

## A PRELIMINARY PHYLOGENY OF THE ‘DIDYMOCARPOID GESNERIACEAE’ BASED ON THREE MOLECULAR DATA SETS: INCONGRUENCE WITH AVAILABLE TRIBAL CLASSIFICATIONS<sup>1</sup>

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The ‘didymocarpoid Gesneriaceae’ (traditional subfam. Cyrtandroideae excluding Epithemateae) are the largest group of Old World Gesneriaceae, comprising 85 genera and 1800 species. We attempt to resolve their hitherto poorly understood generic relationships using three molecular markers on 145 species, of which 128 belong to didymocarpoid Gesneriaceae. Our analyses demonstrate that consistent topological relationships can be retrieved from data sets with missing data using subsamples and different combinations of gene sequences. We show that all available classifications in Old World Gesneriaceae are artificial and do not reflect natural relationships. At the base of the didymocarpoideae are grades of clades comprising isolated genera and small groups from Asia and Europe. These are followed by a clade comprising the African and Madagascan genera. The remaining clades represent the advanced Asiatic and Malesian genera. They include a major group with mostly twisted capsules. The much larger group of remaining genera comprises exclusively genera with straight capsules and the huge genus *Cyrtandra* with indehiscent fruits. Several genera such as *Briggsia*, *Henckelia*, and *Chirita* are not monophyletic; *Chirita* is even distributed throughout five clades. This degree of incongruence between molecular phylogenies, traditional classifications, and generic delimitations indicates the problems with classifications based on, sometimes a single, morphological characters.

**Key words:** *atpB-rbcL* spacer; Bayesian inference analysis; ITS; maximum parsimony; molecular phylogeny; Old World Gesneriaceae; taxonomy; *trnL-F* intron-spacer.

Gesneriaceae is a medium-sized family, comprising between 150 and 160 genera, and over 3200 species (Weber, 2004; Weber and Skog, 2007). The distribution is mainly tropical and subtropical, both in the Old and the New World, with minor outliers to the north (e.g., Pyrenees, Balkan peninsula, central and northern China) and to the south (southeastern Australia, New Caledonia, New Zealand, southern Chile).

While the neotropical Gesneriaceae (subfam. Gesnerioideae, including Coronanthereae, raised to subfamily rank by Wiehler,

1983) have received much attention by molecular systematists and apparently approach consolidation regarding a phylogenetic and formal classification (Smith, 1996, 2000; Smith et al., 1997a, b; Zimmer et al., 2002; Perret et al., 2003; Roalson et al., 2005a, b), the paleotropical Gesneriaceae (subfam. Cyrtandroideae, now Didymocarpoideae) lag behind. The last attempt at a formal (morphological) classification was by Burt and Wiehler (1995), who distinguished five tribes: (1) Cyrtandreae (with indehiscent, berry-like fruits); (2) Trichosporeae (with appendaged seeds); (3) Epithemateae (Klugiaeae and Loxonieae sensu Burt (1963), a group rather difficult to characterize, partly with peculiar anatomical characters such as secretory canals and medullary vascular bundles); (4) Didymocarpeae (the many remaining genera arranged alphabetically to express the wide lack of phylogenetic understanding); and Titanotricheae including the sole genus *Titanotrichum*. Additional tribes (Saintpaulieae, Ramondeae, Rhynchotecheae) have been recognized by Ivanina (1965a, b, 1967) and Wang et al. (1990, 1992).

Detailed morphological investigations (Weber, 1975–1988, as cited in Mayer et al., 2003) and, more recently, a molecular analysis based on cpDNA sequences (Mayer et al., 2003) showed that the Epithemateae form a distinct clade, sister to the remaining Old World Gesneriaceae. Both the morphological investigations and the molecular analyses led to a fairly good understanding of the phylogenetic relationships for six of seven genera of the ‘epithematoid Gesneriaceae’, as they are informally referred to in Weber (2004) and Weber and Skog (2007).

The present paper deals with the diverse non-epithematoid Old World Gesneriaceae, the ‘didymocarpoid Gesneriaceae’. A number of genera had already been included in the molecular

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analysis of Mayer et al. (2003), and preliminary results of the current study formed the basis of the informal classification presented by Weber (2004). In the meantime, the number of samples included in the molecular analysis has considerably increased, and sufficient results are available to make some headway in our understanding of relationships in this group, particularly with respect to previous subdivisions into tribes. Nonetheless, we abstain from a new formal classification at this point because a significant number of genera are not yet included in our analysis.

Effectively analyzing large data sets is challenging and complete analyses computationally intractable (e.g., Savolainen and Chase, 2003). Furthermore, sample-rich analyses including multiple data sets often suffer from missing data or issues of incongruencies between data sets (Sanderson and Shaffer, 2002). Here we address the issue of missing data by analyzing selected subsets of samples with complete data compared to a complete sample set with missing data, and demonstrate that "correct" topologies of phylogenetic trees can be recovered from largely incomplete data matrices.

## MATERIALS AND METHODS

**Plant materials**—Material for DNA extraction came in various forms and from diverse sources, including fresh leaves from research collections (E, HBV), silica-dried leaves of field collections, and herbarium specimens at various herbaria (Appendix 1).

**Outgroup choice**—To produce a reliable phylogeny of the family Gesneriaceae, we included members of major families of basal Lamiales (Oleaceae, Tetrachondraceae, Scrophulariaceae, Plantaginaceae s.l., Calceolariaceae s.s.) (Oxelman et al., 1999; Bremer et al., 2002; AGP II, 2003) in the analysis and used them as outgroups. The trees were rooted on Oleaceae (Olmstead et al., 2000; Tank et al., 2006). Details of outgroup taxa are given in Appendix 1.

**Ingroup taxa**—The samples of Gesneriaceae included 174 species in 78 genera. Their taxonomic classification followed Weber (2004). Twenty-three species were from gesnerioid Gesneriaceae covering 20 (of 53) genera, and six species belonged to coronantheroid Gesneriaceae (six of nine genera). A total of 17 species were sampled from epithematoid Gesneriaceae (six of seven genera); only the monotypic genus *Gyrogynne* W.T.Wang is missing. *Gyrogynne subaequifolia* W.T.Wang is only known from the type collection, and DNA extraction from the isotype failed. Finally, 128 species (from 46 of 78 genera) came from didymocarpid Gesneriaceae. The sample selection covered all tribes recognized by Burtt and Wiehler (1995, Epithemateae as 'Klugieae').

Speciose genera of didymocarpid Gesneriaceae were represented by a higher number of samples; *Aeschynanthus* Jack: five species of ca. 185, *Chirita* Buch.-Ham.: 15 of 80–140, *Cyrtandra* Forst.: six of (perhaps) 450–600, *Didymocarpus* Wall. s.s.: seven of ca. 70, *Henckelia*: five of ca. 180, *Paraboea*: 14 of 87, *Streptocarpus*: 10 of ca. 140.

In addition, *Jerdonia* Wight (monotypic with *J. indica* Wight) and *Titanotrichum* Solereder (monotypic with *T. oldhamii* Solereder) were included in the analysis, the former described in Weber (2004) with uncertain familial affiliations and the latter as excluded from Gesneriaceae. *Jerdonia* was originally described in Gesneriaceae (Wight, 1850), but referred to Scrophulariaceae by Burtt (1977). *Titanotrichum* was originally described in Scrophulariaceae, but recent molecular analyses suggest it to belong to Gesneriaceae, closely allied to New World taxa (Wang et al., 2004).

**DNA extraction, PCR, and direct sequencing**—Genomic DNA was extracted using a modified CTAB procedure (Doyle and Doyle, 1987, 1990) and/or the Qiagen DNeasy DNA Isolation Kit (Crawley, UK) following the manufacturer's protocol.

PCR amplification of the *trnL-F* intron-spacer region (*trnL-F*) and *atpB-rbcL* spacer (*atpB-rbcL*) followed Mayer et al. (2003). Some *trnL-F* and ITS sequences were obtained as follows: PCR amplification was performed using primers 'c' and 'f' (Taberlet et al., 1991) for *trnL-F* or primers 5P and 8P (Möller and Cronk, 1997) for ITS on an MJ Research PTC-200 DNA Engine

thermal cycler. The 50  $\mu$ L reactions contained 5  $\mu$ L  $10\times$   $\text{NH}_4$  reaction buffer (Bioline, UK), 5  $\mu$ L dNTPs (2 mM), 2.5  $\mu$ L  $\text{MgCl}_2$  (50 mM), 1.5  $\mu$ L of each primer (10  $\mu$ M), 32.25  $\mu$ L  $\text{dH}_2\text{O}$ , 1.25 U Biotaq polymerase (Bioline, UK) and 1.0  $\mu$ L DNA template DNA. The PCR thermocycle profile for *trnL-F* was: initial denaturation for 4 min at 94°C; followed by 30 cycles of 45 s at 94°C, 45 s at 55°C and 3 min at 72°C; with a final extension step for 10 min at 72°C. ITS amplifications were carried out using the PCR profile described in White et al. (1990) or Möller and Cronk (1997).

PCR products were run on 1% agarose gels to check for amplification success and quality. Amplified fragments were purified using QIAquick PCR purification kits (Qiagen, Crawley, UK) following the manufacturer's protocol, and sequenced using the dideoxy chain-termination method. Cycle sequencing was performed as in Mayer et al. (2003), or in 10  $\mu$ L reactions containing 4  $\mu$ L DTCS Quickstart mix (Beckman Coulter, High Wycombe, UK), 1  $\mu$ L primer (10  $\mu$ M), 3  $\mu$ L  $\text{dH}_2\text{O}$  and 2  $\mu$ L purified PCR product, under the following PCR conditions: 35 cycles of 96°C for 20 s, 50°C for 20 s and 60°C for 4 min. Sequencing primers were identical to those used for PCR with the addition that primer 'd' (Taberlet et al., 1991) was also used to sequence the *trnL* intron region. Sequencing *atpB-rbcL* followed Mayer et al. (2003). Sequencing PCR products were purified following the manufacturer's instructions, then run and analyzed on a CEQ 8000 Genetic Analysis System (Beckman Coulter, UK). Editing and assemblage of contigs was performed using the programs CESequence Investigator (Beckman Coulter, High Wycombe, UK) and Sequencher 4.5 (Gene Codes Corp., Ann Arbor, USA), and for *atpB-rbcL* as in Mayer et al. (2003). The cpDNA alignment matrices were assembled manually, based on those of Mayer et al. (2003). ITS sequences were initially aligned using the program CLUSTAL W (Larkin et al., 2007) and the alignment then adjusted manually, as described in Möller and Cronk (1997).

**Phylogenetic analysis**—For this study, 123 *trnL-F*, 59 *atpB-rbcL*, and 65 ITS sequences were newly acquired and submitted to GenBank; the remaining sequences were retrieved from GenBank (Appendix 1). Because a complete overlap of sequences across the three gene regions used could not be achieved, the data were analyzed in three sets:

(1) A combined *trnL-F* and *atpB-rbcL* matrix (cpDNA) that included 129 samples (59 *trnL-F* and 59 *atpB-rbcL* sequences were new, the rest from GenBank) (TreeBase: SN4215–20651). This complete plastid matrix included nine outgroup taxa; 22 gesnerioid, five coronantheroid, and 18 epithematoid samples; and a backbone set of 73 samples for didymocarpid taxa and *Jerdonia* and *Titanotrichum*. The main purpose for this analysis was to resolve out-/in-group relationships and the backbone topology for the Gesneriaceae family, specifically for the basal Asiatic and European taxa. The addition of the two samples of *Tetraphyllum* Griff ex C.B. Clarke, for which no *atpB-rbcL* data were available, did not alter the tree topology in any analysis, and were thus included in a final matrix of 131 samples.

(2) A complete, combined *trnL-F* and ITS data set with 88 didymocarpid samples (71 *trnL-F* and 65 ITS sequences were new, the rest from GenBank) (TreeBase: SN4215–20652). This analysis focused on resolving relationships among the advanced Asiatic and Malesian genera (Weber, 2004), with specific focus on testing the monophyly of members of the tribe Trichosporeae. This analysis was based on results of analysis 1, rooted on African and Madagascan samples. The ITS alignment at higher taxonomic level above these samples was found to be too ambiguous and the sampling too incomplete to be analyzed.

(3) A combined *trnL-F*, *atpB-rbcL*, and ITS data matrix with 201 samples across the family, including missing sequences (201 *trnL-F*, 129 *atpB-rbcL*, 88 ITS) (TreeBase: SN4215–20650). 123 *trnL-F*, 59 *atpB-rbcL* and 65 ITS sequences were new, the rest were from GenBank. The purpose for this analysis was to obtain a topology for all samples included in our study. The topology of major groups in this analysis was basically identical to those of the complete analyses 1 and 2.

The matrices were analyzed by maximum parsimony (MP) implemented in the program PAUP\* version 4.0b10 (Swofford, 2002) and by Bayesian Markov chain Monte Carlo (MCMC) inference (BI; Yang and Rannala, 1997) using the program MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001, 2007) on unordered and equally weighted characters.

Combinability of data sets was determined using the incongruence length difference (ILD) test of Farris et al. (1994, 1995), implemented in PAUP\* as the partition-homogeneity test, on 1000 replicates of repartitioning with tree-bisection-reconnection (TBR) on (Yuan et al., 2005).

