The Enigmatic Tribe Whitfieldieae (Acanthaceae): Delimitation and Phylogenetic Relationships Based on Molecular and Morphological Data

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Abstract. Relationships of the enigmatic genera *Whitfieldia, Chlamydacanthus,* and *Lankesteria* (Acanthaceae) were examined using molecular sequence data for two chloroplast loci (*ndhF* gene, *trnL-trnF* spacer and intron) for these and a sample of taxa representing all major lineages within the family. Morphological data, including pollen structure as imaged using SEM, were also compiled for these three genera, and evaluated in a phylogenetic context. Bremekamp suggested that *Whitfieldia* and *Chlamydacanthus* belonged together as tribe Whitfieldieae, and that *Lankesteria* was closely related to *Pseuderanthemum* in Justicieae. Contra earlier classifications of Acanthaceae, this would result in tribes with multiple corolla aestivation patterns. Our results confirm that *Chlamydacanthus* and *Whitfieldia* are closely related. Unexpectedly, *Lankesteria* is sister to these two genera together and we propose that the three genera comprise an expanded tribe Whitfieldieae. Also unexpectedly, Whitfieldieae is sister to Barlerieae. We propose a number of morphological synapomorphies for Whitfieldieae including concentric rings of ridges on the seeds and a densely granular circular area surrounding the pores of pollen grains. *Chlamydacanthus* and *Whitfieldia* further share biporate, flattened pollen grains that are circular in outline, and seeds with glabrous surfaces. Barlerieae is a large and diverse lineage such that synapomorphies to support aspects of their relationships are difficult to identify. However, hygroscopic trichomes on the seeds may be a synapomorphy for Whitfieldieae plus Barlerieae, with subsequent loss in *Chlamydacanthus, Whitfieldia,* and some *Barleria.* As here circumscribed, Whitfieldieae includes plants with both contort and imbricate corolla aestivation seconding Bremekamp's misgivings about basing classifications entirely upon this character.

The most recent comprehensive classification of Acanthaceae (Bremekamp 1965) differs from earlier efforts (Nees ab Esenbeck 1847; Bentham 1876; Lindau 1895) in placing less emphasis on corolla aestivation as a key character at the supratribal level. Lindau (1895) subdivided Acanthaceae sensu stricto (s.s.; i.e., excluding subfamilies Thunbergioideae, Mendoncioideae, and Nelsonioideae) into two groups ''Contortae'' and ''Imbricatae'' based on aestivation patterns (con-tort buds have all corolla lobes half overlapped, im-bricate buds have one lobe fully overlapped and one lobe fully overlapping). In contrast, Bremekamp (1965) stressed characters such as articulation of shoots, cystoliths, and shape of pollen apertures in subdividing Acanthaceae s.s. into two subfamilies, Ruellioideae and Acanthoideae (articulation of shoots and cystoliths are present in the former and lacking in the latter; pollen apertures are colporate or porate
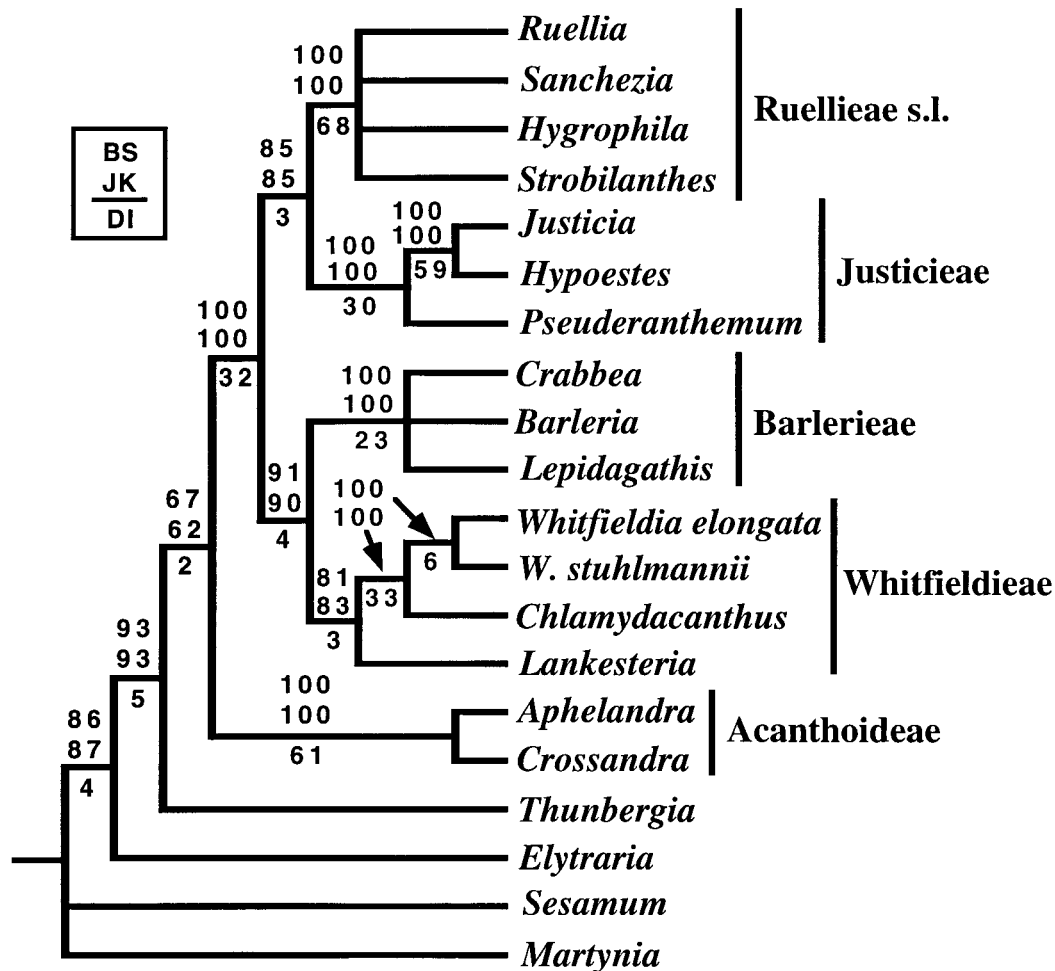
BS
JK
DI

100
100
68 — Ruellia — Sanchezia — Hygrophila — Strobilanthes | Ruellieae s.l.

85
85
3

100 100 — Justicia
100 100 59 — Hypoestes | Justicieae
30 — Pseuderanthemum

100
100
32

100
100 — Crabbea
23 — Barleria | Barlerieae
— Lepidagathis

91
90
4

100
100 — Whitfieldia elongata
6 — W. stuhlmannii
81 33 — Chlamydacanthus | Whitfieldieae
83
3 — Lankesteria

67
62
2

100
100 — Aphelandra
61 — Crossandra | Acanthoideae

93
93
5

86
87
4

— Thunbergia
— Elytraria
— Sesamum
— Martynia

FIG. 1. Strict consensus of eight most parsimonious trees of 1718 steps from the analysis combining *ndhF* and *trnL-trnF* sequences data. CI = 0.750, RI = 0.708. Values above the branches are bootstrap above jacknife indices; decay indices are presented below the branches.

in Ruellioideae, colpate in Acanthoideae). Both of Bremekamp's subfamilies include more than one aestivation type.

Over the course of his systematic research on Acanthaceae, Bremekamp also delimited a number of new tribes, including Whitfieldieae. Bremekamp (1944, 1965) transferred the genera *Whitfieldia* and *Stylarthropus,* with contort aestivation, to Whitfieldieae from Ruellieae, a tribe that also includes plants with contort buds (*Stylarthropus* was synonymized with *Whitfieldia* by Clarke [in Burkill and Clarke 1899–1900] and is currently regarded as congeneric with it, Brummitt 1992). Whitfieldieae, as thus delimited by Bremekamp, was characterized primarily by the presence of lenticular, biporate pollen grains and globose cystoliths.

