Molecular phylogenetics and morphological evolution of Thunbergioideae (Acanthaceae)

Agneta Julia Borg[1*], Lucinda A. McDade[2] & Jürg Schönenberger[1]

[1] *Department of Botany, Stockholm University, Lilla Frescativägen 5, 106 91 Stockholm, Sweden. *borg@botan.su.se (author for correspondence).*
[2] *Rancho Santa Ana Botanic Garden, 1500 North College Avenue, Claremont, California 91711-3157, U.S.A.*

Based on nucleotide sequences from three chloroplast DNA regions (*rps16*, *rpl16*, *trnT-trnL*), we infer detailed phylogenetic relationships within the subfamily Thunbergioideae (Acanthaceae) and among major lineages of the family as a whole. Taxa were sampled to cover the geographic distribution of Thunbergioideae and to include all subgenera of the largest genus *Thunbergia*. All other major lineages of Acanthaceae were sampled to test monophyly and intrafamilial position of Thunbergioideae. Both parsimony and Bayesian analyses support Thunbergioideae as monophyletic. The mangrove genus *Avicennia* is consistently placed as sister to Thunbergioideae although with only moderate support. *Thunbergia* and *Mendoncia* are both monophyletic in all analyses, and *Mendoncia* is placed as sister to *Thunbergia* plus *Pseudocalyx*. Relationships within the two largest genera *Mendoncia* and *Thunbergia* are highly resolved and most branches are strongly supported. Our results suggest that the existing morphology-based classification of *Thunbergia* partially holds, but needs revision. Based on the phylogenetic relationships we found it likely that a twining habit is ancestral for the genus *Thunbergia*. The thecal awns, characteristic for many species in the genus, have probably evolved from unicellular bristles. Longitudinal dehiscence over the whole length of the thecae, which is present in many *Thunbergia* species, most likely evolved from short slits or pores as present in *Pseudocalyx* and *Mendoncia*.

KEYWORDS: Acanthaceae, chloroplast DNA, morphology, phylogeny, Thunbergioideae

INTRODUCTION

Acanthaceae are a large family of mainly tropical and subtropical species within the asterid order Lamiales. The delimitation of the family has been controversial due to morphological differences between the subfamily Acanthoideae (Acanthaceae s.str.), containing the vast majority of all species in the family, and the two other subfamilies, Nelsonioideae and Thunbergioideae sensu Scotland & Vollesen (2000).

The Thunbergioideae comprise five genera, the largest of which, *Thunbergia* Retz., contains about 100 species restricted to tropical and subtropical regions of Africa, Madagascar, Asia, and Australia. The second largest genus, *Mendoncia* Vell. ex Vand. (ca. 60 species), is most diverse in Central and South America with some African representatives and a few species native to Madagascar. The remaining genera are *Pseudocalyx* Radlk. with ca. seven species in Africa and Madagascar, and monotypic *Anomacanthus* R.D. Good in tropical Africa, and monotypic *Meyenia* Nees in India. The subfamily is characterized by a predominantly twining habit, enlarged bracteoles, and a reduced calyx. Furthermore, Thunbergioideae (together with Nelsonioideae) lack the retinaculate fruits found in all Acanthaceae s.str., instead possessing either dry and dehiscent capsules without retinacula (*Thunbergia*, *Pseudocalyx*, *Meyenia*) or fleshy drupes (*Mendoncia*, *Anomacanthus*). While there are no existing subgeneric classifications for *Mendoncia* or *Pseudocalyx* (but see Profice, 1988, for *Mendoncia* in Brazil), more attention has been given to *Thunbergia*. Lindau (1893) subdivided *Thunbergia* into four sections based on morphology and arrangement of flowers. Bremekamp (1955) revised and extended Lindau's subdivision and proposed eight subgenera. His classification largely concurs with a recent study of floral development and structure in *Thunbergia* by Schönenberger (1999), but has never been compared to a hypothesis of phylogenetic relationships based on DNA sequence data.

Thunbergioideae have traditionally been associated with Acanthaceae (Table 1), but their markedly different morphology has led some authors to place them in a separate family Thunbergiaceae together with Nelsonioideae (van Tieghem, 1908a) or even in two distinct families Thunbergiaceae and Mendonciaceae (Bremekamp, 1953; Dahlgren, 1980; Cronquist, 1981). Other authors, however, considered the morphological differences to be insufficient to separate Thunbergioideae from Acanthaceae and instead kept them within the family, either as a tribe (Bentham, 1876), as a subfamily (Brummitt, 1989; Takhtajan, 1997; Scotland & Vollesen, 2000), or as two separate subfamilies Thunbergioideae and Mendoncioideae (Lindau, 1895; Thorne, 1992).

Since molecular methods became widely used, a number of studies have shown that Thunbergioideae and Acanthaceae s.str. are closely related, placing Thunbergioideae either as sister to Acanthaceae s.str. or in a polytomy within or near Acanthaceae s.str. (Hedrén & al., 1995; Scotland & al., 1995; McDade & Moody, 1999). Although based on a minimal taxon sampling, these same studies also indicated that Thunbergioideae (sensu lato) form a natural group. This last was also supported in a study of floral development and structure by Schönenberger & Endress (1998). Nelsonioideae were mostly resolved as sister to all other Acanthaceae (e.g., Scotland & al., 1995; McDade & al., 2000). In a review of morphological and molecular studies in Acanthaceae, Scotland & Vollesen (2000) presented a classification of the family in the broad sense, including both Thunbergioideae and Nelsonioideae. More recently and quite surprisingly, a molecular study by Schwarzbach & McDade (2002) implied that the mangrove genus *Avicennia* L., usually treated as a separate family in Lamiales or as a genus within Verbenaceae, is also part of Acanthaceae. In their study, *Avicennia* is consistently placed as sister group to Thunbergioideae albeit with weak support.

Although the delimitation of the Acanthaceae now seems well supported, exact relationships among Acanthoideae, Thunbergioideae, Nelsonioideae and *Avicennia* remain unclear. Furthermore, despite the number of morphological and molecular studies showing that Thunbergioideae belong in Acanthaceae, no molecular study has so far included more than a couple of representatives from *Thunbergia* and *Mendoncia*. Accordingly, phylogenetic relationships within Thunbergioideae are currently not well understood.

The main goals of the present study are *(1)* to test whether Thunbergioideae are monophyletic, *(2)* to determine the exact position of the Thunbergioideae among the other acanthaceous lineages, *(3)* to elucidate evolutionary relationships within Thunbergioideae, *(4)* to find out whether molecular evidence is congruent with earlier, morphology-based attempts to classify Thunbergioideae as a whole and the genus *Thunbergia* in particular (e.g., Lindau, 1895; Bremekamp, 1955), and *(5)* to discuss the evolution of a number of morphological traits traditionally used for classification of the group.

MATERIALS AND METHODS

Taxon sampling. — We sampled molecular characters from 30 species from the three major genera of Thunbergioideae (*Thunbergia*, *Mendoncia*, *Pseudocalyx*). The sampled *Thunbergia* species (21 species) represent all 8 subgenera circumscribed by Bremekamp (1955) and also cover the geographic range of the genus. The sampling also includes *T. arnhemica*, the only *Thunbergia* species native to Australia. Our sampling furthermore covers the main distribution areas of the genus *Mendoncia* (eight species) with representatives from Tropical West Africa, Madagascar, Central and South America. The small genus *Pseudocalyx* is represented by a single African/Malagasy species.

Sampling of the other acanthaceous lineages include two species from each of the two tribes of the Acanthoideae; the Acantheae and Ruellieae (sensu Scotland & Vollesen, 2000), single representatives of three out of six genera of Nelsonioideae, and three out of eight species of *Avicennia* (sensu Tomlinson, 1995). As an out-group we used the genus *Schlegelia* Miq., which is possibly sister to Acanthaceae (71% jackknife support) as shown in a molecular study of the asterids by Bremer & al. (2002).

