Phylogenetic Relationships among Acantheae (Acanthaceae): Major Lineages Present Contrasting Patterns of Molecular Evolution and Morphological Differentiation

LUCINDA A. McDADE,[1,4] THOMAS F. DANIEL,[2] CARRIE A. KIEL,[1] and KAJ VOLLESEN[3]

[1]Department of Botany, Academy of Natural Sciences, 1900 Ben Franklin Parkway,
Philadelphia, Pennsylvania 19103;
[2]Department of Botany, California Academy of Sciences, 875 Howard Street, San Francisco, California 94103;
[3]Herbarium, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, U.K.
[4]Author for correspondence (mcdade@acnatsci.org)

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ABSTRACT. We used DNA sequence data from four regions ([1] nrITS; the chloroplast [2] *rps16* intron, [3] *trnG-S* spacer, and [4] *trnL-F* intron and spacer) to study phylogenetic relationships within tribe Acantheae (Acanthaceae). Our sample includes 18 of 20 recognized genera and 82 of ca. 500 species (plus two Justicieae as out-groups). Results of parsimony and Bayesian analyses were entirely congruent and provided strong support for monophyly of two sub-lineages of Acantheae, referred to here as the 'one-lipped corolla' and 'two-lipped corolla' lineages, reflecting notable differences in corolla morphology. Subsequent analyses were of the two sublineages separately in order to include all characters (a hypervariable region of *trnG-S* could not be aligned across the full range of taxa but could be aligned within sublineages). The 'one-lipped corolla lineage' comprises six clades of Old World taxa related as follows: [*Crossandra* (*Sclerochiton* clade {*Cynarospermum* [*Blepharis* (*Acanthus* clade + *Acanthopsis*)]})]. All presently recognized genera are strongly supported as monophyletic, except that *Blepharis dhofarensis* is placed with species of *Acanthus*, with strong support from both parsimony and Bayesian inference (monophyly of *Blepharis* was rejected by both parsimony and likelihood). Alternate hypotheses based on calyx and androecial morphology regarding *Crossandrella* and *Streptosiphon* could not be rejected, but placement of these genera with some species of *Crossandra* based on pollen was rejected. The 'two-lipped corolla lineage' is strongly supported and includes one clade of Old World plants (the *Stenandriopsis* clade) that is sister to a strongly supported clade that includes all New World Acantheae as follows: [*Stenandrium* clade (*Neriacanthus* {*Aphelandra* lineage})]. The *Aphelandra* lineage includes the 'armed' *Aphelandra* clade and a polytomy of six unresolved clades: (1) *A. squarrosa*, (2) *Encephalosphaera* clade, (3) *Geissomeria* clade, (4) *A. aurantiaca* clade, (5) *A. pulcherrima* clade, (6) *Rhombochlamys*. In contrast to patterns in the one-lipped lineage, genera in the two-lipped lineage are mostly not monophyletic nor are relationships among them strongly supported by our molecular data or by morphological synapomorphies. We discuss these results in the context of evidence from other sources including macromorphology, palynology, chromosome numbers, and geographic distribution.

The tribe Acantheae (sensu Scotland and Vollesen 2000) includes 20 currently recognized genera with a total of ca. 500 species in both the Old World (OW) and New World (NW) (Table 1). The lineage has been shown to be monophyletic in a number of studies based on DNA sequence data (McDade and Moody 1999; McDade et al. 2000b), and is unique among Acanthaceae in having four monothecate stamens. Although four stamens certainly are plesiomorphic for the family and monothecate anthers have evolved in parallel in a few other distantly related lineages in the family, no other acanths combine the plesiomorphic number with the apomorphic monothecate condition. Acantheae also have colpate pollen grains (Fig. 1A–F) that lack the pore-like endoapertures that are present in most other Acanthaceae and Lamiales; this trait has been interpreted as a synapomorphy by Scotland and Vollesen (2000). The tribe may also be distinguished from its sister group, Ruellieae, by absence of cystoliths. Acantheae and Ruellieae together comprise Acanthoideae (or Acanthaceae s.s.), a lineage of some 4000 species in more than 200 genera that is marked by the synapomorphy of retinacula that subtend the seeds. Within Acantheae, current generic delimitations,

classifications or informal hypotheses of relationship (e.g., Vollesen 1991), and our earlier, morphology-based work on NW (e.g., Daniel 1983, 1985, 1991; McDade 1984, 1992) and OW Acantheae (e.g., Vollesen 1990b, 1994, 2000) provide a series of hypotheses regarding relationships, as outlined below, summarized in Table 2, and tested in this paper.

Most Acantheae can be sorted as NW plants that have bilabiate corollas (e.g., Fig. 2D,E) and OW plants that have corollas with all five lobes directed ventrally (Fig. 2B,C). However, plants of the African and Malagasy species traditionally placed in *Stenandriopsis* S. Moore have corollas with a two-lobed upper lip much like many NW plants (Fig. 2A; Vollesen 1992), specifically those placed in *Stenandrium* Nees. Vollesen (1992) synonymized *Stenandriopsis* with *Stenandrium* and transferred species accordingly. Further, Vollesen suggested that *Stenandrium* (including *Stenandriopsis*) is more closely related to OW Acantheae than to NW Acantheae. The results of McDade et al. (2000b) indicate that New World *Stenandrium* is part of a clade that includes other NW Acantheae, and that OW Acantheae form a separate clade. However, this result was based on limited sampling, especially of OW Acantheae (e.g.,

TABLE 1. Genera, species richness, and range of Old World and New World Acantheae. Total number of species per genus is followed, in parentheses, by number of species sampled here. Note that *Stenandrium* is treated as present in both the Old and New Worlds; OW plants were transferred from *Stenandriopsis* S. Moore to *Stenandrium* by Vollesen (1992).

	No. of species (sampled here)	Range, notes
Old World		
Acanthopsis Harvey	±4 (3)	Southern Africa
Acanthus L.	20 (8)	S Europe, Africa, S. Asia, Malesia, Australasia
Achyrocalyx R. Benoist	3 (1)	Madagascar
Blepharis Jussieu	129 (15)	Africa through Arabia, southern Asia, southeastern Asia
Crossandra Salibury	52 (6)	Africa, Madagascar, Arabia, Indian Subcontinent
Crossandrella C. B. Clarke	2 (1)	Tropical Africa
Cynarospermum Vollesen	1 (1)	India
Sclerochiton Harvey	18 (4)	Tropical & southern Africa
Stenandrium Nees	17 (5)	Africa, Madagascar (= *Stenandriopsis* S. Moore)
Streptosiphon Mildbr.	1 (1)	Tanzania
New World		
Aphelandra R.Br.	180 (22)	Mexico to Peru, Bolivia, Argentina
Cyphacanthus Leonard	1 (0)	Colombia
Encephalosphaera Lindau	3 (1)	Brazil, Colombia, Ecuador, Peru
Geissomeria Lindl.	15 (2)	Tropical America, mostly Brazil
Holographis Nees	16 (3)	Mexico
Neriacanthus Benth.	5 (3)	Panama, Colombia, Venezuela, Ecuador, Peru; Jamaica (*N. purdieanus*)
Orophochilus Lindau	1 (0)	Peru
Rhombochlamys Lindau	2 (1)	Colombia
Salpixantha Hook.	1 (1)	Jamaica
Stenandrium Nees	40–50 (2)	Florida, Texas—Argentina, West Indies

no species of OW *Stenandrium* were included). Here we test monophyly of NW and OW Acantheae with nearly complete sampling at the generic level. We hypothesize that the OW plants with a one-lipped corolla form a clade; as the two-lipped corolla is a symplesiomorphy, we make no hypothesis regarding the phylogenetic status of Acantheae that share this trait. We also treat Vollesen's transfer of species of *Stenandriopsis* to *Stenandrium* as a hypothesis of monophyly and further test his hypothesis that these plants are more closely related to OW than to NW Acantheae.

Vollesen (1991) proposed two groups of OW genera and provided characters to distinguish them. Members of Group 1 (*Crossandra* Salisb., *Crossandrella* C. B. Clarke, *Streptosiphon* Mildbr. and OW *Stenandrium*) have flowers with included stamens with short filaments of texture typical of Acanthaceae. In contrast, members of Group 2 (*Acanthopsis* Harv., *Acanthus* L., *Blepharis* Juss. and *Sclerochiton* Harv.) have flowers with exserted stamens with elongate, bony filaments. The characters distinguishing Group 2 are likely apomorphic and we thus evaluate monophyly of this group as an hypothesis; those of Group 1 are likely plesiomorphic and we do not expect that group to be monophyletic. With regard to other putative synapomorphies, *Crossandrella* and *Streptosiphon* are somewhat enigmatic. With *Acanthopsis*, *Acanthus*, and *Blepharis*, *Crossandrella* shares a distinctive and undoubtedly apomorphic 4-lobed 'fused, sheathing, reduced' calyx

(i.e., the two anterior segments are *fused,* and the large posterior and anterior sepals are external to and *sheathing* the highly *reduced* lateral lobes) (Fig. 2G); other OW Acantheae and all NW Acantheae have a five-lobed calyx with segments that differ only in width (Fig. 2F). With *Acanthopsis*, *Acanthus*, *Blepharis*, and *Sclerochiton*, *Streptosiphon* shares filaments inserted on a thickened flange of the interior corolla surface. Plants of *Streptosiphon*, however, lack the bony filament texture of plants of the other four genera. Finally, with many species of *Crossandra*, plants of *Streptosiphon* and *Crossandrella* share pollen grains that are elongate in equatorial view (i.e., prolate to perprolate), triangular in polar view, and have caveate exine in the apices of the triangle (hereafter 'triangular-caveate,' Figs. 1C, 3; see also figures in Furness 1990, 1994). The last two characteristics are likely apomorphic and we test the hypothesis that these pollen characters mark a lineage. Clearly, all of these interesting morphological features cannot mark monophyletic groups and we thus use our phylogenetic results to shed light on the evolution of these traits.

Among NW Acantheae, all species of *Holographis* Nees and *Stenandrium* for which chromosome counts have been obtained have $x = 13$ and Daniel et al. (1984) hypothesized a close relationship among these plants. We test this hypothesis here along with the idea that *Aphelandra* R. Br. (all vouchered counts to date based on $x = 14$) is more distantly related to the first
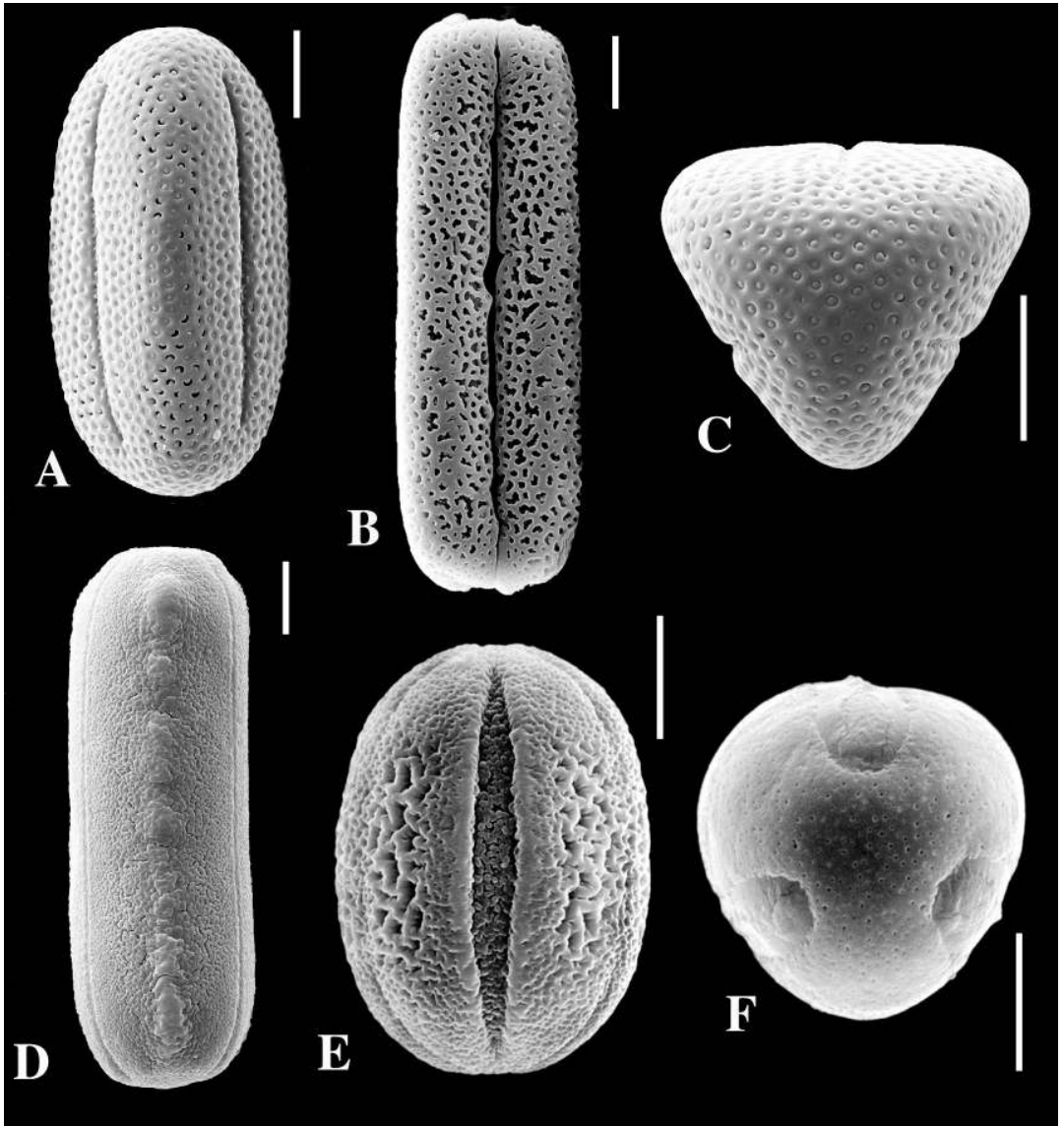
FIG. 1. Tricolpate pollen of Acantheae from the Old World (A—C) and the New World (D—F). A. *Acanthus mollis* (*Daniel s.n.*), intercolpal view. B. *Crossandra pungens* (*Daniel s.n.*), colpal view. C. *Acanthus spinosus* (*Sagorski s.n.*), polar view. D. *Geissomeria tetragona* (*Daniel et al. 10111*), intercolpal view. E. *Aphelandra gigantiflora* (*Breedlove & Daniel 70900*), colpal view. F. *Holographis tamaulipica* (*Woodruff et al. 166*), polar view. Scales = 10 μm.

two genera. Although many OW Acantheae have sharply toothed leaves and/or bracts (with spinescent stems in a few; see, e.g., figures in Vollesen 1990a, 2000), most NW Acantheae are unarmed. About 35 species of *Aphelandra*, some members of the genus *Encephalosphaera* Lindau (including the species included here), one species of *Holographis*, and unispecific *Orophochilus* Lindau have at least sharply toothed leaves and/or bracts and many also have spinescent stems (e.g., Fig. 4). Given the morphological diversity comprised by the large genus *Aphelandra* and the lack of clear characters to distinguish many genera in the NW

(see below), we hypothesize monophyly of a lineage comprising 'armed' plants.

In both NW and OW Acantheae, a small number of genera have >100 species, whereas most genera are smaller and several have fewer than five species (Table 1). Although these latter genera are often diagnosed on the basis of apomorphies, recognition of some of them may render other larger genera paraphyletic. Further, both Daniel (1985, 1991) and Vollesen (1992) have lamented the absence of macromorphological characters to distinguish some genera of Acantheae (e.g., *Aphelandra* and *Stenandrium* in the New World).

TABLE 2. Hypotheses regarding clades and relationships among them. As indicated, hypothesis 2 and parts of hypothesis 10 are supported by the MP (maximum parsimony) and BL (Bayesian likelihood) trees. Other hypotheses were contrasted with the MP result using the parsimony-based Templeton test (z value) and likelihood based Kishino-Haswegawa RELL test (K-H RELL); for parsimony, we report the difference between the shortest trees and those consistent with the constraint (% difference is calculated relative to lengths of the relevant MP trees).

