
PHYLOGENETIC PLACEMENT OF
THE TRIBE RETINIPHYLLEAE
AMONG THE SUBFAMILY
IXOROIDEAE (RUBIACEAE)¹

Rocio Cortés-B.,^{2,5} Piero G. Delprete,³ and
Timothy J. Motley^{4,5}

ABSTRACT

The tribe Retiniphyllae and its single genus *Retiniphyllum* Bonpl. contains 22 species of shrubs and small trees that grow in white sand soils mostly in the Guayana Region of South America. The circumscription of the tribe is based on the diagnostic characteristic of two collateral and pendulous ovules per locule, a rare condition in the Rubiaceae. However, for the same reason, its placement within the family has been controversial. The monophyly and systematic position of the tribe Retiniphyllae and *Retiniphyllum* were tested based on a phylogenetic analysis of *trnL-F* and *rps16* sequence data. The results confirm the monophyly of the tribe and genus *Retiniphyllum*. The tribe is placed sister to the core members of the subfamily Ixoroideae s. str. The genera *Botryarrhena* Ducke and *Scyphiphora* C. F. Gaertn. are not related to the tribe Retiniphyllae, as formerly hypothesized.

Key words: *Botryarrhena*, Guayana Region, Ixoroideae, Retiniphyllae, *Retiniphyllum*, *rps16*, Rubiaceae, *Scyphiphora*, *trnL-F*.

The tribe Retiniphyllae includes only the genus *Retiniphyllum* Bonpl. This genus consists of 22 species of shrubs and small trees that grow on white sand soils in the Neotropics. Most species are distributed in the Guayana Region, and a few reach the Amazon Basin, eastern Andes, and central and eastern Brazil. The Retiniphyllae consists of shrubs or trees characterized by the abundant resin located at apical buds. Each flower is subtended by a bracteole located at the base of the pedicel and an involucrel (calyculus) located at the top of the pedicel. Flowers have corollas with contorted aestivation, stamens reflexed in anthesis, anthers with basal and apical sterile appendages, a (4 to)5(to 6 to 8)-locular ovary

with two collateral pendulous ovules per locule, drupaceous fruits, and pyrenes normally containing one seed due to the abortion of one ovule. In addition, many species exhibit secondary pollen presentation. Some of these characters are not common in the Rubiaceae, especially the condition of two ovules per locule. In this family, most members have one or many ovules per locule. As a consequence, the tribe Retiniphyllae has been clearly defined and isolated in the family, but for the same reason, its placement within the Rubiaceae has been controversial.

In the earliest systems of classification (Kunth, 1818; Roemer & Schultes, 1818; Jussieu, 1820; Richard, 1830; de Candolle, 1830; Bentham, 1841),

¹ We are grateful to Conciencias-Fulbright-LASPAU, Lehman College, and The New York Botanical Garden for supporting the first author during her doctoral studies at the City University of New York; The Lewis B. and Dorothy Cullman Foundation for supporting the work in the laboratory; the International Association for Plant Taxonomy for partially supporting fieldwork; directors of the herbaria BM, BR, C, COAH, COL, F, G, GH, GUYN, K, M, MO, NY, R, U, UDBC, US, VEN, and W for making their specimens available; E. J. Gouda (Utrecht University, The Netherlands), A. Vogel (Botanical Garden, Leiden University, The Netherlands), T. Nanderborcht, and E. Robbrecht (National Botanical Garden, Meise, Belgium) for help in obtaining fresh leaves at the living collections of their institutions; and G. Aymard, P. Berry, R. Evans, and S. Mori for sharing their field observations, specimens, and photographs. This research was undertaken during a fellowship for Visiting Scientist to Piero Delprete from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). A travel grant for the presentation of this work at the Third International Rubiaceae Congress (18–21 September 2006, Katholieke Universiteit, Leuven, Belgium) was provided by the Fund for Scientific Research–Flanders (FWO N. WO.005.05) and the Laboratory of Plant Systematics of Katholieke Universiteit, Leuven, Belgium.

² Herbario Forestal, Universidad Distrital, Campus El Vivero, Avenida Circunvalar–Venado de Oro, Bogotá, Colombia. retiniphyllum@yahoo.com.

³ CNPq Visiting Scientist, Institute of Biological Sciences (ICB-1), Department of General Biology/Botany, Universidade Federal de Goiás, Campus II, 74001-970 Goiânia, Goiás, Brazil. Current address: Institut de Recherche pour le Développement, Botanique et Bioinformatique de l'Architecture des Plantes (AMAP), TA-A51/PS2, Blvd. de la Lironde, 34398 Montpellier Cedex 5, France. pdelprete@hotmail.com.

⁴ Department of Biological Sciences, Old Dominion University, Norfolk, Virginia 23529-0266, U.S.A. tmotley@odu.edu.

⁵ Lewis B. and Dorothy Cullman Program for Molecular Systematics Studies, The New York Botanical Garden, Bronx, New York 10458-5126, U.S.A.

doi: 10.3417/2006198

Table 1. Taxa sampled for the phylogenetic analyses of the *trnL-F* spacer and the *rps16* intron data sets.

