



Reorganization of the Genus *Psychotria* and Tribe Psychotrieae (Rubiaceae) Inferred from ITS and *rbcL* Sequence Data

Author(s): Molly Nepokroeff, Birgitta Bremer, Kenneth J. Sytsma

Source: *Systematic Botany*, Vol. 24, No. 1 (Jan. - Mar., 1999), pp. 5-27

Published by: American Society of Plant Taxonomists

Stable URL: <http://www.jstor.org/stable/2419383>

Accessed: 06/02/2009 09:45

Your use of the JSTOR archive indicates your acceptance of JSTOR's Terms and Conditions of Use, available at <http://www.jstor.org/page/info/about/policies/terms.jsp>. JSTOR's Terms and Conditions of Use provides, in part, that unless you have obtained prior permission, you may not download an entire issue of a journal or multiple copies of articles, and you may use content in the JSTOR archive only for your personal, non-commercial use.

Please contact the publisher regarding any further use of this work. Publisher contact information may be obtained at <http://www.jstor.org/action/showPublisher?publisherCode=aspt>.

Each copy of any part of a JSTOR transmission must contain the same copyright notice that appears on the screen or printed page of such transmission.

JSTOR is a not-for-profit organization founded in 1995 to build trusted digital archives for scholarship. We work with the scholarly community to preserve their work and the materials they rely upon, and to build a common research platform that promotes the discovery and use of these resources. For more information about JSTOR, please contact support@jstor.org.



American Society of Plant Taxonomists is collaborating with JSTOR to digitize, preserve and extend access to *Systematic Botany*.

<http://www.jstor.org>

Reorganization of the Genus *Psychotria* and Tribe Psychotrieae (Rubiaceae) Inferred from ITS and *rbcl* Sequence Data

MOLLY NEPOKROEFF

Laboratory of Molecular Systematics, MRC 534, MSC, Smithsonian Institution, Washington, D.C. 20560

BIRGITTA BREMER

Department of Systematic Botany, Uppsala University, S-752 36, Uppsala, Sweden

KENNETH J. SYTSMA

Department of Botany, University of Wisconsin, 430 Lincoln Dr., Madison, WI 53706

Communicating Editor: James Manhart

ABSTRACT. *Psychotria* is one of the largest genera of angiosperms as currently delimited, with approximately 1650 species estimated worldwide. A molecular phylogenetic study was undertaken using ITS and *rbcl* sequence data to address the generic circumscription of *Psychotria* and its allies in the tribe Psychotrieae and to compare traditional subgeneric groupings with relationships inferred from phylogenetic analysis. ITS sequences from 85 taxa were sampled representing the genus *Psychotria* and members of the tribe Psychotrieae, with *Gaertnera* and *Mitchella* used as outgroups. Sequences from the *rbcl* gene were analyzed for a subset of these taxa, plus genera in the closely related tribes Coussareae, Morindeae, Ophiorrhizeae, and others. Our results suggest that *Psychotria* is broadly paraphyletic and defined by lack of characters used to define other genera in the tribe. Two groups formerly assigned to *Psychotria* (sect. *Notopleura* and subg. *Heteropsychotria* plus *Palicourea*) are more closely related to other genera in the Psychotrieae than they are to other species of *Psychotria*. In addition, subg. *Psychotria* includes species formerly assigned to the Malesian epiphytic myrmecophytes of subtribe Hydnohytinae. We propose that the genus *Psychotria* be restricted to include subg. *Psychotria*, subg. *Tetramerae*, and members of the subtribe Hydnohytinae.

The pantropical genus *Psychotria* is one of the largest genera of flowering plants with between 1,000 to 1,650 species worldwide (Hamilton 1989a; Taylor 1996). *Psychotria* is the largest genus in the Rubiaceae (Hamilton 1989a; Taylor 1996), and is a member of the subfamily Rubioideae, and largely tropical, woody, tribe Psychotrieae. Species of *Psychotria* are mostly understory shrubs, although trees, lianas, herbs, epiphytes, and suffrutices are also known. *Psychotria* and other members of the tribe Psychotrieae are generally characterized by small, whitish flowers that may be pollinated by bees, flies, and moths, although showy inflorescences have been derived in various neotropical lineages (e.g., "*Cephaelis*", section *Notopleura*, *P. ipecacuanha* (Brot.) Stokes and *P. borucana* (A. Molina R.) C.M. Taylor and W.C. Burger, and species of *Palicourea* and *Rudgea*) in conjunction with bird pollination. *Psychotria* contributes importantly to tropical floristic diversity along with other species-rich genera such as *Miconia* (Melastomataceae), *Piper* (Piperaceae), *Ficus* (Moraceae), and *Anthurium* (Araceae), and may comprise a significant proportion of the understory vegetation in wet tropical low-

land forests around the world (Sohmer 1988). For this reason, *Psychotria* has been considered a model genus for inferring patterns and general mechanisms of speciation in the tropics (Hamilton 1989a). Given its large number of species, *Psychotria* may be used to investigate rates of diversification and extinction and origins of evolutionary innovations. Additionally, *Psychotria* species may serve as models for examining the evolution of breeding systems, since it possesses a range of breeding systems: hermaphroditism, distyly, and functional unisexuality.

However, *Psychotria* is taxonomically complex. This complexity is due not only to the large number of species in the genus and tribe, but also to the relative lack of morphological characters available to define groups. *Psychotria* sensu lato is distinguished from other members of the tribe by the presence of an inferior ovary (vs. superior in *Gaertnera* and *Pagamea*); corolla straight at base and usually white or greenish in color (vs. corolla gibbous at base and brightly colored in *Palicourea*); ovary with a single ovule per locule; fruits fleshy and with 2 (–5) pyrenes; pyrenes smooth or ribbed and

hemispherical in cross-section; endosperm often ruminate; seeds with hard, non-oily endosperm, red-brown or purplish testa; stipules seldom pectinate-glandular (vs. pectinate-glandular in *Rudgea*) (Schumann 1891; Petit 1964, 1966; Steyermark 1972; Verdcourt 1976; Robbrecht 1988; Taylor 1996). Indeed, *Psychotria* is defined primarily on the basis of the absence of characters used to define other genera in the tribe. In addition, many of these, and other characters are known to intergrade among other species and genera in the Psychotrieae. For example, the presence of involucre bracts surrounding capitulate inflorescences has been used to define the genera *Cephaëlis* and *Uragoga*, yet this character is likely to have evolved convergently in a diverse array of lineages in Psychotrieae (Schnell 1960; Steyermark 1972; Nepokroeff 1992; Endress 1996; Taylor 1996). One solution to the taxonomic ambiguity in *Psychotria* and the Psychotrieae has been to expand the limits of *Psychotria* to include upwards of 2,000 species. For example, Steyermark (1972) placed many neotropical genera such as *Notopleura*, *Nonatelia*, *Ronabea*, and *Cephaëlis* in *Psychotria*. Another source of ambiguity in the classification of *Psychotria* has been the lack of a comprehensive world-wide treatment; to date, *Psychotria* has not been examined at a worldwide level using an explicitly phylogenetic method.

***Psychotria* Subgeneric Classifications.** Petit (1964, 1966) and Steyermark (1972) recognized three subgenera in *Psychotria* based on morphological characters and geographical distribution. Subgenus *Psychotria* is pantropical in distribution and can be distinguished by leaves drying red-brown to dull green vs. bright green in other groups; stipules sheathing and deciduous, often leaving a fringe of red-brown hairs; and seeds hemispherical in cross-section and with the ventral face either plane or with two longitudinal furrows on the flat ventral side, variously ribbed on the rounded dorsal surface, and lacking in preformed germination slits (adapted from Robbrecht 1989; Hamilton 1989a). Endosperm of the seeds may be highly ruminate. A number of sectional and species groups have been assigned within *Psychotria* subg. *Psychotria* on a regional basis including Africa (Verdcourt 1976), the neotropics (Hamilton 1989a,b,c), and the Pacific (Smith and Darwin 1988). *Psychotria* subg. *Tetrameræ* was originally erected by Petit (1964), based on Hiern's (1877) section *Tetrameræ*, for those species of *Psychotria* in Africa and Madagascar that possess bacterial nodules in the leaves, as well as narrow, biacuminata stipular teeth, variously panic-

ulate inflorescences subtended by small bracts, non-ruminate seeds, and usually tetramerous flowers. Although subg. *Tetrameræ* is confined to Africa, Hamilton (1989c) suggested that the neotropical species *P. microdon* (DC) Urban may be closely related. *Psychotria* subg. *Heteropsychotria* was described by Steyermark (1972) for the remainder of the species of *Psychotria* in the neotropics, including those species formerly referred to *Cephaëlis* and *Uragoga*, as well as a number of segregate genera named by Aublet and Bremekamp, such as *Ronabea*, *Nonatelia*, *Chytropsia*, *Naletonia*, and *Notopleura*. The subgenus is characterized by a heterogeneous set of characters: leaves drying bright green or gray green, habit various, stipules diverse, stipule scars without red-brown hairs, inflorescence various, with bracts prominent or minute, and pyrenes with the ventral face variously keeled, furrowed, or lacking furrows. The naturalness and relationships of the subgenera have not been examined from a worldwide perspective or in a phylogenetic context.

Phylogenetically, *Psychotria* and its relatives in tribe Psychotrieae represent a highly diverse array of lineages, and the identification of apomorphic characters with which to redefine *Psychotria* is an important component to a new phylogenetic classification. A phylogenetic analysis would contribute immensely toward the taxonomic circumscription and possible reorganization of the genus and tribe. Thus, a survey of *Psychotria* and Psychotrieae utilizing molecular characters, as well as those morphological characters previously used, would both constitute a framework for a preliminary phylogenetic classification and serve as a historical framework for testing hypotheses of character evolution. The goals of the present study are to use phylogenetic analysis to (1) address the generic circumscription and infra-generic relationships within *Psychotria* on a worldwide basis, (2) determine the relationships of *Psychotria* to its allies in tribe Psychotrieae, and (3) to determine which morphological characters reflect most accurately the phylogenetic relationships inferred from the molecular analysis. To accomplish these goals, a phylogenetic analysis was conducted on nucleotide sequence data from both the ITS/5.8S region of nuclear rDNA and the chloroplast *rbcl* gene. The ITS region has become a tool of choice for species- and subgenus-level comparisons, and has been used extensively to infer phylogenetic relationships at a wide range of taxonomic ranks (see Baldwin et al. 1995; Sytsma and Hahn 1994, 1996 for reviews). The more conserved *rbcl* has been used for phylogeny reconstruction at a va-

riety of taxonomic levels, including familial (Conti et al. 1993; Olmstead et al. 1993; Bremer et al. 1995), ordinal (Conti et al. 1996; Rodman et al. 1996), and angiosperm-wide (Chase et al. 1993). Use of *rbcL* was implemented to help resolve higher-level taxonomic problems in Psychotrieae after preliminary analysis by Bremer (1996) indicated adequate rates of *rbcL* sequence variation within the tribe.

MATERIALS AND METHODS

Terminal Taxa. In order to determine the placement of *Psychotria* in the tribe Psychotrieae and to determine relationships within *Psychotria*, 85 taxa were sequenced for the ITS region (Table 1). Sixty-four species of *Psychotria* were included in the ingroup, comprising 38 species of pantropical subg. *Psychotria* (from the neotropics, Hawai'i, Africa, Asia and the Pacific), 24 species of neotropical subg. *Heteropsychotria*, and two species of the African subg. *Tetramerae*. In addition, eight species of the Malesian subtribe Hydnophytinae were included—comprising the genera *Hydnophytum*, *Myrmecodia*, *Myrmephytum*, and *Anthorrhiza*, as well as three species of pantropical *Geophila*, two species of neotropical *Rudgea*, one species each of the African endemics *Chazaliella* and *Hymenocoleus*, and three species of neotropical *Palicourea*. The paleotropical genus *Gaertnera* and the genus *Mitchella* (with a disjunct distribution in North America and South East Asia) were used as outgroups in the ITS analysis, based on the placement of these two genera outside the tribe Psychotrieae sensu stricto using *rbcL* evidence (Bremer 1996). The sample includes 18 important genera, with 22 remaining monotypic genera unavailable as poorly known regional endemics. Although the genus *Psychotria* has never been monographed from a worldwide perspective, all three major subgenera are sampled. Portions of the 18S gene, and the entire ITS 1, 5.8S cistron and ITS 2 regions were included in the phylogenetic analysis, with 4.2% of the data scored as missing.

