Systematics and character evolution of Tabernaemontaneae (Apocynaceae, Rauvolfioideae) based on molecular and morphological evidence

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Abstract Tabernaemontaneae (Rauvolfioideae, Apocynaceae) are small trees with mainly animal-dispersed fleshy fruits and arillate seeds represented in the tropics of Africa, Asia, the Pacific and America. The tribe is characterized by complex indole alkaloids, thus its species play a prominent role in traditional medicine. Taxonomically, the Tabernaemontaneae have a convoluted history fraught with contention as to tribal, subtribal, generic and sectional delimitation, with some authors recognizing Ambelanieae and Macoubeeae as separate tribes and others including them in an expanded Tabernaemontaneae s.l. In the species-rich pantropical genus *Tabernaemontana*, seven sections and up to 30 segregate genera have been described during the past 100 years, giving it the dubious distinction of being the most disputed genus in Apocynaceae s.str. Here 420 new chloroplast DNA sequences from 104 species, including representatives of all satellite genera ever recognized in the Tabernaemontaneae, were analyzed phylogenetically to evaluate previous circumscriptions of Tabernaemontaneae and test the sectional treatment of *Tabernaemontana*. The Tabernaemontaneae s.l. as currently circumscribed was shown to be monophyletic. Of the 19 genera recognized in the most recent classification, 15 are maintained. The genera *Bonafousia*, *Stemmadenia*, *Stenosolen* and *Woytokowskia* are nested within *Tabernaemontana*. Of the seven current sections of *Tabernaemontana*, the four that included taxa from more than one continent were found to be para- or polyphyletic. All natural groups recovered within *Tabernaemontana* were found to correspond to geographic areas. Mapping of 29 selected morphological characters onto the molecular tree recovered recurrent suites of co-occurring character states and identified three synapomorphies characterizing the Tabernaemontaneae s.l.: (1) specialized anthers with massive lignified guide-rails; and a differentiated style-head with (2) a five-lobed upper crest and (3) a thickened basal flange. A new combination for the subtribe Ambelaniinae and the necessary new combinations resulting from merging *Stemmadenia* into *Tabernaemontana* are made.

Keywords Ambelanieae; Apocynaceae; classification; Macoubeeae; molecular phylogenetics; morphology; *Tabernaemontana*; Tabernaemontaneae

■ INTRODUCTION

Apocynaceae is one of the largest families of angiosperms, with 375 genera and over 5000 species (Endress, 2004; Endress & al., 2007a). The notable morphological variation in reproductive traits in the family has resulted in distinct interpretations about the appropriate choice of characters for taxonomic classifications. Of the five subfamilies currently recognized in Apocynaceae, four (Apocynoideae, Asclepiadoideae, Periplocoideae, Secamonoideae) have some of the most elaborate and complicated flowers of all the angiosperms, whereas Rauvolfioideae have flowers with a more simple and constant morphology (Endress, 1994; Endress & Bruyns, 2000). Conversely, fruits and seeds in Rauvolfioideae are variable in a number of traits, such as dehiscence, composition of the nutritious portion, size and pericarp consistency, but are almost invariant in the other subfamilies, in which they consist of a pair of dry follicles bearing comose seeds. Not surprisingly, therefore, traditional classifications of Rauvolfioideae were based almost exclusively on fruit and seed characters, whereas those of Apocynoideae, Periplocoideae, Secamonoideae and Asclepiadoideae relied mainly on floral characters (e.g., de Candolle, 1844; Schumann, 1895; Leeuwenberg, 1994a). Recent phylogenetic studies (Potgieter & Albert, 2001; Simões & al., 2007) identified extensive morphological homoplasy among fruit and seed characters within Rauvolfioideae, a pattern reported by other authors in a number of angiosperm families (Johnson & Briggs, 1984 and Conti & al., 1997, for Myrtaceae; Bremer & Eriksson 2002, for Rubiaceae; Smith & Carroll, 1997, for Gesneriaceae; and Clausing & al., 2000, for Melastomataceae).

Within Rauvolfioideae, Tabernaemontaneae are a tribe of particular interest due to a suite of distinctive floral, fruit and seed characteristics. The name Tabernaemontaneae was introduced by Don (1837–8: 70, 87) for taxa of Apocynaceae

with fruits composed of two follicles and seeds neither comose nor winged and with a style-head characterized by an expanded annular base and a bifid apex. His tribe included ten genera, of which six have subsequently been placed in other tribes. In the current delimitation, Tabernaemontaneae comprise 16 genera and about 170 species broadly distributed in the tropics and subtropics, mainly in lowland forest (Endress & al., 2007a). Representatives are all woody and erect; most are small trees or shrubs with fleshy fruits and seeds often surrounded by a colorful aril, which are dispersed mainly by birds and primates (McDiarmid, 1977; Roosmalen, 1985; Baraloto & Forget, 2007). An important characteristic of the tribe are the lignified guide-rails of the anthers, which function in synorganization with the strongly five-angled style-head in guiding the proboscis of pollinating insects into the pollination mechanism (Schick, 1982; Fallen, 1986). Lignified guide rails are a trait otherwise found only in the more derived subfamilies (Endress & Bruyns, 2000). Like the majority of Rauvolfioideae, Tabernaemontaneae contain a broad array of complex indole alkaloids, and thus play an important role in regional folk medicine around the world (Schultes, 1979; Anderson & al. 1985; Bisset, 1985, 1989; Van Beck & al., 1984). They differ from the other tribes of Rauvolfioideae, however, in being characterized by a predominance of indole alkaloids of the ibogan type, the structurally most derived type ("J" type) in the biosynthetic pathways described in Kisakürek & al.'s (1983) system.

Tabernaemontaneae have a contentious taxonomic history, which involves two main centers of dispute: the delimitation of the tribe and the circumscription of its largest genus, *Tabernaemontana*.

Delimitation of the tribe. — The bulk of the controversy at the tribal level can be accounted for by the differing ranks and positions given to four groups: (1) taxa with apocarpous, usually dehiscent fruits and seeds with arils (Tabernaemontaneae s.str.); (2) taxa with syncarpous, berry-like fruits and non-arillate seeds (Ambelanieae); the genera (3) *Macoubea* Aubl. and (4) *Chilocarpus* Blume. In addition, *Geissospermum* and *Eucorymbia* were both at one time considered to belong to the tribe (Schumann, 1895; Stapf, 1905). The rank and position given these six elements in pertinent classifications is compared in Table S1 (in the Electronic Supplement to this article) and discussed below.

One of the important early classifications that included species now ascribed to Tabernaemontaneae was that of de Candolle (1844), who split these species into two groups based on fruit morphology. De Candolle's (1844) fruit-based classification of Rauvolfioideae influenced many taxonomists working in the family thereafter, such as Müller Argoviensis (1860), Bentham & Hooker (1876), Miers (1878), Stapf (1902), Pichon (1948a,b) and Leeuwenberg (1994a). Schumann (1895) followed de Candolle (1844), using syncarpy versus apocarpy for his main tribal division of Rauvolfioideae. In his treatment of the family for tropical Africa, Stapf (1902) initiated a major change in the taxonomy of Tabernaemontaneae s.str. by raising Schumann's (1895) subtribe Tabernaemontaninae to the rank of subfamily. His justification was that flowers in this group of Apocynaceae were distinct in having anthers with

sterile appendages at the base forming two conspicuous wings (= lignified guide-rails). The subfamilial status of Tabernaemontanoideae was maintained thereafter by most taxonomists (Markgraf, 1923, 1935, 1938; Boiteau & Sastre, 1975; Boiteau, 1977; Boiteau & al., 1978; Allorge, 1983; Boiteau & Allorge, 1976).

Pichon (1948a,b) also relied mostly on fruit and seed characters to delimit tribes in Rauvolfioideae. He considered the presence of an aril surrounding the seed to be the most important diagnostic feature of Tabernaemontaneae s.str., although he also cited other potentially diagnostic morphological features, such as fruits composed of two (rarely one) dehiscent mericarps, anthers with sterile basal appendages and ornamentation of the pollen surface. He correctly assessed that *Geissospermum* showed a close relationship with *Aspidosperma*, rather than genera of the Tabernaemontaneae, and removed it from the tribe. He provisionally included *Eucorymbia* Stapf, noting that he had no material available to study, but later, after observing that the anthers and style-head were united, transferred it to Apocynoideae (Pichon, 1949b, 1950). In the tribe Carisseae he proposed the new monogeneric subtribe Chilocarpinae to house *Chilocarpus*, which he later raised to tribal rank (Pichon, 1949a), although he never provided a Latin diagnosis for either. He proposed a new tribe, Ambelanieae, to accommodate a group of Neotropical genera with fruits and seeds similar to those found in species of tribe Carisseae (syncarpous, indehiscent, 2-celled fruits and non-arillate seeds without a deep hilar groove), but flowers with the same distinctive features of Tabernaemontanoideae (anthers with sterile basal appendages), which he divided into two subtribes: Ambelaniinae (including *Ambelania*, *Molongum*, *Neocouma* and *Rhigospira*) and Macoubeinae (including only *Macoubea*).

Pichon (1948a,b) realized that two alternative classifications of Tabernaemontanoideae could be proposed, depending on whether emphasis was given to flower or to fruit and seed characters. *Ambelania* and related taxa could either be considered as a tribe of Plumerioideae or included within a more broadly circumscribed Tabernaemontanoideae s.l. In the absence of strong evidence that could support one of these alternatives, he chose to keep Ambelanieae separate from Tabernaemontanoideae, but suggested that the former might represent a transitional link from a more ancestral Plumerioideae to a more derived Tabernaemontanoideae. This dilemma is particularly evident in his 1948b paper, in which he first maintained the subfamilial status of Tabernaemontanoideae, but by the end of the paper reduced it to a tribe of Rauvolfioideae. In his opinion, the characters that he initially considered sufficient to recognize Tabernaemontanoideae as a subfamily (seeds surrounded by a fleshy aril and with a deep hilar groove) were more appropriate to define lower taxonomic ranks.

Boiteau & Sastre (1975) raised Pichon's (1948a) monogeneric subtribe Macoubeinae to tribal level and transferred it together with Pichon's tribe Chilocarpeae (1949a) from Rauvolfioideae to Tabernaemontanoideae. In 1978, Boiteau & al. raised Ambelaniinae to the rank of tribe and validated it with a Latin diagnosis. Leeuwenberg's (1994a) tribal delimitation

in Rauvolfioideae, based mainly on morphology-based revisions by him or his students (Hallé, 1965, 1971; Beentje, 1978; Barink, 1983; Leeuwenberg, 1985a,b, 1991, 1994b; Vonk & Leeuwenberg, 1989), was similar to that of Pichon (1948a) in maintaining Ambelanieae and Tabernaemontaneae as distinct tribes, although he followed Boiteau & al. (1978) in recognizing both Chilocarpeae (which he validated with a Latin diagnosis) and Macoubeeae as monogeneric tribes.

