
PHYLOGENY OF THE
HERBACEOUS TRIBE
SPERMACOCEAE (RUBIACEAE)
BASED ON PLASTID DNA DATA¹

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ABSTRACT

In its current circumscription, the herbaceous tribe Spermacoceae s.l. (Rubiaceae, Rubioideae) unites the former tribes Spermacoceae s. str., Manettieae, and the *Hedyotis–Oldenlandia* group. Within Spermacoceae, and particularly within the *Hedyotis–Oldenlandia* group, the generic delimitations are problematic. Up until now, molecular studies have focused on specific taxonomic problems within the tribe. This study is the first to address phylogenetic relationships within Spermacoceae from a tribal perspective. Sequences of three plastid markers (*atpB-rbcL*, *rps16*, and *trnL-trnF*) were analyzed separately as well as combined using parsimony and Bayesian approaches. Our results support the expanded tribe Spermacoceae as monophyletic. The former tribe Spermacoceae s. str. forms a monophyletic clade nested within the *Hedyotis–Oldenlandia* group. Several genera formerly recognized within the *Hedyotis–Oldenlandia* group are supported as monophyletic (*Amphiasma* Bremek., *Arcytophyllum* Willd. ex Schult. & Schult. f., *Dentella* J. R. Forst. & G. Forst., *Kadua* Cham. & Schldtl., and *Phylohydrax* Puff), while others appear to be paraphyletic (e.g., *Agathisanthemum* Klotzsch), biphyletic (*Kohautia* Cham. & Schldtl.), or polyphyletic (*Hedyotis* L. and *Oldenlandia* L. sensu Bremekamp). Morphological investigations of the taxa are ongoing in order to find support for the many new clades and relationships detected. This study provides a phylogenetic hypothesis with broad sampling across the major lineages of Spermacoceae that can be used to guide future species-level and generic studies.

Key words: *atpB-rbcL*, *Hedyotis–Oldenlandia* group, Rubiaceae, molecular phylogeny, plastid DNA, *rps16*, Spermacoceae, *trnL-trnF*.

The systematic relationships of the Rubiaceae herbaceous representatives are still unclear at the species and genus levels (Robbrecht & Manen, 2006). Even the higher-level classification in tribes has been the subject of debate. In the last comprehensive classification based on morphology (Robbrecht, 1988, 1993), most herbaceous representatives were assigned to one of the following tribes: Anthospermeae, Argostemmatae, Coccocypseleae, Hedyotideae, Knoxieae, Rubieae, Sipaneeae, Spermacoceae, and Theligoneae. Among these, the Spermacoceae as traditionally delimited (Hooker, 1873; Bremekamp, 1952, 1966; Verdcourt, 1958; Robbrecht, 1988, 1993), referred to in this

paper as Spermacoceae s. str., are characterized by the presence of raphides, fimbriate stipules, uniovulate locules, seeds with an apparent adaxial groove, and the frequent occurrence of pluriaperturate pollen grains. However, molecular data show Spermacoceae s. str. to be deeply nested within the Hedyotideae, making the latter tribe paraphyletic (Bremer, 1996; Andersson & Rova, 1999; Bremer & Manen, 2000; Dessein et al., 2005a). Therefore, Bremer (1996) and later Bremer and Manen (2000) proposed a wider definition for Spermacoceae, in which the former tribes Spermacoceae s. str., Hedyotideae, Manettieae, Knoxieae, and Triainolepideae are merged.

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Based on *rps16* intron data, Andersson and Rova (1999) also found that Hedyotideae is paraphyletic relative to Spermaceae s. str. They did not accept the wide delimitation for Spermaceae as proposed by Bremer (1996), but suggested an emended tribe Knoxiaceae that included a few genera of Hedyotideae (i.e., *Otiophora* Zucc., *Otomeria* Benth., and *Pentas* Benth.) as a more prudent taxonomic approach to handle the information from molecular-based analyses. The latter view was followed by Dessein (2003), who preferred to recognize an emended tribe Knoxiaceae (including Knoxiaceae s. str., Triainolepideae, *Otiophora*, the *Pentas* group of Hedyotideae fide Dessein et al. [2000], and *Carphalea* Juss.) as a sister group of Spermaceae (including Spermaceae s. str., Manettieae, and most of Hedyotideae). Robbrecht and Manen (2006), based on a supertree analysis of the family, came to a similar conclusion and likewise recognized Knoxiaceae s.l. and Spermaceae s.l. The monophyly of the former tribe has also been confirmed by a subsequent molecular study by Kårehed and Bremer (2007). In their taxonomic conspectus, Robbrecht and Manen (2006) listed 33 genera of Spermaceae s.l. for which molecular sequence data are available. Based on morphological data, we recognize 31 of these 33 genera and consider that the tribe should include 30 additional genera; these are listed in Table 1. For each genus, the number of species, the distribution, and the position in Robbrecht's classification of 1988 are given.