The *rbcL* sequences of a subset of the taxa sampled in the ITS data set, representing the most morphologically divergent lineages in the Psychotrieae and species in tribes Morindeae, Coussareae and related taxa were analyzed to determine the circumscription of tribe Psychotrieae. Thirty-five taxa were sampled for the *rbcL* analysis (Table 2) and, of these, ten yielded new sequences reported here. GenBank information

for all accessions is given in Tables 1 and 2. Included were eight species of *Psychotria* subg. *Psychotria* (from the neotropics, Africa, and Hawai'i), two genera of subtribe Hydnophytinae (*Myrmecodia* and *Hydnophytum*), one species of the Pacific segregate genus *Amaracarpus*, five species of *Psychotria* subg. *Heteropsychotria*, one species of *Palicourea*, two species of *Rudgea*, and one species each of *Chazaliella*, *Hymenocoleus*, *Geophila*, *Declieuxia*, and paleotropical *Chasallia*. Additional taxa representing close relatives of Psychotrieae included *Coccocypselum*, *Coussarea*, *Damnacanthus*, *Faramea*, *Gaertnera*, *Lasianthus*, *Mitchella*, *Morinda*, *Pauridiantha*, and *Schradera*. The Indo-Malesian genus *Ophiorrhiza* (tribe Ophiorrhizeae) and the African genus *Pauridiantha* (formerly subfamily Cinchonoideae, cf. Robbrecht 1988) were used as outgroups in the *rbcL* analysis, based on placement of *Ophiorrhiza* at the base of the subfamily Rubioideae, well outside the limits of the tribe Psychotrieae, and placement of *Pauridiantha* as part of the subfamily Rubioideae, and as sister clade to the genus *Lasianthus* (Bremer 1996). Data from the *rbcL* gene was included from basepair 27 through 1428, and 0.3% of the data matrix was scored as missing data. Datasets for ITS and *rbcL* are available from the first author upon request.

DNA Extractions. Leaf material was collected in the field or in botanical gardens and either shipped to the laboratory and frozen at -80°C to await further processing or dried in silica gel. DNA was isolated using $6\times$ CTAB in a modified version (Smith et al. 1991) of the CTAB miniprep of Doyle and Doyle (1977). Vouchers are deposited in herbaria listed in Tables 1 and 2.

PCR Amplification and Sequencing. Double stranded PCR products were amplified for the entire ITS region, using primers "LEU 1" (designed by Lowell Urbatsch, and provided by Bruce Baldwin) and "ITS 4" (White et al. 1990). PCR products were purified using differential centrifugation with either the QIAQuick columns (QIAGEN Inc.) or Millipore Ultrafree-MC tubes (Millipore Corp.). Sequences were obtained for both strands using the primers LEU 1, ITS 3B (Baum et al. 1994) and ITS 4. Dideoxy sequencing was carried out directly from purified PCR products using the dye terminator cycle sequencing protocol (from Applied Biosystems) on a Perkin Elmer 2400 thermocycler. Sequencing reactions were analyzed on an ABI 373 DNA automated DNA sequencer. Chromato-

TABLE 1. Accessions for tribe Psychotrieae used in the phylogenetic analysis of ITS sequences. Asterisks indicate taxa traditionally placed in tribe Psychotrieae (Robbrecht, 1993), but used here as an outgroup based on their placement by Bremer (1996); daggers indicate taxa placed in "group of *Mitchella*" of tribe Morindeae by E. Robbrecht (1993). Vouchers are deposited in herbaria as designated by acronym according to Holmgren et al. (1990).

Taxon	Distribution	Source, voucher and GenBank accession number
Tribe Psychotrieae A. Rich. ex Dum.		
Subtribe Psychotriinae Robbrecht		
1. <i>Amaracarpus</i> sp. = <i>Psychotria hombroniana</i> (Baillon) Fosberg	Pacific	D. Lorence 7844, Micronesia (Kosrae), (PTBG), AF072032
2. <i>Chazalletia abrupta</i> (Hiern) E. Petit & Verdcourt	Africa	B. Bremer 3081, Tanzania (UJS), AF072012
3. <i>Geophila cordifolia</i> Miq.	New World	B. Hammel 18062, Costa Rica (MO), AF072015
4. <i>Geophila macropoda</i> DC.	New World	Natl. Bot. Garden Belgium, acc.# 19841045, AF072017
5. <i>Geophila tenuis</i> (M. Arg.) Standl.	New World	L. Andersson et al. 2025, French Guiana (CAY, GB, S), AF072016
6. <i>Hymenocoleus hirsutus</i> (Benth.) Robbr.	Africa	F. Malaisse 14528, cultivation, Natl. Bot. Gard. Belgium, accession 1995116, AF07201
7. <i>Palicourea guianensis</i> Aubl.	New World	J. Wright s.n., Panama (STRI), AF072010
8. <i>Palicourea leiorrhachis</i> Oerst.	New World	B. Hammel 18027, Costa Rica (MO), AF072009
9. <i>Palicourea paedifolia</i> (Willd. ex Roem. & Schult.) C.M. Taylor & Lorence	New World	B. Hammel 18028, Costa Rica (MO), AF072008
10. <i>Rudgea hostmanniana</i> Benth.	New World	L. Andersson 1954, French Guiana, AF072014
11. <i>Rudgea razeniana</i> W. Burger	New World	B. Hammel 18081, Costa Rica (MO, CR), AF072058, AF072059
<i>Psychotria</i> L. subg. <i>Psychotria</i> Petit emend Steyerf.		
12. <i>Psychotria</i> cf. <i>borjensis</i> H. B. & K.	New World	Cult., BR, Robbrecht s.n. (UPS), AF072041
13. <i>Psychotria brasiliensis</i> Vell.	New World	Cult., Royal Botanic Garden, Edinburgh, acc. 19715317 G09, AF072053
14. <i>Psychotria cadigensis</i> Merrill	Pacific	Cult., Ho'omaluhia Botanic Gardens, Hawaii 82.0104; Philippines (WIS), AF03910
15. <i>Psychotria capensis</i> (Eckl.) Vatke (sect. <i>Flaciflorae</i> Petit)	Africa	M. W. Chase 2268, Transki (K), AF072043
16. <i>Psychotria chagensis</i> Standl.	New World	J. Wright s.n., Panama (STRI), AF072051
17. <i>Psychotria decortifolia</i> Moore	Malasia	cult. Waimea Bot. Gardens, HI, #75P1534 K. Wooliams, Papua New Guinea (WIS), AF034911
18. <i>Psychotria Fitzalanii</i> Benth.	Australia	cult, CSIRO, B. Gray 6235, Queensland, AF072024
19. <i>Psychotria graciliflora</i> Benth.	New World	M. Nepokroeff 508, Costa Rica (WIS), AF0072054, AF072055
20. <i>Psychotria hexandra</i> H. Mann (sect. <i>Pelagomappouria</i>)	Hawaii	D. Lorence 7405, Kauai, Hawaii (PTBG), AF034906
21. <i>Psychotria hobdyi</i> Sohmer (sect. <i>Pelagomappouria</i>)	Hawaii	K. R. Wood # Kauai, Hawaii (PTBG), AF034906
22. <i>Psychotria horizontalis</i> Sw.	New World	J. Wright s.n., Panama (STRI), AF072047
23. <i>Psychotria insularum</i> A. Gray	Pacific	NTBG 890774, T. Flynn 3723, Samoa (PTBG), AF072056, AF072057
24. <i>Psychotria limonensis</i> K. Krause	New World	J. Wright s.n., Panama (STRI), AF072052
25. <i>Psychotria loniceroides</i> Reclinger	Australia	I.R. Telford 8522, New South Wales, Australia, AF072034

TABLE 1. Continued.

Taxon	Distribution	Source, voucher and GenBank accession number
26. <i>Psychotria luzoniensis</i> F. Villar	Philippines	cult., Ho'omaluhia Botanic Gardens, Hawaii; D. Heuschel s.n.; Philippines (WIS), AF072037
27. <i>Psychotria makonii</i> C.H. Wright	Africa	B. Kaplin s.n., Nyungwe Forest, Rwanda (WIS), AF072042
28. <i>Psychotria mapourioides</i> DC.	New World	J. Wright s.n., Panama (STRI), AF072040
29. <i>Psychotria</i> cf. <i>manillensis</i> Bartl. ex DC.	Pacific	cult., Ho'omaluhia Botanic Gardens, Hawaii; D. Heuschel s.n., Mt. Makiling, Philippines (WIS), AF034904
30. <i>Psychotria marginata</i> Sw.	New World	J. Wright s.n., Panama (STRI), AF072049
31. <i>Psychotria martiniana</i> (Cham. & Schlecht.) Fosb. (sect. <i>Straussia</i>)	Hawaii	M. Nepokroeff & T. Flynn 951, Kauai, Hawaii (WIS), AF034905
32. <i>Psychotria mauiensis</i> Fosberg (sect. <i>Straussia</i>)	Hawaii	M. Nepokroeff & J. Lau 915, Moloka'i, Hawaii (WIS), AF034909
33. <i>Psychotria membranacea</i> Humb. & Bonpl. ex Roem. & Schult.	Pacific	cult., Ho'omaluhia Botanic Gardens, Hawaii; D. Heuschel s.n., Philippines (WIS), AF034909
34. <i>Psychotria micrantha</i> H.B.K.	New World	J. Wright s.n., Panama (STRI), AF072048
35. <i>Psychotria rosea</i> Sw.	New World	C. Taylor & J. Miller 10415, Puerto Rico (MO), AF072046
36. <i>Psychotria peduncularis</i> (Salisb.) Steyerem. (sect. <i>Involucratae</i> Petit & Verdc.)	Africa	cult., Edinburgh Bot. Garden acc. Number 19696313, AF072044
37. <i>Psychotria rhombocarpa</i> Kanehira	Pacific	T. Flynn 5996, Kosrae, (PTBG), AF072031
38. <i>Psychotria rubra</i> (Lour.) Poir.	Asia	cult., Edinburgh Bot. Garden, s.n., Hong Kong, AF072022
39. <i>Psychotria serpens</i> Linn.	Asia	cult., NTBG 60619, Hong Kong (PTBG), AF072036
40. <i>Psychotria simmondsiana</i> F.M. Bailey	Australia	L. Bird 210, Australia, AF072023
41. <i>Psychotria submontana</i> Domin	Australia	cult., CSIRO, B. Gray 6221, Australia (QRS), AF072023
42. <i>Psychotria talitensis</i> Drake	Pacific	J. Y. Meyer 421, Tahiti, AF072029
43. <i>Psychotria tenuifolia</i> Sw.	New World	J. Wright s.n., Panama (STRI), AF072050
44. <i>Psychotria</i> sp. (= <i>P.</i> cf. <i>trichocalyx</i>)	Pacific	J. Y. Meyer 415, Tahiti, AF072030
45. <i>Psychotria uawrae</i> Sotner (sect. <i>Straussia</i>)	Hawaii	D. Lorence 7423, Kauai, Hawaii (PTBG) AF034908
46. <i>Psychotria</i> sp.	Marianas	cult., Lyon Arboretum, Hawaii, 82s325, D. Herbst s.n., Tinian Island (WIS), AF072028
48. <i>Psychotria</i> sp. (= <i>Uragoga</i> sp.) (sect. <i>Involucratae</i>)	Africa	B. Bremer 2722, cult. Paris (UPS), AF072033
49. <i>Psychotria</i> sp.	Madagascar	M. Zihra 930, Madagascar (WIS), AF072039
<i>Psychotria</i> subg. <i>Heteropsychotria</i> Steyerem.		
50. <i>Psychotria aubletiana</i> Steyerem.	New World	M. Nepokroeff 757, Costa Rica (WIS), AF072002
51. <i>Psychotria berteriana</i> DC.	New World	J. Miller and C. Taylor 5957, Puerto Rico (MO), AF072003
52. <i>Psychotria brenckampiana</i> Steyerem.	New World	J. Wright s.n., Panama (STRI), AF072000
53. <i>Psychotria brachiata</i> Sw.	New World	C. Taylor & J. Miller 10417, Puerto Rico (MO), AF072001
54. <i>Psychotria brachybotrya</i> Müll. Arg.	New World	J. Wright s.n., Panama (STRI), AF072004

TABLE 1. Continued.