In view of the high number of sequences included, MP starting trees were found using the parsimony ratchet (Nixon, 1999) implemented in the programs PAUPRat (Sikes and Lewis, 2001) and PAUP\*. Only the shortest of the 201

trees saved with PAUPRat were further optimized, with TBR and MulTrees activated, in PAUP\*. For analysis 3, the MaxTree option was set to 100000 trees. Descriptive tree statistics (ensemble consistency index [CI; Kluge and Farris, 1969], ensemble retention index [RI; Farris, 1989a], and ensemble rescaled consistency index [RC; Farris, 1989b]) were obtained using PAUP\*. Statistical branch support analyses were performed twofold, as 10000 replicates of heuristic bootstrap replicates (BS; Felsenstein, 1985) with TBR swapping on and MulTrees off (Spangler and Olmstead, 1999) in PAUP\* and as decay indices (DI; Bremer, 1988), derived from the program AutoDecay version 4.0.2 (Eriksson, 1999) and PAUP\* from 100 replicates of random addition.

Models and parameters priors for the BI analyses were obtained independently for each data set and gene sequence in each analysis using Modeltest (Posada and Crandall, 1998). In the higher level analysis 1 (with 131 samples), the model TVM+I+G was suggested by the Akaike information criterion (AIC; Akaike, 1974) for the *trnL-F* data, and TVM+G for the *atpB-rbcL* data. In analysis 2, the GTR+G model was selected for *trnL-F*, and GTR+I+G for ITS under the AIC. For analysis 3, the models GTR+I+G, TVM+G and GTR+I+G were selected for the *trnL-F*, *atpB-rbcL*, and ITS sequences, respectively.

For analyses 1 and 2, two million generations were run with four MCMC chains in two independent parallel analyses, with one tree sampled every 100 generations (10000 trees). The first 130000 generations or the first 1300 trees for the cpDNA analysis 1 and the first 60000 generations or 600 trees for analysis 2 were discarded as burn-in (generations prior to stationarity of likelihood values). For analysis 3, the number of generations was four million, and the burn-in 400000 generations. A majority rule consensus tree was constructed in PAUP\* from the remaining trees, combined from the two parallel analyses. Although not strictly comparable to bootstrap values (see also Fig. 4) the retrieved posterior probabilities (PP) indicate robustness of clades (Lewis, 2001; Alfaro et al., 2003; but see Cummings et al., 2003; Erixon et al., 2003). Majority rule consensus trees of individual BI runs were identical (except for two branches in analysis 3 with PP values of 0.5 and 0.53). A high correlation of the PP support values was found between the two parallel runs of the Bayesian analysis for all three data sets (Appendix S1, see Supplemental Data with online version of this article).

## RESULTS

**Matrix characteristics**—The combined cpDNA matrix (analysis 1) had 2525 aligned characters (*trnL-F*: 1278 characters, *atpB-rbcL*: 1247 characters), with 366 ambiguous characters excluded, resulting in 2159 characters used for analysis, of which 585 were constant, 176 variable but parsimony uninformative and 386 (33.6%) parsimony informative in the *trnL-F*, and 484, 211, and 317 (31.3%) respectively in the *atpB-rbcL* data.

The combined matrix for analysis 2 consisted of an alignment matrix of 2141 characters (*trnL-F*: 1278 characters; ITS: 863), with 276 ambiguous sites excluded, leaving 1865 characters in the analysis. Of these, 752 were constant, 209 variable but parsimony uninformative, and 186 (16.2%) parsimony informative for *trnL-F* and 248, 127, and 343 (47.8%), respectively, for ITS.

The combination of all three genes (analysis 3) resulted in a matrix of 3652 characters, of which 2877 unambiguously aligned characters were included in the analysis. Of these, 1251 were constant, 528 variable but parsimony uninformative, and 1098 (38.1%) parsimony informative.

**Analysis 1: Tree topology *trnL-F* and *atpB-rbcL***—The cpDNA strict consensus tree, based on 6912 most parsimonious trees of 2636 steps, showed a highly resolved topology with strong backbone support in most areas (Fig. 1). Bootstrap values ranged from 50% to 100% with 64 of 98 internal branches possessing support values of 90% or higher, and decay indices from 1 to 69. The Gesneriaceae were highly supported (BS = 100%; DI = 15) with Calceolariaceae its closest allied family (BS = 90%; DI = 3). A monophyletic coronantheroid clade (BS = 99%; DI = 7) nested within the gesneroid clade. This

clade also included the Old World (OW) genus *Titanotrichum* (BS = 82%; DI = 2). Sister to this group were the other OW samples, which formed a well-supported clade (BS = 96%; DI = 5). Within this clade, the epithematoid (BS = 100%; DI = 13) clade was sister to the remaining OW genera (BS = 98%; DI = 3). Among these, the hitherto unassigned genus *Jerdonia* split off first, followed by several grades and one polytomy for the basal Asiatic and European genera. The African and Madagascan genera formed a monophyletic sister clade (BS = 100%; DI = 11) to the advanced Asiatic and Malesian genera (BS = 65%; DI = 2). The resolution and support among those was not very high, but clades for individual genera were mostly well supported (BS = 98–100%; DI = 2–11), with the exception of samples of *Chirita*, which clustered in five different clades that were closely associated with other genera.

The two genera belonging to tribe Cyrtandreae sensu Burtt (1963) and Burtt and Wiehler (1995), *Cyrtandra* and *Rhyncho-techum* Blume, were resolved separately in distant positions, among the basal Asiatic and European genera (*Rhyncho-techum*) and among the advanced Asiatic and Malesian genera (*Cyrtandra*). Tribe Ramondeae sensu Wang et al. (1990, 1992) was also not monophyletic, with *Ramonda* among the basal Asiatic and European genera, and *Conandron* Siebold & Zucc. among the advanced Asiatic and Malesian genera.

The topology of the majority rule consensus tree of the BI analysis (Appendix S2, see online Supplemental Data) was very similar to the MP strict consensus tree, with only minor differences in terminal clade relationships, which were not highly supported in any analysis. The most obvious discrepancy was a sister rather than grade relationship of the European and part of basal Asiatic genera (including *Leptoboea* Benth., *Platystemma* Wall., *Rhyncho-techum* and *Boeica* C.B. Clarke) (Fig. 1, arrow).

**Analysis 2: Tree topology *trnL-F* and ITS**—The MP analysis yielded 28 most parsimonious trees of 3230 steps. The resulting strict consensus tree was highly resolved with 44 of 78 internal branches with 90% or higher bootstrap branch support and decay values between 1 and 36 (Fig. 2). The trees showed a strongly supported monophyletic ingroup (BS = 100%; DI = 29) of African and Madagascan genera, followed by a clade of mainly twisted-fruited advanced Asiatic and Malesian genera (*Boea* group) (BS = 99%; DI = 8). Among the remaining advanced Asiatic and Malesian genera, the tree topology was resolved but with low backbone support. Several subclades harboring several genera received high branch support (discussed later).

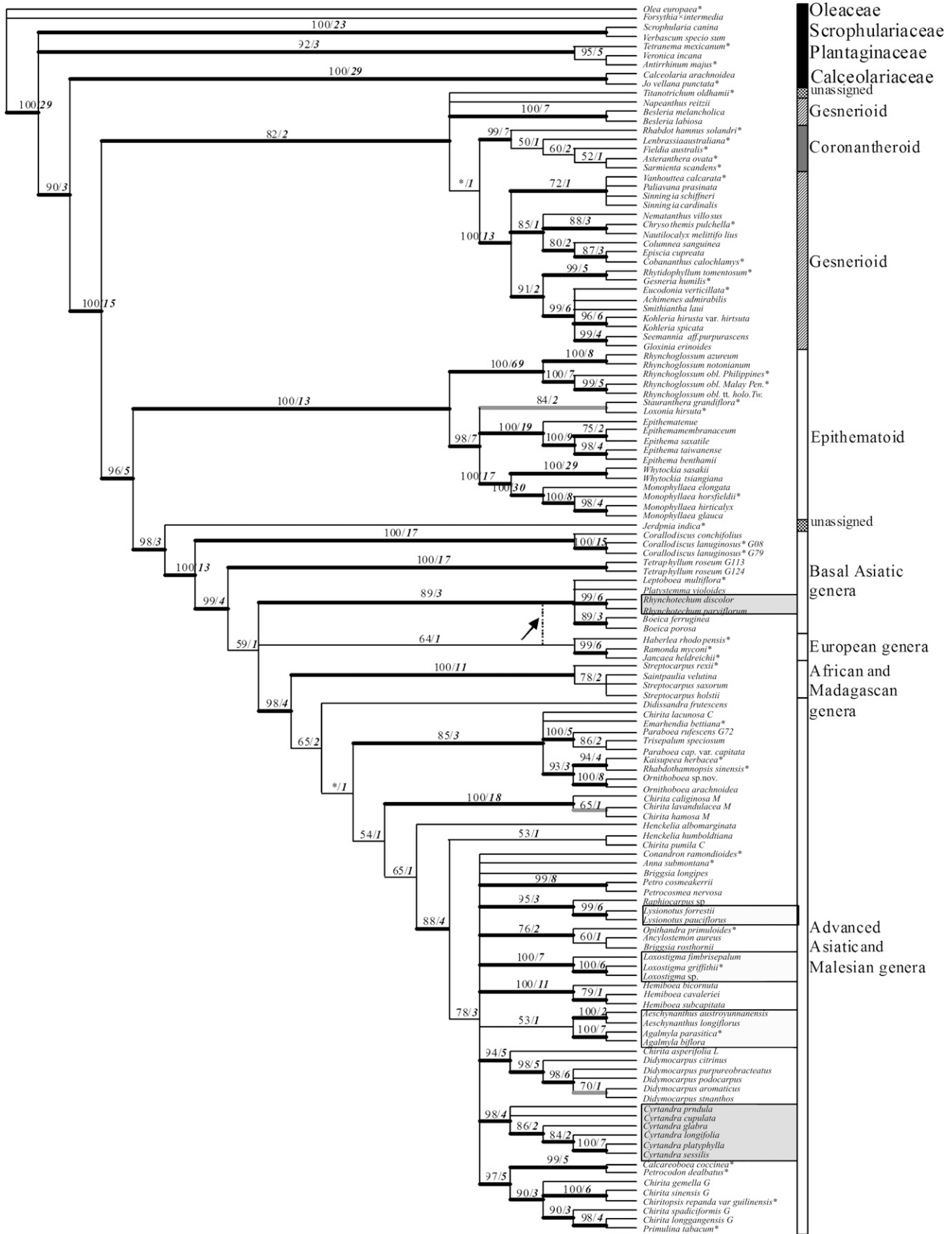
Of the four genera of tribe Trichosporeae sensu Burtt and Wiehler (1995) included in our analysis (*Aeschynanthus*, *Agalmyla* Blume, *Loxostigma* C.B. Clarke, and *Lysionotus* D. Don), the first three fell together in a polytomy (with *Loxostigma* and *Agalmyla* as sister genera), while *Lysionotus* appeared distant to these genera and more closely related to *Hemiboea* C.B. Clarke (Fig. 2).

The BI analysis majority rule consensus tree showed more resolution among the twisted-fruited genera (online Appendix S2), but less resolution for the backbone structure of the straight-fruited genera, though the branches involved generally received low branch support in the MP analysis for the latter.

**Analysis 3: Three-gene tree topology**—The MP analysis on all three-sequence matrices resulted in 18405 most parsimonious trees of 5655 steps (Fig. 3). The topology of the strict con-



Strict



sensus tree was identical to that in the respective areas of the two two-gene MP analyses 1 and 2. The southern hemisphere Coronantheroideae nested within the New World (NW) Gesnerioid genera and also included the OW *Titanotrichum* (BS = 82%; DI = 2). This clade was sister to the rest of the NW didymocarpoid genera (BS = 100%; DI = 15), with the Epithematoid (BS = 100%; DI = 13) sister to the rest (BS = 96%; DI = 5), followed by grades of *Jerdonia* (BS = 98%; DI = 3), *Corallodiscus* Batailin (BS = 100%; DI = 13), *Tetraphyllum* (BS = 99%; DI = 4), and a polytomy of the remaining basal Asiatic genera (BS = 92%; DI = 4) and the European genera (BS = 63%; DI = 1). Next followed the African and Madagascan genera, forming a strongly supported monophyletic clade (BS = 100%; DI = 14), with five genera, the African *Acanthonema* Hook f., *Saintpaulia* Wendl. and *Schizoboea* (K.Fritsch) B.L.Burtt, and the Madagascan *Colpogyne* B.L.Burtt and *Hovanella* A.Weber & B.L.Burtt nesting within the genus *Streptocarpus* Lindl..

Among the remaining advanced Asiatic and Malesian genera, the straight-fruited genus *Didissandra* C.B.Clarke appeared to split the clades of the African (BS = 100%; DI = 14) and Asian genera of the *Boea* group (BS = 85%; DI = 2). The latter including the genera *Boea* Comm. Ex Lam., *Emarhendia* Kiew, A.Weber & B.L.Burtt, *Kaisupeea* B.L.Burtt, *Ornithoboea* Parish ex C.B.Clarke, *Paraboea* Ridl., *Rhabdothamnopsis* Hemsl., and *Spelaeanthus* Kiew, A.Weber & B.L.Burtt. *Trisepalum* C.B.Clarke was found nested within a *Paraboea* clade (BS = 97%; DI = 5). One of the four Asiatic members of *Streptocarpus*, *S. orientalis* Craib included here, was also found in the *Boea* group, as well as the straight-fruited *Henckelia ericii* A.Weber (= *Loxocarpus holttumii* M.R.Hen.) and *Chirita lacunosa* (Hook f.) B.L.Burtt.