Taxa	Voucher	GenBank accession number	
		<i>trnL-F</i>	<i>rps16</i>
<i>Aleisanthia rupestris</i> Ridl.	<i>Tange 45171</i> (AAU)	AF152660 ³	—
<i>Alibertia edulis</i> (Rich.) A. Rich. in DC.	<i>Jansen-Jacobs 3840</i> (GB)	AF201029 ¹	—
<i>Alibertia edulis</i> (Rich.) A. Rich. in DC.	<i>Rova 2288</i> (GB)	—	AF200975 ¹
<i>Amphidasya colombiana</i> (Standl.) Steyerm.	<i>Stähl et al. 3542</i> (GB)	AF152624 ³	—
<i>Amphidasya ambigua</i> (Standl.) Standl.	<i>taxon 61933</i>	—	AF129271 ⁷
<i>Borojoa patinoi</i> Cuatrec.	<i>Persson et al. 2194</i> (GB)	AF201034 ¹	AF200984 ¹
<i>Botryarhena pendula</i> Ducke	<i>Campos 29</i> (NY)	EU821638	—
<i>Calochone redingii</i> (De Wild.) Keay	<i>Chase 3355</i> (K)	AF201036 ¹	AF200986 ¹
<i>Calycophyllum spruceanum</i> (Benth.) Hook. f. ex K. Schum.	<i>Hatschbach 62777</i> (NY)	AY555080 ²	EU821613
<i>Ceriscoides sessiliflora</i> (Wall. ex Kurz) Tirveng.	<i>Maxwell 87-967</i> (AAU)	AF201039 ¹	AF200989 ¹
<i>Cinchona pitayensis</i> Wedd.	<i>Andersson et al. 2109</i> (GB)	AF152684 ³	—
<i>Cinchona pubescens</i> Vahl	<i>taxon 50278</i>	—	AF004035 ⁶
<i>Coffea liberica</i> Hiern	<i>Delprete 7357</i> (NY)	AY555081 ²	EU821614
<i>Condaminea corymbosa</i> (Ruiz & Pav.) DC.	<i>Rova et al. 2084</i> (S)	AF102406 ⁵	—
<i>Condaminea corymbosa</i> (Ruiz & Pav.) DC.	<i>taxon 60042</i>	—	AF004039 ⁶
<i>Duprerrea pavettifolia</i> Pit.	<i>Delprete 7373</i> (NY)	AY555082 ²	EU821615
<i>Fadogia audruana</i> J. M. Fay, J.-P. LeBrun & Stork	<i>Fay 8901</i> (NY)	EU821639	EU821616
<i>Ferdinandusa</i> Pohl sp.	<i>Alves 2267</i> (NY)	AY555083 ²	EU821617
<i>Feretia aeruginescens</i> Stapf	<i>Muanyambo 154</i> (NY)	AY555084 ²	EU821618
<i>Gardenia taitensis</i> DC.	<i>Struwe & Albert 1208</i> (NY)	AF102426 ⁵	—
<i>Gardenia volkensii</i> subsp. <i>spatulifolia</i> Stapf & Hutch.	<i>Rova T011</i> (GB)	—	AF200996 ¹
<i>Genipa americana</i> L.	<i>Delprete 6522</i> (NY)	AF152665 ³	—
<i>Genipa americana</i> L.	<i>Persson & Gustafsson 342</i> (GB)	—	AF200997 ¹
<i>Hippotis brevipes</i> Spruce ex K. Schum.	<i>Woytkowski 5620</i> (NY)	AF152636 ³	—
<i>Hippotis scarlatina</i> Krause	<i>taxon 172217</i>	—	AF331650 ⁴
<i>Ibtralia surinamensis</i> Bremek.	<i>Persson et al. 1930</i> (GB)	AF 201048 ¹	AF201000 ¹
<i>Ixora finlaysoniana</i> Wall. ex G. Don	<i>Delprete 7344</i> (NY)	AY555085 ²	EU821619
<i>Keetia multiflora</i> (Schum. & Thonn.) Bridson	<i>Delprete 7384</i> (NY)	AY555086 ²	—
<i>Kutchubaea</i> Fisch. ex DC. sp.	<i>Rodriguez 59</i> (NY)	AY555087 ²	—
<i>Kutchubaea</i> Fisch. ex DC. sp.	<i>Rodriguez 828</i> (NY)	—	EU821620
<i>Leptactina leopoldi-secundi</i> Büttner	<i>Delprete 7364</i> (NY)	AY555088 ²	EU821621
<i>Limnosipanea spruceana</i> Hook. f.	<i>Jansen-Jacobs et al. 2615</i> (NY)	AY555102 ²	—
<i>Limnosipanea erythraeoides</i> (Cham.) K. Schum.	<i>Macedo 5537</i> (US)	—	EU821622
<i>Macrosphyra longistyla</i> (DC.) Hook. F. ex Hiern	<i>Bagshawe 1457</i> (BM)	AF201051 ¹	AF201004 ¹
<i>Mussaenda pubescens</i> Buch.-Ham.	<i>Delprete 7399</i> (NY)	AY555089 ²	EU821623
<i>Pavetta stenosepala</i> K. Schum.	<i>Delprete 7387</i> (NY)	AY555090 ²	EU821624
<i>Platycarpum acreanum</i> G. K. Rogers	<i>Cid Ferreira 10407</i> (NY)	AY555100 ²	—
<i>Polysphaeria</i> Hook. f. sp.	<i>Groves 529</i> (K)	AF152655 ¹	AF201011 ¹
<i>Posoqueria gracilis</i> (Rudge) Roem. & Schult.	<i>Munzinger 504</i> (NY)	EU821640	—
<i>Pouchetia baumanniana</i> Büttner	<i>Delprete 7359</i> (NY)	AY555091 ²	EU821625
<i>Pseudomussaenda flava</i> Verdc.	<i>Andrews 857</i> (S)	AF152652 ³	—
<i>Psilanthus mannii</i> Hook. f.	<i>Delprete 7349</i> (NY)	AY555092 ²	—
<i>Psychotria</i> L. sp.	<i>Araújo 1054</i> (NY)	AY555079 ²	EU821612
<i>Psydrax schimperiana</i> (A. Rich.) Bridson	<i>Delprete 7388</i> (NY)	EU821641	EU821626
<i>Pyrostria media</i> (A. Rich. ex DC.) Cavaco	<i>Zarucchi 7424</i> (NY)	EU821642	EU821627
<i>Randia nitida</i> (Kunth) DC.	<i>Delprete 7358</i> (NY)	AY555093 ²	EU821628
<i>Retiniphyllum concolor</i> (Spruce ex Benth.) Müll. Arg.	<i>Berry 7093</i> (NY)	EU821643	—
<i>Retiniphyllum concolor</i> (Spruce ex Benth.) Müll. Arg.	<i>Berry 7422</i> (NY)	—	EU821629
<i>Retiniphyllum maguirei</i> Standl.	<i>Evans 3230</i> (MO)	EU821646	EU821632
<i>Retiniphyllum rhabdocalyx</i> Müll. Arg.	<i>Cortés 1648</i> (NY)	EU821644	EU821630
<i>Retiniphyllum schomburgkii</i> (Spruce ex Benth.) Müll. Arg.	<i>Berry 7567</i> (MO)	EU821645	EU821631
<i>Retiniphyllum secundiflorum</i> Bonpl.	<i>Berry 7457</i> (MO)	EU821647	EU821633
<i>Rondeletia inermis</i> (Spreng.) Krug & Urb.	<i>Acevedo et al. 7691</i> (NY)	AF152745 ³	—
<i>Rondeletia portoricensis</i> Krug & Urb.	<i>Taylor 11678</i> (MO)	—	AF243015 ⁴

