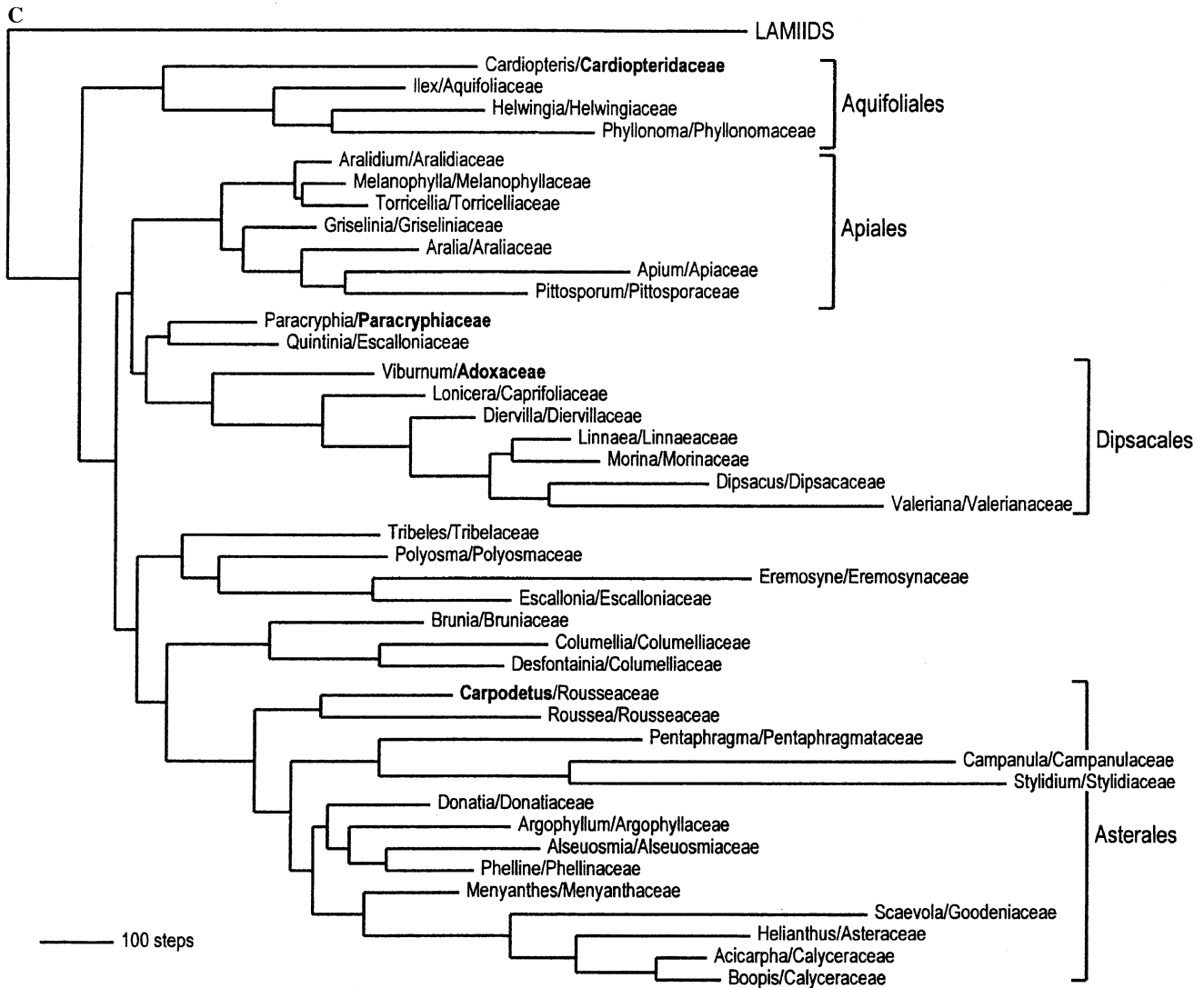


Fig. 2. (continued)

Montiniaceae including all the three genera *Montinia*, *Grevea*, and *Kaliphora* (following APGII, in prep.) but only with low support. The position of the Montiniaceae in Solanales has been disputed and ontogenetic and anatomical data point more to an affinity to Escalloniaceae, according to Decraene et al. (2000).

Most molecular analyses have identified **Lamiales** as a large clade of asterid families (Albach et al., 2001a; Joly et al., 2001; Olmstead et al., 2000, 2001; Soltis et al., 2000), so also in our study. The Lamiales currently comprises 23 families (APGII, in prep.). Within the order the basal branches are strongly supported as in previously published results. Plocospermataceae are sister group to the rest of the Lamiales followed by Oleaceae as sister to the rest (Olmstead et al., 2000, 2001; Oxelman et al., 1999), then Tetrachondraceae as sister to the rest (Oxelman et al., 1999), and subsequently Gesneriaceae as sister to the rest of the order (here including also *Peltanthera* and *Sanango*, Oxelman

et al., 1999). The latter position of Gesneriaceae is also supported by *ndhF* data (Olmstead et al., 2000) alone and by Albach et al.'s (2001a,b) 4-genes analysis but not so by the 3-genes analysis of Soltis et al. (2000). There is strong support for the monophyly of Plantaginaceae and Scrophulariaceae in new circumscriptions.

Plantaginaceae include also *Globularia* and *Antirrhinum*, formerly of Globulariaceae and Scrophulariaceae, respectively (cf. Olmstead et al., 2001; Oxelman et al., 1999). Scrophulariaceae are recircumscribed to include *Myoporum* and *Buddleja* and other genera of Myoporaceae and Buddlejaceae (Olmstead et al., 2001). Interrelationships among the three genera *Buddleja*, *Scrophularia*, and *Selago* are different in our 3-markers analyses (in the non-coding analysis *Buddleja* is sister taxon to *Selago*, with 88% support, while in the coding analysis *Selago* and *Scrophularia* are sister taxa, with 95% support. We have not been able to trace the reason for this incongruity but a close relationship between

Table 2

Classification of asterids following APG (1998) with commented changes

<b>ASTERIDS</b>	
<b>Cornales</b>	
Cornaceae	
Grubbiaceae	
Hydrangeaceae	
Hydrostachyaceae—	not included in this study. Recent analyses indicate that the single genus <i>Hydrostachys</i> is nested in Hydrangeaceae (Soltis et al., 2000)
Loasaceae	
<b>Ericales</b>	
Actinidiaceae	
Balsaminaceae	
Clethraceae	
Cyrillaceae	
Diapensiaceae	
Ebenaceae—	expanded to include Lissocarpaceae from the list of families of uncertain position.
Ericaceae	
Fouquieriaceae	
(Halesiaceae—	included in Styracaceae)
Lecythidaceae	
Maesaceae—	described by Anderberg et al. (2000)
Marcgraviaceae	
Myrsinaceae	
(Pellicieraceae—	included in Tetrameristaceae)
Pentaphylacaceae—	transferred from the list of families of uncertain position
Polemoniaceae	
Primulaceae	
Roridulaceae	
Sapotaceae	
Sarraceniaceae	
Sladeniaceae—	transferred from the list of families of uncertain position
Styracaceae—	expanded to include Halesiaceae
Symplocaceae	
Ternstroemiaceae	
Tetrameristaceae—	expanded to include Pellicieraceae
Theaceae	
Theophrastaceae	
<b>LAMIIDS</b>	
Boraginaceae	
(Plocospermataceae—	transferred to Lamiales)
Icacinaceae—	transferred from the campanulids
Oncothecaceae—	transferred from Garryales
Vahliaceae	
<b>Garryales</b>	
(Aucubaceae—	included in Garryaceae)
Eucommiaceae	
Garryaceae—	expanded to include Aucubaceae
(Oncothecaceae—	transferred to the lamiids without order)
<b>Gentianales</b>	
Apocynaceae	
Gelsemiaceae	
Gentianaceae	
Loganiaceae	
Rubiaceae	
<b>Lamiales</b>	
Acanthaceae	
(Avicenniaceae—	included in Acanthaceae)
Bignoniaceae (Buddlejaceae—	included in Scrophulariaceae)
Byblidaceae	
Calceolariaceae—	re-established from Scrophulariaceae by Olmstead et al. (2001) but not included in this study
Carlemanniaceae—	transferred to Lamiales by Savolainen et al. (2000a) but not included in this study
(Cyclocheilaceae—	included in Orobanchaceae)
Gesneriaceae	
Lamiaceae	
Lentibulariaceae	
(Myoporaceae—	included in Scrophulariaceae)
Martyniaceae—	re-established from synonymy of Pedaliaceae
Oleaceae	
Orobanchaceae—	expanded to include Cyclocheilaceae
Paulowniaceae	
Pedaliaceae	
Phrymaceae	
Plantaginaceae	
Plocospermataceae—	transferred from the lamiids without order
Schlegeliaceae	
Scrophulariaceae—	expanded to include Buddlejaceae and Myoporaceae
Stilbaceae	
Tetrachondraceae	
Verbenaceae	
<b>Solanales</b>	
Convolvulaceae	
Hydroleaceae	
Montiniaceae—	expanded to include Kaliphoraceae from the list of families of uncertain position
Solanaceae	
Sphenocleaceae	
<b>CAMPANULIDS</b>	
(Adoxaceae—	transferred to Dipsacales)
Bruniaceae	
(Carlemanniaceae—	transferred to Lamiales)
Columelliaceae	
Eremosynaceae	
Escalloniaceae	
(Icacinaceae—	transferred to the lamiids without order)
Paracryphiaceae—	transferred from the list of families of uncertain position
Polyosmaceae	
Sphenostemonaceae—	not included in this study
Tribelaceae	
<b>Apiales</b>	
Apiaceae	
Araliaceae	
Aralidiaceae	
Griselinaceae	
Melanophyllaceae	
Pennantiaceae—	circumscribed by Kårehed (2001) but not included in this study
Pittosporaceae	
Toricelliaceae	
<b>Aquifoliales</b>	
Aquifoliaceae	
Cardiopteridaceae—	transferred from the list of families with uncertain position
Helwingiaceae	
Phyllonomaceae	
Stemonuraceae—	described by Kårehed (2001) but not included in this study

Table 2 (continued)

<b>Asterales</b>
Alseuosmiaceae
Argophyllaceae
Asteraceae
Calyceraceae
Campanulaceae
(Carpodetaceae—included in Rouseaceae)
Donatiaceae
Goodeniaceae
Menyanthaceae
Pentaphragmataceae
Phellinaceae
Rouseaceae—expanded to include Carpodetaceae
Stylidiaceae
<b>Dipsacales</b>
Adoxaceae—transferred from the campanulids without order
Caprifoliaceae
Diervillaceae
Dipsacaceae
Linnaeaceae
Morinaceae
Valerianaceae

*Selago* and *Scrophularia* is supported in a more detailed study of *Selago* and close relatives (Kornhall et al., 2001).