Taxon	Distribution	Source, voucher and GenBank accession number
55. <i>Psychotria borucana</i> (A. Molina R.) C.M. Taylor & W.C. Burger	New World	M. Nepokroëff 716, Costa Rica (WIS), AF072021
56. <i>Psychotria capitata</i> Ruiz & Pav.	New World	J. Wright s.n., Barro Colorado Island, Panama, AF072005
57. <i>Psychotria chiriquiensis</i> (Standl.) C.M. Taylor (= <i>P. moliniana</i>) (sect. <i>Cephaelis</i>)	New World	M. Nepokroëff 761, Costa Rica (WIS), AF071999
58. <i>Psychotria deflexa</i> DC.	New World	J. Wright s.n., Panama (STRI), AF072006
59. <i>Psychotria elata</i> (Sw.) Hammel	New World	M. Nepokroëff 510, Costa Rica (WIS), AF072011
60. <i>Psychotria furcata</i> DC.	New World	J. Wright s.n., Panama (STRI), AF071996
61. <i>Psychotria ipecacuanhina</i> (Brot.) Stokes	New World	J. Wright s.n., Panama (STRI), AF072020
62. <i>Psychotria muscosa</i> (Jacq.) Steyererm.	New World	D. Boche 29, Trinidad (WIS), AF071994
63. <i>Psychotria pittieri</i> Standl.	New World	J. Wright s.n., Panama (STRI), AF071998
64. <i>Psychotria poeppigiana</i> Müll. Arg. (sect. <i>Cephaelis</i>)	New World	M. Nepokroëff 602, Costa Rica (WIS), AF071993
65. <i>Psychotria pubescens</i> Sw.	New World	J. Wright s.n., Panama (STRI), AF071997
66. <i>Psychotria racemosa</i> (Aubl.) Raushel	New World	J. Wright s.n., Panama (STRI), AF071995
67. <i>Psychotria urceolata</i> Steyererm.	New World	L. Andersson 1946, French Guiana (CAY, GB, S), AF072007
Section <i>Notopleura</i>		
68. <i>Psychotria dukei</i> Dwyer	New World	M. Nepokroëff 600, Monteverde, Costa Rica (WIS), AF071989
69. <i>Psychotria guadalupensis</i> DC.	New World	C. M. Taylor 10543, Puerto Rico (MO), AF071992
70. <i>Psychotria macrophylla</i> Ruiz & Pavon	New World	M. Nepokroëff 608, Costa Rica (WIS), AF071990
71. <i>Psychotria uliginosa</i> (Sw.) Brem.	New World	M. Nepokroëff 607, Costa Rica (WIS), AF071991
<i>Psychotria</i> subg. <i>Tetrameræ</i> (Hiern.) Petit		
72. <i>Psychotria kirikii</i> Hiern. (= <i>P. bacteriophila</i>)	Africa	cult., Geneva Bot. Garden (WIS), AF072038
73. <i>Psychotria microdon</i> Urb.	New World	B. Hammel s.n., Costa Rica, AF072013
74. <i>Psychotria mucronata</i> Verd.	Africa	cult., Edinburgh Bot. Garden 19500144 (G35, G65), AF072045
Subtribe <i>Hydnophytinae</i> Huxley and Jebb		
75. <i>Hydnophytum formicarum</i> Jack.	Malesia	cult., University of Wisconsin (WIS), AF034912
76. <i>Hydnophytum</i> sp.	Malesia	cult., Oxford Univ., C. H. Lambrick 132/83, AF03914
77. <i>Hydnophytum noseleganum</i> Becc.	Malesia	cult., Oxford Univ., C. H. Lambrick 103/82, AF034913
78. <i>Anthorhiza</i> sp. (= cf. <i>Anthorhiza clemensii</i>)	Malesia	cult., Oxford Univ., C. H. Lambrick 142/83, AF034915
79. <i>Myrmephytum selebianum</i> (Becc.) Becc.	Malesia	cult., Oxford Univ., C. H. Lambrick 24857, AF034916
80. <i>Myrmecodia armata</i> DC.	Malesia	cult., Goteborg Botanical Garden, s.n., AF034917
81. <i>Myrmecodia platyrea</i> Becc.	Malesia	B. Bremer B18, S. coll. 653 (CONIN), AF034918
82. <i>Myrmecodia</i> sp. (= <i>Myrmecodia</i> cf. <i>horrida</i>)	Malesia	cult., Oxford Univ., C. H. Lambrick 142/83, AF071988

TABLE 1. Continued.

	Taxon	Distribution	Source, voucher and GenBank accession number
OUTGROUPS			
83.	<i>Gaertnera paniculata</i> Benth.*	Africa	G. McPherson 16334 (MO), unpublished sequence
84.	<i>Gaertnera psychotrioides</i> (DC.)* Baker	Mauritius	S. Malcomber s.n. (MO), unpublished sequence
85.	<i>Mitchella repens</i> L.†	North America	cult., University of Wisconsin (WIS), AF072019

grams and contiguous alignments were edited using Sequencher vers. 3.0 (Gene Codes Corp.). Boundary regions for the coding regions of the 18S, 5.8S and 26S rDNA and spacer regions were determined by comparison with DNA sequences obtained from previous research on *Psychotria* (Nepokroeff 1992). Sequences from the last 29 base pairs of the 18S gene, ITS 1, 5.8S cistron and the ITS 2 region were included in the analysis. For amplification and sequencing reactions of *rbcL*, we used methods described in Bremer et al. (1995).

Sequence Analysis. A set of ITS sequences including a subset of the ingroup taxa and outgroups was aligned initially using the MALIGN program vers. 1.99 (Wheeler and Gladstein 1992) to produce an initial alignment; remaining sequences were aligned by hand to minimize the the number of single base pair substitutions. In the case of alternative alignments, the one that generated the fewest informative characters was chosen as a conservative measure. Separate analyses excluded the indel sites (i.e., treated indels as missing data) and scored indels as separate characters at the end of the data matrix (see Baum et al. 1994). The phylogenetic utility of indels has been promoted by Lloyd and Calder (1991) and used extensively in phylogenetic analyses of the ITS region in plants (see review in Baldwin et al. 1995). Transition to transversion ratios, GC content, number of informative characters and sequence length were determined using test version 4.0d64 of PAUP*, written by David L. Swofford. Percent nucleotide distance for ITS sequences was calculated using the Hasegawa et al. (1985), or HKY85, model of sequence divergence using PAUP* 4.0d64. *rbcL* sequences were aligned manually to sequences previously obtained for *Psychotria* and related taxa in the family Rubiaceae (Bremer et al. 1995). Pairwise sequence divergence values for *rbcL* sequences were calculated using PAUP* 4.0d64.

Phylogenetic Analyses. A baseline maximum parsimony analysis was performed on ITS sequences with PAUP* 4.0d64 using heuristic searches on the aligned data set (excluding uninformative characters and characters at positions 427–445 bp and 610–652 due to ambiguous alignment at those sites) and assuming unordered character states (i.e., Fitch parsimony). The characters were given equal weight and indels were treated as missing data in this “baseline” analysis. In order to sample the large tree space

TABLE 2. Accessions of *Psychotria*, other genera of tribe Psychotrieae, and related tribes used in phylogenetic analysis of *rbcL* sequences. Vouchers are deposited in herbaria as designated by acronym according to Holmgren et al. (1990).

Taxon	Distribution	Source, voucher, GenBank no.
Tribe Psychotrieae A. Rich. ex Dum subtribe Psychotriinae Robbrecht		
1. <i>Amaracarpus</i> sp.	Indo-Pacific	A. P. Parvis 680, Indonesia (K), AJ002176
2. <i>Clasalia parviflora</i> K. Schum.	Africa	B. Bremer 3067, Tanzania (UPS), Z68790
3. <i>Chazaliella abrupta</i> (Hiern) Petit & Verdcourt	Africa	B. Bremer 3081, Tanzania (UPS), Z68791
4. <i>Declieuxia fruticosa</i> (Willd. ex Roem & Schult.) Kuntze	New World	B. Hammel 20875, Costa Rica (MO, CR), AJ002177
5. <i>Gaertnera</i> sp.	Old World	S. Malcomber 2709, Madagascar (MO), Z68797
6. <i>Geophila repens</i> (L.) I.M. Johnston.	panitropical	B. Bremer 3130, cultivation (UPS), Z68798
7. <i>Hymenocoleus hirsutus</i> (Benth.) Robbrecht	African	F. Malaisse 14528, cult. Natl. Bot. Gardens, Belgium accession 1995116, AJ002178
8. <i>Lasianthus pectunculatus</i> E. A. Bruce	Old World	K. Andreason 71 (UPS), Tanzania, Z68802
9. <i>Palicourea lasiorachis</i> Oerst.	New World	B. Hammel 18028, Costa Rica (MO, CR), AJ002179
10. <i>Rudgea cf. lorentensis</i> Standl.	New World	B. Bremer et al. 3346, Ecuador, (MO, QCA, QCNE), Z68821
11. <i>Rudgea sessiliflora</i> Standl.	New World	B. Bremer 3334, Ecuador, (MO, QCA, QCNE, UPS), AJ002186
<i>Psychotria</i> subg. <i>Psychotria</i> Petit emend Steyerm.		
12. <i>Psychotria</i> cf. <i>borjensis</i> H. B. K.	New World	E. Robbrecht s.n., cultivation (UPS), Z68804
13. <i>Psychotria graciliflora</i> Benth.	New World	M. Nepokroeff 508, Costa Rica (WIS), AJ002183
14. <i>Psychotria martiniana</i> (Cham. & Schlect.) Fosb.	Hawaii	M. Nepokroeff 951, Hawaii (WIS), AJ002184
15. <i>Psychotria nervosa</i> Sw.	New World	C. Taylor & J. Miller 10415 Puerto Rico (MO), AJ002187
16. <i>Psychotria petersii</i> E.Petit	Africa	B. Bremer 3078, Tanzania (UPS), Z68818
17. <i>Psychotria</i> sp.	Africa	B. Bremer 2722 (UPS), Tanzania, Z68817
18. <i>Psychotria</i> sp. ("Cephaelis")	Africa	B. Bremer 2727, cultivation, (UPS), AJ002188
<i>Psychotria</i> subg. <i>Heteropsychotria</i> Steyerm.		
19. <i>Psychotria borucana</i> (A. Molina R.) C. M. Taylor & W. C. Burger	New World	M. Nepokroeff 716, Costa Rica (WIS), AJ002180
20. <i>Psychotria bremekampiana</i> Steyerm.	New World	J. Wright s.n., Panama (STR1), AJ002180
21. <i>Psychotria dukei</i> Dwyer	New World	M. Nepokroeff 600, Costa Rica (WIS), AJ002182
22. <i>Psychotria ipeacacantha</i> (Brot.) Strokes	New World	J. Wright s.n., Panama (STR1), AJ002184
23. <i>Psychotria poeppigiana</i> Muell. Arg.	New World	B. Bremer et al. 3330, Ecuador (UPS, QCA, QCNE), Z68818
<i>Psychotria</i> subg. <i>Tetramerne</i> Petit		
24. <i>Psychotria kirkii</i> Hiern. (<i>bacteriophila</i>)	Africa	B. Bremer 3102, cultivation, (UPS), X83663
Subtribe Hydnophytinae Huxley and Jebb		
25. <i>Hydnophytum formicarium</i> Jack	Malasia	B. Bremer 2701, cultivation, (CONN) X83645
26. <i>Myrmecodia platyrea</i> Becc.	Malasia	B. Bremer 1318, S. coll. 653 (CONN), X87147

TABLE 2. Continued.

Taxon	Distribution	Source, voucher, GenBank no.
Tribe Coccypseleae Bremek.		
27. <i>Coccypselum</i> sp.	New World	B. Bremer 3067 (UPS), X87145
Tribe Coussareae Hook. f.		
28. <i>Coussarea macrophylla</i> Müll. Arg.	New World	B. Bremer 3339, Ecuador (MO, OCA, OCNE, UPS), Y11847
29. <i>Faramea multiflora</i> A. Rich. ex DC.	New World	B. Bremer et al. 3331, Ecuador (MO, QCA, QCNE, UPS), Z68796
Tribe Morindeae Miq.		
30. <i>Dammacanthus indicus</i> Gaertn.	South East Asia	B. Bremer 3107, cultivation (MO), Z68793
31. <i>Morinda citrifolia</i> L.	Old World	B. Bremer 3106, cultivation (UPS), X83651
32. <i>Mitchella repens</i> L.	NW and SE Asia	B. Bremer 2714, cultivation, MO (UPS), Z68805
Tribe Ophiorrhizeae Bremek. ex Verdc.		
32. <i>Ophiorrhiza mungos</i> L.	Old World	Robbrecht s.n., cult. (BR), X83656
Tribe Pauridiantheae Bremek. ex Darwin		
33. <i>Pauridiantha paucineris</i> (Hiern.) Bremek.	Africa	B. Bremer 3090, Tanzania (UPS), Z68804
Tribe Schradereae		
34. <i>Schradera subandina</i> Krause	New World	J. Clark & C. Watt 783, Ecuador (MO, QCNE, UPS), Y11859

and detect the presence of "multiple islands" of most parsimonious trees (Maddison 1991), the four-step search method (Olmstead et al. 1993; Conti et al. 1996) was employed. The tendency for the topology of the ITS trees to vary was explored using gaps recoded in a separate data matrix. Additionally, use of a step matrix of user-defined character types with a weighting strategy of 1:1.1 and 1:2 (transitions relative to transversions) was employed on the nucleotide data, and these were combined with weighting of indel characters. Constraint analyses were used to test alternative hypotheses of relationships in PAUP* 4.0d64.

