POLYPHYLY OF THE GENUS *ECHITES* (APOCYNACEAE: APOCYNOIDEAE: ECHITEAE): EVIDENCE BASED ON A MORPHOLOGICAL CLADISTIC ANALYSIS

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ABSTRACT

A cladistic analysis was performed to test the monophyly of *Echites* (Apocynaceae: Apocynoideae: Echiteae). For the analysis 40 morphological characters were coded for 42 ingroup taxa (22 genera) and three outgroup species (two genera). The results indicate that *Echites* as currently circumscribed is polyphyletic. However, species that fall within the original descriptions of the two subgenera described in *Echites* form monophyletic clades.

KEY WORDS: *Echites*, *Prestonia*, *Thenardia*, and *Thevetia* Apocynaceae, cladistics, morphology, Neo-tropics

RESUMEN

Se realizó un análisis cladístico para comprobar la monofilia de *Echites* (Apocynaceae: Apocynoideae: Echiteae). Para el análisis se codificaron 40 caracteres morfológicos de 42 taxa del grupo (22 géneros) y tres especies como outgroup (dos géneros). Los resultados indican que *Echites* tal como se circunscribe normalmente es polifilético. Sin embargo, las especies que están en las descripciones originales de los dos subgéneros descritos en *Echites* forman clados monofiléticos.

Echites P. Browne was one of the first Neo-tropical Apocynaceae genera established. Consequently, it served as the focal point for the majority of species described in the Neo-tropics. Thus by the mid nineteen-hundreds there were about 375 species of *Echites* described. Woodson (1936) eventually cleared much of the nomenclatural confusion presented by the 300 plus names. In his monumental treatise, Woodson attributed the epithets to an appropriate genus (both newly described and preexisting) and or synonym, recognizing that the species of *Echites* sensu lato represented more than nine different genera. With a foundation established, Woodson (1936, 1938) defined *Echites* sensu Woodson by its twining habit, glabrous salverform corollas without corona, included anthers, and a solitary colleter opposite each of the five sepals. Woodson (ibid) ultimately recognized seven species in the genus that were placed into two subgenera.

Subgenus *Echites* is characterized by having corollas 5–8 cm long, oblique corolla lobes spreading at anthesis, and compact inflorescence with 3–7 flowers (Morales 1997; Williams 2002a). Today four species are recognized in subg.

Echites: E. darienensis J.F. Morales, *E. turrigera* Woodson, *E. umbellata* Jacq. and *E. yucatanensis* Millsp. (Morales 1997; Williams 2002a).

Subgenus *Psuedechites* Woodson is characterized by having corollas 1.25–2.5 m long, narrowly lanceolate corolla lobes that are reflexed at anthesis and a lax inflorescence with 8–20 flowers (Woodson 1936). Woodson included two species in the subgenus: *E. tuxtlensis* Standl. and *E. turbinata* Woodson. A third species of subg. *Pseudechites* was subsequently described by Monachino (1959): *Echites woodsoniana* Monac. This species would later come to have an intertwining history with *Prestonia* sect *Coalitae* (explained below).

Woodson (1936) divided *Prestonia* into four sections: *Coalitae* and *Acutifoliae*, (both characterized by having small and inconspicuous sepals similar to those of *Echites*); and *Annulares* and *Tomentosae* (both characterized by large foliaceous sepals). Woodson (1931, 1936) included *Prestonia agglutinata* (Jacq.) Woodson (=*Echites agglutinata* Jacq.) in *Prestonia* sect. *Coalitae*. Section *Coalitae* was distinguished from the other three sections of *Prestonia* by its lack of an annular corona at the mouth of the corolla. Woodson (1960) would later describe a second species in sect. *Coalitae* (*P. caudata* Woodson). Later, Gentry (1983) transferred *Echites woodsoniana* Monac. to *Prestonia* (*P. woodsoniana* (Monac.) Gentry) placing it as a member of sect. *Coalitae*. Because of the confusing nature of generic delimitation in the Apocynaceae, and the lack of an annular corona in *E. woodsoniana*, Gentry (ibid) was not confident of his transfer. J.K. Williams (1996) maintained *E. woodsoniana* in *Echites* because it lacked an annular corona at the mouth of the corolla. Morales (1997) would later include all species of *Prestonia* sect. *Coalitae* in *Echites* stating that the "narrowly elliptic to almost filiform corolla lobes [of the three species]... characterize *Echites* subg. *Pseudechites*".

The intermingling history of *Echites* subg. *Pseudechites* and *Prestonia* sect. *Coalitae* indicates the problems taxonomists have had in defining genera in the Apocynaceae. *Echites* subg. *Pseudechites* is a taxon that superficially resembles *Prestonia* section *Coalitae*, which make its placement within the family difficult.

A cladistic analysis using morphological characters was performed with two main objectives: 1) to assess the monophyly of *Echites* sensu Woodson and 2) to identify the placement and sister taxon of subg. *Pseudechites*.

