

PHYLOGENY AND CIRCUMSCRIPTION OF THE NEAR-ENDEMIC BRAZILIAN TRIBE MICROLICIEAE (MELASTOMATACEAE)¹

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The members of tribe Microlicieae in the flowering plant family Melastomataceae are nearly all endemic to the cerrado biome of Brazil. Traditional classifications of the Melastomataceae have attributed between 15 and 17 genera to the Microlicieae, but subsequent revisions have circumscribed the tribe more narrowly. The monophyly and intergeneric relationships of the Microlicieae were evaluated through phylogenetic analyses with molecular and morphological data sets. Incorporation of DNA sequences from the intron of the chloroplast gene *rpl16* into a previously generated family-wide data set yielded a clade comprising *Chaetostoma*, *Lavoisiera*, *Microlicia*, *Rhynchanthera*, *Stenodon*, and *Trembleya* (“core Microlicieae”), with *Rhynchanthera* as the first-diverging lineage. The other four genera of Microlicieae sampled are placed in other clades: *Eriocnema* with Miconieae; *Siphanthera* with *Aciotis*, *Nepsera*, and *Acisanthera* of Melastomeae; *Castratella* as sister to *Monochaetum* of Melastomeae; and *Cambessedesia* as part of an unresolved polytomy in a large clade that includes most Melastomataceae. Analyses of the chloroplast genes *rbcL* and *ndhF* that included three core genera produced similar results, as did the combined analysis of all three data sets. Combined parsimony analyses of DNA sequences from *rpl16* and the nuclear ribosomal intergenic transcribed spacer (ITS) region of 22 species of core Microlicieae yielded generally low internal support values. *Lavoisiera*, recently redefined on the basis of several morphological characters, was strongly supported as monophyletic. A morphological phylogenetic analysis of the Microlicieae based on 10 parsimony-informative characters recovered a monophyletic core Microlicieae but provided no further resolution among genera. Penalized likelihood analysis with two calibration time windows produced an age estimate of 3.7 million years for the time of initial divergence of strictly Brazilian core Microlicieae. This date is in general agreement with the estimated age of the most active stage of development of cerrado vegetation and implies an adaptive shift from hydric to seasonally dry habitats during the early evolution of this group.

Key words: cerrado; ITS; Melastomataceae; Microlicieae; *ndhF*; phylogeny; *rbcL*; *rpl16*.

The Microlicieae constitute one of eight major clades of Melastomataceae (Angiospermae: Myrtales) recognized by morphological and molecular characters (Clausing and Renner, 2001). Traditional classifications of the Melastomataceae have attributed between 15 and 17 genera to the Microlicieae (Table 1) based on reniform, oblong seeds with a foveolate testa in combination with capsular fruits, basally prolonged anther connectives, rostrate anther thecae, and glabrous ovaries (Triana, 1871; Cogniaux, 1891). The imprecise circumscription of several genera in the tribe has long been recognized (Hooker, 1867; Triana, 1871; Baillon, 1877; Cogniaux, 1891), but its monophyletic status had not been questioned until the publication of a morphologically based classification of the Melastomataceae in which *Centradenia*, *Poteranthera*, and *Siphanthera* were excluded from the tribe and several other genera were placed into synonymy, thereby reducing it to 11 genera (Renner, 1993; Table 1). More recently, from observations of seed morphology in connection with taxonomic work on

Lavoisiera, the core Microlicieae have been suggested to comprise just six genera (Almeda and Martins, 2001; Table 1).

Of the approximately 275–300 species constituting the Microlicieae, over 90% are endemic to the cerrado biome of Brazil. This biome is characterized by a sparse to dense savanna of shrubs and small trees under the strong influence of seasonal rainfall and fire. Within this biome, the tribe is most diverse in campo rupestre, a habitat of rocky, nutrient-poor, and porous substrates occupied by shrub savannas and grasslands. This habitat is restricted to the higher elevations (generally 1000–2000 m) of southeastern Brazil, especially the Serra do Espinhaço and associated ranges in Minas Gerais, the Chapada Diamantina in Bahia, and the Serra Geral in Goiás. Some Microlicieae also occur in cerrado sensu stricto, a lower-elevation habitat in Brazil with low fertility, well-drained soils, and a deep water table. The woody component is of greater density and height than that of campo rupestre and the flora is generally fire tolerant and fire dependent (Oliveira-Filho and Ratter, 2002).

The age, origin, and dynamics of Neotropical savannas and their environmental determinants continue to be debated (Sarmiento, 1984). Most of the old Precambrian shield of interior Brazil now covered by cerrado has been available for occupation by land plants since before Africa and South America separated from one another between 95 and 100 million years ago (mya; Pitman et al., 1993), but this tells us nothing about the initial availability of eroded substrates that now harbor a

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TABLE 1. Four concepts of the Microlicieae. An asterisk indicates that the genus was not sampled.

Triana (1871)	Cogniaux (1891)	Renner (1993)	Almeda and Martins (2001)
<i>Bucquetia</i> *	<i>Bucquetia</i> *	<i>Bucquetia</i> *	<i>Chaetostoma</i>
<i>Cambessedesia</i>	<i>Cambessedesia</i>	<i>Cambessedesia</i> (incl. <i>Pyramia</i>)	<i>Lavoisiera</i>
<i>Castratella</i>	<i>Castratella</i>	<i>Castratella</i>	<i>Microlicia</i>
<i>Centradenia</i>	<i>Centradenia</i>	<i>Chaetostoma</i>	<i>Rhynchanthera</i>
<i>Chaetostoma</i>	<i>Chaetostoma</i>	<i>Eriocnema</i>	<i>Stenodon</i>
<i>Eriocnema</i>	<i>Eriocnema</i>	<i>Lavoisiera</i>	<i>Trembleya</i>
<i>Lavoisiera</i>	<i>Lavoisiera</i>	<i>Lithobium</i> *	
<i>Lithobium</i> *	<i>Lithobium</i> *	<i>Microlicia</i>	
<i>Meisneria</i> (= <i>Siphanthera</i>)	<i>Microlicia</i>	<i>Rhynchanthera</i>	
<i>Microlicia</i>	<i>Poteranthera</i> *	<i>Stenodon</i>	
<i>Poteranthera</i> *	<i>Pyramia</i> (= <i>Cambessedesia</i>)	<i>Trembleya</i>	
<i>Pyramia</i> (= <i>Cambessedesia</i>)	<i>Rhynchanthera</i>		
<i>Rhynchanthera</i>	<i>Siphanthera</i>		
<i>Stenodon</i>	<i>Stenodon</i>		
<i>Svitramia</i> *	<i>Trembleya</i>		
<i>Trembleya</i>			
<i>Tulasnea</i> (= <i>Siphanthera</i>)			

rich assemblage of Microlicieae. The prototype of present-day cerrado in Brazil possibly existed back in the Cretaceous, but the most dynamic stage of development of this biome is thought to have occurred during the climatic fluctuations that began at the onset of the Quaternary (Oliveira-Filho and Ratter, 2002). Paleoenvironmental studies have confirmed that forests and cerrado vegetation underwent cycles of expansion and contraction in Brazil during the Quaternary with the greatest expansion of cerrado occurring within the last 10000 years, and vegetation resembling present-day cerrado is not known prior to 7000 years before present in central Brazil (Ledru, 2002). As far as we can ascertain, there are no records documenting a paleofloristic assemblage that resembles the current vegetation of campo rupestre.