Later, Bremekamp (1965) suggested that *Chlamydacanthus* also might belong in Whitfieldieae; although plants of this genus have imbricate aestivation, they share the pollen and cystolith characters of *Whitfieldia* (Lindau 1895:343). Bremekamp's emphasis on characters beyond aestivation is further exemplified by his informal suggestion in the 1944 paper to remove *Lankesteria,* with contort aestivation, from tribe Ruellieae to the vicinity of *Pseuderanthemum* in subtribe Odontoneminae of tribe Justicieae (sensu Bremekamp 1965; these plants otherwise have imbricate aestivation). However, Bremekamp never formalized his ideas regarding *Lankesteria* or *Chlamydacanthus,* nor did he return to them in subsequent publications, apparently due to insufficient herbarium material (Bremekamp
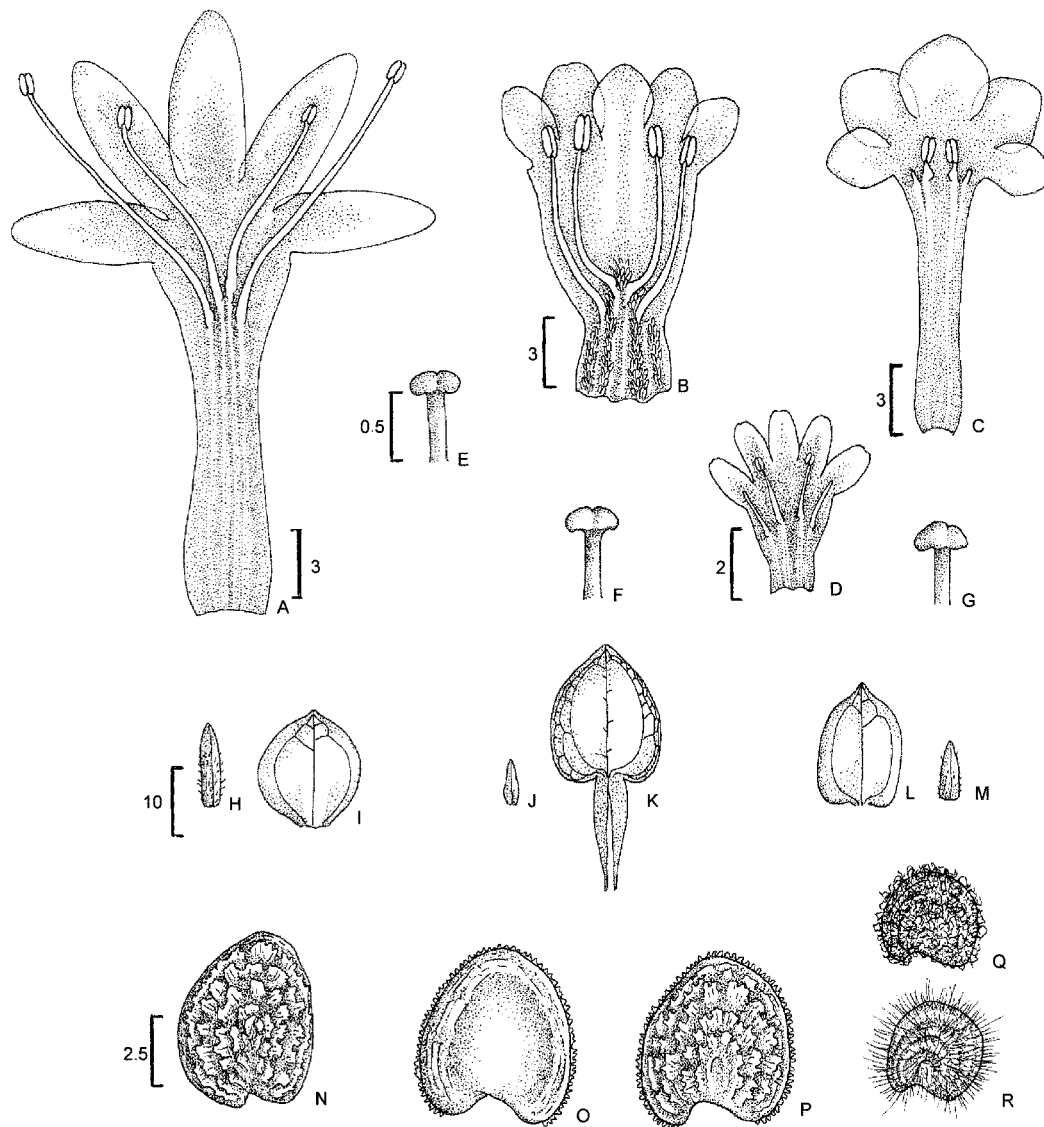
FIG. 2.   Morphology of corollas, stigmas, bracts, bracteoles, and seeds in *Whitfieldia, Chlamydacanthus* and *Lankesteria* (all scale bars in mm). Corollas, opened along median adaxial line to expose the androecium of: A. *Whitfieldia elongata*; B. *W. lateritia*; C. *Chlamydacanthus* sp.; D. *Lankesteria brevior*. Stigmas of: E. *W. lateritia*; F. *C.* sp.; G. *L. brevior*. H, I. Bract and bracteole of *W. lateritia*. J, K. Bract and bracteole of *C.* sp. L, M. Bract and bracteole of *L. brevior*. N. Dry seed of *W. lateritia*, showing ridges (both surfaces the same). O, P. Dry seed of *C. dichrostachyus*, convex surface, and concave surface showing ridges. Q, R. Dry and hydrated seed of *L. brevior*. Figure drawn by M.-J. Balkwill from *Lowe 3513* (*W. elongata*, K); *Lane-Pool 180* (*W. lateritia*, K); *du Puy et al. 230* (*C.* sp., K); *Semsei 804* (*C. dichrostachyus*, K); *Samai SKS 262* (*L. brevior*, K).

1944). The systematic positions of *Whitfieldia, Chlamydacanthus* and *Lankesteria* have not been evaluated since the time of Bremekamp.

Results of recent phylogenetic studies have yielded a framework of relationships within Acanthaceae that permits us to address explicitly the placement of enigmatic genera such as *Whitfieldia, Chlamydacanthus,* and *Lankesteria*. Of particular relevance to the placement of these three genera, these results identify a lineage that includes most of Bremekamp's Ruellieae, but also Trichanthereae and Louteridieae (Scotland et al. 1995; McDade and Moody
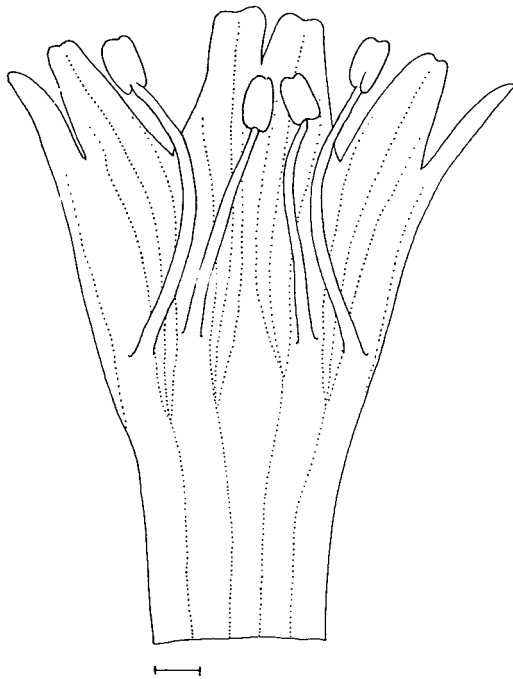
FIG. 3. Corolla of *Chlamydacanthus lindavianus,* opened along the trace of the central lobe of abaxial lip, showing trifurcation of each corolla lobe trace; filament traces not shown. Scale bar = 1 mm. Figure drawn by M. Manktelow from *Borhidi et al. 86464* (UPS).

1999; McDade et al. 2000; Daniel, unpublished data), which were recognized as separate tribes by Bremekamp (1965). All plants placed thus far in this Ruellieae sensu lato (s.l.) have contort aestivation and also a complex 'filament curtain' structure within the corolla (Manktelow 1996, 2000). Barleriinae were treated by Bremekamp (1965) as a subtribe of Ruellieae; these plants, however, have quincuncial aestivation (two corolla lobes fully overlapping the others; Scotland et al. 1995) and lack the filament curtain (Manktelow 2000); recent phylogenetic work places these together with Bremekamp's (1965) tribe Lepidagathideae as a lineage distinct from Ruellieae s.l. (McDade and Moody 1999; McDade et al. 2000). The lineage of Barleriinae plus Lepidagathideae is sister to Ruellieae s.l. plus Justicieae (McDade et al. 2000). The large and heterogeneous lineage Justicieae, as thus far understood, includes plants with imbricate buds and no filament curtain. (Reflecting these advances in our understanding of Acanthaceae, we here use Ruellieae s.l. to refer to the lineage that includes Trichanthereae and Louteridieae but excludes Barleriinae, and Barlerieae for the lineage including at least Barleriinae and Lepidagathideae.)