The first classification of Apocynaceae to include molecular data was that of Endress & Bruyns (2000), who also reunited Apocynaceae s.str. and the traditional Asclepiadaceae. Their classification differed considerably from those of previous authors with regard to the circumscription of tribes. Rather

than fruit characters alone, weight was given to characters of the anthers and style-head, which were complemented by the information available from the phylogenetic studies of the family at that time (e.g., Judd & al., 1994; Struwe & al., 1994; Endress & al., 1996; Sennblad & Bremer, 1996; Sennblad & al., 1998). *Chilocarpus* (Chilocarpeae sensu Leeuwenberg, 1994a) was transferred to Alyxieae, a position strongly supported by a subsequent study based on pollen (van der Ham & al., 2001) and DNA with increased taxon sampling (Endress & al., 2007b). Tabernaemontaneae was circumscribed in a broad sense to include all genera of Rauvolfioideae with anthers characterized by lignified guide-rails, regardless of their fruit type. Thus, for the first time, members of Ambelanieae were included in

Table 1. *Tabernaemontana* and segregate genera as recognized by different authors. Blank space, not recognized by author; –, not described at the

Genus	De Candolle (1844)	Miers (1878)	Schumann (1895–1897)	Stapf (1902)
Anacampta Miers (1878)	–	*Anacampta*		
Anartia Miers (1878)	–	*Anartia*		
Bonafousia A. DC. (1844)	*Bonafousia*	*Bonafousia*	*Tabernaemontana*	
Camerunia (Pichon) Boiteau (1976)	–	–	–	–
Capuronetta Markgr. (1972)	–	–	–	–
Codonema Miers (1878)	–	*Codonema*	*T.* sect. *Eutabernaemontana*	
Conopharyngia G. Don (1837)	*Conopharyngia*		*Tabernaemontana*	*Conopharyngia*
Domkeocarpa Markgr. (1941)	–	–	–	–
Ervatamia (A. DC.) Stapf (1902)	*T.* sect. *Ervatamia*	–	–	*Ervatamia*
Gabunia Schum. ex Stapf (1902)	–	–	*Gabunia*	*Gabunia*
Hazunta Pichon (1948)	–	–	–	–
Leptopharyngia (Stapf) Boiteau (1976)	–	–	–	*C.* sect. *Leptopharyngia*
Merizadenia Miers (1878)	–	*Merizadenia*		
Muntafara Pichon (1948)	–	–	–	–
Ochronerium Baill. (1889)	–	–		
Oistanthera Markgr. (1935)	–	–	–	–
Pagiantha Markgr. (1935)	–	–	–	–
Pandaca Noronha ex Thouars (1806)	*T.* sect. *Rejoua*		*Tabernaemontana*	
Pandacastrum Pichon (1948)	–	–	–	–
Peschiera A. DC. (1844)	*Peschiera*	*Peschiera*	*Tabernaemontana*	
Pterotaberna Stapf (1902)	–	–	*Pterotaberna*	
Phrissocarpus Miers (1878)	–	*Phrissocarpus*	*Tabernaemontana*	*Anacampta*
Protogabunia Boiteau (1976)	–	–	–	–
Quadricasaea Woodson (1941)	–	–	–	–
Rejoua Gaudich. (1828)	*Tabernaemontana*	*Rejoua*	*Tabernaemontana*	
Sarcopharyngia (Pichon) Boiteau (1976)	–	–	–	*C.* sect. *Sarcopharyngia*
Stenosolen (Müll.Arg.) Markgr. (1937)	–	–	–	–
Taberna Miers (1878)	–	*Taberna*	*Tabernaemontana*	
Taberna Markgr. (1938)	*T.* sect. *Taberna*			
Tabernaemontana L. (1753)	*Tabernaemontana*	*Tabernaemontana*	*Tabernaemontana*	
Testudipes Markgr. (1935)	–	–	–	–
Woytkowskia Woodson (1960)	–	–	–	–

Tabernaemontaneae, a position first suggested in a morphological study by Fallen (1986). Nineteen genera were recognized in Tabernaemontaneae s.l.: the six genera of the Ambelanieae, nine genera of Tabernaemontaneae s.str., and the genus *Macoubea* (Macoubeeae, sensu Leeuwenberg, 1994a), as well as three genera that had been merged into *Tabernaemontana* by Leeuwenberg (1994a): *Bonafousia* A. DC., *Stenosolen* (Müll. Arg.) Markgr. and *Woytkowskia* Woodson. Recent phylogenetic studies (Potgieter & Albert, 2001; Sennblad & Bremer, 2002; Simões & al., 2007) strongly support the inclusion of Ambelanieae and Macoubeeae in the tribe and this expanded circumscription was maintained in the latest classification of the family (Endress & al., 2007a).

***Tabernaemontana* and its segregate genera. —** *Tabernaemontana*, with about 100 spp., is by far the largest genus in Tabernaemontaneae, and has a tortuous taxonomic history, being characterized by alternative bouts of lumping (Pichon, 1948c; Leeuwenberg, 1976, 1991, 1994b; Endress & Bruyns, 2000, Endress & al., 2007a) or splitting (Miers, 1878; Stapf, 1902; Markgraf, 1935, 1970, 1972; Boiteau & Allorge, 1976; Table 1). Linnaeus created the genus in 1753 based on three species (*T. alternifolia*, *T. citrifolia*, *T. laurifolia*), the first occurring in tropical Asia and the other two restricted to the West Indies. The genus was further expanded by de Candolle (1844), who described 61 species from the Neotropics and Paleotropics and created two new Neotropical genera morphologically similar to

time of the study.

Markgraf (1935–1972)	Pichon (1948c)	Boiteau, Allorge & collab. (1975–1983)	Leeuwenberg (1991, 1994b)
Anacampta	*T.* subg. *Bonafousia* sect. *Macrosolen*	*Bonafousia*	*T.* sect. *Bonafousia*
Anartia	*T.* subg. *Peschiera* sect. *Anartia*	*Anartia*	*T.* sect. *Bonafousia*
Bonafousia	*T.* subg. *Bonafousia*	*Bonafousia*	*T.* sect. *Bonafousia*
–	*T.* subg. *Sarcopharyngia* sect. *Camerunia*	*Camerunia*	*T.* sect. *Bonafousia*
Capuronetta	–	*Capuronetta*	*T.* sect. *Tabernaemontana*
Anacampta	*T.* subg. Peschiera sect. *Macrosolen*	*Bonafousia* p.p.	*T.* sect. *Bonafousia*
Conopharyngia	*T.* subg. *Pandaca* sect. *Conopharyngia*		*T.* sect. *Pandaca*
Domkeocarpa	*T.* subg. *Leptopharyngia* sect. *Leptopharyngia*	*Domkeocarpa*	*T.* sect. *Pandaca*
Ervatamia	*Ervatamia*	*Ervatamia*	*T.* sect. *Ervatamia*
Gabunia	*T.* subg. *Gabunia*	*Gabunia*	*T.* sect. *Pandaca*
–	*Hazunta*	*Hazunta*	*T.* sect. *Tabernaemontana*
	T. subg. *Leptopharyngia*	*Leptopharyngia*	*T.* sect. *Tabernaemontana*
Bonafousia	*T.* subg. *Peschiera* sect. *Merizadeni*a	*Bonafousia*	*T.* sect. *Bonafousia*
–	*Muntafara*	*Muntafara*	*T.* sect. *Ervatamia*
	T. subg. *Pandaca* sect. *Ochronerium*		*T.* sect. *Pandaca*
Oistanthera	*T.* subg. *Pandaca* sect. *Lepidosiphon*		*T.* sect. *Tabernaemontana*
Pagiantha	*Pagiantha*	*Pagiantha*	*T.* sect. *Pagiantha*
	T. subg. *Pandaca*	*Pandaca*	*T.* sect. *Pandaca*
–	*Pandacastrum*	*Pandacastrum*	*T.* sect. *Pandaca*
Peschiera	*T.* subg. *Peschiera*	*Peschiera*	*T.* sect. *Peschiera*
Pterotaberna	*Pterotaberna*	*Pterotaberna*	*T.* sect. *Ervatamia*
	T. subg. *Bonafousia* sect. *Macrosolen*	*Bonafousia*	*T.* sect. *Bonafousia*
–	–	*Protogabunia*	*T.* sect. *Tabernaemontana*
	T. subg. *Quadricasaea*	*Bonafousia*	*T.* sect. *Bonafousia*
Rejoua	*Rejoua*	*Rejoua*	*T.* sect. *Rejoua*
	T. subg. *Sarcopharyngia*	*Sarcopharyngia*	*T.* sect. *Tabernaemontana* / sect. *Pandaca*
Stenosolen	*T.* subg. *Peschiera* sect. *Stenosolen*	*Stenosolen*	*T.* sect. *Peschiera*
	T. subg. *Peschiera* sect. *Taberna*		*T.* sect. *Ervatamia*
Taberna	*T.* subg. *Bonafousia* sect. *Neotaberna*	*Bonafousia*	*T.* sect. *Bonafousia*
Tabernaemontana	*Tabernaemontana*	*Tabernaemontana*	*Tabernaemontana*
Testudipes			*T.* sect. *Ervatamia*
–	–	*Woytkowskia*	*T.* sect. *Bonafousia*

Tabernaemontana: *Bonafousia* and *Peschiera*. De Candolle's classification, however, failed to provide any diagnostic characters that distinguish these two genera from *Tabernaemontana*. Another important contribution was that of Bentham (1845), who created *Stemmadenia*, a Neotropical genus closely allied to *Tabernaemontana*, but distinguished by flowers with large, infundibuliform corollas with ribs above the anthers.

Miers (1878) contributed significantly to the dismemberment of *Tabernaemontana*, describing five segregate genera from South America. The disintegration of *Tabernaemontana* continued in Stapf's (1902) treatment of the family for the *Flora of tropical Africa*, in which he considered *Tabernaemontana* to be restricted to the Neotropical species and segregated the African species into four genera. This narrow circumscription of *Tabernaemontana* and recognition of several smaller Neotropical or Paleotropical genera was later followed by several other taxonomists in the family, such as Markgraf (1935, 1938), Boiteau & Sastre (1975), Boiteau & Allorge (1976) and Allorge (1983). By the middle of last century the number of new genera closely allied to *Tabernaemontana* published by various authors had turned the taxonomy of the group into something impenetrable (Table 1).

Pichon (1948c) revised the group and proposed the re-establishment of a broad *Tabernaemontana*, encompassing both Neotropical and Paleotropical species. The latest classification of the genus was provided by Leeuwenberg (1991, 1994b), who for the most part followed Pichon's delimitation of *Tabernaemontana*, but additionally reduced all segregate genera described after Pichon's (1948c) treatment into its synonymy (Table 1), as he felt the morphological differences that had been used to delimit the segregate genera (e.g., leaf and corolla texture and shape, inflorescence length, corolla tube shape, follicle texture and shape) were too inconsequential to distinguish genera. Leeuwenberg (1990, 1994b) employed many of the same characteristics, however, to create an infrageneric classification of *Tabernaemontana*, recognizing seven sections, four of which were restricted to the Old World (*T.* sect. *Ervamatia*, 15 spp.; *T.* sect. *Pagiantha*, 7 spp.; *T.* sect. *Pandaca*, 22 spp.; *T.* sect. *Rejoua*, one sp.), one restricted to the New World (*T.* sect. *Peschiera*, 12 spp.) and two including representatives from both the Old and New World (*T.* sect. *Tabernaemontana*, 18 spp.; and *T.* sect. *Bonafousia*, 24 spp.).

In light of the contradictory classifications that have been published for Tabernaemontaneae (Table 1; Table S1), we performed a detailed phylogenetic analysis based on sequences from five chloroplast DNA markers and morphology to address the following questions: (1) Does the phylogeny support the circumscription of Tabernaemontaneae s.l. sensu Endress & al. (2007a) and specifically the inclusion of Ambelanieae and Macoubeeae in the tribe? (2) Does the phylogeny support Leeuwenberg's circumscription of *Tabernaemontana* s.l. (1991, 1994b) or Endress & al.'s (2007a) circumscription? (3) Does the phylogeny support the sectional classification of *Tabernaemontana* proposed by Leeuwenberg (1994b)? (4) Which morphological or secondary chemical characters are congruent with the clades recovered from the molecular phylogenetic analysis? The discussion of our results and how they apply to

the phylogeny of Tabernaemontaneae provides an opportunity to explore how the plasticity of the morphological features that have traditionally been used to define taxonomic ranks has affected previous classifications.