Here we also show that among the remaining families there is one supported group of families comprising Phrymaceae, Paulowniaceae, and Orobanchaceae. *Cyclocheilon*, formerly in a separate family Cyclocheilaceae (APG, 1998) is nested within Orobanchaceae and we consequently here include it in that family. The mangrove genus *Avicennia* (Avicenniaceae) is sister to *Acanthus* of the Acanthaceae and with a more extended sampling of Acanthaceae it turns out that *Avicennia* is nested inside the Acanthaceae such that Avicenniaceae should be reduced to synonymy (B. Bremer et al. and R. Olmstead et al., unpublished data, and L. McDade, pers. comm.). Lamiaceae and Verbenaceae are here sister taxa with medium support (90%) in agreement with pre-molecular systematics (Cronquist, 1981; Dahlgren, 1983). However, after the move of several taxa from Verbenaceae to Lamiaceae (Cantino, 1992; Wagstaff and Olmstead, 1997) no molecular analyses have shown these taxa to be sister groups (e.g., Albach et al., 2001a; Olmstead et al., 2000; Oxelman et al., 1999; Savolainen et al., 2000b; Soltis et al., 2000). Most other relationships between the families are unclear, the support values from our combined analysis (Fig. 1B) are not high enough to establish interrelationships among, for example, Scrophulariaceae, Orobanchaceae, Martyniaceae, Byblidaceae, Lentibulariaceae, Bignoniaceae, Pedaliaceae, Stilbaceae, Acanthaceae, and Lamiaceae + Verbenaceae.

**Campanulids** in this study and in most other molecular studies have been demonstrated to have a basal split between Aquifoliales and the rest of the campanulids (Kårehed, 2001; Olmstead et al., 2000; Soltis et al., 2000)

with strong support. Both clades receive 100% support. Recently, it has been shown that **Aquifoliales** contain not only Aquifoliaceae, Helwingiaceae, and Phyllonomaceae (APG, 1998) but also some former Icacinaceae genera (Soltis et al., 2000). Kårehed (2001) has proposed that many former Icacinaceae belong in Cardiopteridaceae, a family formerly of uncertain position (APG, 1998) but now shown to belong in Aquifoliales. The relationship between the four families of Aquifoliales is fully resolved and strongly supported. Cardiopteridaceae are sister to the rest with Aquifoliaceae as sister to Phyllonomaceae and Helwingiaceae together. This last relationship is different from what has been found by a few other studies in which Aquifoliaceae and Helwingiaceae are sister taxa (Olmstead et al., 2000; Soltis et al., 2000). In our data, five of the six genes support a close relationship between *Phyllonoma* and *Helwingia* and only *ndhF* data indicate *Ilex* as sister to *Helwingia*. The three *ndhF* sequences we have used for these taxa were from GenBank (Olmstead et al., 2000). This example of incongruity may represent a case of mix-up of sequences or misidentification and has to be investigated. From a morphological point of view it seems more plausible that Phyllonomaceae and Helwingiaceae are sister taxa; they share the presence of epiphyllous inflorescences.

The major clade of the campanulids, the sister group to Aquifoliales, contains the three well defined and strongly supported (100%) orders Apiales, Asterales, and Dipsacales, as well as a number of families without order (APG, 1998), namely, Bruniaceae, Columelliaceae, Eremosynaceae, Escalloniaceae, Polyosmaceae, and Tribelaceae. The relationships among these families and the three orders are in most parts still unclear. One clade with medium support (69%) includes Eremosynaceae, Escalloniaceae, Polyosmaceae, and Tribelaceae. Earlier studies including some of these taxa have also failed to give any clear indication of where they belong within the campanulids (Savolainen et al., 2000a; Soltis et al., 2000). A new and strongly supported sister group relationship (99%) is that between *Paracryphia* and *Quintinia*. The former was in APG (1998) listed as a family Paracryphiaceae with uncertain position in the system. The latter is a genus of Escalloniaceae. *Paracryphia* appears as sister to *Sphenostemon* in Savolainen et al.'s (2000a) *rbcL* analysis. *Sphenostemon* is not included in our analyses and *Quintinia* remained in an unresolved position in Gustafsson et al. (1996).

In the **Apiales**, we have investigated taxa representing all seven families of the APG (1998) system. Here for the first time a totally resolved and well supported phylogeny for these seven families is shown. Earlier investigations have indicated the same supported relationship between four of these families (Olmstead et al., 2000). The Apiales are basally split in two branches, one contain Aralidiaceae as sister to Melanophyllaceae and

Toricelliaceae and the other branch contain Griselinaceae as sister to the rest followed by Araliaceae as sister to Pittosporaceae and Apiaceae. Remaining problems not addressed in this study are the circumscriptions and delimitations of Apiaceae, Araliaceae, and Pittosporaceae (e.g., Plunkett and Lowry, 2001).

We have investigated 14 species representing all families of the **Asterales** included in the APG (1998) system. *Carpodetus* and *Roussea* are strongly supported sister taxa and classified together as Rousseeaceae (Lundberg, 2001). They are sister to the rest of the order, however only with low support (61%). An earlier recognised (Cosner et al., 1994; Gustafsson et al., 1996; Michaels et al., 1993) and here strongly supported group contains Asteraceae, Calyceraceae, Goodeniaceae, and Menyanthaceae. There is strong support for Menyanthaceae as sister to the other three families. The relationships among Asteraceae, Calyceraceae, and Goodeniaceae have been uncertain in earlier analyses. With *rbcL* data alone (Gustafsson et al., 1996; Savolainen et al., 2000a) there is bootstrap support for a sister group relationship between Calyceraceae and Goodeniaceae, and the same relationship holds for the 3-genes analysis of *rbcL/atpB/18S* rDNA (Soltis et al., 2000). With somewhat different sampling, however, Asteraceae and Calyceraceae may appear as sister groups with *rbcL* data alone (Gustafsson and Bremer, 1997). With *ndhF* data (Olmstead et al., 2000) or *rbcL* and *ndhF* data combined (Kårehed et al., 1999), Asteraceae and Calyceraceae are sister groups (98% and 99%, respectively) and this relationship is corroborated by our results (88%). Another supported (94%) group of families comprises Argophyllaceae, Alseuosmiaceae, and Phellinaceae (Kårehed et al., 1999). The interrelationships among these three families remain somewhat unclear. Our results have Alseuosmiaceae and Phellinaceae as sister groups with medium support (87%) but in Kårehed et al.'s analysis based on *rbcL* and *ndhF* data Argophyllaceae and Phellinaceae are sister groups also with medium support (78%).

**Dipsacales** are expanded relative to the APG (1998) classification by inclusion of Adoxaceae (Bremer et al., 2001). *Viburnum* representing the latter family is here with 100% support placed as sister group of the Dipsacales as circumscribed by APG (1998). All families are included in our analysis and the interrelationships are completely resolved and in agreement with the first comprehensive *rbcL* analysis of the order (Backlund and Bremer, 1997). All nodes except one are strongly supported (100%). Linnaeaceae and Morinaceae are sister groups with 64% support only. Backlund and Pyck (1998) suggest that Morinaceae are sister to Dipsacaceae and Valerianaceae. However, the high support they refer to comes from a still unpublished analysis. Therefore strongly supported interrelationships among Linnaea-

ceae, Morinaceae, and Dipsacaceae + Valerianaceae remain to be demonstrated.

#### 4.1. Comparison of coding and non-coding sequences

Comparison between the three different analyses shows that even at this higher taxonomic level the phylogenetic utility of the non-coding markers is fully comparable to that of the coding genes. The fraction of parsimony-informative characters to aligned characters (nc/na in Table 1) is somewhat higher for the coding matrix (51.4%) than for the non-coding matrix (47.4%) and the sum of all jackknife support values (TJ in Table 1) is also somewhat higher for the coding results (8550) than for the non-coding results (8009). On the other hand, when the total jackknife support is compared to the number of aligned characters (TJ/na in Table 1), the non-coding analysis actually scored higher than the coding analysis (TJ/na = 1.91 and 1.67, respectively). Supported resolution is the goal of phylogenetic reconstruction and at least in our study the non-coding data thus proved more useful than the coding data when considered in relation to the number of aligned positions. The number of equally parsimonious trees is considerably higher in the non-coding analysis than in the coding analysis (7452 versus 24), but even the higher number is very small compared to what you may obtain in an analysis of 132 taxa, and the strict consensus tree was not very much collapsed. Furthermore, the number of nodes with medium to high support ( $\geq 67\%$ ) is almost the same in the non-coding analysis and in the coding analysis, 78 and 79, respectively (Table 1). The number of strongly supported nodes ( $\geq 95\%$ ) is somewhat higher for the coding analysis than for non-coding analysis, 61 versus 41, respectively. Combining all data in the combined analysis yielded, as expected, even more well supported nodes, 91 nodes with  $\geq 67\%$  and 64 nodes with  $\geq 95\%$  jackknife support. The total support in relation to the number of aligned characters was, however, considerably lower (TJ/na = 0.99).