| Hypothesis	Results of test
H1. Old and New World Acantheae are reciprocally monophyletic	Reject (+26 steps, 0.8%, z = −4.0119, P < 0.0001; K-H RELL P = 0.014)
H2. Old World Acantheae with one-lipped corollas form a clade	Accept (MP and BL trees)
H3. OW and NW *Stenandrium* are together monophyletic exclusive of other Acantheae (except *Achyrocalyx*)	Reject All taxa: (+33 steps, 1.0%, z = −4.7143, P < 0.0001; K-H RELL P < 0.001); Two-lipped lineage only: (+38, 2.2%, z = −5.3571, P < 0.0001; K-H RELL P < 0.001)
H4. OW + NW *Stenandrium* is more closely related to Old World than to New World Acantheae	Reject (+62 steps, 1.8%, z = −6.3948, P < 0.0001; K-H RELL P < 0.001)
H5. OW plants that share bony filaments (i.e., *Acanthopsis, Acanthus, Blepharis, Cynarospermum, Sclerochiton*) form a clade	Cannot reject (+6 steps, 0.3%, z = −1.2792, P = 0.2008; K-H RELL P = 0.173)
H6. Plants that share the distinctive 4-lobed 'fused, sheathing, reduced' calyx form a clade (Fig. 2G; *Acanthopsis, Acanthus, Blepharis, Cynarospermum, Crossandrella*)	Cannot reject (+4 steps, 0.2%, z = −1.0000, P = 0.3173; K-H RELL P = 0.145)
H7. Plants that share filaments inserted on a thickened flange of the interior corolla surface form a clade (i.e., *Acanthopsis, Acanthus, Blepharis, Cynarospermum, Sclerochiton, Streptosiphon*)	Cannot reject (+3 steps, 0.1%, z = −0.6000, P = 0.5485; K-H RELL P = 0.133)
H8. Plants that share 'triangular-caveate' pollen form a clade (Fig. 3; *Crossandra horrida, C. pungens, Crossandrella, Streptosiphon*)	Reject (+32 steps, 1.5%, z = −3.8247, P < 0.0001; K-H RELL, P < 0.001)
H9. *Cynarospermum* is part of *Blepharis*	Cannot reject (+6 steps, 0.3%, z = −1.5000, P = 0.1336; K-H RELL, P = 0.286) (but MP trees consistent with constraint place *Cynarospermum* basal to *Blepharis*)
H10. Recognized genera of OW Acantheae (*Acanthopsis, Acanthus, Blepharis, Crossandra, Sclerochiton*) and NW Acantheae (*Aphelandra, Holographis, Neriacanthus*) are monophyletic	Accept (MP and BL trees): *Acanthopsis, Crossandra, Holographis* (including *Aphelandra verticillata*), *Sclerochiton*
	Cannot reject: *Acanthus* (+6 steps, 0.3%, z = −1.2792, P = 0.2008; K-H RELL, p = 0.172) (but MP trees consistent with constraint place *Blepharis dhofarensis* sister to *Acanthus*)
	Reject: *Aphelandra* including *A. verticillata* (+159 steps, 9.0%, z = −11.0424, P < 0.0001; K-H RELL, P < 0.001); *Aphelandra* except *A. verticillata* (+62 steps, 3.5%, z = −6.3803, P < 0.0001; K-H RELL, P < 0.001); *Blepharis* (+18 steps, 0.9%, z = −3.3376, P < 0.0001; K-H RELL, P = 0.003); *Neriacanthus* (+87 steps, 4.9%, z = −8.7973, P < 0.0001; K-H RELL P < 0.001)
H11. OW *Stenandrium* is monophyletic exclusive of *Achyrocalyx*	Reject (+ 13 steps, 0.7%, z = −2.5019, P = 0.0124; K-H RELL P = 0.025)
H12. *Holographis* and *Stenandrium* form a clade	Accept (MP and BL trees) (Including Jamaican Acantheae)
H13. "Armed" plants in the New World form a clade	Reject (+22 steps, 1.2%, z = −2.9436, P = 0.0032; K-H RELL P < 0.001)
H14. *Aphlendra pulcherrima* clade monophyletic (i.e, exclude *Rhombochlamys*)	Accept (+2 steps, 0.1%; z = −7.071, P = 0.4796; K-H RELL P = 0.254); also weak support for placement of *Rhombochlamys* with *A. pulcherrima* clade (bootstrap < 50%)
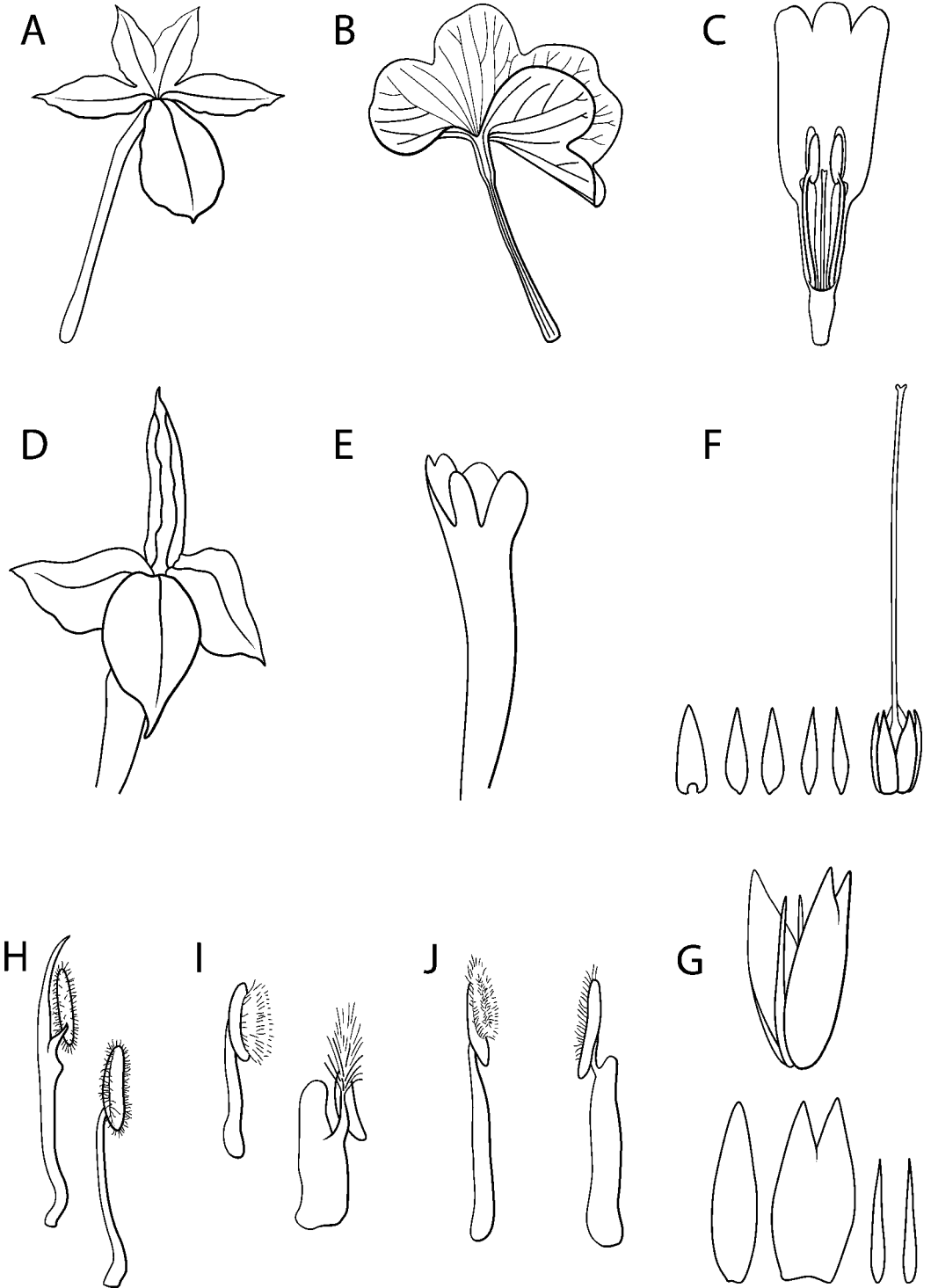
FIG. 2. Corolla, calyx and stamen morphology in Acantheae. A-C, corollas of Old World Acantheae. A. Two-lipped corolla of *Stenandriopsis thomense* (Milne-Redh.) Vollesen (redrawn from Fig. 1F in Vollesen [1992]). B. One-lipped corolla of *Crossandra multidentata* Vollesen, dorsal view, all five corolla lobes are directed ventrally (redrawn from Fig. 3H in Vollesen [1997]). C. One-lipped corolla of *Cynarospermum asperrimum* (Nees) Vollesen, dorsal view, note that all five corolla lobes are directed ventrally and the dorsal corolla tube is extremely short (redrawn from Fig. 1G in Vollesen [1999]). D, E. Corollas of New World Acantheae. D. *Aphelandra aurantiaca*, the upper lip is shallowly two-lobed and folded to form a sheath-like structure (drawn from *McDade 322* [DUKE]). E. *Geissomeria*, Edwards's Botanical Register [1827]). F. Five-lobed calyx characteristic of *Crossandra*, *Sclerochiton*, and *Streptosiphon* and most NW Acantheae. Right, ventral view of intact calyx and gynoecium; left, calyx dissected to show
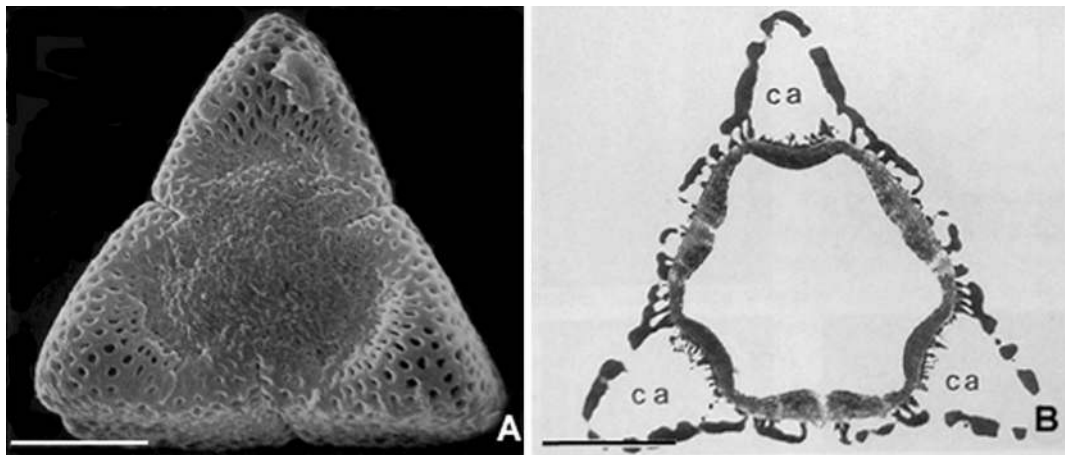
FIG. 3. Triangular-caveate pollen characteristic of some species of *Crossandra*, and of *Crossandrella* and *Streptosiphon*. A, Scanning electron micrograph, polar view, showing triangular shape; B, Transmission electron micrograph, polar view, exine is caveate (ca) at the apices of the triangle. (Reproduced from Fig. 6 in Furness [1990] of *Crossandra sulphurea* G. Taylor). Scales = 10 μm.

Here we test monophyly of these larger genera with species sampled to represent all but two small genera. We also test infrageneric classifications, when these exist.

Here we present results of parsimony and Bayesian likelihood analyses of DNA sequence data from four regions (i.e., [1] nuclear ribosomal ITS; the chloroplast [2] *trnG-S* spacer and [3] *rps16* intron, and [4] *trnL-F* region which includes the *trnL-F* spacer, the *trnL* 3′ exon, and the *trnL* intron). When the hypotheses advanced above are not consistent with the MP and Bayesian likelihood results, we use constraint trees to test for significant differences between the phylogenetic results and the hypothesized relationship. Finally, we discuss these results in the context of evidence from other sources including macromorphology, palynology, chromosome numbers, and geographic distribution.

MATERIALS AND METHODS

Taxon Sampling. We obtained sequences for 82 species representing 18 of the 20 currently recognized genera of Acantheae. The NW genera *Cyphacanthus* Leonard (1 sp.) and *Orophochilus* (1 sp.) are poorly known, and collections are few and old such that

DNA was not available. Also, not included is unispecific *Strobilacanthus* Griseb., described by Grisebach (1858) from Panama, and included in Aphelandreae by Grisebach and also by Lindau (1895). Plants were described as shrubs with quaternate leaves, monothecate anthers, and scaly seeds, a combination of characteristics that is not known among NW Acantheae (indeed, seeds with appressed scales are not known among NW Acantheae; this character is suggestive of *Crossandra*, which may have been cultivated in Panama at that time). No collections were cited in the protologue and none bearing this name have been located. The genus was included among Acantheae by Scotland and Vollesen (2000), but because its identity and disposition remain uncertain it is not considered further here.

Because one of our goals was to test monophyly of the larger genera (e.g., *Aphelandra* [180 spp.], *Blepharis* [129 spp.], *Crossandra* [52 spp.]), we have included species that represent as completely as possible the recognized subgeneric taxa, if any, and the range of morphological diversity and geographic distribution. Appendix 1 lists taxa included here, along with voucher data and sources of sequences that were not newly generated for this project. Two species of Justicieae (Appendix 1) were used as out-groups for purposes of rooting our phylogenetic hypotheses.

Molecular Methods. Fresh leaf material, leaf material dried in silica gel or recently collected herbarium specimens were used as sources of DNA. Total genomic DNA was extracted using the modified CTAB method of Doyle and Doyle (1987). Older herbarium material sometimes gave fragmented DNA or low yields, in which case DNA was extracted using QIAGEN DNeasy® kits for plant tissue. Procedures for purifying genomic DNA and for amplifying the *trnL-F* and nrITS regions were as described in detail

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widest posterior segment, narrower paired anterior segments, and narrowest paired lateral segments. (Redrawn from Fig. 2, McDade [1982] of *Aphelandra golfodulcensis* McDade.) G. Four-lobed calyx characteristic of *Crossandrella*, *Cynarospermum*, *Blepharis*, *Acanthopsis* and *Acanthus*. Above, oblique ventral view of intact calyx with lobes separated to show fused anterior segments, highly reduced (both length and width) lateral segments, and posterior segment; the anterior and posterior segments form a sheath over the lateral segments. Below, posterior, anterior and lateral segments. (Redrawn from Plate 22: 13,14, Heine [1966] of *Crossandrella dusenii* [Lindau] S. Moore.) H-J. *Blepharis* species have the filaments of the anterior pair of stamens thickened, flattened and ornamented with an apical appendage that varies in form as shown here; the densely bearded ventral surface of the thecae is a synapomorphy for the *Cynarospermum—Acanthopsis* clade. H. *Blepharis aspera* Oberm. (Redrawn from Fig. 10L in Vollesen [2000]) I. *Blepharis glumacea* S. Moore (Redrawn from Fig. 31G in Vollesen [2000]) J. *Blepharis maderaspatensis* (L.) Roth. (Redrawn from Fig. 33J in Vollesen [2000]).

FIG. 4. *Aphelandra acanthifolia* Hook., showing armed stems and leaf margins. (Reproduced from Plate 113 in Hooker [1837]).

by McDade and Moody (1999) and McDade et al. (2000b), respectively.

The *rps16* intron was amplified using the 5′ exon and 3′ exon primers and reaction conditions as in Downie and Katz-Downie (1999). Once we had a number of *rps16* sequences for Acanthaceae, we were able to design new primers slightly internal to the above-mentioned primers at both the 5′ (ACA5′rps16: GAGGACARRAT CCGTTGTGGAT) and 3′ (ACA3′rps16: AGACGGCTCATTGGGA TA) termini of the spacer. These worked to amplify and/or sequence *rps16* templates that had failed with the original primers. Reaction conditions were as for *trnL-F* (McDade and Moody 1999) except that cycle duration was adjusted (shorter) for length of the amplicon. PCR products were cleaned using QIA-GEN QiaQuick® PCR purification kits.

For the *trnG-S* sequences, primers were as in Hamilton (1999) and reaction conditions were as for the other cp loci, again adjusting for template length. At the outset of our work with *trnG-S*, a number of DNAs yielded multiple PCR products. We sequenced a number of these products and determined that they were partial fragments of the *trnG-S* spacer. To reduce this problem, we modified PCR conditions by increasing the annealing temperature and reducing the concentration of magnesium chloride. To optimize sequencing results, most PCR products were run on a 1% agarose gel for several hours, the longest band was excised, and the template was purified using QIAGEN QIAQuick® gel extraction kits.

Sequences were generated on ABI or Beckman automated sequencers using initially the same primers as in amplification. The internal 'its2' and 'its3' (Baldwin 1992) and 'd' and 'e' primers (Taberlet et al. 1991) were used for some templates of nrITS and *trnL-F*, respectively, to complete sequences and when the primers used in amplification gave unsatisfactory data. For the *rps16* intron, the additional primers designed for Acanthaceae, as described above, were used to sequence the region when one or both of these primers was used to amplify template and when other primers used in amplification did not give clean sequence data. All *trnG-S* sequences were generated using the same primers as in amplification. For most samples, both strands were sequenced for verification and to complete the sequence. Electropherograms of all sequences were proofread manually. Overlapping portions were reconciled by reverse-complementing one, aligning the two, and double-checking any inconsistencies against the electropherograms; mismatches that could not be resolved were coded as uncertain.

Alignment and Analysis. Sequences for each DNA region were aligned separately by eye in SeqApp 1.9a169 (Gilbert 1992). Sequences were easily aligned across sampled taxa with two exceptions. First, nrITS is both difficult to sequence and highly divergent in some OW Acantheae. We fine-tuned the alignment until further changes had no effect on topology or indices of fit of the data to the tree (i.e., consistency and retention indices). Second, a region near the middle of the *trnG-S* spacer was extremely variable in length (from 15 to 170 bp in these taxa) and in sequence. This region was omitted from the analysis of the full taxon set.

As noted by a number of authors (e.g., Gielly et al. 1996; Kim et al. 1996; McDade and Moody 1999), the non-coding chloroplast regions have a relatively high frequency of parsimony informative indels (i.e., length mutations). Eighteen, ten, and 14 indels were added to the *trnL-F, trnG-S,* and *rps16* data matrices, respectively, as presence/absence characters (no indels were added from the hypervariable region of *trnG-S*). Nine indels were also added to the nrITS data set. The indels scored were identified conservatively (i.e., with common 5′ and 3′ termini) and were parsimony informative (i.e., shared by two or more taxa). Table 3 compares the four DNA regions in terms of length, variability, number of taxa sequenced, and missing data.

Data matrices for the four DNA regions were prepared as Nexus files in MacClade version 4.0 (Maddison and Maddison 2000). As indicated in Appendix 1 and Table 3, sequences for all four loci were not obtained for all taxa for three reasons. First, amplifying and sequencing DNA from older herbarium material was sometimes difficult (e.g., OW *Cynarospermum*, NW *Rhombochlamys*). Second, nrITS was very difficult to amplify and sequence for some Old World Acantheae. Complete or nearly complete (i.e., < 10% missing) nrITS data were obtained for only 20 of 45 OW Acantheae; partial sequences were obtained for 10 additional taxa. In contrast, nrITS was sequenced successfully for 35 of 37 NW Acantheae. Lastly, *trnL-F* varies little among close relatives (Table 3), and our strategy was therefore to sequence this locus for a representative sample of species from each genus. We evaluated the impact of slightly different samples of taxa for each locus by analyzing four data sets that included the full taxonomic range of taxa: (1) all taxa for which sequence data were available from ≥ 2 cp loci, all cp characters except hypervariable region of *trnG-S*; (2) all taxa for which we had nrITS data, nrITS only; (3) all taxa for which sequence data were available from ≥ 2 loci, all characters except hypervariable region of *trnG-S,* (4) all taxa for which sequence data were available for at least one cp locus and for nrITS, all characters except hypervariable region of *trnG-S.* Analyses of all four data sets yielded strong support for reciprocal monophyly of two lineages of Acantheae. Within each of these lineages, the hypervariable region of the *trnG-S* spacer was alignable with confidence. Therefore, to maximize character data (and thus our ability to resolve relationships among close relatives), two partitions of the data were used in subsequent analyses, each of which included all characters. For each of these, out-groups were two relatively basal taxa from the alternative lineage of Acantheae (Appendix 1); the hypervariable region of *trnG-S* was scored as missing for the out-group taxa. Data matrices are deposited in TreeBase (study accession S1358, matrix accessions M2399–2401).