■ MATERIALS AND METHODS

Taxon sampling. — Ninety-one species, including representatives of all the 19 genera currently recognized in Tabernaemontaneae s.l. by Endress & al. (2007a), were defined as the ingroup. For the large pantropical genus *Tabernaemontana*, 64 of the estimated 100 species were chosen, representing all sections proposed by Leeuwenberg (1991, 1994b) and the morphological and geographical breadth of the genus. Due to the unexpected position of *T. aurantiaca* in the phylogeny resulting from the initial sample, two additional samples of this species were included in order to verify that the observed placement was not caused by human error. Five genera each from Vinceae and Willughbeieae, the two tribes that have been shown to be the closest relatives of Tabernaemontaneae in previous studies (Potgieter & Albert, 2001; Sennblad & Bremer, 2002; Simões & al., 2007), were chosen as outgroup taxa. Two species of *Aspidosperma* (*A. australe* and *A. cylindrocarpon*) and one species of *Alstonia* (*A. scholaris*) were used as rooting taxa. The taxa analyzed, voucher information and GenBank accession numbers are listed in Appendix S1 in the Electronic Supplement.

Molecular methods and data matrix composition and alignment. — DNA extraction, amplification and sequencing were performed following the protocols described in Simões & al. (2007). Sequence contigs were manually assembled for each taxon. Multiple sequence alignment was straightforward for *matK*, requiring only a few gaps which, without exception, occurred in multiples of three. Alignment was also straightforward for the *trnK* intron and the *rps16* intron, but proved to be more difficult for the *rpl16* intron and the *trnS-G* intergenic spacer due to the large number of gaps and mononucleotide repeats. Regions of ambiguous alignment were excluded from the analysis (Kelchner, 2000). Unequivocally aligned gaps were coded as binary characters (following the "simple indel coding" method from Simmons & Ochotenera, 2000) using the software Gapcoder (Young & Healy, 2003) for all loci, and then added to the data matrix.

Bayesian inference (BI). — Bayesian posterior probabilities (PP) for branches were calculated using MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). Data were partitioned into the six categories: *matK*, *rpl16* intron, *rps16* intron, *trnK* intron, *trnS-G* spacer and gaps. Because simultaneous analysis of combined data has been proposed as the best approach to phylogenetic inference (Nixon & Carpenter, 1996), we tested the combinability of all partitions by searching for incongruence between individual datasets. For this, we compared the results on a node-to-node basis of all individual datasets with respect to levels of resolution and branch support, as applied by other authors (e.g., Wiens, 1998; Sheahan & Chase, 2000; Whitten & al., 2000; Reeves & al., 2001).

The optimal model of sequence evolution for each partition was selected using Modeltest v.3.7 (Posada & Crandall, 1998) based on the Akaike Information Criterion (AIC, Akaike 1974). A combined dataset of all partitions, including gaps, was analyzed applying separate models and parameters to each data partition. The JC + G model was arbitrarily chosen for the gaps partition because it better reflects uncertainties about relative probabilities of indel events, but allows for the possibility of unequal rates of indel evolution. Starting model parameters were assigned as uniform prior probabilities and further estimated during the analysis, by allowing them to vary independently among data partitions. Five million generations were run using one cold and three incrementally heated Markov chain Monte Carlo (MCMC) chains (Temp = 0.2), with parameters sampled every 5000 generations. Two independent runs (Nruns = 2), starting from different random trees, were performed to ensure that the individual runs had converged to the same result. Based on the inspection of the likelihood scores for each generation, the first 250 sampled generations were considered as burn-in, and thus discarded from subsequent analyses. The post burn-in trees were imported into PAUP* v.4.0b (Swofford, 2000), and a 50% majority-rule consensus tree was then reconstructed to obtain posterior probabilities of the clades. Posterior probabilities ≥0.95 were considered strongly supported, those <0.95 as more weakly supported.

Character mapping. — In order to identify synapomorphies that are congruent with each of the major clades of Tabernaemontaneae retrieved in the molecular tree and to assess the value of characters used in earlier classifications, 29 morphological, anatomical and chemical characters were selected. The character codings were derived mainly from direct observation of herbarium specimens, pickled material, serial sections, codings in previously published studies (Endress & al., 1996, 2007b; Simões & al., 2007) and complemented, when necessary, with information from the literature (Monachino, 1945a,b, 1946; Hallé, 1965, 1971; Boiteau & Sastre, 1975; Beentje, 1978; Allorge, 1983; Barink, 1984; Leeuwenberg, 1985a,b, 1991, 1994b, 1995; Zarucchi, 1987; and Morales & Mendéz, 2005 for morphological characters, and Achenbach & Raffelsberger, 1980; Kisakürek & al., 1983; Van Beck & al., 1984; Anderson & al., 1985; Bisset, 1985, 1989; Van Beck & Van Gessel 1988; and Vonk & Leeuwenberg, 1989 for secondary chemistry). The characters and character states are given in the Appendix. We used the exemplar method, scoring morphological, anatomical and chemical characters in the morphological matrix for the same species as used in the molecular analyses. The complete morphological matrix, coding 29 characters for the 104 taxa, is available in Appendix S2 in the Electronic Supplement. At present, information is not available for the characters of all taxa, especially those that are rare or narrowly distributed. The matrix thus contains some polymorphic codings (e.g., follicle/berry for fruit type) and some missing information (e.g., aril color for species of *Callichilia*). From the 29 optimized morphological characters, 9 were reconstructed as potential synapomorphies for major clades of the ingroup and selected for further discussion (see Figs. 3 and 4 below). Character evolution was reconstructed onto a randomly chosen tree generated in the Bayesian analysis by using the Parsimony Ancestral State Reconstruction Package for Mesquite (Maddison & Maddison, 2005) in Mesquite v.2.05 (Maddison & Maddison, 2007). All morphological characters were considered unordered and unweighted. Alternative optimizations were investigated by checking all possible most parsimonious reconstructions (MPRs) on the selected tree for the nodes of interest and also by tracing character evolution in other Bayesian trees with different topologies.

■ RESULTS

Phylogenetic inference. — The model GTR + g was selected for *trnS-G* spacer and the *rpl16*, *rps16* and 3′ *trnK* introns, the model TVM + g for *matK*. No strongly supported incongruent clades were found between individual partitions, and therefore they were all combined in a single matrix. All further discussion will be based on the majority-rule consensus cladogram obtained from the combined dataset, with PP values estimated from that dataset.

The monophyly of Tabernaemontaneae sensu Endress & al. (2007a) (Tabernaemontaneae s.l. clade) is strongly supported (PP 1.00). The clade is sister to the Willughbeieae clade, but with low support (PP 0.76) (Fig. 1). Within Tabernaemontaneae s.l., an early branching event gave rise to two major clades: Ambelanieae and Tabernaemontaneae s.str. (Fig. 1). The Ambelanieae clade is formed by representatives of all genera of Ambelanieae sensu Zarucchi (1987) and Leeuwenberg (1994a) (*Ambelania*, *Molongum*, *Mucoa*, *Neocouma*, *Rhigospira*, *Spongiosperma*), plus the included representative of Leeuwenberg's Macoubeeae (*Macoubea guianensis*), all of them restricted to the Neotropics. The Tabernaemontaneae s.str. clade is the largest and most diversified within the tribe, and comprises representatives of the nine genera recognized in the tribe by Leeuwenberg (1994b) divided into the *Callichilia* clade (*Callichilia* Stapf, *Calocrater* K. Schum., *Carvalhoa* K. Schum., *Crioceras* Pierre, *Schizozygia* Baill., *Tabernanthe* Baill., *Voacanga*) and the *Tabernaemontana* clade (*Stemmadenia* and *Tabernaemontana*).

The *Callichilia* clade is formed by three strongly supported (PP 1.0), but unresolved subclades: the monotypic genera *Carvalhoa* and *Schizozygia*, plus the sampled species of *Tabernanthe* (*T. iboga* Baill.); *Calocrater*, *Crioceras*, *Callichilia* and *Voacanga*; and the three sampled specimens of *Tabernaemontana aurantiaca* (Fig. 1).