All earlier analyses of asterids, including large samples of taxa, have been based on coding DNA, e.g., *rbcL*, *ndhF*, *atpB*, and 18S rDNA. Even if available, non-coding DNA has not been used, probably due to a preconceived assumption that only coding genes are informative for studies above family level. In e.g., Soltis and Soltis (1998) the taxonomic level of utility for introns and spacers is given as population to family level with a note that these markers may work in some groups within orders. Our study has shown that at least for the asterids, including 10 orders and >100 families, the non-coding markers are almost as good as the coding markers. If the strength of the results is measured in relation to the amount of input data, i.e., as the total jackknife support in the tree divided by the number of aligned nucleotides, the non-coding analysis is even

better. Our results indicate that there are no major differences in the utility of non-coding and coding sequences (given that alignment is possible), at least not for our rather high taxonomic level. For any analysis, independently of taxonomic level, one must have sufficient numbers of variable and informative characters. We submit that there is no logical ground for a pre-conceived assumption that non-coding DNA is less informative at higher taxonomic level. Earlier it was assumed that non-coding DNA is more or less free from constraints and rapidly evolving, randomly and independently (e.g., Böhle et al., 1994; Curtis and Clegg, 1984; Palmer, 1987). Being free from constraints it was assumed that non-coding DNA comparatively rapidly attained saturation of mutations, implying that it should be useless at higher taxonomic levels. We know very little about non-coding DNA evolution, but we do know that there are secondary structures, regulating sequences, and different functions, that all cause constraints on the DNA (e.g., Kelchner, 2000). Hence it is reasonable to assume that non-coding DNA consists of both independently and randomly evolving parts as well as more constrained parts. The latter may well be much more conserved and useful also for high taxonomic levels.

The allegedly randomly evolving non-coding DNA is comparable to third position data in coding DNA, which have been shown to be informative at higher taxonomic levels (e.g., Källersjö et al., 1998; Sennblad and Bremer, 2000). For non-coding DNA Kelchner (2000) argued that there are structural constraints and mechanisms that will make these data less useful and he concluded “if taxonomic level is too high, one would expect saturation of multiple hits and concealment of multiple hit indels in any non-coding region, decreasing its utility as a phylogenetic tool.” However, if there are structural/functional constraints one could just as well argue for the opposite. Constrained DNA markers could be conserved enough to be informative at higher level. For possible mutational “hot spots” and the problem of multiple hits leading to homoplasy, there is no reason to suspect these to be more problematic for non-coding DNA than for coding regions. Our data also show that the level of homoplasy is even lower in the non-coding data, as measured by the consistency and retention indices. Kelchner’s (2000) recommendation that non-coding data should or must be “corrected” by consideration of evolutionary mechanisms in order to be useful in phylogenetic analyses is an interesting approach. However, with very large data set as ours (of more than five hundred thousand bases in the non-coding matrix) this is not possible to do manually. Instead, we excluded all parts where we felt uncertain about the alignment (poly-*N*-sequences, probably results of slipped-strand mispairing). Since the results from non-coding DNA are almost fully congruent with those

from coding DNA, supporting the same groups, we conclude that non-coding DNA are just as useful without a priori corrections.

## 5. In conclusion

This study has provided increased support for resolution within the asterids, demonstrated the utility of non-coding DNA also at higher levels, and contributed to ordinal classification of several families of asterids. We have been able to resolve with strong support the basal interrelationships among Cornales, Ericales, lamiids, and campanulids. Resolution among orders within lamiids and campanulids, respectively, remains partly unclear. Family interrelationships have been fully or almost fully resolved with medium to strong support in Cornales, Garryales, Gentianales, Solanales, Aquifoliales, Apiales, and Dipsacales. Within the three large orders Ericales, Lamiales, and Asterales, family interrelationships remain partly unclear. The three non-coding markers proved almost equally useful as the three coding genes in phylogenetic reconstruction at the high level of orders and families in asterids, and in relation to the number of aligned positions the non-coding markers were even more effective. Our analysis has contributed also to reclassification of several families, e.g., Tetrameristaceae, Ebenaceae, Styracaceae, Montiniaceae, Orobanchaceae, and Scrophulariaceae (by inclusion of Pellicieraceae, Lissocarpaceae, Halesiaceae, Kaliphoraceae, Cyclocheilaceae, and Myoporaceae + Buddlejaceae, respectively), and to the placement of hitherto (APG, 1998) unplaced families, e.g., Sladeniaceae, Pentaphylacaceae, Plocospermataceae, Cardiopteridaceae, and Adoxaceae (in Ericales, Ericales, Lamiales, Aquifoliales, and Dipsacales, respectively), and Paracryphiaceae among campanulids. Several families of euasterids, especially within the campanulids, remain, however, unclassified to order, and require further investigation.

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## Appendix A

List of investigated taxa, with sequence accession numbers and references; for new sequences voucher information is given

Family	Species name with author	Citation/voucher	<i>rbcL</i>	<i>ndhF</i>	<i>matK</i>	<i>trnV</i>	<i>rps16</i>	<i>trnL</i>
Acanthaceae	<i>Acanthus longifolius</i> Host	Erixon and Bremer 44 (UPS)			AJ429326	AJ429679	AH431037	AJ430912
Acanthaceae	<i>Acanthus montanus</i> T. Anderson	Harris 1931 (K)		AJ429115				
Acanthaceae	<i>Acanthus montanus</i> T. Anderson	Hedrén et al. (1995)	L12592					
Acanthaceae	<i>Avicennia marina</i> Vierh.	Bremer 3061 (UPS)			AJ429327	AJ429680	AJ431038	AJ430913
Acanthaceae	<i>Avicennia marina</i> Vierh.	Bruhl 1283 (NE)		AJ429116				
Acanthaceae	<i>Avicennia nitida</i> Jacq.	Wagstaff and Olmstead (1997)	U28868					
Actinidiaceae	<i>Actinidia arguta</i> Miq.	Albach et al. (2001a,b)		AJ236238				
Actinidiaceae	<i>Actinidia chinensis</i> Planch.	Albert et al. (1992)	L01882					
Actinidiaceae	<i>Actinidia kolomikta</i> Maxim.	Erixon and Bremer 26 (UPS)			AJ429279	AJ429640	AJ430992	AJ430869
Adoxaceae	<i>Viburnum rhytidophyllum</i> Graebn.	Backlund 271 (UPS)			AJ429391	AJ429736	AJ431103	AJ430979
Adoxaceae	<i>Viburnum rhytidophyllum</i> Graebn.	Gustafsson et al. (1996)	X87398					
Adoxaceae	<i>Viburnum rhytidophyllum</i> Graebn.	Oxelman et al. (1999)		AF027273				
Alseuosmiaceae	<i>Alseuosmia macrophylla</i> A. Cunn.	Gustafsson et al. (1996)	X87377					
Alseuosmiaceae	<i>Alseuosmia macrophylla</i> A. Cunn.	Mackinder s.n. (UPS)			AJ429378	AJ429725	AJ431091	AJ430965
Alseuosmiaceae	<i>Alseuosmia macrophylla</i> A. Cunn.	Roels, unpublished		AF060157				
Apiaceae	<i>Apium graveolens</i> L.	Albert et al. (1992)	L01885					
Apiaceae	<i>Apium graveolens</i> L.	Erixon and Bremer 45 (UPS)		AJ429124	AJ429370	AJ429716	AJ431081	AJ430956
Apocynaceae	<i>Alstonia scholaris</i> (L.) R. Br.	Fanning 212 (FTG)			AJ429321	AJ429674	AJ431032	AJ430907
Apocynaceae	<i>Alstonia scholaris</i> (L.) R. Br.	Oxelman et al. (1999)		AJ011982				
Apocynaceae	<i>Alstonia scholaris</i> (L.) R. Br.	Sennblad and Bremer (1996)	X91760					
Aquifoliaceae	<i>Ilex crenata</i> Thunb.	Olmstead et al. (2000)		AF130206				
Aquifoliaceae	<i>Ilex mitis</i> (L.) Radlk.	Manen et al. (1998)	X98730					
Aquifoliaceae	<i>Ilex</i> sp.	Erixon and Bremer 52 (UPS)		AJ429128	AJ429376	AJ429722	AJ431088	AJ430962
Araliaceae	<i>Aralia spinosa</i> L.	Chase et al. (1993)	L11166					
Araliaceae	<i>Aralia spinosa</i> L.	Erixon and Bremer 6 (UPS)		AJ429125	AJ429371	AJ429717	AJ431082	AJ430957
Aralidiaceae	<i>Aralidium pinnatifidum</i> Miq.	Plunkett et al. (1997)			U58627			
Aralidiaceae	<i>Aralidium pinnatifidum</i> Miq.	Saleh s.n. (KEP)		AJ429126		AJ429718	AJ431083	AJ430083
Aralidiaceae	<i>Aralidium pinnatifidum</i> Miq.	Xiang and Soltis, unpublished	AF299087					
Argophyllaceae	<i>Argophyllum</i> sp.	Gustafsson et al. (1996)	X87379					
Argophyllaceae	<i>Argophyllum</i> sp.	Kårehed et al. (1999)		AJ238335				
Argophyllaceae	<i>Argophyllum</i> sp.	Telford 5462 (CBG)			AJ429379	AJ429726	AJ431092	AJ430966
Asteraceae	<i>Helianthus annuus</i> L.	Bergqvist 56 (S)			AJ429380	AJ429727	AJ431093	AJ430967
Asteraceae	<i>Helianthus annuus</i> L.	Kanevski et al. (1999)	AF097517					
Asteraceae	<i>Helianthus annuus</i> L.	Kim and Jansen (1995)		L39383				
Balsaminaceae	<i>Impatiens biflora</i> Walt.	Olmstead et al. (2000)		AF130210				
Balsaminaceae	<i>Impatiens capensis</i> Meerb.	Erixon and Bremer 12 (UPS)			AJ429280	AJ429641	AJ430993	AJ430870
Balsaminaceae	<i>Impatiens repens</i> Moon	Morton et al. (1997)	Z80197					
Bignoniaceae	<i>Jacaranda mimosaeifolia</i> D. Don	Erixon and Bremer 28 (UPS)			AJ429328	AJ429681	AJ431039	AJ430914
Bignoniaceae	<i>Jacaranda sparrei</i> A.H. Gen-try	Spangler and Olmstead (1999)	AF102647	AF102631				
Boraginaceae	<i>Borago officinalis</i> L.	Erixon and Bremer 11 (UPS)			AJ429308	AJ429664	AJ431019	AJ430896
Boraginaceae	<i>Borago officinalis</i> L.	Olmstead and Reeves (1995)		L36393				
Boraginaceae	<i>Borago officinalis</i> L.	Olmstead et al. (1992)	L11680					