TABLE 3. Characteristics of four DNA regions used here. To facilitate comparison among regions, statistics b–g are for the data set that included all taxa for which we had both nuclear and chloroplast data (i.e., data set 4, 65 taxa). (a) Aligned length includes all taxa sequenced for each locus. (h) Missing data are reported as number of total of 82 taxa included in the separate analyses of the one-lipped [39 taxa] and two-lipped [43 taxa] lineages and (i) as percent of sites (including only taxa for which any sequence data were available for that locus; because of problems sequencing nrITS for OW Acantheae, we report also % missing data excluding taxa with >10% missing data). Because a hypervariable region of *trnG-S* could not be aligned across all taxa, this region was excluded for calculation of d, f, g. Below the first entries for *trnG-S*, for statistics a–c and e, we report values for the one-lipped and two-lipped lineages separately; these include the hypervariable region but exclude gaps added for insertions present only in taxa belonging to the other lineage. Statistics for the *trnL-F* region include the *trnL-F* spacer, the 3′ *trnL* exon, and the *trnL* intron (most of the missing sequence data are in the extremely conserved exon due to primer placement); those for the nrITS region include 25 and 28 bp of the 18S and 26S ribosomal genes, respectively, that flank ITS1 and ITS2, plus the 5.8S gene.

Characteristic	*trnG-trnS* spacer	*rps 16* intron	*trnL-F* region	nrITS region
a. Aligned length	1121 1-lipped: 1171 2-lipped: 1096	987	1049	788
b. Variable sites (proportion)	423 (0.377) 1-lipped: 351 (0.30) 2-lipped: 394 (0.359)	334 (0.338)	254 (0.242)	436 (0.553)
c. Parsimony informative sites (proportion)	231 (0.206) 1-lipped: 212 (0.181) 2-lipped: 206 (0.188)	147 (0.149)	110 (0.105)	273 (0.346)
d. Parsimony informative indels	10	14	18	9
e. Pairwise distances (range)	0.6–13.8% 1-lipped: 0.2–13.2% 2-lipped: 1.4–15.0%	0.1–8.5%	0.0–7.0%	0.2–16.7%
f. Consistency index	0.662	0.807	0.787	0.562
g. Retention index	0.767	0.879	0.868	0.726
h. Missing data, # taxa (of 82 in-group taxa)	4	8	10	10
i. Missing data (not including taxa for which entire sequence is missing)	4.5%	4.8%	18.7%	20.5% (including 10 partial sequences for OW taxa) 4.0% (taxa with <10% missing data)

Both data partitions were tested for congruence among loci sequenced using the partition homogeneity test (implemented in PAUP* [Swofford 2000] as the Incongruence Length Difference test; 100 replicates, 25 random addition sequences, maxtrees = 10,000). Not unexpectedly, all three chloroplast loci were congruent. When the ILD results indicated incongruence between cp and nrITS data, the sources of incongruence were determined by inspecting the results from analyses of partitions of the data (i.e., cp versus nuclear data) to identify taxa placed differently. All parsimony analyses used rigorous heuristic search strategies designed to find all islands of equally parsimonious trees (i.e., multiple random addition sequences and TBR branch swapping). Bayesian likelihood analyses used MrBayes v3.0B4 (Huelsenbeck and Ronquist 2001; Huelsenbeck et al. 2002; Ronquist and Huelsenbeck 2003), run with settings corresponding to a GTR model with gamma-distributed rate variation and proportion of invariant sites estimated by the program. Three heated and one 'cold' chain were run for > 1,000,000 generations, with trees saved every 100 generations. Analyses that combined cp and nuclear data used a mixed model approach, permitting the model of evolution to be optimized independently for data from the two genomes. Bayesian posterior probability values for branches were determined by opening the tree file produced by MrBayes in PAUP, filtering to remove the pre-burn-in trees from consideration, and then constructing the majority rule consensus tree; this tree is the maximum a posteriori (MAP) tree.

Strength of support for individual branches in the parsimony trees was evaluated using nonparametric bootstrap values (BS; Felsenstein 1985) and decay indices (DI; Bremer 1988; Donoghue et al. 1992). Bootstrap values are from 100 replicates with 10 random addition sequences and TBR branch swapping; for some analyses of the two-lipped corolla clade, maxtrees was set to 10,000 due to very large numbers of MP trees from some bootstrap replicates. DIs for each branch were determined by first using MacClade to prepare a set of trees each with a single branch resolved. These trees were then loaded into PAUP* as constraint trees and the program was asked to find the shortest trees inconsistent with the constraint tree using the same search strategy described above. The difference between the length of these trees and the globally shortest trees is the decay index (DI) for the branch in question.

For both lineages, we examined placement of taxa for which data were missing for ≥ 2 DNA regions with attention to the possible impact of missing data (taxa relevant here are OW *Acanthus longifolius*, *Crossandrella*, *Cynarospermum* Vollesen, *Sclerochiton triacanthus*, *Streptosiphon*, and NW *Rhombochlamys* Lindau). To minimize the impact of missing data, we conducted analyses that included only the portions of sequence data available for these taxa and only taxa that also had complete data for these same regions. The results of these 'no missing data analyses' were then compared to results from analysis of the larger data sets.

Alternative phylogenetic hypotheses (Table 2) were evaluated using MacClade to prepare trees reflecting these relationships. These were loaded into PAUP* as constraint trees using the same search strategy described above except that PAUP* was asked to find the shortest trees consistent with the constraints. One of the resultant MP trees consistent with each constraint was randomly selected and compared to one randomly selected MP tree using Templeton's test in PAUP* (reported as z statistics). The same strategy was used to compare likelihood scores of trees reflecting alternative phylogenetic hypotheses with all likelihood parameters (except base frequencies for which empirical values were used) estimated using one randomly selected MP tree. These parameters were used as the model to compare likelihood scores of the most likely tree to that of trees consistent with each of the alternative phylogenetic hypotheses using the Kishino-Hasegawa RELL test (K-H RELL) as implemented in PAUP*. Tests were one-tailed because an optimal tree was one of the trees compared in each case (Felsenstein 2004:369).

RESULTS

All data matrices that included taxa representing the entire taxonomic range of Acantheae (i.e., the four ma-

trices enumerated and described above) were congruent except as related to differences in taxa included. All results supported monophyly of two lineages of Acantheae with 100% bootstrap (BS) values for both (Fig. 5). One lineage includes only the Old World taxa that share the apomorphy of having all corolla lobes directed ventrally such that there is effectively no upper lip (Fig. 2B,C); this clade will be referred to subsequently as the 'one-lipped corolla lineage.' The second lineage includes a basal clade of Old World plants (OW *Stenandrium* + *Achyrocalyx* R. Benoist) that is sister to all New World Acantheae. Constraining the Old World taxa to monophyly resulted in trees that were significantly less parsimonious and less likely (Hypothesis 1 [H1], Table 2). We have not identified a morphological synapomorphy for the plants belonging to the second, mostly NW lineage, but they are distinguished from plants of the 'one-lipped corolla lineage' by having a two-lipped corolla (i.e., two lobes oriented dorsally, three lobes oriented ventrally, Fig. 2A,D,E) that is typical of Acanthaceae (and Lamiales). This group will be referred to as the 'two-lipped corolla lineage.'

We addressed alternative hypotheses about relationships of the OW and NW plants currently treated as species of *Stenandrium* using the data sets that included taxa representing the entire taxonomic range of Acantheae and that included only the 'two-lipped lineage' (see below). The small Malagasy endemic genus *Achyrocalyx* is strongly supported as part of the lineage that includes all sampled OW species of *Stenandrium* (see below and Fig. 7); we maintained this relationship in constructing the constraint trees because the results would otherwise be confounded by also forcing break up of the *Achyrocalyx* + OW *Stenandrium* relationship. The two sampled species of NW *Stenandrium* are together monophyletic (BS = 100, decay index [DI] = 31) and are strongly supported as part of the basal lineage of NW Acantheae, the *Stenandrium* clade (BS = 100, DI = 28) (Fig. 5). Constraining OW and NW *Stenandrium* to monophyly can be rejected as less parsimonious and less likely than the topology shown in Fig. 5 (H3, Table 2). Likewise, the hypothesis that OW + NW *Stenandrium* are together more closely related to OW than to NW Acantheae is rejected by both parsimony and likelihood as well (H4, Table 2).

Subsequent analyses were of separate data sets for taxa from the 'one-lipped' and 'two-lipped' lineages in order to include all characters (i.e., including the hypervariable region of *trnG-S*), as described in the methods.

One-Lipped Corolla Lineage. The ILD test indicated significant incongruence between the cp and nrITS data (P = 0.01), but the sum of the 'best' random partition was only 1 step longer than the original partition and the sum of the "worst" partition was only 9 steps
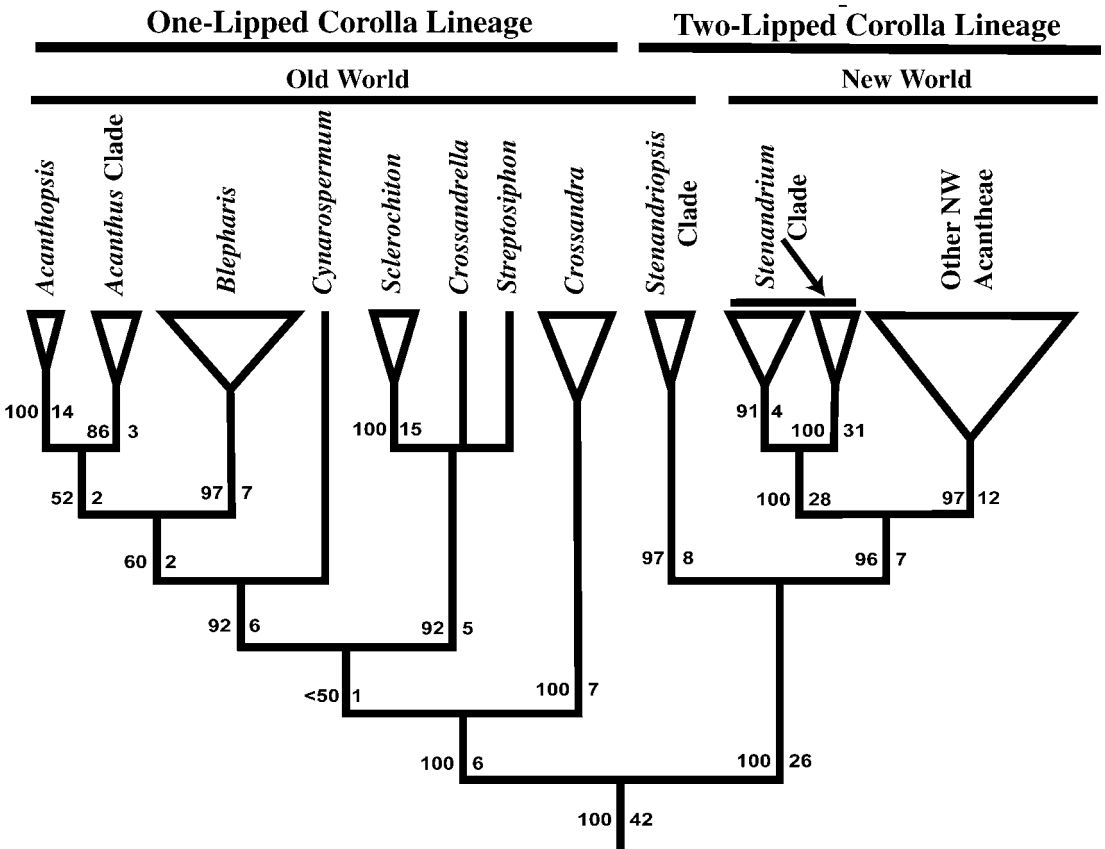
FIG. 5. Relationships among clades of Acantheae. Strict consensus of 15 MP trees (length = 3352, CI = 0.655, RI = 0.777; 950 parsimony informative characters) from analysis of data set (3) which included all taxa for which sequence data were available from ≥ 2 loci and all characters except hypervariable region of *trnG-S* (all four data sets that included the full range of taxa yielded identical results regarding clades of Acantheae and relationships among them; see text for full explanation of data sets). Triangles scaled to indicate relative sizes of clades both in terms of sampling and of described species. Bootstrap values to the left, decay indices to the right of clades. Arrow indicates position of two sampled species of New World *Stenandrium* within the *Stenandrium* clade.

longer. Inspection of the MP trees from analyses of the cp and nrITS data sets separately indicated that only one taxon, *Acanthus montanus*, was placed differently. This species shifted position relative to other species of *Acanthus* (details presented below). Removal of *A. montanus* yielded a non-significant ILD test (P = 0.31), indicating congruence of the cp and nrITS data for all other taxa. Subsequent analyses of taxa in the 'one-lipped lineage' were therefore of a data matrix that combines all four DNA regions.

The strict consensus of MP trees and the maximum a posteriori (MAP) Bayesian tree are entirely congruent and provide considerable resolution and support for relationships (Fig. 6). *Crossandra* is the basal clade of the 'one-lipped lineage' and is strongly supported as monophyletic (bootstrap [BS], decay index [DI], Bayesian posterior probability [BPP] = 100, 20, 99). There is weak support for monophyly of all other members of the 'one-lipped lineage' (BS, DI, BPP =

<50, 1, 79). *Sclerochiton* is monophyletic with strong support (BS, DI, BPP = 100, 15, 100). *Crossandrella* and *Streptosiphon* form a clade with *Sclerochiton* (BS, DI, BPP = 94, 3, 99), with no support for resolution of relationships among the three genera that compose the *Sclerochiton* clade. There is strong support for monophyly of members of the 'one-lipped lineage' above *Crossandra* and the *Sclerochiton* clade (BS, DI, BPP = 96, 6, 99), with unispecific *Cynarospermum* placed basally in this clade. There is weak support for monophyly of the clade sister to *Cynarospermum* (i.e., including *Blepharis*, *Acanthus*, and *Acanthopsis*; BS, DI, BPP = 70, 2, 66 plus 3 length mutations, all *rps16*). With the exception of *Blepharis dhofarensis*, all sampled species of *Blepharis* form a clade with strong support (BS, DI, BPP = 98, 9, 100). *Acanthopsis* and *Acanthus* are sister taxa with weak support (BS, DI, BPP = 60, 2, 66). All sampled species of *Acanthus* plus one of *Blepharis* (*B. dhofarensis*) form a monophyletic group (BS, DI, BPP = 91, 9, 98).
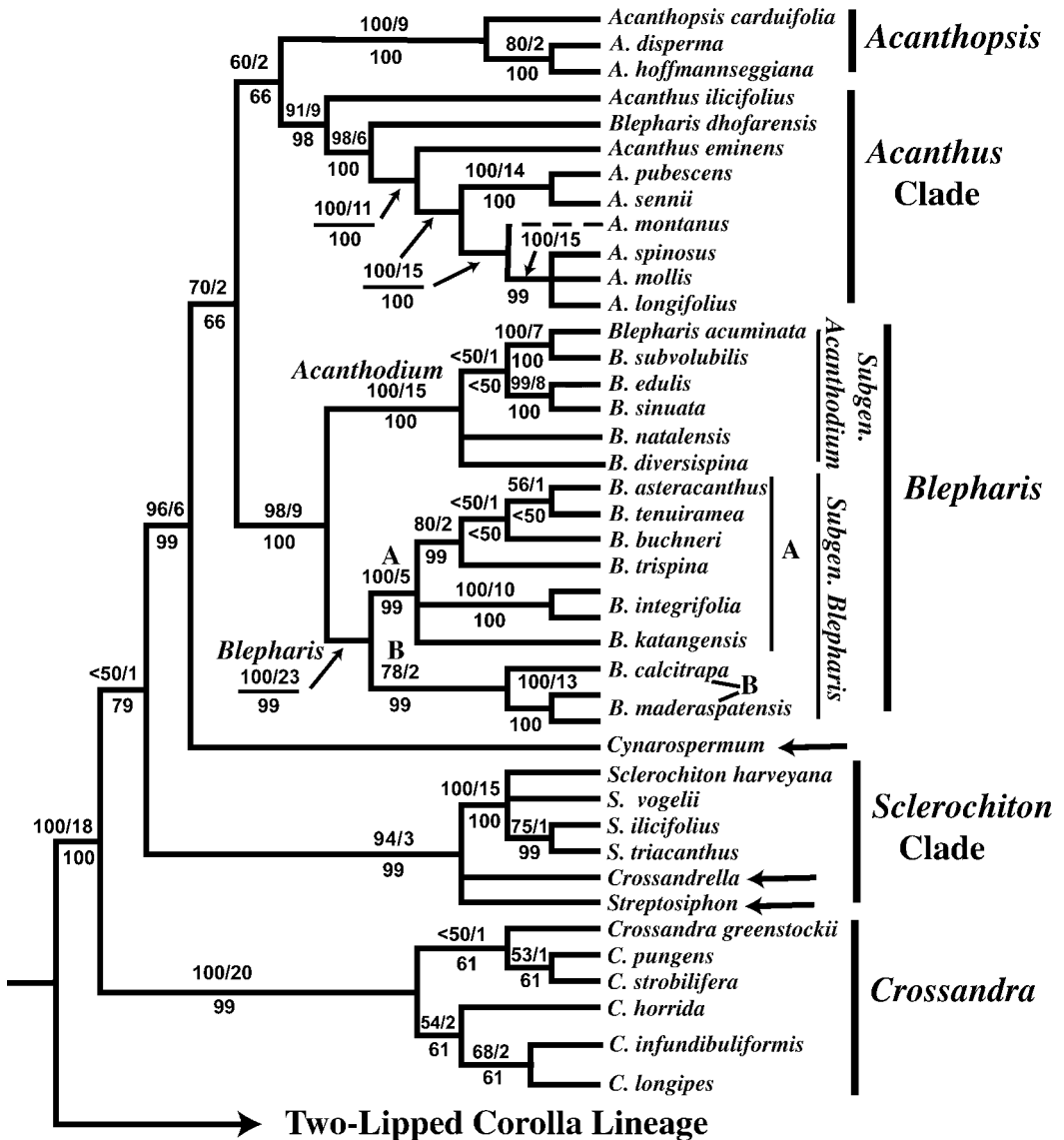
FIG. 6.   Relationships among species of the One-lipped Corolla Lineage of Acantheae. Strict consensus of 24 MP trees (length = 2042, CI = 0.764, RI = 0.812; 659 parsimony informative characters, 39 in-group taxa [+one additional accession of each of two species of *Blepharis* and one *Crossandra*]). Except for branches with <95% posterior probability, this tree is identical to the Bayesian MAP tree. Bootstrap / decay index values are above and Bayesian posterior probabilities are below clades. The dashed branch to *Acanthus montanus* indicates uncertainty about placement of this species (see text for full explanation); horizontal arrows highlight the placement of taxa represented by a single species.

Finally, *Acanthopsis* is monophyletic with strong support (BS, DI, BPP = 100, 9, 100).