Spermaceae s.l. forms a primarily herbaceous lineage that is generally characterized by fimbriate stipules and 4-merous flowers. Floral characters (Fig. 1), as well as seeds and fruits, are highly variable. Morphologically, three main groups can be identified within Spermaceae s.l. The first, the *Hedyotis*-*Oldenlandia* group, is characterized by multiovulate locules and comprises the large genera *Hedyotis* L. and *Oldenlandia* L. and their presumed relatives. Most of these taxa were formerly placed in the tribe Hedyotideae. The generic delimitations of the *Hedyotis*-*Oldenlandia* group have been the subject of controversy for many years. The main issue is whether most species of the complex should be lumped into *Hedyotis* (advocated by inter alia Merrill & Metcalf, 1946; Wagner et al., 1989; Fosberg & Sachet, 1991; Dutta & Deb, 2004) or whether many small genera should be recognized in addition to a narrow circumscription of *Hedyotis* and *Oldenlandia* (supported for African taxa by Bremekamp, 1952; for Neotropical taxa by Terrell et al., 1986; Terrell, 1991, 2001a, b, c; and for Asian taxa by Terrell & Robinson, 2003).

The second well-marked group within Spermaceae s.l. is Spermaceae s. str., which is character-

ized by uniovulate locules. According to Dessein (2003), this group contains 19 genera of which *Spermaceae* L. is by far the largest with an estimated 275 species. Within Spermaceae s. str., controversy has focused on the delimitation of its nominal genus *Spermaceae*. The main question is whether *Spermaceae* should be limited to species with the same type of fruit dehiscence as *S. tenuior* L., the type species of the genus. In this species, fruits open asymmetrically, resulting in one closed and one open fruit part. If this narrow delimitation for *Spermaceae* (referred to as *Spermaceae* s. str.) is accepted, most other species in the tribe Spermaceae s. str. must be included in *Borreria* G. Mey.

A third well-defined group within Spermaceae s.l. comprises only two American genera, *Bouvardia* Salisb. and *Manettia* Mutis ex L. Bremekamp (1952) considered *Bouvardia* closely related to *Heterophyllaea* Hook. f., *Hindsia* Benth. ex Lindl., and *Lecanosperma* Rusby. Robbrecht (1988) placed these genera together with inter alia *Manettia* in a group with uncertain affinities, because their winged seeds suggest a relation to Cinchoneae, while the presence of raphides indicates a relation to Hedyotideae. In the classification of Bremer and Manen (2000), only *Bouvardia* and *Manettia* belong to Spermaceae s.l., because *Hindsia* and *Heterophyllaea* (including *Lecanosperma*) are included in Coussareeae. *Manettia* is similar to *Bouvardia* in many characters, but its winding shoots and corneous endosperm separate it from *Bouvardia*, which is erect and has fleshy endosperm. These differences were the basis for Bremekamp (1934) to place *Manettia* in its own tribe, Manettieae.

Until now, molecular studies within Spermaceae s.l. have focused on particular taxonomic problems, such as the circumscription and biogeography of *Arcytophyllum* Willd. ex Schult. & Schult. f. (Andersson et al., 2002), the generic status of *Houstonia* L. (Church, 2003), the delimitation of *Pentanopsis* Rendle, the affinities of *Phylohydrax* Puff (Thulin & Bremer, 2004), and the taxonomic position of *Gomphocalyx* Baker (Dessein et al., 2005a). In the present paper, we aim to present a phylogenetic hypothesis of Spermaceae s.l. based on the analysis of three plastid markers (*atpB-rbcL*, *rps16*, and *trnL-trnF*) with the broadest sampling to date. More specifically, we want to address the following questions: (1) Is Spermaceae s.l. as circumscribed by Robbrecht and Manen (2006) monophyletic? (2) What are the relationships among members of Spermaceae s. str. and genera of the former tribes Hedyotideae and Manettieae? (3) What are the major clades within the *Hedyotis*-*Oldenlandia* group?