Table 1. Continued.

Taxa	Voucher	GenBank accession number	
		<i>trnL-F</i>	<i>rps16</i>
<i>Rosenbergiodendron densiflorum</i> (K. Schum.) Fagerl.	Jansen-Jacobs et al. 3977 (GB)	AF201061 ¹	—
<i>Rosenbergiodendron densiflorum</i> (K. Schum.) Fagerl.	Gustafsson et al. 1994 (GB)	—	AF201014 ¹
<i>Rytigynia senegalensis</i> Blume	Madsen 6176	—	EU821637
<i>Scyphiphora hydrophyllacea</i> C. F. Gaertn.	Larsen 43134 (NY)	EU821648	EU821634
<i>Sipanea stahelii</i> Bremek.	Rova et al. 2068 (GB)	—	AF243023 ⁴
<i>Sipanea wilson-brownei</i> R. S. Cowan	Mori 25056 (NY)	EU821649	—
<i>Sipaneopsis rupicola</i> (Spruce ex K. Schum.) Steyerl.	Wurdack & Adde 43253 (NY)	AF152678 ³	—
<i>Stachyarrhena harleyi</i> J. H. Kirkbr.	Thomas 12032 (NY)	EU821650	—
<i>Stachyarrhena</i> sp.	Jansen-Jacobs et al. 4707 (GB)	—	AF201021 ¹
<i>Tarenna drummondii</i> Bridson	Delprete 7406 (NY)	AY555097 ²	EU821635
<i>Tocoyena williamsii</i> Standl.	Ståhl 3028 (GB)	AF201071 ¹	—
<i>Tocoyena</i> Aubl. sp.	Jansen-Jacobs et al. 3976 (GB)	—	AF201016 ¹
<i>Vangueria madagascariensis</i> J. F. Gmel.	Delprete 7383 (NY)	AY555098 ²	EU821636

GenBank sequences were originally published in ¹Persson (2000b), ²Delprete & Cortés-B. (2004), ³Rova et al. (2002), ⁴Rova (unpublished), ⁵Struwe et al. (1998), ⁶Andersson & Rova (1999), and ⁷Piesschaert et al. (2000).