The remaining advanced Asiatic and Malesian genera with straight fruits formed a weakly supported clade (BS = 62%; DI = 2) with fairly well-resolved relationships, but with low or no internal branch support. A highly supported clade (BS = 100%; DI = 16) of *Chirita* species of section *Microchirita* was sister to the remaining samples, followed by a clade of two SE Asian *Henckelia* Spreng. species (BS = 99%; DI = 6) and a mixed clade of *Chirita* and *Henckelia* from China and Sri Lanka (BS = 70%; DI = 2).

The remainder of the samples fell into two large clades, but with little backbone support, one including the genera *Anna* Pellegr., *Briggsia* Craib, *Calcareaoboea* C.Y.Wu, *Chirita*, *Chiritopsis* W.T.Wang, *Hemiboea*, *Lysionotus*, *Petrocodon* Hance, *Petrocosmea* Oliv., *Primulina* Hance, *Raphiocarpus* Chun and *Ridleyandra* A.Weber & B.L.Burtt. In this clade, *Primulina* was sister to *Chirita longgangensis* W.T.Wang (BS = 96%; DI = 5), nested deep inside a *Chirita* section *Gibbosaccus* clade (BS = 73%; DI = 1). *Chiritopsis* also nested within this clade (BS = 97%; DI = 2).

The second large clade included *Aeschynanthus*, *Agalmyla*, *Ancylostemon* Craib, *Briggsia*, *Chirita asperifolia* (Blume) B.L.Burtt, *Conandron*, *Cyrtandra*, *Didymocarpus*, *Loxostigma*, *Opithandra* B.L.Burtt, and *Oreocharis* Benth. *Chirita asperifolia* (the type species of *Chirita* section *Liebigia*, reestablished

by Hilliard, 2004), was sister to *Didymocarpus* (BS = 92%; DI = 10).

The four samples of *Briggsia* included here did not form a monophyletic group and the two sister pairs fell into different clades, one closely allied to *Ancylostemon*, *Opithandra* and *Oreocharis* (BS = 96%; DI = 3), the other sister to a group of genera including *Raphiocarpus* sp., *Anna*, *Lysionotus*, and *Hemiboea*, but with little branch support.

The genus *Chirita* was highly polyphyletic, falling in four places among the straight-fruited, advanced Asiatic and Malesian genera and once in the *Boea* group.

The BI analysis was largely congruent with the MP analysis for strongly supported branches but differed in areas that received low or no BS support in the MP analysis (Fig. 3; online Appendix S2).

## DISCUSSION

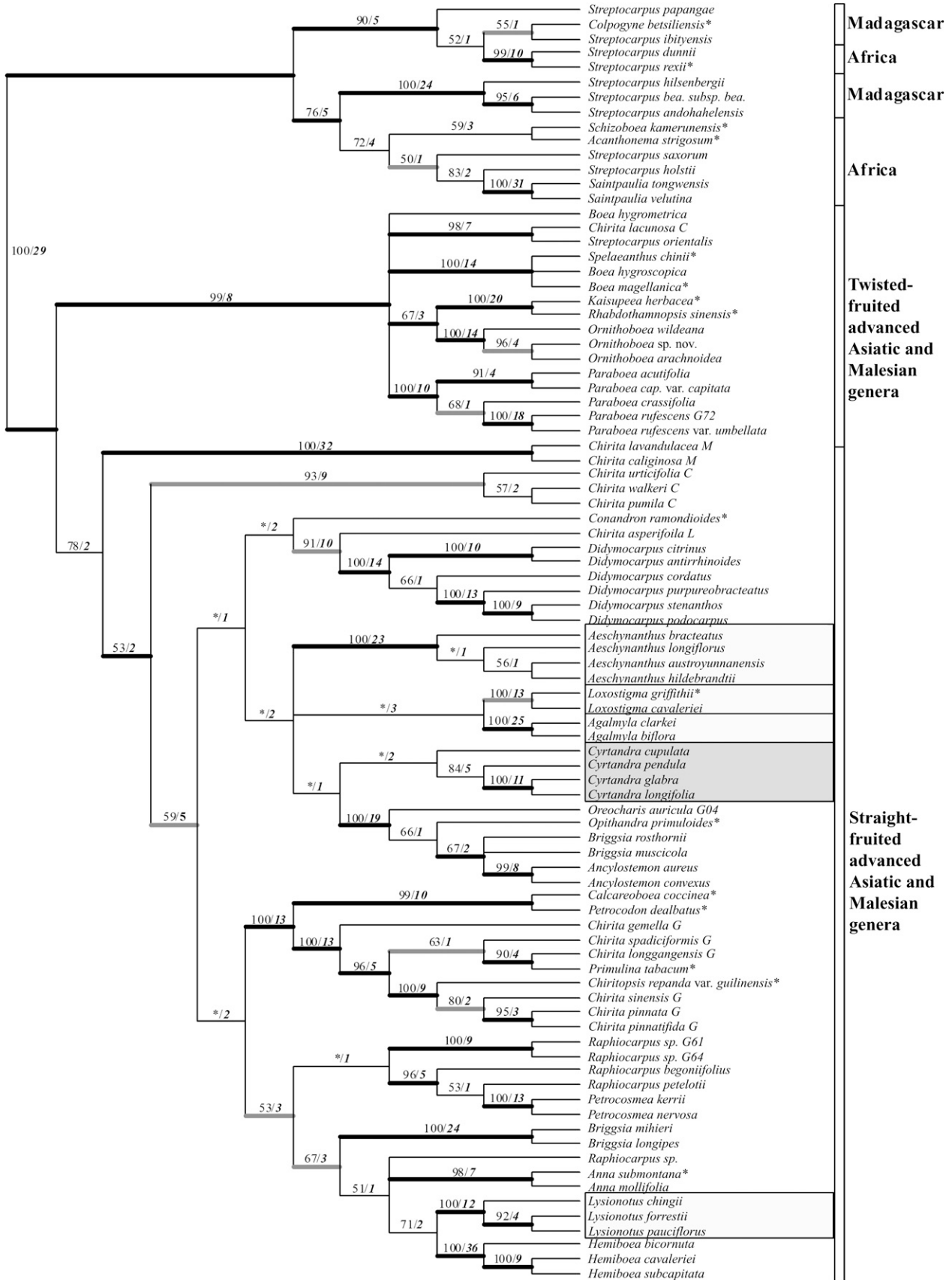
**Phylogenetic analysis**—Our MP analyses on 190 Gesneriaceae samples resulted in well-resolved and stable topologies across the complete two-gene analyses (analyses 1 and 2), and gave a highly resolved strict consensus tree in the three-gene analysis (analysis 3), irrespective of missing sequences for some samples in the latter. This finding is in line with previous studies that suggested that even highly incomplete matrices can yield accurate phylogenetic topologies (Qiu et al., 1999; Murphy et al., 2001; Kearney, 2002; Wiens, 2003, 2006), and the effects of missing sequences can be minor (Wiens and Reeder, 1995). In fact, in our three-gene analysis, the more conserved cpDNA data supported the backbone of the trees, particularly at the higher taxonomic level, while the faster evolving ITS sequences resolved and supported relationships at lower taxonomic levels. This complementarity has previously been observed in other data sets (Qiu et al., 1999; Long et al., 2000; Sinclair et al., 2002). Furthermore, undesired effects such as long-branch attraction (Felsenstein, 1978), suspected to be problematic in combining incomplete data sets (Wiens, 2006) were not found in our analyses, as seen by the high similarity of topologies between analyses using different gene combinations.

The ILD test for combinability of our matrices showed some incongruence between the data sets, but the probability values were above the  $P = 0.05$  threshold. The applicability of the ILD test as a determinant of combinability of different data sets has been discussed controversially, with Cunningham (1997) being a proponent, while others opposed its use (Barker and Lutzoni, 2002; Hipp et al., 2004). However, the consistencies of our tree topologies across the three analyses suggest that the hierarchy retrieved from the three combined genes reflects reasonably well the relationships of the genera included here.

Controversy surrounds the interpretation of posterior probabilities (PP) and their comparison to other branch support values (Wilcox et al., 2002; Suzuki et al., 2002; Cummings et al., 2003; Erixon et al., 2003). We investigated this issue by plotting the bootstrap against PPs (Fig. 4), and it becomes clear that

←  
Fig. 1. Strict MP consensus tree of 6912 most parsimonious trees of 2636 steps based on combined *trnL-F* and *atpB-rbcL* sequences of 131 samples (CI = 0.62, RI = 0.84, RC = 0.52). Bootstrap values and decay indices (bold and italics) are given above branches. Light gray boxes = Trichosporeae, dark gray boxes = Cyrtandreae; systematics (right side) follows Weber 2004; black bars = outgroups, hatched bars = gesnerioid, lined bars = coronantheroid, open bars = didymocarpoid, crosshatched = unassigned. Branches in bold are supported by posterior probabilities of 1.00 (black) or 0.99 (gray) in the Bayesian analysis. Asterisks indicate generic types. Arrow indicates a relationship found in the Bayesian analysis.

Strict



the PP values were over most parts significantly higher than bootstrap values, with branches receiving BS values as little as 51% having PPs of 0.99 or 1.00. Thus, while Bayesian inference analysis here often results in more resolved trees with high PP values, these high PP values do not necessarily reflect strong robustness of the BI tree topologies, irrespective of missing data (see Fig. 4E for data set 2 without missing data, and Fig. 4F for data set 3 with missing data). Our interpretations consequently rely predominantly on the more conservative MP topology and its branch support values.

We observed only few differences between the MP and BI analyses with some bearing on our main objectives relating to the monophyly of current tribes; one concerns the relationships between the European and part of the basal Asiatic genera, the other involves a sister relationship (BI) of *Aeschynanthus* and *Agalmyla* as opposed to such a relationship of the latter to *Loxostigma* (MP). In neither case is monophyly of the tribe Trichosporeae supported (discussed later), and thus these incongruities have no effects on the interpretation of our data and the conclusions drawn.

Highly supported relationships among the straight-fruited, advanced Asiatic and Malesian genera remained elusive, but it must be noted that over 30 didymocarpoid genera (most are likely to belong to this group) have not been included in our analysis yet. Their addition may well stabilize the tree topology in this area. It is not lack of sequence variation but lack of topology congruencies and synapomorphies (shared evolved characters) that causes the problem as inferable from individual phylograms (online Appendix S3).

It was interesting to see that the distribution of branch support was greatly partitioned across the tree. While intergeneric branches received low or medium support, branches leading to genera were highly supported (Table 1). The latter indicates that the genera included (except nonmonophyletic genera) represented strongly defined genetic entities. Nonetheless, further data are required to better resolve the relationships of the didymocarpoid genera.

**Tribal classification of Old World Gesneriaceae**—The traditional family classifications of Gesneriaceae are those of Bentham (1876) and Fritsch (1893–1894). The latter represents the most detailed classification available (2 subfamilies, 18 tribes, 9 subtribes). Fritsch's (1893–1894) classification was largely based on formal morphological characters, and many insufficiencies became apparent on closer inspection. Burt (1954) even qualified Fritsch's classification as "negative in quality" and a retrogression. In the Old World Gesneriaceae subfam. Cyrtandroideae, Burt reduced the number of tribes to five: Cyrtandreae, Trichosporeae, Klugieae, Loxonieae, and Didymocarpeae. Later, based on morphological data of Weber (1975–1988, cited in Mayer et al., 2003), he united Klugieae and Loxonieae into a single tribe (informally in Burt, 1977, formally in Burt and Wiehler, 1995), which is presently known as Epithemateae (Burt, 1997). Apart from Didymocarpeae, each of the tribes contained only a small number of genera. The strong asymmetry in species number was paralleled by the dif-

ficulties in recognizing generic relationships. Burt and Wiehler (1995) thus listed the genera of Didymocarpeae simply in alphabetical order.

The five-tribe classification of OW Gesneriaceae of Burt and Wiehler (1995) is reflected in our analyses only to a very low degree. Though morphologically not easy to define, the Epithemateae are a well-supported monophyletic group, which is sister to the remaining Old World genera (Mayer et al., 2003; Wang et al., 2004). The tribes Cyrtandreae and Trichosporeae were supported in a morphological cladistic analysis of Smith (1996), but not by molecular investigations (Smith et al., 1997a, b). In principal agreement with the latter data, we found both tribes nested within tribe Didymocarpeae sensu Burt and Wiehler (1995). Furthermore, genera from neither tribe Cyrtandreae or Trichosporeae formed monophyletic groups, suggesting that the morphological features considered characteristics at tribal level (Cyrtandreae with indehiscent fruits and Trichosporeae with seed appendages) have repeatedly and independently evolved.

Tribe Cyrtandreae included three genera in Burt and Wiehler (1995): *Cyrtandra*, *Sepikea* Schltr., and *Rhynchotechum*. Because the monospecific *Sepikea* is probably just an abnormally tetrandrous *Cyrtandra* (perhaps even based on a misobservation, Burt, 1998), only *Cyrtandra* and *Rhynchotechum* remain. According to our data, *Rhynchotechum* has its place among other basal Asiatic didymocarpooids, while *Cyrtandra* occupies a position within the advanced Asiatic and Malesian didymocarpooids. Therefore, there is no basis for retaining the tribe.

Tribe Trichosporeae was thought to contain six genera in Burt and Wiehler (1995). From these, two genera (*Oxychlamys* Schltr., Van Royen, 1983; *Micraeschynanthus* Ridl., Middleton, 2007) have been sunk into synonymy, thus leaving the four remaining genera *Aeschynanthus*, *Agalmyla*, *Loxostigma*, and *Lysionotus*. All these were included in our analysis. Some (but not all) of our trees suggest a closer, though unsupported, affinity between the genera *Agalmyla*, *Loxostigma*, and to some degree *Aeschynanthus*, but *Lysionotus* was always more closely associated with *Hemiboea* than with any other genus of Trichosporeae. Both the position within the advanced Asiatic and Malesian genera and the partial nonaffinity of the genera require disbandment of the Trichosporeae.