TESTING THE PLACEMENT OF TAXA WITH PARTIAL DATA. For *Acanthus longifolius*, we have only *rps16* data from Genbank (Appendix 1). Analysis of the *rps16* data alone indicates 100% bootstrap support for placement of this species in a trichotomy with *A. mollis* and *A. spinosus*, confirming the results from the analysis of all data (Fig. 6). Likewise, *Sclerochiton triacanthus* is sister to *S. ilicifolius* in the analysis of all data (Fig.

6; BS, DI, BPP = 75, 1, 99) and in the analysis that included only the region for which we have data for this taxon, *trnG-S* (results not shown). *Cynarospermum* is placed as in Fig. 6 (analysis of all data) by an analysis that included only those data for which we have sequence for *Cynarospermum* (i.e., most of *trnG-S*; partial sequences of *rps16* and *trnL-F*): distal to *Crossandra* and the *Sclerochiton* clade and sister to the clade that includes *Blepharis*, *Acanthus* and *Acanthopsis*, with strong support (BS = 95; not shown). Finally, an anal-

ysis that included only *trnG-S* and *trnL-F* data placed *Streptosiphon* and *Crossandrella* with *Sclerochiton* with strong support (BS, BPP = 100, 100; not shown), as in the analysis including all data (Fig. 6).

TESTING ALTERNATIVE HYPOTHESES OF RELATIONSHIPS AMONG GENERA. The enigmatic genera *Crossandrella* and *Streptosiphon* have mosaics of putative morphological synapomorphies, and are central to several alternative hypotheses of relationship. First, the hypothesis that plants that share bony filaments (i.e., *Acanthopsis*, *Acanthus*, *Blepharis*, *Cynarospermum*, *Sclerochiton*) form a clade cannot be rejected (H5, Table 2). In fact, the MP trees consistent with this hypothesis differ from the MP and Bayesian likelihood trees only in that *Crossandrella* and *Streptosiphon*, both of which have filaments of normal texture, are placed between *Sclerochiton* and *Crossandra* rather than with *Sclerochiton* as in the MP and MAP trees. Second, the hypothesis that the apomorphic four-lobed 'fused, sheathing, reduced' calyx (Fig. 2G) marks a monophyletic group including *Acanthopsis*, *Acanthus*, *Blepharis*, *Cynarospermum*, and *Crossandrella* cannot be rejected (H6, Table 2). Trees consistent with this constraint differed from the MP and MAP trees only in that *Crossandrella* is placed between the *Sclerochiton* clade and the *Cynarospermum—Acanthopsis* lineage. Third, monophyly of the genera that share the putative synapomorphy of filaments inserted into a thickened flange of the internal corolla surface (i.e., *Acanthopsis*, *Acanthus*, *Blepharis*, *Cynarospermum*, and *Streptosiphon*) cannot be rejected (H7, Table 2). Again, trees consistent with this constraint differ from the MP and MAP trees only by shifting *Crossandrella*, which lacks the thickened flange, to below the *Sclerochiton* clade. Fourth, monophyly of taxa that share the 'triangular-caveate' pollen type (i.e., *Crossandrella*, *Streptosiphon* and many species of *Crossandra*, here represented by *C. horrida* Vollesen, *C. pungens* Lindau) is both less parsimonious and less likely than the MP and MAP trees (H8, Table 2). In sum, although both parsimony and Bayesian likelihood place *Crossandrella* and *Streptosiphon* with *Sclerochiton* with strong support, placement of these genera at nodes adjacent to their MP and MAP position cannot be rejected by our data. However, we can reject placing them with those species of *Crossandra* with which they share pollen morphology.

The MP and Bayesian likelihood results place *Cynarospermum* sister to (*Blepharis* (*Acanthus* + *Acanthopsis*)). However, constraining *Cynarospermum* to monophyly with the sampled species of *Blepharis* is not less parsimonious or likely (H9, Table 2) than the MP and ML results (Fig. 2). All trees consistent with this constraint placed *Cynarospermum* basal to all sampled *Blepharis* species except *B. dhofarensis*.

MONOPHYLY OF RECOGNIZED GENERA. The genera *Crossandra*, *Sclerochiton*, and *Acanthopsis* are strongly supported as monophyletic in the MP and MAP trees and, in the absence of alternative hypotheses about these genera, we accept their monophyly. Unique length mutations in the *trnL-F* and *trnG-S* loci provide additional support for monophyly of *Sclerochiton* and of *Acanthopsis*, respectively. The placement of *Blepharis dhofarensis* with *Acanthus* is both very strongly supported by our data (including both cp and partial nrITS data) and also unexpected based on morphology. To confirm this result, we re-extracted DNA and also obtained material from a second collection of this species (Appendix 1); all DNAs yielded identical data for the loci sequenced. Constraining *B. dhofarensis* to monophyly with the other sampled species of *Blepharis* is significantly less parsimonious and less likely (H10, Table 2) than placement of the species with *Acanthus*. Monophyly of *Acanthus* cannot be rejected by our data (H10, Table 2), but all trees that are consistent with this constraint simply move *B. dhofarensis* one node down, below *A. ilicifolius*, which does not change the result that this species of *Blepharis* is more closely related to *Acanthus* than to other *Blepharis*.

RELATIONSHIPS WITHIN GENERA. Despite providing strong support for monophyly of *Crossandra*, our data provide little evidence for relationships among species. The two clades from the basal node (i.e., *C. greenstockii—C. strobilifera* and *C. horrida—C. longipes*) are each supported by one length mutation in the *rps16* intron. On the other hand, two indels (both in *rps16*) contradict these same two clades. Two accessions of *C. infundibuliformis*, one cultivated and one from a field-collected specimen (Appendix 1), are nearly identical for nrITS; this species is most closely related to *C. longipes* from Madagascar. The two Malagasy species (*C. longipes*, *C. strobilifera*) are not sister taxa, nor do the African species (i.e., the other four) form a clade. In fact, our data provide little variation among these taxa across the four DNA regions: interspecific distances are only 1.5%-3.4% (uncorrected p-distances as reported by PAUP*).

As for *Crossandra*, our data do not provide resolution among species of *Sclerochiton* despite strong support for monophyly of the genus, except that *S. ilicifolius* and *S. triacanthus* are sister taxa with especially strong support from Bayesian likelihood (BS, DI, BPP = 75, 1, 99). Again, there is little variation among these taxa across the four DNA regions: interspecific distances are only 1.0%-1.5%.

Our data strongly support monophyly of *Blepharis* (except *B. dhofarensis*) and provide considerable resolution among the species sampled. Notably, there is more than twice as much variation among species of *Blepharis* as of *Crossandra* or *Sclerochiton*: interspecific pairwise distances were 0.6%− 8.3%. There is strong support for two clades within the genus; these conform quite closely to Vollesen's (2000) subgenera *Ble-*

pharis (BS, DI, BPP = 100, 23, 99) and *Acanthodium* (BS, DI, BPP = 100, 15, 100), as indicated in Fig. 6. Monophyly of these two clades is further supported by one (subgen. *Blepharis*) and five (subgen. *Acanthodium*) unique length mutations. Within subgenus *Blepharis*, clade A is monophyletic with strong support (BS, DI, BPP = 100, 5, 99) and includes B. *asteracanthus*—*B. katangensis* (Fig. 6). Clade B, comprising the Malagasy species *B. calcitrapa* Benoist and two accessions of *B. maderaspatensis*, is only moderately supported by parsimony (BS, DI, BPP = 78, 2, 99). Within subgenus *Blepharis*, two accessions of each of two species are nearly identical (pairwise distance = 0.2%); notably one of our sources of *B. maderaspatensis* was from South Africa (*McDade et al. 1292*) and the other was from Ethiopia (*Friis et al. 7275*), supporting monophyly of this wide-ranging species (i.e., throughout sub-Saharan Africa, India, SE Asia north of Peninsular Malaya; see maps 37 and 38 in Vollesen 2000). In subgenus *Acanthodium*, there is strong support for two pairs of sister species: *B. acuminata* + *B. subvolubilis* (BS, DI, BPP = 98, 7, 100; also one length mutation, *rps16*) and *B. edulis* + *B. sinuata* (BS, DI, BPP = 99, 8, 100; also one length mutation, nrITS).

Our results provide strong support for the resolution of relationships among the sampled species of *Acanthus* except among *A. mollis*, *A. spinosus* and *A. longifolius*. These three taxa form a strongly supported clade (BS, DI, BPP = 100, 15, 99) which is sister to *Acanthus pubescens* + *A. sennii* (BS, DI, BPP = 100, 14, 100). As noted above, *Acanthus montanus* was the source of incongruence between cp and nuclear data. The cp data place *Acanthus montanus* sister to [(*A. pubescens* + *A. sennii*)(*A. mollis*, *A. spinosus*, *A. longifolius*)]. In contrast, the nrITS data place *A. montanus* sister to *A. spinosus*, with *A. mollis* sister to these two species together (note that nrITS data are not available for *A. longifolius*). Not surprisingly, analysis of combined data sets (cp + nrITS) yields an intermediate result, placing *A. montanus* below (*A. mollis*, *A. spinosus*, *A. longifolius*) with strong support (BS, DI, BPP = 100, 15, 100), with these four species together sister to (*A. pubescens* + *A. sennii*) (monophyly of this entire group of six species is further supported by one length mutation in *rps16*). *Acanthus eminens*, *Blepharis dhofarensis* and the odd mangrove species, *A. ilicifolius*, are placed as a pectinate series below *A. mollis*—*A. sennii*, with all nodes strongly supported (BS, DI, BPP = 98–100, 6–11, 100). Our data provide nearly as much variation among *Acanthus* species (pairwise distances = 1.3%−7.1%) as among species of *Blepharis*.

The small (± 4 species) South African genus *Acanthopsis* is represented by three species, with moderate support for monophyly of *A. disperma* + *A. hoffmannseggiana* (BS, DI, BPP = 84, 2, 100). As for *Crossandra* and *Sclerochiton*, there is little sequence divergence among *Acanthopsis* species (pairwise distances = 0.5%−1.1%).

Two-Lipped Corolla Lineage. As noted above, analysis of all data sets inclusive of the full taxonomic range of Acantheae provided strong support for monophyly of Acantheae that share the plesiomorphic two-lipped corolla. Analysis of these taxa alone, including the hypervariable region of *trnG-S,* indicated conflict between the nrITS and cp data (ILD test, P = 0.01). Examination of the trees produced by separate analyses of the nuclear and cp data sets clearly pointed to two problematic taxa: *Aphelandra dolichantha* and *A. aurantiaca*. Removal of these two taxa results in congruence between the nuclear and cp data (ILD test, P = 0.40). We present the results of analysis of a data matrix that combines all four DNA regions and omits these two problematic taxa; their relationships and possible causes for incongruence between data sets are detailed below.

The strict consensus of MP trees and the Bayesian MAP tree were congruent except where support is weak from both methods. The *Stenandriopsis* clade, including the OW species of *Stenandrium* plus the one sampled species of *Achyrocalyx*, is the basal lineage with especially strong support for monophyly from Bayesian likelihood (Fig. 7; BS, DI, BPP = 82, 4, 100). There is strong support for monophyly of the sister group to the *Stenandriopsis* clade, a lineage that includes all NW members of Acantheae (BS, DI, BPP = 94, 7, 100). The *Stenandrium* clade is basal among NW Acantheae and includes all sampled species of *Stenandrium* and *Holographis* plus both Jamaican Acantheae, *Neriacanthus purdieanus* and unispecific *Salpixantha*, and one species of *Aphelandra*, *A. verticillata*, with strong support for monophyly of this taxonomically heterogeneous clade (BS, DI, BPP = 100, 28, 100). This clade is also supported by length mutations from three of the four loci sequenced (2 from *trnL-F*; 1 each from *rps16* and nrITS). The *Stenandrium* clade is sister to a strongly supported clade (BS, DI, BPP = 99, 7, 100) that includes all other NW Acantheae. The two mainland species of the small genus *Neriacanthus* Benth. are monophyletic (BS, DI, BPP = 80, 3, 94) (the third sampled species, from Jamaica, was placed with the *Stenandrium* clade, as just described). Bayesian likelihood supports monophyly of the *Aphelandra* lineage but parsimony provides little support for this relationship (BS, DI, BPP = 58, 1, 97). The 'armed' clade is monophyletic with strong support (BS, DI, BPP = 92, 5, 100) and the remaining members of the *Aphelandra* lineage together are its sister group (BS, DI, BPP = 96, 6, 100). There is no support for resolution among the five remaining clades of the *Aphelandra* lineage: (1) *A. squarrosa*, (2) *Encephalosphaera* + *A. maculata* (BS, DI, BPP = 98, 6, 100), (3) the *Geissomeria* clade (BS, DI, BPP = 100, 19, 100; also three length mutations), (4) the *A. aurantiaca*
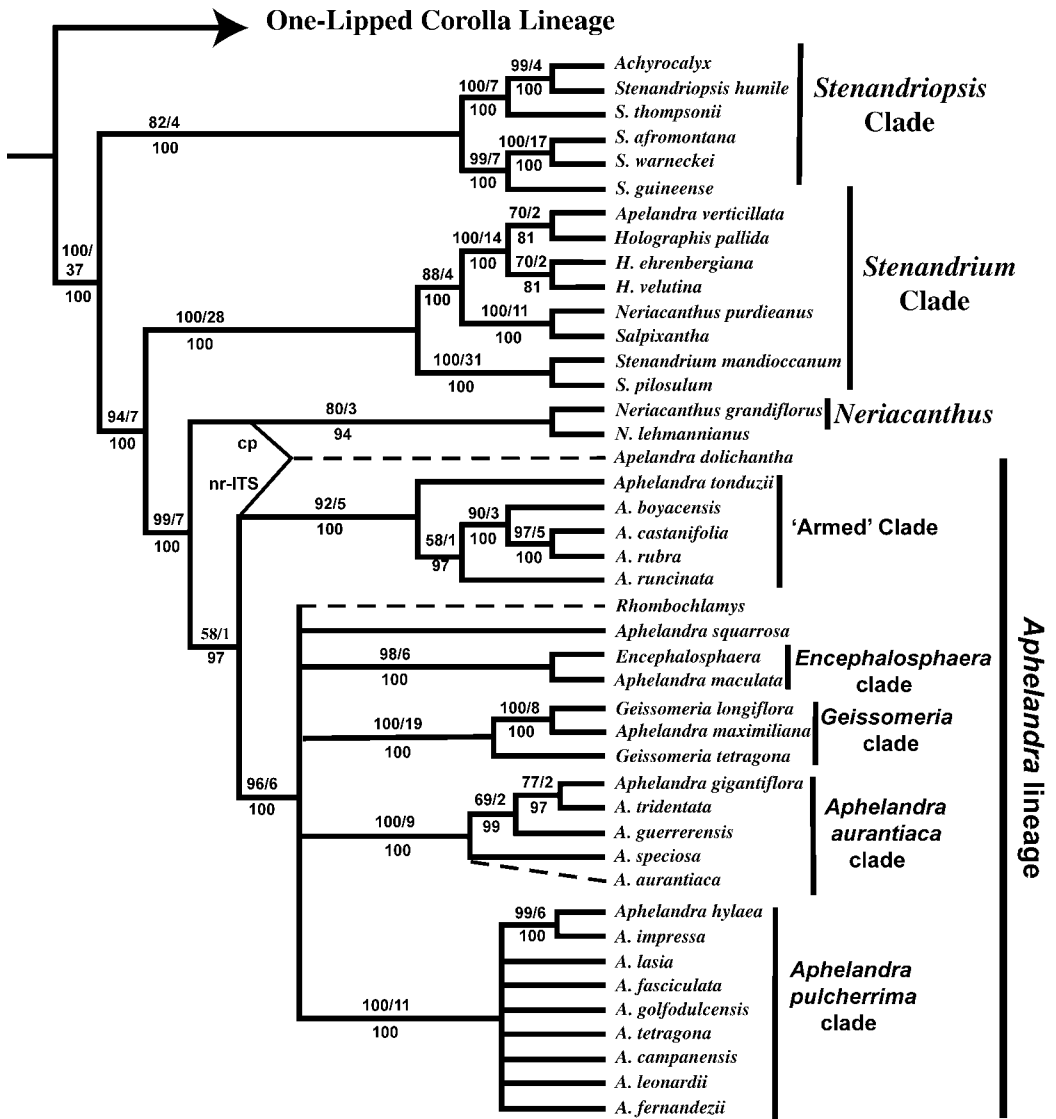
FIG. 7. Relationships among species of the Two-lipped Corolla Lineage of Acantheae. Bayesian MAP tree and strict consensus of 10 MP trees (length = 1759, CI = 0.717, RI = 0.739; 502 parsimony informative characters, 41 in-group taxa); branches with weak support from both parsimony (BS<70%) and Bayesian likelihood (BPP<95%) not shown. Bootstrap / decay index values are above and Bayesian posterior probabilities are below clades. Taxa whose positions are indicated by dashed branches are those for which data from only one locus were available (*Rhombochlamys*) or that were placed differently by cp and nrITS data (*Aphelandra dolichantha* and *A. aurantiaca*); see text for full explanation.

clade (BS, DI, BPP = 100, 9, 100), and (5) the *A. pulcherrima* clade (BS, DI, BPP = 100, 11, 100; also two length mutations). There is weak support from parsimony and no support from Bayesian inference for placement of *A. squarrosa* with the *Encephalosphaera* clade (not shown).

OMITTED TAXA. The cp data weakly support placement of *Aphelandra dolichantha* with *Neriacanthus* whereas the nrITS data place this species above *Neriacanthus* in a polytomy with the other clades of the *Aphelandra* lineage (Fig. 7). The case of *A. aurantiaca* is similar in

that, whereas the cp data place this species in a polytomy with all other clades of the *Aphelandra* lineage (results not shown), the nrITS data place it as in Fig. 7 as part of the clade that bears its name (BS, DI, BPP = 93, 3, 100).

PLACING THE TAXON WITH MANY MISSING DATA. Due presumably to low quality DNA from one of the few herbarium specimens of *Rhombochlamys*, we were able to sequence only *rps16* for this plant. This sequence places *Rhombochlamys* sister to *Aphelandra hylaea* within the *A. pulcherrima* clade. However, excluding

Rhombochlamys from a monophyletic *A. pulcherrima* complex is neither less parsimonious nor less likely than the optimal result (H14, Table 2).