Additional insight into the origin and development of the vegetation of cerrado and campo rupestre can be gained through phylogenetic study of the plant groups endemic to these habitats, but few such groups have been studied in this context. Here we use DNA sequences from the nuclear ribosomal internal transcribed spacer (ITS) region, the chloroplast genes *rbcL* and *ndhF*, the chloroplast *rpl16* intron, and a data set based on gross morphology to evaluate the monophyly, generic composition, and generic relationships of the Microlicieae. We then use the molecular data to estimate the time of initial divergence of the Microlicieae with which to make inferences on the paleoecology of cerrado vegetation.

MATERIALS AND METHODS

Taxon sampling and DNA sequencing—Total genomic DNA was isolated from desiccant-dried leaf tissue with DNeasy plant extraction kits (Qiagen, Valencia, California, USA). Desiccant-dried leaf tissue was pulverized by high-speed action in a Wig-L-Bug grinding mill (REFLEX Analytical, Ridge-wood, New Jersey, USA). Polymerase chain reaction (PCR) amplification was performed with standard methods (Dieffenbach and Dveksler, 1995) and BIO-LASE (Bioline USA, Randolph, Massachusetts, USA) as the DNA polymerase. The PCR products were purified with the Wizard PCR Preps DNA Purification System (Promega, Madison, Wisconsin, USA), cycle sequencing was performed with the ABI Prism BigDye Terminators v2.0 Cycle Sequencing Reaction Kit (Applied Biosystems, Foster City, California, USA) by using 1/4-scale reaction mixtures in a model 9600 PCR System thermal cycler (Perkin-Elmer, Boston, Massachusetts, USA), and sequences were determined with an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Forward and reverse reads were obtained for all samples. Sequences were edited with

the computer program Sequencher (3.0 and 4.1; Gene Codes, Ann Arbor, Michigan, USA) and all sequences have been deposited in GenBank (Appendix; see Supplemental Data accompanying the online version of this article).

To evaluate the monophyly of the Microlicieae, we incorporated nine of the 11 genera of this tribe sensu Renner (1993; Table 1) into a previously generated data set of *rpl16* sequences representing all tribes and major clades of Melastomataceae (Clausing and Renner, 2001). The Microlicieae samples included seven newly generated *rpl16* sequences and the three sequences from Clausing and Renner (2001; *Rhynchanthera* was represented by two samples). A newly generated sequence from *Siphanthera* was also included on the basis of Cogniaux (1891), who placed this genus in the Microlicieae (Table 1). Two species of Memecylaceae (*Memecylon edule* and *Mouriri helleri*) served to root the tree on the basis of Clausing and Renner (2001). We employed “rpl-mel-exon1” (5′-GCTATGCTTAGCGTGTGACTCGT-3′) and “L16 exon2” (Downie et al., 2000) as amplification primers. We sequenced *rpl16* with the amplification primers plus the internal primers “rpl-mel-748F” (5′-TAA-CAAAAATACATTCTTCAT-3′) and “rpl-mel-666R” (5′-CTTCACGGGC GAATATTGAC-3′).

To attempt to improve phylogenetic resolution and statistical support for the placement of “non-core” genera of Microlicieae beyond that recovered from analysis of *rpl16* sequences (see Results), we generated DNA sequences of the chloroplast genes *rbcL* and *ndhF* for *Cambessedesia*, *Castratella*, and *Eriocnema* and an *rbcL* sequence from *Siphanthera* (Appendix; see Supplemental Data accompanying the online version of this article; *Siphanthera* was not sampled for *ndhF* because it failed to amplify despite repeated attempts). We then included these sequences in previously generated data sets of each gene comprising many of the same samples from the *rpl16* analysis, including three genera of “core” Microlicieae. The first half of the *rbcL* gene was amplified and sequenced with primers 1F and 724R; the second half was amplified and sequenced with 724F (i.e., the reverse-complement of 724R) and 1460R (5′-CTTTTAGTAAAAGATTGGGCCGAG-3′; Olmstead et al., 1992; Fay et al., 1997). The *ndhF* gene was amplified by following the protocol of Clausing and Renner (2001) with primers developed by Olmstead and Sweere (1994), except that for some samples the gene was amplified in sections with various combinations of internal and external primers. Outgroups were the same as those employed for *rpl16*.

To assess phylogenetic relationships within core Microlicieae (see Results), we expanded the sample of core-genera *rpl16* sequences to 26. This encompassed eight species of *Lavoisiera*, nine of *Microlicia*, four of *Trembleya*, and one each of *Chaetostoma*, *Rhynchanthera*, and *Stenodon* (Appendix; *R. grandiflora* and *T. parviflora* were each represented by two samples). All of these, except three from Clausing and Renner (2001), were newly generated for this study. *Rhynchanthera* served as outgroup on the basis of the family-wide analyses (see Results). We combined the *rpl16* data with 24 newly generated core Microlicieae DNA sequences of the intergenic transcribed spacer

TABLE 2. List of characters and their states used in the morphological analysis of Microlicieae.

1. Habit shrub (0); habit suffrutescent (1); habit rosulate (2); habit annual (3).
2. Flower isomerous (0); flower anisomerous (1).
3. Flower 3-merous (0); flower 4-merous (1); flower 5-merous (2); flower >5-merous (3).
4. Staminal pedoconnective present (0); staminal pedoconnective absent (1).
5. Staminal appendage present (0); staminal appendage absent (1).
6. Ovary cell number 5–8 (0); ovary cell number 4 (1); ovary cell number 3 (2); ovary cell number 2 (3).
7. Seed reniform (0); seed lacrimiform (1); seed cuneate-clavate (2); seed linear-oblong (3); seed cochleate (4).
8. Raphe terminal (0); raphe lateral (1).
9. Testa foveolate or lacunate-reticulate (0); testa areolate (1); testa tuberculate (2); testa colliculate (3).
10. Testa cells noninterdigitating (0); testa cells interdigitating (1).

(ITS) region of nuclear ribosomal DNA representing 24 species (Appendix). The ITS amplification and sequencing employed primers from Swensen et al. (1998) and proceeded as described earlier. Because taxonomic knowledge of *Microlicia* is poor, five of the *Microlicia* samples included in the analysis are designated only as “sp.” and two are designated with “aff.” (affinity). At least some of these likely represent undescribed species.

Molecular data analysis—The *rpl16*, *ndhF*, and ITS sequences were aligned manually; the *rbcl* sequences required no alignment. Gaps introduced into the alignment were treated as missing data. All final data matrices are available through TreeBASE (<http://www.herbaria.harvard.edu/treebase>).

Phylogenetic analyses of the various data sets employed maximum parsimony with the computer program PAUP* 4.0b10 (Swofford, 1998). The search strategy for finding the most parsimonious trees followed that of Fritsch et al. (2001), except that steepest descent was deselected. Relative support for individual clades was estimated with the parsimony bootstrap method (Felsenstein, 1985) as implemented in PAUP* under the conditions described in Fritsch et al. (2001).