**Other tribes used in recent classifications**—Early definitions of most tribes are problematic, but some have been revived, redefined, or newly established in recent work. These are discussed here in some detail.

**Ramondeae (Fritsch, 1893–1894)**—Originally, this tribe included the European genera (*Ramonda* Rich., *Jancaea* Boiss., *Haberlea* Pohl ex Barker), *Corallodiscus*, and *Petrocosmea* from Asia, and *Saintpaulia* from Africa. The key character for defining this group is straight fruits with septicidal dehiscence. Wang et al. (1990) redefined this tribe to include all OW genera with actinomorphic flowers (*Ramonda*, *Thamnocharis* W.T. Wang, *Tengia* Chun, *Bournea* Oliv., and *Conandron*). Neither definition

← Fig. 2. Strict MP consensus tree of 29 most parsimonious trees of 3275 steps based on combined ITS and *trnL-F* sequences of 89 sampled of advanced Asiatic genera rooted on African genera (CI = 0.43, RI = 0.63, RC = 0.27). Bootstrap values and decay indices (bold and italics) are given above branches. Light gray boxes = Trichosporeae, dark gray boxes = Cyrtandreae; systematics (right side) follows Weber 2004; open bars = didymocarpoid. Branches in bold are supported by posterior probabilities of 1.00 (black) or 0.99 (gray) in the Bayesian analysis. Asterisks indicate generic types.









of the tribe is supported in our analyses (see also Möller et al., 1999).

**Saintpaulieae (Ivanina, 1965b)**—This tribe included the straight-fruited African genera *Saintpaulia*, *Carolofritschia* Engl. (now *Acanthonema*: Burt, 1982), *Acanthonema*, and *Linnæopsis* Engl. (now *Streptocarpus*: Darbyshire, 2006). Our data show that the genera included in our analysis do not form a coherent group (Figs. 2, 3), but are scattered among *Streptocarpus* (see also Möller and Cronk, 1997, 2001a, b).

**Streptocarpeae (Fritsch, 1893–1894)**—This tribe (with *Phylloboea* Benth., *Boea*, *Ornithoboea*, and *Streptocarpus*) was reduced to subtribe Streptocarpeae of tribe Didymocarpeae by Ivanina (1965b) and expanded to include also *Paraboea*, *Rhabdothamnopsis*, and *Trisepalum*. All, except most *Streptocarpus* species, are distributed in Asia and indeed form a coherent alliance in our analyses: the *Boea* group. The majority of *Streptocarpus* species, as currently circumscribed, occur in Africa, Madagascar, and the Comoro Islands (~140 species Hilliard and Burt, 1971), but four species have been described from Asia: *S. burmanicus* Craib (Burma), *S. orientalis* (Thailand), *S. sumatranus* B.L.Burt (Sumatra), and *S. clarkeanus* (Hemsl.) Hilliard & B.L.Burt (China) (cf. Hilliard and Burt, 1971). At first sight, the occurrence of *Streptocarpus* species in Asia seems to support the idea of a transcontinental distribution of a group comprising *Streptocarpus* and the Asiatic genera with twisted fruits. However, Hilliard and Burt (1971) have doubted that the Asiatic species of *Streptocarpus* are placed in the correct genus. They placed these in the genus with considerable reservations because of the lack of distinguishing morphological features. Indeed, one of them, *S. clarkeanus*, has been recently transferred to *Boea* by Wang et al. (1990). Another, *S. orientalis*, was examined molecularly in the current study and cytologically by M. Kiehn (University of Vienna, unpublished data). Both the molecular and the cytological data ( $2n = 18$ ) indicated that there is no relationship between the Asian *S. orientalis* and the African and Madagascan species. We believe that it is simply a question of time before the remaining Asiatic species follow suit.

The African and Madagascan species of *Streptocarpus* plus the straight-fruited compatriots formed a highly supported monophyletic clade in our analyses. If retained in a future classification, tribe or subtribe Streptocarpeae (-inae) will most probably form a group comprising exclusively African and Madagascan genera and species.

**Rhynchotecheae (Ivanina, 1965a)**—This tribe included *Rhynchotechum* and *Isanthera* Nees (included in *Rhynchotechum* by Burt 1962a). Ivanina indeed was right to separate *Rhynchotechum* from the Cyrtandreae, but she still thought there would be a close relationship between the two tribes (Ivanina, 1965b: fig. 14). As shown here, this is not the case. Therefore, the indehiscent fruit characterizing *Cyrtandra* and *Rhynchotechum* has no taxonomic bearing. It is simply a convergence.

**Indication of para/polyphyly of some genera**—Our data indicate that a number of genera are artificial and polyphyletic: *Streptocarpus* (see discussion above), *Chirita*, *Henckelia*, *Briggsia*, and possibly *Paraboea*, *Boea*, and *Raphiocarpus*.

**Chirita**—Species of this large genus (see revisions of Wood, 1974, and Wang, 1985a, b) appear distributed over at least five clades in our analysis. Some conform to current sections (sects. *Chirita*, *Microchirita*, *Gibbosaccus*, *Liebigia*), but others do not (*C. lacunosa*). Because of its straight fruits, *C. lacunosa* (S Thailand, NW Peninsular Malaysia) seems misplaced among the predominantly twisted-fruited *Boea* group. But it must be remembered that some species of *Paraboea* also possess straight fruits (similar to non-*Streptocarpus* taxa among the African and Madagascan taxa), suggesting that loss of fruit twist can occur frequently. The polyphyly of *Chirita* species over different parts of the didymocarpoid Gesneriaceae, however, is not erratic, but strongly indicates the artificial nature of the genus and requires a thorough (re) examination and (re)definition of the entire genus and its future segregates.

**Henckelia**—This genus was reestablished when splitting the large genus *Didymocarpus* into smaller and more natural entities (Weber and Burt, 1998b). As to the present data, the split of *Didymocarpus* proves more than justified. Neither ‘African’ nor ‘South Indian’ nor ‘Malesian *Didymocarpus*’ (as discussed in Weber and Burt, 1998b) are parts of, or closely allied to ‘*Didymocarpus* s.s.’, which has its center of distribution in the Himalaya region, but a possible Malay Peninsula origin (Palee et al., 2006). Because ‘South Indian’ and ‘Malesian’ *Didymocarpus* as well as *Loxocarpus* R.Br. appeared somewhat linked, the genus *Henckelia* was revived and used in an inclusive sense for their accommodation (Weber and Burt, 1998b). The present data give a first indication, though, that *Henckelia* is not monophyletic.

**Briggsia**—This genus has over 20 species, ranging from the Himalayas to Vietnam. Species number, the wide distribution, and the wide range of morphologies (including rosette and caulescent habits, differences in indumentum and anther morphology) allow the prediction that this rather variable taxon is not monophyletic. Therefore, it is not too surprising that our molecular data confirm this prediction.

**Paraboea**—This genus appears to be paraphyletic, with *Trisepalum* sharing the polytomy with *Paraboea* clades and species in our analysis 3 (Fig. 3). The close relationship of *Paraboea* and *Trisepalum* is undisputed because both have the characteristic arachnoid indumentum.

**Boea**—The species analyzed formed two clades in analysis 3, with *Spelaeanthus* seemingly linked to the Australian / Papua New Guinean *Boea* species in one strongly supported clade, the other in a basal polytomy of the *Boea* group. However, more

← Fig. 3. Strict MP consensus tree of 10977 most parsimonious trees of 5694 steps based on combined ITS, *trnL-F* and *atpB-rbcL* sequences of 202 samples (CI = 0.47, RI = 0.75, RC = 0.35). Bootstrap values and decay indices (bold and italics) are given above branches. Light gray boxes = Trichosporeae, dark gray boxes = Cyrtandreae; black bars = outgroups, hatched bars = gesneroid, lined bars = coronantheroid, open bars = didymocarpoid; numbers in bars = number of anthers; p, posterior pair, rest with two anterior anthers. Branches in bold are supported by posterior probabilities of 1.00 (black) or 0.99 (gray) in the Bayesian analysis. Asterisks indicate generic types. Arrows indicate relationship suggested in the Bayesian analysis.

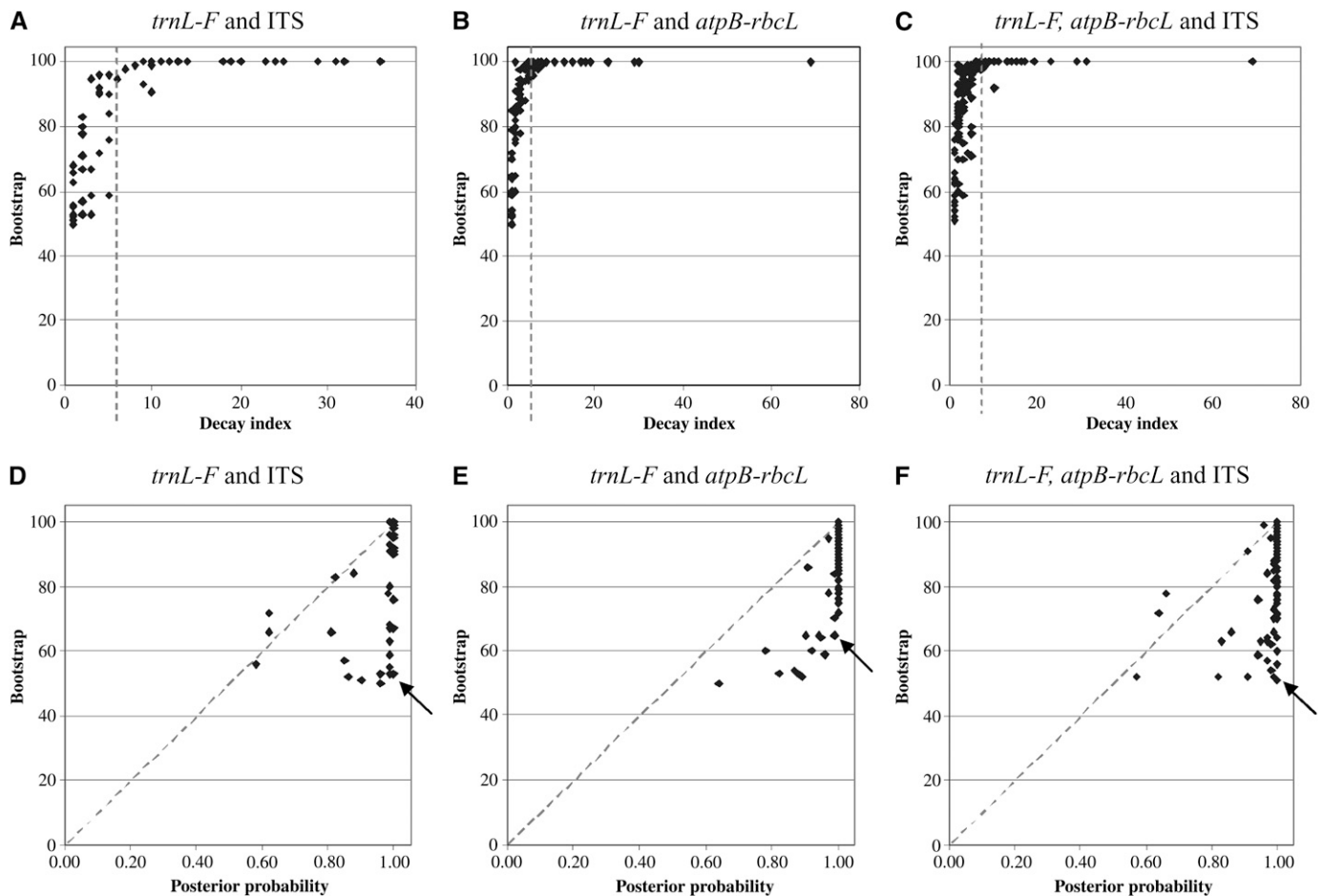


Fig. 4. Plots of branch support values, (A–C) bootstrap vs. decay index and (D–F) posterior probabilities of (A, D) the combined *trnL-F* and ITS; (B, E) the *trnL-F* and *atpB-rbcL*, and (C, F) the *trnL-F*, *atpB-rbcL*, and ITS data sets (bootstrap values >50% included only). Only decay values >5 receive constantly >90% bootstrap values (vertical dotted red lines, top). In the bottom graphs, the diagonal line indicates the hypothetical 1 : 1 relationship between bootstrap and posterior probabilities. However, in the *trnL-F* and ITS analysis, branches with bootstrap values as low as 51% receive PP values of 1.0 (arrow), for the *trnL-F* and *atpB-rbcL* dataset bootstrap values as low as 65%, and for the three-gene analysis branches with bootstrap values as low as 51% receive PP values of 0.99 (arrows), illustrating the overoptimism of PP values.

species need to be included and missing data completed in a future analysis to elucidate the exact relationships in the *Boea* group as a whole.

*Raphiocarpus*—This genus, revived by Weber and Burt (1998a) for the accommodation of a number of Sino-Vietnamese species of *Didissandra*, was noted as potentially artificial even at its inception. Moreover, the identity of the *Raphiocarpus* sp. samples included in our analysis cannot be ascertained at present; these may well be misidentified. Thus, a more detailed analysis is needed for clarification here.