Understanding the delimitation and morphological synapomorphies of the major lineages of Acanthaceae s.s. clearly depends on the placement of *Whitfieldia, Chlamydacanthus,* and *Lankesteria.* For example, if *Whitfieldia* and *Lankesteria,* with contort aestivation, belong within Ruellieae s.l., then contort aestivation would be synapormophic for the entire lineage. If these genera are placed elsewhere, then aestivation type would not provide a unique derived character for Ruellieae s.l. Instead, the complex filament curtain structure within the corolla would uniquely characterize Ruellieae s.l. (*Whitfieldia* and *Lankesteria* lack a filament curtain, Manktelow 2000). If *Lankesteria* is related to *Pseuderanthemum* in Justicieae, as suggested by Bremekamp (1965), then our concept of Justicieae must be expanded to include plants with contort aestivation. Further, the phylogenetic position of *Chlamydacanthus,* with imbricate aestivation and no filament curtain, must also be established in order to understand the evolutionary history of both characters among Acanthaceae. In this paper, we examine phylogenetic relationships of *Whitfieldia, Chlamydacanthus* and *Lankesteria,* using molecular sequence data from the chloroplast genome (the *ndhF* gene and the *trnL-trnF* spacer and intron) and morphological characters.

MATERIALS AND METHODS

Taxon Sampling. The appendix lists taxa included in both the molecular and morphological components of this work. For the molecular analysis, *Chlamydacanthus lindavianus, Lankesteria brevior,* and two species of *Whitfieldia* are included. *Whitfieldia elongata* and *W. stuhlmannii* represent the merged genera *Whitfieldia* and *Stylarthropus,* respectively. All three genera are African: *Chlamydacanthus* (4 spp.) occurs on Madagascar and in East Africa, *Lankesteria* (7 spp.) on Madagascar and in tropical Africa, and *Whitfieldia* (10 spp.) in tropical Africa (Mabberley 1997). Additional species of all three genera were examined for morphological data (see below). As described above, Bremekamp (1944, 1965) proposed relationships of these genera to tribes Ruellieae s.l. and Justicieae. Taxa chosen to represent these tribes include the most distantly related groups within Ruellieae s.l. and Justicieae for which we were able to obtain sequences. This should have the effect of ''breaking'' long branches, thus increasing confidence in our results (see Gray-