## Appendix A (continued)

Family	Species name with author	Citation/voucher	<i>rbcL</i>	<i>ndhF</i>	<i>matK</i>	<i>trnV</i>	<i>rps16</i>	<i>trnL</i>
Boraginaceae	<i>Pholisma arenarium</i> Nutt.	Colwell CAP76P-2 (MO)	AJ428894	Missing	AJ429309	Missing	AJ431020	AJ430897
Bruniaceae	<i>Brunia albiflora</i> Phillips	Backlund and Bremer (1997)	Y10674					
Bruniaceae	<i>Brunia albiflora</i> Phillips	Gustafsson 239 (UPS)			AJ429361	AJ429707	AJ431072	AJ430948
Bruniaceae	<i>Brunia albiflora</i> Phillips	Roels, unpublished		AF060159				
Byblidaceae	<i>Byblis liniflora</i> Salisb.	Albert et al. (1992)	L01891					
Byblidaceae	<i>Byblis liniflora</i> Salisb.	Qiu 95128 (IND)		Missing	AJ429354	AJ429533	AJ431070	AJ430941
Calyceraceae	<i>Acicarpha tribuloides</i> Juss.	Gustafsson 207 (UPS)		AJ429129	AJ429381	AJ429728	AJ431094	AJ430968
Calyceraceae	<i>Acicarpha tribuloides</i> Juss.	Gustafsson et al. (1996)	X87376					
Calyceraceae	<i>Boopis anthemoides</i> Juss.	Hunziker 25258 (CORD)						AJ430969
Calyceraceae	<i>Boopis anthemoides</i> Juss.	Kim and Jansen (1995)		L39384				
Calyceraceae	<i>Boopis anthemoides</i> Juss.	Michaels et al. (1993)	L13860					
Calyceraceae	<i>Boopis graminea</i> Phil.	DeVore 1442 (OS)			AJ429382	AJ429729	AJ431095	
Campanulaceae	<i>Campanula elatines</i> L.	Erixon and Bremer 49 (UPS)			AJ430387	AJ430391		AJ430970
Campanulaceae	<i>Campanula ramosissima</i> Sibth. and Sm.	Kim and Jansen (1995)		L39387				
Campanulaceae	<i>Campanula ramosissima</i> Sibth. and Sm.	Michaels et al. (1993)	L13861					
Caprifoliaceae	<i>Lonicera orientalis</i> Lam.	Backlund 267 (UPS)			AJ430196	AJ429737	AJ431104	AJ430980
Caprifoliaceae	<i>Lonicera orientalis</i> Lam.	Gustafsson et al. (1996)	X87389					
Caprifoliaceae	<i>Lonicera orientalis</i> Lam.	Oxelmann et al. (1999)		AF027274				
Cardiopteridaceae	<i>Cardiopteris quinqueloba</i> Hassk.	Meebold 16830 (S)		AJ312963	AJ429310	AJ429665	AJ431021	AJ430898
Cardiopteridaceae	<i>Cardiopteris quinqueloba</i> Hassk.	Savolainen et al. (2000a)	AJ402936					
Carpodetaceae	<i>Carpodetus serratus</i> Forst.	Gustafsson and Bremer (1997)	Y08461					
Carpodetaceae	<i>Carpodetus serratus</i> Forst.	Cameron s.n. (UPS)			AJ429383	AJ429535	Missing	AJ430971
Carpodetaceae	<i>Carpodetus serratus</i> Forst.	Kårehed et al. (1999)		AJ238336				
Clethraceae	<i>Clethra alnifolia</i> L.	Albach et al. (2001a,b)		AJ236242				
Clethraceae	<i>Clethra alnifolia</i> L.	Erixon and Bremer 5 (UPS)			AJ429281	AJ429526	AJ430994	AJ430871
Clethraceae	<i>Clethra alnifolia</i> L.	Kron and Chase (1983)	L12609					
Columelliaceae	<i>Columellia oblonga</i> Ruiz and Pav.	Backlund and Bremer (1997)	Y10675					
Columelliaceae	<i>Columellia oblonga</i> Ruiz and Pav.	Roels, unpublished		AF060160				
Columelliaceae	<i>Desfontainia spinosa</i> Ruiz and Pav.	Bremer et al. (1994)	Z29670					
Columelliaceae	<i>Desfontainia spinosa</i> Ruiz and Pav.	Oxelmann et al. (1999)		AJ011988				
Columelliaceae	<i>Columellia oblonga</i> Ruiz and Pav.	Bremer et al. 3374 (UPS)			AJ429362	AJ429708	AJ431073	AJ430949
Columelliaceae	<i>Desfontainia spinosa</i> Ruiz and Pav.	Bremer 2739 (UPS)			AJ429363	AJ429709	AJ431074	AJ430950
Convolvulaceae	<i>Ipomoea batatas</i> (L.) Lam.	Erixon and Bremer 18 (UPS)			AJ429355	AJ429702	AJ431071	AJ430942
Convolvulaceae	<i>Ipomoea coccinea</i> L.	Olmstead et al. (1993)	L14400					
Convolvulaceae	<i>Ipomoea coccinea</i> L.	Olmstead et al. (1993)		U08918				
Cornaceae	<i>Cornus mas</i> L.	Bremer 3318 (UPS)			AJ429275	AJ429636	AJ430988	AJ430866
Cornaceae	<i>Cornus mas</i> L.	Roels, unpublished		AF060161				
Cornaceae	<i>Cornus mas</i> L.	Xiang et al. (1993)	L11216					
Cyrtillaceae	<i>Cyrtilla racemiflora</i> L.	Albert et al. (1992)	L01900					
Cyrtillaceae	<i>Cyrtilla racemiflora</i> L.	Anderberg et al. (2002)		AF421051				
Cyrtillaceae	<i>Cyrtilla racemiflora</i> L.	Anderberg 7389 (S)			AJ429282	AJ429527	AJ430995	AJ430872
Diapensiaceae	<i>Diapensia lapponica</i> L.	Beier 122 (UPS)			AJ429283	Missing	Missing	AJ430873
Diapensiaceae	<i>Diapensia lapponica</i> L.	Anderberg et al. (2002)		AF421052				
Diapensiaceae	<i>Diapensia lapponica</i> L.	Kron and Chase (1983)	L12612					
Diervillaceae	<i>Diervilla rivularis</i> Gatt.	Erixon and Bremer 39 (UPS)			AJ429392	AJ429738	AJ431105	AJ430981
Diervillaceae	<i>Diervilla sessilifolia</i> Buckl.	Bremer et al. (1994)	Z29672					