All sampled species of *Tabernaemontana* except *T. aurantiaca*, together with all species of *Stemmadenia* included in this study, form a large and strongly supported clade (*Tabernaemontana* clade, PP 1.00; Figs. 1 and 2). A major split at the base of this clade gave rise to two lineages, one containing all Neotropical species of *Tabernaemontana* plus *Stemmadenia* (Neotabernaemontana clade), and the other containing the remaining species of *Tabernaemontana*, all from the Paleotropics (Paleotabernaemontana clade; Fig. 2). Within the Neotabernaemontana clade, the six sampled species of *Stemmadenia* are strongly supported (PP 1.00) as sister to the 26 sampled species of Neotropical *Tabernaemontana*. The Paleotabernaemontana clade, formed exclusively by the sampled representatives of the
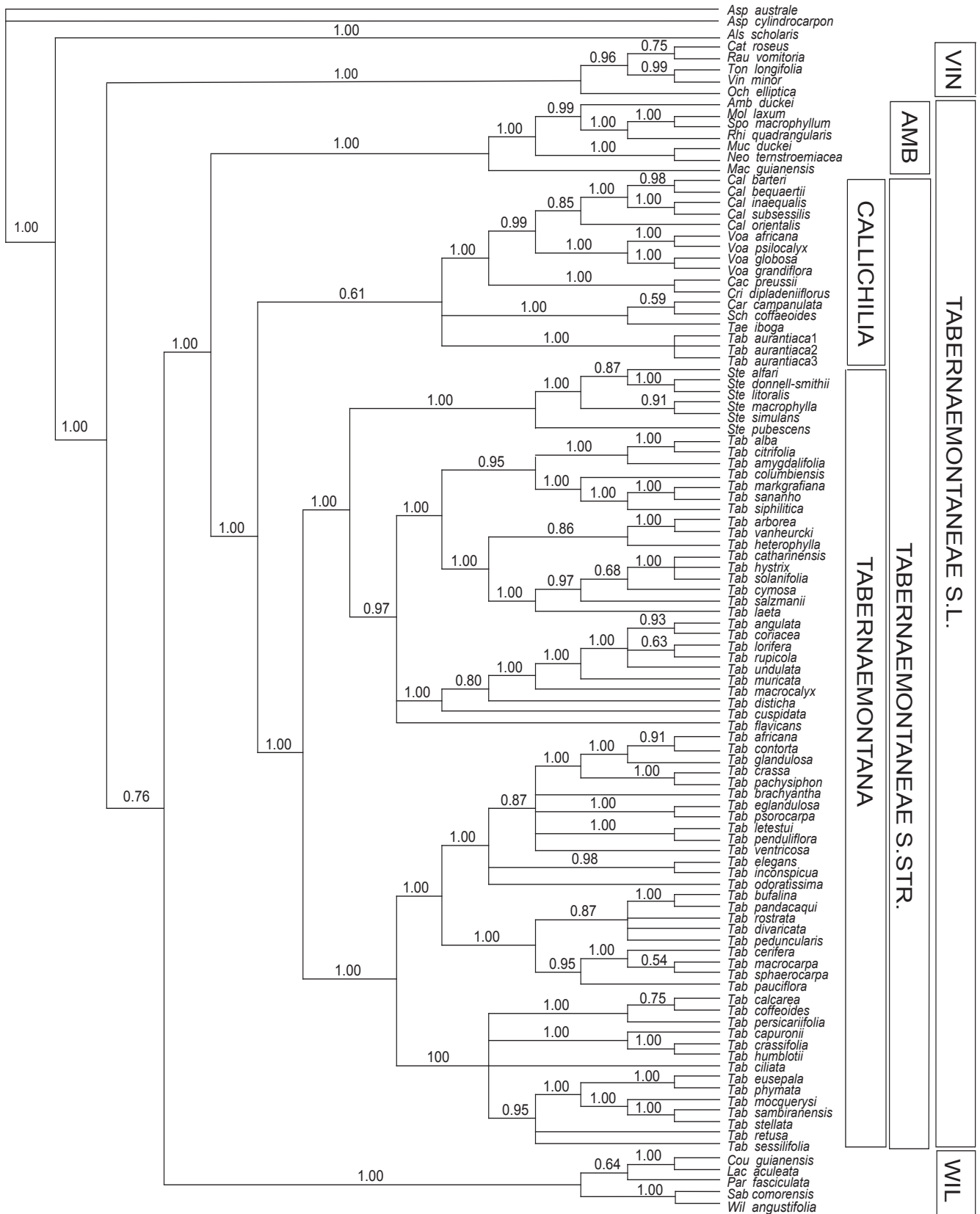
Fig. 1. Majority rule consensus tree based on the Bayesian analysis of the combined molecular dataset. Posterior probabilities are indicated above the branches. Full taxon names are given in the Supplementary Appendix S1 in the Electronic Supplement. AMB: Ambelanieae clade; VIN: Vinceae clade; WIL: Willughbeieae clade.
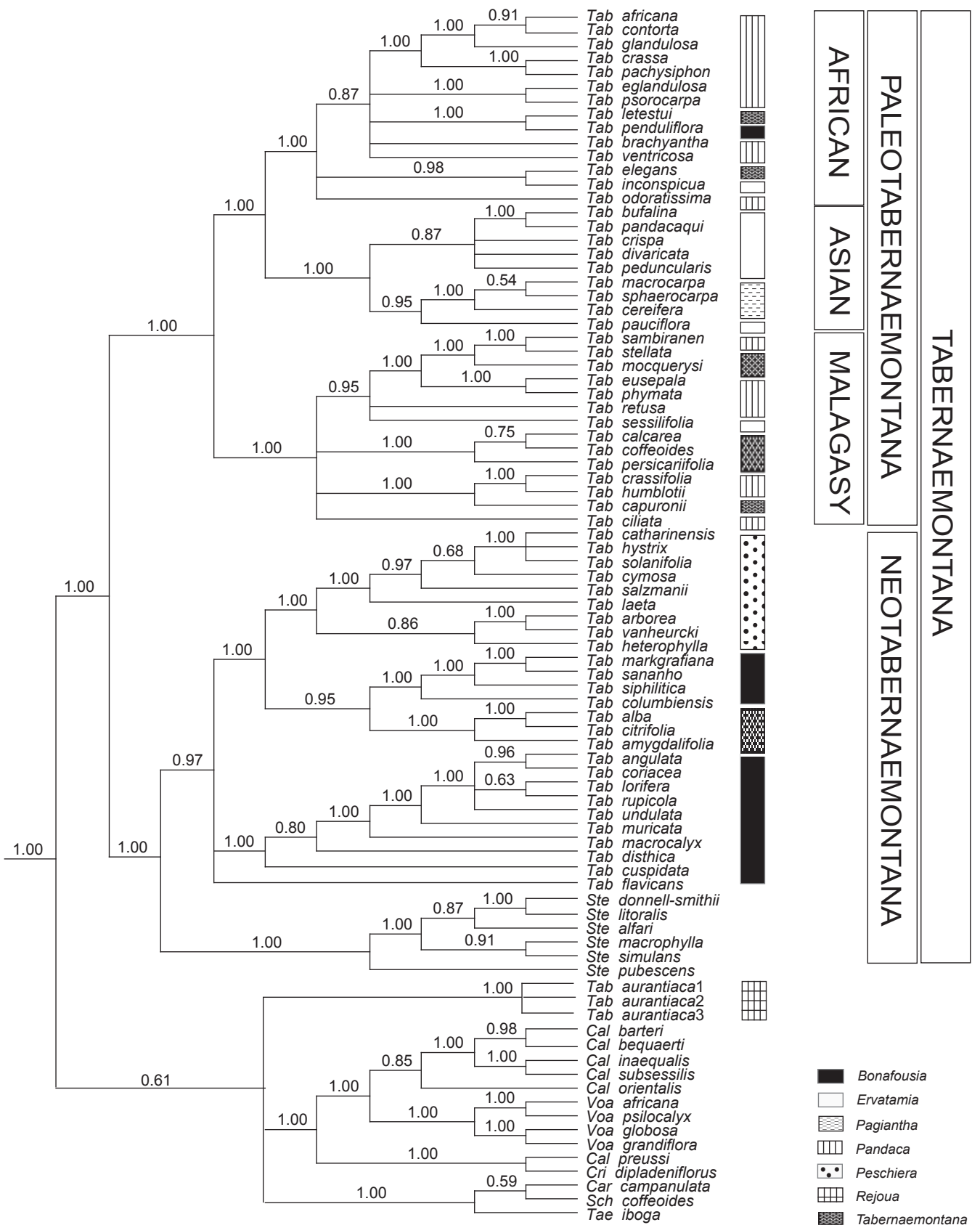
Fig. 2. Tabernaemontaneae s.str. clade from the majority rule consensus tree with the most recent sectional classification of the genus *Tabernaemontana* (Leeuwenberg, 1994b) mapped onto it.

genus *Tabernaemontana*, comprises three lineages (all with PP 1.00), which are largely coincident with geographic areas (Fig. 2). The first (Malagasy clade) is sister to the other two and comprises 14 species from the West Indian Ocean, 12 of which are Madagascan endemics. The second (Asian clade) includes 9 species that occur in tropical Asia, both continental and insular, and is sister to the third (African clade), composed of 14 species that are restricted to continental tropical Africa.

■ DISCUSSION

Circumscription of Tabernaemontaneae. — The Tabernaemontaneae s.l. clade is characterized by three synapomorphies: (1) specialized anthers with massive lignified guide-rails; and a differentiated style-head with (2) a five-lobed upper crest and (3) a thickened basal flange and is divided into two main clades, which are unequal in size: (i) the small Ambelanieae clade, including seven Neotropical genera; (ii) the large and pantropical Tabernaemontaneae s.str. clade, which is further divided into two subclades: the *Tabernaemontana* clade, including the species-rich, pantropical genus *Tabernaemontana* and the neotropical genus *Stemmadenia*, and the *Callichilia* clade, including seven genera, all but one restricted to Africa. Both Leeuwenberg's (1994a) and Endress & Bruyns' (2000) circumscription of Tabernaemontaneae, corresponding to the Tabernaemontaneae s.str. and the Tabernaemontaneae s.l. clades, respectively, are equally strongly supported as monophyletic by our results (Fig. 1), depending upon whether the Ambelanieae clade and the Tabernaemontaneae s.str. clade are recognized as two distinct tribes or as sister lineages within a larger tribe.

We consider the broad circumscription of Tabernaemontaneae proposed by Endress & Bruyns (2000) the more satisfactory, as it underscores the important morphological and chemical similarities among the genera that form the Tabernaemontaneae s.l. clade. Thus defined, the tribe is supported by three potential morphological synapomorphies identified by ancestral state reconstruction (Fig. 3). The most recent common ancestor (MRCA) of the Tabernaemontaneae clade was unambiguously reconstructed as having calycine colleters arranged in one to several rows spread across the base of the calyx lobes (char. 4: 1; Appendix) (Boiteau & Allorge, 1978; Leeuwenberg, 1991, 1994a,b; Endress & Bruyns, 2000; Simões & al., 2007); anthers with lignified guide rails (char. 13: 1); and style-head with a five- to ten-lobed upper crest (char. 15: 2) (Fallen, 1986)

(Fig. 3). Within the Tabernaemontaneae s.str. clade, the loss of lignified guide-rails (char. 13) is always linked with a reversal to a morphologically simple style-head (i.e., lacking both upper crest and basal flange; chars. 15 and 16). This evolved in parallel four times: in *Tabernaemontana aurantiaca* (Pacific Basin), in the MRCA of the Asian *Tabernaemontana* clade, in *T. inconspicua* (Africa) and in *T. coffeoides* and *T. sessilifolia* (Madagascar). Flowers with this combination of traits are characteristic for all species of the Asian clade, but the same suite of traits has occurred independently in *Tabernaemontana aurantiaca* (Pacific Basin), *T. coffeoides* and *T. sessilifolia* (Madagascar).

A flange (a thickened projection of the style-head body base) (char. 16; Appendix) is characteristic for the majority of genera in the Tabernaemontaneae s.l. clade, but is absent in several genera of the *Callichilia* clade as well as sporadically in different clades within the genus *Tabernaemontana*. The reconstruction of this character is ambiguous: it could be a synapomorphy for the Tabernaemontaneae s.l. clade, and then have been lost independently in some genera of the *Callichilia* clade and in parallel in some species of *Tabernaemontana* (see Fig. 3), or the basal flange could have been absent in the MRCA of the Tabernaemontaneae s.l. clade, and then have originated in parallel in the Ambelanieae, *Callichilia* and *Tabernaemontana* clades, with subsequent losses in the latter. It is noteworthy that loss of the basal flange is almost always linked with a concomitant loss of the upper crest of the style-head as well (except in the *Callichilia* clade), resulting in a morphologically simple style-head.

In addition to the morphological characters mentioned above, genera of the traditional Ambelanieae, Macoubeeae and Tabernaemontaneae (here the Tabernaemontaneae s.l. clade) are characterized by the presence of highly evolved indole alkaloids of the ibogan type (Achenbach & Raffelsberger, 1980; Kisakürek & al., 1983; Van Beck & al., 1984; Van Beck & Van Gessel, 1988), which are otherwise very rare in the family. Unfortunately, we lacked data on the secondary chemistry of the sister group, Willughbeieae, and thus were unable to feasibly use this character for ancestral state reconstruction (see char. 29, Appendix). The MRCA of the Tabernaemontaneae s.str. clade was unambiguously reconstructed as having seeds with a ruminate endosperm (char. 27: 1) and a deep hylar groove (char. 28: 1; Fig. 4). Ruminate endosperm is otherwise rare in the family, but is characteristic for certain genera of tribe Alyxieae (Endress & al., 2007b), for example, *Chilocarpus*, which prompted Boiteau & Sastre (1975) to transfer *Chilocarpus* to subfamily Tabernaemontanoideae.