*Toward a new classification*—As discussed, none of the available classifications and definition of tribes agree with the data presented here. Obviously, a revised classification needs to be established. In view of the many isolated genera and cascades of clades (= grades), a formal classification will prove difficult at this point. In the present paper, we do not attempt to establish such a formal classification, particularly because the position of a considerable number of genera (over 30) is still unknown or uncertain. Such a revised classification would also

require a detailed morphological discussion of many groups which cannot be presented here.

Informally, the didymocarpoid Gesneriaceae can be divided into three groups: (1) the ‘basal Asiatic and European group’; (2) the ‘African and Madagascar group’; (3) the ‘advanced Asiatic and Malesian group’.

Our phylogenetic results support that the phylogeography and distribution patterns form an essential component in the understanding of the evolutionary diversification of the didymocarpoid Gesneriaceae. Now we have to discuss whether these groupings make sense in morphological respects.

‘*Basal Asiatic and European group*’—This group comprises a number of mono- or oligogeneric clades that form grades or polytomies at the base of the didymocarpoid Gesneriaceae clade. It is not a single taxonomic entity.

Their morphology is varied, some are flat-rosette plants (*Jerdonia*, *Corallo-discus*, *Ramonda*, *Haberlea*, *Jancaea*), some are tall, shrubby (lignescant) plants with decussate (rarely alternate) leaves (*Tetraphyllum*, *Leptoboaea*, *Boeica*, *Rhyncho-techum*), and one (*Platystemma*) is a unifoliate herb. Floral form includes



TABLE 1. Comparison of different branch support values for branches between genera (intergeneric), branches supporting genera (generic, except nonmonophyletic genera) and within genera (intrageneric) for the three-gene analysis of combined *trnL-F*, *atpb-rbcL*, and ITS data.

Index	Branch support values		
	Intergeneric	Generic	Intrageneric
Decay index	3.57	<b>10.12</b>	3.84
SE	0.40	2.16	0.45
N	82	33	38
Bootstrap	82.78	<b>94.85</b>	88.28
SE	2.04	1.91	2.19
Branches <50% support	18	0	2
N	64	33	36
Posterior probability	0.96	<b>0.99</b>	0.97
SE	0.01	0.01	0.01
Branches <0.5 support	13	1	1
N	69	32	37

Notes: SE = standard error, N = number of branches.

a full range from strongly zygomorphic with a distinct tube (*Corallodiscus*) through slightly zygomorphic with a short, broad tube ('campanulate' = e.g., *Jancaea*, *Rhynchotechum*) to (sub)actinomorphic and flat-faced with very short tube ('saintpaulioid' = *Ramonda*, *Platystemma*). Thus, morphologically, there is little evidence that the basal Asiatic and European Gesneriaceae belong together or that they are particularly primitive. However, there are morphological features that are characteristic for the group and (at least partly) can be qualified as plesiomorphic: (1) Presence of four fertile stamens (tetrandry). Tetrandry is characteristic of the coronantheroid, gesnerioid, and epithematoid (except for *Epithema* Blume and Asian *Rhynchoglossum* Blume, which possess two fertile anthers in posterior positions, unlike all other diandrous didymocarpoid taxa where the anthers are in anterior position. This character state difference for taxa with two stamens was also overlooked by Smith, 1996) and is clearly ancestral within the family (Fig. 3). The presence of four stamens is also characteristic for the European group (except *Ramonda* with five). The African and Madagascan group (except for *Acanthonema*) and the twisted-fruited advanced Asiatic and Malesian group stamen number is always two, the derived state, while in the straight-fruited advanced Asiatic and Malesian group both tetrandrous and diandrous genera/groups occur. (2) Straight capsules with septicidal dehiscence (sometimes combined with loculicidal dehiscence). This type of fruit opening has already been regarded as primitive by Fritsch (1893–1894). This assumption was merely for morphological reasons (dehiscence along the lines of carpel fusion), but can be supported by the fact that it also occurs in presumed basal members of the New World coronantheroid Gesneriaceae (*Coronanthera* Vieill. ex C.B. Clarke, *Depanthus* S. Moore; combined with loculicidal dehiscence in *Negria* F. Muell. and *Rhabdothamnus* A. Cunn.) and in the basal gesnerioid tribe Beslerieae (*Anetanthus* Hiern ex Benth. & Hook f. and probably others). (3) Seed cell ornamentation. As can be concluded from the work of Beaufort-Murphy (1983) and personal observations of the last author (A.W.), the genera of the basal Asiatic and European group have seeds without testa cell ornamentation. Reticulate and striate seeds, without ornamentation on the cell surfaces, are also characteristic of the coronantheroid, gesnerioid, and epithematoid Gesneriaceae and are thus considered

plesiomorphic. These groups also share a twisted testa cell arrangement. Ornamented seeds are found frequently in the African and Madagascan group as well as in the advanced Asiatic and Malesian group and are therefore derived. Thus, the basal Asiatic and European genera with plesiomorphic unornamented seeds and straight testa cell arrangement form a perfect transition to the higher groups of didymocarpoid Gesneriaceae.

'*African and Madagascan group*'—While in earlier classifications the African and Madagascan genera appeared scattered over several tribes, Burt suggested on many occasions (e.g., in Hilliard and Burt, 1971) that they are closely related. In all our phylogenetic trees, they indeed formed a well-supported clade and can be considered a coherent taxonomic group. Despite the huge variation in vegetative and floral characters (even in the single genus *Streptocarpus* in the restricted sense), they are tied together by at least three synapomorphies: (1) Diandry. (2) Twisted fruits, with loculicidal dehiscence; the most parsimonious interpretation of the straight capsules found in *Acanthonema*, *Colpogyne*, *Hovanella*, *Saintpaulia*, and *Schizoboea* suggests that they are independent reversals to the ancestral state in the family. (3) Ornamented seeds with verruculose surface pattern (but with a reversal to the ancestral, reticulate state in most derived taxa in *Streptocarpus* subg. *Streptocarpus*). Before taxonomic changes can be made concerning this group, the status of the Asian *Streptocarpus* species needs to be addressed (i.e., their unrelatedness to African *Streptocarpus* species demonstrated).

*Advanced Asiatic and Malesian group*—This group is the largest of the three groups. It needs detailed discussion, which is postponed to a future, more complete analysis. This group comprises, *inter alia*, the well-known and species-rich genera *Didymocarpus*, *Aeschynanthus*, and *Cyrtandra*. These genera, the leading genera of different tribes in former classifications, thus prove surprisingly closely related. Their characteristic features, including the appendaged seeds of *Aeschynanthus* and the indehiscent fruits of *Cyrtandra*, are obviously convergences, characterizing small groups or single genera, but have no major classificatory significance. Like the basal Asiatic and European group, it consists of a number of larger clades, starting with *Didissandra* (sensu Weber and Burt, 1998a). This genus has tetrandrous flowers, capsules of a very special type (tardily loculicidally dehiscent, with the valves finally disintegrating along the sclerified vascular bundles (see Weber and Burt, 1998a), and seeds with mostly knobby ornaments along the testa cell margins (see Sontag and Weber, 1998).

A well-supported major clade includes the genera with predominantly twisted capsules, the *Boea* group (*Boea*, *Emarhenda*, *Kaisupeea*, *Ornithoboea*, *Paraboea* p.p., *Rhabdothamnopsis*, *Trisepalum*; only *Senyumia* Kiew, A. Weber & B.L. Burt has not been included in the analysis, but probably has also its place here), but scattered within this group are also species with straight fruits (*Paraboea* p.p., *Chirita lacunosa*, *Henckelia ericii*). This group will be discussed in more detail in a forthcoming paper.

The remainder of clades in the advanced Asiatic and Malesian group are not strongly supported, though many monophyletic genera are (Fig. 3), and the intergeneric relationships of this group will be addressed comprehensively in another publication.

*Phytogeography*—It is still enigmatic in which part of the world Gesneriaceae originated. Burt (1998) proposed the hypothesis that the family is of southern hemisphere (Gondwana) origin, with the coronantheroid Gesneriaceae representing a sur-

vivors of the oldest group. This gave rise both to the gesnerioid Gesneriaceae (invading South America via the Antarctic and southern South America) and the didymocarpoid Gesneriaceae (by migrating northwards, ‘dropping’ representatives in Africa and Madagascar and finally reaching the Eurasiatic continent and spreading from there to the Sunda Islands and the Pacific).

This hypothesis faces difficulties both from the geological time scale and the molecular data. The age of the family has been variously estimated, from 65 million years (Raven and Axelrod (1974) to 71 million years (Bremer et al., 2004, though *Peltanthera* was used erroneously as a member of Gesneriaceae here, thus the family maybe younger), but nowhere near a Gondwana origin (Gondwana break-up began 150 Ma, Storey et al. 1995). As far as the didymocarpoid Gesneriaceae are concerned, the most basal members are found on the Eurasian continent, especially on the Indian subcontinent: *Jerdonia* (mountains of SW India), *Corallo-discus* (Himalayas and China), *Tetraphyllum*, *Leptoboëa*, *Boëica* (Himalayas and adjacent areas). Only *Rhynchotechum*, with around 15 species being the largest genus of the group, has a wider distribution. It spreads from the Himalayas to the Malay archipelago and one species even reaches New Guinea. This island spreading would have been aided by the possession of fleshy fruits of the genus, as in *Cyrtandra*, that are putatively bird dispersed (Cronk et al., 2005). The basal Asiatic genera thus can be marked as an essentially ‘Indian group’ with most genera represented in the Sino-Himalayan area. This point is where the molecular and phytogeographical data and parts of Burt’s hypothesis meet. These genera may well be relicts of a group that had its origin on the Indian plate. Transgression to the west (Europe) was apparently very early, followed by a transgression to the south (perhaps first Madagascar and then Africa; the highest morphological diversity occurs in Madagascar, and a subset may have entered the African mainland 25–35 Ma; Möller and Cronk, 2001b) and—under explosive radiation and diversification—to the east and southeast (Indochina, Malesia, Pacific). The case of the widespread *Rhynchotechum* of the basal Asiatic and European group suggests that a migration from the Himalayas to the east may have involved several lineages in parallel.

**Conclusions**—The present work represents a major step forward in our understanding of the largest group of Old World Gesneriaceae, the ‘didymocarpoid Gesneriaceae’. The molecular data strongly suggest that none of the available current classifications properly reflect phylogeny. Neither the classification into tribes nor the delimitation of the tribes established so far is reflected in the molecular phylogenies. All characters that have been considered to be of major taxonomic value, such as actinomorphic flowers, diandry, indehiscent fruits, appendaged seeds, were found to be homoplastic, i.e., having evolved several times independently. Thus, apart from perhaps fruit twist, most other morphological characters and states are not helpful in shaping a new classification for the family.

The informal classification proposed by Weber (2004), which was partly based on unpublished molecular data, is essentially confirmed here on a much larger data set. No formal taxonomy is presented at this point, but a new classification and an understanding of the evolutionary pathways is emerging, and it is the biogeography that is best reflected in the resulting phylogenies so far.

This study will provoke a reappraisal of the approach for a classification of the OW Gesneriaceae if it is to reflect our results obtained here. Even though the epithematoid, basal Asiatic and European, and African and Madagascan groups are

well defined and supported, relationships among the advanced Asiatic and Malesian taxa are far from resolved. It is now paramount to obtain a stable phylogeny for this group of Gesneriaceae by adding more taxa and more data.

At present, molecular data for a considerable number of genera of didymocarpoïds are still lacking. The bulk will be relevant for the advanced Asiatic and Malesian Gesneriaceae. We are confident that their inclusion will result in a better resolution of this group and stabilize relationships. Their addition will also contribute to a better definition of particular genera and solve problems of generic delimitation. Even now it is clear that some genera are not monophyletic, that some genera have to be reduced to synonymy and that new genera have to be established. Much work is waiting.

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APPENDIX 1. List of taxa included in the phylogenetic analysis of Gesneriaceae, including voucher number, deposition of voucher, origin and GenBank sequence information. (An asterisk [\*] denotes generic types; [C] = *Chirita* section *Chirita*; [G] = *Chirita* section *Gibbosaccus*; [M] = *Chirita* section *Microchirita*; [L] = *Chirita* section *Liebigia*.)