## Appendix A (continued)

Family	Species name with author	Citation/voucher	<i>rbcL</i>	<i>ndhF</i>	<i>matK</i>	<i>trnV</i>	<i>rps16</i>	<i>trnL</i>
Diervillaceae	<i>Diervilla sessilifolia</i> Buckl.	Roels, unpublished		AF060164				
Dipentodontaceae	<i>Dipentodon sinicus</i> Dunn	Tsai 58398 (A)	AJ428890	AJ429102	AJ429273	AJ429634	AJ430986	AJ430865
Dipsacaceae	<i>Dipsacus sativus</i> Garsault	Erixon and Bremer 46 (UPS)			AJ429393	AJ429739	AJ431106	AJ430982
Dipsacaceae	<i>Dipsacus sativus</i> Garsault	Michaels et al. (1993)	L13864					
Dipsacaceae	<i>Dipsacus sativus</i> Garsault	Olmstead et al. (2000)		AF130190				
Donatiaceae	<i>Donatia fascicularis</i> Forst.	Gustafsson et al. (1996)	X87385					
Donatiaceae	<i>Donatia fascicularis</i> Forst.	Laurent et al. (1998)		AJ225074				
Donatiaceae	<i>Donatia fascicularis</i> Forst.	Swenson 301 (UPS)			AJ429384	Missing	AJ431096	AJ430972
Ebenaceae	<i>Diospyros kaki</i> Thunb.	Erixon and Bremer 19 (UPS)			AJ430197	AJ429642	AJ430996	AJ430874
Ebenaceae	<i>Diospyros kaki</i> Thunb.	Morton et al. (1997)	Z80185					
Ebenaceae	<i>Diospyros texana</i> Scheele	Olmstead et al. (2000)		AF130213				
Eremosynaceae	<i>Eremosyne pectinata</i> Endl.	Annels and Hearn 4795 (UWA)		AJ429120	AJ429364	AJ429710	AJ431075	AJ430951
Eremosynaceae	<i>Eremosyne pectinata</i> Endl.	Hibsch-Jetter et al., unpublished	L47969					
Ericaceae	<i>Erica australis</i> L.	Kron and Chase (1983)	L12617					
Ericaceae	<i>Erica carnea</i> L.	Erixon and Bremer 32 (UPS)		AJ429105	AJ429284	Missing	AJ430997	AJ430875
Escalloniaceae	<i>Escallonia coquimbensis</i> Remy in Gay	Morgan and Soltis (1993)	L11183					
Escalloniaceae	<i>Escallonia rubra</i> Pers.	A. Backlund, no voucher			AJ429365	AJ429711	AJ431076	AJ430952
Escalloniaceae	<i>Escallonia x langleyensis</i> Vilm. and Bois	Roels, unpublished			AF060165			
Escalloniaceae	<i>Quintinia verdonii</i> F. Muell	Telford 3244 (CBG)			AJ429366	AJ429712	AJ431077	AJ430953
Escalloniaceae	<i>Quintinia verdonii</i> F. Muell	Kårehed et al. (1999)	AJ238344					
Escalloniaceae	<i>Quintinia verdonii</i> F. Muell	Gustafsson et al. (1996)	X87394					
Eucommiaceae	<i>Eucommia ulmoides</i> Oliver	Olmstead 97-141 (WTU)		AJ429113				
Eucommiaceae	<i>Eucommia ulmoides</i> Oliver	Albert et al. (1992)	L01917					
Eucommiaceae	<i>Eucommia ulmoides</i> Oliver	Olmstead 97-11 (WTU)			AJ429317	Missing	AJ431028	AJ430905
Fouquieriaceae	<i>Fouquieria digueti</i> I.M. Johnst.	Erixon and Bremer 25 (UPS)			AJ429285	AJ429643	AJ430998	AJ430876
Fouquieriaceae	<i>Fouquieria splendens</i> Engelm.	Albach et al. (2001a,b)		AJ236249				
Fouquieriaceae	<i>Fouquieria splendens</i> Engelm.	Olmstead et al. (1992)	L11675					
Garryaceae	<i>Aucuba japonica</i> Thunb.	Erixon and Bremer 30 (UPS)			AJ429318	AJ429672	AJ431029	AJ430906
Garryaceae	<i>Aucuba japonica</i> Thunb.	Roels, unpublished		AF060158				
Garryaceae	<i>Aucuba japonica</i> Thunb.	Xiang et al. (1993)	L11210					
Garryaceae	<i>Garrya elliptica</i> Dougl. ex Lindl.	Albert et al. (1992)	L01919					
Garryaceae	<i>Garrya elliptica</i> Dougl. ex Lindl.	Olmstead et al. (2000)		AF147714				
Garryaceae	<i>Garrya elliptica</i> Dougl. ex Lindl.	Rancho Santa Ana Bot. Gard., 11829, no voucher			AJ429319	AJ429529	AJ431030	Missing
Gelsemiaceae	<i>Gelsemium sempervirens</i> Ait.	Bremer 3026 (UPS)			AJ429322	AJ429675	AJ431033	AJ430908
Gelsemiaceae	<i>Gelsemium sempervirens</i> Ait.	Oxelman et al. (1999)		AJ011984				
Gelsemiaceae	<i>Gelsemium sempervirens</i> Ait.	Olmstead et al. (1993)	L14397					
Gentianaceae	<i>Gentiana procera</i> Holm	Olmstead and Reeves (1995)		L36400				
Gentianaceae	<i>Gentiana procera</i> Holm	Olmstead et al. (1993)	L14398					
Gentianaceae	<i>Gentiana purpurea</i> L.	Struwe 1009 (UPS)			AJ429323	AJ429676	AJ431034	AJ430909
Gesneriaceae	<i>Androya decaryi</i> H. Perrier	Oxelman et al. (1999)	AJ001756	AF027276				
Gesneriaceae	<i>Androya decaryi</i> H. Perrier	Philipson et al. 3747 (MO)			AJ429329	AJ429530	AJ431040	AJ430915
Gesneriaceae	<i>Peltanthera floribunda</i> Benth.	Hammel 19855 (MO)			AJ429330	AJ429682	AJ431041	AJ430916
Gesneriaceae	<i>Peltanthera floribunda</i> Benth.	Oxelman et al. (1999)	AJ001762	AF027281				
Gesneriaceae	<i>Sanago</i> sp.	Bremer et al. 3352 (UPS)			Missing	AJ429683	AJ431042	AJ430917
Gesneriaceae	<i>Sanago</i> sp.	Oxelman et al. (1999)	AJ001763					
Gesneriaceae	<i>Sanago</i> sp.	Oxelman et al. (1999)		AF027283				
Gesneriaceae	<i>Streptocarpus caulescens</i> Vatke	Erixon and Bremer 35 (UPS)			AJ429331	AJ429684	AJ431043	AJ430918

## Appendix A (continued)

Family	Species name with author	Citation/voucher	<i>rbcL</i>	<i>ndhF</i>	<i>matK</i>	<i>trnV</i>	<i>rps16</i>	<i>trnL</i>
Gesneriaceae	<i>Streptocarpus holstii</i> Engl.	Olmstead and Reeves (1995)		L36415				
Gesneriaceae	<i>Streptocarpus holstii</i> Engl.	Olmstead et al. (1993)	L14409					
Goodeniaceae	<i>Scaevola frutescens</i> K. Krause	Kim and Jansen (1995)		L39385				
Goodeniaceae	<i>Scaevola frutescens</i> K. Krause	Michaels et al. (1993)	L13932					
Goodeniaceae	<i>Scaevola</i> sp.	Lundberg 55 (UPS)			AJ429385	AJ429730	AJ431097	AJ430973
Griselinaceae	<i>Griselinia littoralis</i> (Raoul) Raoul	Tibell NZ3 (UPS)			AJ429372	AJ429719	AJ431084	AJ430958
Griselinaceae	<i>Griselinia lucida</i> (J.R. Forst. and G. Forst) G. Forst	Olmstead et al. (2000)		AF130205				
Griselinaceae	<i>Griselinia lucida</i> (J.R. Forst. and G. Forst) G. Forst	Plunkett et al. (1997)			U58628			
Griselinaceae	<i>Griselinia lucida</i> (J.R. Forst. and G. Forst) G. Forst	Xiang et al. (1993)	L11225					
Grubbiaceae	<i>Grubbia rosmarinifolia</i> Berg.	Chase 5704 (K)		AJ429104	AJ429276	AJ429637	AJ430989	AJ430867
Grubbiaceae	<i>Grubbia tomentosa</i> (Thunb.) Harms	Morton et al. (1996)	Z83141					
Helwingiaceae	<i>Helwingia japonica</i> (Thunb. ex Murray) F. Dietrich	Olmstead et al. (2000)		AF130207				
Helwingiaceae	<i>Helwingia japonica</i> (Thunb. ex Murray) F. Dietrich	Peng et al. 17408 (S)			AJ430195	AJ429723	AJ431089	AJ430963
Helwingiaceae	<i>Helwingia japonica</i> (Thunb. ex Murray) F. Dietrich	Xiang et al. (1993)	L11226					
Hydrangeaceae	<i>Hydrangea aspera</i> Buch.-Ham. ex D. Don	Erixon and Bremer 4 (UPS)			AJ429277	AJ429638	AJ430990	Missing
Hydrangeaceae	<i>Hydrangea macrophylla</i> (Thunb.) Ser.	Morgan and Soltis (1993)	L11187					
Hydrangeaceae	<i>Hydrangea macrophylla</i> (Thunb.) Ser.	Olmstead et al. (2000)		AF130218				
Hydroleaceae	<i>Hydrolea ovata</i> Nutt.	Ferguson (1998)		AF013999				
Hydroleaceae	<i>Hydrolea ovata</i> Nutt.	Olmstead et al. (1993)	L14293					
Hydroleaceae	<i>Hydrolea ovata</i> Nutt.	Torrey s.n. (UPS)			AJ429356	AJ429703	Missing	AJ430943
Icacinaceae	<i>Apodytes dimidiata</i> E. Mey. ex Bernh.	Bremer 3770 (UPS)	AJ428895	AJ429109	AJ429311	AJ429666	AJ431022	AJ430899
Icacinaceae	<i>Cassinopsis ilicifolia</i> (Hochst.) Sleumer	Bremer 3769 (UPS)	AJ428896	AJ429110	AJ429312	AJ429667	AJ431023	AJ430900
Icacinaceae	<i>Icacina senegalensis</i> Juss.	Jongkind 2012 (UPS)		AJ429111				
Icacinaceae	<i>Icacina senegalensis</i> Juss.	Pettersson 3026 (UPS)	AJ428897		AJ429313	AJ429668	AJ431024	AJ430901
Icacinaceae	<i>Pyrenacantha grandifolia</i> Engl.	Bremer 3795 (UPS)			AJ429314	AJ429669	AJ431025	AJ430902
Icacinaceae	<i>Pyrenacantha malvaefolia</i> Engl.	Kårehed (2001)		AJ312952				
Icacinaceae	<i>Pyrenacantha malvaefolia</i> Engl.	Savolainen et al. (2000a,b)	AJ402995					
Lamiaceae	<i>Lamium album</i> L.	Erixon and Bremer 15 (UPS)			AJ429332	AJ429685	AJ431044	AJ430919
Lamiaceae	<i>Lamium purpureum</i> L.	Kaufmann and Wink (1996)	Z37403					
Lamiaceae	<i>Lamium purpureum</i> L.	Wagstaff and Olmstead (1997)		U78694				
Lecythidaceae	<i>Barringtonia asiatica</i> (L.) Kurz	Anderberg et al. (2002)		AF421044				
Lecythidaceae	<i>Barringtonia asiatica</i> (L.) Kurz	Chung and Anderberg 1417 (HAST)			AJ429286	AJ429644	AJ430999	AJ430877
Lecythidaceae	<i>Barringtonia asiatica</i> (L.) Kurz	Morton et al. (1997)	Z80174					
Lentibulariaceae	<i>Pinguicula caerulea</i> Walt.	Albert et al. (1992)	L01942					
Lentibulariaceae	<i>Pinguicula</i> sp.	Erixon and Bremer 54 (UPS)		Missing	AJ429333	AJ429686	AJ431045	AJ430920
Linnaeaceae	<i>Linnaea borealis</i> L.	A. Backlund, no voucher			AJ429394	AJ429740	AJ431107	AJ430983
Linnaeaceae	<i>Linnaea borealis</i> L.	Olmstead s.n. (WTU)	AJ428899					
Linnaeaceae	<i>Linnaea borealis</i> L.	Roels, unpublished		AF060166				