Phylogenetic analysis of the 35 *rbcL* sequences was conducted using maximum parsimony, with equal character weights and with transversions weighted relative to transitions using estimated biases for each codon position (Albert et al. 1993). The four-step search strategy described above for the ITS data was employed with the *rbcL* data to detect the presence of multiple islands of most-parsimonious trees. Support for internal branches in trees resulting from analysis of the ITS and *rbcL* datasets was evaluated using the "parsimony jackknife" procedure of Farris et al. (1996) using 100,000 replicates of the fast search option in PAUP*, emulating JAC resampling with 36.8% nominal deletion and "collapse=amb" under the condense trees option. Bremer support (Bremer 1988) was calculated using the enforce topological constraints option in PAUP* vs. 4.0d64.

Morphological characters. To explore the phylogenetic utility of morphological characters used in traditional classifications of the Psychotriaceae, character state evolution of three morphological characters, showy involucre bracts, pyrene germination slits and habit was reconstructed using the assumptions of maximum parsimony with the "Trace Character" facility in MacClade vers. 3.04 (Maddison and Maddison 1992). The lineages represented in this analysis comprise all major groups bearing the features in question, based on the work of Steyermark (1972) and others, assuming monophyly of the groups. Additionally, these lineages are well-defined morphologically and anatomically, and for the scale of traits investigated, have been sampled such that the traits are tightly linked to the placeholder taxa. Ancestral character states of the core Psychotriaceae were reconstructed at internal nodes and branches using the simplified

cladogram inferred from ITS sequences. In the event of equivocal reconstructions, all most parsimonious states were shown at each node rather than arbitrarily resolving with ACCTRAN or DELTRAN (Maddison and Maddison 1992) optimization.

RESULTS

Sequence Analysis. The range of lengths of all of ITS 1, ITS 2 spacers and the 5.8S cistron is similar to that found in other angiosperms (Baldwin et al. 1995). The 5.8S cistron is 164 bp in length for almost all taxa. The insertion of 36 gaps was required to align the ITS sequences from species of *Psychotria* and relatives in the tribe Psychotriaceae and the outgroup species of *Gaertnera*. Twenty of these indels could be scored as phylogenetically informative. Percent nucleotide distance values showed a tremendous range of sequence divergence from 0.85% between congeneric species to 37.8% between *Mitchella* and *Psychotria serpens* Linn. This value of ITS sequence divergence is higher than that reported in other studies (Baldwin et al. 1995), but similar to values obtained for the comparably large legume genus *Astragalus* (Sanderson and Wojciechowski 1996), the family Agavaceae (Bogler and Simpson 1996), and subfamily Apioideae of Apiaceae (Downie and Katz-Downie 1996). Most pairwise distances were below 14% within well-supported clades described below, but were 10%–30% between those clades. The transition–transversion ratio (based on only unambiguous sequences) calculated for the entire ITS 1, 5.8S and ITS 2 region is 1.48, similar to that obtained for subfamily Apioideae (1.43; Downie and Katz-Downie 1996), but slightly higher than that reported for *Epilobium* (Baum et al. 1994). The GC content for the entire ITS region is 54.3%, similar to other reported values for ITS (Baldwin et al. 1995). For the entire ITS region, 639 nucleotide sites were unambiguously aligned and included in the analysis. Of these sites, 105 were constant and 534 were variable, with more variable sites (216) occurring in the ITS 2 spacer region than in the ITS 1 region (175); 18 sites were variable in the included 29 characters of the 18S gene, and 125 sites were variable in the 5.8S cistron. Of the variable sites, 329 were phylogenetically informative with 7 phylogenetically informative characters occurring in the included 29 base pairs of the 18S gene, 147 in the ITS 1

spacer, 35 in the 5.8S cistron and 140 in the ITS 2 spacer regions.

The *rbcL* data set consisted of 1402 nucleotides, from position 27 to 1428 of the *rbcL* gene, with 187 characters phylogenetically informative for 35 terminal taxa. Pairwise sequence divergence values for the Psychotrieae and closest relatives calculated using the Kimura 2-parameter model of sequence divergence ranged from 1.10% for the least divergent pair of taxa (*Psychotria nervosa* and *Psychotria peterii*) to 8.2% for the most divergent pair of taxa (*Psychotria graciliflora* and *Faramea multiflora*). The highest pairwise sequence divergence between the members of the tribe Psychotrieae and closest relatives is slightly higher than values reported for the family Onagraceae (Conti et al. 1993).

Phylogenetic Analysis of ITS. The baseline ITS analysis resulted in 264 shortest trees with a length of 2,142 steps (when uninformative characters are not included, CI' = 0.373, RI = 0.674, for 85 terminal tax) and 2,307 steps when uninformative characters are included (CI = 0.325). The strict consensus of these trees is well-resolved, as indicated by solid lines on the branches of tree shown in Fig. 1. Weighting transitions relative to transversions 1:1.1 results in 48 trees, these being a subset of the 264 trees obtained in the unweighted baseline analysis. One of these 48 trees is shown in Fig. 1, with branch lengths, Bremer support, and parsimony jackknife values indicated. Using a slightly higher weighting scheme of 1:2 (transitions to transversions) resulted in 216 shortest trees (not shown) with lengths of 2,152 steps under Fitch parsimony, or ten steps longer than the trees derived from unweighted or weighted analyses using a 1.1:1 weight. Maximum parsimony analysis that included indels (weighted equally with the substitution characters) resulted in the same 264 shortest trees obtained under Fitch assumptions, but with a length of 2,220 steps (or 2,385 when uninformative characters are included). Using 1:1.1 weighting (transitions to transversions) and indels resulted in the same set of 48 trees obtained with weighting alone.

A summary of the major relationships of the Psychotrieae inferred from the parsimony analyses of ITS data is shown in Figure 2. The ITS trees indicate that the core Psychotrieae comprise two major clades: (1) consisting of *Psychotria* subg. *Psychotria* (from the neotropics, Australasia and Africa), subg. *Tetrameræ*, a "Pacific

clade" comprised of some members of Pacific subg. *Psychotria*, the segregate genus *Amaracarpus*, and the subtribe Hydnohytinae—this first clade hereafter referred to as "*Psychotria sensu stricto*"; and (2) consisting of other genera of tribe Psychotrieae, plus those species currently assigned to the neotropical subg. *Heteropsychotria* (Figs. 1 and 2). *Psychotria sensu lato* is broadly paraphyletic, with species falling into at least five different clades, the "*Psychotria sensu stricto*" clade, the "*Heteropsychotria* plus *Palicourea* clade" (neotropical *Psychotria* subg. *Heteropsychotria* and two species of *Palicourea*), and at least three different monophyletic lineages more closely related to other genera in the tribe than to *Psychotria sensu stricto*. These three lineages include *Psychotria microdon* Urb. and its sister clade, *Chazaliella* (Africa), the neotropical *Psychotria* sect. *Notopleura*, and a smaller clade consisting of the neotropical *P. ipeacuanha* and *P. borucana* (see Fig. 2). To test the alternative hypothesis that *Psychotria* subg. *Psychotria*, subg. *Heteropsychotria* and subg. *Tetrameræ* form a monophyletic group, trees were constrained using the enforce topological constraints option in PAUP*. When subtribe Hydnohytinae and the three representatives of *Palicourea* were excluded from the monophyletic "*Psychotria*," the shortest tree was 2,310 steps (excluding uninformative characters) or 158 steps longer than the most parsimonious trees, which represents a 7.3% increase in tree length over the shortest trees.

Phylogenetic Analysis of *rbcL*. The unweighted heuristic four-step search of the *rbcL* sequences resulted in 24 shortest trees with lengths of 577 steps and CI' and RI of 0.437 and 0.646, respectively (including uninformative characters, the tree is 725 steps with a CI of 0.552). Weighted parsimony analysis using the weighting scheme of Albert et al. (1993) resulted in a single most parsimonious tree, which is one of the 24 obtained from the unweighted (Fitch) analysis. This single, weighted parsimony tree is depicted in Fig. 3; branches that collapse in the strict consensus of the 24 Fitch trees are shaded in gray. These results indicate that tribe Psychotrieae as currently recognized (cf. Robbrecht 1988) is paraphyletic, with *Declieuxia*, *Gaertnera*, and *Lasianthus* more closely related to other genera in a number of tribes than they are to the "core" monophyletic Psychotrieae (Fig. 3). The neotropical genera *Coussarea*, *Faramea*, and *Coccyoselum* (Coccyoseleae) and *Declieuxia* (Psy-

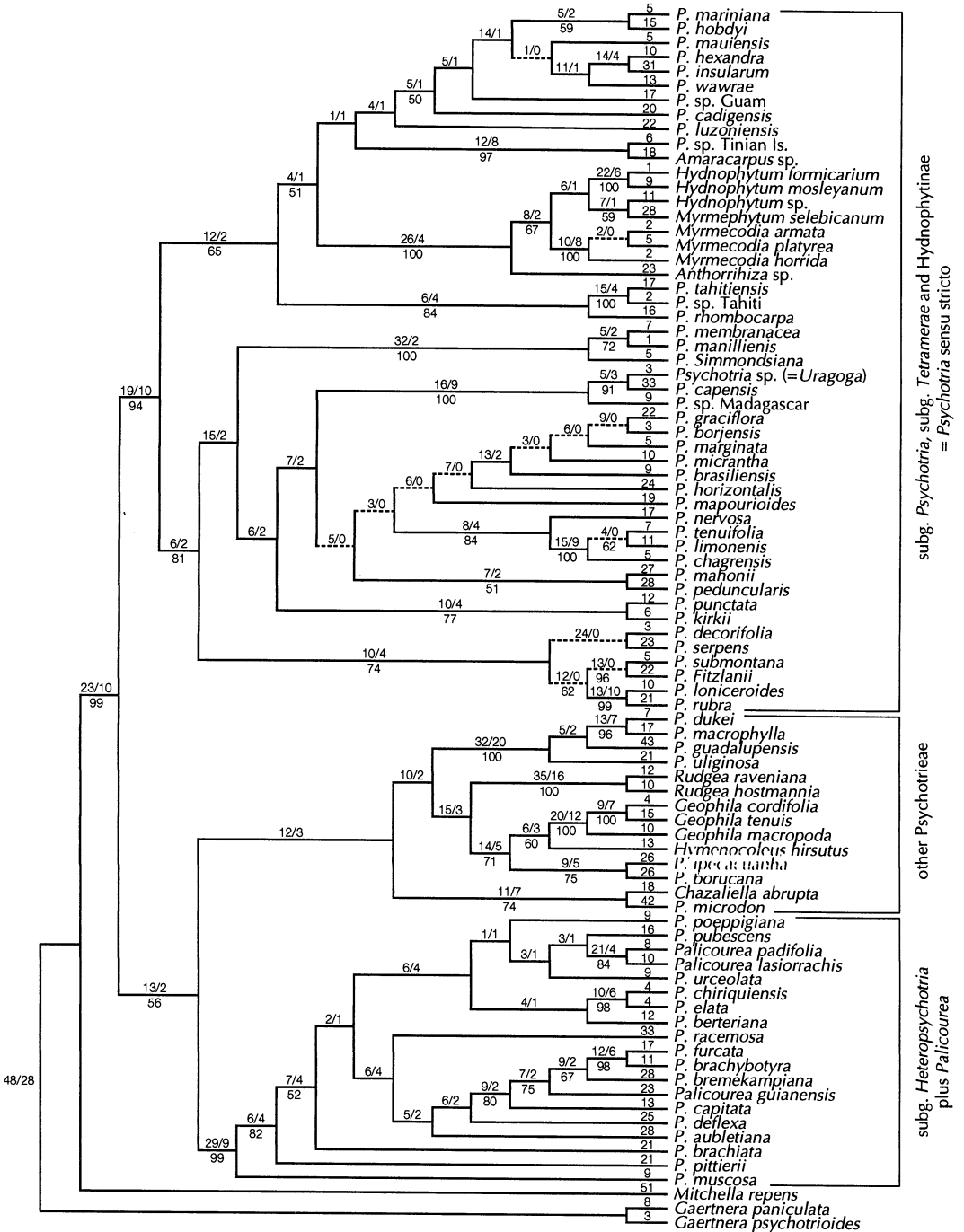


FIG. 1. One of 48 trees resulting from maximum parsimony analysis of ITS sequences, weighting transversions to transitions 1.1 to 1, also one of 264 trees resulting from Fitch parsimony analysis (no weighting). Branch lengths and Bremer support are shown above branches and parsimony jackknife support greater than 50% is shown below branches. Branches that collapse in the strict consensus of the 264 trees are indicated with dashed lines. The genus *Psychotria* is broadly paraphyletic with members of subg. *Heteropsychotria* more closely related to other genera of the tribe than other *Psychotria* species.