Taxon	Voucher number	Deposited in	Origin	<i>atpB-rbcL</i>	<i>trnL-F</i>	ITS1/ITS2
<b>Oleaceae</b>						
<i>Olea europaea</i> L.*	A.Dahl 703 ( <i>trnL-F</i> ); cult RBGE 19832265 ( <i>atpB-rbcL</i> )	E	Cyprus; Troodos	FJ501367	AF231866	—
<i>Forsythia xintermedia</i> Zabel	A.Dahl702 ( <i>trnL-F</i> ); cult RBGE 19687553 ( <i>atpB-rbcL</i> )	E	cultivar	FJ501368	AF231824	—
<b>Tetrachondraceae</b>						
<i>Polypremum procumbens</i> L.	Struwe 1000	UPS	—	—	AJ430938	—
<i>Tetrachondra patagonica</i> Skottsbo.	Martinsson and Swenson 314	UPS	—	—	AJ430939	—
<b>Scrophulariaceae</b>						
<i>Scrophularia canina</i> L.	Perret S1.119	G	—	AY423105	AY423123	—
<i>Verbascum speciosum</i> Opiz	cult. HBV; Kiehn s.n.	WU	Austria, exact origin unknown	AJ490885	AJ492271	—
<b>Plantaginaceae</b>						
<i>Antirrhinum majus</i> L.*	cult. HBV	no voucher	unknown origin	AJ490883	AJ492270	—
<i>Tetranema roseum</i> (Martens & Galeotti) Standl. & Steyerl. [= <i>T. mexicanum</i> Benth. ex Lindl.*]	cult. HB München, Dec. 1997; Kiehn s.n. (27.4.98)	WU	Mexico; exact origin unknown	AJ490884	AJ492272	—
<i>Veronica incana</i> L.	cult. HB Bonn ex HB Mainz no. XX-0-MJG-19-47720; Albach 155	WU	unknown origin	—	AY486449	—
<i>Veronica incana</i> L.	unknown	—	unknown origin	AY818908	—	—
<b>Calceolariaceae</b>						
<i>Calceolaria arachnoidea</i> Graham	cult. RBGE 19912379	E	Chile, Los Lagos	AY423108	AY423126	—
<i>Jovellana punctata</i> Ruiz & Pavon*	cult. RBGE 19980599	E	Chile, Biobío, Prov. de Arauco:	AY423109	AY423127	—
<b>Gesnerioid</b>						
<i>Achimenes admirabilis</i> Wiehler	cult. CJBG, Nov. 1997, Chautems & Perret 01-033	G	unknown origin	AJ439982	AJ439827	—
<i>Besleria labiosa</i> Hanst.	cult. RBGE 19822666; Wiehler & Steyermark 72453	E	Venezuela; Distrito Federal, Cerro Narguata.	AY423110	AY423128	—
<i>Besleria melancholica</i> (Vell.) C.V.Morton	Chautems, Leitman & Martinelli 240, 21.5.1987	G	Brazil, Rio de Janeiro, Macaé de Cima	AJ490923	AJ492310	—
<i>Chrysothemis pulchella</i> (Donn ex Sims) Decne.*	cult. RBGE 19802568; Wiehler	E	unknown origin	AJ490925	AJ492312	—
<i>Cobananthus calochlamys</i> (Donn. Sm.) Wiehler*	cult. RBGE 19822676; H.Wiehler 7553	E	Guatemala, Coban	AJ490926	AJ492313	—
<i>Columnnea sanguinea</i> (Pers.) Hanst.	cult. HBV, ex HB St. Gallen 1987; Kiehn s.n.	WU	unknown origin	AJ490927	AJ492314	—
<i>Episcia cupreata</i> (Hook.) Hanst.	cult. RBGE 19570361	E	unknown origin	AJ490928	AJ492315	—
<i>Eucodonia verticillata</i> (Martens & Galeotti) Wiehler*	cult. RBGE 19782220	E	unknown origin	FJ501369	—	—
<i>Gesneria humilis</i> L.*	Skog 7714	US	USBRG 86-097	—	AY047120	—
<i>Glossoloma bolivianum</i> (Britt.) Wiehler	Chautems & Perret 97-020	G	cult. CJBG, Oct. 1997	AJ439976	AJ439821	—
<i>Gloxinia erinoides</i> (DC.) Roalson & Boggan	Mendoza-T. et al. 506	US	unknown origin	—	AY047156	—
<i>Kohleria hirsuta</i> (Kunth) Regel var. <i>hirsuta</i> [ <i>K. eriantha</i> (Benth.) Hanst.]	Chautems & Perret 01-034	G	cult. CJBG, Nov. 1997	AJ439983	AJ439828	—
<i>Kohleria spicata</i> (Kunth) Oerst.	cult. RBGE 19821486; M.Koehnen	E	Ecuador, unknown locality	AY423114	AY423132*	—
<i>Napeanthus reitzii</i> (L.B.Smith) B.L.Burt ex Leeuwenb.	Chautems & Perret 97-018	G	cult. CJBG	AJ439975, part	AJ439820	—
<i>Nautilocalyx melittifolius</i> (L.) Wiehler	A. Cervi & al. AC479	UPCB	Brazil, PR, Morretes, Route de la Graciosa, Volta Grande	AJ493036	AJ492321	—
<i>Nematanthus villosus</i> (Hanst.) Wiehler	Chautems & Perret 01-025	G	unknown origin	AJ439984, part	AJ439829	—
<i>Paliavana prasinata</i> (Ker Gawl.) Benth.	Perret 99-041	G	cult. CJBG, Sept. 1998	AJ439980	AJ439825	—
<i>Rhytidophyllum tomentosum</i> (L.) Mart.*	Chautems & Perret 00-013	G	cult. CJBG	AJ490932	AJ492319	—
<i>Seemannia</i> aff. <i>purpurascens</i> Rusby	cult. HBV; Kiehn s.n.	WU	unknown origin	AJ490930	AJ492317	—
<i>Simmingia cardinalis</i> (Lehm.) H.E.Moore	Chautems & Perret 97-019	G	cult. CJBG, Oct. 1998	AJ439977	AJ439822	—
<i>Sinningia schiffneri</i> Fritsch	Chautems & Perret 97-015	G	cult. CJBG,	AJ490931	AJ492318	—
<i>Smithiantha lauii</i> Wiehler	Chautems & Perret 97-010	G	cult. CJBG Oct. 1997	AJ439900	AJ439745	—
	cult. GRF (Gesneriad Research Foundation, Sarasota, Fl., USA), cult. ID = G-3588 Sequence by M. Perret	G	unknown origin	AJ439978	AJ439823	—

## APPENDIX 1. Continued.

Taxon	Voucher number	Deposited in	Origin	<i>atpB-rbcL</i>	<i>trnL-F</i>	ITS1/ITS2
<i>Vanhouttea calcarata</i> Lem.*	Carvalho et al. 526	CEPEC	Brazil, State of Rio de Janeiro, Nova Friburgo	AJ490933	AJ492320	—
<b>Coronantheroideae</b>						
<i>Asteranthera ovata</i> (Cav.) Hanst.*	cult. RBGE 19980608; UCEXC 362	E	Chile, Los Lagos, Prov. de Palena, Chaitén	FJ501371	FJ501427	—
<i>Fieldia australis</i> Cunn.*	RBGE 19696862	E	Australia, unknown locality	AY423112	AY423130	—
<i>Lenbrassia australiana</i> (C.T.White) G.W.Gillett var. <i>australiana</i> *	cult. RBGE19970901; P.D.Hint 6654	—	Australia, Queensland	AJ490921	AJ492308	—
<i>Mitraria coccinea</i> Cav.*	cult. RBGE 19792696; M.Mason	E	Argentina, Bariloche Region	—	AY423131	—
<i>Rhabdothamnus solandri</i> Cunn.*	cult. RBGE 19660192	E	New Zealand, North Island	FJ501370	FJ501426	—
<i>Sarmienta scandens</i> (J.D.Brandis) Pers.*	cult. RBGE 19882757; M.Gardner & S.Knees 4033	E	Chile, Región X Los Lagos, Prov. de Osorno	AJ490922	AJ492309	—
<b>Epithematoideae</b>						
<i>Epithema benthamii</i> C.B.Clarke	cult RBGE 19972563; Philippine Expedition 1997 SM9	E	Philippines, Luzon, Isabela	AY423118	AY423135	—
<i>Epithema membranaceum</i> (King) Kiew	Weber 860908–2/1	WU	Peninsula Malaysia, Pahang, Jerantut distr.	AJ490887	AJ492274	—
<i>Epithema saxatile</i> Blume	Weber & Anthonyamy 870521–3/2 (WU); cult. HBV	WU	Peninsula Malaysia, Perak, Kinta distr.; Sg. Siput Selatan	AJ490888	AJ492275	—
<i>Epithema taiwanense</i> S.S.Yin	C.-N. Wang & al.	TNU	Taiwan, Kaohsiung Hsien: Tengshih	AJ490889, part	AJ492276	—
<i>Epithema tenue</i> C.B.Clarke	cult. RBGE ex DTH 5815	E	Cameroon, Kupe village	AJ490890	AJ492277	—
<i>Loxonia hirsuta</i> Jack*	Weber 870602–1/5	WU	Peninsula Malaysia, Pahang; Pulau Tioman	AJ490891	AJ492278	—
<i>Monophyllaea elongata</i> B.L.Burtt	Weber & Antonyamy 870518–1/1	WU	Peninsula Malaysia, near Sungai Siput Selatan	AJ490892	AJ492279	—
<i>Monophyllaea glauca</i> C.B.Clarke	Vogel & Weber 790106–1/1	WU	Borneo, Sarawak, Bkt. Mentawa	AJ490893	AJ492280	—
<i>Monophyllaea hirticalyx</i> Franch.	Chin & Weber, Chin 2107 (KLU) = Vogel & Weber 790801	WU	Peninsula Malaysia, Selangor, Gua Batu	AJ490894	AJ492281	—
<i>Monophyllaea horsfieldii</i> R.Br.*	cult. HBV, seeds rec. from RBGE	WU	Peninsula Malaysia, Selangor, Batu Caves	U91315	AJ492269	—
<i>Rhynchoglossum azureum</i> (Schltdl.) B.L.Burtt	Huber & Weissenhofer 722	WU	Costa Rica, Prov. Alajuela; Valle Virgen	AJ490895	AJ492282	—
<i>Rhynchochlossum notonianum</i> (Wall.) B.L.Burtt	cult. HB München	no voucher	unknown origin	AJ490896	AJ492283	—
<i>Rhynchoglossum obliquum</i> Blume* [Malay Peninsula]	Weber 870510–1/3	WU	Peninsula Malaysia, Pahang, Lipis district	AJ490897	AJ492284	—
<i>Rhynchoglossum obliquum</i> Blume* [Philippines]	Mendum & al. 25349	E	Philippines, Palawan, betw. San Rafael and Cleopatra Needle	AJ490898	AJ492285	—
<i>Rhynchoglossum obliquum</i> Blume var. <i>hologlossum</i> (Hayata) W.T.Wang [Taiwan]	C.-N. Wang & al.	TNU	Taiwan, Kaohsiung Hsien: Tengshih	AJ490899	AJ492286	—
<i>Stauranthera grandiflora</i> Benth.*	Weber 870602–1/1	WU	Peninsula Malaysia, Pahang; Pulau Tioman	AJ490900	AJ492287	—
<i>Whytockia purpurascens</i> Y.Z.Wang	MMO 01–87	E, WU	China, Yunnan, Maguan county	—	FJ501428	—
<i>Whytockia sasakii</i> (Hayata) B.L.Burtt	C.-N. Wang & al.	TNU & E	Taiwan, Hualian Hsien, Hsiulin Hsiang	AJ490901	AJ492288	—
<i>Whytockia tsiangiana</i> (Hand.-Mazz.) A.Weber	1986 Sino-Amer. Expedition Nr. 200	WU	China, Guizhou Prov., Jiangkou county	AJ490902	AJ492289	—
<b>Didymocarpoideae</b>						
<i>Acanthonema strigosum</i> Hook.f.*	B.Macinder 49	K	Cameroon, Kupe village	—	FJ501454	FJ501306
<i>Aeschynanthus austroyunnanensis</i> W.T.Wang	MMO 01–79	E, WU	China, Yunnan, Hekou county,	FJ501396	FJ501500	—
<i>Aeschynanthus austroyunnanensis</i> W.T.Wang	cult RBGE 19951561; A.Reid & J.Fernie 004	E	China; Yunnan, Xishuangbanna Dai Aut. Pref.	—	—	AF349218 / AF349299
<i>Aeschynanthus bracteatus</i> Wall. ex DC.	Wang 991113	PE	China, Yunnan, Xichou	—	FJ501501	—
<i>Aeschynanthus bracteatus</i> Wall. ex DC.	cult RBGE 19970165; R.Cherry 123	E	Viet Nam; Lao Cai	—	—	AF349203 / AF349284
<i>Aeschynanthus hildebrandtii</i> Hemsl.	Skog 7777	US	unknown origin	—	AY047099	AY047040