Molecular methods. — Total genomic DNA was extracted from leaf material dried in silica gel or from recently collected herbarium specimens, either using DNeasy kits (Qiagen), or following the CTAB protocol by Doyle & Dickson (1987). CTAB samples were cleaned with QIAquick PCR Purification Kit (Qiagen). Three chloroplast (cp) DNA regions, the *rps16* intron, the *rpl16* intron, and the *trnT-trnL* intergenic spacer, were amplified and sequenced for all taxa. The *rps16* intron is a widely used cp DNA region, which has been shown to be informative also among Acanthaceae (McDade & al., 2005). The *rps16* intron was amplified using the primers of McDade & al. (2005). To amplify the *rpl16* intron, primers F71 (Jordan & al., 1996) and R1516 by Baum & al. (1998) were used. The *trnT-trnL* intergenic spacer was amplified using the trnA2 primer of Cronn & al. (2002) and the b primer of Taberlet & al. (1991). Polymerase chain reaction (PCR) amplifications for all three regions used the following thermal cycling protocol: preheating at 94°C for 2 min 30 s followed by 35 cycles of denaturation

Table 1. Classification of Acanthaceae.

Lindau, 1895	Bremekamp, 1953	Scotland & Vollesen, 2000
Acanthoideae	Acanthaceae (s.str.)	Acanthoideae
Thunbergioideae	Thunbergiaceae	Thunbergioideae (s.l.)
Mendoncioideae	Mendonciaceae	Nelsonioideae
Nelsonioideae	Nelsonioideae referred to Scrophulariaceae	

at 94°C for 45 s, annealing at 52°C for 1 min, and extension at 74°C for 1 min 20 s. A final 10 min extension at 72°C was followed by cooling to 4°C. Amplified PCR products were purified by vacuum filtration using MultiScreen Vacuum Manifold (Millipore). The same primer pairs were used for the sequencing reactions together with the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing was accomplished on an automated capillary ABI 3100 Genetic Analyzer (Applied Biosystems). Both strands of the three regions were sequenced for all taxa to verify the complementary strands against each other. All sequences were proofread and double-checked against electropherograms and then assembled using the Staden Software Package version 1.6.0 (http://staden.sourceforge.net/).

Alignment and analysis. — Sequences were aligned by eye after an initial alignment was created with Clustal W (Thompson & al., 1994) in BioEdit version 7.0.1 (Hall, 1999). A region near the middle of the *rpl16* intron was extremely variable in length (1 to 235 base pairs) and we were unable to align the sequences with confidence. This region was therefore omitted from all further analyses.

Phylogenetic reconstruction. — Phylogenetic analyses were performed using maximum parsimony (MP) as well as Bayesian inference of phylogeny. MP analyses were carried out in PAUP version 4.0b10 (Swofford, 2002). All characters and character states were weighted equally and gaps were treated as missing characters. Four datasets were analyzed; one for each cp DNA region and one combined dataset including all three regions. The four matrices were analyzed by employing a heuristic search strategy with 10,000 replicates of random taxon addition, holding 100 trees at each step during stepwise addition, using the tree-bisection-reconnection branch-swapping algorithm, saving multiple equally parsimonious trees, and with the steepest descent option in effect. The resulting MP trees from each analysis were used to produce strict consensus trees. Relative branch support was measured by nonparametric bootstrap analysis (Felsenstein, 1985) using a heuristic search strategy, 10,000 bootstrap replicates with ten random sequence additions and holding one tree at each step during stepwise addition.

Bayesian analyses (BA) were carried out using MrBayes version 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). The software program MrAIC version 1.4.2 (Nylander, 2004) was used to compare 24 nucleotide substitution models based on the Akaike and Bayesian information criteria (BIC). We let the program evaluate models for the three separate data partitions as well as for the combined dataset. The same models were chosen with the same ranking for all datasets and we therefore proceeded with the combined data set only. The two best fitting models were applied to the data in separate analyses, and each analysis was carried out in MrBayes as follows: Two parallel runs (default in MrBayes version 3.1.2), each using one cold and three heated chains, were run for 5,000,000 generations with sample trees saved every 100 generations. The first 12,500 (25%) of the sample trees from each run were discarded (burn in), and a maximum a posteriori tree was constructed by summarizing the remaining 75,000 trees from parallel runs in a majority rule consensus tree, thus yielding the posterior probability (PP) values for each clade.

RESULTS

Table 2 presents dataset characteristics for the three cp regions and the combined data set. The combined dataset included 2,899 aligned positions, of which 1,063 were variable and 597 were parsimony informative. The *trnT-trnL* intergenic spacer provided the highest percentage of parsimony informative characters (22.1%), followed by the *rpl16* intron (21.4%) and the *rps16* intron (18.2%).

Parsimony analyses. — MP analyses of the three separate cp regions yielded congruent results and the strict consensus trees did not present any topological conflicts (trees not shown here, see Table 2). The combined MP analysis resulted in eight most parsimonious trees, summed up in a strict consensus tree (Fig. 1A). Combining

Table 2. Description of datasets and trees resulting from maximum parsimony analyses (excluding uninformative characters).

Characteristic	*rps16* intron	*rpl16* intron	*trnT-trnL* spacer	Combined
Aligned length	959	987	953	2,899
Variable positions (%)	316 (33.0)	369 (37.4)	378 (39.7)	1,063 (36.7)
Parsimony informative (%)	175 (18.2)	211 (21.4)	211 (22.1)	597 (20.6)
Consistency index	0.7143	0.6506	0.7088	0.6816
Retention index	0.8801	0.8285	0.8813	0.8608
Number of shortest trees	184	162	1,166	8
Length of shortest tree	478	615	578	1,675

the three data matrices improved overall tree resolution and branch support, but the combined analysis did not find any clades not present in at least one of the strict consensus trees of the individual datasets.

The deepest split in the phylogeny is between a monophyletic Nelsonioideae (BS = 100) and the remaining ingroup taxa (BS = 89). Acanthoideae, Thunbergioideae and *Avicennia* are all well supported monophyletic groups (BS = 97, 100 and 100, respectively). *Avicennia* is moderately supported as sister to Thunbergioideae (BS = 68) and Acanthoideae are sister to the two latter taxa. The MP analysis fully supports the monophyly of Thunbergioideae as a whole and clearly shows that *Thunbergia* as well as *Mendoncia* are monophyletic (both BS = 100). *Pseudocalyx* is consistently resolved as sister to *Thunbergia* (BS = 99) and *Mendoncia* is in turn sister to these two genera.

Bayesian analyses. — The same models were preferred using the Akaike information criterion (AIC) and Bayesian information criterion (BIC). Two models fitted the data (together corresponding to a cumulative Akaike weight of 1.000). The best scored model for all four datasets was a general time-reversible model with gamma-distributed rates (GTR+Γ). The second best model for all datasets was a GTR model with gamma-distributed rate and a proportion of invariant sites (GTR+Γ+I). Since the same model ranking was proposed for each of the three individual datasets as well as the combined data set, we decided to analyze the three data matrices as a single partition.