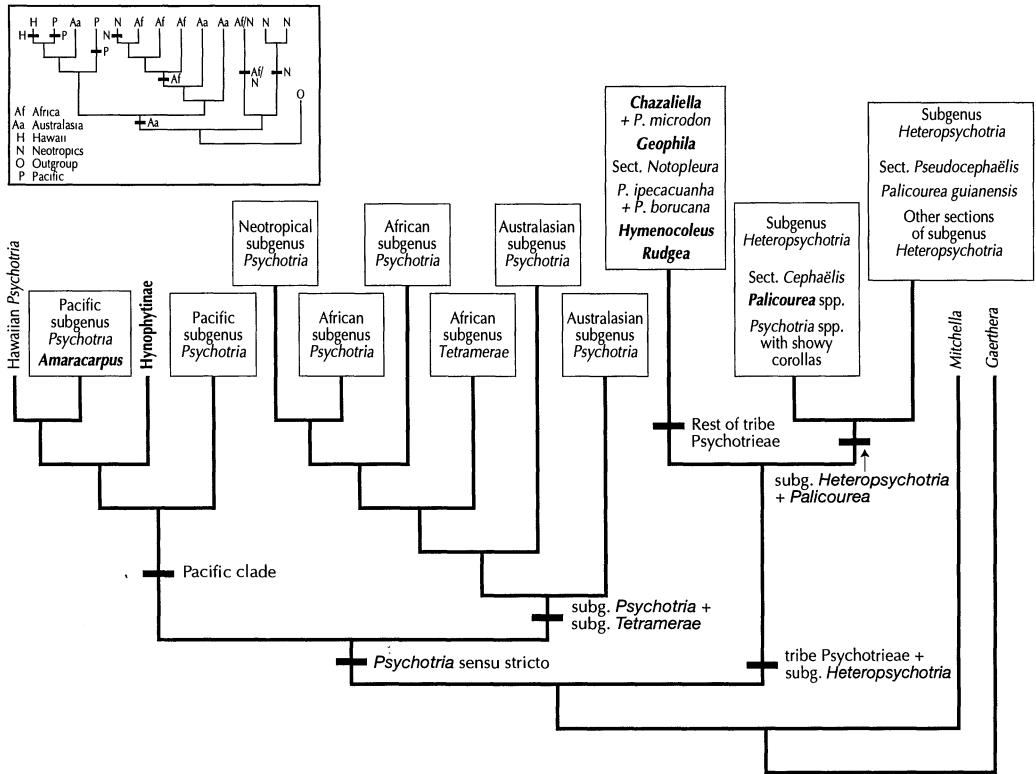


FIG. 2. Summary of principal relationships among Psychotrieae inferred from phylogenetic analysis of ITS sequences. Box in upper left depicts biogeographical history of Psychotrieae. The Psychotrieae are an ancestrally Australasian group with one radiation occurring in the neotropics and subsequently to Africa (tribe Psychotrieae plus subg. *Heteropsychotria*). A second radiation (the *Psychotria sensu stricto* lineage) is comprised of two smaller lineages, with one radiation occurring in Africa and subsequently the neotropics, and another occurring in the Pacific (including the Malesian Hydnophytinae) and Hawaii.

chotrieae) form a monophyletic lineage that is sister to the "core" Psychotrieae. The core Psychotrieae form two major clades with one consisting of members of *Psychotria* subg. *Psychotria*, subg. *Tetramerae*, *Amaracarpus*, and the Hydnophytinae (= the *Psychotria sensu stricto* clade), and with the second consisting of other "core" Psychotrieae (represented in this analysis by *Chasallia*, *Geophila*, *Hymenocoleus*, *Rudgea*, *Palicourea*, and various members of *Psychotria* subg. *Heteropsychotria*).

Although the taxonomic samples in the *rbcl* and ITS analyses are not completely overlapping, relationships within the Psychotrieae derived from the *rbcl* analysis are completely consistent with those obtained using ITS. Congruency between the ITS- and *rbcl*-derived phylogenies includes: (1) paraphyly of *Psychotria*, with

Psychotria subg. *Heteropsychotria* more closely related to other genera of tribe Psychotrieae than to subg. *Psychotria*, (2) paraphyly of subg. *Heteropsychotria*, and recognition of a number of distinct monophyletic sublineages including *Psychotria* sect. *Notopleura*, *P. ipecacuanha* and *P. borucana*, and subg. *Heteropsychotria* plus *Palicourea*, and (3) paraphyly of the *Psychotria sensu stricto* clade with respect to the ant-plants of subtribe Hydnophytinae.

DISCUSSION

The results presented here represent the first explicitly phylogenetic analysis of *Psychotria* and tribe Psychotrieae. This analysis indicates that *Psychotria*, as currently circumscribed, is broadly paraphyletic and in need of reorganization to

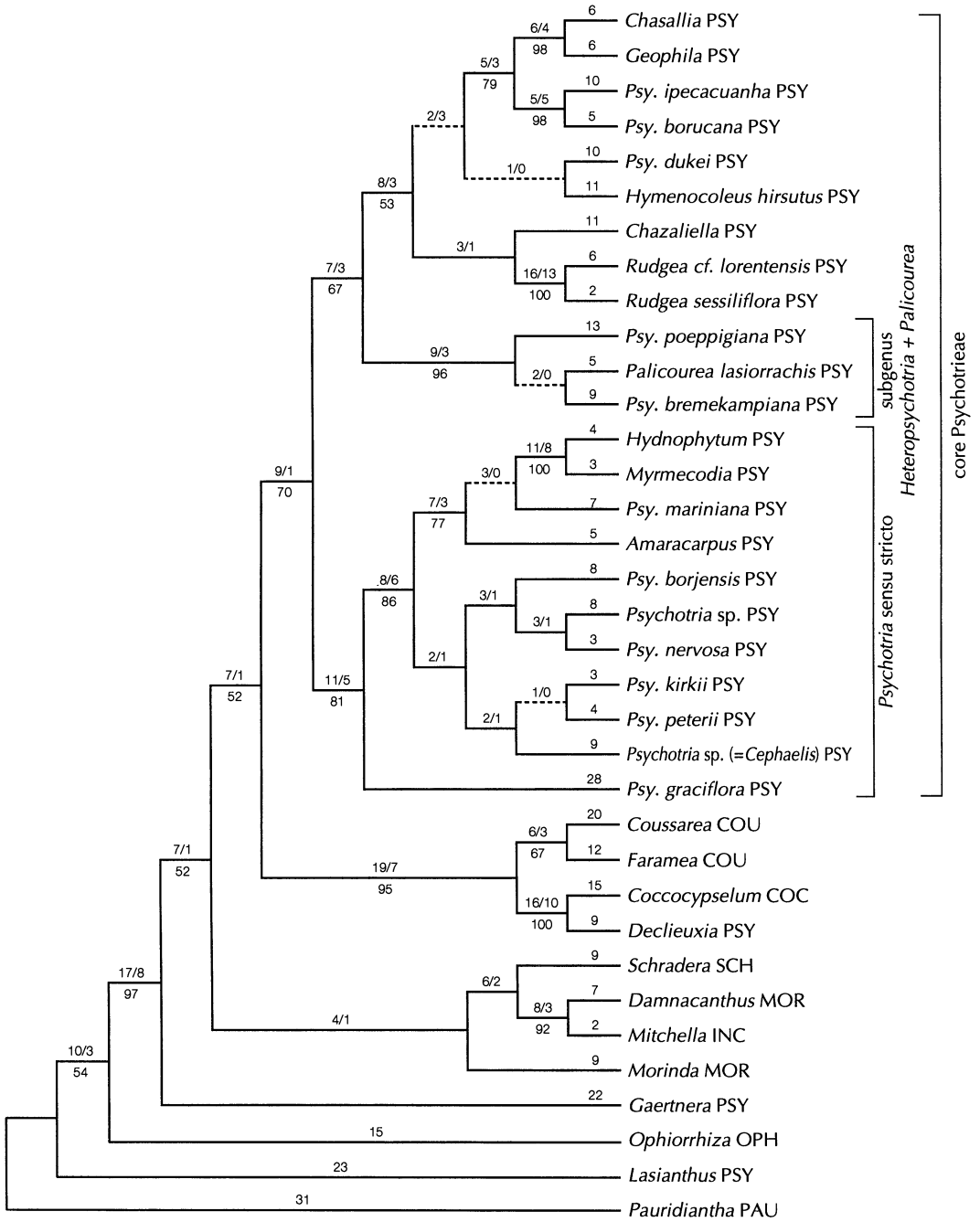


FIG. 3. Single tree resulting from weighted analysis of *rbcL* sequences from Psychotriaceae and related taxa. The tree is also one of 24 resulting from unweighted analysis of the same *rbcL* data. Branch lengths and Bremer support are indicated above the branches and parsimony jackknife support below. Branches that collapse in the strict consensus of the trees resulting from unweighted analysis are indicated with dashed lines.

better reflect natural groupings based on phylogenetic relationships. Although some authors (e.g., Robbrecht 1989) have suggested that *Psychotria* be subdivided into smaller segregate genera, a sound basis for the division of *Psychotria* has been largely lacking. The results of this phylogenetic analysis support the idea that *Psychotria* sensu stricto be reduced and reorganized to include: (1) *Psychotria* subg. *Psychotria* from the neotropics, Australasia, and Africa, (2) subg. *Tetrameræ* from Africa, (3) subtribe Hydnophytinae from Malesia, and (4) the segregate Pacific and Asian genus *Amaracarpus* (Figs. 1 and 2). Species of subg. *Heteropsychotria* and sect. *Notopleura* are more closely related to *Chazaliella*, *Geophila*, *Hymenocoleus*, *Palicourea*, and *Rudgea* than they are to other members of *Psychotria* in the strict sense, and should be elevated to generic rank. Relatively short branch lengths and moderate levels of support for the lineage comprising *Psychotria* subg. *Heteropsychotria* plus *Palicourea* and the remainder of tribe Psychotrieae may have resulted from rapid radiation of the lineage. Presented below is a more detailed examination of the two major clades (and their subclades) identified by ITS and *rbcL* analyses—the *Psychotria* sensu stricto clade, and the clade comprising the remainder of the core Psychotrieae (including all other genera in the tribe, plus those *Psychotria* species currently assigned to the neotropical subg. *Heteropsychotria*).

Relationships and Monophyletic Groups within the *Psychotria* sensu stricto Clade. The monophyly of *Psychotria* sensu stricto is strongly supported by parsimony jackknife values of 94% in the ITS analysis and 81% in the *rbcL* analysis and has been supported by several lines of morphological evidence. Steyermark (1972) characterized subg. *Psychotria* by stipules deciduous, leaves drying red-brown or black (due to the presence of alkaloids), presence of hairs at the base of the stipule scar, the ventral face of the pyrene either planar or with two fissures, and the dorsal surface of the pyrene with four or five lobes. Hamilton (1985) monitored growth form in species of subg. *Psychotria* and subg. *Heteropsychotria* in the neotropics and found that all examined species of subg. *Psychotria* share the same type of branching patterns, whereas members of subg. *Heteropsychotria* possess different and variable architectures. Johansson (1992) concluded that subg. *Psychotria* in the neotropics and Africa and subg. *Tetrameræ* comprise a

monophyletic clade based on shared similarities of pollen architecture. Additionally, based on the lack of preformed germination slits in pyrenes in *Psychotria* sensu stricto, Robbrecht (1989) suggested a narrow circumscription for *Psychotria*. Similarly, Solis et al. (1995) demonstrated the presence of a special kind of indole-type alkaloid found in subg. *Psychotria* but not in subg. *Heteropsychotria*. Thus, there are numerous morphological characters in (i.e., potential synapomorphies) supporting monophyly of *Psychotria* sensu stricto.

Members of the subtribe Hydnophytinae (*Hydnophytum*, *Myrmecodia*, *Myrmephytum*, and *Anthorrhiza*) are most closely related to a group of *Psychotria* species from Micronesia and French Polynesia (*Psychotria* aff. *trichocalyx*, *P. tahitiensis*, and *P. rhombocarpa*)—rendering *Psychotria* sensu stricto paraphyletic unless subtribe Hydnophytinae is included. Huxley and Jebb (1990) recognized the five epiphytic myrmecophilous genera (including *Squamellaria*) as subtribe Hydnophytinae of the Psychotrieae. A close relationship of Hydnophytinae and *Psychotria* had been recognized by Huxley and Jebb (1991a,b). Indeed, Sohmer (1988) had hypothesized that the Hydnophytinae were derived directly from climbing species of Malesian *Psychotria*—a group of poorly known species. A thorough cladistic analysis of molecular and morphological characters in the Hydnophytinae is clearly needed. The extent of nomenclatural reorganization of the tribe Psychotrieae implied by the results of the phylogenetic analysis is exemplified by the inclusion of a subtribe (the Hydnophytinae) within a genus (*Psychotria*). If monophyletic, Hydnophytinae may be better ranked as a subgenus of *Psychotria*.