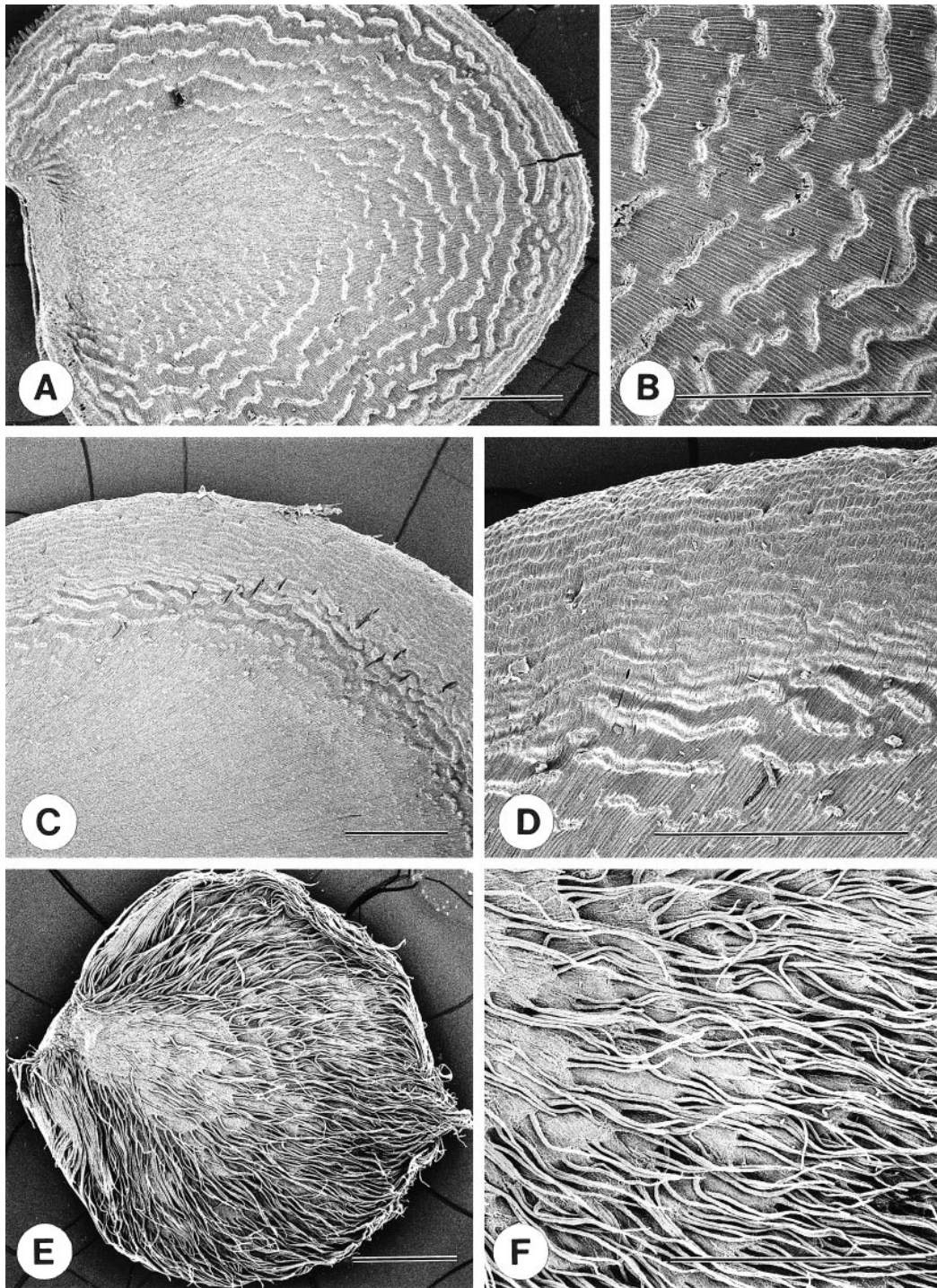
Fig. 4.   Scanning electron photomicrographs of seeds. A, B. *Chlamydacanthus dichrostachyus* (*Polhill & Lovett 4913*). A. Seed surface showing incomplete concentric ridges that increase in density toward edge of seed. B. Detail of incomplete ridges. C, D. *Whitfieldia elongata* (*Fanshawe 4648*). C. Concentric ridges at edge of a seed. D. Detail of ridges. E, F. *Lankesteria brevior* (*Leeuwenberg 2521*). E. Seed with trichomes covering the surface. F. Detail of trichomes. All scale bars = 1 mm.
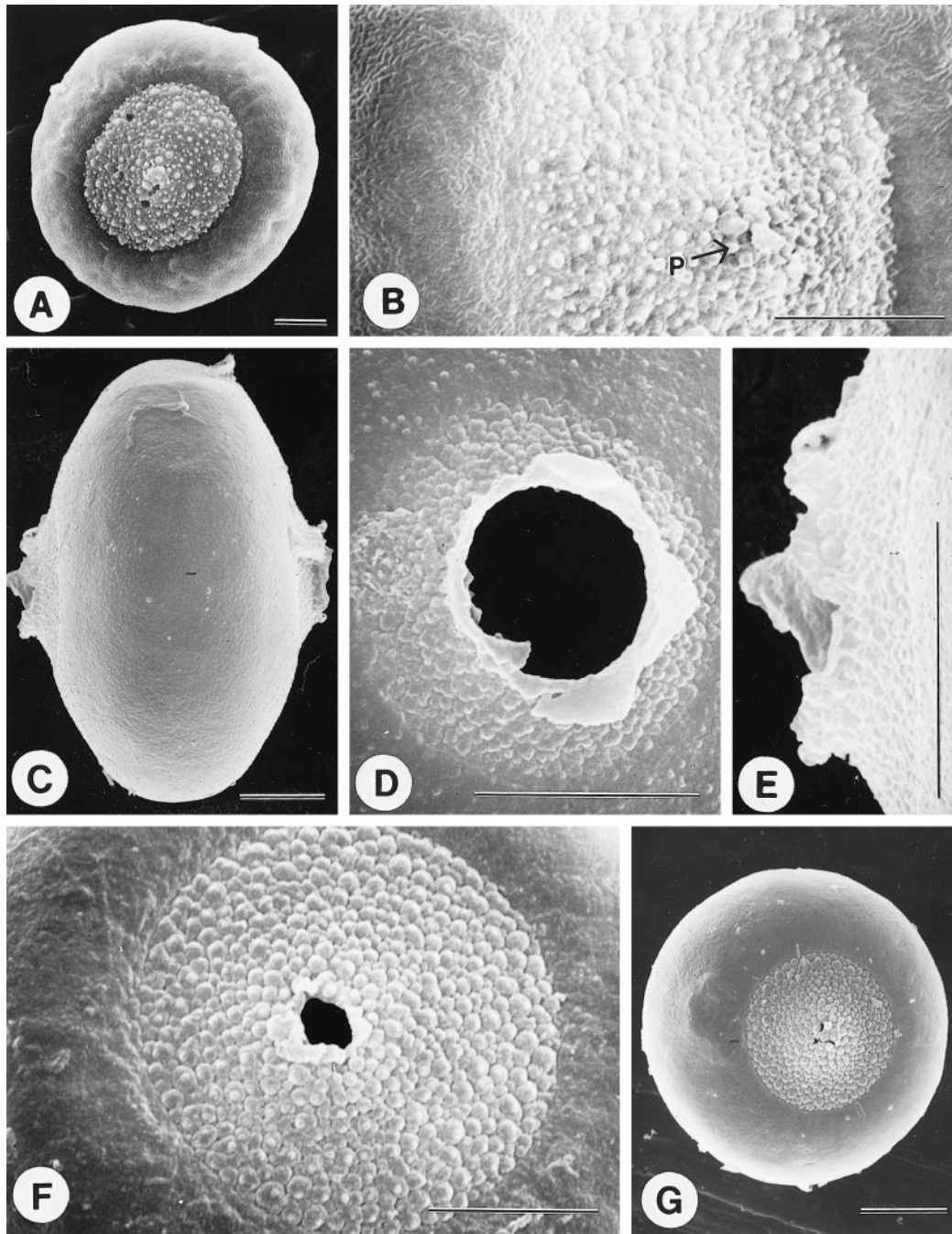
FIG. 5. Scanning electron photomicrographs of pollen. A,B. *Chlamydacanthus dichrostachyus* (*Polhill & Lovett 4913*). A. Whole grain, showing one apertural face. B. Detail of the circular microechinate surface surrounding the pore (p), which is covered by a membrane. C-E. *Whitfieldia arnoldiana* (*Leonard 158*). C. Whole grain, with two equatorial pores. D. Detail of a large pore with a ruptured membrane, and the surrounding granular surface. E. Detail of one pore in C. F. *W. lateritia* (*Dawe 406*). Circular granular surface surrounding a small pore. G. *W. letestui* (*Le Testu 2066*). Whole grain showing one apertural face, a membrane almost covers the pore. All scale bars = 10 μm.
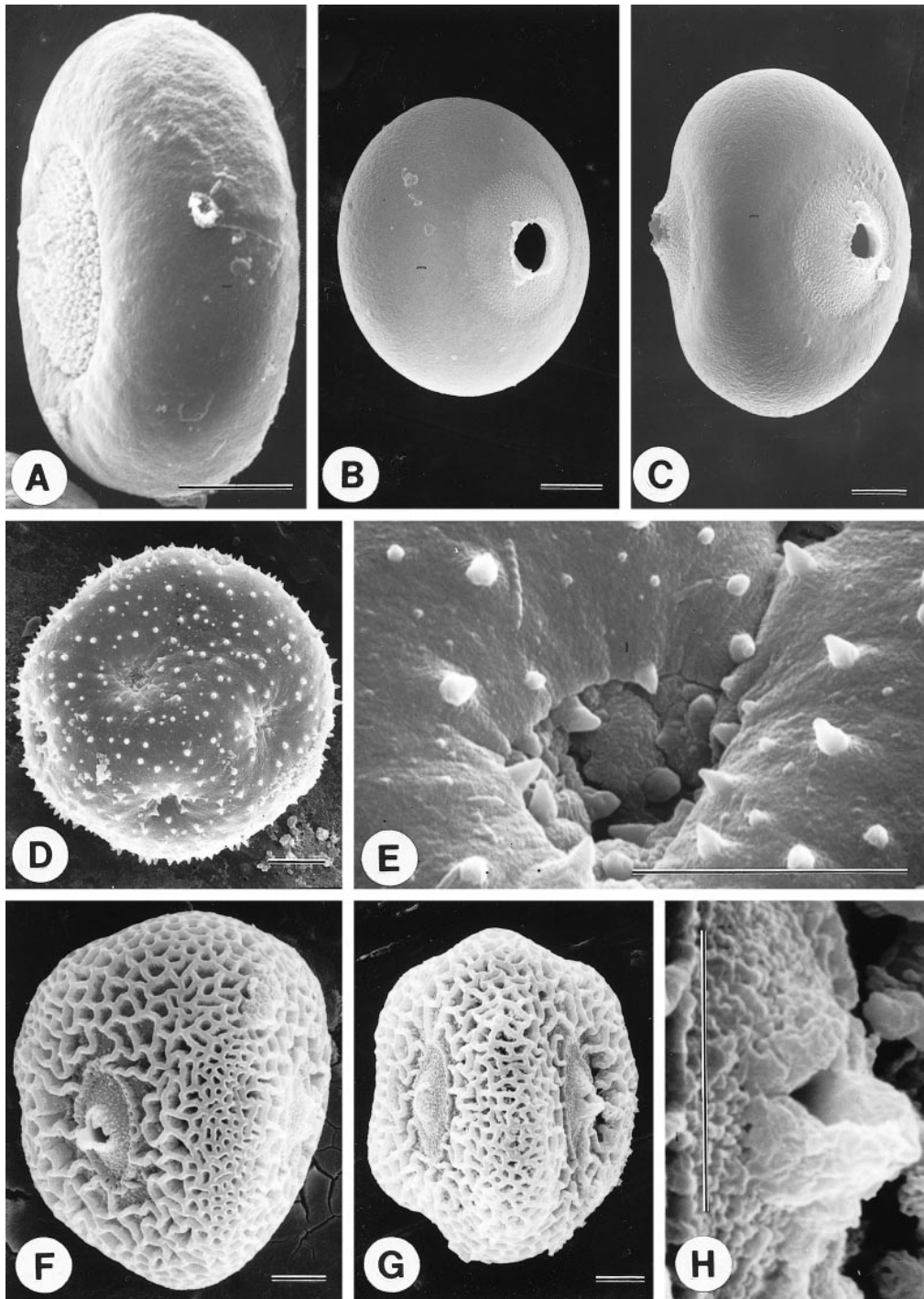
Fig. 6.   Scanning electron photomicrographs of pollen. A. *Whitfieldia letestui* (*Le Testu 2066*). Oblique view of a whole grain showing one granular apertural face and the psilate non-apertural area. B, C. *W. colorata* (*Fidao s.n.*). B. Biporate grain showing one pore. C. Triporate grain showing two pores. D, E. *W. elongata* (*Thomas 2015*). D. Spheroidal pantoporate

beal 1998). From Justicieae, we specifically included *Pseuderanthemum* in order to evaluate Bremekamp's (1944) ideas regarding placement of *Lankesteria* with that genus. Members of the other two major lineages of Acanthaceae were also included: Barlerieae (in Ruellioideae) and Acanthoideae. We also included representatives of two lineages that are the closest relatives of Acanthaceae s.s. (McDade et al. 2000), *Thunbergia* and *Elytraria.* Members of Pedaliaceae and Martyniaceae, which have been shown to be close relatives of Acanthaceae within Lamiales sensu Olmstead et al. (1993), were used as outgroups.