The Bayesian analysis provided a tree topology almost identical to the MP tree (Figs. 1B, 2), the difference being that the BA majority rule tree is somewhat better resolved than the MP strict consensus tree. The PP values are generally high. The Bayesian analysis found four

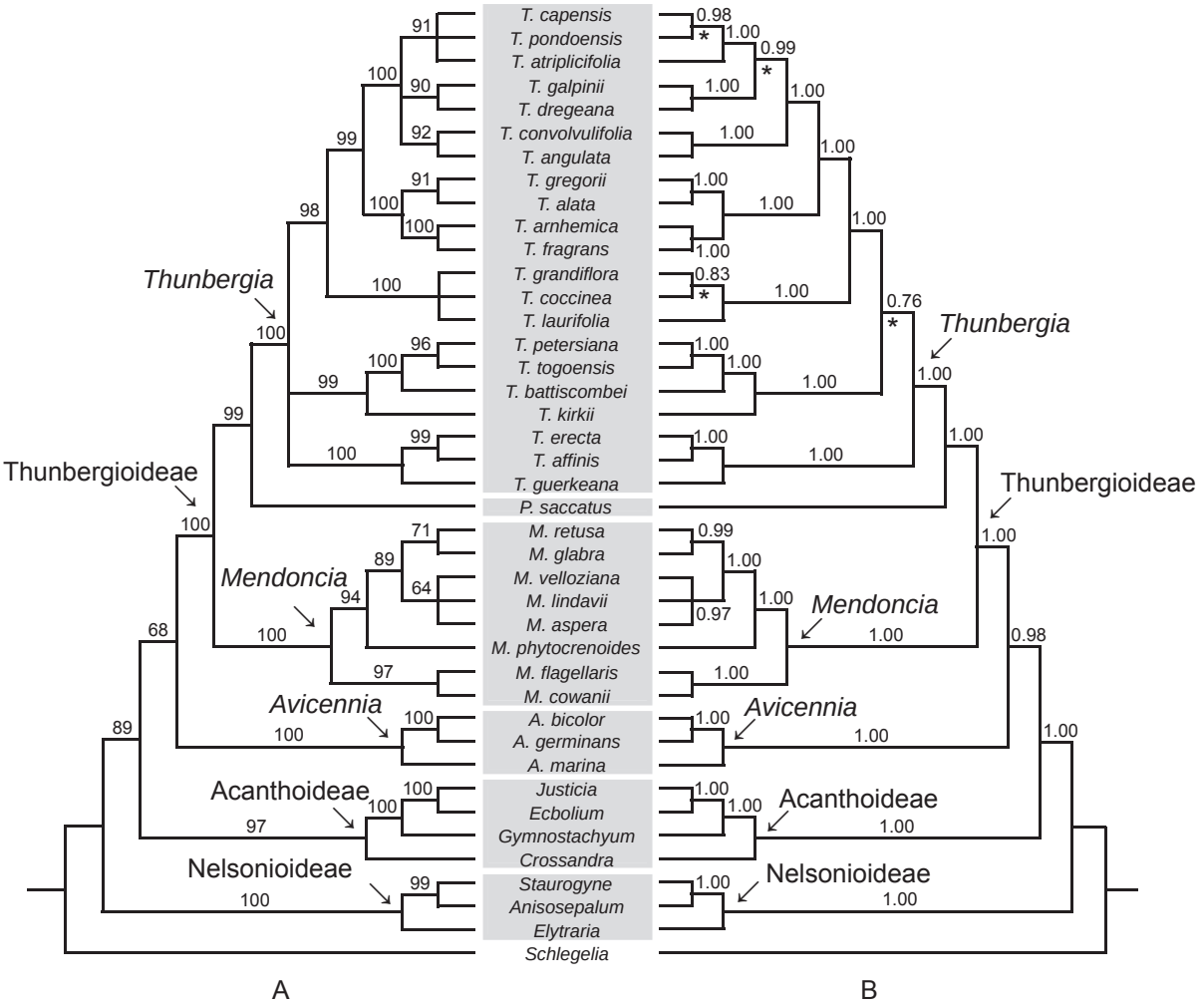

Fig. 1. Phylogenetic trees based on analyses of the combined dataset. A, strict consensus of eight shortest trees resulting from maximum parsimony (MP) analysis; numbers above branches are bootstrap values. B, majority rule consensus tree resulting from Bayesian analysis; numbers above branches are Bayesian posterior probabilities; asterisks indicate nodes not present in MP tree.

branches that are not present in the MP tree: *(1)* The basal trichotomy in *Thunbergia* is resolved, suggesting that the clade containing *T. erecta, T. affinis* and *T. guerkeana* (PP = 1) is sister to all other *Thunbergia* species (PP = 0.76); *(2) T. coccinea* and *T. grandiflora* are more closely related to each other (PP = 0.83) than to *T. laurifolia*; *(3) T. capensis* and *T. pondoensis* are more closely related to each other (PP = 0.98) than to *T. atriplicifolia*, and *(4)* together these three species constitute the sister group to *T. dregeana* and *T. galpinii* (PP = 0.99) while *T. angulata* and *T. convolvulifolia* have diverged earlier on.

DISCUSSION

Monophyly and subdivision of Thunbergioideae. — Our results suggest that Thunbergioideae are monophyletic (BS = 100; PP = 1.0). Uncertainties remain regarding the two monotypic genera *Anomacanthus* and *Meyenia*, of which we were unable to get material. However, the inclusion of these species is unlikely to change the circumscription of the subfamily as both genera show all general characteristics of Thunbergioideae (Good, 1923; Brummitt, 1989), and the single *Meyenia* species