Fig. 3. Parsimony optimization of five floral characters for the Tabernaemontaneae, Tabernaemontaneae s.str. and *Tabernaemontana* clades on one of the trees generated in the Bayesian analysis and randomly chosen from the pool of trees. Solid bars denote unambiguous reconstructions (with one single most parsimonious reconstruction for a given tree) for characters 4, 10, 13 and 15, and one of the most parsimonious reconstructions for character 16. A more detailed explanation on the evolution of character 16 within the *Callichilia* clade is given in the Discussion. All numbers above the symbols correspond to the character numbers, and numbers below the symbols correspond to the character state as listed in the Appendix. Character numbers and states are as follows: 4, Inner surface of the calyx. 0: naked; 1: with several to numerous colleters in one or more rows; 2: with five colleters quincuncially arranged. 10, Corolla lobe tips in bud. 0: not inflexed; 1: inflexed. 13, Lignified guide rails. 0: absent; 1: present. 15, Style-head body apex: (0) undifferentiated; (1) with un-lobed upper wreath; (2) with deeply 5- to 10-lobed upper crest. 16, Style-head body base: (0) undifferentiated; (1) with membranous collar; (2) with thickened expanded flange. Thicker branches indicate those branches strongly supported by PP values of 0.95 or higher. Clade names are indicated as follows: Amb: Ambelanieae clade; Cal: *Callichilia* clade; Tab: *Tabernaemontana* clade; Tsl: Tabernaemontaneae s.l. clade; Tss: Tabernaemontaneae s.str. clade.
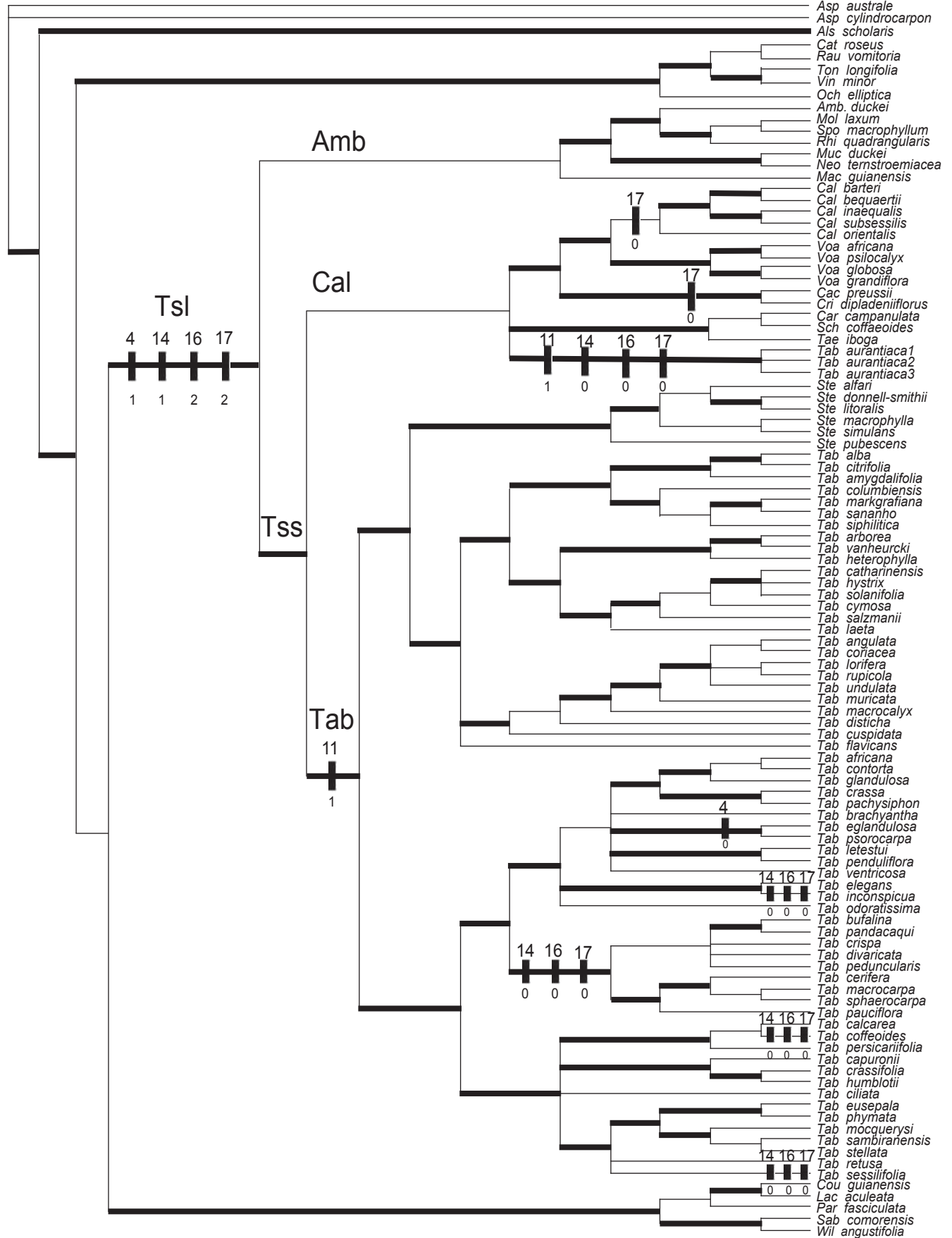
The Ambelanieae clade. — This strongly supported (PP 1.00) clade includes the six species-poor genera of tribe Ambelanieae sensu Zarucchi (1987) (*Ambelania, Molongum, Mucoa, Neocouma, Rhigospira, Spongiosperma*), together with *Macoubea*, the sole member of tribe Macoubeeae sensu Boiteau & Sastre (1975) (Fig. 1). Within the Ambelanieae clade, the tribe Ambelanieae sensu Zarucchi (1987) forms a strongly supported (PP 1.00) subclade sister to *Macoubea* (Fig. 1), a genus treated by Leeuwenberg (1994a) in the monogeneric tribe Macoubeeae. If Leeuwenberg's tribal classification is followed, either two or three tribes must be circumscribed within the Tabernaemontaneae clade, depending on whether *Macoubea* is recognized as the representative of Macoubeeae or is included in Ambelanieae. This clade is sister to the Tabernaemontaneae s.str. clade.

All genera in the Ambelanieae clade are small to medium-sized trees with fleshy white salverform corollas, and are restricted to lowland tropical forest in Amazonia except *Macoubea*, for which one species (*M. mesoamericana* J.F. Morales) has been discovered in the pluvial forest of Panama and Costa Rica (Morales, 1999a). All members of the Ambelanieae clade have syncarpous, indehiscent fruits and seeds without an aril. Earlier, these taxa were considered to be more "primitive" than those with dehiscent fruits and arillate seeds and thought to be allied with genera included in "Carisseae" (= Willughbeieae in current classifications; Schumann, 1895; Monachino, 1945a,b, 1946). Pichon (1948a) recognized that these taxa share three important features with the genera from the Tabernaemontaneae s.str. clade: (1) specialized anthers with massive lignified guide-rails; and a differentiated style-head with (2) a five-lobed upper crest and (3) a thickened basal flange (representing the three synapomorphies characterizing the Tabernaemontaneae s.l. clade identified in this study; see Fig. 3). Because they resemble taxa of Willughbeieae in their fruit and seed morphology, but Tabernaemontaneae s.str. in their specialized anther and style-head structure, they were considered to fall somewhere between the "primitive" Willughbeieae and the "advanced" Tabernaemontaneae s.str. (Monachino, 1945b) and were therefore assigned tribal status by Pichon (1948a). In classifications that recognized the taxa included here in the Tabernaemontaneae s.str. clade as a distinct subfamily, i.e., Tabernaemontanoideae (e.g., Markgraf, 1938; Boiteau & al., 1978; Allorge & al., 1981), the taxa from the Ambelanieae clade were always segregated in a separate subfamily, Plumerioideae (= Rauvolfioideae; Table S1 in the Electronic Supplement). The first person to suggest that the tribes Ambelanieae and Tabernaemontaneae should be united was Fallen (1986: 280), a position that has been supported in various phylogenetic

studies since then (Endress & al., 1996, 2007b; Sennblad & Bremer, 1996, 2002; Potgieter & Albert, 2001; Simões & al., 2007) and that was incorporated in the family classification of Endress & Bruyns (2000) and Endress & al. (2007a). The sister relationship of the Ambelanieae and Tabernaemontaneae s.str. clades observed in our study gives additional support to their circumscription as a single tribe within Rauvolfioideae.

Macoubea is a small genus of three species, restricted to lowland tropical forest in Central and South America. In the cpDNA tree, the sampled species of the genus (*M. sprucei*) is strongly supported as sister to the other genera from the Ambelanieae clade (Fig. 1). All species are trees with sweet-scented, white, salverform flowers superficially resembling those of *Couma* Aubl. (Willughbeieae). However, the anthers of *Macoubea* have massive lignified guide-rails and the style-head has a five-lobed upper crest and thick flange at the base as in other members of the Tabernaemontaneae s.l. clade, but lacking in *Couma*. Although the gynoecium of *Macoubea* is apocarpous or hemi-syncarpous, development of the two carpels is unequal. In *M. guianensis* Aubl. often only one of the two carpels develops normally, so that at maturity the fruit is composed of a single indehiscent carpel with the remnant of the second carpel visible in mature fruits as a small bump at the base and thus superficially resembles the syncarpous, indehiscent fruits found in Ambelanieae. In the case of *M. sprucei* (Müll.-Arg.) Markgraf, the mature fruit is composed of one larger and one smaller, broadly divergent carpel that are fused to about halfway, so that at maturity the fruit has an irregular shape resembling a Dutch clog.

Because of the specialized structure of the anthers and style-head, and the apocarpous yet indehiscent fruits, *Macoubea* has usually been considered to occupy a linking position between the congenitally syncarpous, indehiscent taxa of Ambelanieae and the apocarpous, dehiscent taxa of Tabernaemontaneae. It has most commonly been included in tribe Tabernaemontaneae (Markgraf, 1938; Monachino, 1945a; Boiteau & Sastre, 1975; Boiteau & al., 1978) or earlier in the genus *Tabernaemontana* (de Candolle, 1844; Müller Argoviensis, 1860), and sometimes in tribe Ambelanieae (Pichon, 1948a), or as its own tribe, Macoubeeae (Leeuwenberg, 1994a).

A source of dispute is whether or not the seeds of *Macoubea* are arillate. The seeds are completely covered by a viscous, translucent outer layer, which breaks down during fruit maturation, so that in the ripe fruits it forms a sweet, liquid syrup, which collects in the indehiscent, thick-walled fruits and is appreciated by primates (Monachino, 1945b; Boiteau & Sastre, 1975; Schultes, 1979; Roosmalen, 1985). Boiteau