For ten genera, *trnL-trnF* and *ndhF* sequences were not available for the same species, requiring that these genera be represented by two different species (*Aphelandra, Barleria, Crabbea, Crossandra, Elytraria, Hypoestes, Justicia, Lepidagathis, Pseuderanthemum,* and *Ruellia*; see Appendix). Because we do not seek to address relationships at the species level, this should not affect our results. Further, in all cases, the species whose sequences were combined are part of monophyletic groups (McDade et al. 2000, unpubl. data). For example, *Justicia longii* and *J. americana* are members of New World *Justicia,* a monophyletic group that includes more than three hundred species but within which there is little molecular divergence (McDade et al. 2000).

Molecular Methods.   Fresh leaf materials dried in silica gel or herbarium specimens were used as the sources of DNA. For the *trnL-trnF* sequences, total genomic DNA was extracted using the modified CTAB method of Doyle and Doyle (1987). A fragment comprising the *trnL* intron, the 3′ *trnL* exon, and the intergenic spacer between this exon and the *trnF* gene of the chloroplast genome (Taberlet et al. 1991) was amplified using the ''c'' and ''f'' primers designed by these same authors. Standard PCR techniques were used to amplify double-stranded DNA.

Many of the *ndhF* sequences were produced by Scotland et al. (1995) and were retrieved from Genbank. The *ndhF* sequence for *Martynia annua* was generated in the Olmstead lab (Univ. of Washington). These were supplemented by seven new sequences generated specifically for this study. Total genomic DNA was extracted as described in Oxel-

man et al. (1997) and purified using GeneClean™ Spin kits. The primers cited by Oxelman et al. (1999) were used to amplify ca. 2100 bp of coding sequence using standard PCR techniques.

PCR products were purified with Qiagen™ Qiaquick spin-columns to remove primers and unincorporated dNTPs. Sequences were generated on ABI automated sequencers using the same primers as in amplification; internal primers were used for some of the *ndhF* sequences (see Oxelman et al. 1999). 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 were coded as uncertain.

Alignment and Analysis.   Sequences for each locus were aligned separately by eye in SeqApp 1.9a169 (Gilbert 1992). As noted by McDade and Moody (1999) for Acanthaceae, and by others for other groups (e.g., Gielly et al. 1996; Kim et al. 1996), the *trnL-trnF* sequences have a relatively high frequency of informative indels. Scotland et al. (1995) demonstrated that some of the few indels in *ndhF* were also phylogenetically informative. Sixteen (*trnL-trnF*) and four (*ndhF*) indels were added to the data matrix as presence/absence characters. The indels treated in this way were identified conservatively (i.e., with common 5′ and 3′ termini) and were parsimony informative (i.e., shared by two or more taxa). As expected given that *ndhF* is a protein-coding locus, all indel lengths were multiples of three; indels in the non-coding *trnL-trnF* region were not constrained in length.
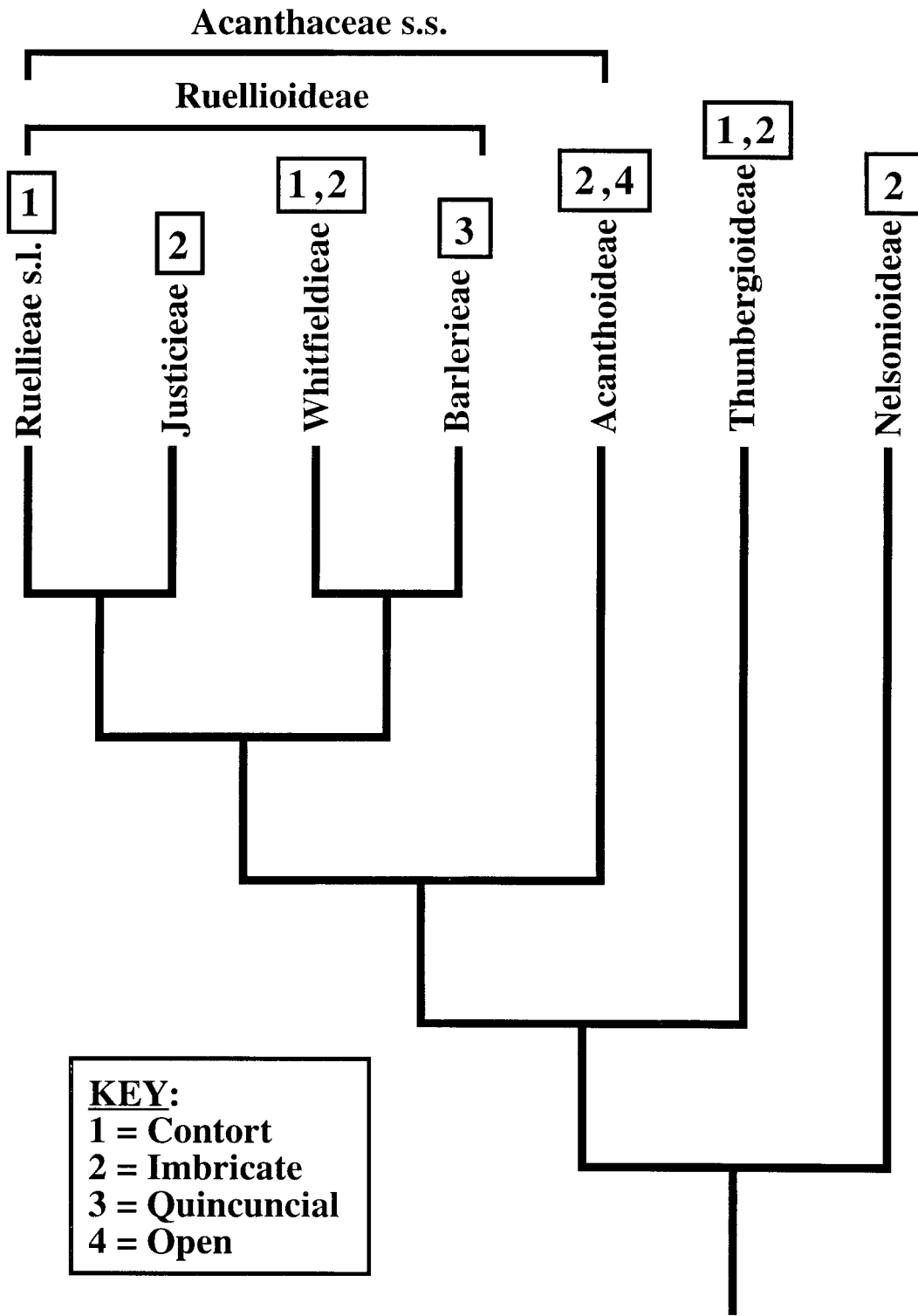
Data matrices for the two loci were prepared separately in MacClade version 4.0a10 (Maddison and Maddison 1999) and are available on request from MM or LAM. Matrices were analyzed separately in PAUP* 4.0b2 (Swofford 1999) on a MAC Power PC, with the PAUP* default settings for heuristic searches using parsimony except that addition sequence was set to random with 20 replicates. Multiple most parsimonious (MP) trees were combined as strict consensus trees.

The strength of support for individual branches

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grain with scattered microechinae. E. Detail of a pore and the microechinate surface. F-H. *Lankesteria hispida* (*Adam 3848*). F, G. Triporate grain showing two equatorial pores and a reticulate surface. F. Oblique equatorial view. G. Equatorial view. H. Detail of a pore and the surrounding granular surface. All scale bars = 10 μm.

Acanthaceae s.s.

Ruellioideae

| 1 | 2 | 1,2 | 3 | 2,4 | 1,2 | 2 |

Ruellieae s.l. | Justicieae | Whitfieldieae | Barlerieae | Acanthoideae | Thunbergioideae | Nelsonioideae

KEY:
1 = Contort
2 = Imbricate
3 = Quincuncial
4 = Open

was estimated using decay indices (DI; Bremer 1988; Donoghue et al. 1992), and bootstrap (BS; Felsenstein 1985) and jackknife values (JK; Felsenstein 1985; Farris et al. 1996). 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. BS values reported are from 200 ''full heuristic'' replicates with five random sequence addition replicates and TBR branch swapping. Jackknifing used 50% of characters deleted per replicate, 200 replicates with five random sequence addition replicates each, and TBR branch swapping.