## APPENDIX 1. Continued.

Taxon	Voucher number	Deposited in	Origin	<i>atpB-rbcL</i>	<i>trnL-F</i>	ITS1/ITS2
<i>Aeschynanthus lancilimbus</i> W.T.Wang	Wang S-10868	PE	China, unknown locality	—	FJ501499	—
<i>Aeschynanthus longiflorus</i> (Blume) DC.	Weber 950905 (photo record)	WU	Peninsula Malaysia, Perak, Larut distr.	AJ490920	AJ492307	—
<i>Aeschynanthus longiflorus</i> (Blume) DC.	cult. RBGE 19680624	E	Peninsula Malaysia	—	—	FJ501333
<i>Agalmyla biflora</i> (Elmer) O.M.Hilliard & B.L.Burt	cult. RBGE 19980287, DNA no. AG04	E	Philippines, Palawan, Near summit of Cleopatra Needle	FJ501421	FJ501541	-
<i>Agalmyla biflora</i> (Elmer) O.M.Hilliard & B.L.Burt	cult. RBGE 19980292, RBGE-PNHE1998-25517	E	Philippines; Palawan, near Thumb Peak	—	—	FJ501361
<i>Agalmyla clarkei</i> (Elmer) B.L.Burt	cult. RBGE 19991911, Royal Botanic Garden Edinburgh-Philippine National Herbarium Expedition 1999(P99) 13	E	Philippines, Leyte, Leyte Island, Mt. Lobi,	—	FJ501540	—
<i>Agalmyla clarkei</i> (Elmer) B.L.Burt	cult. RBGE 19972530A,	E	Philippines, Luzon, Barangay Penicuason	—	—	FJ501360
<i>Agalmyla parasitica</i> (Lam.) Kuntze*	5.9.95 Weber	WU	Peninsula Malaysia; Maxwell's Hill	FJ501420	FJ501539, part	—
<i>Ancylostemon aureus</i> (Franch.) B.L.Burt	MMO 01-153	E, WU	China, Yunnan, Binchuan county,	FJ501398	FJ501505	FJ501336
<i>Ancylostemon convexus</i> Craib	MMO 01-176	E, WU	China, Yunnan, Dali Co., Yu Dai Lu, Cang Shan,	—	FJ501506	FJ501337
<i>Anna mollifolia</i> (W.T.Wang) W.T.Wang & K.Y.Pan	MMO 01-146	E, WU	China, Guangxi, Napo county	—	FJ501543	AF055050 / AF055051
<i>Anna submontana</i> Pellegr.*	MMO 01-85	E, WU	China, Yunnan, Maguan county	FJ501422	FJ501542	FJ501362
<i>Boea hygrometrica</i> (Bunge) R.Br.	Gu 01-6184	KUN	China, unknown locality	—	FJ501476	FJ501319
<i>Boea hygroskopica</i> F.Muell.	Weber 810808-1/1	WU	Australia, N Queensland, Palmerston N.P.	—	FJ501577	—
<i>Boea hygroskopica</i> F.Muell.	B. Tan, R.G.Coveny & E.A.Brown 443, cult 19970386	E	Australia, N Queensland, Tchupala Falls	—	—	FJ501320
<i>Boea magellanica</i> Lam.*	Lambinon 87/830	L	Papua New Guinea, Morobe Province	—	FJ501478	FJ501321
<i>Boeica ferruginea</i> Drake	MMO 01-182B ex Zhang Chang Qin 200012	E, WU	China, SE Yunnan	FJ501379	FJ501440	—
<i>Boeica porosa</i> C.B.Clarke	Gu 99-705	KUN	China, unknown locality	FJ501380	FJ501441	—
<i>Briggsia longipes</i> (Hemsl. ex Oliv.) Craib	MMO 01-122	E, WU	China, Yunnan, Xichou county	FJ501423	FJ501545	AF055052 / AF055053
<i>Briggsia mihieri</i> Craib	Wang 11315B	PE	China, Chongqing, Nanchuan	—	FJ501544	FJ501363
<i>Briggsia muscicola</i> (Diels.) Craib	Kew (1995-2229)	K	unknown origin	—	FJ501548	FJ501366
<i>Briggsia rosthornii</i> (Diels) B.L.Burt	Sino-Amer. Guizhou Botanical Expedition 398 (US 229325)	US	China, Guizhou Prov., Jiangkou Xian	FJ501425	FJ501547	FJ501365
<i>Calcareoboea coccinea</i> C.Y.Wu ex H.W.Li*	MMO 01-141	E, WU	China, Guangxi, Napo county	FJ501406	FJ501516	FJ501365
<i>Chirita asperifolia</i> (Blume) B.L.Burt [L]	P.Woods 1071, 30.4.1968 (C6570)	E	Indonesia, Java, forest above Tjibodas Garden	FJ501419, part	FJ501538	FJ501359
<i>Chirita caliginosa</i> C.B.Clarke [M]	cult. HBV GS-96-02 ex HB München-Nymphenburg; Kiehn & Pfosser 2000-1	WU	Peninsula Malaysia	FJ501391	FJ501488	FJ501325
<i>Chirita flavimaculata</i> W.T.Wang [G]	cult. Smithsonian 94-085, Skog 7735 (US 590933)	US	China, Guangxi, leg. in US 11.03.1996	—	FJ501525	—
<i>Chirita gemella</i> D.Wood [G]	cult. RBGE 19941913, Averyanov, L. 1987	E	Viet Nam, Hong Quang Special Region, Cat Hai	FJ501408	FJ501523	FJ501345
<i>Chirita hamosa</i> R.Br. [M]	Panigrahi 12231, 1969 (C8032H)	E	India, Allahabad, Meizapus	FJ501392	FJ501489	—
<i>Chirita lacunosa</i> (Hook f.) B.L.Burt [C]	cult. RBGE 19972897	no voucher	Peninsula Malaysia, Pahang, Lipis distr., Gua Rusa	FJ501384, part	FJ501458	FJ501308
<i>Chirita lavandulacea</i> Stapf. [M]	cult. RBGE 20000897	E	China	FJ501390	FJ501487	FJ501324
<i>Chirita longgangensis</i> W.T.Wang [G]	cult. RBGE 19941915. Takhtajan, A. & Aruzytov, N. 1975	E	Viet Nam	AJ490903	AJ492290	FJ501347
<i>Chirita pinnata</i> W.T.Wang [G]	Expedition Beijing 896526 (US 294374)	US	China, Guangxi, Rongshui Xian	—	FJ501526	FJ501349
<i>Chirita pinnatifida</i> (Hand.-Mazz.) B.L.Burt [G]	Xie Qingjian J-037 (US 422838)	US	China, Guangdong Prov., Lianxian county	—	FJ501527	FJ501350
<i>Chirita pumila</i> D.Don [C]	cult. RBGE 19962271, Gaoligong Shan Expedition 1996 7938	E	China, Yunnan, Nujiang Lisu Aut. Pref., Fugong county	FJ501393, part	FJ501491	FJ501327

## APPENDIX 1. Continued.

Taxon	Voucher number	Deposited in	Origin	<i>atpB-rbcL</i>	<i>trnL-F</i>	ITS1/ITS2
<i>Chirita sinensis</i> Lindl. [G]	cult. RBGE 19791050. Godfrey, T.C. 369	E	China, Hong Kong	FJ501409, part	FJ501524	FJ501348
<i>Chirita spadiciformis</i> W.T.Wang [G]	ex Smithsonian Institute 94– 087, cult. RBGE 19951205	E	China	AJ490904	AJ492291	FJ501346
<i>Chirita urticifolia</i> Buch.-Ham. ex D.Don* [C]	EMAK 109 H 20.9.1991 (Edinburgh-Makalu Expedition 1991)	E	Nepal, Sankhuwasabha distr., Arun valley	—	FJ501492	FJ501328
<i>Chirita walkeri</i> Gardner [C]	Skog 7736 (US 590934)	US	cult. Smithsonian 94–250, origin: Sri Lanka; leg. in US 11.03.1996	—	FJ501490	FJ501326
<i>Chiritopsis repanda</i> W.T.Wang var. <i>gulinensis</i> W.T.Wang*	cult. RBGE 19951206	E	China, Guangxi, Zhuang Aut. Reg.	AJ490905	AJ492292	FJ501351
<i>Colpogyne betsiliensis</i> B.L.Burtt*	MM 9894C	E	Madagascar, Fianarantsoa	—	FJ501445	FJ501302
<i>Conandron ramondioides</i> Sieb. & Zucc.*	cult. RBGE 19691267; Takeda Herbal Garden Kyoto MMO 01–138	E	Japan	FJ501405	FJ501515	FJ501340
<i>Corallodiscus lanuginosus</i> (Wall. ex R. Br.) B.L.Burtt (G79)*	Sino-America Bot. Expedition 1429	E, WU	China, Yunnan, Xichou— Napo	FJ501374	FJ501432	—
<i>Corallodiscus lanuginosus</i> (Wall. ex R. Br.) B.L.Burtt (G8)*	cult. RBGE 19943415A, AGS Expedition 1994–1622	US	China, Yunnan Prov., Kunming Municipality	FJ501373	FJ501431	—
<i>Corallodiscus</i> sp.		E	China, Yunnan, Dêqên Zang Aut. Reg., Zhongdian county	—	FJ501430	—
<i>Corallodiscus conchifolia</i> Batalin	Wang, Hong et al 105	E	China, unknown locality	FJ501375	FJ501433	—
<i>Cyrtandra cupulata</i> Ridl.	Weber 840806–2/4	WU	Peninsula Malaysia, Perak, Maxwell's Hill	FJ501414	FJ501532	AY818826 / AY818861
<i>Cyrtandra glabra</i> Banks ex Gaertn.	Cronk & Percy T91	E	French Polynesia: Society Is.: Tahiti: Mt. Tearoa Col	AY423119	AY423136*	FJ501353
<i>Cyrtandra longifolia</i> (Wawra) Hillebr. ex C.B.Clarke	cult. HBV; Kiehn 920825–2/1	WU	USA, Hawaii, Kauai	FJ501413	FJ501531	AY818846 / AY818881
<i>Cyrtandra pendula</i> Blume	cult. HBV; Weber & Anthonysamy 860730–1/2	WU	Peninsula Malaysia	FJ501412	FJ501530	FJ501354
<i>Cyrtandra platyphylla</i> A.Gray	cult. HBV; Smith 3905/GES	SRP	USA, Hawaii, Maui, East Maui	FJ501410	FJ501528	—
<i>Cyrtandra sessilis</i> H.St.John	Kapua & al. s.n.	SRP (photo voucher)	USA, Hawaii, Oahu	FJ501411	FJ501529	—
<i>Didissandra frutescens</i> (Jack) C.B.Clarke	Weber 840805–1/2 (DI01)	WU	Peninsula Malaysia, Perak, Maxwell's Hill	U91313	FJ501521	—
<i>Didissandra frutescens</i> (Jack) C.B.Clarke	Weber 840805–1/2 (MB)	WU	Peninsula Malaysia, Perak, Maxwell's Hill	—	FJ501522	—
<i>Didymocarpus antirrhinoides</i> A.Weber	cult. RBGE 19650167, Jong 9009	E	Peninsula Malaysia, Perak, Bujong Melakah, Ipoh.	—	FJ501513	DQ912671
<i>Didymocarpus aromaticus</i> Wall. ex D. Don	Poelt s.n. sub GZU Inv.-Nr. 109–86	GZU	Nepal, Langtang Area (N Kathmandu)	FJ501402	FJ501511	—
<i>Didymocarpus citrinus</i> Ridl.	cult. RBGE 19830510; P.Davis 69437	E	Peninsula Malaysia, Perlis, Kedat Peak	AJ490906	AJ492293	DQ912669
<i>Didymocarpus cordatus</i> Wall. ex DC.	Weber 860816–2/1	WU	Peninsula Malaysia, Perak, Maxwell's Hill	—	AJ492294	DQ912673
<i>Didymocarpus podocarpus</i> C.B.Clarke	Noltie, Pradhan, Sherub & Wangdi 193, NPSW 193	E	Bhutan, Deothang District	FJ501404	FJ501514	DQ912688
<i>Didymocarpus purpureobracteatus</i> W.W.Sm.	Wang 991106	PE	China, Yunnan, Pingbian	FJ501401	FJ501510	—
<i>Didymocarpus purpureobracteatus</i> W.W.Sm.	MMO 01–70	CM	China: Yunnan, Pingbian	—	—	DQ912676
<i>Didymocarpus stenanthos</i> C.B.Clarke	MMO 01–156	E, WU	China, Yunnan, Binchuan county	FJ501403	FJ501512	DQ912687
<i>Emarhendia bettiana</i> (M.R.Hend.) Kiew, A. Weber & B.L.Burtt*	Weber & Anthonysamy 860825–1/1; cult. HBV.	WU	Peninsula Malaysia, Pahang, Kuantan distr.	AJ490908	AJ492295	—
<i>Haberlea rhodopensis</i> Friv.*	cult. RBGE 19754106	E	(Greece)	AJ490909	AJ492296	—
<i>Hemiboea bicornuta</i> (Hayata) Ohwi	cult. RBGE 19951207	E	unknown origin	FJ501416	FJ501534	FJ501356
<i>Hemiboea cavaleriei</i> H.Lev.	Gu G3	KUN	China, unknown locality	FJ501415	FJ501533	FJ501355
<i>Hemiboea gracilis</i> Franch.	Wang 11317	PE	China, Chongqing, Nanchuan	—	FJ501536	—
<i>Hemiboea subcapitata</i> C.B.Clarke	Wang 11306	PE	China, Chongqing, Chengkou	FJ501417	FJ501535	FJ501357
<i>Henckelia albomarginata</i> (Hemsl.) A.Weber	Weber 840805–1/12	WU	Peninsula Malaysia, Perak, Maxwell's Hill; base	AJ490910	AJ492297	—