RELATIONSHIPS WITHIN LINEAGES, PHYLOGENETIC STATUS OF RECOGNIZED GENERA, AND TESTS OF ALTERNATIVE HYPOTHESES OF RELATIONSHIP. Within the *Stenandriopsis* clade, the five sampled species of OW *Stenandrium* are not monophyletic. Instead, *Achyrocalyx* is part of a sublineage that includes the two sampled species of Malagasy *Stenandrium* (*S. humile, S. thompsonii*) with strong support for monophyly of this geographically defined clade (BS, DI, BPP = 100, 7, 100) and for a sister relationship between *Achyrocalyx* and *S. humile* (BS, DI, BPP = 99, 4, 100). The three African *Stenandrium* are monophyletic (BS, DI, BPP = 99, 7, 100) and sister to the Malagasy lineage; *S. afromontana* and *S. warneckei* are sister taxa (BS, DI, BPP = 100, 17, 100). Constraining OW *Stenandrium* to monophyly is both less parsimonious and less likely than the MP and MAP result (H11, Table 2) even though trees consistent with the constraint merely move *Achyrocalyx* to a position basal to the five OW *Stenandrium* species.

Monophyly of a group comprised of OW + NW *Stenandrium* is rejected by the data set for NW Acantheae, as it was by the data set for all Acantheae (H3, Table 2). Our results support the hypothesis of a close relationship between *Stenandrium* and *Holographis*, and a more distant relationship between these two genera and *Aphelandra* (H12, Table 2).

The two sampled species of NW *Stenandrium* are sister taxa with extremely strong support (BS, DI, BPP = 100, 31, 100; also three length mutations), and together are the basal lineage of the *Stenandrium* clade with strong support for monophyly of the remaining members of the clade (BS, DI, BPP = 88, 4, 100). Within this group, the two Jamaican Acantheae, *Neriacanthus purdieanus* and *Salpixantha*, are strongly supported as sister taxa (BS, DI, BPP = 100, 11, 100). Placement of these two Jamaican Acantheae as sister taxa was unanticipated and is strongly supported by both the cp and nuclear data. Further, the other two species of *Neriacanthus* that we sampled are not part of this clade (see below). We tested the placement of Jamaican *N. purdieanus* by re-extracting DNA from the original sample and by acquiring DNA from a different collection of this species (Appendix 1). Sequences from all DNA samples of this species were essentially identical. The Jamaican clade is sister to the three sampled species of *Holographis* + *Aphelandra verticillata* with very strong support for monophyly of these last four taxa together (BS, DI, BPP = 100, 14, 100; also two length mutations). Both parsimony and Bayesian likelihood weakly support two sister group pairs within *Holographis* s.l.: *H. pallida* + *A. verticillata* and *H. velutifolia* + *H. ehrenbergiana*.

Two mainland species of *Neriacanthus* sampled here are sister taxa, with moderate support. As noted above, the third species of *Neriacanthus* that we sampled, Jamaican *N. purdieanus* is sister to *Salpixantha* and part of the *Stenandrium* clade. Constraining the three *Neriacanthus* species to monophyly is less parsimonious and less likely than the MP and MAP trees (H10, Table 2).

The *Aphelandra* clade includes all sampled members of this large genus except *A. verticillata*, plus sampled species of *Geissomeria, Encephalosphaera,* and *Rhombochlamys*. Constraining *Aphelandra* to monophyly is strongly rejected by both parsimony and likelihood, as is constraining all species of *Aphelandra* except *A. verticillata* to monophyly (H10, Table 2). Thus, our results strongly support placement of *A. verticillata* distant from other *Aphelandra* and inclusion of taxa that have been placed in several other genera as part of a monophyletic group that includes the other species of *Aphelandra*.

The 'armed' *Aphelandra* clade includes the four sampled aphelandras with spines and/or sharply toothed leaves and bracts, plus the unarmed *A. tonduzii* as the basal member. This clade does not include *Encephalosphaera lasiandra*, a species with sharply toothed leaves. Constraining this species to monophyly with the 'armed' *Aphelandra* can be rejected (H13, Table 2). Within the 'armed' *Aphelandra* clade, *A. castanifolia* and *A. rubra* are sister taxa with strong support (BS, DI, BPP = 97, 5, 100), and *A. boyacensis* is sister to these two together (BS, DI, BPP = 90, 3, 100).

The lineage that includes most species of *Aphelandra* consists of five clades (six including *Rhombochlamys* for which we have data for only *rps16*). The southeastern Brazilian species *Aphelandra squarrosa* is not closely related to any of the other taxa included here nor can *Rhombochlamys* be placed precisely with the available data. The single sampled species of the small genus *Encephalosphaera* (*E. lasiandra*) is closely related to *Aphelandra maculata* (BS, DI, BPP = 98, 6, 100). *Geissomeria*, with two species sampled, is monophyletic with inclusion of *A. maximiliana* (BS, DI, BPP = 100, 19, 100); in fact, this species of *Aphelandra* is nested within *Geissomeria* as sister to *G. longiflora* (BS, DI, BPP = 100, 8, 100). The *Aphelandra aurantiaca* clade is monophyletic (BS, DI, BPP = 100, 9, 100); inclusion of *A. aurantiaca*, nrITS data only, weakens support for monophyly of the clade somewhat (BS, DI, BPP = 93, 3, 100; results not shown), probably due to missing data. Within the *A. aurantiaca* clade, *A. gigantiflora* and *A. tridentata* are weakly supported as sister taxa (BS, DI, BPP = 77, 2, 97), and *A. guerrerensis* is sister to these, again with weak support (BS, DI, BPP = 69, 2, 99). Within the *Aphelandra pulcherrima* complex, there is strong support for sister taxon status of *A. hylaea* and *A. impressa* (BS = 99, DI = 6, BPP = 100); relationships among the other species are not resolved with confidence.

DISCUSSION

The present study did not test monophyly of Acantheae, which has been shown in a number of studies that sampled widely from Acanthaceae and close relatives (e.g., McDade and Moody 1999; McDade et al. 2000b; Schwarzbach and McDade 2002). However, noteworthy patterns emerge from our study of comparative morphology of Acantheae. As noted in the introduction, Acantheae share the synapomorphy of monothecate anthers. A few other distantly related lineages of Acanthaceae have monothecate stamens (e.g., core Isoglossinae of Justicieae, McDade et al. 2000a) but, in these lineages, the loss of one theca per stamen seems to have occurred after reduction to two stamens. In contrast, Acantheae retain the plesiomorphic trait of four stamens, and this combination of stamen (four) and thecae (one) number is, to our knowledge, unique among Acanthaceae. Most NW Acantheae, members of the *Stenandriopsis* clade, *Crossandra*, *Sclerochiton*, and *Streptosiphon*, have a five-lobed calyx with at least slightly unequal segments: the dorsal lobe is widest, the paired anterior lobes are narrower and the paired lateral lobes are narrowest (Fig. 2F). We propose that the unequal, 1+2+2 configuration of the calyx is a synapomorphy for Acantheae. The trait has been further modified in the members of the one-lipped clade that have a four-lobed calyx (Fig. 2G), and also in at least some members of the *Stenandrium* clade that have narrow, apparently equal segments. At least the basal members of the other main lineages of Acanthaceae s.s. have a calyx of five equal lobes, although there are further modifications in some lineages, notably Barlerieae.

One-Lipped Clade. Within Acantheae, as predicted, our results indicate that OW members that share one-lipped corollas are monophyletic. This is a very distinctive trait that is approached in Acanthaceae only by plants of a few distantly related genera (e.g., *Barleria* L., *Eremomastax* Lindau). In Acantheae, the trait is further elaborated distal to the phylogenetically basal genus *Crossandra*, as described below, and there are no reversals to the plesiomorphic condition. The basal members of the one-lipped clade, *Crossandra*, *Streptosiphon*, and *Sclerochiton*, have seeds that are densely covered with pectinate scales. These are not known on seeds from plants of the two-lipped lineage. If this trait is a synapomorphy for the one-lipped clade, then it is further modified distal to these phylogenetically basal members as seeds of *Crossandrella* have minute tubercles (Vollesen 1994), *Cynarospermum* has seeds with trigonous tubercles, *Acanthopsis* + *Blepharis* have seeds with hygroscopic trichomes, and seeds of *Acanthus* are glabrous or puberulous.

Given that they have the plesiomorphic two-lipped corolla (Fig. 2A), it is not surprising that OW *Stenan-*

driopsis and *Achyrocalyx* are not part of this lineage. Clearly, many molecular characters place these plants as the basal lineage of an otherwise NW group of plants that share the plesiomorphic corolla, but morphological synapomorphies for this lineage remain to be discovered. The two-lipped lineage is discussed below.

The sampled members of *Crossandra* form a monophyletic group that is the basal clade of the 'one-lipped lineage.' Vollesen (1990b) noted that plants of at least one species of *Crossandra* (African *C. flava* Hook.) have a corolla that is not as clearly one-lipped as most members of this clade. We lack DNA material of this species but our data suggest a phylogenetically cohesive *Crossandra*. Vollesen (1990b) suggested that the 2-veined and 2-toothed dorsal calyx lobe is a diagnostic morphological character for at least African species of *Crossandra*; this character is sometimes indistinct and is apparently lacking or dimorphic in some Malagasy species (Vollesen 1997).

Our data do not convincingly resolve relationships among the sampled species of *Crossandra* (indeed, data from more rapidly evolving DNA regions will be necessary to resolve relationships with confidence). However, there is no indication that either the African or the Malagasy species form monophyletic groups. Although Vollesen (1997) did not place Malagasy species of *Crossandra* in the sections that he recognized for African species (Vollesen 1990b), he did note that *C. longipes* shares a number of characters with African species of *Crossandra*. We have neither the sampling density nor the resolution to test monophyly of the sections recognized by Vollesen (1990b). However, the two sampled species of Vollesen's (1990b) section *Dentatae*, *C. greenstockii* and *C. pungens*, are weakly supported as monophyletic together with Malagasy *C. strobilifera*. Somewhat surprisingly, our data place the two sampled species with triangular-caveate pollen (*C. horrida* and *C. pungens*; see Fig. 3) in separate sublineages. If homoplasy with regard to these pollen characters is supported with further sampling and better supported resolution, it will add confidence to our conclusion that this pollen type has evolved independently also in *Crossandrella* and *Streptosiphon* as discussed below.

Members of the 'one-lipped corolla lineage' other than *Crossandra* form a weakly supported group. Discussion of morphological character evolution distal to *Crossandra* requires examination of the disparate taxa placed in the *Sclerochiton* clade. Our data support monophyly of this clade, composed of *Sclerochiton*, *Crossandrella*, and *Streptosiphon*. *Crossandrella*, a West African genus of two species, and *Streptosiphon*, a unispecific genus from Tanzania, present mosaics of putatively derived characters (Vollesen 1990b, 1994). *Crossandrella* is unique among Acantheae in having beaked capsules that crack transversely at maturity. The re-

supinate corolla of *Streptosiphon*, with the tube twisted through 180°, is likewise autapomorphic among Acantheae. Morphological support for the *Sclerochiton* clade is subtle at best. *Streptosiphon* and *Crossandrella* both have the stigma bent anteriorly (i.e., toward the lower lip) through 180°. Plants of at least some *Sclerochiton* species have the stigma curved in the same direction (e.g., see Fig. 7J in Vollesen 1991). This character has not been studied across Acantheae and further work is needed before it can be hypothesized as a synapomorphy for this clade. *Streptosiphon* and *Crossandrella* also share a nearly entire, unlobed (lower) corolla lip, a condition approached in some other Acantheae (e.g., some *Blepharis* that have the lower lip reduced to only three lobes).

Several other characters are less subtle but all suggest relationships for these two genera that require homoplasy; of these, only the relationship with some species of *Crossandra* based on pollen morphology is rejected by our data (H8, Table 2). Among those that cannot be rejected, calyx morphology is perhaps the most compelling, in part because this character seems to evolve conservatively among Acanthoideae (sensu Scotland and Vollesen 2000). Large lineages (e.g., the two-lipped lineage) have little or no variation in calyx morphology other than degree of fusion and dimensions of the segments. The four-lobed, 'fused, sheathing, reduced' calyx (Fig. 2G) shared by *Crossandrella* and members of the *Cynarospermum—Acanthopsis* clade is notable also for the fact that it represents at least three changes: fusion of the anterior segments, marked reduction in size of the lateral segments, and sheathing posture of the anterior and posterior segments to enclose the laterals. These calyx characters argue for placement of *Crossandrella* sister to the *Cynarospermum—Acanthopsis* clade, a placement that could not be rejected by our molecular data (H6, Table 2). This placement is also consistent with the loss of dense pectinate scales on the seeds, a trait shared by *Crossandra*, *Sclerochiton*, and *Streptosiphon*. However, placing *Crossandrella* distal to the *Sclerochiton* clade might require homoplasy with regard to the stigma and corolla lip characters just described, as well as the other characters that link members of the *Streptosiphon* clade in conflicting patterns to other members of the one-lipped clade (i.e., filament texture and nature of the corolla tube at the point of filament insertion, see below).

Streptosiphon, *Sclerochiton*, and the *Cynarospermum—Acanthopsis* clade share insertion of the filaments into a thickened flange of corolla tissue. Both the MP/MAP placement of *Crossandrella* as part of the *Sclerochiton* clade, and placement of this genus above *Sclerochiton* based on calyx morphology, as discussed above, require loss of this thickened flange in *Crossandrella*. The filament texture character presents a similar problem except that both *Streptosiphon* and *Crossandrella* lack

this trait and have filaments of normal texture. Flowers of both of these genera have short filaments that are inserted distally in the corolla tube whereas plants of *Sclerochiton* and the *Cynarospermum—Acanthopsis* clade have elongate filaments that are inserted near the base of the corolla tube. Placement of *Streptosiphon* distal to *Crossandra* but below *Sclerochiton* (Fig. 8) permits mapping of all of the morphological characters just discussed with homoplasy only in *Crossandrella*. Constraining *Streptosiphon* to this placement is barely accepted by parsimony ($z = -1.8074$, P = 0.0707) and barely rejected by likelihood (KH RELL, P = 0.031). Notably, all trees consistent with this constraint place *Crossandrella* distal to *Sclerochiton* as in Fig. 8. Given the short filaments and small, apparently delicate corollas of *Crossandrella* (Heine 1966), reversal of the two characters associated with the androecium does not seem as unlikely as parallel evolution of the remarkable 'fused, sheathing, reduced' calyx. It merits noting that, for both *Crossandrella* and *Streptosiphon*, we have data only from cp loci. Organellar data can mislead consistently (i.e., across multiple loci) and strongly, and it is possible that nuclear data would place these taxa differently. We hope to obtain fresh collections of these plants which should improve prospects for sequencing nuclear loci.

Our data provide strong support for monophyly of *Sclerochiton*, a mostly eastern and southern African genus (Vollesen 1991). A possible synapomorphy for *Sclerochiton* is the presence of a small prickle on the medial ventral surface of the anthers. The four species included here belong to both subgenera recognized by Vollesen, and to two of five sections in his subgenus *Isacanthus*, and thus constitute a reasonable test of monophyly of the genus. However, our sample is too small and variation too limited to test Vollesen's (1991) classification other than section *Ilicifolia* of subgen. *Isacanthus*: two of three species, *S. ilicifolius* and *S. triacanthus*, are included here and are sister taxa.

There is strong support for monophyly of the lineage that is sister to the *Sclerochiton* clade, i.e., the *Cynarospermum—Acanthopsis* clade (Fig. 6). These plants share further reduction of the upper lip so that the dorsal portion of the tube is extremely short compared to the length of the ventral portion (Fig. 2C). In these plants, the bony filaments and dorsal calyx lobe seem to take the place of the upper lip structurally and perhaps also in pollination. These plants also share anthers that are densely bearded along the ventral edge (Figs. 2H-J); anthers are finely puberulous on various surfaces in many other Acantheae but not bearded as in these plants.

Cynarospermum was treated as a species of *Blepharis* until Vollesen (1999) noted that it lacks the distinguishing characters of *Blepharis* (i.e., it lacks leaves in pseudowhorls and appendages near the apices of the an-
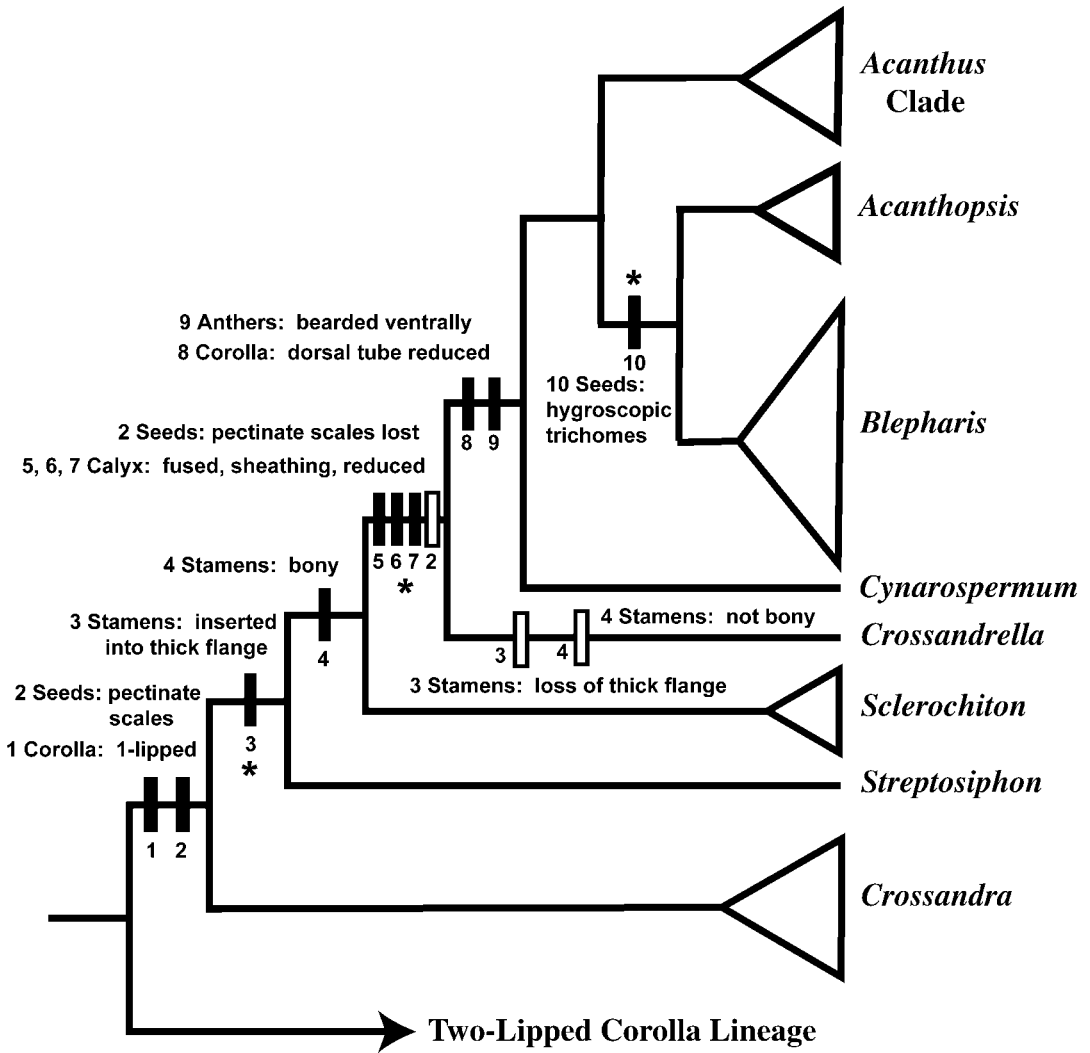
FIG. 8.   Morphologically informed phylogeny of the one-lipped corolla clade; solid bars mark gains of characters, open bars mark subsequent losses (apomorphies of genera not shown). This hypothesis differs from the MP and Bayesian likelihood tree at nodes marked by asterisks (compare to Fig. 6).

terior pair of filaments), and is marked by a number of autapomorphies (i.e., inflorescences reduced to solitary or paired flowers in the axils of vegetative leaves, three-fid bracteoles, and seeds with conical tubercles). Our results concur in that *Cynarospermum* is placed sister to (*Blepharis* (*Acanthus* + *Acanthopsis*)). However, there is weak support for monophyly of the clade that comprises these last three genera, and trees that place *Cynarospermum* as the basal member of the *Blepharis* clade are not significantly less parsimonious or less likely than the MP and MAP trees (H9, Table 2). Further, although our molecular data resolve relationships as *Blepharis* sister to (*Acanthus* + *Acanthopsis*), this topology is not strongly supported. It is thus best to view relationships among these three genera plus *Cynarospermum* as unresolved. In fact, plants of *Blepharis*

and *Acanthopsis* share branched hygroscopic trichomes on the seeds (Fig. 8), a trait that is otherwise absent among Acantheae (although hygroscopic trichomes have evolved at least twice in more distantly related lineages of Acanthaceae [i.e., Barlerieae, Manktelow et al. 2001; Ruellieae, Grubert 1974, Scotland et al. 1995, Manktelow 1996). The seeds of plants of *Cynarospermum* and *Acanthus* lack these trichomes.