There were almost no missing data in the *trnL-trnF* data set (1.3%) but a number of the *ndhF* sequences that were retrieved from Genbank were incomplete, resulting in 11.4% missing data in that data set. To evaluate the impact of these missing data, we repeated analyses without the portions of the *ndhF* sequences that had the most missing data. The results were identical to those from the analysis of the entire data set except that support values were slightly lower (results not shown).

The *ndhF* and *trnL-trnF* data sets were combined into a single NEXUS file using the file editing capabilities of PAUP*. The two data sets were tested for congruence using Farris et al.'s (1995) Incongruence Length Difference test (implemented in PAUP* as the partition homogeneity test). Phylogenetic analyses of the combined data set were conducted as described above, including generation of DI, BS, and JK values.

Alternative phylogenetic hypotheses were evaluated by using MacClade to prepare trees that reflect the alternate 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

topology in question. The difference between the length of these trees and the globally shortest trees provides an indication of the parsimony cost (in terms of additional evolutionary transitions) involved in accepting the alternative hypothesis.

Morphology. All specimens of *Chlamydacanthus, Lankesteria,* and *Whitfieldia* from K were studied; this was augmented by examination of additional specimens at UPS (Appendix; herbarium abbreviations follow Holmgren et al. 1990). Flowers, fruits, and seeds were studied under a dissecting microscope. Corollas were rehydrated prior to dissection. Seeds were hydrated with Libsorb™ fluid and observed under the dissecting microscope for any hygroscopic response. Morphological characters were also extracted from the literature (Burkill and Clarke 1899–1900; Heine 1963, 1966; Evrard and DeMillecamps 1992). Comparison of these plants to Barlerieae benefits from earlier exhaustive studies by MJB of holdings of pertinent genera at a number of herbaria (list available on request from MJB).

Surface detail on seeds from one species each of *Chlamydacanthus, Lankesteria,* and *Whitfieldia* (see Appendix) was examined using scanning electron microscopy (SEM). Seeds were removed from herbarium specimens at K, mounted on specimen stubs with the abaxial surface uppermost, sputter coated with platinum, and viewed using a Hitachi S-2400 SEM. Pollen was examined from a total of 13 species of the three genera (see Appendix) using SEM. Pollen was removed from herbarium specimens at K, acetolyzed according to standard techniques (Erdtman 1960), air-dried onto stubs from 90% ethanol, sputter coated with platinum and examined using a Hitachi S-510 SEM. For light microscopy (LM), slides were prepared by mounting pollen in glycerol jelly and examined using a Nikon Labophot.

RESULTS

Phylogenetic Relationships. The *trnL-trnF* and *ndhF* data sets were not significantly incongruent: 75 of 100 random partitions of the combined data

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FIG. 7. Distribution of aestivation patterns in Acanthaceae, following Scotland et al. (1994) and Schönenberger and Endress (1998); phylogeny from this paper and McDade et al. (2000). Imbricate aestivation no doubt merits further subdivision; for example, Scotland et al. (1994) has shown that imbricate aestivation in Nelsonioideae differs from that in other Acanthaceae s.l. Note that, within Acanthaceae s.s., only (3) quincuncial and (4) open aestivation patterns are candidates for non-homplasious synapomorphies; the evolutionary history of other aestivation types must involve homoplasious transitions.

had summed tree lengths shorter than the sum of the lengths of the original partition. Further, the topology obtained from the combined data set (Fig. 1) differs from those obtained from the separate data sets (not shown) only in degree of resolution (e.g., the *trnL-trnF* data do not resolve relationships among the *Whitfieldia* species and *Chlamydacanthus*) or in weakly supported portions (e.g., the *ndhF* tree resolves relationships among Ruellieae s.l., but with BS < 50 and DI = 1). As expected, given congruence of the data sets and increased number of characters in the combined data set (377 and 165 parsimony informative characters in the *ndhF* and *trnL-trnF* data sets, respectively, for a total of 532 in the combined data set), branch support increases markedly in the combined topology. For all of these reasons, discussion of relationships is based on the outcome of the combined analysis (Fig. 1).

Whitfieldia, Chlamydacanthus, and *Lankesteria* together form a well supported monophyletic group (BS=81, JK=83, DI=3; Fig. 1); we will subsequently refer to this group as Whitfieldieae. Among these, *Whitfieldia* is monophyletic (BS=100, JK=100, DI=6), and *Chlamydacanthus* is more closely related to *Whitfieldia* than is *Lankesteria* (Fig. 1). Surprisingly, these three genera are placed with representatives of tribe Barlerieae (i.e., *Lepidagathis, Barleria, Crabbea*), with strong support (BS=91, JK=90, DI=4; Fig. 1). Ruellieae s.l. and Justicieae are each strongly supported as monophyletic and are each others' closest relatives (BS=85, JK=85, DI=3). The Whitfieldieae plus Barlerieae lineage is sister to these last two together, with very strong support (BS=100, JK=100, DI=32, Fig. 1). Acanthoideae are sister to all other members of Acanthaceae s.s., and *Thunbergia* and *Elytraria* are sequentially more distantly related to Acanthaceae s.s.

Constraining *Lankesteria* and *Pseuderanthemum* to be sister taxa requires 54 additional steps (3.1% of total tree length). When *Lankesteria* is constrained to monophyly with all Justicieae (i.e., including *Pseuderanthemum* but not specifically as sister to this taxon), the shortest trees are 15 steps (0.9%) longer than the MP trees.

Morphology. Because these plants are poorly known, we here provide a general description of flower, fruit, seed, and pollen characters. The phylogenetic status of these characters will be assessed in the discussion. Like other Acanthaceae, *Whitfieldia, Chlamydacanthus,* and *Lankesteria* have inflorescences that are basically thyrses. In these genera, the thyrses are narrow and the individual cymes are three-flowered in some species or reduced to a single flower in others. In *Chlamydacanthus* and *Whitfieldia,* each flower is subtended by one narrow bract and two ovate bracteoles (Fig. 2H-K) whereas in *Lankesteria,* the floral bract is linear to ovate and the paired bracteoles are inconspicuous and linear (Fig. 2L,M). The calyx has a short tube and then is evenly five-lobed in *Chlamydacanthus* and *Lankesteria.* In *Whitfieldia,* one of the five calyx lobes is markedly smaller than the others; the four equal lobes are obovate whereas the fifth is linear to narrowly elliptic. The corollas of the investigated species of *Chlamydacanthus* have a relatively short, broad tube (Fig. 2D); they are slightly (*C. lindavianus*) to strongly (*C. euphorbioides* Lindau) zygomorphic. In *Whitfieldia,* most species also have a slightly zygomorphic corolla with a relatively short and broad tube (Fig. 2B). In contrast, *W. elongata* has a subactinomorphic corolla with a narrow, elongated tube (Fig. 2A). The corollas in *Lankesteria* are subactinomorphic and infundibuliform with a narrow tube (Fig. 2C). Corolla morphology thus suggests a range of pollinator relationships from bees (*Chlamydacanthus,* most *Whitfieldia*) to moths (*W. elongata*) or butterflies (*Lankesteria*).

Beyond differences likely associated with pollinators, the corollas of species in all three genera have a trifurcation of each corolla lobe trace (Fig. 3), no filament curtain, four filaments (two stamens are reduced to staminodes in *Lankesteria* [Fig. 2C] and in *Chlamydacanthus* sp. [Fig. 2D]; some flowers of *W. elongata* appear to have a fifth staminal element which is staminodal), anther thecae positioned at equal height within each stamen, and a capitate stigma (Fig. 2E-G). The trifurcation of the corolla lobe traces is conspicuous in *Chlamydacanthus* and *Whitfieldia* whereas, in *Lankesteria,* this structure is faint. Further, the capitate stigma of plants in the former two genera is symmetrical, whereas it is oblique in *Lankesteria.*

Capsules are two-seeded in *Chlamydacanthus* and *Lankesteria,* and two- to four-seeded in *Whitfieldia.* The surfaces of seeds of *Chlamydacanthus* and *Whitfieldia* are glabrous (Figs. 2N-P, 4A-D), whereas those of *Lankesteria* are covered with whitish trichomes that show hygroscopic movement (Figs. 2Q,R; 4E,F). Seeds of *Chlamydacanthus* also have a ring of very short trichome-like protrusions around the rim of the seeds (Figs. 2O,P; 4A); these are sometimes apically reflexed, and are quite unlike the trichomes that cover seeds of *Lankesteria.* It was not possible to test these protrusions for hygroscopic movement due to their small size. In addition, seeds from plants of all three genera are cov-

ered with concentric rings of ridges (these are limited to one surface in *Chlamydacanthus*) (Figs. 2N-R, 4). The trichomes of *Lankesteria* seeds appear to be attached to these ridges (Fig. 4F).