MATERIALS AND METHODS

Taxa analyzed.—Included in this analysis are representative genera of Apocynoideae known from Mexico and Central America. In addition, Old World genera have been included in the study in order to expand the morphological variation and broad geographic range of the Apocynoideae. I did not intend to test the monophyly of the tribes recognized within the Apocynoideae, which is why a larger sampling of genera was not included. However, the genera that

were selected represent four (Apocyneae, Echiteae, Mesechiteae, and Wrightieae) of the five tribes in the Apocynoideae recognized by Endress & Bruyns (2000).

Thevetia L. and *Cerbera* L. were selected as outgroups for the analysis. In previous cladistic studies (Endress et al. 1996; Sennblad & Bremer 1996; Sennblad et al. 1998; Potgieter & Albert 2001) *Thevetia* was indicated as one of the closer relatives to the Apocynoideae, and is appropriate for rooting the tree.

Selection of characters.—A total of 45 taxa, representing 25 genera, were included in the present study. Forty characters, representing 105 character states (Table 1), were scored for every taxon presented in this analysis. Character states were selected from those utilized in previous studies (Struwe et al. 1994; Endress et al. 1996; Sennblad et al. 1998; Potgieter & Albert, 2001; Williams 2002b). New characters not included in the above works, but uncovered during the course of this study were also included. Fifteen of the characters were vegetative and the other 25 were floral or reproductive. Analysis indicates that none of the characters are uninformative. The characters and their rationale are discussed in Appendix 1.

Sampling.—Character measurements and states for the data matrix (Table 2) were obtained from herbarium sheets (specimens and label data) and field observations for every representative species included in this study except *Tintinnabularia gratissima* J.F. Morales, and four species of *Parsonsia* R. Br. (*P. heterophylla; P. latifolia* (Benth.) S.T. Blake; *P. praeruptis* Heads & de Lange; *P. purpurascens* J.B. Williams). Data for *T. gratissima* was obtained from Morales (1996). The species of *Parsonsia* were included in the study in order to better represent the diversity of *Parsonsia* (a genus with many superficial similarities to *Thenardia* H.B.K.). Morphological data for the four species of *Parsonsia* were obtained from literature descriptions (J.B. Williams 1996; Heads & de Lange 1998).

With the exception of the species of *Parsonsia*, and *Tintinnabularia gratissima*, a representative specimen is deposited at the Plant Resources Center (TEX) for each of the species examined in the morphological cladistic analysis. Further observations and data were collected from material borrowed from or observed at the following herbaria: BM, BRIT, CHAPA, F, FLAS, G, GH, K, MA, METPEC, MEXU, MO, NY, P, SHST, TAMU, TEX, US, WIS.

The pollen of all genera was studied using a light microscope as well as a scanning electron microscope (Philips 515). All genera were examined and measured under the SEM at the Cell Research Center of the University of Texas at Austin.

Cladistic analysis.—The characters and character states (Table 2) used in the analysis were entered into a data matrix using MacClade 3.0 (Maddison & Maddison 1992). A phylogenetic analysis was then performed in PAUP 3.1 (Swofford 1993). A heuristic search by stepwise addition of random trees was performed with 100 replicates and the ACCTRAN, MULPARS and TBR options

TABLE 1. Characters and character states used in the cladistic analysis.

1. Latex
   0-milky
   1-watery
2. Predominate growth habit
   0-woody shrub
   1-liana
   2-suffruticose herb
   3-herb
3. Leaf arrangement
   0-opposite
   1-alternate
4. Colleters around the stem
   0-absent
   1-present
5. Colleters at base of upper leaf blade surface
   0-absent
   1-present
6. Colleters along the upper leaf blade surface
   0-absent
   1-present
7. Leaves with domatia
   0-absent
   1-present
8. Secondary venation of leaves
   0-visible
   1-obsure
9. Tertiary venation of leaves
   0-visible
   1-obsure
10. Calyx size
    0-minute (0-3 mm)
    1-foliaceous (5-15 mm)
11. Calycine colleters
    0-absent
    1-numerous and alternate with the sepals
    2-solitary and opposite the sepals
12. Aestivation
    0-sinistrorse
    1-dextrorse
    2-valvate
13. Corolla shape
    0-salverform
    1-urceolate
    2-infundibuliform
    3-rotate

14. Corolla color
    0-white
    1-yellow
    2-maroon
15. Corolla with epistaminal appendages
    0-absent
    1-reduced to a callused ridge
    2-extended into a linear protuberance resembling a filament
16. Corona between petal sinuses
    0-absent
    1-present
17. Infrastaminal appendages
    0-absent
    1- present
18. Corolla tube size
    0-minute (1–4 mm)
    1-small (6–10 mm)
    2-medium (11–20 mm)
    3-large (21–50 mm)
19. Filaments
    0-minute (0–1 mm)
    1-medium (3–6 mm) and running along the style
    2-long (10 mm and greater) and separate from the style
20. Anthers from ribs
    0-no
    1-yes
21. Stamen exposure
    0-included
    1-anther tips exserted
    2-stamens fully exserted
22. Anthers with apical appendages
    0-absent
    1-present
23. Anther dehiscence
    0-introrse
    1-latrorse
24. Anther morphology
    0- Connective enlarged, theca displaced laterally
    1- Connective not enlarged, theca not displaced, bases rounded and sterile
    2- Connective not enlarged, theca not displaced, bases forked sterile
    3- Connective not enlarged, theca not