## Appendix A (continued)

Family	Species name with author	Citation/voucher	<i>rbcL</i>	<i>ndhF</i>	<i>matK</i>	<i>trnV</i>	<i>rps16</i>	<i>trnL</i>
Lissocarpaceae	<i>Lissocarpa guianensis</i> Gleason	Anderberg et al. (2002)	AF421094	AF421062				
Lissocarpaceae	<i>Lissocarpa guianensis</i> Gleason	Maas and Westra 3999 (S)			AJ429287	AJ429645	Missing	Missing
Loasaceae	<i>Loasa loxensis</i> Humb. Bonpl. and Kunth	Hempel et al. (1995)	U17876					
Loasaceae	<i>Loasa triphylla</i> Juss.	Erixon and Bremer 42 (UPS)			AJ429278	AJ429639	AJ430991	AJ430868
Loasaceae	<i>Loasa vulcanica</i> André	Roels, unpublished		AF060167				
Loganiaceae	<i>Logania</i> sp.	Backlund et al. (2000)		AJ235837				
Loganiaceae	<i>Logania vaginalis</i> F. Muell.	Bremer 1996	Z68826					
Loganiaceae	<i>Logania vaginalis</i> F. Muell.	Bremer 3013 (UPS)			AJ429324	AJ429677	AJ431035	AJ430910
Maesaceae	<i>Maesa myrsinoides</i> Leveille	Morton et al. (1997)	Z80203					
Maesaceae	<i>Maesa tene</i> ra Mez	Stähl s.n. (S)			AJ429288	Missing	AJ431000	AJ430878
Maesaceae	<i>Maesa tene</i> ra Mez	Källersjö et al. (2000)		AF213750				
Marcgraviaceae	<i>Marcgravia rectiflora</i> Triana and Planch.	Albach et al. (2001a,b)		AJ236263				
Marcgraviaceae	<i>Marcgravia rectiflora</i> Triana and Planch.	Morton et al. (1996)	Z83148					
Marcgraviaceae	<i>Marcgravia</i> sp.	Anderberg s.n. (S)			AJ429289	AJ429646	AJ431001	AJ430879
Martyniaceae	<i>Proboscidea fragrans</i> (Lindl.) Decne.	Erixon and Bremer 41 (UPS)			AJ429334	AJ430388	AJ431046	AJ430921
Martyniaceae	<i>Proboscidea louisianica</i> (Mill.) Thell.	Albach et al. (2001a,b)		AJ236267				
Martyniaceae	<i>Proboscidea louisianica</i> (Mill.) Thell.	Albert et al. (1992)	L01946					
Melanophyllaceae	<i>Melanophylla alnifolia</i> Baker	Albach et al. (2001a,b)		AJ236244				
Melanophyllaceae	<i>Melanophylla alnifolia</i> Baker	Plunkett et al. (1996)	U50254					
Melanophyllaceae	<i>Melanophylla</i> sp.	Thulin et al. 10282 (UPS)			AJ429373	AJ430390	AJ431085	AJ430959
Menyanthaceae	<i>Menyanthes trifoliata</i> L.	Kim and Jansen (1995)		L39388				
Menyanthaceae	<i>Menyanthes trifoliata</i> L.	No voucher			AJ429386	AJ429731	AJ431098	AJ430974
Menyanthaceae	<i>Menyanthes trifoliata</i> L.	Olmstead et al. (1993)	L14006					
Montiniaceae	<i>Grevea</i> sp.	Thulin et al. s.n. (UPS)	AJ428898	AJ430426	AJ429357	AJ429704	AJ431066	AJ430944
Montiniaceae	<i>Kaliphora madagascariensis</i> Hook. f	Savolainen et al. (2000a)	AJ402963					
Montiniaceae	<i>Kaliphora madagascariensis</i> Hook. f	Schatz et al. 3568 (MO)		AJ431206				
Montiniaceae	<i>Kaliphora madagascariensis</i> Hook. f	Bremer et al. 4081-B81 (UPS)			AJ429358	AJ429705	AJ431067	AJ430945
Montiniaceae	<i>Montinia caryophyllacea</i> Thunb.	Bremer 3521 (UPS)			AJ429359	AJ429706	AJ431068	AJ430946
Montiniaceae	<i>Montinia caryophyllacea</i> Thunb.	Morgan and Soltis (1993)	L11194					
Montiniaceae	<i>Montinia caryophyllacea</i> Thunb.	Olmstead et al. (2000)		AF130178				
Morinaceae	<i>Morina coulteriana</i> Royle	Backlund and Bremer (1997)	Y10706					
Morinaceae	<i>Morina longifolia</i> Wall.	Erixon and Bremer 38 (UPS)		AJ429130	AJ429395	AJ429741	AJ431108	AJ430984
Myrsinaceae	<i>Myrsine africana</i> L.	Anderberg et al. (1998)	U96652					
Myrsinaceae	<i>Myrsine africana</i> L.	Källersjö et al. (2000)		AF213751				
Myrsinaceae	<i>Myrsine africana</i> L.	Erixon and Bremer 17 (UPS)			AJ429290	AJ429647	AJ431002	AJ430880
Oleaceae	<i>Olea europaea</i> L.	Erixon and Bremer 34 (UPS)			AJ429335	AJ429687	AJ431047	AJ430922
Oleaceae	<i>Olea europaea</i> L.	Oxelman et al. (1999)	AJ001766	AF027288				
Oncothecaceae	<i>Oncotheca balansae</i> Baill.	Jaffre 3238 (NOU)		AJ429114	AJ429320	AJ429673	AJ431031	AJ430529, AJ430530
Oncothecaceae	<i>Oncotheca balansae</i> Baill.	Savolainen et al. (2000a,b)	AJ131950					
Orobanchaceae	<i>Cyclocheilon somaliense</i> Oliver	Thulin et al. 8364 (UPS)		AJ429117	AJ429336	AJ429688	AJ431048	AJ430923
Orobanchaceae	<i>Cyclocheilon somaliense</i> Oliver	Wagstaff and Olmstead (1997)	U28871					
Orobanchaceae	<i>Lindenbergia</i> sp.	Thulin 8079 (UPS)			AJ429337	AJ429689	AJ431049	AJ430924