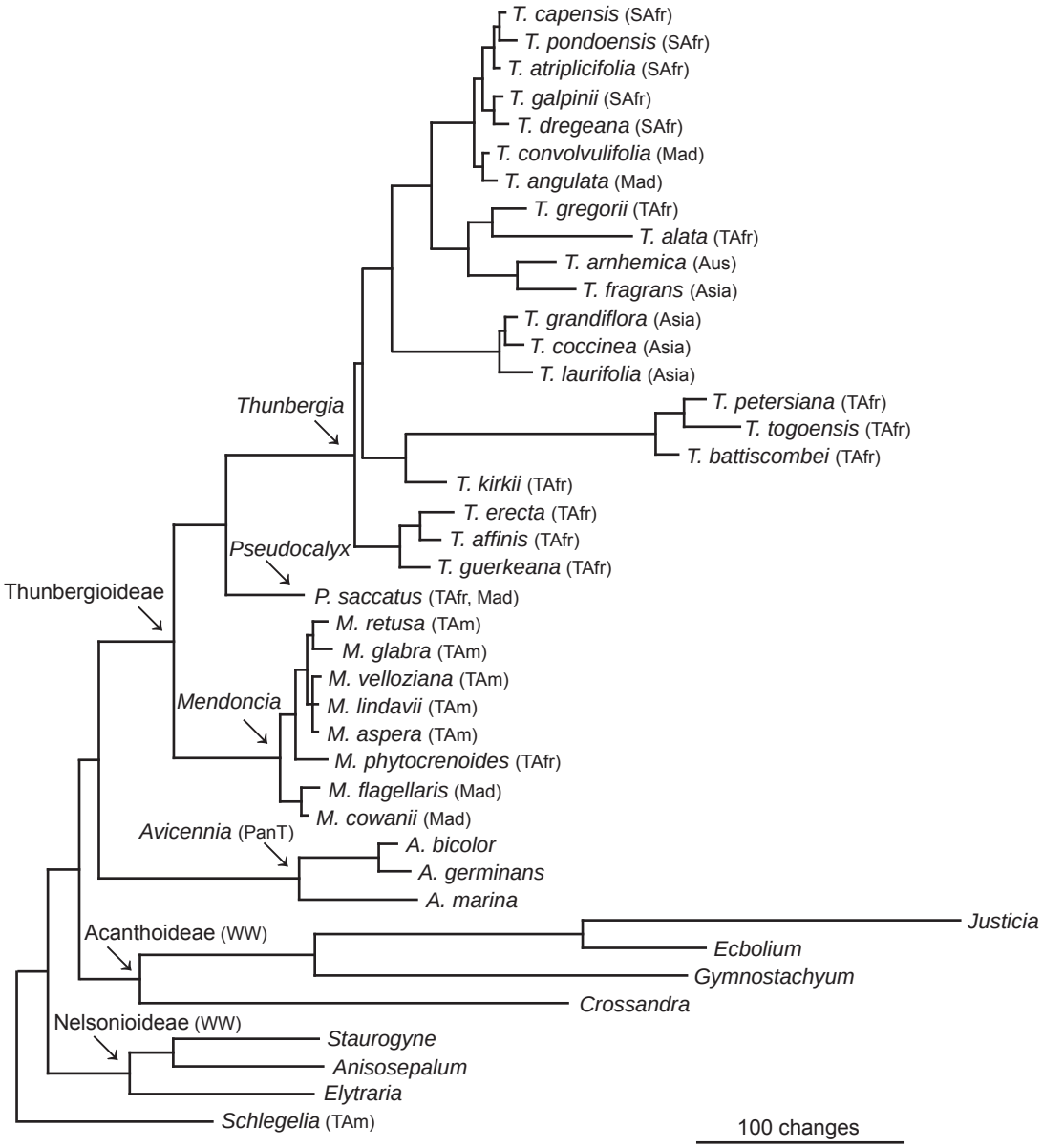

Fig. 2. Branch lengths and geographical distribution. Phylogram resulting from Bayesian analysis of the combined dataset. Branch lengths are proportional to number of changes. Abbreviations: Aus, Australia; Mad, Madagascar; PanT, Pantropical; SAfr, South Africa; TAfr, Tropical Africa; TAm, Tropical America; WW, Worldwide.

was originally described as belonging to *Thunbergia* by Wallich (1826; see also Matthew, 1983). Nevertheless, we aim to explore the exact positions of *Anomacanthus* and *Meyenia* in future studies as they may have bearing on the delimitation of the larger genera.

The monophyly of Thunbergioideae is also supported by morphological characters. The subfamily is characterized by having flowers subtended by two large persistent bracteoles (Fig. 3A–D), strongly reduced calyces (Fig. 3E–H), and a tendency to twine. Other characters

Fig. 3. Floral morphology. A–D, flowers of Thunbergioideae. Scale bars = 1 cm: A, *Thunbergia convolvulifolia*; B, *T. petersiana*; C, *Pseudocalyx saccatus*; D, *Mendoncia retusa*. E–H, calyx types in *Thunbergia*. Scale bars = 1 mm: E, many-lobed (*T. convolvulifolia*, corolla removed); F, six-lobed (*T. petersiana*, corolla removed); G, irregularly truncate (*T. coccinea*, corolla removed); H, truncate (*T. laurifolia*). I–L, anther appendages and dehiscence in *Thunbergia*. Scale bars = 1 mm: I, multicellular bristles and short slits in upper half (*T. erecta*); J, multicellular awns and short slits in lower half (*T. togoensis*); K, multicellular awns (indicated by arrowheads) and long slits (*T. angulata*); L, anther appendages absent and long slits (*T. fragrans*). M–P, Stigma types in *Thunbergia*. Scale bars = 0.5 mm: M, adaxial lobe folded and abaxial spreading (*T. convolvulifolia*); N, both lobes equal and folded (*T. guerkeana*); O, funnel-shaped with short lobes and trichome tufts (indicated by arrowhead, *T. petersiana*); P, funnel-shaped and adaxial lobe more folded than abaxial lobe (*T. coccinea*).

connecting *Thunbergia*, *Mendoncia* and *Pseudocalyx* are lignified unicellular bristles on the anthers (Figs. 3I, 4), an ephemeral endothecium, and similar inflorescences (Schönenberger & Endress, 1998).

Based on the present taxon sampling, *Thunbergia* and *Mendoncia* are two monophyletic genera and all analyses suggest that *Thunbergia* and *Pseudocalyx* are sister groups (BS = 99; PP = 1.0), and together they are sister to *Mendoncia*. There are several morphological traits that demonstrate the close relationship between *Thunbergia* and *Pseudocalyx*. The most obvious one is perhaps that *Thunbergia* and *Pseudocalyx* have dry capsules, like all other Acanthaceae, whereas *Mendoncia* possesses fleshy drupes; an exceptional character in Acanthaceae only shared with *Anomacanthus*. Another difference in the fruit is that *Thunbergia* and *Pseudocalyx* have two fertile locules, while in *Mendoncia* only one of two initiated locules develops fully (Schönenberger & Endress, 1998).

The dissimilarities prompted Lindau (1895) to place *Mendoncia* in a separate subfamily Mendoncioideae