Plants of *Blepharis*, the largest genus of OW Acantheae (Table 1), are quite distinct in having leaves in pseudowhorls and dimorphic filaments, with the anterior pair laterally flattened and bearing a remarkable appendage distally (i.e., near the anther) that is usually tooth- or finger-like but is sometimes rounded, and the posterior pair narrower and unappendaged (Fig. 2H-J). As just noted, these plants also have branched hy-

groscopic trichomes on seeds (Fig. 8N in Vollesen 2000), which may be a synapomorphy with *Acanthopsis*. Subgenus *Blepharis* (Fig. 6) likewise is well marked by synapomorphies: inflorescences are reduced, the corolla limb has three (rather than five) lobes, and the fertile portion of each anther locule is reduced to 1/2–2/3 of anther length. Sections *Scorpioidea* and *Blepharis* correspond to clades A and B in our analysis except that *B. integrifolia*, which was placed by Vollesen in section *Blepharis* (i.e., clade B), is placed by our data with five species from section *Scorpioidea* (i.e., clade A). Vollesen (1999) suggested that subgenus *Acanthodium* is the least advanced in the genus; although we cannot identify morphological synapomorphies, the clade is strongly supported as monophyletic by our data and thus clearly marked by many molecular synapomorphies. Our data provide considerable resolution among sampled species of *Blepharis*, yielding a framework for further study of phylogeny and morphological evolution in this morphologically complex and species-rich genus.

Acanthus is apparently unique among members of the one-lipped clade in having glabrous or rarely puberulous seeds (*Blepharis dhofarensis*, nested within *Acanthus* in our results, is the exception, as discussed below). *Acanthus* is also notable for its extremely wide geographic range, including the Mediterranean, southern Europe, India, the western Pacific, and Australia. Relationships among *A. montanus*, *A. spinosus*, *A. mollis*, and *A. longifolius* are problematic, perhaps due to missing data (*A. longifolius*) and hybridization (*A. montanus*). Our source of DNA of *A. montanus* was a cultivated plant (Appendix 1); as *Acanthus* species are reported to hybridize extensively in cultivation (R. Brummitt, pers. comm.), it is possible that we worked with a plant of hybrid origin. Within the *Acanthus* clade, the mangrove thistle, *A. ilicifolius*, is basal. This plant represents one polymorphic and very wide-ranging species (India to the Philippines and Australia) or one of several species, all true mangroves (Tomlinson 1986). The monophyly of this complex of entities could certainly be tested with these data. More broadly, there is no existing treatment or subgeneric classification for *Acanthus*, but given support values for branches within this clade, sequence data could be useful in recognizing such infrageneric groups.

Our results place *B. dhofarensis* with *Acanthus* with strong support. Indeed, it is nested within *Acanthus* (i.e., distal to *A. ilicifolius*) and, although monophyly of *Acanthus* cannot be rejected by our data, all trees consistent with this constraint place *B. dhofarensis* basal to *Acanthus* rather than with *Blepharis*. Placement with *Blepharis* is strongly rejected by our data (Table 2). Vollesen (1999) has noted that this species is remarkable in *Blepharis* in a number of characters including habit, and size of the anthers, capsules, and seeds. However,

these plants have a number of the synapomorphies of *Blepharis*, notably dimorphic filaments and hygroscopic trichomes on seeds. It is possible that this species is transitional among *Cynarospermum*, *Blepharis*, and *Acanthus*, and denser sampling of these latter two genera might help to clarify relationships. It also should be noted that we were able to obtain clean sequence for only part of nrITS for *B. dhofarensis*; these results should be tested with complete data from a nuclear locus.

The small southern African genus *Acanthopsis* is monophyletic in our results. These plants have unusual bracts with 3–5 apical spines that are themselves spinescent, and branched hygroscopic trichomes on the seeds (the latter might be interpreted as a synapomorphy for *Acanthopsis* + *Blepharis*, as discussed above). *Acanthopsis disperma* and *A. hoffmannseggiana* are sister taxa; plants of these species are both basal rosette-forming plants that seem to "skeletonize" during the dry season (McDade pers. obs.).

Two-Lipped Corolla Lineage. Although it is not unexpected that OW Acantheae that have the plesiomorphic corolla trait of a two-lobed upper and three-lobed lower lip are not part of the one-lipped lineage, our results unexpectedly show that these are part of a lineage that otherwise includes only NW plants. The strong support for this lineage indicates that these plants share many molecular synapomorphies (including three length mutations), but we are unable to identify morphological synapomorphies that link them. Certainly, among Acantheae, the two-lipped corolla distinguishes this lineage from other Acantheae but this is a symplesiomorphy that does not support monophyly.

The small Malagasy genus *Achyrocalyx* was recognized by Benoist (1930, 1967) as distinct from *Stenandriopsis* on the basis of having a bilabiate corolla versus one with the five lobes nearly equal. As many African *Stenandriopsis* have corollas that are as zygomorphic as those of *Achyrocalyx*, this would not seem to be a valid distinction between the two genera. Further, plants of *Achyrocalyx* share with Malagasy *Stenandriopsis* the character of having leaves in pseudowhorls, a trait that Vollesen (1992) used in placing African and Malagasy species of *Stenandriopsis* into different sections. Thus it is not surprising that our data place *Achyrocalyx* within the clade of Malagasy *Stenandriopsis*. Notably, our results for the *Stenandriopsis* clade concur with Vollesen's treatment of the African and Malagasy species as two sections, *Cordifolia* and *Hirtiflora*, respectively. If reciprocal monophyly of the African and Malagasy clades of *Stenandriopsis* is confirmed with inclusion of additional taxa, this would suggest that the process that resulted in the disjunct geographic distribution occurred near the time of origin of the genus as a whole.

Our results strongly support monophyly of all New

World Acantheae. This lineage is clearly marked by numerous molecular synapomorphies and it is remarkable that we can identify no morphological synapomorphies for this clade.

Our results confirm the prediction of Daniel et al. (1984) of a close relationship between *Stenandrium* and *Holographis* based on chromosome data ($x = 13$ vs. $x = 14$ for other NW Acantheae that have been counted), and add at least three other taxa to the *Stenandrium* clade. Daniel (1991) noted that, among Mexican *Aphelandra*, *A. verticillata* is as much like *Holographis* as like other aphelandras. In part, this reflects the paucity of morphological synapomorphies among lineages of NW Acantheae, but the quaternate leaves and pollen with colpi that bifurcate toward the poles of *A. verticillata* (Fig. 9A) are otherwise unknown in *Aphelandra* (except occasional grains of *A. golfodulcensis*, McDade 1984) but occur among species of *Holographis* (Fig. 9B). Our results strongly reject placement of *A. verticillata* with other *Aphelandra*, confirm a close relationship with *Holographis*, and further lead us to predict a chromosome number based on $x = 13$ for plants of this species. With the inclusion of *A. verticillata*, our data indicate that *Holographis* is monophyletic.

The two Jamaican Acantheae, *Salpixantha* and *Neriacanthus purdieanus*, are sister taxa and are together sister to *Holographis* in the *Stenandrium* lineage. Chromosome counts are not available for these species and, morphologically, they are unlike *Holographis* or *Stenandrium*, or indeed each other. *Salpixantha coccinea* has small bracts and diurnal ''scarlet or crimson'' (Adams 1972) corollas with a funnel-shaped tube and short lobes (Hooker 1845, plate 4158). Notably, based on corolla morphology, Grisebach (1864) and Bentham and Hooker (1876) treated *Salpixantha* as a synonym of *Geissomeria*, a placement that is not borne out by our results. In marked contrast, plants of *N. purdieanus* have relatively large, imbricate bracts and nocturnal pale-colored corollas that have a very narrow tube and well-developed lobes, traits that it shares with mainland species of *Neriacanthus*. These taxa do share the trait of having 'islands' or 'bridges' of exine within the colpi (Fig. 9C,D); this is certainly unusual among Acantheae but further study is necessary to determine if it can be claimed as a synapomorphy. These two species are the only Acantheae on Jamaica; our results suggest that the island was reached by the common ancestor of these two taxa and that they have differentiated there via a process likely involving specialization for different pollinators. As for *A. verticillata*, placement of the Jamaican Acantheae with the *Stenandrium* clade leads us to predict chromosome numbers based on $x = 13$ for them.

Although we have a small sample of species from the fairly large genus *Stenandrium*, the two species essentially span the morphological range of the genus and thus suggest monophyly of *Stenandrium* as a whole and that this genus is sister to the remaining members of the *Stenandrium* lineage. Vollesen (1992) argued that OW *Stenandriopsis* and NW *Stenandrium* cannot be distinguished morphologically and we concur. However, these plants do not share apomorphic characters that conflict with our results and, instead, it seems likely that these plants retain the plesiomorphic conditions for Acantheae of many macromorphological characters. In sum, our results argue for recognition of the traditional genera, OW *Stenandriopsis* and NW *Stenandrium*, and direct us to look harder for relevant morphological characters. We intend to test monophyly of NW *Stenandrium* by richer taxon sampling before recommending nomenclatural changes.

Neriacanthus has been distinguished from other NW Acantheae by the combination of white or pink bracts, and corollas with subequal lobes and slender tubes. These traits are shared with the Jamaican species, *N. purdieanus*, but our data strongly reject monophyly of the three sampled *Neriacanthus* together (H10, Table 2). Further, none of these characters is unique to species of *Neriacanthus* and none is clearly a synapomorphy. In this context, it is interesting that our data do support monophyly of the mainland species of *Neriacanthus*. Pollen of these two species of *Neriacanthus* appears similar: both *N. lehmannianus* (Scotland 1990) and *N. grandiflorus* (Fig. 9E) have tricolpate pollen with each colpus flanked by a pair of pseudocolpi. Pseudocolpi are not otherwise known in Acantheae and thus appear to be a synapomorphy for mainland *Neriacanthus*.

Aphelandra dolichantha was placed by the cp data with mainland *Neriacanthus* and by the nuclear data as an unresolved member of the *Aphelandra* lineage (Fig. 7). Plants of this species have a number of characters that are readily accommodated in *Neriacanthus*: large, imbricate pale bracts, pale flowers with a long, slender tube and corolla lobes that are subequal. Given the morphological diversity encompassed by the *Aphelandra* lineage, it is difficult to point to characters that associate *A. dolichantha* with plants of this lineage. In this context, it is notable that we have observed pollen abnormalities in *A. dolichantha*: a number of the samples we studied include collapsed grains as well as apparently normal tricolpate grains that are almost round and have foveolate exine, together with grains with the colpi tending to close off irregularly polygonal to rectangular regions of the exine (Fig. 9F, G). Grains with the polygonal patterns are reminiscent of those that characterize *Encephalosphaera* (Fig. 9I, see below). Further, nrITS was extremely difficult to sequence in this species suggesting the presence of multiple versions of the locus. All of these observations suggest a hybrid origin for this species which merits further investigation. Data from a low copy nuclear region might also permit a more confident assessment
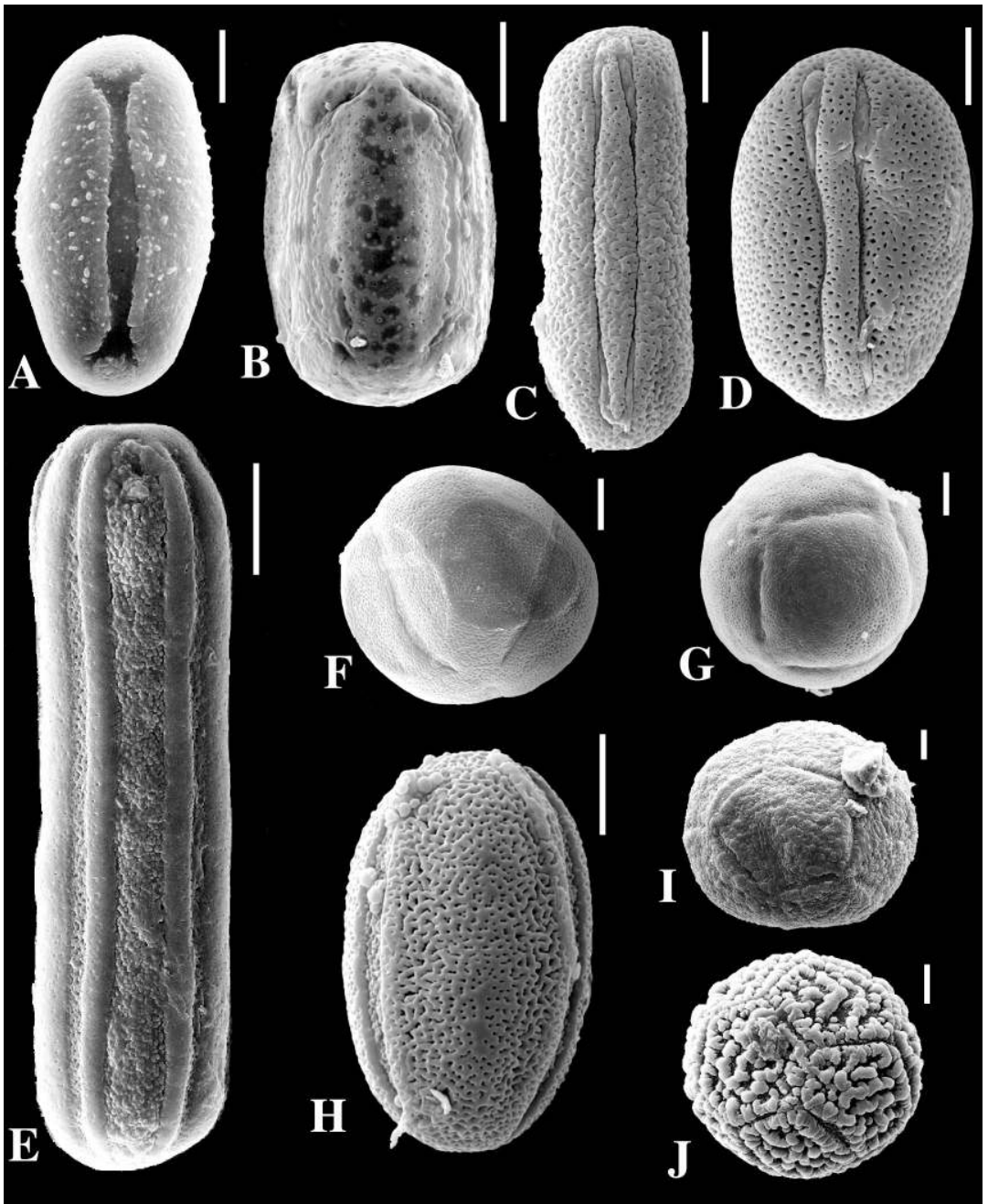
FIG. 9.   Variation among pollen of New World Acantheae. A. *Aphelandra verticillata* (*Daniel et al. 3295*), colpal view; note colpi bifurcating near poles. B. *Holographis virgata* (*Carter & Ferris 3819*), intercolpal view; note colpi bifurcating near poles. C. *Neriacanthus purdieanus* (*Proctor 20760*), colpal view; note large 'island' of apparently normal exine within the colpus. D. *Salpix-antha coccinea* (*Webster & Proctor 5635*), colpal view; note 'bridge' of apparently normal exine within the colpus. E. *Neriacanthus grandiflorus* (*Daniel et al. 8152*), colpal view; note pseudocolpi that flank each margin of the colpus. F. *A. dolichantha* (*Worthington 13651*), subpolar view, apparently normal grain. G. *A. dolichantha* (*Daniel et al. 5512*), undetermined view, apparently abnormal grain; note colpi tending to close off irregularly polygonal regions of the exine. H. *Rhombochlamys elata* (*Lehmann 2891*), inter-colpal view; note that grain is tricolpate as typical of Acantheae (compare to Fig. 1). I. *Encephalosphaera lasiandra* (*Daly et al. 7787*), undetermined view; note exine divided into polygonal regions by short, colpi-like ''apertures.'' J. *A. flammea* (*McDade 1117*), undetermined view; note exine divided into polygonal regions by short, colpi-like ''apertures.'' Scales = 10 μm.

of both relationships of this species and of what process has led to cp and nuclear data that place the species discordantly. *Aphelandra dolichantha* is one of a small number of species in the genus from southern Central America and Colombia that share the bract and floral characters noted above and also have a very strongly reduced calyx. Palynological data are not available for other members of this group. Clearly, more study is needed to understand the evolutionary history of this species and its relatives.