displaced, bases rounded, anthers uniformly fertile
25. Anther-style head relationship
 0-anthers free from style head
 1-anthers fused to style head
26. Pistil head
 0- Pistil head short, pentagonal; *Thevetia*-type
 1- Pistil head elongated pentagonal; *Mandevilla*-type
 2-Pistil head fusiform; *Echites*-type
27. Nectary
 0-absent
 1–5 free nectaries
 2-nectaries fused into a cup, *Echites*-type
 3-nectaries fused into a cup *Thevetia*-type
28. Inflorescence position
 0-axillary
 1-terminal
29. Inflorescence morphology
 0-raceme
 1-corymbose
 2-reduced cyme
30. Inflorescence branching
 0-absent
 1-present
31. Fruit type
 0-linear follicle (2–15 mm in diameter)
 1-robust follicle (30–60 mm diameter)
 2-drupe
32. Follicle orientation
 0-Two follicles developing from one flower, both spreading
 1-Two follicles developing from one

flower, both fused only at the apical tips
 2-Two follicles developing from one flower, both fused throughout entire length
 3-One follicle developing from one flower
33. Follicles moniliform
 0-no
 1-yes
34. Follicle color
 0-tan
 1-red
 2-black
35. Fruit texture
 0-herbaceous
 1- woody
 2-leathery
36. Seeds with coma
 0-absent
 1-present and sessile
 2-present and rostrate
37. Pollen apertures
 0-tricolporate
 1-triporate
38. Exine pattern
 0-smooth
 1-microreticulate
39. Pollen shape
 0-spherical
 1-triangular
40. Pollen diameter
 0–20–35 μm
 1–40–75 μm
 2–75–110 μm

in effect. The option for maximum trees stored was set at 10,000. Taxa with multi-state characters were recognized as polymorphic for those characters. Characters were treated as unordered and of equal weight. At the end of the analysis the stored trees were rooted, with both the outgroup and ingroup directed as monophyletic. A strict consensus (Fig. 1) and a majority rule consensus (Fig. 2) tree of the stored trees were produced. Bootstrap values were calculated using 1,000 replicates with the PAUP settings at: full heuristic search, starting trees obtained via stepwise addition, random search set for additional sequences with 10 replicates, branches collapse if maximum branch length is zero, include groups compatible with 50% majority-rule consensus, include only informative characters. The majority rule tree (Fig. 2) is presented along with bootstrap values near or higher than 50%.

TABLE 2. Data matrix of the 40 informative characters used in the phylogenetic analysis[a,b] presented in this study.

Species	Character number and character states			
	0000000091 1234567890	11111111112 1234567890	2222222223 1234567890	3333333334 1234567890
Adenium obesum	0010000000	112b010200	0102120110	000001000?
Angadenia berterii	0101000000	1121000200	0002122010	0100120001
Apocynum cannabinum	0300000000	0110010000	0002121111	0000010000
Cerbera odollam	0010000111	1000101201	0010203111	2—1201112
Echites agglutinata	1100000010	2101000100	0002121011	0110020000
Echites turbinata	1100000110	2101300110	0002121011	0110020000
Echites turrigera	0100000000	2100000200	0001121010	0100120001
Echites umbellate	0100000000	2100000200	0001122010	0000120001
Echites woodsoniana	1100000110	2101000100	0002121011	0110020000
Echites yucatanensis	0100000000	2100000200	0001122010	0000120001
Fernaldia pandurata	0100000000	2120000200	0001121010	0100120001
Forsteronia acouci	0100101000	1130000010	2001121111	0000110000
Forsteronia myriantha	0100101001	1130000020	2001121111	0000110000
Forsteronia peninsularis	0101001001	1130000020	2002121111	0000110000
Forsteronia spicata	0100100001	1130000020	2001121111	0200110000
Laubertia contorta	1100000000	0102310210	1002121010	0110010001
Mandevilla acutiloba	0101100000	1101000100	0003111000	0100010001
Mandevilla foliosa	0201100000	1101000100	0003111000	0110010001
Mandevilla hirsuta	0101110001	1121000200	0003111000	0110010002
Mandevilla subsagittata	0101110000	1101000200	0003111000	0110010002
Mesechites trifida	0101100000	1101000200	0003111011	0110010001
Nerium oleander	0000000000	112b010200	0102120110	0100010000
Odontadenia macrantha	0100000001	1121000200	0002121010	1300120001
Parsonsia latifolia	0101000000	3230000010	2002121111	0200110000
Parsonsia heterophylla	1101000000	323a000000	0002121a11	0200111000
Parsonsia praeruptis	1001000110	3230000000	2002121a11	0200110000
Parsonsia purpurascens	1101000000	3231000000	0002121111	0200010000
Parsonsia straminae	1101000000	3231000000	1002121a11	0200110002
Pentalinon andrieuxii	0101000000	1121000200	0102122010	0100120001
Prestonia acutifolia	0101000000	2101210110	1002121011	0100110001
Prestonia mexicana	0101000001	2101310210	1002121011	1000110002
Prestonia tomentosa	0101000001	2101210210	1002121011	1000110001
Prestonia portobellensis	0101000001	2102210210	1002121011	0100110002
Rhabdadenia biflora	0100000001	0120000200	0001121020	0000110002
Strophanthus kombe	0100000000	1120010200	1002120111	000001000?
Telosiphonia brachysiphon	0200100001	1120000300	0003111120	0100010001
Thenardia chiapensis	1100000010	2130000010	2002121011	0210010000
Thenardia floribunda	1100000010	2130000010	2002121011	0210010001
Thoreauea paneroii	0100000000	2110010010	0002121011	0??0?10000
Thevetia ovata	0010000011	1021101201	0010203111	2—2201112
Thevetia ahouai	0010000111	1001101201	0010203111	2—1201112
Tintinnabularia mortonii	0101101001	1121000220	0103111011	0??0?10001