Fig. 4. Parsimony optimization of four fruit and seed morphological characters for Ambelanieae, Tabernaemontaneae s.l., Tabernaemontaneae s.str. and *Tabernaemontana* clades on one of the trees generated in the Bayesian analysis and randomly chosen from the pool of trees. The two most parsimonious reconstructions for character 25 are denoted by solid and dotted figures, respectively. All numbers above the symbols correspond to the character numbers, and numbers below the symbols correspond to the character state as listed in the Appendix. Subsequent changes from follicles (0) to berries (2) in character 21 within the *Tabernaemontana* clade are indicated by asterisks. Character numbers and states are as follows: 21, Fruit type. 0: follicular; 1: drupaceous; 2: baccate. 25, Aril on seed. 0: absent; 1: present. 27, Endosperm. 0: not ruminate; 1: ruminate. 28, Deep hylar groove on seeds. 0: absent; 1: present. Thicker branches indicate those branches strongly supported by PP values of 0.95 or higher. Clade names are indicated as follows: Amb: Ambelanieae clade; Cal: *Callichilia* clade; Tab: *Tabernaemontana* clade; Tsl: Tabernaemontaneae s.l. clade; Tss: Tabernaemontaneae s.str. clade; Wil: Willughbeieae clade.
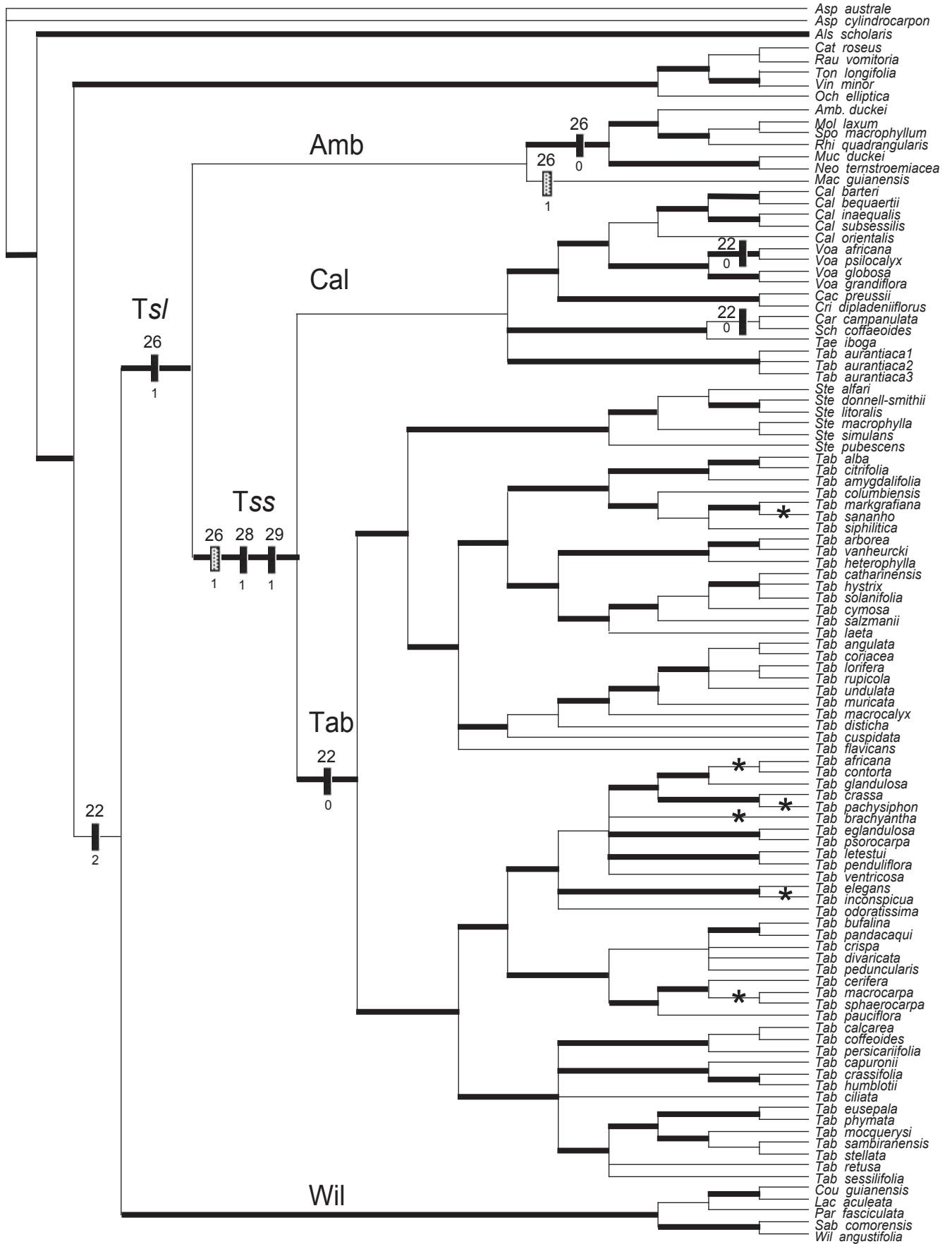
& Sastre (1975) interpreted this outer layer as an aril and its presence as justification for transferring *Macoubea* from Willughbeieae to Tabernaemontanoideae, where they assigned it tribal status. In contrast, Leeuwenberg (1994a) did not consider the mucilaginous covering on the seeds to be an aril. Like Pichon (1948a), he found that "the seeds lack the aril and the hilar groove, and the endosperm is not ruminate." The interpretation of the outer covering of the seeds also plays a key role in evaluating character evolution in tribe Tabernaemontaneae. If it is interpreted to be an aril, the MRCA of the Tabernaemontaneae s.l. clade possessed an aril and this was subsequently lost in the remainder of the Ambelanieae clade or arils have evolved twice, once in the MRCA of the Tabernaemontaneae s.str. clade and independently in *Macoubea* (Fig. 4; char. 25). If it is not interpreted as an aril, then arils arose only once in the MRCA of the *Tabernaemontana* s.str. clade. Without developmental studies, it is impossible to determine whether the outer covering of the seeds of *Macoubea* is an aril or not. It is undisputed, however, that the translucent, mucilaginous outer layer completely covering the seeds of *Macoubea* looks very different than the fleshy, non-viscous and often brightly colored arils that often only partly cover the seed, and which together with a hylar groove and ruminate endosperm, characterize the Tabernaemontaneae s.str. clade. Since *Macoubea* lacks both a hylar groove and ruminate endosperm, this suggests that the thin, viscid covering on the seeds in *Macoubea* is most likely not homologous to the fleshy arils found in Tabernaemontaneae s.str., which is not surprising, since no other members of the Ambelanieae clade display these traits.

The *Callichilia* clade. — This poorly supported clade is formed by the three following unresolved subclades: (1) *Callichilia*, *Voacanga*, *Calocrater* and *Crioceras*; (2) *Carvalhoa*, *Schizozygia* and *Tabernanthe*; and (3) *Tabernaemontana aurantiaca* (Fig. 1). The MRCA of the *Callichilia* clade is unambiguously reconstructed as having indehiscent, baccate fruits, with two subsequent shifts to follicular fruits: once in the ancestor of the African species of *Voacanga*, and again in the ancestor of the *Schizozygia* + *Carvalhoa* clade (char. 21, Appendix; Fig. 4). Additionally, in all genera of the clade (except *T. aurantiaca*), the base of the ovary is surrounded by a distinct disc nectary (char. 17), which is indistinct or adnate in all members of the Ambelanieae clade and most of the *Tabernaemontana* clade (see Appendix S2 in the Electronic Supplement). Further, the style-head lacks the basal flange (char. 16; Fig. 3) in *Callichilia*, *Calocrater*, *Crioceras* and in *Tabernaemontana aurantiaca*. It is uncertain whether this loss occurred once in the MRCA of the *Callichilia* clade and was then regained in *Voacanga*, *Carvalhoa*, *Schizozygia* and *Tabernanthe*, or two independent losses occurred: once in the MRCA of *Callichilia*, *Voacanga*, *Calocrater* and *Crioceras*, with a subsequent reversal in *Voacanga*, and a second loss in *Tabernaemontana aurantiaca*.

Within the first subclade of the *Callichilia* clade, the two rare, monotypic genera with a narrow geographic distribution in tropical West Africa, *Calocrater* and *Crioceras*, have traditionally been considered to be closely allied (Hallé 1965, 1971; Leeuwenberg, 1995), a relationship that is strongly supported here (PP 1.00; Fig. 1). Both are shrubs or small trees growing in the understory of forests, and are characterized by large white, sweet-scented flowers with infundibuliform, delicate corollas. *Crioceras* has probably the most spectacular flowers in the tribe. The infundibuliform corolla is up to 19 cm long and individual flowers are pendent, dangling on peduncles up to 8 cm long and sheathed in two large leafy bracts up to 11 cm long (Hallé, 1971).

Calocrater + *Crioceras* are strongly supported (PP 1.00) as sister to *Callichilia* + *Voacanga*. The latter two genera are also strongly supported (PP 0.99) as sisters, a relationship that has not previously been suggested (Fig. 1). *Callichilia* is a genus of seven species restricted to rainforest and riverine forest in tropical Africa; all are small shrubs except *C. inaequalis*, which is described as a woody climber. *Callichilia orientalis*, originally described as the genus *Ephippiocarpa* (Markgraf, 1923), is moderately supported (PP 0.85) as sister to the other four species of *Callichilia* included in this study. Previously *Callichilia* was considered to have the closest affinities to *Calocrater* and *Crioceras* (Beentje, 1978), whereas the closest relative of *Voacanga* was thought to be *Tabernaemontana* (Leeuwenberg, 1985b). These are reasonable conclusions based on morphology. Like *Calocrater* and *Crioceras*, the flowers of *Callichilia* have large, infundibuliform corollas and are borne in long-peduncled, pendent, few-flowered inflorescences (Beentje, 1978). The ellipsoid, thin-walled, berry-like fruits of *Callichilia*, *Calocrater* and *Crioceras* are also similar.

Voacanga comprises twelve species, seven restricted to Africa and five widespread in the islands of Malesia, of which one, *V. grandiflora*, reaches Queensland, Australia (Leeuwenberg, 1985b). In this study two species each from Asia (*V. globosa*, *V. grandiflora*) and Africa (*V. africana*, *V. psilocalyx*) form a strongly supported (PP 1.00) clade (Fig. 1). All species are shrubs or trees with long-pedunculate, but not pendent inflorescences. The calyx lobes in most species are connate at the base, forming a campanulate tube that sheaths the lower corolla tube; the salverform to narrowly expanded corolla often has a thick and fleshy texture and the tube is strongly twisted in most species. The fruits are globose to sub-globose and mostly thick-walled and some are apparently indehiscent. Leeuwenberg (1985b) notes that the mericarps of the tropical West African species *V. caudiflora* Stapf, *V. pachyceras* Leeuwenberg and *V. psilocalyx* Pierre ex Stapf have only a faint line of dehiscence, and David Middleton, who has done extensive field work in tropical Asia notes "I would be very surprised if any Asian *Voacanga*s were dehiscent" (pers. comm., Oct. 2008).

The second subclade of the *Callichilia* clade is comprised of three small genera. Two of them, *Schizozygia* and *Carvalhoa*, are monotypic and restricted to a relatively small region in tropical East Africa; the third, *Tabernanthe*, with two recognized species, is found in Central and tropical West Africa. It is difficult to find meaningful morphological traits that unite the three genera, other than that they have much smaller flowers than those of the genera in the other two subclades. *Tabernanthe* and *Schizozygia* both have a narrow salverform corolla that is constricted at the throat, but have completely different fruit types, those of *Tabernanthe* being soft, indehiscent and berry-like, whereas those of *Schizozygia* are leathery to thinly

woody and dehiscent. Neither the campanulate flowers nor the thinly fleshy, dehiscent fruits of *Carvalhoa* resemble either *Tabernanthe* or *Schizozygia*, which is not surprising, since its relationship to the latter is poorly supported (PP = 0.59).

Tabernaemontana aurantiaca is a shrub or small tree with sweet-scented white flowers, found on the Moluccas, New Guinea, the Solomon Islands and Western Pacific Islands. Morphologically, *T. aurantiaca* resembles much more *Tabernaemontana* than it does any of the taxa in the *Callichilia* clade. The anthers lack lignified guide-rails and the style-head is undifferentiated, with neither an upper crest nor basal flange, as is also typical for the other *Tabernaemontana* species from Asia and the Pacific Basin. The fruit is composed of two indehiscent mericarps filled with a white spongy tissue containing the arillate seeds. The spongy tissue could function as an aid in saltwater dispersal, particularly considering the widespread distribution of this species on Pacific islands. *Tabernaemontana aurantiaca* was originally described as a distinct genus, *Rejoua* (Gaudichaud, 1826), based mainly on its odd, sometimes tear-drop-shaped, indehiscent fruits, and was assigned to its own monotypic section in *Tabernaemontana* by Leeuwenberg (1994b). Additional collections, however, have shown that the fruits of *T. aurantiaca* are quite variable in shape, ranging from globose to very narrowly pyriform, and furthermore, that a number of other species of *Tabernaemontana* also have indehiscent fruits (Leeuwenberg, 1994b; Middleton, 2007). The position of this species within the *Callichilia* clade, rather than with the rest of the species of *Tabernaemontana*, was completely unexpected. However, it should be noted that the entire *Callichilia* clade is poorly supported (PP 0.61) and comprises an unresolved polytomy, of which the three samples of *T. aurantiaca* form one subclade. Since the *Callichilia* clade is poorly supported, we cannot exclude the possibility that the addition of other species of *Tabernaemontana* from the Pacific basin might result in topological changes affecting the position of *T. aurantiaca*. Because of its strong morphological similarity to *Tabernaemontana*, rather than to any other genus of the *Callichilia* clade, and because its position is unresolved, we prefer to maintain *T. aurantiaca* as a taxon of uncertain position within the tribe until additional data become available.