Morphological analysis—A data set of 10 parsimony-informative morphological characters assessed for 10 of the 11 genera of Microlicieae sensu Renner (1993) plus *Siphanthera* was constructed to provide a phylogenetic comparison with the molecular-based results (Tables 2 and 3). *Bucquetia* has been placed confidently within the tribe Merianieae by Solt and Wurdack (1980; based on chromosome number) and Whiffin and Tomb (1972; based on seed morphology). Thus, *Bucquetia* was not included in the analysis. The genus *Tibouchina* (tribe Melastomeae) was employed as outgroup on the basis of prior results in which the sister group of Microlicieae is a clade comprising members of the tribes Rhexieae and Melastomeae (Clausing and Renner, 2001). Phylogenetic analysis was conducted with parsimony as described earlier, except that in cases where characters had multiple character states the taxa were treated as polymorphic rather than uncertain. Characters were unordered and equally weighted, and multistate characters were coded as non-additive.

Divergence time estimates—We estimated the time of initial divergence of both core Microlicieae and non-*Rhynchanthera* core-Microlicieae (see Results) using penalized likelihood rate smoothing (Sanderson, 2002) as implemented in the shareware computer program r8s. Penalized likelihood permits deviations from a molecular clock and uses cross-validation to determine the best level of clock enforcement (“smoothing”) with the given data. The input branch lengths (calculated from genetic distances) for r8s were determined under the General-Time-Reversible model of sequence evolution with the rate matrix, gamma parameter, and proportion of invariable sites all estimated on the single most parsimonious tree obtained from combined *rbcl*, *ndhF*, and *rpl16* data after exclusion of all gapped or ambiguous nucleotide sites. The

TABLE 3. Data matrix used for morphological phylogenetic analysis of Microlicieae. See Table 2 for character list.

Taxon	1	2	3	4	5	6	7	8	9	10
<i>Cambessedesia</i>	01	1	2	1	1	012	2	1	2	1
<i>Castratella</i>	2	0	1	1	1	1	3	0	3	1
<i>Chaetostoma</i>	0	1	2	0	0	012	0	0	0	0
<i>Eriocnema</i>	2	1	2	1	1	3	2	0	3	1
<i>Lavoisiera</i>	0	01	23	0	0	0	0	01	03	0
<i>Lithobium</i>	2	0	0	0	0	3	2	1	3	1
<i>Microlicia</i>	0	1	2	0	0	3	0	01	0	0
<i>Rhynchanthera</i>	0	01	2	0	0	012	0	0	0	0
<i>Siphanthera</i>	13	0	1	0	01	4	1	0	1	0
<i>Stenodon</i>	0	1	23	0	0	1	0	0	0	0
<i>Trembleya</i>	0	1	2	0	0	012	0	0	03	01
<i>Tibouchina</i>	01	0	12	0	0	0	4	0	2	1

two most distant outgroups were pruned from the r8s divergence time analysis (see Sanderson, 2002).

The genetic distances were calibrated in three alternative ways. (1) The root of crown Melastomataceae was constrained to be minimally 53 million years (my) old and maximally 90 my old. The minimum age is based on the oldest known fossil of the family, a leaf from the Golden Valley formation (Hickey, 1977; a similar leaf fossil is depicted in Renner et al., 2001). The maximum age is based on the oldest fossils of the order Myrtales, 88-my-old pollen of Myrtaceae (Morley, 2000), to which Melastomataceae belong. Allowing maximum ages of 100 or 130 my did not significantly influence the results. (2) The age of crown Melastomeae was constrained to 24 my on the basis of the oldest (highly distinct and well documented) seeds of that clade, which are between 23 and 26 my old (Renner et al., 2001). (3) A third calibration was based on an Upper Oligocene *Meriania*-like leaf from north-western Colombia (Huertas, 1977; the strata from which this leaf comes have been dated to the Upper Oligocene, not Eocene as suggested in the original publication; L. N. Parra, University of Medellín, personal communication, 2001). This leaf constrains crown Merianieae to 30 my old.

RESULTS

Alignment of the 50 sampled *rpl16* sequences of Melastomataceae resulted in a data set of 1145 characters. After the exclusion of parsimony-uninformative positions and positions 756–807 because of alignment ambiguity, the data set comprised 232 characters with 3.1% missing values. Parsimony analysis yielded 17056 equally most parsimonious trees (length = 566; consistency index [CI] = 0.59; retention index [RI] = 0.81). The strict consensus exhibits a clade comprising *Chaetostoma*, *Lavoisiera*, *Microlicia*, *Rhynchanthera*, *Stenodon*, and *Trembleya* (100% bootstrap support; Fig. 1). This core Microlicieae clade and a clade comprising *Rhexia* and *Arthrostemma* (99%) group together (57%) as sister (100%) to a clade comprising the rest of Melastomeae (58%). The other four genera of Microlicieae that were sampled fall outside of this clade: *Eriocnema* together with the representatives of Miconieae (100%), *Siphanthera* as sister to a clade comprising *Aciotis*, *Nepsera*, and *Acisanthera* of the Melastomeae clade (100%), *Castratella* as sister to *Monochaetum* of Melastomeae (97%), and *Cambessedesia* as part of an unresolved polytomy in a large clade that includes most Melastomataceae. Within the core Microlicieae clade, the sister relationship between *Rhynchanthera* and the rest of the clade has strong support (100%), as does a clade comprising *Lavoisiera*, *Microlicia*, and *Stenodon* (89%).

The three-gene parsimony analysis comprised 36 samples of Melastomataceae. Alignment of the *ndhF* sequences resulted in a matrix of 1015 characters. After the deletion of par-