Table 2. (continued)

Species	Character number and character states			
	0000000091 1234567890	1111111112 1234567890	2222222223 1234567890	3333333334 1234567890
Tintinnabularia gratissima	0101101001	1121000200	0003111011	0110?1000?
Tintinnabularia mullaraensis	0101101000	1121000220	1103111011	0??0?10001
Trachelospermum difforme	0101000000	1100000100	0002121111	0000?10000

a Character numbers and character states correspond to those in Table 1.
b Polymorphic character states are represented by letters as follows: a=0,1; b=0,3; (within the data matrix character states for polymorphic characters were entered as 0/1 etc. Letters are used here for the convenience of aligning the table).

RESULTS

The data matrix (Table 2) of 45 taxa and 40 characters included no characters that were uninformative. Of the 1800 cells in the matrix 14 (.77%) were scored with a question mark for unknown character states. The data matrix included six characters coded as polymorphic constituting .33% of the entries.

The heuristic search yielded a total of 48 equally parsimonious trees of 159 steps and a consistency index (C.I.) of 0.434 and a retention index (R.I.) of .767. The low consistency index reflects the high level of homoplasy (Kitching et al. 1998) within the characters selected. The high level of homoplasy probably also accounts for the lower bootstrap values in the basal branches (Fig. 2), thereby reducing stability in the basal clades. Stability is seen in the terminal branches, which is reflected by the higher bootstrap values (Fig. 2). The discrepancy in support for the basal clades versus the terminal clades is acceptable considering that the main focus of this study was to test the monophyly of genera in the Apocynaceae, in particular *Echites*.

The ingroup taxa formed two large clades. The first large clade, Clade I, is comprised of two clades. In the first of these, the Wrightieae clade is sister to two subclades: one is represented solely *Apocynum* (Apocyneae); the other, the *Prestonia* subclade, is comprised of three smaller subclades of genera from Echiteae, in which the genus *Thoreauea* is sister to the other two; one of these is a subclade composed of *Echites* subgen. *Pseudechites* and the two species of *Thenardia*; the other subclade is composed of *Laubertia* and *Prestonia*. The second group of clades in Clade I is comprised of the genus *Trachelospermum*, which is sister to two subclades: the *Forsteronia* subclade, and the *Parsonsia* subclade, which are comprised solely of these two genera, respectively, the first of which is in Apocyneae and the latter in Echiteae.