The segregate Indo-Pacific genus *Amaracarpus* was described by Blume (1826) based on the feature of axillary inflorescences and small-leaved habit. Recent authors have not agreed on the systematic value of inflorescence position, with some reorganizing the genus (Darwin 1979) and others placing *Amaracarpus* within *Psychotria* (Fosberg and Sachet 1991; Fosberg et al. 1993). Fosberg and Sachet (1991) noted that species assigned to *Amaracarpus* in Papua New Guinea and Indonesia share the characters of small leaves and single, axillary flowers, distinct from the form seen in Micronesia. Thus, it is not possible to establish synonymy of all species currently assigned to *Amaracarpus* pending inclu-

sion of additional taxa from Malesia. Results of both the ITS and *rbcl* analyses support the view that *Amaracarpus*—represented by *Psychotria hombroniana*—should be placed within *Psychotria*.

The ITS analysis also identifies a group of western Pacific species, from Micronesia, the Philippines, and Samoa, as the closest relatives to Hawaiian *Psychotria* species. Sohmer (1977, 1978) postulated that the closest relatives of the Hawaiian species originated in the Philippines and Papua New Guinea, as these regions are high in *Psychotria* diversity and are considered the source area for many other tropical elements of the Hawaiian flora (Carlquist 1981). A more detailed phylogenetic analysis (Nepokroeff 1997), utilizing all endemic species and multiple populations of Hawaiian *Psychotria*, has determined the number of separate introductions of the genus into Hawaii and established patterns of colonization and speciation.

The *Psychotria* subg. *Heteropsychotria* plus *Palicourea* Clade. Results of the ITS analysis strongly support the idea that species of the entirely neotropical *Psychotria* subg. *Heteropsychotria* together with neotropical *Palicourea* species may be better considered a separate genus. *Palicourea*, as currently defined (Taylor 1997), is a polyphyletic group whose circumscription is based on the characters of colorful inflorescences and corollas with gibbous swelling at the base and colorful inflorescences—a suite of characters adapted to hummingbird pollination. The type species of the genus, *Palicourea guianensis*, is shown here to be embedded within subg. *Heteropsychotria* (of sect. *Pseudocephaelis*), whereas the other two species of *Palicourea* represented in the analysis are more closely related to members of sect. *Cephaelis* and other sections of subg. *Heteropsychotria* (Fig. 1). Most recent treatments (e.g., Steyermark 1972; Taylor 1996, 1997), however, acknowledge the potential homoplasious nature of the floral characters used to define *Palicourea*. Some *Psychotria* species (e.g., *P. berteriana* and *P. pubescens*) and *Palicourea* exhibit a color spectrum in the inflorescence and corolla and the characteristic swollen, corolla-tube base.

Constraining the heuristic searches so that all the *Palicourea* species are monophyletic resulted in a tree that is 13 steps longer than the most parsimonious trees otherwise found. The polyphyletic nature of *Palicourea* is further supported by certain morphological characters. First, *Palicourea padifolia* and *Pal. lasiorrachis* form a sister

clade to *Psychotria pubescens*, which possesses yellow rather than the typical white flowers of other species of subg. *Heteropsychotria*. Second, some species of *Palicourea* and *Psychotria berteriana*, and other members of subg. *Heteropsychotria* show a similar pattern of pyrene germination slits—two or three slits on the dorsal side of the pyrene (F. Piesschart, National Botanical Garden, Belgium, also confirms this observation in independent analysis, pers. comm.). Thus, characters traditionally used to define *Palicourea* apart from *Psychotria* intergrade and can be found in taxa currently assigned to *Psychotria*. Further detailed morphological studies, especially pyrene germination slit patterns, may yield important new characters to define monophyletic lineages in the *Heteropsychotria*–*Palicourea* clade. Additional sampling in *Palicourea* should indicate the number of separate lineages comprising this unnatural group.

While the re-classification presented here of neotropical lineages currently recognized as *Psychotria* is radical, it is not novel—similar relationships, in part, were suggested by Müller-Argovienensis (1881) and by Taylor (1996). Additionally, Taylor (1989) suggested that species of *Palicourea* are most closely related to members of subg. *Heteropsychotria* based on their shared persistent and continuously connate stipules, versus the deciduous stipules found in other *Psychotria* species (e.g., subg. *Psychotria*). Müller-Argovienensis (1881) considered subg. *Psychotria* to be “*Mapouria*” and subg. *Heteropsychotria* together with *Palicourea* to comprise the genus “*Psychotria*”—a division that was later rejected by Steyermark (1972), Hamilton (1989a), and others.

***Psychotria microdon* and *Chazaliella*.** The neotropical *Psychotria microdon* is supported as the sister group to the African *Chazaliella* with a parsimony jackknife value of 74% and Bremer support of seven steps. *Psychotria microdon* is an unusual species among neotropical Psychotriaceae, and has been considered a lineage of the African *Psychotria* subg. *Tetramerae* (Hamilton 1989c; Taylor 1996). While the placement of *P. microdon* with *Chazaliella* is unexpected, in retrospect the two taxa share many similarities including strongly dorsally flattened pyrenes with two marginal germination slits, and pale seeds. The close relationship between the two taxa suggests that *P. microdon* may either be a disjunct member of *Chazaliella* or a neotropical sister genus of *Chazaliella*. More complete sampling of *Chazaliella*

and the neotropical taxa thought to be closely related to *P. microdon* should clarify these issues.

Psychotria ipecacuanha, P. borucana, Geophila, and Hymenocoleus. Two neotropical species currently assigned to subg. *Heteropsychotria*, *P. ipecacuanha* and *P. borucana*, appear more closely related to the assemblage of paleo- and neotropical genera of Psychotrieae than to the subg. *Heteropsychotria* plus *Palicourea* clade. The neotropical *P. borucana* is strongly supported as sister to *P. ipecacuanha* in the ITS analyses, with parsimony jackknife support of 75% and Bremer support of five steps. *Psychotria ipecacuanha* and *P. borucana* share many morphological similarities, including pyrene anatomy, a succulent, subwoody, and unbranched habit, and densely capitate inflorescences subtended by showy involucre bracts. Pyrene anatomical studies conducted independently by F. Piesschart (National Botanic Garden, Belgium, pers. comm.) suggest strong affiliation between *P. ipecacuanha* and the pantropical *Geophila*, a genus of herbaceous creeping herbs. The results of the present phylogenetic analysis place *P. ipecacuanha* and *P. borucana* as the sister to the clade containing *Geophila* and *Hymenocoleus*, supporting the classification based on pyrene characters.

Psychotria sect. Notopleura. The group of neotropical species currently treated as members of *Psychotria* sect. *Notopleura* (subg. *Heteropsychotria*) has been long recognized as a morphologically distinct group (Bentham 1852; Bremekamp 1934; Hamilton 1985; Nepokroeff 1992; Nepokroeff and Sytsma, in press). Characters such as suffrutescent growth form (and succulent epiphytes) and pseudo-axillary inflorescences have set members of sect. *Notopleura* apart from other species of neotropical *Psychotria*, leading Bremekamp (1934) to give the section generic status. Results of the ITS phylogenetic analysis presented here strongly support sect. *Notopleura* as a monophyletic group with parsimony jackknife values of 100% and Bremer support of 20 steps. Section *Notopleura* is closely related to other genera of tribe Psychotrieae, including neotropical *Rudgea*, African *Chasallia* and *Chazaliella*, pantropical *Geophila*, African *Hymenocoleus*, and neotropical *Psychotria microdon*, *P. ipecacuanha*, and *P. borucana*. Additionally, pyrene characters are highly diagnostic, with members of sect. *Notopleura* characterized by strongly dorsally flattened pyrenes and two marginal germination slits (Robbrecht 1989; F. Piesschart, pers. comm.).

This pyrene morphology represents a uniquely derived character state (or character states) supporting the view of *Notopleura* as a separate genus of Psychotrieae. Transfer of species of *Psychotria* sect. *Notopleura* to the genus *Notopleura* will be undertaken in a separate paper.

Relationships in Tribe Psychotrieae and Related Tribes. Evidence from phylogenetic analysis of the chloroplast *rbcL* gene (Fig. 3) supports the same relationships among genera and monophyletic groups and a circumscription of tribe Psychotrieae that resulted from phylogenetic analysis of ITS sequences. Of the taxa sampled, the pantropical *Lasianthus*, traditionally placed in tribe Psychotrieae, is most closely related to the African *Pauridiantha* (tribe Pauridianthaeae) and occupies the most basal position in the tree. Additionally, the paleotropical *Gaertnera*, also traditionally placed in Psychotrieae (Robbrecht 1988), occupies a position in the tree that is much farther removed from the tribe Psychotrieae than had previously been suggested. Although *Pagamea* was not included in the present study, the results of the phylogenetic analyses may suggest a placement of *Gaertnera* and *Pagamea* in their own tribe, or in a tribe other than, but closely related to, Psychotrieae. A clade comprising the pantropical *Morinda* and the disjunct *Mitchella* (both placed in "group of *Mitchella*" of tribe Morindeae according to Robbrecht 1993), the Southeast Asian *Damnanthus* (Morindeae), and *Schradera* (tribe Schradereae) diverge later, and are thus more closely related to Psychotrieae than are *Lasianthus* or *Gaertnera*. The neotropical *Coussarea*, *Faramea* (Coussareae), *Coccocypselum* (Coccocypseleae), and *Declieuxia* (Psychotrieae) form a monophyletic lineage that diverges next, and a lineage that represents the sister to the bulk of the taxa comprising tribe Psychotrieae.

A lineage that includes the bulk of Psychotrieae (Psychotrieae sensu stricto) branches off subsequently and forms the two major clades of *Psychotria* and relatives discussed above. As in the ITS trees, the *rbcL*-supported clade that represents subg. *Psychotria* comprises a group containing both African and neotropical taxa, and another group containing Pacific species of *Psychotria*, *Amaracarpus*, and members of the *Hynodophytinae*. However, in contrast with the ITS results, the neotropical *Psychotria graciliflora* is sister to this clade, rather than part of a monophyletic neotropical clade, although its position in the ITS tree is poorly supported. A long branch

length for *Psychotria graciliflora* may contribute to the incongruent placement of this taxon in the *rbcL* trees. Additionally, the clade comprising the rest of the Psychotrieae plus subg. *Heteropsychotria* is topologically consistent with the results of the ITS analysis. *Psychotria poeppigiana* and *P. bremekampiana* (subg. *Heteropsychotria*) together with a representative of *Palicourea* form a monophyletic group (subg. *Heteropsychotria* plus *Palicourea* clade). The neotropical subg. *Heteropsychotria* is polyphyletic, with some members (*P. ipeacacuanha*, *P. borucana*, and *P. dukei*) more closely related to other genera of Psychotrieae than they are to each other or other species currently assigned to subg. *Heteropsychotria*.

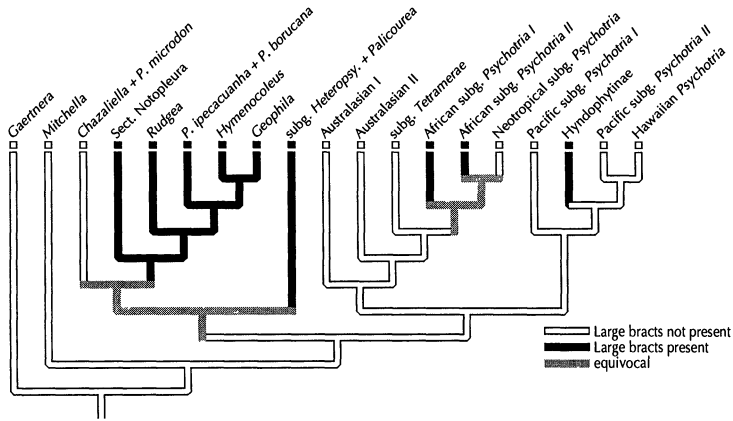
Because many regional endemic genera were not included in the present study, the delimitation and relationships of tribe Psychotrieae inferred from the phylogenetic analysis of ITS and *rbcL* sequences should not be over-interpreted. However, taxa not included in our analysis are largely monotypic, endemic genera of remote locations, such as genera from the Guayana Highlands in southern Venezuela (*Aphanocarpus*, *Coryphothamnus*, *Coccochondra*, and *Pagameopsis*), the Amazonian monotypic genus *Stachyococcus*, endemic genera from Madagascar (*Cremonocarpus*, *Psathura*, *Pyragra*, *Saldinia*, and *Trigonopyren*), and Fijian endemics (*Gillespeia*, *Hedstromia*, *Readea*, and *Squamellaria*). Thus, full representation of the tribe Psychotrieae will require considerable further effort.