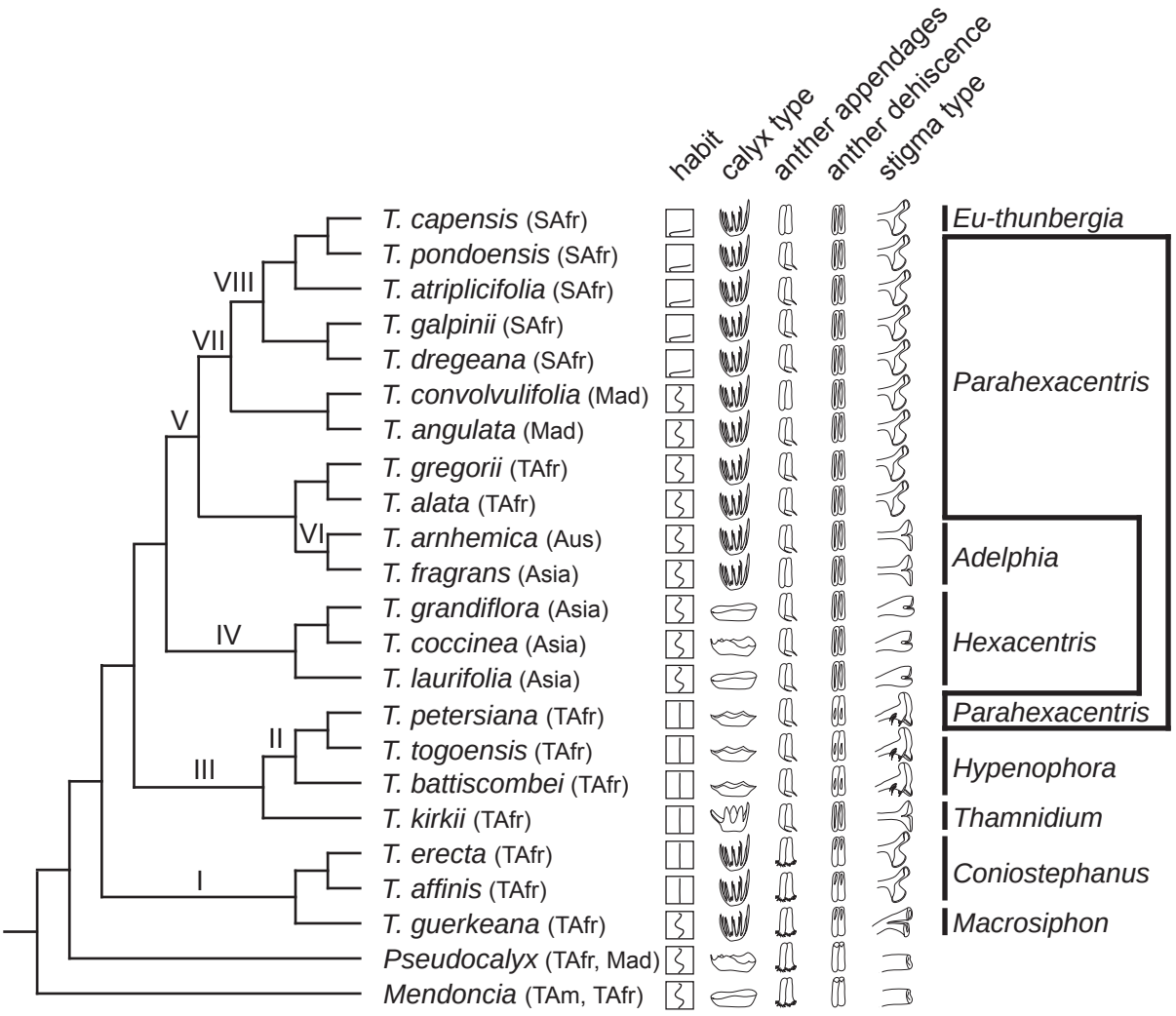

Fig. 4. Distribution of morphological characters in Thunbergioideae on the majority rule consensus tree resulting from Bayesian analysis of the combined dataset,. Bremekamp's (1955) subgeneric classification of *Thunbergia* is given on the right and geographic ranges are indicated after taxon names. Abbreviations: Aus, Australia; Mad, Madagascar; SAfr, South Africa; TAfr, Tropical Africa; TAm, Tropical America. Habit: creeping, twining, erect. Calyx type: many-lobed, truncate, irregularly truncate, five-lobed, six-lobed. Anther appendages: none, multicellular awns, unicellular bristles. Anther dehiscence: Long slits, short slits in lower half of theca, short slits in upper half of theca, pores. Stigma type (Schönenberger, 1999): adaxial lobe folded and abaxial spreading, funnel-shaped and both lobes equal, funnel-shaped and adaxial lobe more folded than abaxial lobe, funnel-shaped with short lobes and trichome tufts, both lobes equal and folded, both lobes very short. Data from Barker, 1986; Braz & al., 2002; Bremekamp, 1955; Breteler, 1998; Clarke, 1912; Heine, 1966; Retief & Reyneke, 1984; Schönenberger 1999; Turrill, 1919; A.J. Borg pers. obs.

(Table 1). Bremekamp (1953) went even further and raised Lindau's Mendoncioideae to family rank, mainly due to the divergent fruit characters. However, several authors have pointed out characters linking *Mendoncia* to Thunbergioideae s.str. and in particular to *Pseudocalyx*, which shares several features with *Mendoncia* including stigma shape and anther dehiscence (Fig. 4), indumentum (Brummitt, 1989; Schönenberger & Endress, 1998), and relative size of corolla lobes (Radlkofer, 1883). Brummitt (1989) noted that *Pseudocalyx* has important characters in common with both *Thunbergia* and *Mendoncia*, and considered it impossible to place *Mendoncia* in a separate family. Later, Schönenberger & Endress (1998) confirmed this and showed that the drupaceous fruits in *Mendoncia* and the dry capsules in *Thunbergia* and *Pseudocalyx* actually have an identical bauplan and that differences arise late during floral development. The main differences between *Thunbergia* and *Pseudocalyx* were thought to lay in bracteole characteristics, stigma structure, and anther dehiscence (i.e., longitudinal vs. porate; Bremekamp, 1955). However, Schönenberger & Endress (1998) showed that the differences in dehiscence mode are not clear cut between the two genera as intermediate dehiscence patterns occur in both groups.

Subgeneric relationships in *Mendoncia* and *Thunbergia*. — There is no comprehensive subgeneric taxonomic treatment of *Mendoncia*, although some of the species have earlier been ascribed to two isolated genera. Three of the *Mendoncia* species in the present study, *M. flagellaris*, *M. cowanii* and *M. phytocrenoides*, have earlier been ascribed to *Monachochlamys* Baker (*M. flagellaris* by Baker 1883; *M. cowanii* and *M. phytocrenoides* by Moore, 1929) and the latter two have also been ascribed to *Afromendoncia* Gilg (*A. phytocrenoides* by Gilg in Lindau, 1893; *A. cowanii* by Moore, 1906). Both genera were sunk back into *Mendoncia* by Benoist (1944), who argued that the few existing divergent characters in *Monachochlamys* are insufficient to distinguish them as a genus, and that some of the observations underlying the separation of *Afromendoncia* were actually incorrect. Our data show that neither *Monachochlamys* nor *Afromendoncia* are monophyletic. However, relationships among *Mendoncia* species correspond well to the geographic distribution of the genus (Fig. 4). The genus displays a trans-Atlantic disjunction, and the present results support a clade consisting entirely of American species, while *M. phytocrenoides,* which is sister to the American group, occurs in tropical regions of the African continent and the sister group to these two lineages is endemic to Madagascar.

Bremekamp (1955) presented a subgeneric classification of *Thunbergia*, with eight subgenera determined mainly by stigma and anther morphology, leaf and inflorescence features as well as growth form. A study of structure and development of flowers in *Thunbergia* (Schönenberger, 1999) largely agrees with this classification. The present molecular phylogenetic study partly supports Bremekamp's subgenera.

Based on the present taxon sampling, the subgenera *Macrosiphon* and *Coniostephanus* constitute a monophyletic group (clade I in Fig. 4) of African plants with a 10–15-toothed calyx (Fig. 3E) and unicellular lignified bristles at the base of the thecae (Fig. 3I). Furthermore, the thecae open by longitudinal slits that are restricted to the upper half, a characteristic not found elsewhere among examined species of *Thunbergia* (Fig. 3I). What separates the two subgenera is that in *Coniostephanus*, the upper stigmatic lobe is folded and the lower is flat and spreading, whereas in *Macrosiphon* the two stigmatic lobes are more or less equal in shape (Fig. 3M–N). Bremekamp (1955) commented that *Macrosiphon* and *Coniostephanus* must be closely related, and our molecular data strongly support them as sister groups (BS = 100; PP = 1.0). Moreover, Bayesian analyses suggest that the two subgenera are sister to the clade containing all other *Thunbergia* species.

Subgenus *Hypenophora* is represented in our study by *T. togoensis* and *T. battiscombei*. According to our results they are indeed closely related, but *T. petersiana*, referred by Bremekamp (1955) to subgenus *Parahexacentris*, is sister to *T. togoensis.* This classification by Bremekamp most likely is a mistake, since the species would be placed in *Hypenophora* by the characters he used for his classification. The clade of *Hypenophora* and *T. petersiana* (clade II in Fig. 4) is rather distinct: all are erect herbs, have a basically 6-lobed calyx (Fig. 3F), anthers that open by short slits in the lower half of the thecae, and anthers provided with curved awns (Fig. 3J). In addition, as Bremekamp (1955) noted, a remarkable feature of *Hypenophora* (and *T. petersiana*) is the funnel shaped stigma with a tuft of trichomes emerging on each side (Fig. 3O). This combination of characters is not present in any other *Thunbergia* clade and the floral morphology of this clade stands out against the rest of the genus. The distinctiveness of the clade is also apparent at the molecular level as the branch in the phylogeny leading to this clade is particularly long, i.e., has accumulated a relatively large amount of nucleotide substitutions (Fig. 2).

Subgenus *Thamnidium* consists of only two species, represented here by *T. kirkii*, which is characterized by having a distinctly pentamerous calyx and stigmas without lateral tufts, as opposed to its closest relatives in the subgenus *Hypenophora* (including *T. petersiana*). Also, *Thamnidium* have thecae that open by long slits extending over the whole length of the thecae, and in this respect is more similar to most other *Thunbergia* species than they are to subgenus *Hypenophora*. *Thamnidium* and *Hypenophora*, including *T. petersiana*, occur in tropical regions in Africa and form a well supported clade (III) in the present analysis (BS = 99; PP = 1.0).

Subgenus *Hexacentris* (clade IV) is a rather well defined group of Asian woody climbers with irregular, more or less truncate calyces (Fig. 3G–H), conspicuously awned thecae opening by long slits (Fig. 3K), and funnel shaped stigmas with short lobes (Fig. 3P). *Hexacentris* has earlier been proposed by some authors to constitute a separate genus (Nees von Esenbeck, 1847; Van Tieghem, 1908b), and the group is also strongly supported as being monophyletic by our data (BS = 100; PP = 1.0). Bremekamp (1955), however, found no reason to raise *Hexacentris* to genus level, and molecular data also show that it is deeply nested within *Thunbergia*.