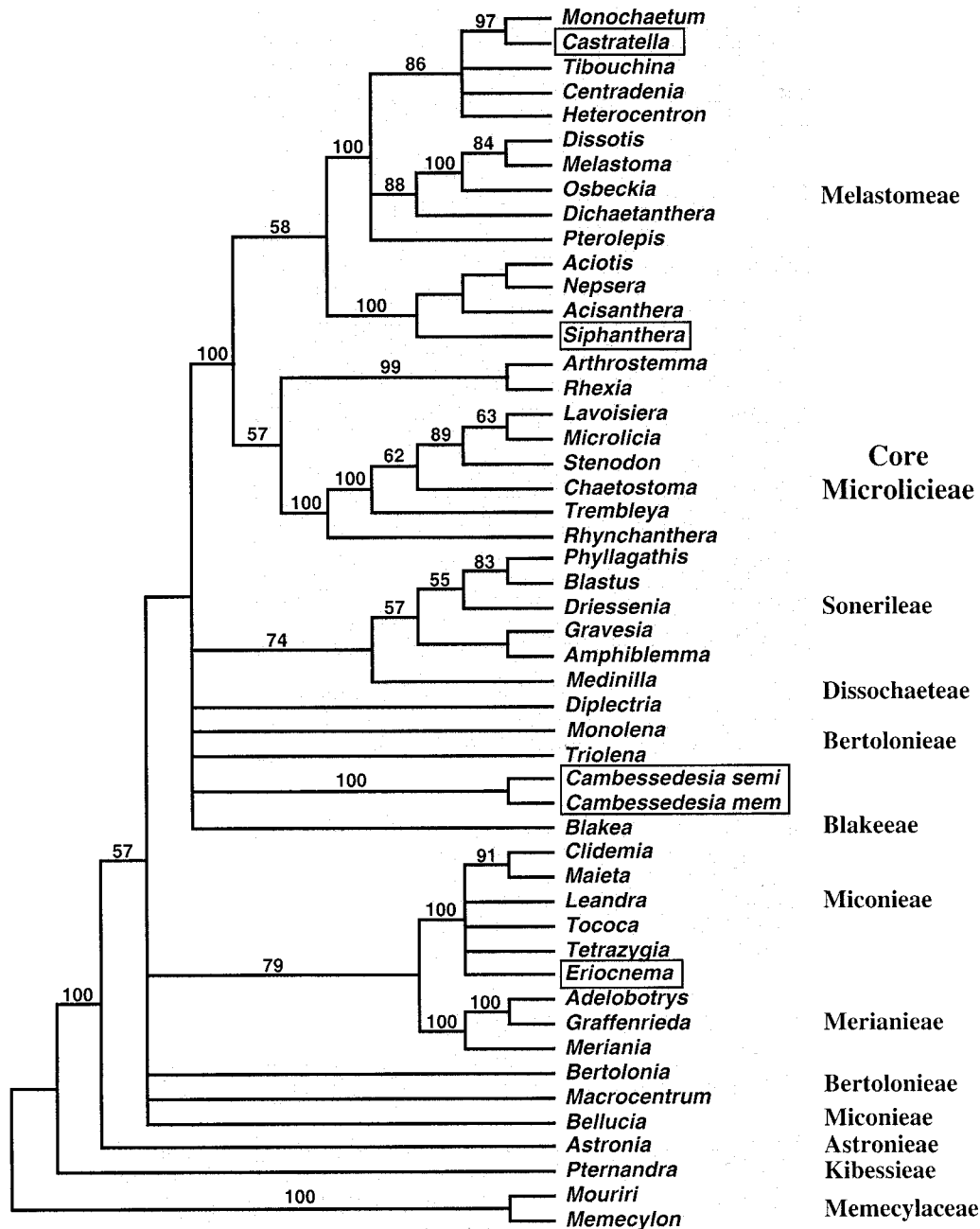


Fig. 1. Strict consensus of 17 056 equally most parsimonious trees for Melastomataceae from *rpl16* data (length = 566; CI = 0.59; RI = 0.81). Numerals indicate bootstrap values. Tribe names, indicated at right, are based on Renner (1993) and Clausing and Renner (2001). Non-core Microlicieae are boxed.

simony-uninformative characters and four base-ambiguous positions (645–648), the *ndhF* matrix comprised 216 characters. The *rbcL* matrix comprised 1427 characters. After the deletion of parsimony-uninformative positions and the first 30 positions because of a large proportion of missing data, the *rbcL* matrix comprised 118 characters. The combined data set comprised 528 parsimony-informative characters with 1.9% missing values. Analysis yielded seven equally most parsimonious trees (length = 1254; CI = 0.58; RI = 0.74). In the strict consensus, the core Microlicieae clade (100% bootstrap support) and a clade comprising *Rhexia* and *Arthrostemma* (100%) group together (62%) as sister (100%)

to a clade comprising the rest of Melastomeae (100%; Fig. 2). *Eriocnema* is placed as sister to the tribe Miconieae (82%), *Castratella* is sister to *Monochaetum* (100%) well within the Melastomeae clade, and *Cambessedesia* forms part of an unresolved polytomy with *Blakea* (tribe Blakeeae), *Diplectria* (tribe Dissochaeteae), and a clade comprising *Medinilla* (tribe Dissochaeteae), *Gravesia*, *Amphiblemma*, and *Driessenia* (tribe Sonerileae; $\leq 50\%$).

Alignment of the 26 *rpl16* core Microlicieae sequences resulted in a data set of 1045 characters. The addition of four unambiguously aligned gaps scored as binary characters resulted in a total of 1049 characters, 45 of which were parsimony-