The *Aphelandra* lineage includes all sampled species of *Aphelandra* (except *A. verticillata*) plus *Geissomeria*, *Encephalosphaera*, and *Rhombochlamys*. Although this clade is supported by Bayesian likelihood, it is only weakly supported by parsimony and slight modifications of the data set (taxa or characters) yield trees that associate the 'armed' *Aphelandra* clade with *Neriacanthus*. The data strongly reject alternative hypotheses regarding the genus *Aphelandra* (i.e., monophyly of *Aphelandra* whether inclusive or exclusive of *A. verticillata*, Table 2, H10). This result is perhaps not surprising given that, although some clades of the *Aphelandra* lineage are marked by morphological synapomorphies, nothing to our knowledge marks *Aphelandra* exclusive of these other genera. Further, species of *Aphelandra* have been treated in at least eight other genera indicating that generic delimitations in the *Aphelandra* lineage are a work in progress. Data available to date indicate that these plants have chromosome numbers based on $x = 14$ (which is likely symplesiomorphic). Counts have not been made for *Encephalosphaera*, *Geissomeria* or *Rhombochlamys*, but their phylogenetic placements suggest $x = 14$.

The 'armed' *Aphelandra* that are included in our sample comprise a monophyletic group. There are about 20 species of *Aphelandra* that have spiny stems and serrate leaves (e.g., Fig. 4), and another ± 15 that have sharply toothed leaves but no spines. Our sample includes two of each, suggesting monophyly of the entire group. It is interesting that one spiny-stemmed (*A. rubra*) and one toothed-leaves only (*A. castaniflolia*) species are each other's closest relatives, and that their sister taxon is a species with only toothed leaves (*A. boyacensis*). This suggests a complex pattern of morphological evolution with regard to these traits, although richer taxon sampling and a faster evolving genic region will be necessary to explore these patterns. In the *Aphelandra* lineage as a whole, defensive structures have evolved at least twice because *Encephalosphaera lasiandra*, with sharply toothed leaves, is not part of the 'armed' *Aphelandra* clade. This is perhaps not surprising given that the genus *Encephalosphaera* is marked by a clear pollen synapomorphy (see below) and the other species placed in this genus are not armed. Further, at least two additional NW Acantheae are armed: *Orophochilus*, with spiny stems and armed leaf margins,

and *Holographis ilicifolia* with sharply toothed leaves. We lack DNA material of both of these but predict that *H. ilicifolia* will be placed with *Holographis*, as treated by Daniel (1983), thus constituting a third transition from unarmed to armed plants among NW Acantheae. We cautiously predict that *Orophochilus* will be part of the 'armed' *Aphelandra* clade because this clade contains the only sampled NW Acantheae with spiny stems.

Remarkably, *A. tonduzii* is the basal member of the 'armed' *Aphelandra* clade with strong support from both parsimony and Bayesian likelihood. Plants of this species are entirely unarmed and are similar in habit and floral morphology to members of *Stenandrium*. Interestingly, placement of this species in *Aphelandra* is supported by its chromosome number of $n = 14$ (Daniel 2000). Several other *Aphelandra* species are of diminutive stature (to the point of being essentially basal rosette forming plants) and have small *Stenandrium*-like flowers (e.g., *A. seibertii*, *A. arnoldii*); placing these plants phylogenetically will advance our understanding of the delimitation of *Stenandrium* and *Aphelandra*, and of habit evolution among NW Acantheae. Inclusion of additional taxa might also clarify the placement of the 'armed' *Aphelandra* clade as a whole; our data do not provide strong support for placement of this clade.

The remaining clades of the *Aphelandra* lineage are together monophyletic but we cannot identify morphological characters that distinguish these plants from other members of the two-lipped clade. Our data do not resolve relationships among the five remaining clades with confidence and *Rhombochlamys* should be treated as a sixth clade of uncertain relationships (see below).

The southeastern (SE) Brazilian species, *Aphelandra squarrosa*, is not clearly part of any of the multi-species clades of the *Aphelandra* lineage. More than a dozen species of *Aphelandra* have ranges restricted to SE Brazil; of these, our sample includes only two, *A. squarrosa* and *A. maximiliana*. These plants are not closely related in our analysis (*A. maximiliana* is part of the *Geissomeria* clade, see below) and it would be interesting to determine whether the other SE Brazilian species are members of the same two clades or whether other lineages of *Aphelandra* have radiated in SE Brazil.

The two sampled species of *Geissomeria* plus *Aphelandra maximiliana* form a strongly supported clade. *Geissomeria* is a genus of the southern margin of the tropics in South America (Brazil, Bolivia, Argentina), although poorly documented species have been described from elsewhere in the Neotropics. *Aphelandra maximiliana*, from southern Brazil is geographically at home in the clade. Compared to other members of the *Aphelandra* lineage, these plants have a distinctive corolla with the tube relatively much longer than the lips

(Fig. 2E). Again, *A. maximiliana* shares this trait and is at home in this clade.

The genus *Encephalosphaera* has been distinguished from *Aphelandra* on the basis of pollen grains that are round (versus prolate to perprolate), with the exine divided into numerous polygonal regions by short, colpilike ''apertures'' (versus tricolpate) (Fig. 9I). Unfortunately, we were successful in acquiring sequences for only one species of *Encephalosphaera* and thus cannot test monophyly of plants that share this pollen morphology. Interestingly, a number of species currently treated in *Aphelandra* have similar pollen (e.g., *A. flammea* [fig 9J] and *A. variegata*, see also Figs. 49–51 in Wasshausen 1975) and, in future work, we will seek to place these species within the *Aphelandra* lineage, hypothesizing that they are related to *Encephalosphaera*. The one species of *Aphelandra* that our data clearly align with *Encephalosphaera*, *A. maculata*, has tricolpate pollen grains typical of *Aphelandra* (see Fig. 14 in Wasshausen 1975). If pollen with polygonal regions outlined by short colpi-like apertures is synapomorphic, then it marks a more limited clade than that discovered here. At least one species of *Encephalosphaera* (*E. lasiandra*, included here) has sharply toothed leaves, as discussed above.

The *Aphelandra aurantiaca* clade includes the very wide-ranging namesake species (Mexico through Central America to NW South America, the Guyanas, Brazil and Bolivia) and, so far as we know at present, several Mexican and Central American taxa each with quite limited distribution. Plants of *A. aurantiaca* and *A. tridentata* are morphologically similar and also share some morphological traits with the Mexican and northern Central American species (i.e., upper lip of the corolla more or less folded to form a sheath-like structure around the anthers and stigma at anthesis, lower lip with lateral lobes slightly to strongly reduced compared to the central lobe, Fig. 2D). However, the clade is not macromorphologically or palynologically homogeneous (e.g., exine sculpturing varies among species; Daniel 1991; Wasshausen 1975:154). The validity of the *A. aurantiaca* clade should be tested with more complete taxon sampling and, ideally, resolution of the conflict between cp and nuclear data in *A. aurantiaca*. Our data suggest that the cp of *A. aurantiaca* had a different evolutionary history than that indicated by the nuclear and morphological data. In fact, we had considerable trouble obtaining high quality sequences from DNA from other accessions of this species, suggesting that this wide-ranging species may have an interesting pattern of intraspecific evolution.

The *Aphelandra pulcherrima* lineage is a long recognized group (Leonard 1953) that has been hypothesized to be monophyletic based on macromorphological data (McDade 1984, 1992). These plants share a suite of morphological synapomorphies: extrafloral nectaries on the floral bracts (McDade and Turner 1997), lateral lobes of the lower lip strongly reduced and lobes of upper lip folded over to form a pocket that conceals the reproductive organs at anthesis (figured in McDade 1984) and that opens to reveal these structures when the flower is manipulated by a legitimately visiting hummingbird. Within the *A. pulcherrima* clade, our results provide little resolution. *Aphelandra hylaea* and *A. impressa* are sister taxa; these plants were hypothesized by McDade (1984, 1992) to be part of a four species clade that is basal within the *A. pulcherrima* complex on the basis of morphological characters (relatively primitive morphology of both the extrafloral nectaries and the corolla).

Limited data for *Rhombochlamys* place this taxon sister to *A. hylaea* within the *A. pulcherrima* complex. However, these plants lack the morphological synapomorphies of that clade and excluding *Rhombochlamys* yields trees that are not less parsimonious or likely. This small South American genus was distinguished from *Aphelandra* on the basis of having pollen with broader colpi. In his key to the Colombian genera of series Imbricatae, Leonard (1953:119) indicates that pollen of members of *Aphelandra* often and of *Rhombochlamys* apparently always are colporate. In fact, pollen of *R. elata* (Fig. 9H) is similar to that of most other Acantheae in being tricolpate (e.g., Fig. 1). To our knowledge, endoapertures have never been documented for pollen of any Acantheae and, in fact, this is one of the characters that distinguishes Acantheae (colpate pollen) from its sister group, Ruellieae (colporate pollen). Leonard (1953:285) wrote that ''. . . in all other respects the two species . . . of *Rhombochlamys* are typical of *Aphelandra* . . .'' Until more complete data can be obtained, it is best to consider *Rhombochlamys* as one of the unresolved clades that compose the *Aphelandra* lineage.

Biogeography. Our results point to an OW distribution for the common ancestor of Acantheae. More precise resolution of the ancestral range is not possible because the basal lineages of both the one- and two-lipped lineages (i.e., the *Crossandra* and *Stenandriopsis* clades, respectively) have members in Africa and Madagascar. New World plants are a strongly supported lineage suggesting a single colonization event of the NW, at least for extant Acantheae. This geographic pattern must be considered in the context of the ages of the lineages involved. As part of an effort to date lineages of Asteridae using molecular data and fossils belonging to clades distant from Lamiales, Bremer et al. (2004) estimated 106 and 67 million years (MY) for the stem lineages of Lamiales and Acanthaceae, respectively, and ca. 54 MY for the crown node of Acanthaceae (i.e., age of extant lineages within the family). These estimates are older than those based on fossils that can be assigned with confidence to Lamiales,

which are middle Eocene in age (48–37 MY; Pigg and Wehr 2002). The fossil record of related clades yields an inferred age for Lamiales of lower Eocene (ca. 50 MY) (Magallón et al. 1999). Palynomorphs that unequivocally demonstrate the existence of Acanthaceae are known from the upper Miocene (oldest = ca. 22 MY) (Germeraad et al. 1968; Medus 1975). These represent pollen characteristic of relatively derived lineages within Acanthaceae and thus likely underestimate the age of the family.

Given the position of Acanthaceae within Lamiales (i.e., not among the first lineages to radiate, see Olmstead et al. 1992, 2000; Oxelman et al. 1999; Schwarzbach and McDade 2002), the estimated clade ages reported above, and the dates of the confirmed acanth fossils, the radiation of acanth lineages is not likely explained by continental drift. The break-up of Gondwanaland began more than 100 MYBP and, by ca. 50 MYBP, the relevant landmasses were widely separated (Scotese 2003). Dispersal is thus implicated as likely responsible for the current distribution of Acantheae. This is remarkable given that, with few exceptions, dispersal is via explosively dehiscent capsules with no secondary dispersal mechanisms, indicating limited vagility of these plants (among OW Acantheae, there are two exceptional mechanisms of seed dispersal [see below] but these occur in plants that are phylogenetically distant from those that are the closest relatives of NW Acantheae and thus are unlikely to have been involved in dispersal to the New World). Whether dispersal was transoceanic or overland during periods when subtropical and tropical habitats extended into high latitudes as has been suggested for Malpighiaceae (Davis et al. 2004) is not known. Also important is that many additional amphi-Atlantic disjunctions occur in Acanthaceae (e.g., four are listed by Renner 2004; the single tribe Justicieae includes at least four more [McDade et al. 2000a]) such that full investigation of acanth biogeography will require a broader taxonomic perspective.

Lack of strongly supported resolution and, to a lesser degree, taxon sampling preclude inferences about biogeography among NW Acantheae. However, among OW Acantheae, our results permit some inferences. Notably, although Acantheae are much less diverse on Madagascar and in Asia than in Africa, multiple clades of the tribe occur in both of the former regions. Regarding Malagasy Acantheae, our data suggest monophyly of members of the *Stenandriopsis* clade, whereas Malagasy *Crossandra* seem to represent at least two lineages. Several *Blepharis* species also occur on Madagascar (represented here by *B. calcitrapa*), including the wide-ranging *B. maderaspatensis*; additional sampling of Malagasy plants could determine whether all Malagasy *Blepharis* share a unique common ancestor with Malagasy *B. maderaspatensis* or whether these

species represent two or more lineages, each with closer relatives elsewhere. Notably, all Malagasy *Blepharis* are members of section *Blepharis* subgenus *Blepharis* sensu Vollesen (2000). Although most species of *Blepharis* have fruits that remain on the parent plant until wetted, plants placed in this section seem to have predehiscence dispersal via epizoochory. Fruits are enclosed in bracts that have terminal and marginal barbed, glochidiate and recurved bristles and they readily dislodge from the plant when mature to stick to socks and trousers (L. McDade, pers. obs., *B. maderaspatensis*). About plants in section *Blepharis*, Vollesen (2000:159) noted that [fruits are] ''. . . often dropping whole when mature . . . '' The fact that all SE Asian and most Indian species of *Blepharis* are also members of section *Blepharis* is consistent with the hypothesis that fruits of plants of this taxon are transported occasionally over long distances by birds or mammals.

One or a few members of three genera, *Crossandra*, *Blepharis*, and *Acanthus*, along with unispecific *Cynarospermum*, occur in Asia. The low diversity of Acantheae in Asia compared to Africa is consistent across all four clades with Asian species. In two cases, the phylogenetic positions of Asian plants suggest that recent arrival in Asia is not likely to explain low species diversity. *Cynarospermum* is sister to, and thus of the same age as, a clade with about 150 species. Similarly, the sole Asian *Acanthus*, *A. ilicifolius*, is basal to all other *Acanthus* sampled here. This plant is a mangrove, with secondary seed dispersal via water (Tomlinson 1986), perhaps explaining the extreme wide range of this species (India to Australia and the Philippines). Low speciation rates or high extinction rates (or a combination of the two) must thus explain the paucity of species of Acantheae in Asia.

Contrasting Patterns of Evolution in Acantheae. The two major lineages of Acantheae present remarkably different phylogenetic patterns. With the exception of the placement of *Blepharis dhofarensis* with *Acanthus*, traditionally recognized genera of the one-lipped lineage are monophyletic with strong support from sequence data and, in most cases, clear morphological synapomorphies. Relationships among these genera are not entirely resolved, but the sequence data do resolve some aspects and remarkable morphological synapomorphies largely corroborate and extend the sequence data. Further, within some genera, our data permit resolution of relationships among the included species.

In contrast, the two-lipped lineage is notable for the paucity of clear morphological synapomorphies for clades, and macromorphological characters supporting the 'backbone' of relationships among clades cannot be identified. No doubt related to this paucity of diagnostic morphological characters, many traditional genera are not monophyletic. Contrasting with the lack of

morphological signal, our molecular data do provide strong support both for monophyly of the basal clades (i.e., the *Stenandriopsis* and *Stenandrium* clades) and for their phylogenetic placement as a series of clades basal to other members of the two-lipped lineage. However, distal to these basal lineages, and especially within the species-rich *Aphelandra* lineage, with few exceptions, neither molecular nor morphological data provide evidence for relationships among clades. The fact that some of the clades within the *Aphelandra* lineage are supported by both molecular and morphological data (e.g., the *Geissomeria* and *A. pulcherrima* clades) does offer some hope that it may be possible to place most species into clades, if not to resolve relationships among them. On the other hand, our inability to resolve relationships among the sampled members of the *A. pulcherrima* complex suggests that phylogenetic patterns may be elusive closer to the species level as well as among clades. The four regions that comprise the present data set offer little promise of resolving relationships among and within the clades of the *Aphelandra* lineage.

The contrasting patterns of morphological and molecular diversity between the main lineages of Acantheae seem to be mirrored by chromosomes: a diversity of base chromosome numbers has been reported for OW taxa (e.g., $x = 28$ in *Acanthus*, $x = 10$ in *Crossandra*, Daniel and Chuang 1998; $x = 21$ in *Sclerochiton* based on the only known count in the genus, Mangenot and Mangenot 1962); whereas in NW plants there is comparatively little diversity of base numbers ($x = 13$, $x = 14$; Daniel et al. 1984, Daniel et al. 1990, Daniel 2000). However, this contrasting pattern does not hold in terms of species richness or of morphological diversity: NW Acantheae are as species-rich as the OW lineage, and are also extremely diverse morphologically (e.g., in terms of habit and floral morphology). In particular, with ca. 200 species, the *Aphelandra* lineage is one of the most species-rich and morphologically diverse lineages of Acantheae. Monophyly of the two Jamaican Acantheae, with their markedly different floral traits and likely pollinators, suggests that selection on ecologically important characters can readily produce disparate morphological traits between close relatives. NW Acantheae are one of several species-rich, morphologically diverse lineages of Acanthaceae within which it has been difficult to elucidate patterns of relationship (e.g., Strobilanthinae, Moylan et al. 2004; NW *Justicia*, McDade et al. 2001a). Such groups may have radiated rapidly, with clades established over a relatively short time period thus leaving short branches among clades that will be difficult to reconstruct without recourse to more rapidly evolving loci than are currently in our tool chest.

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APPENDIX 1

Taxa, Genbank accession numbers (*trnG-S, rps16, trnL-F,* nrITS; —= sequence not obtained), sources of plant materials from which DNA was extracted for sequencing and lineage and clade(s) where placed by the analyses presented here (i.e., as depicted in Figs. 6 and 7). To facilitate data location, taxa are listed in alphabetical order by genus and species. Sequences previously generated by us and reported in earlier papers are so indicated: (1) McDade and Moody (1999); (2) McDade et al. 2000b). (GB) = sequence downloaded from Genbank. When plants in cultivation were used, we provide information on native range in parentheses. Abbreviations for herbaria follow Holmgren et al. (1990). (OG) = used as one of out-groups for analyses of the other sublineage of Acantheae.