Hexacentris is sister to a large clade (V) consisting of species which also have thecae that open by long slits, but they differ from *Hexacentris* by having calyces with 10–15 lobes (Fig. 3E) Bremekamp (1955) referred the species in this clade to three subgenera: *Parahexacentris*, *Eu-thunbergia* and *Adelphia*. *Parahexacentris* is the largest subgenus within *Thunbergia* containing African and Malagasy species. It is characterized by two well developed stigma lobes (Fig. 3M) in combination with rather long thecal awns (Fig. 3K). The same type of stigma is present in the small subgenus *Eu-thunbergia*. The thecal bases, however, are blunt in *Eu-thunbergia* (Fig. 3L). Molecular data reveal that *Eu-thunbergia*, represented by *T. capensis*, is in fact nested within *Parahexacentris*. It is worth mentioning, however, that there is at least one other species in *Parahexacentris* which also lacks thecal appendages, i.e., the Malagasy *T. convolvulifolia* (pers. obs.), which Bremekamp probably was not able to study himself (he mentions that his classification is partially based on descriptions; Bremekamp, 1955). The third subgenus, *Adelphia*, contains a number of Asian/Australian species, represented by *T. arnhemica* and *T. fragrans* in the present analyses. The clade formed by these two species (VI) is also nested within subgenus *Parahexacentris. Adelphia* has a different kind of stigma morphology with funnel shaped stigmata and equally sized lobes and thecae usually lacking appendages (Bremekamp, 1964). Flowers of this subgenus exhibit a hawkmoth pollination syndrome with only weakly monosymmetric flowers, a white corolla, and narrow corolla throat and tube (Schönenberger, 1999). The species included by Bremekamp in *Adelphia* have often been regarded as varieties of *T. fragrans* (Bremekamp, 1955), but whereas *T. fragrans* originates from India and Sri Lanka, *T. arnhemica* is only known from northern Australia, and is the only *Thunbergia* native to Australia (Bremekamp, 1964; Barker, 1986). At the molecular level, differences in nucleotide sequences between *T. fragrans* and *T. arnhemica* are as or more numerous than between many other species pairs in *Thunbergia* (Fig. 2).

Thus, Bremekamp's (1955) classification partially stands, but the large subgenus *Parahexacentris* is clearly paraphyletic with both *Eu-thunbergia* and *Adelphia* nested within it. Instead, a number of other monophyletic groups involving representatives of Bremekamp's subgenus *Parahexacentris* can be distinguished based on our analysis: A South African clade (clade VII in Fig. 4) comprising *Eu-thunbergia* and *Parahexacentris* p.p., all with a creeping habit (Retief & Reyneke, 1984) finds strong support in the Bayesian analysis (PP = 0.99). Together with two twining Malagasy representatives of *Parahexacentris*, i.e., *T. convolvulifolia* and *T. angulata,* the latter species form a strongly supported clade (clade VIII; BS = 100; PP = 1.0). The clade (BS = 91; PP = 100) with the two remaining species of Bremekamp's *Parahexacentris*, i.e., *T. alata* and *T. gregorii*, is sister to subgenus *Adelphia*.

Habit, calyx morphology, and anther dehiscence patterns seem to reflect molecular evolutionary relationships particularly well among *Thunbergia* species (Fig. 4), whereas the presence or absence of anther appendages and stigma structure are somewhat more labile and most likely are closely correlated with differences in pollination biology (see Schönenberger, 1999, for discussion).

The position of Thunbergioideae among the other acanthaceous lineages. — The results agree with the intrafamilial position of Thunbergioideae proposed in earlier higher-level studies (e.g., Hedrén & al., 1995; Scotland & al., 1995; McDade & al., 2000), with generally high bootstrap support values and Bayesian posterior probabilities. According to our analyses, the closest relative of Thunbergioideae is the pantropical mangrove genus *Avicennia*. This relationship is supported by high Bayesian PP values (0.98) and moderate bootstrap support (BS = 68). Schwarzbach & McDade (2002) showed that *Avicennia* belong in Acanthaceae and they too found consistent molecular evidence for a sister relationship between Thunbergioideae and *Avicennia* (max. BS = 78) using parsimony and maximum likelihood analyses of chloroplast and nuclear DNA regions. They also considered morphological traits but these did not provide evidence for a closer relationship of *Avicennia* to Thunbergioideae than to any other acanthaceous lineage. *Avicennia* appears to be morphologically highly specialized due to adaptations to the mangrove habitat, and exhibit many convergent characters found in several mangrove taxa (Sanders, 1997; Schwarzbach & McDade, 2002). Taxonomic adjustments concerning *Avicennia* will have to await future studies, both at the molecular and morphological level.

Acanthoideae are sister to Thunbergioideae plus *Avicennia* with strong support (PP = 1.00; BS = 89). This was also found by Schwarzbach & McDade (2002). Acanthoideae are distributed from the tropics to temperate regions and are recognized by their unique fruit type, an explosively dehiscent capsule with retinacula functioning as a lever ejecting the seeds from the fruits.

Our analyses place the subfamily Nelsonioideae, here represented by one species from each of its two major

genera (*Staurogyne*, *Elytraria*) plus one smaller genus (*Anisosepalum*), as sister to the clade comprising all other Acanthaceae (BS = 89; PP = 1.0). These findings are consistent with previous molecular studies (e.g., Scotland & al., 1995; McDade & al., 2000). Based on our limited taxon sampling, the Nelsonioideae appear monophyletic (BS = 100; PP = 1.0). However, Scotland & Vollesen (2000) were unable to find unambiguous morphological characters supporting the monophyly of Nelsonioideae, and no molecular study so far has included all genera of the subfamily. Nelsonioideae differ in several important aspects from other Acanthaceae. Like Thunbergioideae, they lack the retinaculate fruits characterizing Acanthoideae. Furthermore, Nelsonioideae have alternate bracts in the inflorescence and persistent endosperm as opposed to decussate bracts and seeds without endosperm in other Acanthaceae. Like Acanthoideae, Nelsonioideae are widespread in tropical and warm temperate regions.

Molecular data has certainly enhanced the understanding of evolutionary relationships among the first branching lineages of Acanthaceae, but some questions remain. The traditionally conflicting views regarding the taxonomy and systematic position of Thunbergioideae reflect the fact that indisputable morphological synapomorphies linking Thunbergioideae to other Acanthaceae have yet to be discovered. More molecular work as well as detailed comparative morphological studies are needed to shed light on structural evolution of *Avicennia*, and molecular work on Nelsonioideae will provide further information about the early evolution of Acanthaceae. The geographic ranges of genera and species in the basal lineages of Acanthaceae (see Fig. 2) raise some questions regarding the geographic origin of the various taxa. The disjunction in *Mendoncia* and a number of other trans-Atlantic disjunct genera are listed by Renner (2004), and are suggested to have occurred by long-distance dispersal or, in older groups (i.e., higher taxonomic ranks), may be attributed to the breakup of continents. The test of these and of more detailed hypotheses on the biogeographic history of Acanthaceae and in particular of Thunbergioideae and its subclades will, however, have to await future phylogenetic analyses based on a broader taxon sampling.