## Appendix A (continued)

Family	Species name with author	Citation/voucher	<i>rbcL</i>	<i>ndhF</i>	<i>matK</i>	<i>trnV</i>	<i>rps16</i>	<i>trnL</i>
Polemoniaceae	<i>Polemonium pulcherrimum</i> Hook.	Erixon and Bremer 8 (UPS)			AJ429292	AJ429649	AJ431004	AJ430882
Polemoniaceae	<i>Polemonium reptans</i> L.	Olmstead et al. (1992)	L11687					
Polyosmaceae	<i>Polyosma cunninghamii</i> Benn.	Bremer 3941 (UPS)		AJ429122	AJ429368	AJ429714		
Polyosmaceae	<i>Polyosma cunninghamii</i> Benn.	Cejie 22179 (UNSW)					AJ431079	AJ430954
Polyosmaceae	<i>Polyosma cunninghamii</i> Benn.	Savolainen et al. (2000a,b)	AJ402992					
Primulaceae	<i>Primula sieboldi</i> E. Morr	Källersjö et al. (2000)		AF213757				
Primulaceae	<i>Primula sieboldi</i> E. Morr	Anderberg et al. (1998)	U96657					
Primulaceae	<i>Primula veris</i> L.	Erixon and Bremer 33 (UPS)			AJ429293	AJ429650	AJ431005	AJ430883
Roridulaceae	<i>Roridula gorgonias</i> Planch.	Albach et al. (2001a,b)		AJ236270				
Roridulaceae	<i>Roridula gorgonias</i> Planch.	Albert et al. (1992)	L01950					
Roridulaceae	<i>Roridula gorgonias</i> Planch.	Linder, cult., no voucher			AJ429294	AJ429651	AJ431006	AJ430884
Rousseaceae	<i>Roussea simplex</i> Sm.	From Soltis (MAU)			AJ429389	AJ429734	AJ431101	AJ430977
Rousseaceae	<i>Roussea simplex</i> Sm.	Koontz and Soltis (1999)	AF084477					
Rousseaceae	<i>Roussea simplex</i> Sm.	Lundberg (2001)		AJ277384				
Rubiaceae	<i>Luculia grandifolia</i> Ghose	Bremer et al. (1995)	X83648					
Rubiaceae	<i>Luculia gratissima</i> Sweet	CONN 870064, no voucher			AJ429325	AJ429678	AJ431036	AJ430911
Rubiaceae	<i>Luculia gratissima</i> Sweet	Oxelman et al. (1999)		AJ011987				
Sapotaceae	<i>Manilkara zapota</i> (L.) van Royen	Albert et al. (1992)	L01932					
Sapotaceae	<i>Manilkara zapota</i> (L.) van Royen	Anderberg et al. (2002)		AF213732				
Sapotaceae	<i>Manilkara zapota</i> (L.) van Royen	Erixon and Bremer 23 (UPS)			AJ429295	AJ429652	AJ431007	AJ430885
Sarraceniaceae	<i>Sarracenia flava</i> L.	Albert et al. (1992)	L01952					
Sarraceniaceae	<i>Sarracenia purpurea</i> L.	Erixon and Bremer 21 (UPS)		Missing	AJ429296	AJ429653	AJ431008	AJ430886
Schlegeliaceae	<i>Schlegelia parviflora</i> (Oerst.) Monach.	Gentry 14221 (MO)			AJ429345	AJ429696	AJ431057	AJ430932
Schlegeliaceae	<i>Schlegelia parviflora</i> (Oerst.) Monach.	Olmstead and Reeves (1995)	L36448	L36410				
Scrophulariaceae	<i>Buddleja asiatica</i> Lour.	Bremer 3500 (UPS)			AJ429346	AJ429697	AJ431058	AJ430933
Scrophulariaceae	<i>Buddleja davidii</i> Franch.	Olmstead and Reeves (1995)		L36394				
Scrophulariaceae	<i>Buddleja davidii</i> Franch.	Oxelman et al. (1999)	AJ001757					
Scrophulariaceae	<i>Myoporum mauritianum</i> A. DC.	Olmstead and Reeves (1995)	L36445	L36403				
Scrophulariaceae	<i>Myoporum montanum</i> R. Br.	Bremer 3927 (UPS)			AJ429347	AJ429698	AJ431059	AJ430934
Scrophulariaceae	<i>Selago thomsoni</i> Rolfe ex Oliver	Bremer 3095 (UPS)			AJ429348	AJ429699	AJ431060	AJ430935
Scrophulariaceae	<i>Selago thunbergii</i> Choisy	Olmstead and Reeves (1995)	L36450	L36412				
Scrophulariaceae	<i>Scrophularia arguta</i> Sol. ex Ait.	Thulin and Gifri 8633 (UPS)			AJ429349	AJ429531	AJ431061	AJ430936
Scrophulariaceae	<i>Scrophularia</i> sp.	Olmstead and Reeves (1995)	L36449	L36411				
Sladeniaceae	<i>Sladenia celastrifolia</i> Kurz	Bartholomen et al. 1636 (A)			AJ429297	AJ429654	AJ431009	AJ430081
Sladeniaceae	<i>Sladenia celastrifolia</i> Kurz	Savolainen et al. (2000a,b)	AJ403004					
Sladeniaceae	<i>Sladenia celastrifolia</i> Kurz	Anderberg et al. (2002)		AF421081				
Solanaceae	<i>Nicotiana tabacum</i> L.	Olmstead et al. (1993)		L14953				
Solanaceae	<i>Nicotiana tabacum</i> L.	Shinozaki et al. (1986)	Z00044		Z00044	Z00044	Z00044	Z00044
Sphenocleaceae	<i>Sphenoclea zeylanica</i> Gaertn.	Cosner et al. (1994)	L18798					
Sphenocleaceae	<i>Sphenoclea zeylanica</i> Gaertn.	Madsen 5986 (S)		AJ429119	AJ429360			
Sphenocleaceae	<i>Sphenoclea zeylanica</i> Gaertn.	Thulin et al. s.n. (UPS)				AJ429534	AJ431069	AJ430947
Stilbaceae	<i>Stilbe albiflora</i> E. Mey.	Oxelman et al. (1999)		AF027287				
Stilbaceae	<i>Stilbe ericoides</i> L.	Bremer 3731 (UPS)			AJ429350	AJ429532	AJ431062	AJ430937
Stilbaceae	<i>Stilbe vestita</i> Berg.	Bremer (1996)	Z68827					

## Appendix A (continued)

Family	Species name with author	Citation/voucher	<i>rbcL</i>	<i>ndhF</i>	<i>matK</i>	<i>trnV</i>	<i>rps16</i>	<i>trnL</i>
Styliaceae	<i>Styidium bulbiferum</i> Benth.	Bremer and Gustafsson 44 (UPS)			AJ429390	AJ429735	AJ431102	AJ430978
Styliaceae	<i>Styidium calcaratum</i> R. Br.	Laurent et al. (1998)	AJ225053	AJ225079				
Styracaceae	<i>Halesia carolina</i> L.	Mullens and Rodgers 64036 (S)			AJ429298	AJ429655	AJ431010	AJ430082
Styracaceae	<i>Halesia carolina</i> L.	Morton et al. (1997)	Z80190					
Styracaceae	<i>Halesia tetraptera</i> L.	Olmstead et al. (2000)		AF130214				
Styracaceae	<i>Pterostyrax hispidum</i> Sieber and Zucc.	Erixon and Bremer 37 (UPS)	AJ428892	AJ429107	AJ429299	AJ429656	Missing	AJ430887
Styracaceae	<i>Styrax americanum</i> Lam.	Kron and Chase (1983)	L12623					
Styracaceae	<i>Styrax americanum</i> Lam.	Olmstead et al. (2000)		AF130215				
Styracaceae	<i>Styrax officinale</i> L.	Lundqvist 15364 (S)			AJ429300	AJ429657	AJ431011	AJ430888
Symplocaceae	<i>Symplocos bogotensis</i> Brand	Anderberg et al. (2002)		AF421074				
Symplocaceae	<i>Symplocos bogotensis</i> Brand	Harling et al. 26476 (S)			AJ429301	AJ429658	AJ431012	AJ430889
Symplocaceae	<i>Symplocos costata</i> Choisy ex Zoll.	Morton et al. (1997)	Z80192					
Ternstroemiaceae	<i>Ternstroemia gymnanthera</i> Sprague	Erixon and Bremer 20 (UPS)			AJ429302	AJ429659	AJ431013	AJ430890
Ternstroemiaceae	<i>Ternstroemia gymnanthera</i> Sprague	Anderberg et al. (2002)		AF421076				
Ternstroemiaceae	<i>Ternstroemia stahlii</i> Krug and Urb.	Morton et al. (1997)	Z80211					
Tetrachondraceae	<i>Polypremum procumbens</i> L.	Oxelmann et al. (1999)	AJ011989	AJ011986				
Tetrachondraceae	<i>Polypremum procumbens</i> L.	Struwe 1000 (UPS)			AJ429351	AJ429700	AJ431063	AJ430938
Tetrachondraceae	<i>Tetrachondra hamiltonii</i> Petrie	Wagstaff and Olmstead (1997)	U28885					
Tetrachondraceae	<i>Tetrachondra patagonica</i> Skottsb.	Martinsson and Swenson 314 (UPS)			AJ429352	AJ430389	AJ431064	AJ430939
Tetrachondraceae	<i>Tetrachondra patagonica</i> Skottsb.	Oxelmann et al. (1999)		AF027272				
Tetrameristaceae	<i>Pelliciera rhizophoreae</i> Planch. and Triana	Anderberg et al. (2002)		AF421069				
Tetrameristaceae	<i>Pelliciera rhizophoreae</i> Planch. and Triana	Pennington et al. 586 (K)	AJ428893		AJ429303	AJ429660	AJ431014	AJ430891
Tetrameristaceae	<i>Tetramerista</i> sp.	Coode 7925 (K)		AJ429108	AJ429304	AJ429528	AJ431015	AJ430892
Tetrameristaceae	<i>Tetramerista</i> sp.	Morton et al. (1997)	Z80199					
Theaceae	<i>Camellia japonica</i> L.	Olmstead et al. (2000)		AF130216				
Theaceae	<i>Camellia japonica</i> L.	Kron and Chase (1983)	L12602					
Theaceae	<i>Camellia sinensis</i> Kuntze	Erixon and Bremer 40 (UPS)			AJ429305	AJ429661	AJ431016	AJ430893
Theaceae	<i>Schima superba</i> Gardn. and Champ.	Anderberg et al. (2002)		AF421073				
Theaceae	<i>Schima superba</i> Gardn. and Champ.	Chung and Anderberg 1410 (HAST)			AJ429306	AJ429662	AJ431017	AJ430894
Theaceae	<i>Schima superba</i> Gardn. and Champ.	Morton et al. (1997)	Z80208					
Theophrastaceae	<i>Theophrasta americana</i> L.	Anderberg et al. (1998)	U96649					
Theophrastaceae	<i>Theophrasta americana</i> L.	Erixon and Bremer 24 (UPS)			AJ429307	AJ429663	AJ431018	AJ430895
Theophrastaceae	<i>Theophrasta americana</i> L.	Källersjö et al. (2000)		AF213762				
Toricelliaceae	<i>Toricellia tiliaefolia</i> DC.	Xiang and Soltis, unpublished	AF299089					
Toricelliaceae	<i>Toricellia tiliaefolia</i> DC.	Y.-L. Tu 1991 s.n. no herbarium listed		AJ429127	AJ429375	AJ429721	AJ431087	AJ430961
Tribelaceae	<i>Tribeles australis</i> Phil.	Gardner and Knees 3879 (K)		AJ429123	AJ429369	AJ429715	AJ431080	AJ430955
Tribelaceae	<i>Tribeles australis</i> Phil.	Savolainen et al. (2000a,b)	AJ403010					
Vahliaceae	<i>Vahlia capensis</i> Thunb.	Bremer 3785 (UPS)		AJ429112	AJ429316	AJ429671	AJ431027	AJ430904
Vahliaceae	<i>Vahlia capensis</i> Thunb.	Morgan and Soltis (1993)	L11208					
Valerianaceae	<i>Valeriana officinalis</i> L.	Bremer 3316 (UPS)						AJ431680
Valerianaceae	<i>Valeriana fauriei</i> Briquet	Olmstead et al. (2000)		AF130192				
Valerianaceae	<i>Valeriana hirtella</i> Kunth	Backlund and Bremer (1997)	Y10699					