In Clade II, *Rhabdadenia* is sister to two clades. The first, the *Echites* clade, includes a subclade of *Angadenia* and *Pentalinon* (Echiteae) and *Odontadenia*
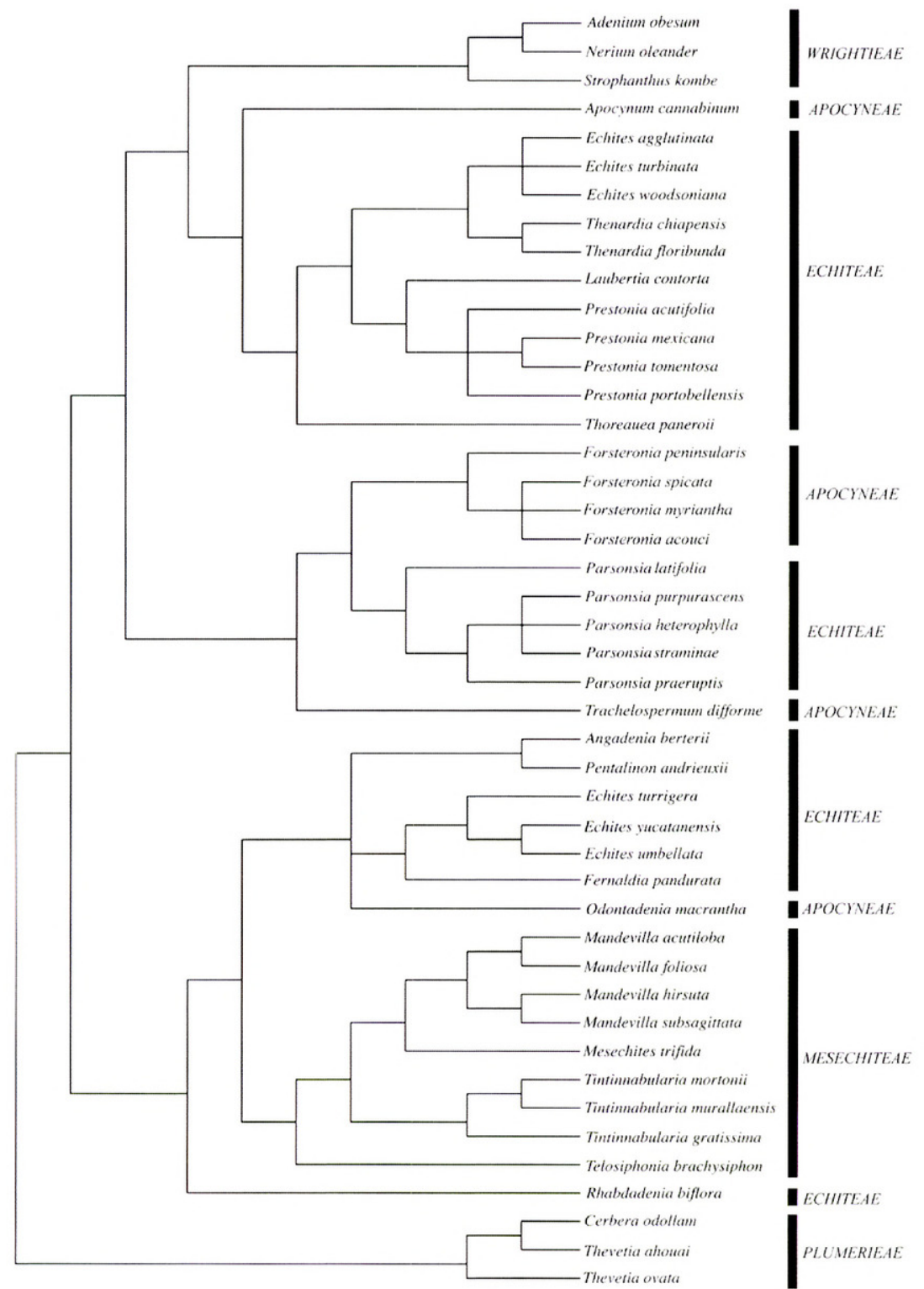
Fɪɢ. 1. Strict consensus tree. Taxa in capital letters and to the right of the cladogram indicate Tribes recognized in Endress & Bruyns (2000). Note: *Thoreauea* (Williams, 2002b) was described after Endress & Bruyns (2000) and therefore not included in their treatment. However, *Thoreauea*, as discussed in Williams (2002b), corresponds to the description of the Echiteae and is included in this tribe
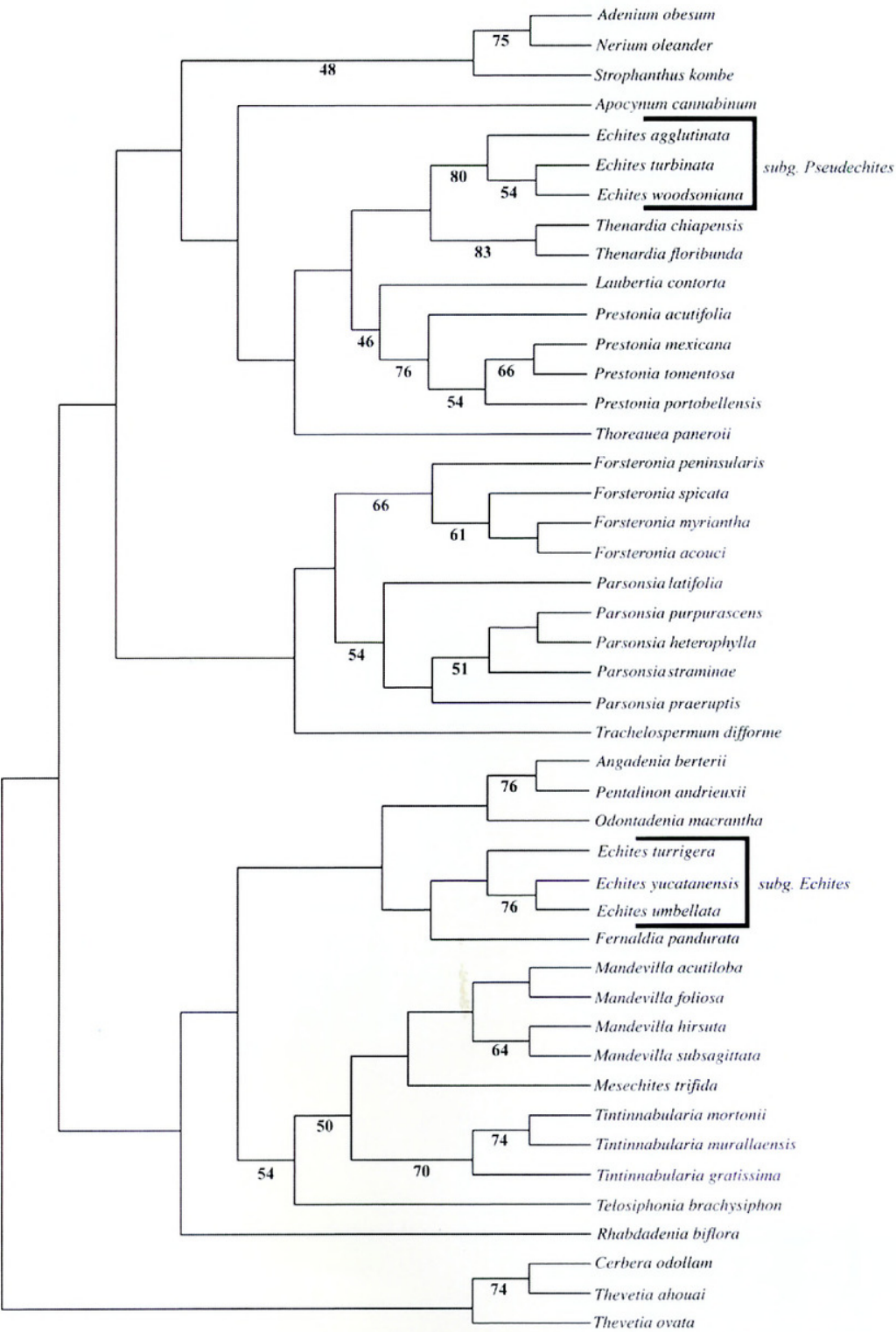
Fig. 2. Majority rule consensus tree calculated from 48 most parsimonious trees (length 165, CI =.434, RI=.767, RC = .333). Numbers below the branches are bootstrap values near or greater than 50%.