Pollen of *Chlamydacanthus* (Fig. 5A,B) and most *Whitfieldia* species (Figs. 5C-G, 6A-C) is biporate and circular in shape in apertural view (Figs. 5A,C,G; 6A,B). The position of the pores (equatorial or polar) is not known because the tetrad stage of development has not been examined (see Furness and Vollesen 1991). By analogy with other acanthaceous grains and with other eudicots, an equatorial position is most likely. The apertural faces are flattened and may collapse after acetolysis. Each pore is surrounded by a densely granular to microechinate circular area (Fig. 5B,D,F) and a granular to microechinate membrane may almost completely cover the pore, as in *Chlamydacanthus* (Fig. 5A,B), or rupture to reveal a pore that varies in size among species (Fig. 5D,F,G). The exine between apertures is almost psilate, or with faintly scabrate ornamentation (e.g., Fig. 5B). Occasional triporate grains, with a triangular outline in polar view, were observed with biporate grains in *W. colorata* (Fig. 6B,C). *Whitfieldia elongata* is exceptional in our survey in having spheroidal pollen with 6–9 scattered pores (Fig. 6D,E). The surface has scattered microchinae and each pore has a microechinate membrane but is not surrounded by a region of differentiated exine.

Lankesteria hispida has triporate pollen (Fig. 6F-H), with three equatorial pores (although tetrads have not been examined). It is triangular in polar view and the apertural faces are flattened. Each pore is surrounded by a densely granular circular area and a densely granular membrane covers the pore but usually ruptures in acetolysis. The remainder of each apertural face has a coarse reticulum, which is finer in the interapertural areas.

DISCUSSION

Our results support Bremekamp's (1944, 1965) suggestions regarding exclusion of *Whitfieldia* from tribe Ruellieae s.l. and a close relationship between *Chlamydacanthus* and *Whitfieldia.* However, they refute the suggestion of a close relationship between *Lankesteria* and *Pseuderanthemum* (Bremekamp 1944). Instead, our data indicate that *Lankesteria* is sister to *Chlamydacanthus* plus *Whitfieldia,* and thus a member of an extended Whitfieldieae including these three genera. Further conclusions from our data are that tribe Whitfieldieae is sister to tribe

Barlerieae, rather than being closely related to tribe Ruellieae s.l., that the synonymization of *Whitfieldia* and *Stylarthropus* is tenable, and that Lepidagathideae should be included in an expanded tribe Barlerieae.

Given the enigmatic nature of the three genera studied here, it is perhaps not surprising that morphological synapomorphies are subtle. We propose that the unusual concentric ridges protruding from the testa of the seeds represent a synapormorphy for all three genera of Whitfieldieae. These are somewhat masked by hygroscopic trichomes in *Lankesteria*; seeds of most Barlerieae have hygroscopic hairs like those of *Lankesteria* but lack the ridges (M.-J. Balkwill, pers. obs.). Pollen grains of *Chlamydacanthus, Whitfieldia* and *Lankesteria* share the densely granular circular area surrounding each pore. Our data suggest secondary loss of this circular area in *W. elongata*; however, Scotland (1993) described pollen of this species as having a densely microechinate area encircling or covering each pore.

Morphological synapomorphies for *Chlamydacanthus* plus *Whitfieldia* include seeds with glabrous surfaces, perhaps representing loss of hygroscopic trichomes. K. Vollesen (pers. comm.) has suggested that retention of the concentric ridges on the seed surfaces of *Chlamydacanthus* and *Whitfieldia* supports the notion that these plants have lost the hygroscopic trichomes that are attached to those ridges on seeds of *Lankesteria. Chlamydacanthus* plus *Whitfieldia* are marked also by presence of biporate, flattened pollen grains that are circular in outline (the pollen morphology of *W. elongata* can only be interpreted as autapomorphic given that both morphology and molecular sequence data otherwise support placement of this species with other *Whitfieldia*). Although relative shape of bracts and bracteoles varies considerably among Acanthaceae, the combination of narrow bracts and ovate bracteoles is unusual and not found among close relatives of these plants. The trifurcation of each corolla lobe trace occurs also in *Lankesteria* and in at least some Barlerieae (see below), but its conspicuousness in *Chlamydacanthus* and *Whitfieldia* may be an additional synapomorphy.

The lineage comprised of Whitfieldieae plus Barlerieae is a large (ca. 430 species; Lindau 1895; Mabberley 1997) and heterogeneous group of plants such that identification of morphological synapomorphies is difficult. The hygroscopic trichomes on seeds of *Lankesteria* are like those that occur on seeds of most Barlerieae (M.-J. Balkwill, pers. obs.). We hypothesize that these are homologous and syn-

apomorphic for Whitfieldieae plus Barlerieae (interpreting their absence in *Whitfieldia* and *Chlamydacanthus* as due to subsequent loss). The mucilaginous hairs found in Ruellieae s.l. are different and apparently not homologous to those in any genera of Barlerieae or Whitfieldieae (Grubert 1974; Scotland et al. 1995; Manktelow 1996). The trifurcation of the corolla lobe traces occurs in some Barlerieae (e.g., *Barleria prionitis,* M. Manktelow, pers. obs.), as well as in the three genera of Whitfieldieae. This character has not been thoroughly surveyed in other Acanthaceae, but it is not conspicuously present in groups with which we are familiar. In all taxa of Whitfieldieae plus Barlerieae (except *Barleria*) that we surveyed, the stigma appears capitate (although it is two-lobed). Balkwill and Balkwill (1996, 1997) report a rich diversity of stigma morphology in *Barleria,* from two-lobed capitate to one-lobed filiform. Furthermore, some genera in Justicieae have a capitate stigma (Lindau, 1895). A detailed survey of stigma morphology in Acanthaceae is needed to determine whether the type of capitate stigma found in Whitfieldieae plus Barlerieae is a synapomorphy for this clade, with further modifications in *Barleria.* Pollen morphology is exceedingly variable among genera of Barlerieae (Raj 1961) and we know of no pollen synapomorphies that support monophyly of that lineage nor that support the sister group relationship proposed here between Whitfieldieae and Barlerieae.

Regarding alternative hypotheses that have been advanced for placement of the genera here treated as Whitfieldieae, corolla morphology of *Lankesteria* is reminiscent of some *Pseuderanthemum* species but such traits vary at low taxonomic levels within Acanthaceae. The pollen grains of *Lankesteria* are markedly different from the tricolporate hexapseudocolpate grains found in species of *Pseuderanthemum* and relatives (e.g., Sharma and Vishnu-Mittre 1963, Daniel 1998). We also do not know of any Ruellieae with pollen grains like those of *Whitfieldia* or *Chlamydacanthus.* Indeed, the only other occurrence in Acanthaceae of biporate, circular pollen grains like those found in Whitfieldieae is in *Isoglossa* and relatives, which are members of *Justicieae* (see McDade et al., in press). However, *Isoglossa* pollen has spinules over the entire surface and the walls have distinct columellae, compared with the granular wall structure in *Whitfieldia* and *Chlamydacanthus* (C. Furness, pers. obs.). The similarity in aperture number and shape among pollen grains of these plants is evidently homoplasious. The complex filament curtain structure, a synapomorphy for

Ruellieae s.l. (Manktelow 2000), is absent from flowers of Whitfieldieae. Other similarities shared by *Whitfieldia, Chlamydacanthus* and Ruellieae (e.g., four bithecous stamens with parallel thecae), and by *Lankesteria* and *Pseuderanthemum* are likely symplesiomorphic for all Acanthaceae.

The combination of retinacula and contort aestivation is not diagnostic for a monophyletic lineage in Acanthaceae, as suggested by Scotland et al. (1995), because this character combination appears in both Ruellieae s.l. and Whitfieldieae, as defined here. Synapomorphic characters for Ruellieae s.l. are instead the presence of a filament curtain (Manktelow 2000) and a stigma with unequal lobes (apparently reversed to equal in only a few species of the derived genus *Louteridium;* Bremekamp 1965; Daniel 1995, pers. comm.).