Phylogenetic Utility of Some Morphological Characters for Psychotria and Psychotrieae. SHOWY INVOLUCRAL BRACTS. The presence of showy, extra-floral bracts subtending capitate or densely contracted inflorescences has been used to establish the segregate genera *Cephaëlis* and *Uragoga* (Molina 1953). The type species of *Cephaëlis* (*C. muscosa* = *Psychotria muscosa*) and the neotropical representatives of *Cephaëlis*—including the well-known “hot-lips” plants such as *C. tomentosa* (= *P. poeppigiana*)—were treated by Steyermark (1972) as members of *Psychotria* subg. *Heteropsychotria*. However, results of the ITS and *rbcL* analyses support an extreme case of polyphyly with regard to *Cephaëlis*, with taxa assigned to *Cephaëlis* or *Uragoga* distributed throughout the Psychotrieae in such diverse lineages as the *Heteropsychotria* + *Palicourea* clade, *P. ipeacacuanha* + *P. borucana* clade, *Geophila*, *Rudgea*, *Psychotria* sect. *Notopleura*, *Hymenocoleus*, the *Psychotria* sensu stricto clade (the Af-

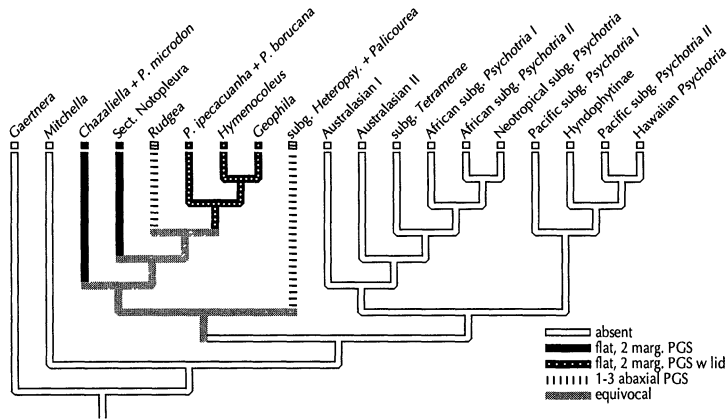
rican sect. *Involuncratae*, and other African lineages), and even in the Pacific clade. Convergent evolution in the development of condensed inflorescences with involucre bracts appears to have occurred multiple times in the Rubiaceae, as among the close relatives of Psychotrieae sensu stricto. Schnell (1960) notes that *Schraderea* (*Schradereae*) also exhibits capitate inflorescences subtended by involucre, and species of *Morinda* (*Morindeae*) have a tendency toward capitulization. Additionally, there is a tendency toward development of non-petaloid organs as attractants in diverse lineages of Rubiaceae. Calcophylls, or enlarged sepals with attractive function, are known in at least 28 genera of the family (Bremer and Jansen 1991; Albert et al. 1997; B. Bremer and H. Lantz, pers. comm.), although development of “pseudanthia” via “hyperfloral differentiation” is confined only to tribe Psychotrieae and closely related tribes such as in *Faramea* (*Coussareae*) (Robbrecht 1988). Figure 4A depicts the most parsimonious derivation of showy extrafloral bracts in the principal lineages of the Psychotrieae. Showy bracts are gained at least three times, and may have been lost as well (in the *Chazaliella* + *P. microdon* and the neotropical subg. *Psychotria* lineages). The character of strongly contracted inflorescences subtended by involucre bracts is derived convergently or in parallel in multiple lineages of Psychotrieae and appears not to be phylogenetically useful at the generic level within the tribe.

PYRENE ANATOMY. Diagnostic features of Psychotrieae may have been found in the pyrenes that forms part of the mature fruit structure. Most genera of Psychotrieae have fleshy, drupaceous fruits in which the endocarp has differentiated into two hard, bony, structures each enclosing one seed. The bony endocarp plus the seed is termed the “pyrene.” Pyrenes are believed to have a protective function, given that many of the fruits are dispersed by birds (or mammals) and therefore pass through the digestive tract of the animal. Pyrenes are variously hemispherical in cross section or dorsally flattened in Psychotrieae. In addition, the embryo is aided in the germination process by the presence of weak lines of breakage in the pyrene wall, “preformed germination slits” (PGS) (Robbrecht 1989). Petit (1964) was one of the first to use pyrene characters in the classification of African Psychotrieae. Robbrecht (1989) determined that the number and position of the PGS have high

A. Showy involucre bracts



B. Pyrene germination slits



C. Habit

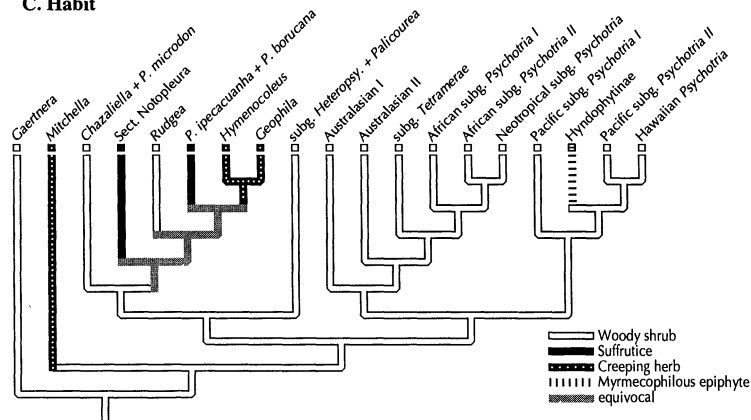


FIG. 4. A most parsimonious reconstruction of character state evolution for three characters used in traditional classifications of tribe Psychotrieae, showy involucre bracts, pyrene morphology, and habit. Patterns of evolution were inferred from a simplified cladogram summarizing principal lineages of Psychotrieae (from Fig. 2), using assumptions of maximum parsimony in MacClade 3.04 (Maddison and Maddison 1992). All most parsimonious reconstructions of character states are shown, in place of arbitrarily resolving using ACCTRAN or DELTRAN optimization. A. Minimum pattern of evolution of showy involucre bracts. The character state of showy bracts present was coded for any lineage in which at least some members exhibited the trait. B. Minimum pattern of pyrene germination slit (PGS) evolution. C. Minimum pattern of habit evolution.

diagnostic value at the generic level. While species of *Psychotria* sensu stricto (subg. *Psychotria* + subg. *Tetramerae* + Hydnophytinae) have relatively thin-walled pyrenes and lack PGS, members of other major lineages within Psychotrieae (such as sect. *Notopleura*, *Hymenocoleus*, and *Chazaliella*) have thick-walled pyrenes and possess PGS.

Although the classification of the neotropical Psychotrieae is in disarray, an a posteriori investigation of PGS characteristics in monophyletic groups such as *Psychotria* sect. *Notopleura*, *Chazaliella* + *P. microdon* clade, and the *Heteropsychotria* + *Palicourea* clade reveals that PGS characters are useful in defining monophyletic groups. Evolution of pyrene characteristics in Psychotrieae is reconstructed in Fig. 4B. Although pyrene anatomy for *P. borucana* is unknown, the trait was scored as is found in *P. ipecacuanha* (i.e., flat, with 2 marginal PGS with lid; F. Piesschart, pers. comm.). Pyrene anatomy in subg. *Heteropsychotria* + *Palicourea* is not well characterized, but preliminary observations (M. Nepokroeff, unpubl. data; F. Piesschart, pers. comm.) suggest that some members of the group exhibit 3 to 4 dorsal PGS. The subg. *Heteropsychotria* + *Palicourea* clade was therefore coded as having 3–4 dorsal PGS. Similarly, all examined species of sect. *Notopleura* are characterized by having two marginal and one short ventral median PGS. Species of *Psychotria* subg. *Psychotria* examined so far have a very thin endocarp and lack PGS, a character state which may represent a synapomorphy for the *Psychotria* sensu stricto lineage (Robbrecht 1989 and pers. comm.)

HABIT. The Psychotrieae are predominantly woody shrubs. However, creeping herbs, suffrutices, and succulent epiphytes are also known. Fig. 4C depicts the most parsimonious evolution of habit type in the tribe. The ancestral habit for the Psychotrieae is most parsimoniously reconstructed as branched woody shrub. Herbaceousness is derived in *Geophila* and *Hymenocoleus*, and the tuberous, myrmecophilous epiphytic habit is derived in the Hydnophytinae. Epiphytes and suffrutices are also derived (via ancestral suffruticose forms) in the neotropical sect. *Notopleura* (Nepokroeff 1992; Nepokroeff and Sytsma, in press). Suffrutices are also independently derived in the *P. ipecacuanha* and *P. borucana* lineage. Additional sampling and floristic work in the neotropics may reveal other members belonging to this previously cryptic

monophyletic lineage. Modifications of the woody, multi-branched habit are more common in the clade containing the rest of the tribe Psychotrieae than they are in the *Psychotria* sensu stricto clade.

Redefinition of the Genus *Psychotria*. The absence of floral diversity and the large size of *Psychotria* have contributed to the difficulty in addressing phylogenetic relationships among Psychotrieae. The traditional classification of *Psychotria* is based on absence of morphological characters found in other genera, or on characters that intergrade considerably among other closely related genera in the tribe. Taxa based on such criteria often form paraphyletic groups; in fact the problem of paraphyly may be extremely widespread in many large genera. However, an a posteriori analysis suggests that the overlooked character of pyrene surface morphology (PGS) may be highly diagnostic at the genus level within Psychotrieae. The genus *Psychotria* should be redefined to include members of subg. *Psychotria*, subg. *Tetramerae*, and members of subtribe Hydnophytinae. A new description of the genus should include lack of preformed germination slits on the pyrenes. Other monophyletic groups can be defined based on this molecular phylogenetic analysis, including some formerly cryptic groups (e.g., *Psychotria ipecacuanha* and *P. borucana*) and associations between neotropical and African genera (e.g., *Chazaliella* and *P. microdon*). Additionally, elevation to generic rank for *Psychotria* sect. *Notopleura* is well supported by our analysis, as is inclusion of *Palicourea* within *Psychotria* subg. *Heteropsychotria*, and elevation of this clade to genus rank. Taylor (1996) has argued that the name for such a genus would be *Psychotrophum* based on rules of priority.

As to estimated numbers of species, our phylogenetic analysis indicates that approximately 1,200 species would be included in *Psychotria* sensu stricto including 200 neotropical species, 250 African species, 700 species in Asia and Oceania, and 50 species of the former subtribe Hydnophytinae. The clade containing subg. *Heteropsychotria* plus *Palicourea* (or *Psychotrophum*) would contain 550 to 650 species including 350–450 neotropical species formerly placed in subg. *Heteropsychotria* and 200 species formerly placed in *Palicourea*. *Notopleura* (formerly sect. *Notopleura*) would contain approximately 45 neotropical species. Nomenclatural transfers for *Notopleura* will be presented elsewhere.

Lastly, the biogeographical history of the tribe and genus can be inferred based on the results of these analyses. Psychotriaceae and *Psychotria* are largely Australasian in origin (Fig. 2, Box), with major radiations occurring in the neotropics (*Palicourea* plus *Heteropsychotria*) and subsequently to Africa among other Psychotriaceae genera. *Psychotria* as a genus is also ancestrally Australasian, with major radiations occurring in Africa, and subsequently the neotropics (African taxa are sister to the neotropical taxa sampled), and also the Pacific (including the Malesian myrmecophytes of the subtribe Hydnophytinae) and Hawaii.

ACKNOWLEDGEMENTS. The authors thank the many people and institutions who have provided leaf material for this project: David Lorence and Ken Wood (National Tropical Botanical Garden, Lawai, Hawaii); Waimea Arboretum (Hawaii); Honolulu Botanical Gardens (Hawaii); Joe Wright (Smithsonian Tropical Research, Panama); Barry Hammel and Charlotte Taylor (Missouri Botanical Garden); Elmar Robbrecht (National Botanical Garden, Belgium); Lennart Andersson (University of Goteberg, Sweden); Christopher Puttock (Australian National Herbarium, Canberra); Simon Malcomber (Washington University, St. Louis); Jean-Yves Meyer (Haleakala National Park, Hawaii); Mark Chase (Royal Botanical Gardens, Kew); Murray Rixon (Royal Botanic Gardens, Edinburgh); Jean-Francois Manen (Conservatoire et Jardin Botanique de la Ville de Genève, Switzerland); and Camilla Huxley-Lambrick (Oxford University, England). Frederick Piesschart (National Botanical Garden, Belgium) provided unpublished data on pyrene characters. David Swofford kindly gave permission to KJS to use PAUP* for data analysis. We thank Steven Darwin and an anonymous reviewer for helpful comments which greatly improved the manuscript. Kandis Elliot provided artwork and Jessica Rönnholm assisted with *rbcl* sequencing. This work was supported by NSF grant DEB-9423590 to KJS and MN, NFR (Swedish Natural Science Research Council) BU-01487-319 to BB, and grants from the American Society of Plant Taxonomists, and the Garden Club of America/World Wildlife Fund to MN.