MATERIAL AND METHODS

PLANT MATERIAL AND SAMPLING

The aim was to obtain a broad sampling covering most of the geographic and taxonomic diversity of Spermaceae and to enable identification of the principal clades within the tribe. We included a total of 128 species representing 32 of the 61 genera within Spermaceae. Three taxa belonging to the Knoxiaceae (*Batopedina pulvinellata* Robbr., *Carphalea madagascariensis* Lam., and *Pentanisia parviflora* Stapf ex Verdc.) were chosen as outgroup following Robbrecht and Manen (2006) and Kårehed and Bremer (2007). For *rps16* and *trnL-trnF*, we used 40 and seven previously published sequences, respectively (Andersson & Rova, 1999; Andersson et al., 2002; Dessein et al., 2005a). Two hundred seventy-two sequences are newly generated (100 *atpB-rbcL* sequences, 67 *rps16* sequences, 105 *trnL-trnF* sequences) using dried silica and herbarium material. Appendix 1 lists all taxa included in this study with voucher information and GenBank accession numbers.

DNA EXTRACTION, POLYMERASE CHAIN REACTION
AMPLIFICATION, AND SEQUENCING

DNA was extracted from silica-dried and herbarium material using the CTAB method as described by Janssens et al. (2006). Amplification of the *atpB-rbcL* spacer was done with oligonucleotides two and five as primers (Manen et al., 1994). Specific amplification products could be obtained with a touchdown polymerase chain reaction (PCR) with two cycles with an annealing temperature of 53°C, then 12 cycles with an annealing temperature of 52.5°C declining 0.5°C every cycle, followed by 16 cycles with an annealing temperature of 47°C. The *rps16* intron was amplified with the *rps16F* and *rps16R2* primers described by Oxelman et al. (1997). For the *trnL-trnF* intergenic spacer, we used the primers *e* and *f* of Taberlet et al. (1991). Both *rps16* and *trnL-trnF* were amplified using standard PCR techniques with an annealing temperature of 55°C. The PCR reaction mixture was cleaned using a Nucleospin Extraction II Kit (Machery-Nagel, Dren, Germany) according to the manufacturer's instructions. Sequencing was mostly done on an ABI 310 Genetic Analyzer (Applied Biosystems, Lennik, Belgium). Some PCR products were sequenced by Macrogen (Seoul, South Korea) sequencing facilities.

SEQUENCE ASSEMBLY, ALIGNMENT, AND GAP CODING

The assembling and editing of sequences were conducted using the Staden Package (Staden et al.,

1998). Sequences were initially aligned with ClustalX (Thompson et al., 1997) applying the default parameters. Further adjustments of the preliminary aligned data matrices were done manually with MacClade 4.04 (Maddison & Maddison, 2001). Parsimonious informative gaps were coded manually according to the conservative simple indel coding method described by Simmons and Ochoterena (2000).

PHYLOGENETIC ANALYSES

Phylogenetic analyses were conducted using both parsimony (MP) and Bayesian inference (BI). The three plastid regions were first analyzed separately and then combined.

Equally weighted MP analyses were performed using Nona 2.0 (Goloboff, 1993) launched through WinClada 1.00.08 (Nixon, 2002). Heuristic searches for the shortest trees were performed using the parsimony ratchet (Nixon, 1999). Ratchet runs of 200 iterations each, holding one tree per iteration and randomly weighting 10% of the potentially informative characters, were carried out until the most parsimonious trees (MPTs) were repeatedly found. A strict consensus tree was calculated using the trees obtained in the parsimony ratchet analyses. In order to evaluate the relative support of the clades, jackknife and bootstrap analyses were executed using 1000 replicates with 10 initial trees holding five trees per random addition, doing tree bisection-reconnection (TBR) to hold 1000 trees, and calculating a consensus on each repetition. Frequency values were plotted onto the consensus of the MPTs.

For the BI analyses, a substitution model was selected for each DNA region with Modeltest 3.06 (Posada & Crandall, 1998) under the Akaike Information Criterion (AIC). Modeltest selected the GTR+I+G model of evolution for the *atpB-rbcL* spacer and the GTR+G model for the two remaining markers. Indels were not included in the BI analyses. In the combined analysis, a mixed-model approach was used (Ronquist & Huelsenbeck, 2003). The combined data were partitioned and the same models of evolution were used on the partitions as selected for the single analyses. The BI analyses were conducted with MrBayes 3b4 (Huelsenbeck & Ronquist, 2001). Four Markov chains (one cold, three heated) starting with a random tree were run simultaneously for one million generations, sampling trees at every 100 generations. The first 2500 sampled trees (25%) were regarded as burn-in and discarded. PAUP* version 4b10 (Swofford, 2002) was used to calculate a 50% majority rule tree and to report the posterior probabilities for each clade. Only posterior probabilities above 0.95 are considered (Suzuki et al., 2002).