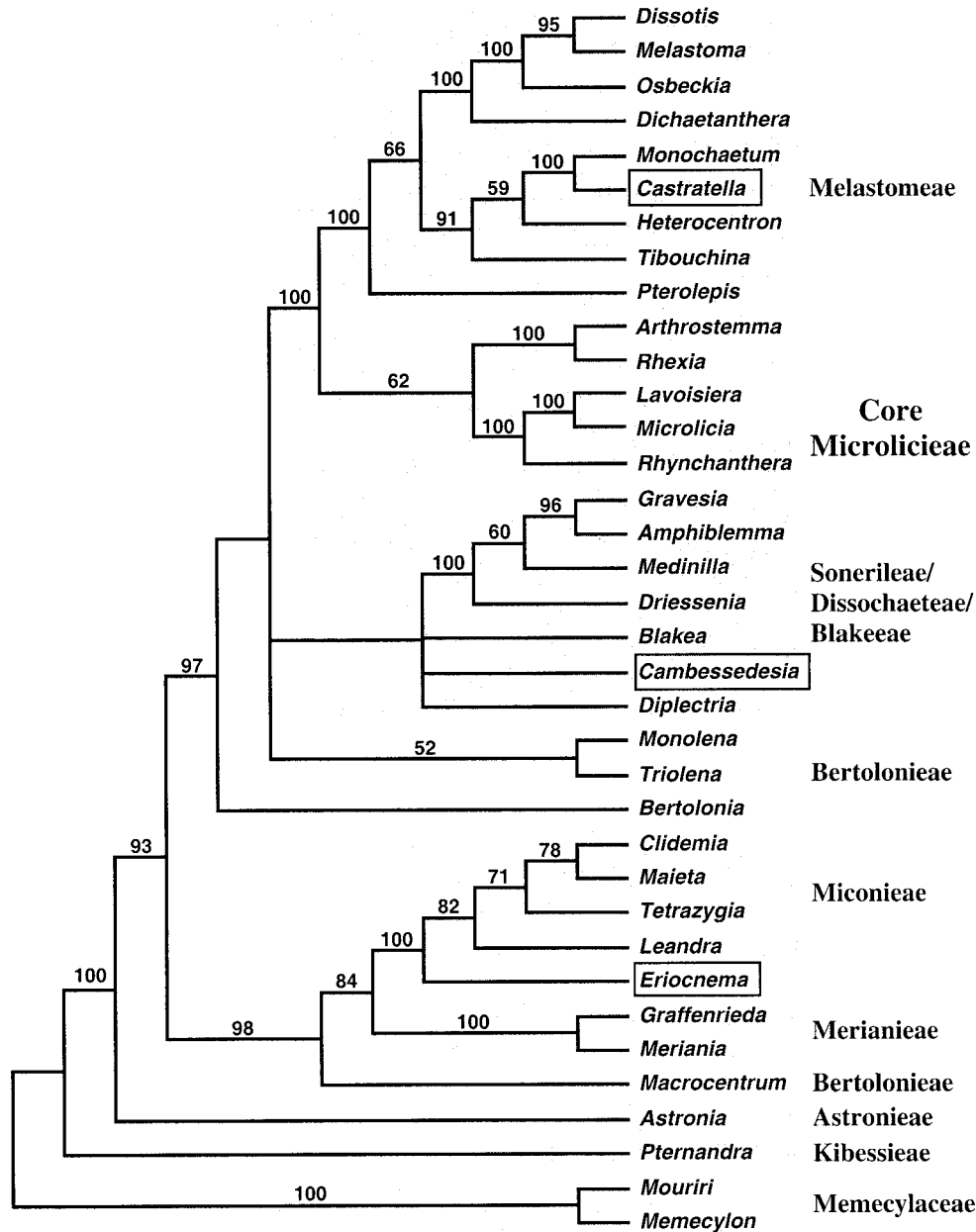


Fig. 2. Strict consensus of seven equally most parsimonious trees for Melastomataceae from combined *rpl16*, *rbcl*, and *ndhF* data (length = 1254; CI = 0.58; RI = 0.74). Numerals indicate bootstrap values. Tribe names, indicated at right, are based on Renner (1993) and Clausen and Renner (2001). Non-core Microlicieae are boxed.

mony-informative (0.3% missing values). Parsimony analysis yielded 15 equally most parsimonious trees (length = 54; CI = 0.93; RI = 0.95). The strict consensus has low clade support nearly throughout the ingroup topology; only the grouping of *Microlicia* aff. *tomentella* with *M. sp. 5* and that of the two accessions of *Trembleya parvifolia* are well supported (88% bootstrap support for both; Fig. 3).

Alignment of the 24 ITS core Microlicieae sequences resulted in a data set of 631 characters, 17 of which were parsimony-informative (no missing values). Analysis yielded 138 equally most parsimonious trees (length = 29; CI = 0.69; RI = 0.88). The strict consensus has low clade support nearly throughout the tree; only the monophyly of *Lavoisiera* and the sister-group relationship between *L. crassifolia* and the rest of

Lavoisiera are well supported (95% and 93% bootstrap support, respectively; Fig. 4).

The combined *rpl16* + ITS data set of core Microlicieae consisted of 22 sequences and 1680 characters, 28 of which were parsimony-informative (0.2% missing values). Parsimony analysis yielded one most parsimonious tree (length = 49; CI = 0.70; RI = 0.84; Fig. 5). *Lavoisiera* and *Trembleya* are supported as monophyletic (92% and 81% bootstrap support, respectively); *Microlicia* is supported as monophyletic ($\leq 50\%$) with the exception of *M. sp. 3*, which is placed as sister to all other taxa except *Rhynchanthera* ($\leq 50\%$). The only other well-supported areas of the tree are the placement of *L. crassifolia* as sister to the rest of *Lavoisiera* (93%) and the sister-group relationship between *M. aff. tomentella* and *M. sp. 5*.

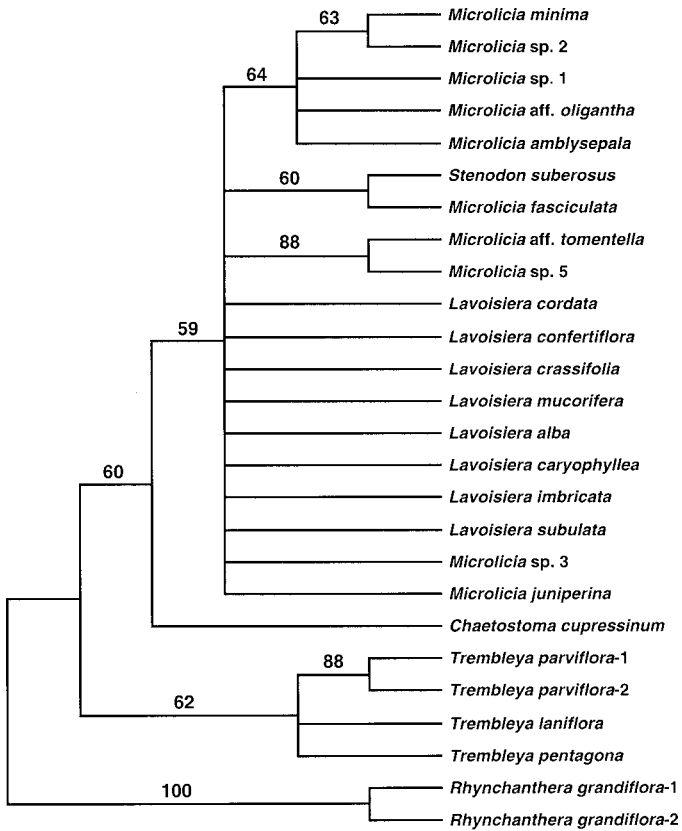


Fig. 3. Strict consensus of 15 equally most parsimonious trees for Microlicieae from *rpl16* data (length = 54; CI = 0.93; RI = 0.95). Numerals indicate bootstrap values.

The morphological phylogenetic analysis of Microlicieae yielded 191 equally most parsimonious trees (length = 48; CI = 0.85; RI = 0.70; Fig. 6). The genera of core Microlicieae form an unresolved monophyletic group that is sister to a clade comprising non-core Microlicieae. No clade is supported by bootstrap values >50%. Character state 9–0 (testa foveolate or lacunate-reticulate) is placed as a synapomorphy for core Microlicieae in all optimizations of all equally most parsimonious trees; character state 7–0 (seed reniform) is placed as a synapomorphy for core Microlicieae in approximately 90% of all optimizations of all equally most parsimonious trees.

As expected from similar data sets in Renner and Meyer (2001) and Renner et al. (2001), a maximum likelihood ratio test with the combined *rpl16* + *rbcL* + *ndhF* data for 36 taxa (after exclusion of all gapped or ambiguous sites) rejected a molecular clock, albeit barely (as long as distant outgroups were excluded). Nonparametric rate smoothing with the cross-validated optimal level of smoothing estimated to be approximately 300 yielded age estimates of 18 my for core Microlicieae crown group diversification (*Lavoisiera*, *Microlicia*, and *Rhynchanthera*) and 3.7 my for *Lavoisiera* + *Microlicia* crown group diversification.