LITERATURE CITED

- ALBERT, V. A., M. W. CHASE, and B. D. MISHLER. 1993. Character-state weighting for cladistic analysis of protein coding DNA sequences. *Annals of the Missouri Botanical Garden* 80: 752-766.
- , M. H. G. GUSTAFSSON, and L. DI LAURENZIO. 1997. Ontogenetic systematics, molecular developmental genetics and the angiosperm petal. in *Molecular Systematics of Plants II*, eds. D. Soltis, P. Soltis, and J. Doyle. New York, NY: Chapman and Hall (in press).
- BALDWIN, B., M. J. SANDERSON, J. M. PORTER, M. F. WOJCIECHOWSKI, C. S. CAMPBELL, and M. J. DONOGHUE. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247-277.
- BAUM, D. A., K. J. SYTSMAN, and P. C. HOCH. 1994. A phylogenetic analysis of *Epilobium* (Onagraceae) based on nuclear ribosomal DNA sequences. *Systematic Botany* 19: 363-388.
- BENTHAM, G. 1852. in Orsted: Centralamerikas Rubiaceae. 15. Videnskabelige Meddelelser den naturhistoriske Foreningi Kjöbenhavn.
- BLUME, C. L. 1826. *Bijdragen tot de flora van Nederlandsch Indië*. Batavia.
- BOGLER, D. J. and B. B. SIMPSON. 1996. Phylogeny of the Agavaceae based on ITS rDNA sequence variation. *American Journal of Botany* 83: 1225-1235.
- BREMEKAMP, C. E. B. 1934. Notes on the Rubiaceae of Surinam. *Recueil des Travaux Botaniques Neerlandais* 31: 248-290.
- BREMER, B. 1996. Phylogenetic studies within Rubiaceae and relationships to other families based on molecular data. *Opera Botanica Belgica* 7: 33-50.
- , and R. K. JANSEN. 1991. Comparative restriction site mapping of the chloroplast DNA implies new phylogenetic relationships within the Rubiaceae. *American Journal of Botany* 78: 198-213.
- , D. OLSSON, and K. ANDREASON. 1995. Testing Rubiaceae classification with *rbcl* data. *Annals of the Missouri Botanical Garden* 82: 383-397.
- BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogeny reconstruction. *Evolution* 42: 795-803.
- CARLQUIST, S. 1981. Chance dispersal. *American Scientist* 69: 509-516.
- CHASE, M. W., D. E. SOLTIS, R. G. OLMSTEAD, D. MORGAN, D. H. LES, B. D. MISHLER, M. R. DUVALL, R. PRICE, H. G. HILLS, Y. QIU, K. A. KRON, J. H. RETTIG, E. CONTI, J. D. PALMER, J. R. MANHART, K. J. SYTSMAN, H. J. MICHAELS, W. J. KRESS, K. G. KAROL, W. D. CLARK, M. HEDREN, B. S. GAUT, R. K. JANSEN, K.-J. KIM, C. F. WIMPEE, J. F. SMITH, G. R. FURNIER, S. H. STRAUSS, Q.-Y. XIANG, G. M. PLUNKETT, P. S. SOLTIS, S. M. SWENSON, S. E. WILLIAMS, P. A. GADEK, C. J. QUINN, L. E. EGUIARTE, E. GOLENBERG, G. H. LEARN, JR., S. W. GRAHAM, S. DAYANANDAN, and V. A. ALBERT. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcl*. *Annals of the Missouri Botanical Garden* 80: 528-580.
- CONTI, E., A. FISCHBACH, and K. J. SYTSMAN. 1993. Tribal relationships in Onagraceae: implications from *rbcl* sequence data. *Annals of the Missouri Botanical Garden* 80: 672-685.
- , A. LITT, and K. J. SYTSMAN. 1996. Circumscription of the Myrtales and their relationships to other rosids: evidence from *rbcl* sequence data. *American Journal of Botany* 83: 221-233.

- DARWIN, S. P. 1979. A synopsis of the indigenous genera of Pacific Rubiaceae. *Allertonia* 2: 1–44.
- DOWNIE, S. R. and D. S. KATZ-DOWNIE. 1996. A molecular phylogeny of Apiaceae subfamily Apioideae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany* 83: 234–251.
- DOYLE, J. J. and J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.
- EMDRESS, P. K. 1996. Homoplasy in angiosperm flowers. Pp. 303–325 in *Homoplasy: the recurrence of similarity in evolution*, eds. M. J. Sanderson and L. Hufford. San Diego, CA: Academic Press.
- FARRIS, J. S., V. A. ALBERT, M. KÄLLERSJÖ, D. LIPSCOMB, and A. G. KLUGE. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12: 99–124.
- FOSBERG, F. R. and M. SACHET. 1991. Studies in the Indo-Pacific Rubiaceae. *Allertonia* 6: 191–278.
- , ———, and R. L. OLIVER. 1993. Flora of Micronesia, 5: Bignoniaceae-Rubiaceae. *Smithsonian Contributions to Botany*, Number 81.
- HAMILTON, C. W. 1985. Architecture in the Neotropical *Psychotria* L. (Rubiaceae): dynamics of branching and its taxonomic significance. *American Journal of Botany* 72: 1081–1088.
- . 1989a. A revision of Mesoamerican *Psychotria* subgenus *Psychotria* (Rubiaceae). Part I: Introduction and species 1–16. *Annals of the Missouri Botanical Garden* 76: 67–111.
- . 1989b. A revision of Mesoamerican *Psychotria* subgenus *Psychotria* (Rubiaceae). Part II: Species 17–47. *Annals of the Missouri Botanical Garden* 76: 386–429.
- . 1989c. A revision of Mesoamerican *Psychotria* subgenus *Psychotria* (Rubiaceae). Part III: Species 48–61. *Annals of the Missouri Botanical Garden* 76: 886–916.
- HASEGAWA, M., H. KISHINO, and T. YANO. 1985. Dating of the human ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* 21: 160–174.
- JIERN, W. P. 1877. Rubiaceae. Pp. 33–247 in *Flora of tropical Africa III*, ed. D. Oliver. Ashford, Reeve and Co.
- HOLMGREN, P. K., N. H. HOLMGREN, and L. C. BARNETT. 1990. *Index Herbariorum. Part I: The Herbaria of the World, 8th edition*. New York, NY: New York Botanical Garden.
- HUXLEY, C. R. and M. H. P. JEBB. 1990. New taxa in the myrmecophilous Psychotriaceae (Rubiaceae). *Bulletin Jardin Botanique National de Belgique* 60: 415–421.
- and ———. 1991a. The tuberous epiphytes of the Rubiaceae 1: a new subtribe—the Hydnohytinae. *Blumea* 36: 1–20.
- and ———. 1991b. The tuberous epiphytes of the Rubiaceae 3: a revision of *Myrmephytum* to include *Myrmedoma*. *Blumea* 36: 43–52.
- JOHANSSON, J. T. 1992. Pollen morphology in *Psychotria* (Rubiaceae, Rubioideae, Psychotriaceae) and its taxonomic significance. A preliminary survey. *Opera Botanica* 115: 1–71.
- LLOYD, D. G. and V. L. CALDER. 1991. Multi-residue gaps, a class of molecular characters with exceptional reliability for phylogenetic analyses. *Journal of Evolutionary Biology* 4: 9–21.
- MADDISON, D. P. 1991. The discovery and importance of multiple islands of most parsimonious trees. *Systematic Zoology* 40: 315–328.
- MADDISON, W. P. and D. P. MADDISON. 1992. *MacClade vs. 3.0: Analysis of Phylogeny and Character Evolution*. Sinauer Associates, Inc., Sunderland, MA.
- MOLINA, A. 1953. Revisión de las especies de *Cephaelis* en México, Centro America y las Antillas. *Ceiba* 4: 1–38.
- MÜLLER (-ARGOVIENSIS), J. 1881. *Flora Brasiliensis*, Vol. 6 (Part 5): 383–427, 457–466.
- NEPOKROEFF, M. 1992. Relationships among *Psychotria* section *Notopleura* and related taxa in the tribe Psychotriaceae (Rubiaceae): determining monophyletic groups based on ITS sequence data. Masters thesis, University of Wisconsin, Madison.
- . 1997. Systematics of the tropical shrub genus *Psychotria* L. (Rubiaceae): origins, speciation and breeding systems in Hawai'i. Ph.D. thesis, University of Wisconsin, Madison.
- and K. J. SYTSA. 1999. Speciation in cloud forest *Psychotria*. in *The natural history of Monteverde*, eds. N. Nadkarni and N. Wheelwright. Oxford, Oxford University Press (in press).
- OLMSTEAD, R. G., H. J. MICHAELS, K. M. SCOTT, and J. D. PALMER. 1993. A parsimony analysis of the Asteridae and identification of their major lineages inferred from DNA sequences of *rbcl*. *Annals of the Missouri Botanical Garden* 80: 700–722.
- PETTIT, E. 1964. Les espèces africaines du genre *Psychotria* L. (Rubiaceae). I. *Bulletin du Jardin Botanique de Bruxelles* 34: 1–229.
- . 1966. Les espèces africaines du genre *Psychotria* L. (Rubiaceae). II. *Bulletin du Jardin Botanique de Bruxelles* 36: 65–189.
- ROBBRECHT, E. 1988. Tropical Woody Rubiaceae. *Opera Botanica Belgica* 1: 1–271.
- . 1989. A remarkable new *Chazaliella* (African Psychotriaceae), exemplifying the taxonomic value of pyrene characters in the Rubiaceae. *Bulletin du Museum National d'histoire Naturelle. Section B, Adansonia, Botanique Phytochimie* 4: 341–349.
- . 1993. Supplement to the 1988 outline of the classification of the Rubiaceae Index to genera. *Opera Botanica Belgica* 6: 173–196.
- RODMAN, J. E., K. G. KAROL, R. A. PRICE, and K. J. SYTSA. 1996. Molecules, morphology, and Dahlgren's expanded order Capparales. *Systematic Botany* 21: 289–307.
- SANDERSON, M. J. and M. F. WOJCIECHOWSKI. 1996. Divergence in a temperate legume clade: are there "so many species" of *Astragalus* (Fabaceae)? *American Journal of Botany* 83: 1488–1502.

- SCHNELL, R. 1960. Note sur le genre *Cephaelis* et le problème de l'évolution parallèle chez les Rubiacées. Bulletin du Jardin Botanique de Bruxelles 30: 357–373.
- SCHUMANN, K. 1891. Rubiaceae. Pp. 1–156 in *Die natürlichen Pflanzenfamilien* 4(4), eds. A. Engler and K. Prantl. Leipzig, Germany.
- SMITH, A. C. and S. P. DARWIN. 1988. Family 168. Rubiaceae. in *Flora Vitiensis Nova*, Vol. 4. Kauai, HI: Pacific Tropical Botanical Garden.
- SMITH, J. F., K. J. SYTSMA, J. S. SHOEMAKER, and R. L. SMITH. 1991. A qualitative comparison of total cellular DNA extraction protocols. Phytochemical Bulletin 23: 2–9.
- SOHMER, S. 1977. *Psychotria* L. (Rubiaceae) in the Hawai'ian islands. *Lyonia* 1: 103–186.
- . 1978. Morphological variation and its taxonomic and evolutionary significance in the Hawai'ian *Psychotria* (Rubiaceae). *Brittonia* 30: 256–264.
- . 1988. *The non-climbing species of the genus Psychotria (Rubiaceae) in New Guinea and the Bismarck Archipelago*. Bishop Museum Press, Honolulu, HI.
- SOLIS, P. N., C. LANG'AT, M. P. GUPTA, G. C. KIRBY, D. C. WARHURST, and J. D. PHILLIPSON. 1995. Bioactive compounds from *Psychotria camponutans*. *Planta Medica* 61: 62–65.
- STEYERMARK, J. 1972. Botany of the Guayana Highlands. *Memoirs of the New York Botanical Garden* 23: 227–832.
- SYTSMA, K. J. and W. J. HAHN. 1994. Molecular systematics: 1991–1993. *Progress in Botany* 55: 307–333.
- and ———. 1996. Molecular systematics: 1994–1995. *Progress in Botany* 58: 470–499.
- TAYLOR, C. M. 1989. Revision of *Palicourea* (Rubiaceae) in Mexico and Central America. *Systematic Botany Monographs* 261: 1–102.
- . 1996. Overview of the Psychotrieae (Rubiaceae) in the Neotropics. *Opera Botanica Belgica* 7: 261–270.
- . 1997. Conspectus of the genus *Palicourea* (Rubiaceae: Psychotrieae) with the description of some new species from Ecuador and Colombia. *Annals of the Missouri Botanical Garden* 84: 224–262.
- VERDCOURT, B. 1976. Rubiaceae. (Part 1). Pp. 1–134 in *Flora of tropical East Africa*, ed. R. M. Polhill. London: Crown Agents for Oversea Governments and Administrations.
- WHEELER, W. and D. GLADSTEIN. 1992. *MALIGN: a multiple sequence alignment program, vers. 2.0*. New York: The American Museum of Natural History.
- WHITE, T. J., T. BRUNS, S. LEE, and J. TAYLOR. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315–322 in *PCR protocols: a guide to methods and applications*, eds. M. Innis, D. Gelfand, J. Sninsky, and T. White. San Diego, CA: Academic Press.