## Appendix A (continued)

Family	Species name with author	Citation/voucher	<i>rbcL</i>	<i>ndhF</i>	<i>matK</i>	<i>trnV</i>	<i>rps16</i>	<i>trnL</i>
Valerianaceae	<i>Valeriana hirtella</i> Kunth	Bremer et al. 3396 (UPS)			AJ429396	AJ429742	AJ431109	
Verbenaceae	<i>Verbena bracteata</i> Cav. ex Lag. and Rodr.	Olmstead and Reeves (1995)		L36418				
Verbenaceae	<i>Verbena officinalis</i> L.	Kaufmann and Wink (1996)	Z37473					
Verbenaceae	<i>Verbena rigida</i> Spreng.	Erixon and Bremer 9 (UPS)			AJ429353	AJ429701	AJ431065	AJ430940
Vitaceae	<i>Vitis aestivalis</i> Michx.	Albert et al. (1992)	L01960					
Vitaceae	<i>Vitis vinifera</i> L.	Bremer and Bremer 4091 (UPS)		AJ429103	AJ429274	AJ429635	AJ430987	AJ430864

## Appendix B

Primers used for new sequences in this study. Positions of primer corresponding to chloroplast DNA of tobacco (Shinozaki et al., 1986). All primers except those marked with A–F are constructed at the Department of Systematic Botany, Uppsala University; A = Zurawski, DNAX Research Institute, B = Kim and Jansen pers. comm., C = Oxelman et al., 1999, D = Oxelman et al., 1997, E = Sang et al., 1997, F = Taberlet et al., 1991

Name of DNA marker and primer	Primer sequence from the 5' end	Primer position in tobacco (GenBank Z00044)
<i>rbcL</i> gene		
FORWARD		
<i>rbcL</i> _5F	5'ATG TCA CCA CAA ACA GAA ACT AAA GC	(57595-57620)
<i>rbcL</i> _bs427F	5'GCT TAT ATT AAA ACC TTC CAA GGC CCG CC	(58021-58049)
<i>rbcL</i> _Z674F	5'TTT ATA AAT CAC AAG CCG AAA CTG GTG AAA TC	(58268-58299) A
<i>rbcL</i> _Z895F	5'GCA GTT ATT GAT AGA CAG AAA AAT CAT GGT	(58492-58521) A
REVERSE		
<i>rbcL</i> _Z1020R	5'ATC ATC GCG CAA TAA ATC AAC AAA ACC TAA AGT	(58650-58618) A
<i>rbcL</i> _Z674R	5'GAT TTC GCC TGT TTC GGC TTG TGC TTT ATA AA	(58299-58268) A
<i>rbcL</i> _Z895R	5'ACC ATG ATT CTT CTG CCT ATC AAT AAC TGC	(58518-58487) A
<i>rbcL</i> _Z1204R	5'CCC TAA GGG TGT CCT AAA GTT TCT CCA CC	(58829-58801) A
<i>rbcL</i> _Z1375R	5'AAT TTG ATC TCC TTC CAT ATT TCG CA	(58994-58969) A
<i>rbcL</i> _3'R	5'CTT TTA GTA AAA GAT TGG GCC GAG	(59154-59131)
<i>ndhF</i> gene		
FORWARD		
<i>ndhF</i> _1F (-47)	5'AGG TAA GAT CCG GTG AAT CGG AAA C	(114344-114320) B, C
<i>ndhF</i> _15F (1)	5'ATG GAA CAG ACA TAT CAA TAY GSR TG	(114292-114267) B, C
<i>ndhF</i> _3F (396)	5'TAC TTC CAT GTT GGG ATT AGT TAG TAG	(113897-112871) B
<i>ndhF</i> _4F (590)	5'TTG GAT AAC GGG GAG TTT CGA ATT T	(113702-113678) B, C
<i>ndhF</i> _7F (1201)	5'AGG TAC ACT TTC TCT TTG CGG TAT TCC	(113093-113067) B, C
<i>ndhF</i> _7bF	5'AGG TAC ACT TTC TCT TTG YGG TAT TCC	(113093-113067)
<i>ndhF</i> _9F (1427)	5'TTC TAT TCA ATA TCT CTA TGG GGT	(112876-112853) B, C
<i>ndhF</i> _10F (1600)	5'ATC CTT ATG AAT CGG ATA ATA CTAT G	(112692-112667) B
<i>ndhF</i> _1738F (1658)	5'TTT GTT CGT TGG ATY YWT AGG AAT	(112634-112611)
REVERSE		
<i>ndhF</i> _8dR	5'GTA AAT AGA TCC GAA ACA TAT AAA ATG	(112939-112965)
<i>ndhF</i> _8R (1350R)	5'ATA GAT CCG ACA CAT ATA AAA TGC GGT TC	(112943-112970) B, C
<i>ndhF</i> _925R (953R)	5'CCT CTC TTA ATG TCT TTT TGA GCA AGA GCT	(113340-113369)
<i>ndhF</i> _2040R (1947R)	5'CTA TGT AAG CMC GAT TAT MYG ACC AA	(112306-112331)
<i>ndhF</i> _215 OR	5'CCA ACY CCA TTY GTA ATT CCA TCA AT	(112203-112228)
<i>ndhF</i> _2133R (209R)	5'CAG GAA CAA GAG GGA TCC ACC GAA	(111860-111883)
<i>ndhF</i> _2130R	5' CTA CTG ATT TGA TAC CCT CTC CTA	(112162-112185)
<i>matK</i> gene		
FORWARD		
<i>matK</i> _1Fb	5'TAT ATC CAC TTA TNT TTC AGG AGT	(2603-2580)
<i>matK</i> _5F	5'GGT ACG GAG TCA AAT KCT AGA AAA T	(2618-2594)



## Appendix B (continued)

Name of DNA marker and primer	Primer sequence from the 5' end	Primer position in tobacco (GenBank Z00044)
<i>matK_3F</i>	5'AAG ATG CCT CTT CTT TGC AT	(3141-3122) E
<i>matK_4F</i>	5'CTT CGC TAY TGG GTA AAA GAT GC	(3157-3135)
<i>matK_2F</i>	5'GTT CAC TAA TTG TGA AAC GT	(3495-3476) E
<i>matK_1F</i>	5'ACT GTA TCG CAC TAT GTA TCA	(3727-3707) E
REVERSE		
<i>matK_1R</i>	5'GAA CTA GTC GGA TGG AGT AG	(1834-1853)E
<i>matK_5R</i>	5'GCC AAA GTT CTA GCA CAA GAA ACT CG	(2325-2350)
<i>matK_3R</i>	5'GAT CCG CTG TGA TAA TGA GA	(2391-2410) E
<i>matK_6R</i>	5'TTC TAG MAT TTG ACT CCG TAC C	(2597-2618)
<i>matK_4R</i>	5'GCA TCT TTT ACC CAR TAG CGA AG	(3135-3157)
<i>trnT-F</i> intron and intergenic spacers		
FORWARD		
<i>trnT-F_aF</i>	5'CAT TAC AAA TGC GAT GCT CT	(48546-48565) F
<i>trnT-F_a1F</i>	5'ACA AAT GCG ATG CTC TAA CC	(48550-48469)
<i>trnT-F_rF</i>	5'GTT ATA ACT AAT GAG ACA TTC C	(48953-48974)
<i>trnT-F_cF</i>	5'CGA AAT CGG TAG ACG CTA CG	(49306-49325) F
<i>trnT-F_eF</i>	5'GGT TCA AGT CCC TCT ATC CC	(49862-49881)F
<i>trnT-F_jF</i>	5'GTT CTA ACA AAT GGA GTT GG	(49493-49512)
REVERSE		
<i>T-F_bR</i>	5'TCT ACC GAT TTC GCC ATA TC	(49318-49299) F
<i>trnT-F_iR</i>	5'CCA ACT CCA TTT GTT AGA AC	(49508-49495)
<i>trnT-F_dR</i>	5'GGG GAT AGA GGG ACT TGA AC	(49882-49863) F
<i>trnT-F_fR</i>	5'ATT TGA ACT GGT GAG ACG AG	(50299-50280) F
<i>trnV</i> intron (Complementary strand)		
FORWARD		
<i>trnV_1bF</i>	5'GAA CCG TAG ACC TTC TCG GTA AAA CAG ATC	(52772-53798)
<i>trnV_1F</i>	5'TAG GGC TAT ACG GAG TCG AAC CG	(53755-53777)
<i>trnV_3F</i>	5'GTG TAA ACG AGT TGC TCT ACC	(54361-54381)
<i>trnV_5F</i>	5'CAT ACG GCG GGA GTC ATT GGT TC	(54622-54644)
REVERSE		
<i>trnV_2R</i>	5'GGT AGA GCA ACT CGT TTA CAC	(54381-54361)
<i>trnV_4R</i>	5'GAA CCA ATG ACT CCC GCC GTA TG	(54644-54622)
<i>trnV_6bR</i>	5'GAA GAA ATG ACC TTA AAT CTT TGT G	(55290-55266)
<i>trnV_6R</i>	5'GGA GAG CAA TTT GAA GAA ATG	(55282-55302)
<i>rps16</i> intron		
FORWARD		
<i>rps16_F</i>	5'GTG GTA GAA AGC AAC GTG CGA CTT	(6187-6164) D
<i>rps16_2F</i>	5'GAA GGA CAC GAT CCG YTG TGG AT	(6162-6140)
REVERSE		
<i>rps16_2R</i>	5'TCG GGA TCG AAC ATC AAT TGC AAC	(5271-5294) D
<i>rps16_R3</i>	5'CGA TAG ACG GCT CAT TGG GAT A	(5299-5320)

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