DISCUSSION

Our results support the restriction of Microlicieae to the six genera proposed by Almeda and Martins (2001). These genera all possess reniform ellipsoid or elongate seeds with a foveolate or lacunate-reticulate testa, and our data strongly suggest

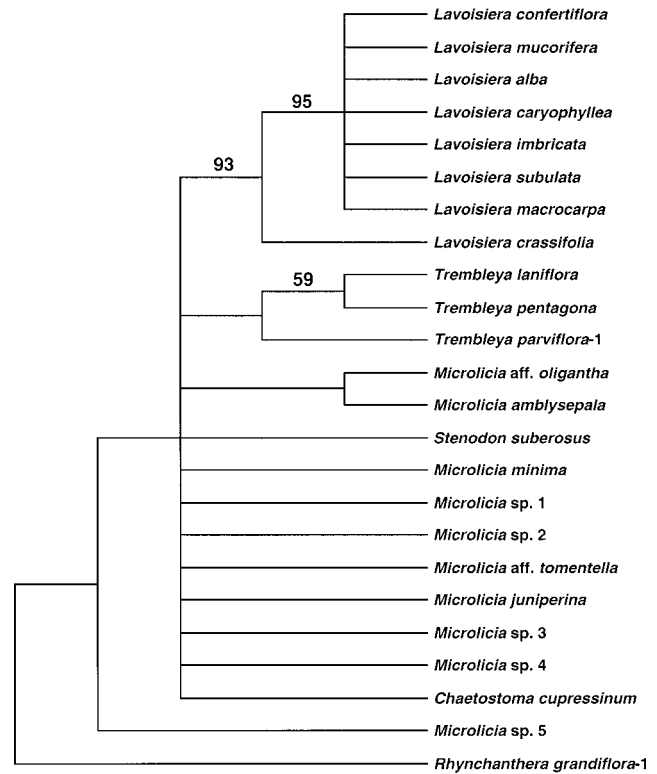


Fig. 4. Strict consensus of 138 equally most parsimonious trees for Microlicieae from ITS data (length = 29; CI = 0.69; RI = 0.88). Numerals indicate bootstrap values.

that these characters are synapomorphies that unite this group (Fig. 7). *Cambessedesia*, *Castratella*, *Eriocnema*, and *Siphnanthera* can now confidently be excluded from the Microlicieae on the basis of our morphological and molecular results, and seed morphology in these four genera can be accommodated in other tribes.

Non-core Microlicieae—*Cambessedesia* is a genus of 21 species distinguished by the combination of bicolored petals (red-orange and yellow or rarely completely yellow), linear-oblong anthers with thickened connectives that are bluntly appendiculate dorso-basally, and clavate or oblong-pyramidate seeds with a tuberculate and papillate surface pattern (Martins, 1984; Fig. 7G). Our samples of this genus grouped strongly within the clade comprising core Microlicieae and the tribes Melastomeae, Sonerileae, Dissochaeteae, Bertolonieae (in part), and Blakeeae. Further resolution of its placement will require a broader generic sampling of Neotropical Melastomataceae and more phylogenetically informative data, although our data indicate that it can be confidently excluded from within Melastomeae and the clade consisting of Sonerileae and Dissochaeteae.

The well-supported grouping of *Eriocnema* with representatives of the Miconieae is surprising and unexpected because of its fleshy subcaulescent rhizomatous habit (vs. woody habit in Miconieae), capsular fruit (vs. usually a berry), and cuneate-clavate seeds with an inconspicuously colliculate testa (vs. ovoid, pyramidate to lunate seeds with a testa that varies from smooth to muriculate or complexly costate-tuberculate; Fig. 7H). *Eriocnema* is similar to many Miconieae, however, in its unappendaged anther connectives and a chromosome number

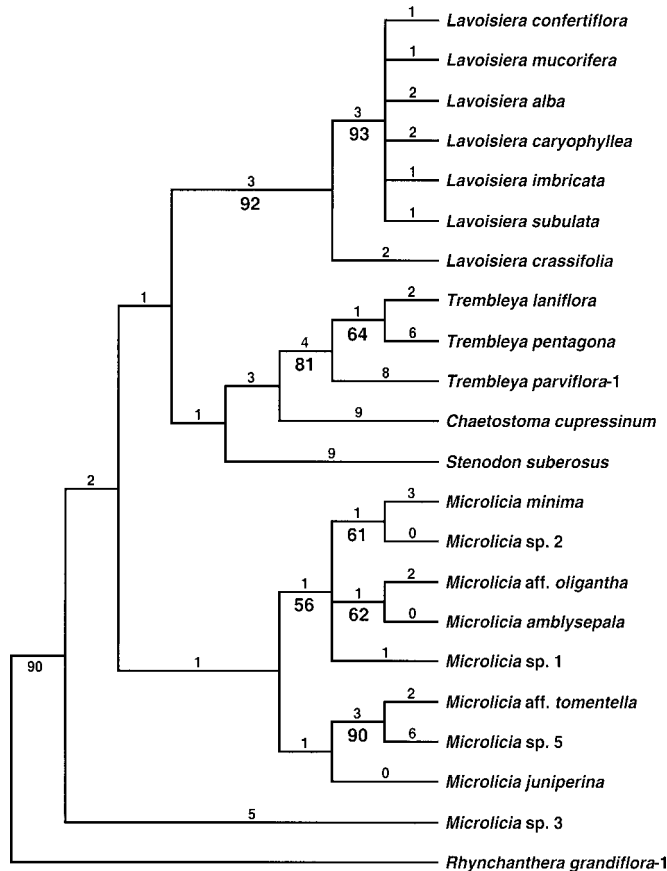


Fig. 5. The single most parsimonious tree for core Microlicieae from combined *rpl16* and ITS data (with uninformative characters excluded: length = 49; CI = 0.70; RI = 0.84). Numerals above branches indicate branch lengths under DELTRAN optimization and all variable characters included (total length = 179); those below branches indicate bootstrap values.

of $n = 17$ (E. R. Forni-Martins, Universidade Estadual de Campinas, personal communication; Almeda, 1997a), and our data suggest that these two characters are synapomorphies for Miconieae + *Eriocnema*. One or more of the members of tribe Bertolieae may eventually be found to group with *Eriocnema*. The herbaceous habit, cuneate-clavate seeds with a colliculate testa, and chromosome number are possible derived characters shared between *Eriocnema* and some members of Bertolieae, a Neotropical tribe that remains poorly sampled for molecular data, and it may be significant that one species of *Bertonia* was originally described in *Eriocnema* (Baumgratz, 1989/1990: p. 116). Bertolieae is polyphyletic, with *Macrocentrum* grouping well away from the rest of the members of the tribe sampled for molecular data (Fig. 2; Clausen and Renner, 2001). The strongly supported placement of *Eriocnema* in the clade that includes *Macrocentrum* in the three-gene analysis (Fig. 2) may be significant in this regard.

Molecular data are particularly useful in assessing the placement of *Castratella*, a genus comprising two species of rosulate herbaceous perennials confined to the Andean páramos of Colombia and Venezuela. *Castratella* groups strongly with *Monochaetum*, another montane genus with a center of diversity in the Andes, and the clade comprising these two genera is strongly supported within Melastomeae. Characters possibly synapomorphic for *Castratella* and *Monochaetum* are four-