(Apocyneae), which is sister to a clade comprised of *Fernaldia* and *Echites* subgen. *Echites* (all Echiteae). The second main clade, the Mesechiteae clade, is composed of representatives of *Mandevilla*, *Mesechites*, *Tintinnabularia* and *Telosiphonia*. Comparing the results obtained here to the latest classification of the family by Endress and Bruyns (2000), the Wrightieae and Mesechiteae are supported as monophyletic, whereas the Apocyneae and Echiteae are polyphyletic.

DISCUSSION

Monophyly of Echites.—The results presented here indicate that *Echites*, as currently circumscribed, is not monophyletic. The placement of subg. *Pseudechites* in a clade both distant and distinct from subg. *Echites* renders *Echites* polyphyletic. This result was not unexpected, since the taxa of subg. *Pseudechites* are morphologically distinct from subg. *Echites* in at least thirteen observable characters (Table 3; three vegetative and 10 floral or reproductive). However, the species that fall within the original description of subg. *Pseudechites* form a well supported (bootstrap 80%) monophyletic clade.

Because of the polyphyletic nature of *Echites* sensu Woodson, based on the number of differences between subg. *Echites* and subg. *Pseudechites*, and the strongly supported monophyletic clade of subg. *Pseudechites*, it is suggested that the species of subg. *Pseudechites* be transferred to a genus separate from *Echites* sensu stricto The new genus and appropriate name combinations are proposed in Morales & Williams (2004).

Phylogeny of subg. Pseudechites.—Both the strict (Fig. 1) and the majority rule (Fig. 2) trees show subg. *Pseudechites* sister to *Thenardia*. A relationship between *Thenardia* and subg. *Pseudechites* was suggested in Williams (1998). The taxa share in common watery sap, leaves with inconspicuous secondary veins, and pollen of similar size. It should be noted however, that despite the similarities in morphology the bootstrap support for the clade comprising *Thenardia* and subg. *Pseudechites* is low (37%). Nevertheless, based on previous observations (Williams 1998) and the data presented here, the hypothesized relationship between *Thenardia* and subg. *Pseudechites* appears relatively sound.

Monophyly and phylogeny of subg. Echites.—Both the strict (Fig. 1) and the majority rule (Fig. 2) trees show subg. *Echites* sister to *Fernaldia* Woodson. There is only one major character difference between subg. *Echites* and *Fernaldia* (salverform vs. infundibuliform corollas; Table 3). It was hypothesized before the analysis that *Fernaldia* might branch with *E. turrigera* Woodson, due to their similar fruit types (follicles fused at the apex; character 32:1) rendering subg. *Echites* paraphyletic. Indeed, bootstrap support (Fig. 2) is 76% for the two subg. *Echites* species with spreading follicles (*E. umbellata* and *E. yucatanensis*) while support for the branch basal to the subg. *Echites* clade is below 50%. Regardless,

TABLE 3. Morphological comparisons of *Echites* subg. *Echites*, subg. *Pseudechites* and *Fernaldia*.

	subg. *Pseudechites*	subg. *Echites*	*Fernaldia*
Latex	Watery	Milky	Milky
Secondary veins visible	No/yes	Yes	Yes
Tertiary veins visible	No	Yes	Yes
Inflorescence branched	1–3x	0–1x	0x
Corolla	Salverform	Salverform	Infundibuliform
Corolla length	4–9 mm	30–70 mm	35–50 mm
Corolla color	Yellow	White	White
Anther length	3–5 mm	5–9 mm	5–9 mm
Anther bases	Sagittate	Obtuse	Obtuse
Corolline corona			
behind the anthers	Yes/No	No	No
Follicles fused at apex	Yes	No/Yes	Yes
Pollen diameter	25–30 μm	45–50 μm	45–50 μm
Pollen aperture diameter	3–4.5 μm	5–8 μm	5–8 μm

as presented here both the strict and majority rule indicate subg. *Echites* to be monophyletic.