Table 1. List of genera associated with Spermaceoaceae s.l., their distribution, and species number following Govaerts et al. (2006), except when stated otherwise. Genera in boldface were listed by Robbrecht and Manen (2006); other genera are here based on morphological similarities. Synonymous taxa are as given by Robbrecht (1988), except when stated otherwise.

Genus	Robbrecht, 1988	Native distribution	No. of species	Sampled
Agathisanthemum Klotzsch	Hed	tropical and S Africa, Comoros	4	yes
Amphasma Bremek.	Hed	tropical and S Africa	7	yes
<i>Anthospermopsis</i> (K. Schum.) J. H. Kirkbr.	Spe	NE Brazil	1	no
Arcytophyllum Willd. ex Schult. & Schult. f.	Hed	Mexico to W South America	17	yes
<i>Astiella</i> Jovet	Hed	Madagascar	1	no
Bouardia Salisb.	Cin/Hed	S U.S.A., Mexico to C America	42	yes
<i>Bradea</i> * Standl. ex Brade	Hed	SE Brazil	5	no
<i>Carterella</i> Terrell	Hed	Mexico	1	no
Conostomium (Stafp) Cufod.	Hed	Ethiopia to S Africa	5	yes
Crusea Cham. & Schldtl.	Spe	Arizona, New Mexico, Mexico to C America	14	yes
Dentella J. R. Forst & G. Forst.	Hed	tropical and subtropical Asia to SW Pacific	8	yes
<i>Diacrodon</i> Sprague	Spe	Brazil	1	no
Dibrachionostylus Bremek.	Hed	E Tropical Africa	1	yes
<i>Denscantia</i> E. L. Cabral & Bacigalupo	Spe	E Brazil	4	no
<i>Diodella</i> Small ⁽¹⁾	Spe	S U.S.A. to S America	16	yes
Diodia L. ⁽¹⁾	Spe	S U.S.A. to S America	5	no
<i>Dolichometra</i> K. Schum.	Hed	Tanzania	1	no
Emmeorhiza Pohl ex Endl.	Spe	S tropical America and Trinidad	1	yes
Ernodea Sw. ⁽²⁾	Spe	Florida, Mexico to C America, Caribbean	4	yes
Galianthe Griseb. ⁽³⁾	Spe	S and C America	50	yes
Gomphocalyx Baker	Spe	Madagascar	1	yes
Hedyotis L.	Hed	tropical and subtropical Asia to NW Pacific	ca. 115	yes
<i>Hedythyrus</i> Bremek.	Hed	C and E tropical Africa	2	yes
Houstonia L. ⁽⁴⁾	Hed	N and C America	20	yes
Hydrophylax L. f.	Spe	India, Sri Lanka, Thailand	1	no
<i>Kadua</i> Cham. & Schldtl. (incl. <i>Gouldia</i> A. Gray and <i>Wiegmannia</i> Meyen) ⁽⁵⁾	Spe	Hawaiian Islands to S Pacific	28	yes
Kohautia Cham. & Schldtl. ⁽⁶⁾	Hed	Africa, Madagascar, and Asia	31	yes
Lelya Bremek.	Hed	tropical Africa	1	yes
<i>Leptomischus</i> * Drake	Hed	Assam to China	7	no
<i>Leptoscela</i> Hook. f.	Hed	NE Brazil	1	no
<i>Lucya</i> DC.	Hed	Caribbean	1	no
Manettia Mutis ex L.	Cin/Hed	tropical America	124	yes
Manostachya Bremek.	Hed	C and E tropical Africa	3	yes
<i>Micrasepalum</i> Urb.	Spe	Caribbean	2	no
Mitracarpus Zucc. ex Schult. & Schult. f.	Spe	tropical America, naturalized elsewhere	58	yes
Mitrasacmopsis Jovet	Hed	C and E tropical Africa and Madagascar	1	yes
<i>Neanotis</i> W. H. Lewis	Hed	tropical and subtropical Asia	33	no
<i>Neohymenopogon</i> * Bennet	Cin/Hed	E Himalaya, Tibet, SC China, N Indo-China	3	no
Nesohedyotis (Hook. f.) Bremek.	Hed	St. Helena	1	yes
<i>Nodocarpaea</i> A. Gray	Spe	Cuba	1	no
Oldenlandia L. (incl. <i>Eionitis</i> Bremek., <i>Exallage</i> Bremek., and <i>Theorchus</i> Bremek.)	Hed	pantropical	ca. 240	yes
<i>Oldenlandiopsis</i> Terrell & W. H. Lewis	Hed	tropical and subtropical America	1	no
Pentanopsis Rendle	Hed	Ethiopia to N Kenya	2	yes
Pentodon Hochst.	Hed	tropical and S Africa, Arabian Pen., W Indian Ocean, naturalized in America	2	yes
<i>Phyllocrater</i> Wernham	Hed	Borneo	1	no
Phylohydrax Puff	Spe	coastal Tanzania to S Africa, Madagascar	2	yes

Table 1. Continued.