the bi-ovulated locules of *Retiniphyllum* were misinterpreted, resulting in its association with tribes currently placed in the subfamily Rubioideae. Hooker (1873) established the tribe Retiniphyllae to include *Retiniphyllum* and, by an incorrect interpretation of the fruit, the genus *Kutchubaea* Fisch. ex DC. In the classic system of classification proposed by Schumann (1891), the tribe Retiniphyllae was not recognized, and *Retiniphyllum* was placed in the tribe Gardenieae of the subfamily Cinchonoideae. Verdcourt (1958) recognized the tribe Retiniphyllae, and maintained it in the subfamily Cinchonoideae. Bremekamp (1966), who proposed one of the most important systems of classification for the Rubiaceae in the 20th century, criticized previous placements of *Retiniphyllum* and simply called it an “aberrant” genus. According to Bremekamp (1966), the absence of secondary pollen presentation, a defining character of the tribe Gardenieae and his subfamily Ixoroideae, was absent in *Retiniphyllum*. Robbrecht (1988, 1993) maintained *Retiniphyllum* in the tribe Retiniphyllae and placed it in the subfamily Antirheoideae, a subfamily that was not supported by molecular data (Bremer & Jansen, 1991; Bremer & Struwe, 1992; Bremer et al., 1995; Bremer, 1996; Rova, 1999; Rova et al., 2002). Andersson and Rova (1999), in their phylogenetic study that focused on the subfamily Rubioideae using *rps16*, sampled *Retiniphyllum* for the first time and placed it in the subfamily Ixoroideae. This placement was also supported by Rova (1999) and Rova et al. (2002) in their study of the Condamineae–Rondeletiae–Sipaneae complex. In these analyses, *Retiniphyllum* was located in an isolated clade of the Ixoroideae related to Paleotropical representatives, sister to a clade with members of the tribes Coffeeae,

Gardenieae, Octotropideae, Pavetteae, Rondeletiae, and Vanguerieae. In the most recent Rubiaceae classification, the traditional Cinchonoideae and Ixoroideae were merged in a single subfamily, and the tribe Retiniphyllae was placed in the supertribe Ixoridinae of the subfamily Cinchonoideae (Robbrecht & Manen, 2006).

The genera *Botryarrhena* Ducke and *Scyphiphora* C. F. Gaertn. were tentatively included in the tribe Retiniphyllae by Robbrecht (1988). *Botryarrhena* comprises two species distributed in the Amazon Basin and Guayana Region. Ducke (1932) pointed out the resemblance of *Botryarrhena* to *Retiniphyllum* because they share bisexual flowers, terminal, racemose inflorescences, and two (rarely three or four) ovules per locule. On the other hand, the Asian genus *Scyphiphora* includes only *S. hydrophyllacea* C. F. Gaertn., the only mangrove species in the Rubiaceae. It has unique placentation with two ovules per locule, one of which is pendulous and the other erect. Robbrecht (1988) thought that this peculiar placentation is perhaps a form derived from the condition in *Retiniphyllum*.

The main goals of this study are to: (1) test the monophyly of the tribe Retiniphyllae, (2) evaluate the phylogenetic position of the Retiniphyllae within the subfamily Ixoroideae, and (3) evaluate the relationship of the genera *Botryarrhena* and *Scyphiphora* with *Retiniphyllum*.

METHODS

TAXON SAMPLING

A total of 49 taxa representing most tribes or groups recognized in the subfamily Ixoroideae s.l. were used

to test the monophyly of the Retiniphyllae: the Condamineae complex clade (Rova et al., 2002), Henriquezieae (Rogers, 1984), Posoquerieae (Delprete et al., 2004), Sipaneeae (Delprete & Cortés-B., 2004), Mussaendeae (Bremer & Tulin, 1998), Ixoreae (Andreasen & Bremer, 2000), Vanguerieae (Lantz et al., 2002), Octotropideae (Robbrecht, 1988), Coffeae, Pavetteae (Andreasen & Bremer, 2000), the *Alibertia* group (Persson, 2000a), and Gardenieae s.l. (Robbrecht, 1988).

In the phylogeny of *Retiniphyllum* (Cortés-B. et al., in prep.), most of the species are resolved in three main clades. In the present study, five representative species of *Retiniphyllum* were selected, including at least one from each clade: *R. concolor* (Spruce ex Benth.) Müll. Arg., *R. maguirei* Standl., *R. rhabdocalyx* Müll. Arg., *R. schomburgkii* (Benth.) Müll. Arg., and *R. secundiflorum* Bonpl.

Leaf samples were collected in the Botanical Gardens of Bruxelles (BR), Leiden (L), and Wageningen (W) by the second author, or obtained from herbarium material. We used chloroplast DNA (cpDNA) sequences of the *trnL-F* intergenic spacer and the *rps16* intron. Of the total number of sequences used in the analyses, 41% were original; the rest were downloaded from GenBank from Persson (2000b) [22%], Delprete and Cortés-B. (2004) [20%], and Rova et al. (2002) [9%], and the remaining 8% from Rova (unpublished), Struwe et al. (1998), Andersson and Rova (1999), and Piesschaert et al. (2000). Voucher information is presented in Table 1.

OUTGROUP SELECTION

Four members of the subfamilies Rubioideae and Cinchonoideae were selected as outgroup. *Psychotria* L. and *Amphidasya* Standl. have been shown to be part of the subfamily Rubioideae (Bremer & Manen, 2000; Rova et al., 2002), while *Cinchona* L. and *Rondeletia* L. are members of the subfamily Cinchonoideae (Bremer & Thulin, 1998; Rova et al., 2002).