ACKNOWLEDGEMENTS

We thank Luis Diego Gomez, Eugenio González and Francisco Campos from the Organization for Tropical Studies (OTS) for help with collection permits in Costa Rica; Barry Hammel, Nelson Zamora and other staff at the National Biodiversity Institute (INBio) in Costa Rica for assistance and help in the field; Sylvain Razafimandimbison for organizing a field trip to Madagascar; and Carrie Kiel and Anbar Khodabandeh for sharing their knowledge in the molecular lab. For providing leaf material, we are grateful to Alexandre Antonelli, Thomas F. Daniel, Paul I. Forster and Quentin Luke. Finally, we thank two anonymous reviewers for critically reviewing the manuscript. Financial support for this research was received from the Jessup-McHenry Fund (Philadelphia Academy of Natural Sciences) and Hammarlundfonden (Stockholm University).

LITERATURE CITED

Baker, J.G. 1883. Contributions to the Flora of Madagascar Part 2: Monopetalae. *J. Linn. Soc., Bot.* 20: 159–236.

Barker, R.M. 1986. A taxonomic revision of Australian Acanthaceae. *J. Adelaide Bot. Gard.* 9: 1–292.

Baum, D.A., Small, R.L. & Wendel, J.F. 1998. Biogeography and floral evolution of baobabs (*Adansonia*, Bombacaceae) as inferred from multiple data sets. *Syst. Biol.* 47: 181–207.

Benoist, R. 1944. Contribution à la connaissance des Acanthacées africaines et malgaches. *Notul. Syst. (Paris)* 11: 137–151.

Bentham, G. 1876. Acanthaceae. Pp. 1060–1122 in: Bentham, G. & Hooker, J.D. (eds.), *Genera plantarum*, vol. 2. Reeve, London.

Braz, D.M., Carvalho-Okano, R.M. & Kameyama, C. 2002. Acanthaceae da reserva florestal Mata do Paraíso, Viçosa, Minas Gerais. *Revista Brasil. Bot.* 25: 495–504.

Bremekamp, C.E.B. 1953. The delimitation of the Acanthaceae. *Proc. Kon. Ned. Akad. Wetensch., Ser. C, Biol. Med. Sci.* 56: 533–546.

Bremekamp, C.E.B. 1955. The *Thunbergia* species of the Malesian area. *Verh. Kon. Akad. Wetensch., Afd. Natuurk., Sect. 2* 50(4): 1–90.

Bremekamp, C.E.B. 1964. On the systematic position of the Australian *Nelsonias* and *Thunbergias* and of the *Ruellia* species which by Domin were referred to *Aporuellia* Clarke. *Proc. Kon. Ned. Akad. Wetensch., Ser. C, Biol. Med. Sci.* 67: 301–306.

Bremer, B., Bremer, K., Heidari, N., Erixon, P., Olmstead, R.G., Anderberg, A.A., Källersjö, M. & Barkhordarian, E. 2002. 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. *Molec. Pylog. Evol.* 24: 274–301.

Breteler, F.J. 1998. Novitates Gabonenses 33. A new species of *Pseudocalyx* (Acanthaceae) from Gabon with a synopsis of all species of this genus. *Adansonia* 20: 271–280.

Brummitt, R.K. 1989. Against separating Mendonciaceae from Acanthaceae. *Acanthus* 5: 1–3.

Clarke, C.B. 1912. Acanthaceae. Pp. 1–92 in: Thiselton-Dyer, W.T. (ed.), *Flora Capensis*, vol. 5. Lovell Reeve, London.

Cronn, R.C., Small, R.L., Haselkorn, T. & Wendel, J.F. 2002. Rapid diversification of the cotton genus (*Gossypium*: Malvaceae) revealed by analysis of sixteen nuclear and chloroplast genes. *Amer. J. Bot.* 89: 707–725.

Cronquist, A. 1981. *An Integrated System of Classification of Flowering Plants*. Columbia University Press, New York.

Dahlgren, R. 1980. A revised system of classification of the angiosperms. *Bot. J. Linn. Soc.* 80: 91–124.

Doyle, J.J. & Dickson, E.E. 1987. Preservation of plant-samples for DNA restriction endonuclease analysis. *Taxon* 36: 715–722.

Felsenstein, J. 1985. Confidence-limits on phylogenies – an approach using the bootstrap. *Evolution* 39: 783–791.

Good, R.D. 1923. *Anomacanthus*: A new genus of Acanthaceae. *J. Bot.* 61: 161–164.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows95/98/NT. *Nucleic Acids Symp. Ser.* 41: 95–98.

Hedrén, M., Chase, M.W. & Olmstead, R.G. 1995. Relationships in the Acanthaceae and related families as suggested by cladistic analysis of *rbcL* nucleotide sequences. *Pl. Syst. Evol.* 194: 93–109.

Heine, H. 1966. Acanthacées. Pp. 1–250 in: Aubréville, A. (ed.), *Flore du Gabon*, vol. 13. Museum National d'Histoire Naturelle, Paris.

Huelsenbeck, J.P. & Ronquist, F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754–755.

Jordan, W.C., Courtney, M.W. & Neigel, J.E. 1996. Low levels of intraspecific genetic variation at a rapidly evolving chloroplast DNA locus in North American duckweeds (Lemnaceae). *Amer. J. Bot.* 83: 430–439.

Lindau, G. 1893. Übersicht über die bisher bekannten Arten der Gattung *Thunbergia* L. f. *Bot. Jahrb. Syst.* 41: 31–41.

Lindau, G. 1895. Acanthaceae. Pp. 274–354 in: Engler, A. & Prantl, K. (eds.), *Die natürlichen Pflanzenfamilien*, vol. 3b. Engelmann, Leipzig.

Matthew, K.M. 1983. *The Flora of the Tamilnadu Carnatic*. Rapinat Herbarium, Tiruchirapalli.

McDade, L.A., Daniel, T.F., Kiel, C.A. & Vollesen, K. 2005. Phylogenetic relationships among Acantheae (Acanthaceae): major lineages present contrasting patterns of molecular evolution and morphological differentiation. *Syst. Bot.* 30: 834–862.

McDade, L.A., Masta, S.E., Moody, M.L. & Waters, E. 2000. Phylogenetic relationships among Acanthaceae: evidence from two genomes. *Syst. Bot. 25*: 106–121.

McDade, L.A. & Moody, M.L. 1999. Phylogenetic relationships among Acanthaceae: evidence from noncoding trnL-trnF chloroplast DNA sequences. *Amer. J. Bot.* 86: 70–80.

Moore, S. 1906. Alabastra diversa. Part XIII. Acanthaceae. *J. Bot.* 44: 150–151.

Moore, S. 1929. Some new or rare African Acanthaceae. *J. Bot.* 67: 225–231.

Nees von Esenbeck, C.G. 1847. Acanthaceae. Pp. 46–519 in: De Candolle, A. (ed.), *Prodromus systematis naturalis regni vegetabilis*. Masson, Paris.

Nylander, J.A.A. 2004. *MrAIC.pl*. Program distributed by the author. Evolutionary Biology Center, Uppsala University.

Profice, S.R. 1988. *Mendoncia* Vell. ex Vand. (Acanthaceae): espécies ocorrentes no Brasil. *Arq. Jard. Bot. Rio de Janeiro* 29: 201–279.

Radlkofer, L. 1883. Ein Beitrag zur africanischen Flora. *Abh. Naturwiss. Vereine Bremen* 8: 369–442.

Renner, S. 2004. Plant dispersal across the tropical Atlantic by wind and sea currents. *Int. J. Pl. Sci.* 165: S23–S33.

Retief, E. & Reyneke, W.F. 1984. The genus *Thunbergia* in southern Africa. *Bothalia* 15: 107–116.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Sanders, R.W. 1997. The Avicenniaceae in the Southeastern United States. *Harvard Pap. Bot.* 10: 81–92.

Schönenberger, J. 1999. Floral structure, development and diversity in *Thunbergia* (Acanthaceae). *Bot. J. Linn. Soc.* 130: 1–36.