Our data are in accord with earlier studies that have indicated that aestivation is an evolutionarily labile character in at least some lineages of Acanthaceae (Fig. 7; Schönenberger and Endress 1998). Within the clade of Whitfieldieae plus Barlerieae three different aestivation patterns are found: contort, imbricate and quincuncial. Other lineages in Acanthaceae in which aestivation pattern varies include Acanthoideae, with imbricate and open aestivation (Scotland et al. 1994), and Thunbergioideae, with imbricate and contort aestivation (Schönenberger and Endress 1998). Contort aestivation thus occurs in three distantly related groups within Acanthaceae s.l.: Thunbergioideae, Whitfieldieae, and Ruellieae s.l., and among these it is consistent only in Ruellieae s.l. (Fig. 7). It thus seems that Bremekamp (1938) was correct in suggesting that the use of aestivation as the cardinal character at the supratribal level ''has but little to recommend itself, and is better dropped.'' Indeed, the notion of seeking a single, completely reliable character upon which to base a classification is clearly misguided. Corolla aestivation, like most other characters, provides unique and unreversed synapomorphies in some lineages and at some taxonomic levels, but is homoplastic at others. Distinguishing these patterns of character evolution is the continuing task of systematics.

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APPENDIX 1. Taxa, Genbank accession numbers and voucher or reference citations for *trnL-trnF* sequences followed by *ndhF* sequences (NA = not available); (below) taxa and voucher specimens studied for morphological, seed and pollen characters. Abbreviations for herbaria follow Holmgren et al. (1990). Classification of Acanthaceae is as presented in the text, and reflects Bremekamp (1965), McDade et al. (2000), and the results of this study.

Acanthaceae
  Ruellioideae
    Whitfieldieae
      Chlamydacanthus lindavianus H. Winkl.: AF194435 (*Borhidi et al. 86464,* UPS); AJ249408 (*Borhidi et al. 86464,* UPS)
      Lankesteria brevior C.B. Clarke: AF194436 (*Manktelow et al. 117,* UPS); AJ249409 (*Manktelow et al. 117,* UPS)
      Whitfieldia elongata (P. Beauv.) De Wild. & Th. Dur.: AF195518 (*Manktelow 682,* UPS); AJ249406 (*Manktelow 682,* UPS)
      W. stuhlmannii (Lindau) C.B. Clarke: AF194437 (*Iversen et al. 87520,* UPS); AJ249407 (*Iversen et al. 87520,* UPS)
    Ruellieae s.l.
      Hygrophila corymbosa Lindau: AF063120 (Missouri Botanical Garden, Accession No. 897223); U12661 (Scotland et al. 1995)
      Ruellia californica (Rose) I.M. Johnst.: AF063115 (*McDade 1157,* ARIZ); NA
      Ruellia ciliosa Pursh: NA; U12664 (Scotland et al. 1995)
      Sanchezia speciosa Leonard: AF063113 (Duke University greenhouse, Accession No. 66-462); U12665 (Scotland et al. 1995)
      Strobilanthes dyeriana Mast.: AF195519 (*MacDougal 5061,* MO); U12666 (Scotland et al. 1995)
    Justiceae
      Hypoestes taeniata Benoist: NA; U12662 (Scotland et al. 1995)
      H. phyllostachya Baker: AF195520 (*McDade 1173,* ARIZ); NA
      Justicia americana (L.) Vahl: NA; U12663 (Scotland et al. 1995)
      J. longii Hilsenb.: AF063135 (*Van Devender 87-307,* ARIZ); NA
      Pseuderanthemum subviscosum (C.B. Clarke) Stapf: NA; AJ249411 (*Borhidi et al. 84378,* UPS)
      Pseuderanthemum alatum (Nees) Radlk.: AF063130 (Duke University greenhouse, Accession No. 84-055); NA
    Barlerieae
      Barleria oenotheroides Dum.: AF063116 (*Freeh & Johnson 94-012,* ARIZ); NA
      B. prionitis L.: NA; U12653 (Scotland et al. 1995)

Crabbea reticulata C.B. Clarke: NA; U12655 (Scotland et al. 1995)

C. velutina S. Moore: AF195521 (*Manktelow 613,* UPS); NA

Lepidagathis andersoniana Lindau: NA; AJ249410 (*Steiner 636,* UPS)

L. villosa Hedrén: AF063121 (Scotland et al. 1995); NA

Acanthoideae

Aphelandra dolichantha Donn. Sm.: AF063111 (*McDade 243,* Duke); NA

A. squarrosa Nees: NA; AJ249405 (*McDade 1174,* ARIZ)

Crossandra nilotica Oliv.: NA; U12656 (AJ249410)

C. pungens Lindau: AF061825 (Duke University greenhouses, Accession No. 91-036); NA

Thunbergioideae

Thunbergia alata Bojer ex Sims: AF061820 (*McDade 1175,* ARIZ); U12667 (Scotland et al. 1995)

Nelsonioideae

Elytraria crenata Vahl: NA; U12657 (Scotland et al. 1995)

E. imbricata (Vahl) Pers.: AF061819 (*McDade & Jenkins 1155,* ARIZ); NA

Martyniaceae

Martynia annua L.: AF067065 (*Jenkins 97-149,* ARIZ); AF190906 (*Jenkins 97-149,* ARIZ)

Pedaliaceae

Sesamum indicum L.: AF067067 (*Jenkins 97-141,* ARIZ); L36413 (Olmstead & Reeves 1995)

Additional material studied for morphological (M), seed (S), and pollen (P) characters (note that all specimens of these genera at K were examined; we cite below those on which detailed morphological observations were made, from which illustrations were prepared, and from which material was taken for examination of seeds or pollen):

Whitfieldieae

Chlamydacanthus dichrostachyus Mildbr. (*Semsei 804,* K; M) (*Polhill & Lovett 4913,* K; S, P)

C. lindavianus H. Winkl. (*Adams 72, Drummond & Hemsley 3592,* K; M)

C. sp. (*Du Puy et al. 230,* K; M)

Lankesteria brevior C.B. Clarke (*Deighton 312, 4095, Samai SKS 262,* K; *Manktelow et al. 117,* UPS; M) (*Leeuwenberg 2521,* K; S)

L. elegans (P. Beauv.) T. Anderson (*Obile et al. 20541,* K; M)

L. hispida (Willd.) T. Anderson (*Adam 3848,* K, *Daniel 435,* UPS; M) (*Adam 3848,* K; P)

Whitfieldia arnoldiana Wildem. & Th.Dur. (*Leonard 158,* K; P)

W. brazzei C.B. Clarke (*Fidao s.n.,* K; P)

W. colorata C.B. Clarke (*King 196B,* K; P)

W. elongata (P. Beauv.) De Wild. & Th. Dur. (*Lowe 3513, Callens 2766, Thomas 2015,* K; *Lebrun 1771,* UPS; M) (*Thomas 2015,* K; P) (*Fanshawe 4648,* K; S)

W. lateritia Hook. (*Lane-Pool 180,* K; *Afzelius s.n.,* UPS; M) (*Dawe 406,* K; P)

W. Letestui R. Benoist (*Le Testu 2066,* K; P)

W. preussii C.B. Clarke (*Zenker 4668,* K; P)

W. rutilans Heine (*Heine 8858,* K; P)

W. striata (S. Moore) Vollesen (*Gossweiler 8049,* K; P)

W. stuhlmannii (Lindau) C.B. Clarke (*Faulkner 1795,* K; *Iversen et al. 87520,* UPS; M); (*Faulkner 1795,* K; P)

W. sp. nov. (*Baldwin 10754,* K; P)

Barlerieae

Barleria prionitis L. (*Zúmer 289,* UPS; M)

Crabbea velutina S. Moore (*Pawek 7739,* K, *Hedrén 653,* UPS; M)

Lepidagathis alopecuroidea (Vahl) R. Br. (*Morton GC 9170,* K)

L. calycina Nees (*Gilbert & Ermias 8487,* UPS; M)

L. formosensis C.B. Clarke (*Furuse 5448,* K; M)

Lophostachys sessilifolia Pohl (*Philcox & Freeman 4733,* K; M)

L. villosa Pohl (*Brookes & Reeves 544,* K; M)