   Tribal and other generic circumscriptions.—As stated in the "taxa analyzed" section it was not the intention of this study to test the monophyly of the tribes recognized by Endress & Bruyns (2000). However, the results presented in the strict consensus trees (Fig. 1) indicate that the tribes Mesechiteae and Wrightieae sensu Endress & Bruyns are monophyletic (each with bootstrap support near or over 50%; Fig. 2) and that the tribes Apocyneae and Echiteae sensu Endress & Bruyns are polyphyletic (Fig. 1). Furthermore the results presented suggest that *Thevetia* is not monophyletic, supporting Potgieter and Albert (2001). However, the results here show *Thevetia* to be paraphyletic, while Potgieter and Albert (ibid) show *Thevetia* to be polyphyletic. The discrepancy in results is due in large part to the much larger sampling of taxa in the Rauvolfiodeae by Potgieter and Albert (2001).

   Despite the evidence indicating polyphyly in Apocyneae and Echiteae sensu Endress & Bruyns and paraphyly in *Thevetia*, tribal and generic reconstruction in these taxa is beyond the scope of the present paper and thus the discussion is left to further study and evaluation.

APPENDIX 1

Discussion of the characters utilized in the morphological cladistic analysis of the Apocynaceae. Characters in bold indicate newly uncovered and utilized characters during this study, and have lengthier discussions. The character number is given in parenthesis and corresponds to the character and character states in Table 1.

Latex (1). This character has not been utilized or discussed as a character for cladistic studies in the Apocynaceae. However, field observations of most of the genera of Echiteae presented in this work

indicate that some taxa consistently have watery sap, *Laubertia*, *Echites* subg. *Pseudechites*, and *Thenardia*, versus the typical milky sap typical of most Apocynaceae. *Parsonsia*, which has approximately 40 species, is polymorphic for this character.

Predominate growth habit and leaf arrangement (2–3, respectively). These two characters were utilized in three previous cladistic studies (Struwe et al. 1994; Endress et al. 1996; Potgieter and Albert, 2001). All of the genera of the Apocynoideae included in this study have opposite leaves (whorled in *Nerium*), except *Adenium*.

Colleters (4–6 & 11). Thomas and Dave (1991) provided a discussion of the systematic implications of colleters in the Apocynaceae that will not be repeated here. Endress et al. (1996) and Sennblad et al. (1998) utilized calycine colleters in their studies (character 12 here). I have expanded the use of colleters by including the presence or absence of colleters on other parts of the plant. Character 4, colleters around the stem; Character 5, colleters present on the apex of the leaf petiole of the upper leaf surface, appears to be convergent as it is shared by members of the "*Mandevilla*" clade and the distantly related *Forsteronia*. Character 6, colleters along the upper leaf midrib are only possessed by two species of *Mandevilla* studied here. This character is a synapomorphy which unites *M. subsagittata* and *M. hirsuta*.

Domatia (7). Domatia are only present in two of the genera studied here, *Tintinnabularia* and *Forsteronia*. This character appears convergent, haven arisen in two separate clades.

Venation (8 & 9). Distinctness of the secondary venation of leaves and tertiary venation of leaves has not been utilized in a morphological analysis. Observation in the field coupled with herbarium studies indicates that certain genera have inconspicuous lateral venation. The lack of secondary veins is a character uniting *Thevetia ahouai* with *Cerbera*. The lack of tertiary veins is a character that unites *Thenardia* and *Echites* subg. *Pseudechites*.

Calyx size (10). The majority of taxa in the Apocynoideae have sepals 1–3 mm long, a few have sepals much larger, 5–15 mm. Overall this character is highly variable with large sepals occurring randomly throughout the representative taxa. However, large sepals appear to unify a few of the species of *Prestonia*.

Aestivation (12). With the exception of *Parsonsia* (valvate), dextrorse aestivation is present in all of the taxa in the Apocynoideae included in this study. Aestivation type is one of the few synapomorphies that distinguishes the Apocynoideae from the Rauvolfioideae (sinistrorse aestivation).

Corolla shape ,**color and size**(13, **14**, **18**). Corolla shape was utilized by Endress et al. (1996), color and tube size are new characters. Most of the taxa presented in this work have yellow or white corollas. However, some have maroon corollas. Color was used because *Laubertia* and *Prestonia portobellensis* possess maroon corollas. Consequently the character was utilized to test if the species paired, thereby testing the monophyly of *Prestonia*. In addition, the two subgenera of *Echites* have different corolla colors. In this instance color was utilized to test the monophyly of *Echites*. Corolla tube size is added as an augment to the variability that exists in corolla shape. Utilization of tube size helps to emphasize that although both subg. of *Echites* have salverform corollas, there exists a considerable difference in the lengths of the corollas.