## APPENDIX 1. Continued.

Taxon	Voucher number	Deposited in	Origin	<i>atpB-rbcL</i>	<i>trnL-F</i>	ITS1/ITS2
<i>Henckelia corrugata</i> Mendum	cult RBGE 19981788, RBGE-PNH Expedition 1998, DNA no. D12	E	Philippines, Palawan, Cleopatra Needle	—	FJ501484	—
<i>Henckelia ericii</i> A. Weber [= <i>Loxocarpus holttumii</i> M.R.Hend.]	Weber 840723–1/2	WU	Malaysia, Malaya	—	FJ501479	—
<i>Henckelia floccosa</i> (Thwaites) A. Weber & B.L.Burtt	G 157 Jang	WU	Sri Lanka	—	FJ501486	—
<i>Henckelia humboldtiana</i> (Gardner) A. Weber & B.L.Burtt	Kostermans 28519	L	Sri Lanka, Gombiya Ridge	FJ501389	FJ501485	—
<i>Hovanella madagascariensis</i> (C.B. Clarke) A. Weber & B.L.Burtt*	MM 9880A	E	Madagascar, Antsiranana Prov.	—	FJ501451	—
<i>Hovanella</i> sp. nov.	T.Sieder & M.Pfossen 101; 9.2.2000	WU	Madagascar, Toamasina	—	FJ501452	—
<i>Jancaea heldreichii</i> Boiss.*	cult. RBGE 19771605	photo E	Greece, Mt Olymp	FJ501378	FJ501439	—
<i>Kaisupeeia herbacea</i> (C.B. Clarke) B.L.Burtt*	cult. RBGE 19972918; K.Larsen 44272, 6 Nov 1993.	E	Thailand, Prov. Chachoengsao, Khao Tak Groep	FJ501385	FJ501459	FJ501309
<i>Leptoboea multiflora</i> (C.B. Clarke) Gamble* subsp. <i>grandifolia</i> B.L.Burtt	Larsen et al. 32065, 26.8.1972	E	Thailand, SE, Khaso Phra Bat, N of Chanthaburi	FJ501381	FJ501442	—
<i>Loxostigma cavalieri</i> (H.Lev. & Van.) B.L.Burtt	MMO 01–131	E, WU	China, Yunnan, Xichou Co., Far Dou	—	FJ501509	FJ501355
<i>Loxostigma fimbrisepalum</i> K.Y.Pan	Wang 991005	PE	China, Yunnan, Jinping	FJ501399	FJ501507	—
<i>Loxostigma griffithii</i> (Wight) C.B. Clarke*	cult. RBGE 19892473A; Kew/Edinburgh Kanchenjunga Expedition (1989) 940.	E	Nepal, Yamphudin	FJ501400	FJ501508	FJ501338
<i>Loxostigma</i> sp.	cult. RBGE 19962309, Gaoligong Shan Expedition 1996 GSE96–7668	E	China, Yunnan, Nujiang Lisu Aut. Pref., Gongshan	AY423137	AY423137	—
<i>Lysionotus chingii</i> Chun ex W.T.Wang	Wang S-10669	PE	China, unknown locality	—	FJ501498	FJ501332
<i>Lysionotus forrestii</i> W.W.Sm.	cult. RBGE 19962269A, Gaoligong Shan Expedition 1996 7925	E	China, Yunnan, Nujiang Lisu Aut. Pref.,	FJ501394	FJ501495	AF349152 / AF349233
<i>Lysionotus pauciflorus</i> Maxim.	MMO 01–101	E, WU	China, Yunnan, road to Xichou, Cheng Jia Po	FJ501395	FJ501497	FJ501331
<i>Lysionotus petelotii</i> Pellegr.	MMO 01–100	E, WU	China, Yunnan, road to Xichou, Cheng Jia Po	—	FJ501496	—
<i>Opithandra primuloides</i> (Miq.) B.L.Burtt*	cult. RBGE 19842178A	E	Japan, unknown locality	FJ501424	FJ501546	FJ501364
<i>Oreocharis aurea</i> Dunn.	Wang S-10725	PE	China, unknown locality	—	FJ501483	—
<i>Oreocharis auricula</i> (S. Moore) C.B. Clarke G03	Luo Lin-bo 0125	WU	China, Hunan Prov., Xining county	—	FJ501482	—
<i>Oreocharis auricula</i> (S. Moore) C.B. Clarke G04	Sino-America Expedition 1832	WU	China, Guizhou Prov. Yinjiang county	—	FJ501481	—
<i>Oreocharis auricula</i> (S. Moore) C.B. Clarke G04	MMO 03–304	E	China; Guizhou, Jiangkou	—	—	FJ501323
<i>Ornithoboea arachnoidea</i> (Diels) Craib	cult RBGE 19972903	E	Thailand, Chiang Mai, Doi Chiang Dao	FJ501387	FJ501461	FJ501312
<i>Ornithoboea wildeana</i> Craib	Wang 00401	PE	China, Yunnan, Xichou	—	FJ501462	FJ501313
<i>Ornithoboea</i> sp. nov.	MMO-04–439	E	Thailand; Chiang Mai, Grasshopper cave	FJ501386	FJ501460	FJ501311
<i>Paraboea acutifolia</i> (Ridl.) B.L.Burtt	Weber 86805–2/1	WU	Peninsula Malaysia, Kedah, Pulau Langkawi, Bukit Terbak	—	FJ501464	FJ501314
<i>Paraboea brachycarpa</i> (Ridl.) B.L.Burtt	Weber 870508–2/6	WU	Peninsula Malaysia, Pahang, Lipis distr., Gua Bama	—	FJ501465	—
<i>Paraboea capitata</i> Ridl. var. <i>capitata</i>	Weber 870522–5/2; cult. HBV.	WU	Peninsula Malaysia, Perak, Kinta distr.	AJ490911	AJ492298	FJ501315
<i>Paraboea crassifolia</i> (Hemsl.) B.L.Burtt	MMO 01–83	E, WU	China, Yunnan, Maguan county	—	FJ501472	FJ501318
<i>Paraboea dictyoneura</i> (Hance) B.L.Burtt	Xie Qingjian J-040 (US 422817)	US	China, Guangdong Prov, Lianxian county	—	FJ501463	—
<i>Paraboea ferruginea</i> (Ridl.) Ridl.	Weber 860806–1/2	WU	Peninsula Malaysia, Kedah, Pulau Langkawi, Selat Panchar	—	FJ501471, part	—
<i>Paraboea lanata</i> (Ridl.) B.L.Burtt	Weber 860807–1/2	WU	Peninsula Malaysia, Kedah, Pulau Langkawi, Pulau Dayang Bunting	—	FJ501467	—
<i>Paraboea laxa</i> Ridl.	C 4197	E	Peninsula Malaysia, Kedah, Pulau Langkawi	—	FJ501466	—



## APPENDIX 1. Continued.

Taxon	Voucher number	Deposited in	Origin	<i>atpB-rbcL</i>	<i>tmL-F</i>	ITS1/ITS2
<i>Paraboea rufescens</i> (Franch.) B.L.Burt (G23b)	Sino-Amer. Bot. Exped 1566 (US 64646)	US	China, Yunnan Prov., Lunan Xian	—	FJ501468	—
<i>Paraboea rufescens</i> (Franch.) B.L.Burt (G72)	MMO 01–99	E, WU	China, Yunnan, Xichou, Cheng Jia Po	FJ501388	FJ501469	FJ501316
<i>Paraboea rufescens</i> (Franch.) B.L.Burt var. <i>umbellata</i> (Drake) K.Y.Pan	MMO 01–147	E, WU	China, Guangxi, Napo, Nong Bu	—	FJ501470	FJ501317
<i>Paraboea sinensis</i> (Oliv.) B.L.Burt (G20)	Wen He Qun W049 (US 329798)	US	China, Guangxi Prov., Napo county	—	FJ501473	—
<i>Paraboea sinensis</i> (Oliv.) B.L.Burt (G21b)	Xie Qingjian J-003 (US 422825)	US	China, Guangxi Prov. Jingxi county	—	FJ501474	—
<i>Paraboea swinhoei</i> (Hance) B.L.Burt	Wagner 6640 (US 427725)	US	Taiwan, Taoyuan Hsien	—	FJ501475	—
<i>Petrocodon dealbatus</i> Hance*	Xie Qingjian J-042 (US 422841)	US	China, Guangdong Prov., Lianxian county	FJ501418	FJ501537	FJ501358
<i>Petrocosmea kerrii</i> Craib	cult. RBGE 19715592	E	unknown origin	FJ501397, part	FJ501502	FJ501334
<i>Petrocosmea minor</i> Hemsl.	Sino-Amer. Bot. Exped. 1574 (US 56119)	US	China, Yunnan Prov., Lunan Xian	—	FJ501504	—
<i>Petrocosmea nervosa</i> Craib	cult. RBGE 19933232, SL.78–057	E, US	China, N Yunnan	AJ490912	AJ492299	FJ501335
<i>Petrocosmea sericea</i> C.Y.Wu ex H.W.Li	Gu 99–1104	KUN	China, unknown locality	—	FJ501503	—
<i>Platystemma violoides</i> Wall.	Projektteam 197–241	WU	Nepal, SE Kathmandu Pulchoki	FJ501382	FJ501443	—
<i>Primulina tabacum</i> Hance*	cult. RBGE 19951540, Xie, Q.J. & Ye, C.X.	E	China, Guangdong, Lian River	AJ490913	AJ492300	FJ501352
<i>Ramonda myconi</i> (L.) Rchb.*	cult. RBGE 19711477	E	Spain, Pyrenees	AJ490914	AJ492301	—
<i>Ramonda nathaliae</i> Panc. & Petr.	cult. RBGE 19784020	E	Macedonia, unknown locality	—	FJ501438	—
<i>Raphiocarpus begoniifolius</i> (H.Lev.) B.L.Burt	Wang 991108	PE	China, Yunnan, Yuanyang	—	FJ501517	FJ501342
<i>Raphiocarpus</i> sp. (G61)	MMO 01–55	E, WU	China, Yunnan, Pingbian Co., Dar Wei Shang	—	FJ501493	FJ501329
<i>Raphiocarpus</i> sp. (G64)	MMO 01–69	E, WU	China, Yunnan, Pingbian Co., Dar Wei Shang	—	FJ501494	FJ501330
<i>Raphiocarpus</i> sp.	Beijing Youth team 572	PE	China, Kwangsi (Guangxi)	FJ501407	FJ501519	FJ501344
<i>Raphiocarpus petelotii</i> (Pellegr.) B.L.Burt	cult RBGE 19982405; S.Goodwin & R.Cherry 92/208	E	Viet Nam, Lao Cai Prov.	—	FJ501518	FJ501343
<i>Rhabdothamnopsis sinensis</i> Hemsl.*	ex Kew 1988 4866	K	China, unknown locality	AJ490915	AJ492302	FJ501310
<i>Rhynchochotum discolor</i> (Maxim.) B.L.Burt	RBGE 1997 2562; RBGE-PNH Expedition 1997/SM8	E	Philippines; Luzon, Isabela	FJ501376	FJ501436	—
<i>Rhynchochotum parviflorum</i> Blume	M.Mendum, G.Argent, Hendrian 00148; coll 25.2.2000	E	Central Sulawesi, Mt Sojol.	FJ501377	FJ501437	—
<i>Ridleyandra porphyrantha</i> (Kiew & A. Weber) A. Weber	Weber 870420–2/4	WU	Malaysia, Pahang, side ridge of Gunung Bunga Buah	—	FJ501520	—
<i>Saintpaulia tongwensis</i> B.L.Burt	cult. RBGE 19850668, I.C.Mather 2	E	Tanzania, Tanga Region	—	FJ501446	FJ501303
<i>Saintpaulia velutina</i> B.L.Burt	cult. RBGE 19872179	E	Tanzania; unknown locality	AJ490916	AJ492303	FJ501304
<i>Schizoboea kamerunensis</i> K.Fritsch (B.L.Burt)*	J.Lewalle 6693, 9.4.1972	E	Burundi, Muramvya, Mount Teza	—	FJ501453	FJ501305
<i>Spelaeanthus chinii</i> Kiew, A.Weber & B.L.Burt*	Weber 860709–2/2	WU	Peninsula Malaysia, Pahang, Jerantut distr., Taman Negara	—	FJ501457	FJ501307
<i>Streptocarpus andohahelensis</i> Humbert	MM 9717	E	Madagascar, Tuléar Prov., Ranomafana	—	FJ501449	AF316903
<i>Streptocarpus beampingaratrensis</i> Humbert var. <i>beampingaratrensis</i>	MM 9715	E	Madagascar, Tuléar Prov., Ranomafana	—	FJ501448	AF316905
<i>Streptocarpus dumii</i> Hook.f.	cult. RBGE 19941745, Isobel La Croix	E	Swaziland, Mbabane	—	FJ501456	AF316951
<i>Streptocarpus hilsenbergii</i> R.Br.	cult. RBGE 19631505	E	Madagascar, Mandrake Valley	—	FJ501450	AF316907
<i>Streptocarpus holstii</i> Engl.	cult. RBGE 19592272	E	Tanzania, unknown locality	AJ490917	AJ492304	AF316917
<i>Streptocarpus ibityensis</i> Humbert	cult. RBGE 19932867, E.Fischer 250/93	E	Madagascar, Antananarivo Prov.	—	FJ501455	AF316926

## APPENDIX 1. Continued.

Taxon	Voucher number	Deposited in	Origin	<i>atpB-rbcL</i>	<i>trnL-F</i>	ITS1/ITS2
<i>Streptocarpus orientalis</i> Craib	A.Weber & M.Kiehn 29.9.98	E, WU	Thailand, Prov. Chiang Mai	—	FJ501480	FJ501322
<i>Streptocarpus papangae</i> Humbert	MM 9718	E	Madagascar, Tuléar Prov., Ranomafana	—	FJ501444	AF316929
<i>Streptocarpus rexii</i> Lindl.*	cult. RBGE 19870333, K.Jong	E	South Africa, NE Cape Prov., Grahamstown	AJ490918	AJ492305	AF316979
<i>Streptocarpus saxorum</i> Engl.	Chautems & Perret 01–023	MP	cult. CJBG	FJ501383	FJ501447	—
<i>Streptocarpus saxorum</i> Engl.	cult. RBGE 19721499; I.C.Mather 4		Tanzania, Tanga region	—	—	AF316914
<i>Tetraphyllum roseum</i> Stapf (G113)	Kurzweil HK 798	WU	Thailand, Krabi Prov., c. 20km N of Krabi	—	FJ501434	—
<i>Tetraphyllum roseum</i> Stapf (G124)	Larsen & al. 31190	E	Thailand, Lam Tok Lam Pae	—	FJ501435	—
<i>Trisepalum speciosum</i> (Ridl.) B.L.Burt	Weber 860805–1/1	WU	Peninsula Malaysia, Kedah, Pulau Langkawi, Bukit Puteh	AJ490919	AJ492306	—
Unassigned:						
<i>Jerdonia indica</i> Wight*	G 155 Jang	no voucher	India	FJ501372	FJ501429	—
<i>Titanotrichum oldhamii</i> (Hemsl.) Soler.*	cult. RBGE 19973433	E	Taiwan, unknown locality	AY423111	AY423129	—