DNA EXTRACTION, AMPLIFICATION, AND SEQUENCING

Total genomic DNA was isolated from approximately 1 cm² of dried leaf tissue desiccated in silica gel, or from herbarium material, using a modified CTAB methodology (Motley et al., 2005).

DNA was amplified using the polymerase chain reaction (PCR) following Motley et al. (2005). For amplification of the *trnL-F* spacer, the primers “e” (5'-GGTCAAGTCCCTCTATCCC-3') and “f” (5'-ATTTGAAGTGGTGACACGAG-3') of Taberlet et al. (1991) were used. The *rps16* intron was amplified using the primers *rpsF* (5'-CTGCTAGAAAG-

CAACGTGCGACTT-3') and *rpsR2* (5'-TCGGGATC-GAACATCAATTGCAAC-3') designed by Oxelman et al. (1997). The PCR conditions were: hold 94°C for 3 min., 32 cycles of 94°C for 45 sec., 52°C for 30 sec., 72°C for 1 min. 30 sec., and hold 74°C for 7 min., hold 4°C. Cross-contamination was controlled by using negative controls in the PCR reactions. In addition, DNA from two individuals per species was extracted, amplified, and sequenced, when possible. Amplified products were purified with spin columns from the QIAquick PCR purification kit (Qiagen, Valencia, California, U.S.A.) following protocols provided by the manufacturer. Cycle sequencing conditions, gene cleaning using hydrated Sephadex G-50 DNA Grade F columns (Amersham Pharmacia Biotech Inc., Piscataway, New Jersey, U.S.A.), and the visualization separation of fragments were run on an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, California, U.S.A.) following the protocols described in Motley et al. (2005).

PHYLOGENETIC ANALYSIS

The sequences were first edited in Sequencher 3.1.2 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.) and preliminarily aligned with ClustalX (Thompson et al., 1997) using the default settings. They were then manually edited using BioEdit (Hall, 1999).

Parsimony analyses with equal character weights and unordered characters were performed with NONA (Goloboff, 1993) in concert with WinClada (Nixon, 2002). In the analyses, gaps were treated as missing values. Five heuristic searches were performed holding a maximum of 100,000 trees per search. In each search, 500 replications were carried out, keeping five trees per replication under the option mult*max*. The trees obtained were used to calculate a strict consensus tree. In order to evaluate the relative support of the clades, bootstrap (BS) and jackknife (JK) analyses were executed using 1000 replicates.

RESULTS

The combined analysis of the *trnL-F* and *rps16* data matrices had a total of 52 taxa and 1428 characters, 282 of which were parsimony informative. The heuristic search resulted in 54 most parsimonious trees of 651 steps in length, with a consistency index (CI) of 0.61 (when excluding uninformative characters) and a retention index (RI) of 0.82. Figure 1 shows the strict consensus tree obtained in the heuristic search with BS and JK support values.

In the strict consensus tree resulting from the maximum parsimony analysis, a well-supported clade