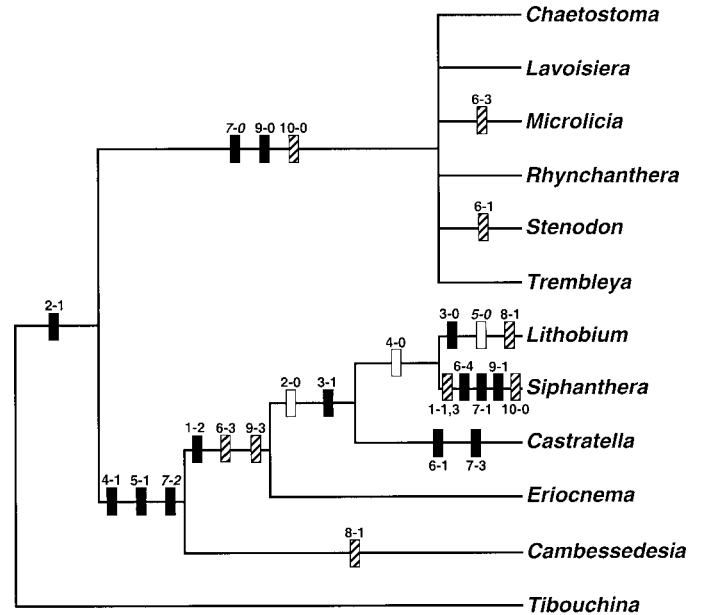


Fig. 6. One of 191 equally most parsimonious trees from phylogenetic analysis of Microlicieae with morphological characters (length = 48; CI = 0.85; RI = 0.70). Character state changes (DELTRAN optimization) are indicated by bars (solid = unique, hatched = state arises more than once, open = loss or reversal). Numerals indicate characters; all states are binary except where indicated by a dash and the state, e.g., 6-3 along the branch subtending *Microlicia*. Italicized numerals indicate character states with alternative positions under ACCTRAN optimization. Changes occurring within polymorphic terminal nodes are not shown. This topology is identical to the strict consensus of the 191 trees.

merous flowers, a four-locular ovary, and interdigitating cells of the seed testa. Unlike most members of Melastomeae, *Castratella* lacks both staminal pedoconnectives and appendages, features likely to be derived within the tribe. In contrast to other Melastomeae, however, it has the apparent autapomorphies of linear-oblong seeds and a colliculate testa (vs. cochleate seeds and a tuberculate, costate, or foveolate testa in Melastomeae; Fig. 7F).

Our data agree with Renner's (1993) reassignment of *Siphanthera* to the Melastomeae. Like *Aciotis* and other members of Melastomeae (Solt and Wurdack, 1980; Almeda, 1997b), *Siphanthera* has ventral staminal appendages and a base chromosome number of $x = 10$ (F. Almeda and O. Robinson, unpublished data), but it has unusual lacrimiform seeds with an areolate testa (Fig. 7E). This seed type is clearly an autapomorphy within Melastomataceae, being unknown in the rest of the family.

Among other genera traditionally placed in the Microlicieae, only *Lithobium* and *Bucquetia* remain to be studied in a molecular phylogenetic context. *Lithobium* of central Brazil is a monotypic subcaulescent herb of wet sites with trimerous flowers, unappendaged anther connectives, and clavate-obovoid colliculate seeds. *Bucquetia*, an Andean genus of three species, has four-merous flowers, unappendaged or dorsally appendiculate anthers, and oblong-cuneate smooth seeds (Fig. 7I). From morphology, we predict that molecular and cytological evidence will support their removal from the Microlicieae.

Core Microlicieae—Our data provide strong evidence for the placement of *Rhynchanthera* as sister to the remaining core

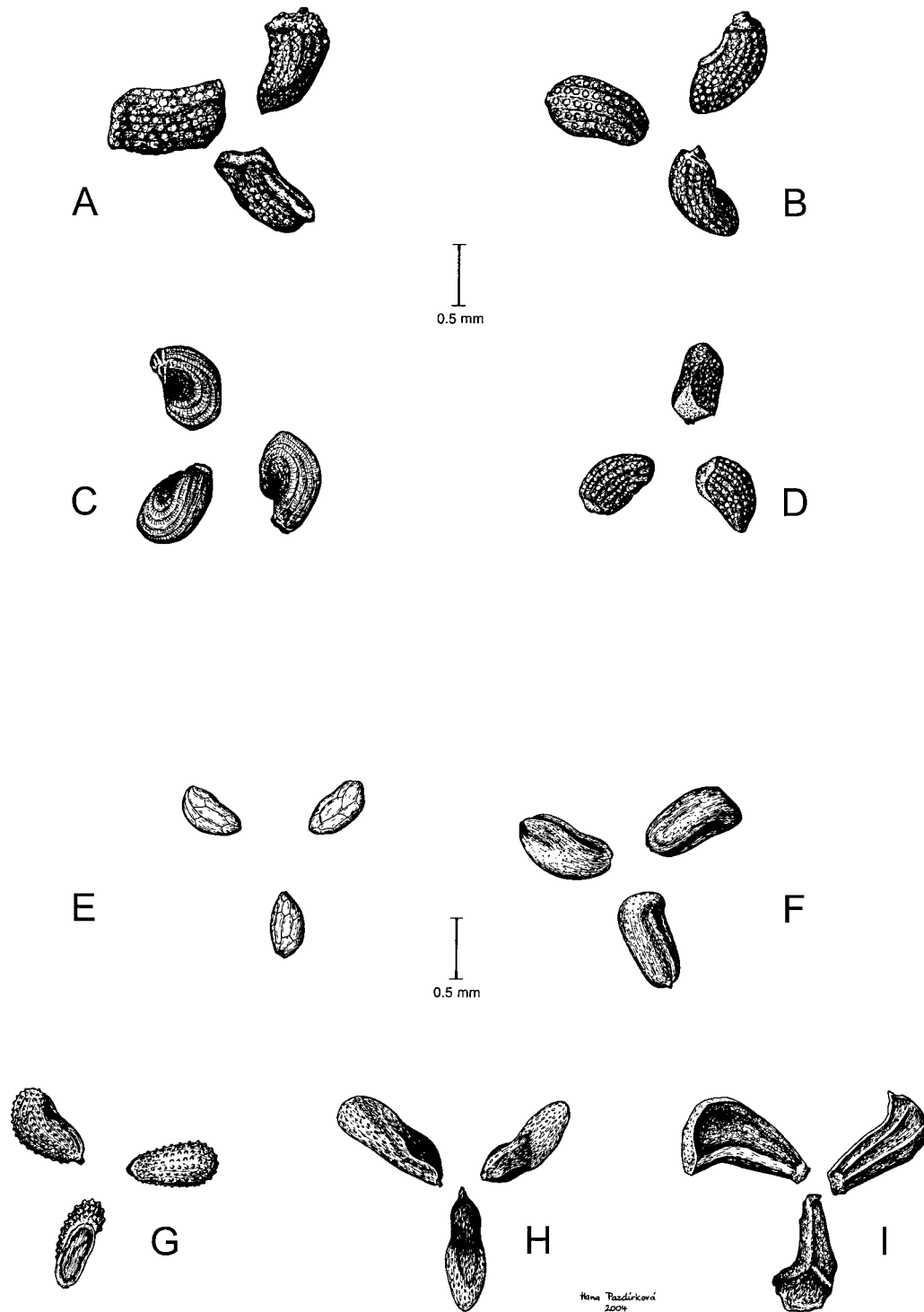


Fig. 7. Seeds of Microlicieae. (A–D) Core Microlicieae; (E–I) Non-core Microlicieae. (A) *Rhynchanthera paludicola*. (B) *Microlicia amplexicaulis*. (C) *Lavoisiera subulata*. (D) *Chaetostoma pungens*. (E) *Siphanthera arenaria*. (F) *Castratella piloselloides*. (G) *Cambessedesia hilariana*. (H) *Eriocnema fulva*. (I) *Bucquetia vernicosa*.

group. The consistent presence of filamentous staminodia distinguishes *Rhynchanthera* from all other members of core Microlicieae (Renner, 1990). Filamentous staminodia are otherwise uncommon among Neotropical Melastomataceae and largely confined to dioecious species of *Miconia* section *Cremanium* (*Siphanthera* is polymorphic for staminode presence

and this parsimony-uninformative character was not included in our analysis). Moreover, *Rhynchanthera* has a base chromosome number of $x = 10$ (Solt and Wurdack, 1980; F. Almeida et al., unpublished data), whereas other genera of core Microlicieae (except *Stenodon*, which remains to be studied cytologically) have a base chromosome number of $x = 12$ (F.