Delimitation of *Tabernaemontana*. — The *Tabernaemontana* clade has a deep basal split into a Paleotropical and a Neotropical lineage, both strongly supported (PP 1.00; Fig. 2). Its MRCA is unambiguously reconstructed as having inflexed corolla lobe tips in bud (char. 10: 1; Fig. 3) and dehiscent, follicular fruits (char. 21: 0; Fig. 4). At least three independent reversals to indehiscent baccate fruits have occurred within the genus (Fig. 4). Although there seems to be a potential correlation between indehiscent fruits and arils that are white or cream-colored (as opposed to the more typical red or orange arils in the majority of species with dehiscent fruits), data on aril color (char. 26; Appendix) are lacking for critical genera in the *Callichilia* and *Tabernaemontana* clades, so we were unable to make a reasonable reconstruction of this potentially very interesting character.

The *Tabernaemontana* clade largely corresponds to Leeuwenberg's broad circumscription of the genus *Tabernaemon

tana*, with two exceptions: (1) one species, *T. aurantiaca*, is weakly supported as part of the sister clade (*Callichilia* clade); (2) all sampled species of *Stemmadenia* form a strongly supported clade deeply nested within the *Tabernaemontana* clade. To render *Tabernaemontana* monophyletic, three different approaches could be followed. One option would be to expand the circumscription of the genus to encompass all representatives of the *Tabernaemontana* clade, thus including *Stemmadenia*. A second option would be to restrict the genus to the species of the Neotabernaemontana clade (including *Stemmadenia*) and recognize the species of the Paleotabernaemontana clade as a separate genus. The third option would be to recognize each major lineage of the *Tabernaemontana* clade as a distinct genus, which would maintain the generic status of *Stemmadenia* but would require the resurrection of several genera that were included in the synonymy of *Tabernaemontana* by Leeuwenberg (1991, 1994b), such as *Bonafousia*, *Ervatamia*, *Pandaca* and *Peschiera*. Since we found no reliable morphological synapomorphies, either to distinguish species of the Paleotabernaemontana clade from those of the Neotabernaemontana clade, or to justify generic recognition of the various subclades, we feel that the fragmentation of *Tabernaemontana* is untenable. We have thus chosen a broad circumscription of *Tabernaemontana* sensu Leeuwenberg (1991, 1994b), but including *Stemmadenia* as well.

Species of *Stemmadenia* are shrubs or (usually small) trees, mostly with large showy flowers, occurring mainly in wet tropical forest, although some species (e.g., *S. donnell-smithii*) are prominent members of dry or seasonally dry forest, with a center of distribution in Mesoamerica. Of the 17 currently recognized species, only one does not occur in Mesoamerica. *Stemmadenia* and Neotropical species of *Tabernaemontana* are morphologically very similar. All species have massive lignified guide-rails and a differentiated style-head with a five-lobed upper crest and thick flange at the base, characteristics that set them apart from all other genera of Rauvolfioideae in the New World.

It seems somewhat surprising that the differences, rather than the similarities between *Tabernaemontana* and *Stemmadenia* have been stressed in the past. *Stemmadenia* is distinguished by a usually very large corolla, with the tube differentiated into a lower, often strongly twisted, cylindrical part and an expanded upper throat characterized by five vertical ridges above the stamens, calyx lobes usually large, foliaceous and unequal in size, while *Tabernaemontana* has smaller, salverform corollas, usually without a twisted tube and never with vertical ridges above the stamens, and calyx lobes never foliaceous (Markgraf, 1938). In Woodson's (1940) treatment of Apocynaceae of the Yucatan Peninsula, *Stemmadenia* was distinguished from *Tabernaemontana* based on its large, imbricate, conspicuously foliaceous or petaloid bracts and calyx lobes, corolla often infundibuliform, thin and delicate in texture and calycine colleters in several rows (versus: small bracts and calyx lobes, corolla salverform and often more fleshy in texture, and calycine colleters in a single row in *Tabernaemontana*).

A broader sampling of *Tabernaemontana*, including species from South America, however, shows that a number of species

also have large, showy white or pink, imbricate calyx lobes (e.g., *T. cerea* (Woods.) Leeuwenberg, *T. macrocalyx*) or a strongly twisted corolla tube (e.g., *T. siphilitica*). Conversely, several species of *Stemmadenia* have only a single row of colleters and a number of species have a narrow, salverform corolla, in particular, the recently described *S. simulans*, of which the author states: "The specific name recalls that *Stemmadenia simulans* looks like a species of *Tabernaemontana*" (Morales, 1999b). In more recent taxonomic treatments, the only constant feature given to distinguish *Stemmadenia* from *Tabernaemontana* is the vertical ribs on the corolla tube above the anthers (Allorge, 1983; Leeuwenberg, 1994b; Morales & Méndez, 2005). The inclusion of *S. macrophylla* as a synonym of *S. litoralis*, as proposed in the revision of the genus by Morales & Méndez (2005), is not supported by our study. Instead, *S. litoralis* is strongly supported (PP 1.00) as sister to *S. donnell-smithii*, whereas *S. macrophylla* is more weakly supported (PP 0.91) as sister to *S. simulans* (Figs. 1 and 2). Although our study only included 6 of the 17 recognized species of *Stemmadenia*, these results suggest that the status of *S. macrophylla* needs to be re-examined.

Infrageneric classification of *Tabernaemontana*. — Our broad sampling of the large genus *Tabernaemontana*, including 64 of the estimated 100 species (Leeuwenberg 1991, 1994a), allows us to elucidate phylogenetic relationships among its major lineages and compare the results obtained with the most recent classification of the genus proposed by Leeuwenberg (1991, 1994b). Of the seven sections of *Tabernaemontana* proposed by Leeuwenberg (1994b), only three (*Peschiera*, *Pagiantha* and the monotypic *Rejoua*) were found to be monophyletic (Fig. 2). The most extreme cases of polyphyly are his sections *Pandaca* and *Tabernaemontana*. The sampled species of *T.* sect. *Pandaca* are distributed throughout the Malagasy and African clades of Paleotropical *Tabernaemontana* (Fig. 2), together with species from *T.* sects. *Bonafousia*, *Ervatamia*, *Pagiantha* and *Tabernaemontana*. Species from *T.* sect. *Tabernaemontana* are scattered across three major clades. The Neotropical species *T. alba*, *T. amygdalifolia*, and *T. citrifolia* form a strongly supported clade (PP 1.00) deeply nested within the Neotabernaemontana clade, whereas *Tabernaemontana calcarea*, *T. capuronii*, *T. coffeoides*, *T. mocquerysi*, *T. persicariifolia* and *T. stellata* (all restricted to Madagascar and the Mascarene islands), are included within the Malagasy clade and the two species from continental Africa, *T. elegans* and *T. letestui*, are included in the African clade (Fig. 2). Most of the sampled species from *T.* sect. *Ervatamia* form a strongly supported clade (PP 1.0) together with the three sampled species of *T.* sect. *Pagiantha*. Two species, however, were found to belong to other clades: *Tabernaemontana inconspicua* is part of the African clade, and *T. sessilifolia* is sister to a group of Malagasy species.

Based on our phylogeny, combined with morphological data, the tribe Tabernaemontaneae as delimited by Endress & Bruyns (2000) can be divided into two subtribes: the Tabernaemontaninae (which has already been published) and the Ambelaniinae. In addition, within the Tabernaemontaneae s.str. clade, *Stemmadenia* must be included in the synonymy of *Tabernaemontana*. The necessary taxonomic adjustments are presented below.

■ TAXONOMIC CONSEQUENCES

Tabernaemontaneae G. Don, Gen. Hist. 4: 70, 87. 1837–8 – Type: *Tabernaemontana* L., Sp. Pl. 1: 210. 1753. 16 genera.

Trees or shrubs, rarely lianas, with milky latex. Leaves opposite, sometimes anisophyllous. Inflorescences axillary, terminal, or (usually paired) in the forks of branches; calycine colleters mostly present and centered on the lower part of the sepal, often multiseriate, rarely absent, very rarely (*Tabernanthe*) alternisepalous; corolla usually salverform, less often tubular-campanulate or infundibuliform; corona absent; corolla lobe aestivation almost always sinistrorse (dextrorse in *Schizozygia*, 1 sp. of *Callichilia* and 2 spp. of *Tabernaemontana*); stamens mostly sessile or nearly so, anthers mostly with lignified guide-rails (these absent in some species of *Tabernaemontana*); style-head with the stigmatic region beneath a basal flange and usually with an (often five-ribbed) upper crest or the style-head subglobose and without a basal collar and upper crest and body uniformly receptive; ovary syncarpous or apocarpous; disc surrounding the base of the ovary, adnate, indistinct or free. Fruit with fleshy pericarp, usually an indehiscent berry with the seeds embedded in pulp or pair of dehiscent follicles with arillate seeds; seed testa often wrinkled, pitted or with longitudinal ridges, often with a long hilar groove. Pollen 3–5-colporate, sometimes zono-colporate; in *Callichilia* in tetrads. Secondary chemistry highly evolved indole alkaloids of the heynean type. (Description extracted from Simões & al., 2007.)

Subtr. Ambelaniinae (Pichon ex Boiteau & al.) A.O. Simões & M.E. Endress, **stat. nov.** ≡ Ambelanieae Pichon ex Boiteau & al. in Adansonia, sér. 2, 18(2): 276. 1978 – Type: *Ambelania* Aubl., Hist. Pl. Guiane: 265, t. 104. 1775.

Anthers with lignified guide-rails; style-head with the stigmatic region beneath a basal flange and usually with a five-ribbed upper crest; ovary syncarpous (apocarpous in *Macoubea*), 1- or 2-celled. Fruit an indehiscent berry with the seeds embedded in pulp; seed testa often ranging from smooth to tortuous and spongy, often without a long hilar groove. Seven genera, northern South America.

Ambelania Aubl., Hist. Pl. Guiane: 265, t. 104. 1775.

Macoubea Aubl., Hist. Pl. Guiane Suppl.: 17, t. 378. 1775.

Molongum Pichon in Mém. Mus. Natl. Hist. Nat. 24: 167. 1948.

Mucoa Zarucchi in Agric. Univ. Wageningen Pap. 87(1): 40. 1988.

Neocouma Pierre in Bull. Mens. Soc. Linn. Paris, sér. 2, 1: 33. 1898.

Rhigospira Miers, Apocyn. S. Amer.: 67, t. 10A. 1878.

Spongiosperma Zarucchi in Agric. Univ. Wageningen Pap. 87(1): 48. 1988.