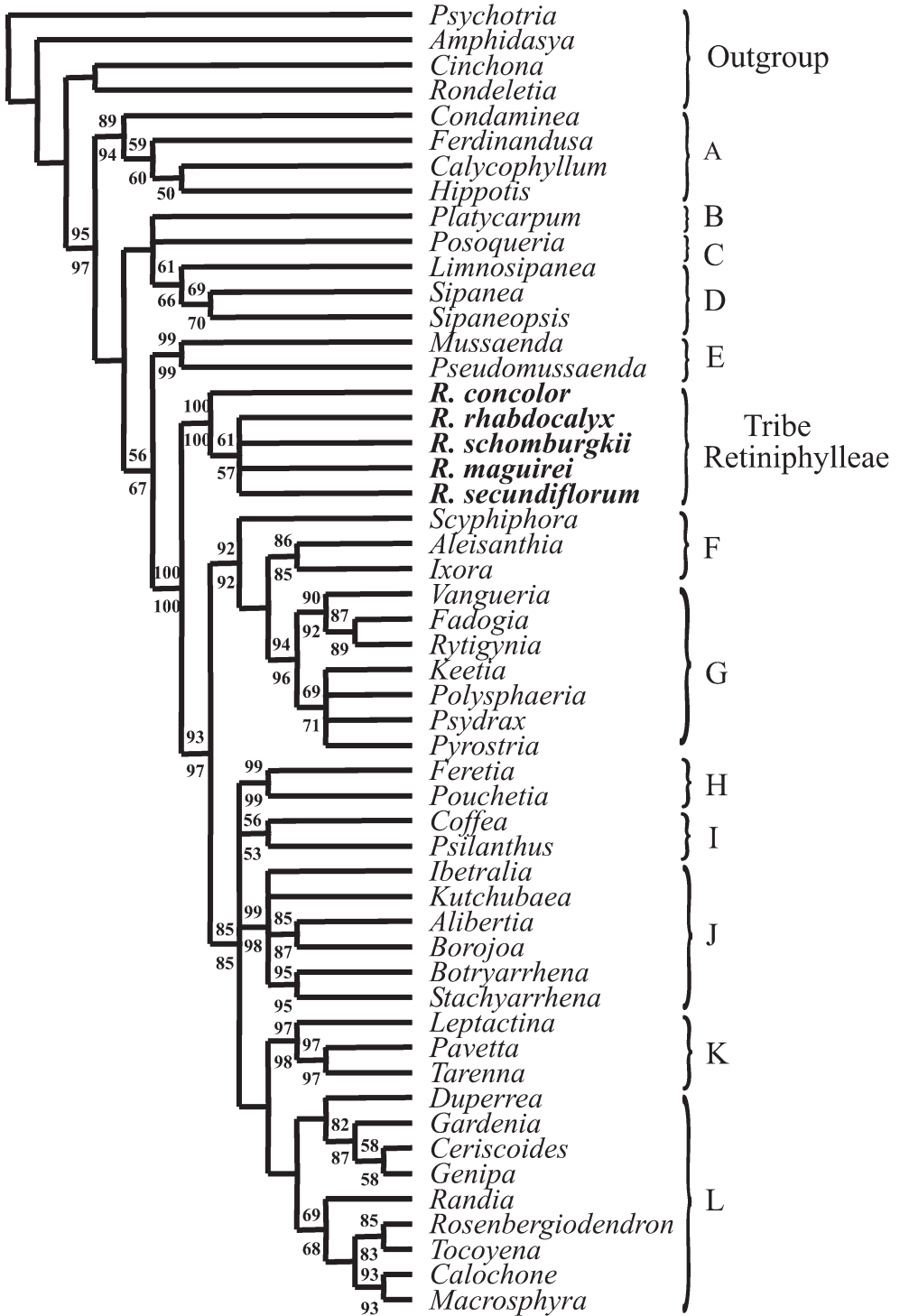


Figure 1. Strict consensus tree of the 54 most parsimonious trees from the combined *trnL-F* and *rps16* analysis. —A. Condamineae complex clade (Rova et al., 2002). —B. Henriquezieae (Rogers, 1984). —C. Posoquerieae (Delprete et al., 2004). —D. Sipaneae (Delprete & Cortés-B., 2004). —E. Mussaendeae (Bremer & Thulin, 1998). —F. Ixoreae (Andreasen & Bremer, 2000). —G. Vanguerieae (Lantz et al., 2002). —H. Octotropideae (Robbrecht, 1988). —I. Coffeae. —J. *Alibertia* group (Persson, 2000a). —K. Pavetteae (Andreasen & Bremer, 2000). —L. Gardenieae s.l. (Robbrecht, 1988). Bootstrap (BS) and jackknife (JK) support values are indicated above and below branches, respectively.

(BS = 89%, JK = 94%) was retrieved containing genera *Condaminea* DC., *Ferdinandusa* Pohl, *Calycophyllum* DC., and *Hippotis* Ruiz & Pav., which had been placed in the subfamily Cinchonoideae in previous systems of classification (Verdcourt, 1958; Bremekamp, 1966; Robbrecht, 1988, 1993). This clade was sister to the rest of the sampled genera. Members of the tribes Sipaneeae, Henriquezieae, and Posoquerieae are together in an unsupported clade sister to the members of the tribe Mussaendeae (BS = 56%, JK = 67%) and the clade that includes *Retiniphyllum* species. All the species sampled of *Retiniphyllum* are together in a strongly supported clade (BS = 100%, JK = 100%), sister to a clade that includes Ixoreae, Vanguerieae, Octotropideae, Coffeae, Pavetteae, *Alibertia* group, and Gardenieae s.l.

DISCUSSION

THE MONOPHYLY AND POSITION OF THE TRIBE RETINIPHYLLEAE IN THE SUBFAMILY IXOROIDEAE

Because species of *Retiniphyllum* formed a strongly supported monophyletic lineage (BS = 100%, JK = 100%) in the independent (not shown) and combined chloroplast analyses (Fig. 1), the monophyly of the genus *Retiniphyllum* is confirmed. Similarly, the isolated position of *Retiniphyllum* in the cladogram also confirms the monophyly of the tribe Retiniphyllae.

The tribe Retiniphyllae is placed as a clade within the subfamily Ixoroideae s.l., sister to the tribes that correspond for the most part to the Ixoroideae sensu Bremekamp (Bremekamp, 1966). Although the systematics of the subfamily Ixoroideae have been largely modified since Bremekamp's proposal, his tribes Gardenieae, Ixoreae, and Vanguerieae correspond to the core of the Ixoroideae in its original circumscription. The results presented here also support those previously reported by Rova (1999) and Rova et al. (2002).

Robbrecht (1988) included the tribes Retiniphyllae and Vanguerieae in the subfamily Antirrhoideae, suggesting that morphological similarities of their fruits and seeds supported this relationship. In addition, Robbrecht and Manen (2006) considered that these similarities were consistent with their placement in their supertribe Ixoridinae. According to our results, the tribe Retiniphyllae is not closely related to the Vanguerieae.

THE PLACEMENT OF *BOTRYARRHENA*

Botryarrhena was resolved within the *Alibertia* group, sister to *Stachyarrhena* Hook. f. in a clade with the genera *Ibetrulia* Bremek., *Kutchubaea*,

Alibertia A. Rich. ex DC., and *Borojoa* Cuatrec. (Fig. 1).