Almeda et al., unpublished data). A putative base number for the family is $x = 12$ (Almeda, 1997c), suggesting that all core Microlicieae except *Rhynchanthera* have retained the primitive chromosome number for the family. We have been unable to detect consistent non-molecular characters that would be synapomorphic for the non-*Rhynchanthera* core Microlicieae clade.

Other strongly supported major clades are those corresponding to *Lavoisiera* and *Trembleya*. *Lavoisiera* has been recently redefined to include species with a partially inferior ovary, laterally flattened placental intrusions, capsular fruits that dehisce from the base to the apex, and a persistent columella (Almeda and Martins, 2001). The latter two characters are unique in the family. We can detect no morphological characters that support the placement of *L. crassifolia* as the strongly supported first-diverging lineage within *Lavoisiera*. *Trembleya*, a genus of 19 species, is defined by a three- to five-locular ovary, deciduous columella, subpeltate placental intrusions, and pedicellate or sessile mostly five-merous flowers borne in compound bracteolate dichasia or reduced modifications thereof. Although the samples of *Trembleya* form a monophyletic group in our analysis with relatively strong support, data from more samples are desirable to confirm the monophyly of this group.

The positions of the remaining genera of core Microlicieae are only weakly supported. *Microlicia* is the largest genus of core Microlicieae, with possibly over 175 species, and is poorly understood taxonomically. It has five-merous (rarely six- or eight-merous) flowers, a three-locular (rarely four- or five-locular) ovary, magenta, pink, white, or yellow petals, dimorphic stamens within a flower, and well-developed ventral staminal appendages. *Chaetostoma*, a genus of 12 species, is one of the most distinctive members of core Microlicieae. It is recognized by a ring of appressed adnate hairs that crown the outer hypanthial apex (a character unique in the family), uniformly ericoid leaves, and mature capsules that conspicuously exceed the hypanthium in length. More samples and more phylogenetically informative data will be required to adequately assess the monophyly and phylogenetic relationships of both *Microlicia* and *Chaetostoma*. *Stenodon*, with two species, is found only in cerrado sensu stricto. It superficially resembles *Microlicia* but has six- to seven-merous flowers, a four- to five-locular ovary, red-magenta petals, stamens that are only slightly unequal within a flower, and inconspicuous ventral staminal appendages. *Stenodon* is also unlike *Microlicia* in having thick, woody branches that decorticate like other cerrado shrubs.

The origin and historical biogeography of core Microlicieae—It is clear that core Microlicieae have originated in South America, for two reasons. First, the vast majority of core Microlicieae are endemic to South America; the only species of core Microlicieae known outside of South America are three species of *Rhynchanthera* found in Mexico and Central America (Renner, 1990). Second, of these three species, the diploid *R. grandiflora* ($n = 10$; F. Almeda et al., unpublished data) is also widely distributed in South America, and *R. paludicola* (Donn. Sm.) Gleason, endemic to southern Central America, has a chromosome number of $n = 20$ (Davidse, 1970; F. Almeda et al., unpublished data). This number is clearly derived from $n = 10$ and implicates *R. paludicola* as a tetraploid derivative species. These data suggest migration of *Rhynchanthera* into Mesoamerica from South America,

most likely via the Panama land bridge that formed about 3 mya (Nisancioglu et al., 2003).

The phylogenetic position of core Microlicieae provides insight into the evolution of drought-tolerance in the Melastomataceae. Species of the family usually thrive under conditions of high humidity and precipitation. The major exception to this in the Neotropics is found in the Microlicieae, nearly endemic to the Brazilian cerrado. The well-nested placement of core Microlicieae within the family (see also Clausen and Renner, 2001) supports a shift from a generally hydric habitat to the seasonally dry cerrado along the stem leading to core Microlicieae, followed by high diversification in the cerrado.

This scenario is corroborated by the phylogenetic position of *Rhynchanthera* as sister to the remaining members of core Microlicieae. In contrast to the cerrado endemism displayed by most species in other genera of core Microlicieae, *Rhynchanthera* is distributed from Mexico to Paraguay where it occurs in open habitats under locally wet conditions. Cerrado species of *Rhynchanthera*, for example, prefer seasonal, valley-side marshes (veredas) where the water table extends nearly to the surface during the rainy season (Renner, 1990; Oliveira-Filho and Ratter, 2002); most species in other genera of Microlicieae avoid such wet areas or occur only peripherally near them. Nonetheless, the habitat of *Rhynchanthera* experiences successive periods of soil waterlogging and pronounced water deficit. In the savannas of Venezuela and central Amazonia, for example, *R. grandiflora* and *R. dichotoma* (Desr.) DC. colonize grassy areas that are burned during the dry season (Renner, 1990). Thus, the habitat of *Rhynchanthera* can be considered transitional between the hydric habitats typical of most Neotropical Melastomataceae and the seasonally dry cerrado habitats to which most species of the core Microlicieae are restricted. The phylogenetic position of *Rhynchanthera* as the first-diverging lineage within core Microlicieae is precisely what one would predict on the basis of both its widespread distribution and this aspect of its habitat.

The widespread geographic distribution of *Rhynchanthera*, as well as its preference for wet habitats, suggests that the most specialized adaptations to cerrado within core Microlicieae occurred after the divergence of *Rhynchanthera* from the rest of the tribe. In the context of the minimum estimated age of the core Melastomataceae (53 my; Renner et al., 2001), our relatively recent age estimate of 3.7 my for the crown node of the branch leading to the non-*Rhynchanthera* core Microlicieae clade is comparable to the time of the most active stage of development for cerrado vegetation beginning near the Tertiary/Quaternary boundary 1.8 mya (Oliveira-Filho and Ratter, 2002), implying an adaptive shift from hydric to seasonally dry habitats in the stem lineage of this group. A Bayesian analysis of *rbcL*, *ndhF*, and *rpl16* sequences from 52 Melastomataceae and outgroups that used multiple fossil and geological calibrations (S. S. Renner, unpublished manuscript) independently arrived at an age estimate of 16–19 my for core Microlicieae crown group diversification, corroborating our results.

Cerrado vegetation has successively expanded and contracted in response to the climatic fluctuations of the Quaternary (Ledru, 2002). This periodicity combined with the shallow, low-fertility soils, seasonal drought, and periodic fires that characterize the cerrado undoubtedly were potent forces impelling diversification of the Microlicieae. The time of origin of the cerrado and the adaptations of plants to this unique biome can be further assessed by comparing the phylogenetic

data and derived estimates of divergence times presented here with those of other cerrado endemics.

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