Corolla with epistaminal appendages, corona between petal sinuses, corolla with infrastaminal appendages (15, 16, 17). These three characters were utilized and discussed by Endress et al. (1996).

Filament length (19). An examination of the filaments of the taxa in this analysis indicates that filament length appears to be positively correlated with generic relationships. Short filaments are typical of the taxa in the subg. *Echites* clade (Fig. 1), while medium length filaments are consistent with the "*Prestonia*" clade (Fig. 1). Long filaments are only present in two of the three species of *Tintinnabularia*. In this instance the character was utilized to test the monophyly of *Tintinnabularia*.

Anthers from ribs, stamen exposure, and anther dehiscence (20, 21, 23). Anther ribs are only present in *Thevetia* and *Cerbera* and are used mainly to establish the monophyly of the outgroup. Anther exposure and dehiscence were utilized and discussed in Endress et al. (1996).

Apical appendages on anthers (22). It was presumed before the analysis was conducted that this character was highly convergent. However, it was included as a reference for testing the monophyly of *Tintinnabularia*, which has two species with and one species without elongate apical anther appendages.

Anther morphology, anther-pistil head relationship, pistil head type (24, 25, 26). At least five different types of anthers and pistil heads are exhibited in the Apocynaceae. The different anther and pistil head types have been discussed in Woodson (1930) and Fallen (1986). The important traits that characterize the different anther types are the connective, theca positioning and fertility and the base of the anther body. The union of the anthers and the pistil head is a synapomorphy that unifies the Apocynoideae.

Nectary (27). Several types of nectaries are exhibited by the Apocynaceae. Three different types are here recognized: five free nectaries, nectaries fused into a cup, and nectaries fused into a cup type two. Type two refers to the nectaries of *Thevetia* and *Cerbera*, that are twice as large and twice as wide as those found in the Apocynoideae.

Inflorescence position, inflorescence morphology, inflorescence branching (28, 29, 30). The structure of the inflorescence has not been used in a morphological cladistic analysis of the Apocynaceae. Woodson (1935) has provided a detailed account of the inflorescence types in the Apocynaceae that will not be repeated here. Within the taxa examined only the Wrightieae and Apocyneae (*Apocynum*) have terminal inflorescences, with all of the Echiteae possessing an axillary inflorescence. Inflorescence branching is one of the characters separating subg. *Echites* (not branched) from subg. *Pseudechites* (branched).

Fruit type, follicle orientation, follicles moniliform, follicle color, fruit dehiscence, **fruit texture** (31–36). Of the above characters, only fruit dehiscence has been utilized in a cladistic study (Endress et al. 1996). Fruits have been an underutilized resource in the systematics of the Apocynaceae. This is mainly due to the paucity of fruiting herbarium specimens. Collecting trips were made by the author specifically in the latter part of the flowering season, for the purpose of collecting fruits. From these observations, a pattern emerged. Many of the taxa with presumed relationships had similar fruit types. Characters observed were the union of the follicles, fused at apex, follicles spreading, or fused throughout. The fusion of the follicles, is a useful character in distinguishing species within genera (e.g. *Echites*), but overall the cladistic analysis indicated that follicle union is a convergent character, with spreading and fused follicles occurring throughout the Apocynoideae. In addition, follicle texture was noted. Some follicles were membranous while others were firm and woody. This character was useful in indicating *Echites* as polyphyletic (subg. *Echites* with woody follicles and subg. *Pseudechites* with herbaceous follicles). Lastly, it was noticed that some taxa had straight follicles and others were moniliform. Moniliform follicles occur more frequently in the "*Prestonia*" clade. Fruit color was used to test the monophyly of *Thevetia*. This character is a synapomorphy uniting *T. ahouai* and *Cerbera*, indicating that *Thevetia* as currently circumscribed is paraphyletic.

Seeds with coma (37). This character was utilized by Endress et al. (1996) and by Potgieter and Albert (2001) and subsequently discussed by them.

Pollen apertures, pollen exine pattern, **pollen shape, pollen size** (39–42). Pollen apertures and exine pattern were utilized in Endress et al. (1996). The Rauvolfioideae and the Apocynoideae are distinguished by the apertures of the pollen, with tri-porate pollen as a synapomorphy uniting the Apocynoideae. In addition, the pollen of taxa in the Apocynoideae is consistently smooth vs. the Rauvolfioideae which has various exine patterning. Pollen shape, also helps to distinguish the Rauvolfioideae from the Apocynoideae. In general, the Rauvolfioideae have triangular-rounded pollen vs. the Apocynoideae that are consistently spherical. Pollen size was useful for determining intergeneric and intrageneric relationships. For instance, pollen size supports *Echites* as polyphyletic (subg. *Echites*, 45–60 μm vs. subg. *Pseudechites*, 23–35 μm). In addition, within *Mandevilla*, pollen size indicated *M. hirsuta* and *M. subsagittata* to be closely related.

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