Ducke (1932) suggested an affinity between the genera *Botryarrhena* and *Stachyarrhena*. They share racemose inflorescences, but differ because *Stachyarrhena* has unisexual flowers and a dioecious breeding system (Ducke, 1932). *Stachyarrhena* is now placed within the *Alibertia* group, a clade within the tribe Gardenieae (Persson 2000a, b). Subsequently, unisexual flowers have been observed in *Botryarrhena* (Persson & Delprete, pers. comm.), providing further support for the placement of *Botryarrhena* in this lineage.

The relationship between *Botryarrhena* and *Retiniphyllum* suggested by Ducke (1932) was based on flower sexuality, inflorescence morphology, and number of ovules per locule. However, it is important to note that Standley was not able to see the berry-like fruits of *Botryarrhena*, a common fruit type of many genera in the Ixoroideae but not in *Retiniphyllum*.

THE PLACEMENT OF *SCYPHIPHORA*

According to our results, the genera *Retiniphyllum* and *Scyphiphora* are resolved in two distinct clades, indicating that the bi-ovulate condition has evolved independently in the subfamily.

Puff and Rohrhofer (1993) studied the morphology of *Scyphiphora* in detail and found no characters suggesting a close relationship to *Retiniphyllum*. They tentatively placed *Scyphiphora* in the subtribe Diplosporinae, tribe Gardenieae s.l., based on the presence of tracheidal idioblasts in *Scyphiphora*, which are similar to the mesophyll sclereids in the Gardenieae. Andreassen and Bremer (2000), using morphological and molecular data, placed *Scyphiphora* as sister to the tribe Ixoreae, and they tentatively included it in the Ixoreae.

Our results indicate that *Scyphiphora* is sister to a clade that includes the tribe Ixoreae and also Vanguerieae (Fig. 1). This indicates that *Scyphiphora* is neither a member of the tribe Gardenieae s.l. as Puff and Rohrhofer (1993) suggested, nor a member of the tribe Ixoreae as Andreassen and Bremer (2000) hypothesized.

Literature Cited

- Andersson, L. & J. H. E. Rova. 1999. The *rps16* intron and the phylogeny of the Rubioideae (Rubiaceae). *Pl. Syst. Evol.* 214: 161–186.
- Andreassen, K. & B. Bremer. 2000. Combined analysis in the Rubiaceae–Ixoroideae: Morphology, nuclear and chloroplast DNA data. *Amer. J. Bot.* 87: 1731–1748.
- Bentham, G. 1841. Contributions towards a Flora of South America—Enumeration of plants collected by Mr. Schomburgk in British Guiana. *J. Bot. (Hooker)* 3: 212–250.