Subtr. Tabernaemontaninae K. Schum. in Engler & Prantl, Nat. Pflanzenfam. 4(1): 145. 1891 – Type: *Tabernaemontana* L., Sp. Pl. 1: 210. 1753.

Anthers mostly with lignified guide-rails (absent in some species of *Tabernaemontana*); style-head with the stigmatic region beneath a basal flange and usually with an (often five-ribbed) upper crest or the style-head subglobose and without a basal collar and upper crest and body uniformly receptive; ovary apocarpous, more rarely hemisyncarpous or syncarpous. Fruit a pair of dehiscent follicles or berries, more rarely one, with arillate seeds; seed testa often wrinkled, pitted or with longitudinal ridges, often with a long hilar groove. Eight genera, paleotropical.

Callichilia Stapf in Thiselton-Dyer, Fl. Trop. Afr. 4(1): 130. 1902.

Calocrater K. Schum. in Engler & Prantl, Nat. Pflanzenfam. 4(2): 175. 1895.

Carvalhoa K. Schum. in Engler & Prantl, Nat. Pflanzenfam. 4(2): 189. 1895.

Crioceras Pierre in Bull. Mens. Soc. Linn. Paris 1: 1311. 1897.

Schizozygia Baill. in Bull. Mens. Soc. Linn. Paris 1: 752. 1888.

Tabernaemontana L., Sp. Pl. 1: 210. 1753.

Tabernanthe Baill. in Bull. Mens. Soc. Linn. Paris 1: 782. 1888.

Voacanga Thouars, Gen. Nov. Madagasc:. 10. 1806.

New combinations

Tabernaemontana abbreviata (J.F. Morales) A.O. Simões & M.E. Endress, **comb. nov.** ≡ *Stemmadenia abbreviata* J.F. Morales in Novon 9: 236. 1999 – Type: Costa Rica, Heredia, Finca La Selva, the OTS Field Station on the río Puerto Viejo just E of its junction with the río Sarapiquí, 13 Apr 1982, *Hammel 11680* (holotype, INB; isotype, DUKE).

Tabernaemontana allenii (Woodson) A.O. Simões & M.E. Endress, **comb. nov.** ≡ *Stemmadenia allenii* Woodson in Ann. Missouri Bot. Gard. 28: 461–462. 1941 – Type: Panama, Coclé, N of El Valle de Antón, 14 Jul 1940, *Allen 2187* (holotype, MO; isotypes, MICH, US, photo WAG).

Tabernaemontana brasiliensis (Leeuwenb.) A.O. Simões & M.E. Endress, **comb. nov.** ≡ *Stemmadenia brasiliensis* Leeuwenb., Revis. Tabernaemontana 2: 401, 403–405, f. 101, map. 54. 1994 – Type: Brazil, Pará, Oriximiná, 7 km N of Cachoeira Porteira, 20 Aug 1986, *C.A. Cid Ferreira & al. 7935* (holotype, INPA; isotypes, NY, WAG).

Tabernaemontana eubracteata (Woodson) A.O. Simões & M.E. Endress, **comb. nov.** ≡ *Stemmadenia eubracteata*

Woodson in Ann. Missouri Bot. Gard. 15(4): 368, t. 49, f. 2. 1928 – Type: Guatemala, Santa Rosa, Volcán Tecuamburro, Feb 1893, *Heyde & Lux 4538* (holotype, GH; isotypes, BM, F, K, M, US).

Tabernaemontana hannae (M. Méndez & J.F. Morales) A.O. Simões & M.E. Endress, **comb. nov.** ≡ *Stemmadenia hannae* M. Méndez & J.F. Morales in Candollea 60: 354, f. 8A. 2005 – Type: Costa Rica, Guanacaste: Hojancha, Nicoya, reserva forestal Monte Alto, río Nosara y quebrada Huacas, 19 Apr 2003 (fl., fr.), *J.F. Morales & M. Méndez 10339* (holotype, INB; isotypes, K, MO).

Tabernaemontana oaxacana (L.O. Alvarado-Cárdenas) A.O. Simões & M.E. Endress, **comb. nov.** ≡ *Stemmadenia oaxacana* L.O. Alvarado-Cárdenas in Candollea 62: 190–191. 2007 – Type: Mexico, Oaxaca, Dpto. Miahuatlán, 1 km al N de San J. Coatlán, cañada de San Jerónimo, 20 Apr 1990 (fl.), *A. Campos V., A. García M. & J. Reyes S. 3061* (holotype, MEXU; isotype, HT).

Tabernaemontana odontadeniiflora A.O. Simões & M.E. Endress, **nom. nov.** ≡ *Stemmadenia pubescens* Bentham, Bot. Voy. Sulphur: 125. 1845 ≡ *Bignonia (?) obovata* Hook. & Arn., Bot. Beechey Voy.: 439. 1841., nom. illeg., non Spreng. (1825) ≡ *Stemmadenia obovata* K. Schum. in Engler & Prantl., Nat. Pflanzenfam. 4(2): 149. 1895, nom. illeg., Art. 52 – Type: Mexico, San Luis Potosí, Realejo, fecha perdida, *Sinclair s.n.* (holotype, K, photo INB).

A new species name is necessary, since the name *Taberaemontana pubescens* R. Br. (1810) already exists. The species name chosen reflects the similar appearance of the large golden yellow infundibuliform corolla in this taxon to those of the genus *Odontadenia*.

Tabernaemontana pauli (Leeuwenb.) A.O. Simões & M.E. Endress, **comb. nov.** ≡ *Stemmadenia pauli* Leeuwenb., Revis. Tabernaemontana 2: 428–430, f. 107, photos 12–13, map 50. 1994 – Type: Costa Rica, Puntarenas, Reserva Florestal Golfo Dulce, península de Osa, cantón de Sierpe, cerca de Rancho Quemado, camino a Quebrada Quebradón, 6 Feb 1991, *P.J.M. Maas 7850* (holotype, U; isotypes, INB, MO, WAG).

Tabernaemontana robinsonii (Woodson) A.O. Simões & M.E. Endress, **comb. nov.** ≡ *Stemmadenia robinsonii* Woodson in Ann. Missouri Bot. Gard. 15: 369, f. 2A, 3B, 10A, 11A. 1928 – Type: Costa Rica, Limón, Valley of Yorquín, Talamanca Mts, *Pittier 8617* (holotype, US; isotypes, BR, CR, G, photo WAG).

Tabernaemontana simulans (J.F. Morales & Q. Jiménez) A.O. Simões & M.E. Endress, **comb. nov.** ≡ *Stemmadenia simulans* J.F. Morales & Q. Jiménez in Novon 9: 236, f. 11B, 12. 1999 – Type: Costa Rica, San José, Cantón de Puriscal, Parque Nacional La Cangreja, Faja Costeña del Valle de Parrita, Mastatal de Puriscal, Río Negro, 290–320 m, 3

Mar 1994, *J.F. Morales, D.A. Smith & G. Smith 2414* (holotype, INB; isotypes, B, BR, C, CAS, CR, F, G, GH, INB, K, MEXU, MICH, MO, NY, P, UPS, US, USF, W, WAG, WIS, Z).

Tabernaemontana stenoptera (Leeuwenb.) A.O. Simões & M.E. Endress, **comb. nov.** ≡ *Stemmadenia stenoptera* Leeuwenb., Revis. Tabernaemontana 2: 432, f. 108, map 54. 1994 – Type: Mexico, Colima, Mun. Manzanillo, Playa Miramar, 19 km NW of Manzanillo, 9 March 1981, *Lott & al. 359* (holotype, USF; isotypes, MO, WAG).

Tabernaemontana tomentosa (Greenm.) A.O. Simões & M.E. Endress, **comb. nov.** ≡ *Stemmadenia tomentosa* Greenm. in Proc. Amer. Acad. Arts 35: 310. 1900 – Type: Mexico, Jalisco, near Zapotlan, *Pringle 4370* (holotype, GH; isotypes, BM, BR, E, F, G, HBG, K, M, MO, MU, NY, P, S, UC, US, WU, Z, photo WAG).

Tabernaemontana venusta (J.F. Morales) A.O. Simões & M.E. Endress, **comb. nov.** ≡ *Stemmadenia venusta* J.F. Morales in Candollea 60: 360–361, f. 13, 14. 2005 – Type: Mexico, Oaxaca, Palomares, Juchitan, Montan de la Pedrera, 14–17 Apr 1970 (fl.), *MacDougall s.n.* (holotype, NY).

■ ACKNOWLEDGEMENTS

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Appendix. List of morphological and chemical characters and their states employed for ancestral state reconstruction. The character states are denoted as (0), (1) and (2).

1. Habit: (0) Trees and shrubs; (1) Lianas or vines; (2) Perennial herbs. **2. Phyllotaxis:** (0) Leaves opposite; (1) Leaves alternate; (2) Leaves mainly whorled, occasionally opposite at some nodes. **3. Inflorescence architecture:** (0) Lax; (1) Congested. **4. Inner surface of the calyx**: (0) naked; (1) with several to numerous colleters in one or more rows; (2) with five colleters quincuncially arranged.. **5. Corolla aestivation:** (0) Dextrorse; (1) Sinistrorse. **6. Corolla shape:** (0) Salverform; (1) Funnelform; (2) Campanulate. **7. Corolla consistency:** (0) Thin; (1) Thick. **8. Torsion of corolla tube:** (0) Not twisted; (1) Twisted. **9. Vertical ridges on corolla tube above insertion of stamens:** (0) Absent; (1) Present. **10. Corolla lobe tips in bud:** (0) Not inflexed; (1) Inflexed. **11. Anthers:** (0) Atop distinct filaments that arise from the corolla tube; (1) Sessile or nearly so on thickened ribs of the corolla tube. **12. Insertion of the stamens on the corolla tube:** (0) Lower third; (1) Upper third; (2) Middle. **13. Lignified guide rails:** (0) Absent; (1) Present. **14. Junction of filament and anther connection:** (0) Flat; (1) With a globose swelling. **15. Style-head body apex:** (0) Undifferentiated; (1) With un-lobed upper wreath; (2) with deeply 5- to 10-lobed upper crest.. **16. Style-head body base:** (0) Undifferentiated; (1) With membranous collar; (2) With thickened expanded flange. **17. Nectary:** (0) Indistinct or adnate to the outer wall of ovary, possibly absent; (1) Encircling base of carpels; (2) Two distinct lobes alternating with carpels.. **18. Ovary fusion:** (0) Congenitally syncarpous; (1) Hemisyncarpous; (2) Apocarpous. **19. Placentation:** (0) Parietal; (1) Marginal ; (2) Axile. **20. Ovules per carpel:** (0) 1–2(–10); (1) Numerous. **21. Fruit type:** (0) Follicular; (1) Drupaceous; (2) Baccate. **22. Fruit shape:** (0) Globose to subglobose; (1) Ovoid to ellipsoid; (2) Narrow and elongate. **23. Mesocarp consistency:** (0) Fleshy or spongy; (1) Dry or woody. **24. Epicarp surface:** (0) Smooth; (1) Warty or muricate. **25. Aril on seed:** (0) Absent; (1) Present. **26. Aril color:** (0) Orange to red; (1) White to cream; (2) Translucent. **27. Endosperm:** (0) Not ruminate; (1) Ruminate. **28. Deep hylar groove on seeds:** (0) Absent; (1) Present. **29. Indole alkaloids:** (0) Characteristic indole alkaloids present those with non-rearranged secologanin skeleton; (1) Characteristic indole alkaloids present including those with rearranged secologanin skeleton of the eburnan and/or plumeran type (2) Characteristic indole alkaloids present including those with rearranged secologanin part of the ibogan type.