Acanthopsis carduifolia Shinz; DQ059216, DQ059140, DQ054850,—; southern Africa, *Ward & Seely 10243* (K); One-Lipped Corolla Lineage, *Acanthopsis.* *Acanthopsis hoffmannseggiana* C. B. Clarke; DQ059217, DQ059141, DQ054851,—; South Africa, *Balkwill et al. 11763* (J); One-Lipped Corolla Lineage, *Acanthopsis.* *Acanthopsis disperma* Nees; DQ059218, DQ059142,—, DQ028411; South Africa, *Balkwill et al. 11780* (PH); One-Lipped Corolla Lineage, *Acanthopsis.*

Acanthus eminens C. B. Clarke; DQ059220, DQ059144, DQ054853,—; Ethiopia, *Friis et al. 9760* (C); One-Lipped Corolla Lineage, *Acanthus* Lineage. *Acanthus ilicifolius* L.; DQ059219, DQ059143, DQ054852, DQ028412; Cultivated, Geneva, Accession No. 19700568/0 (G) (native to India, SE Asia, Australia); One-Lipped Corolla Lineage, *Acanthus* Lineage. *Acanthus longifolius* Host.;—, AJ431037 (GB),—,—; One-Lipped Corolla Lineage, *Acanthus* Lineage. *Acanthus mollis* L.; DQ059221, DQ059145, AF061824 (1), DQ028416; Cultivated, U. Arizona campus, *Freeh & Johnson 94–029* (ARIZ) (widely distributed in S Europe, Asia Minor, N Africa; extensively cultivated such that native range uncertain); One-Lipped Corolla Lineage, *Acanthus* Lineage. *Acanthus montanus* (Nees) T. Anders.;—,—, AF061823 (1), AF169756 (2); Cultivated, Duke University greenhouses, Accession No. 86–169 (DUKE) (native to tropical W Africa); One-Lipped Corolla Lineage, *Acanthus* Lineage. *Acanthus montanus* (Nees) T. Anders.; DQ059222, DQ059146,—,—; cultivated, National Botanic Garden of Belgium, Accession No. 07–3575 (BR) (native to tropical W Africa); One-

Lipped Corolla Lineage, *Acanthus* Lineage. *Acanthus pubescens* (Oliver) Engler; DQ059223, DQ059147, DQ054855, DQ028414; cultivated, Royal Botanic Gardens Kew, Accession No. 1986–2701 (K) (native to East Africa); One-Lipped Corolla Lineage, *Acanthus* Lineage. *Acanthus sennii* Chiovenda; DQ059224, DQ059148, DQ054856, DQ028415; Ethiopia, *Friis et al. 7006* (C); One-Lipped Corolla Lineage, *Acanthus* Lineage. *Acanthus spinosus* L.; DQ059225, DQ059149, AF1673301 (2), AF169757 (2); Cultivated, San Francisco Botanical Garden at Strybing Arboretum, *Anderson 3696* (CAS) (native to S. Europe, Asia Minor); One-Lipped Corolla Lineage, *Acanthus* Lineage.

Achyrocalyx decaryi Benoist; DQ059256, DQ059177, DQ054875, DQ028432; Madagascar, *Lorence 1947* (K); Two-Lipped Corolla Lineage, *Stenandriopsis* Clade.

Aphelandra aurantiaca Lindl.;—,—,—, DQ028459; Guatemala, *Lundell & Contreras 19840* (PH) (note that sequences were obtained for the cp loci used here but because they conflict with the nuclear and morphological evidence, we do not use them in results reported here and posting them to Genbank awaits further study); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Aphelandra aurantiaca* Clade. *Aphelandra boyacensis* Leonard; DQ059275, DQ059196, AF061828 (1), AF169759 (2); Colombia, *McDade 989* (DUKE); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, ''Armed'' *Aphelandra* Clade. *Aphelandra campanensis* Durkee;—,—, AF061829 (1), AF169760 (2); Panama, *McDade 852* (DUKE); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Aphelandra pulcherrima* Clade. *Aphelandra castanifolia* Britton; DQ059276, DQ059197, DQ054891, DQ028447; Bolivia, *Daniel 10175* (CAS); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, ''Armed'' *Aphelandra* Clade. *Aphelandra dolichantha* Donn. Sm.; DQ059274, DQ059195, AF063111 (1), DQ028446; Costa Rica, *McDade 243* (DUKE); Two-Lipped Corolla Lineage, Incertae sedis. *Aphelandra fasciculata* Wassh.;—,—, DQ054898, DQ028454; Venezuela, *McDade 659* (DUKE); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Aphelandra pulcherrima* Clade. *Aphelandra fernandezii* Leonard; DQ059284, DQ059203,—, DQ028455; Colombia, *McDade 999* (DUKE); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Aphelandra pulcherrima* Clade. *Aphelandra golfodulcensis* McDade; DQ059285, DQ059204,—, DQ028456; Costa Rica, *McDade 251* (DUKE); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Aphelandra pulcherrima* Clade. *Aphelandra gigantiflora* Lindau; DQ059289, DQ059207, DQ054901, DQ028460; Mexico, *Daniel 8368* (CAS); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Aphelandra aurantiaca* Clade. *Aphelandra guerrerensis* Wassh.; DQ059290,—, DQ054902, DQ028461; Mexico, *Daniel & Ton 6163* (CAS); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Aphelandra aurantiaca* Clade. *Aphelandra hylaea* Leonard; DQ059282, DQ059202, DQ054897, DQ028452; Ecuador, *McDade 1089* (DUKE); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Aphelandra pulcherrima* Clade. *Aphelandra impressa* Lindau; DQ059283,—,—, DQ028453; Colombia, *McDade 911* and *McDade 935* (DUKE); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Aphelandra pulcherrima* Clade. *Aphelandra lasia* Leonard, DQ059286,—, DQ054899, DQ028457; Colombia, *McDade 1003* (DUKE); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Aphelandra pulcherrima* Clade. *Aphelandra leonardii* McDade; DQ059287, DQ059205, AF063112 (1), AF169761 (2); Costa Rica, *McDade 310* (DUKE); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Aphelandra pulcherrima* Clade. *Aphelandra maculata* (Tafalla ex Nees) Voss; DQ059281, DQ059201, DQ054896, DQ028451; cultivated, National Botanic Garden of Belgium Accession No. 19550109 (BR); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Encephalosphaera* Clade. *Aphelandra maximiliana* (Nees) Bentham; DQ059295, DQ059213, DQ054906, DQ028466; Brazil, *Wasshausen 2326* (US); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Geissomeria* Clade. *Aphelandra rubra* Wassh.; DQ059277, DQ059198, DQ054892, DQ028448; Bolivia, *Roca 332* (CAS); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, ''Armed'' *Aphelandra* Clade. *Aphelandra runcinata* Klotzsch in DC ex Nees; DQ059278, DQ059199, DQ054893, DQ028449; Colombia, *McDade*

& *Lundberg 1137* (DUKE); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, ''Armed'' *Aphelandra* Clade. *Aphelandra speciosa* Brandegee; DQ059291, DQ059208,—, DQ028462; Guatemala, *McDade 212* (DUKE); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Aphelandra aurantiaca* Clade. *Aphelandra squarrosa* Nees; DQ059279, DQ059200, DQ054894, DQ028450; cultivated, *McDade 1173* (ARIZ) (native to Brazil); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, Incertae sedis. *Aphelandra tetragona* Nees; DQ059288, DQ059206, DQ054900, DQ028458; Venezuela, *McDade et al. 642* (DUKE); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Aphelandra pulcherrima* Clade. *Aphelandra tonduzii* Leonard; DQ059273, DQ059194, DQ054890, DQ028445; Panama, *Daniel et al. 8105* (CAS); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, ''Armed'' *Aphelandra* Clade. *Aphelandra tridentata* Hemsl.; DQ059292, DQ059209, DQ054903, DQ028463; Costa Rica, *Haber & Zuchowski 8680* (MO); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Aphelandra aurantiaca* Clade. *Aphelandra verticillata* Nees; DQ059262, DQ059183, DQ054881, DQ028436; Mexico, *Daniel et al. 3295* (CAS) (OG); Two-Lipped Corolla Lineage, *Stenandrium* Clade.

Blepharis acuminata Oberm.; DQ059227, DQ059151, DQ054858,—; South Africa, *McDade et al. 1272* (J); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis asteracanthus* C. B. Clarke; DQ059228, DQ059152,—,—; east Africa, *Faden et al. 96/204* (K); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis buchneri* Lindau; DQ059229, DQ059153,—,—; south central Africa, *Faden et al. 96/307* (K); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis calcitrapa* Benoist; DQ059230, DQ059154,—, DQ028422; Madagascar, *Daniel et al. 10403* (CAS); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis dhofarensis* A. G. Miller; DQ059231, DQ059155, DQ054859, DQ028413; Yemen, *Thulin et al. 9715* (K); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis diversipina* (Nees) C. B. Clarke; DQ059232,—, DQ054865,—; South Africa, *McDade et al. 1269* (J); One-Lipped Corolla Lineage, *Acanthus* Lineage. *Blepharis dhofarensis* A. G. Miller; DQ061158,—,—,—; Oman, *A. G. Miller 2552* (K) (second accession used to verify data for this species; only one genic region sequenced); One-Lipped Corolla Lineage, *Acanthus* Lineage. *Blepharis edulis* (Forssk.) Pers.; DQ059233, DQ059156,—, DQ028418; north and east Africa, Arabia, *Friis 6735* (K); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis integrifolia* (L.f.) E. Mey. ex Schinz; DQ059234, DQ059157, DQ054860,—; South Africa, *Balkwill et al. 11814* (J); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis integrifolia* (L.f.) E. Mey. ex Schinz; DQ059235, DQ059158, DQ054861,—; South Africa, *Balkwill et al. 11656* (J); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis katangensis* De Wild.; DQ059236, DQ059159,—, DQ028421; east Africa, *Bidgood et al. 3521* (K); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis maderaspatensis* (L.) Roth.; DQ059237, DQ059160,—, DQ028423; South Africa, *McDade et al. 1292* (PH); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis maderaspatensis* (L.) Roth.; DQ059238, DQ059161,—,—; Ethiopia, *Friis et al. 7275* (K); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis natalensis* Oberm.; DQ059239, DQ059162, DQ054862, DQ028420; South Africa, *Balkwill et al. 11667* (J); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis sinuata* (Nees) C. B. Clarke; DQ059240, DQ059163, DQ054863, DQ028419; South Africa; *McDade & Dold 1193* (PH); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis subvolubilis* C. B. Clarke; DQ059241, DQ059164, DQ054864, DQ028417; South Africa, *Balkwill et al. 10850* (J); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis tenuiramea* S. Moore; DQ059242, DQ059165,—,—; Tanzania, *Bidgood et al. 3869* (K); One-Lipped Corolla Lineage, *Blepharis* Lineage. *Blepharis trispina* Napper; DQ059243, DQ059166,—,—; Tanzania, *Bidgood et al. 1102* (K); One-Lipped Corolla Lineage, *Blepharis* Lineage.

Crossandra greenstockii S. Moore; DQ059250, DQ059171, DQ054871, DQ028427; South Africa, *McDade & Balkwill 1241* (J) (OG); One-Lipped Corolla Lineage, *Crossandra*. *Crossandra horrida* Vollesen; DQ059251, DQ059172, DQ054872, DQ028428; Somalia,

Thulin et al. 8949 (K); One-Lipped Corolla Lineage, *Crossandra*. *Crossandra infundibuliformis* (L.) Nees; DQ059252, DQ059173, AF061826 (1), AF169754 (2); Cultivated, U. Arizona, *McDade 1162* (ARIZ) (native to India and Africa); One-Lipped Corolla Lineage, *Crossandra*. *Crossandra infundibuliformis* (L.) Nees;—,—,—, DQ028429; Ethiopia, *Gilbert et al. 8241* (K); One-Lipped Corolla Lineage, *Crossandra*. *Crossandra longipes* S. Moore; DQ059253, DQ059174, DQ054873, DQ028430; Madagascar, *Hearn Mad-62* (PH); One-Lipped Corolla Lineage, *Crossandra*. *Crossandra pungens* Lindau; DQ059254, DQ059175, AF061826 (1), AF169755 (2); Cultivated, San Francisco Conservatory of Flowers, *Daniel s.n.* (CAS) (native to Kenya, Tanzania); One-Lipped Corolla Lineage, *Crossandra*. *Crossandra strobilifera* (Lam.) Benoist; DQ059255, DQ059176, DQ054874, DQ028431; Madagascar, *Hearn Mad-49* (PH); One-Lipped Corolla Lineage, *Crossandra*.

Crossandrella dusenii (Lindau) S. Moore; DQ059248, DQ059170, DQ054869,—; Zaire (now the Democratic Republic of the Congo), *Lisowski 45706* (BR); One-Lipped Corolla Lineage, *Sclerochiton* Clade.

Cynarospermum asperrimum (Nees) Vollesen; DQ059226, DQ059150, DQ054857,—; India, *Townsend 73/9* (K); One-Lipped Corolla Lineage, *Cynarospermum*.

Encephalosphaera lasiandra Mildbr.; DQ059280,—, DQ054895,—; Peru, *Graham & Schunke Vigo 0958* (F); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Encephalosphaera* Clade.

Geissomeria longiflora Lindl.; DQ059293, DQ059211, DQ054904, DQ028465; Brazil, *Wasshausen 2337* (US); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Geissomeria* Clade. *Geissomeria tetragona* Lindau; DQ059294, DQ059212, DQ054905, DQ028464; Bolivia, *Daniel 10103* (CAS); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, *Geissomeria* Clade.

Holographis ehrenbergiana Nees; DQ059263, DQ059184, DQ054882, DQ028437; Mexico, *Daniel & Baker 3712* (CAS); Two-Lipped Corolla Lineage, *Stenandrium* Clade. *Holographis pallida* Leonard & Gentry; DQ059264, DQ059185, DQ054883, DQ028438; Mexico, *Daniel et al. 8510* (CAS); Two-Lipped Corolla Lineage, *Stenandrium* Clade. *Holographis velutifolia* (House) T. F. Daniel; DQ059265, DQ059186, DQ054884, DQ028439; Mexico, *Salinas T. et al. 4779* (CAS); Two-Lipped Corolla Lineage, *Stenandrium* Clade.

Justicia adhatoda L.; DQ059296, DQ059214, AF289773, AF289734; Cultivated, Univ. Arizona campus, Tucson, Arizona, U.S.A., *Barr 60–393* (ARIZ) (native to southern Asia); Out-Group (Justicieae).

Neriacanthus grandiflorus Leonard; DQ059271, DQ059192, DQ054888, DQ028443; Panama, *Daniel et al. 8152* (CAS); Two-Lipped Corolla Lineage, *Neriacanthus*. *Neriacanthus lehmannianus* Lindau; DQ059272, DQ059193, DQ054889, DQ028444; Colombia, *Wood 5379* (CAS); Two-Lipped Corolla Lineage, *Neriacanthus*. *Neriacanthus purdieanus* Benth.; DQ059266, DQ059187, DQ054885, DQ028442; Jamaica, *Gillis 14978* (BM); Two-Lipped Corolla Lineage, *Stenandrium* Clade. *Neriacanthus purdieanus* Benth.; DQ059267, DQ059188,—,—; Jamaica, *Proctor 24475* (US) (second accession used to verify data for this species; only two DNA regions sequenced); Two-Lipped Corolla Lineage, *Stenandrium* Clade.

Odontonema tubaeforme (Bertol.) Kuntze; DQ059297, DQ059215, AF169748, AF063127; Cultivated, Duke University greenhouses, Durham, North Carolina, U.S.A., Accession No. 66–153, *McDade 1182* (ARIZ) (native to Mexico and Central America); Out-Group (Justicieae).

Rhombochlamys rosulata Lindau;—, DQ059210,—,—; Ecuador, *J. L. Clark et al. 5063* (US); Two-Lipped Corolla Lineage, *Aphelandra* Lineage, Incertae sedis.

Salpixantha coccinea Hook.; DQ059268, DQ059189, DQ054886, DQ028441; Jamaica, *Webster & Proctor 5635* (BM); Two-Lipped Corolla Lineage, *Stenandrium* Clade.

Sclerochiton harveyanus Nees; DQ059244, DQ059167, DQ054866, DQ028424; South Africa, *Balkwill 12274* (J); One-Lipped Corolla Lineage, *Sclerochiton* Clade. *Sclerochiton ilicifolius* Meeuse; DQ059245, DQ059168, DQ054867,—; South Africa, *McDade et al.*

1297 (PH); One-Lipped Corolla Lineage, *Sclerochiton* Clade. *Sclerochiton triacanthus* Meeuse; DQ059246,—,—,—; South Africa, *McDade & Balkwill 1255* (J); One-Lipped Corolla Lineage, *Sclerochiton* Clade. *Sclerochiton vogelii* (Nees) T. Anderson; DQ059247, DQ059168, DQ054868, DQ028425; Tanzania, *Kayombo 2938* (K) (OG); One-Lipped Corolla Lineage, *Sclerochiton* Clade.

Stenandrium afromontanum (Mildbr.) Vollesen (= *Stenandriopsis afromontana* (Mildbr.) Benoist); DQ059257, DQ059178, DQ054876, DQ028433; Tanzania, *Lovett 274* (CAS); Two-Lipped Corolla Lineage, *Stenandriopsis* Clade. *Stenandrium guineensis* (Nees) Vollesen (= *Stenandriopsis guineense* (Nees) Benoist); DQ059258, DQ059179, DQ054877, DQ028434; W and Central Africa, Cultivated, Palm House, Royal Botanic Gardens, Kew, Accession No. 1990–2299 (K) (OG); Two-Lipped Corolla Lineage, *Stenandriopsis* Clade. *Stenandrium humile* Benoist Vollesen (= *Stenandriopsis humile* Benoist); DQ059259, DQ059180, DQ054878,—; Madagascar, *DuPuy et al.*

MB587 (CAS); Two-Lipped Corolla Lineage, *Stenandriopsis* Clade. *Stenandrium mandioccanum* Nees; DQ059269, DQ059190, DQ054887, DQ028442; Bolivia, *Wasshausen & Wood 2201* (CAS); Two-Lipped Corolla Lineage, *Stenandrium* Clade. *Stenandrium pilosulum* (S.F.Blake) T.F. Daniel; DQ059270, DQ059191, AF061827 (1), AF169758 (2); Mexico, *Van Devender & Reina G. 97–434* (ARIZ); Two-Lipped Corolla Lineage, *Stenandrium* Clade. *Stenandrium thompsonii* (S. Moore) Vollesen (= *Stenandriopsis thompsonii* S. Moore); DQ059260, DQ059181, DQ054879,—; Madagascar, *Schatz 2714* (K); Two-Lipped Corolla Lineage, *Stenandriopsis* Clade. *Stenandrium warneckei* (S. Moore) Vollesen (= *Stenandriopsis warneckei* (S. Moore) Napper); DQ059261, DQ059182, DQ054880, DQ028435; Tanzania, *Steiner 403* (UPS); Two-Lipped Corolla Lineage, *Stenandriopsis* Clade.

Streptosiphon hirsutus Mildbr.; DQ059249,—, DQ054870, DQ028426; Tanzania, *Bidgood et al. 1568* (K); One-Lipped Corolla Lineage, *Sclerochiton* Clade.