Schönenberger, J. & Endress, P.K. 1998. Structure and development of the flowers in *Mendoncia*, *Pseudocalyx*, and *Thunbergia* (Acanthaceae) and their systematic implications. *Int. J. Pl. Sci.* 159: 446–465.

Schwarzbach, A.E. & McDade, L.A. 2002. Phylogenetic relationships of the mangrove family Avicenniaceae based on chloroplast and nuclear ribosomal DNA sequences. *Syst. Bot.* 27: 84–98.

Scotland, R.W., Sweere, J.A., Reeves, P.A. & Olmstead, R.G. 1995. Higher-level systematics of Acanthaceae determined by chloroplast DNA-sequences. *Amer. J. Bot.* 82: 266–275.

Scotland, R.W. & Vollesen, K. 2000. Classification of Acanthaceae. *Kew Bull.* 55: 513–589.

Swofford, D.L. 2002. *PAUP: Phylogenetic Analyses Using Parsimony (*and Other Methods)*, vers. 4.0b10. Sinauer, Sunderland.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991. Universal primers for amplification of 3 noncoding regions of chloroplast DNA. *Pl. Mol. Biol.* 17: 1105–1109.

Takhtajan, A. 1997. *Diversity and Classification of Flowering Plants*. Columbia University Press, New York.

Thompson, J.D., Higgins, D.G. & Gibson, T.J. 1994. Clustal-W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673–4680.

Thorne, R.F. 1992. Classification and geography of the flowering plants. *Bot. Rev.* 58: 225–348.

Tomlinson, P.B. 1995. *The Botany of Mangroves*. Cambridge University Press, Cambridge.

Turrill, W.B. 1919. A revision of the genus *Mendoncia*. *Bull. Misc. Inform. Kew* 1919: 407–425.

Van Tieghem, M.P. 1908a. Structure du pistil et de l'ovule, du fruit et de la graine des Acanthacées. *Ann. Sci. Nat., Bot.* 7: 1–24.

Van Tieghem, M.P. 1908b. Restauration du genre *Hexacentre* dans la famille nouvelle des Thunbergiacées. *Ann. Sci. Nat., Bot.* 7: 111–116.

Wallich, N. 1826. *Tent. Fl. Napal.*, vol. 49. **publisher, city? full title?**

Appendix. Taxon; location; voucher; GenBank accession numbers *rps16*, *rpl16*, *trnT-trnL*.

Anisosepalum alboviolaceum (Benoist) E. Hossain; cultivated; National Botanic Garden of Belgium 95-0025-35 (BR); EU315785, EU315826, EU315867. *Avicennia bicolor* Standl.; Costa Rica; *Borg 10* (S); EU315786, EU315827, EU315868. *Avicennia germinans* (L.) Stearn; U.S.A.; *Schönenberger 725* (voucher in ethanol) (S); EU315787, EU315828, EU315869. *Avicennia marina* (Forssk.) Vierh.; Madagascar; *Borg 35* (S); EU315788, EU315829, EU315870. *Crossandra strobilifera* (Lam.) Benoist; Madagascar; *Borg*

Appendix. Continued.

24 (S); EU315789, EU315830, EU315871. *Ecbolium madagascariense* Vollesen; Madagascar; *Borg 37* (S); EU315790, EU315831, EU315872. *Elytraria imbricata* Pers.; Bolivia; *Daniel 10108* (CAS); EU315791, EU315832, EU315873. *Gymnostachyum ceylanicum* Arn. & Nees; cultivated; *Borg 55* (S); EU315792, EU315833, EU315874. *Justicia rizzinii* Wassh.; cultivated; *Borg 51* (S); EU315793, EU315834, EU315875. *Mendoncia aspera* Nees; Bolivia; *Schönenberger 416* (Z); EU315794, EU315835, EU315876. *Mendoncia cowanii* (S. Moore) Benoist; Madagascar; *Schönenberger A-49* (UPS); EU315795, EU315836, EU315877. *Mendoncia flagellaris* Benoist; Madagascar; *Borg 47* (S); EU315796, EU315837, EU315878. *Mendoncia glabra* Nees; Bolivia; *Schönenberger 440* (Z); EU315797, EU315838, EU315879. *Mendoncia lindavii* Rusby; Honduras; *Daniel 9510* (CAS); EU315798, EU315839, EU315880. *Mendoncia phytocrenoides* Benoist; Cameroon; *Schönenberger 50* (K); EU315799, EU315840, EU315881. *Mendoncia retusa* Turrill; Panama, *Daniel 8061* (CAS); EU315800, EU315841, EU315882. *Mendoncia velloziana* Mart.; Brazil; *Antonelli & Andersson 287* (GB); EU315801, EU315842, EU315883. *Pseudocalyx saccatus* Radlk.; Madagascar; *Schönenberger A-168* (UPS); EU315802, EU315843, EU315884. *Schlegelia fastigiata* Schery; Costa Rica; *Borg 4* (S); EU315784, EU315825, EU315866. *Staurogyne letestuana* Benoist; cultivated; National Botanic Garden of Belgium 20000119-77 (BR); EU315803, EU315844, EU315885. *Thunbergia affinis* S. Moore; cultivated; National Botanic Garden of Belgium 95-0081-92 (BR); EU315804, EU315845, EU315886. *Thunbergia alata* Bojer ex Sims; cultivated; *Borg 50* (S); EU315805, EU315846, EU315887. *Thunbergia angulata* Hils. et Bojer ex Hook.; Madagascar; *Borg 26* (S); EU315806, EU315847, EU315888. *Thunbergia arnhemica* F. Muell.; Australia; *Forster 11819* (BRI); EU315807, EU315848, EU315889. *Thunbergia atriplicifolia* E. Mey.; South Africa; *Daniel 9350* (CAS); EU315808, EU315849, EU315890. *Thunbergia battiscombei* Turrill; cultivated; *Daniel s.n.* (CAS); EU315809, EU315850, EU315891. *Thunbergia capensis* Retz.; cultivated; *Daniel s.n.* (CAS); EU315810, EU315851, EU315892. *Thunbergia coccinea* Wall.; cultivated; *Schönenberger 144* (Z); EU315811, EU315852, EU315893. *Thunbergia convolvulifolia* Baker; Madagascar; *Borg 44* (S); EU315812, EU315853, EU315894. *Thunbergia dregeana* Nees; South Africa; *McDade & Balkwill 1242* (PH); EU315813, EU315854, EU315895. *Thunbergia erecta* T. Anderson; cultivated; *Borg 49* (S); EU315814, EU315855, EU315896. *Thunbergia fragrans* Roxb.; cultivated; *Schönenberger 129* (Z); EU315815, EU315856, EU315897. *Thunbergia galpinii* Lindau; South Africa; *McDade & Balkwill 1250* (PH); EU315816, EU315857, EU315898. *Thunbergia grandiflora* Roxb.; cultivated; *Daniel s.n.* (CAS); EU315817, EU315858, EU315899. *Thunbergia gregorii* S. Moore; cultivated; *Daniel s.n.* (CAS); EU315818, EU315859, EU315900. *Thunbergia guerkeana* Lindau; Kenya; *Luke & al. 6197* (EA); EU315819, EU315860, EU315901. *Thunbergia kirkii* Hook. f.; cultivated; Botanic Garden of the University of Zürich 1988-1164 (Z); EU315820, EU315861, EU315902. *Thunbergia laurifolia* Lindl., cultivated; *Schönenberger 16* (Z); EU315821, EU315862, EU315903. *Thunbergia petersiana* Lindau; cultivated; Sweden, *Schönenberger 147* (UPS); EU315822, EU315863, EU315904. *Thunbergia pondoensis* Lindau; South Africa; *Daniel 9331* (CAS), EU315823, EU315864, EU315905. *Thunbergia togoensis* Lindau; cultivated; Royal Botanic Gardens Kew 1966-50003; EU315824, EU315865, EU315906.