- Bremekamp, C. E. B. 1966. Remarks on the position, the delimitation, and the subdivision of the Rubiaceae. *Acta Bot. Neerl.* 15: 1–33.
- Bremer, B. 1996. Phylogenetic studies within Rubiaceae and relationships to other families based on molecular data. *Opera Bot. Belg.* 7: 33–50.
- & R. K. Jansen. 1991. Comparative restriction site mapping of the chloroplast DNA implies new phylogenetic relationships within the Rubiaceae. *Amer. J. Bot.* 78: 198–213.
- & L. Struwe. 1992. Phylogeny of the Rubiaceae and the Loganiaceae: Congruence or conflict between morphological and molecular data? *Amer. J. Bot.* 79: 1171–1184.
- & M. Thulin. 1998. Collapse of Isertieae, re-establishment of Mussaendeae, and a new genus of Sabiceae (Rubiaceae); phylogenetic relationships based on *rbcL* data. *Pl. Syst. Evol.* 211: 71–92.
- & J. F. Manen. 2000. Phylogeny and classification of the subfamily Rubioideae (Rubiaceae). *Pl. Syst. Evol.* 225: 47–72.
- , K. Andreasen & D. Olsson. 1995. Subfamilial and tribal relationships in the Rubiaceae based on *rbcL* sequence data. *Ann. Missouri Bot. Gard.* 82: 383–397.
- Candolle, A. P. de. 1830. Rubiaceae. *Prodromus Systematis Naturalis Regni Vegetabilis*, Vol. 4: 341–362. Treuttel & Würtz, Paris.
- Delprete, P. G. & R. Cortés-B. 2004. A preliminary phylogenetic study of the tribe Sipaneeae (Rubiaceae, Ixoroideae), using *trnL-F* and ITS sequence data. *Taxon* 53: 347–356.
- , L. B. Smith & R. B. Klein. 2004. Rubiaceae, 1. *Alseis* at 19. *Galium*. Pp. 1–344 in A. Reis (editor), *Flora Ilustrada Catarinense*, Vol. 1: Gêneros de A–G. Herbario Barbosa Rodrigues, Itajaí, Santa Catarina, Brazil.
- Ducke, A. 1932. Neue Arten aus der *Hyalea* Brasiliens. *Notizbl. Bot. Gard.* 11: 471–483.
- Goloboff, P. A. 1993. NONA version 2.0. Program and documentation distributed by the author, Tucumán, Argentina.
- Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids Symp. Ser.* 41: 95–98.
- Hooker, J. D. 1873. Ordo LXXXIV. Rubiaceae. Pp. 7–151 in G. Bentham & J. D. Hooker (editors), *Genera Plantarum*, Vol. 2. Lovell Reeve & Co., London.
- Jussieu, A. L. de. 1820. Sur la famille des plantes Rubiacées. *Mém. Mus. Hist. Nat.* 6: 365–410.
- Kunth, C. S. 1818. *Retiniphyllum*. Pp. 421–422 in F. H. A. Humboldt & A. J. de Bonpland (editors), *Nova genera et species plantarum,* Librariae graeco-latini-germanicae, Paris.
- Lantz, H., K. Andreasen & B. Bremer. 2002. Nuclear rDNA ITS sequence data used to construct the first phylogeny of Vanguerieae (Rubiaceae). *Pl. Syst. Evol.* 230: 173–187.
- Motley, T. J., K. J. Wurdack & P. G. Delprete. 2005. Molecular systematics of the Chiococceae–Catesbaeae Complex (Rubiaceae): Flower and fruit evolution and systematic implications. *Amer. J. Bot.* 92: 316–329.
- Nixon, K. C. 2002. WinClada Vers. 1.00.08. Published by the author, Ithaca, New York.
- Oxelmann, B., M. Lidén & D. Berglund. 1997. Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Pl. Syst. Evol.* 206: 393–410.
- Persson, C. 2000a. Phylogeny of the Neotropical *Alibertia* group (Rubiaceae), with emphasis on the genus *Alibertia*, inferred from ITS and 5S ribosomal DNA sequences. *Amer. J. Bot.* 87: 1018–1028.
- . 2000b. Phylogeny of Gardenieae (Rubiaceae) based on chloroplast DNA sequences from the *rps16* intron and *trnL(UAA)-F(GAA)* intergenic spacer. *Nord. J. Bot.* 20: 257–269.
- Piesschaert, F., L. Andersson, S. Jansen, S. Dessein, E. Robbrecht & E. Smets. 2000. Searching for the taxonomic position of the African genus *Collettoecema* (Rubiaceae): Morphology and anatomy compared to an *rps16*-intron analysis of the Rubioideae. *Canad. J. Bot.* 78: 288–304.
- Puff, C. & U. Rohrhofer. 1993. The character states and taxonomic position of the monotypic mangrove genus *Scyphiphora* (Rubiaceae). *Opera Bot. Belg.* 6: 143–172.
- Richard, A. 1830. Mémoire sur la famille des Rubiacées. Imprimerie de J. Tatsu, Paris. (Reimpr. *Mém. Soc. Hist. Nat. Paris*, ser. 3 5: 81–224. 1834).
- Robbrecht, E. 1988. Tropical Woody Rubiaceae. *Opera Bot. Belg.* 1: 1–271.
- . 1993 [1994]. Supplement to the 1988 outline of the classification of the Rubiaceae. Index to genera. Pp. 173–196, in E. Robbrecht (editor), *Advances in Rubiaceae Macro-systematics*. *Opera Bot. Belg.* Vol. 6.
- Richard, A. & J. F. Manen. 2006. The major evolutionary lineages of the coffee family (Rubiaceae, angiosperms). Combined analysis (nDNA and cpDNA) to infer the position of *Coptosapelta* and *Luculia*, and supertree construction based on *rbcL*, *rps16*, *trnL-trnF*, and *atpB-rbcL* data. A new classification in two subfamilies, Cinchonoideae and Rubioideae. *Syst. Geogr. Pl.* 76: 85–146.
- Roemer, J. J. & J. A. Schultes. 1818. *Systema vegetabilium* 3: 255. J. G. Cotta, Stuttgart.
- Rogers, G. K. 1984. *Gleasonia*, *Henriquezia*, and *Platyca-rpum* (Rubiaceae). *Fl. Neotrop. Monogr.* 39: 1–135.
- Rova, J. H. E. 1999. The Rondeletieae–Condamineae–Sipaneeae Complex (Rubiaceae). Ph.D. Dissertation, University of Göteborg, Göteborg, Sweden.
- , P. G. Delprete, L. Andersson & V. A. Albert. 2002. A *trnL-F* cpDNA sequence study of the Condamineae–Rondeletieae–Sipaneeae complex with implications on the phylogeny of the Rubiaceae. *Amer. J. Bot.* 89: 145–159.
- Schumann, K. 1891. Rubiaceae. Pp. 1–156 in A. Engler & K. Prantl (editors), *Die natürlichen Pflanzenfamilien*, Vol. 4(4). Engelmann, Leipzig.
- Struwe, L., M. Thiv., J. W. Kadereit, A. R. Pepper, T. J. Motley, P. J. White, J. H. E. Rova, K. Potgieter & V. A. Albert. 1998. *Saccifolium* (Saccifoliaceae), an endemic of Sierra de la Neblina on the Brazilian-Venezuelan border, is related to a temperate-alpine lineage of Gentianeae. *Harvard Pap. Bot.* 3: 199–214.
- Taberlet, P., L. Gielly, G. Patou & J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Pl. Molec. Biol.* 17: 1105–1109.
- Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin & D. G. Higgins. 1997. The Clustal X Windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.* 24: 4876–4882.
- Verdcourt, B. 1958. Remarks on the classification of the Rubiaceae. *Bull. Jard. Bot. État Bruxelles* 28: 209–281.