Genus	Robbrecht, 1988	Native distribution	No. of species	Sampled
<i>Pleiocraterium</i> Bremek.	Hed	tropical Asia	4	no
<i>Polyura</i> * Hook. f.	Hed	E Himalaya to Assam	1	no
<i>Pseudonesohedyotis</i> Tennant	Hed	Tanzania	1	no
<i>Psyllocarpus</i> Mart. & Zucc.	Spe	Brazil	9	no
<i>Richardia</i> L.	Spe	tropical and subtropical America, naturalized elsewhere	16	yes
<i>Sacosperma</i> * G. Taylor	Hed	W and C tropical Africa	2	no
<i>Schwendenera</i> K. Schum.	Spe	Brazil	1	no
<i>Spermaceo</i> L. (incl. <i>Borreria</i> G. Mey. and <i>Hemidiodia</i> K. Schum.) ⁽⁷⁾	Spe	panropical	250–300	yes
<i>Staelia</i> Cham. & Schltdl.	Spe	Mexico and S tropical America	14	no
<i>Stenaria</i> (Raf.) Terrell	Hed	C and E U.S.A. to Mexico, Bahamas	5	yes
<i>Stenotis</i> Terrell	Hed	Mexico (Baja California)	7	no
<i>Stephanococcus</i> Bremek.	Hed	WC tropical Africa	1	no
<i>Synaptantha</i> Hook. f.	Hed	Australia	2	yes
<i>Tobagoa</i> Urb.	Spe	Panama to Tobago	1	no
<i>Tortuella</i> Urb.	Spe	Île de la Tortue (Haiti)	1	no

Hed, Hedyotideae; Spe, Spermaceace s. str.; Cin, Cinchoneae.

⁽¹⁾ = Bacigalupo & Cabral (1999); ⁽²⁾ = Negrón-Ortiz & Hickey (1996); ⁽³⁾ = Cabral (1991); ⁽⁴⁾ = Terrell (1996); ⁽⁵⁾ = Terrell et al. (2005); ⁽⁶⁾ = Mantell (1985); ⁽⁷⁾ = Dessein (2003).

* Tentatively included.

RESULTS

Sequence data from the aligned *atpB-rbcL*, *rps16*, and *trnL-F* regions were analyzed independently and in a combined analysis (Table 2). Individual plastid sequence analyses were topologically congruent. Therefore, only the results from the MP and BI analysis of the combined matrix are presented (Figs. 2–4). Compared to the topologies of the individual plastid sequence analyses, the combined plastid trees show increased resolution and branch support. The Bayesian tree is somewhat better resolved than the consensus of the MP analysis, but more resolved lineages have low posterior probabilities.

Spermaceace s.l., as delimited in the introduction (Table 1), form a well-supported monophyletic group (jackknife support [JS] = 100, bootstrap support [BS] = 100, posterior probability [PP] = 1), as can be seen in Figure 2. A highly supported pentamerous-flowered clade including *Dentella* J. R. Forst. & G. Forst. and *Pentodon* Hochst. (JS = 100, BS = 99, PP = 1) is resolved as sister to the rest of the tribe (Fig. 2). The remaining ingroup taxa are part of a clade that lacks significant jackknife and bootstrap support and has only weak posterior probability (PP = 0.84). Within this clade, stars with Roman numerals I to III are assigned to the three deeper internal nodes that have reasonable support. These three clades are discussed in the following paragraphs.

Clade I in Figure 2 (JS = 88, BS = 77, PP = 1) includes a *Kohautia* subg. *Kohautia* Verdc. clade sister to a clade that includes *Pentanopsis* and allied genera. This *Pentanopsis* clade (JS = 95, BS = 95, PP = 1) is similar to that proposed by Thulin and Bremer (2004). However, our larger sampling resulted in a broader circumscription adding *Gomphocalyx*, *Oldenlandia affinis* (Roem. & Schult.) DC., *O. herbacea* (L.) Roxb., and *O. rosulata* K. Schum. Our results support the monophyly of both *Amphiasma* Bremek. (JS = 98, BS = 98, PP = 1) and *Phylohydrax* (JS = 93, BS = 95, PP = 1).

In clade II (JS = 88, BS = 83, PP = 1) of Figure 2, all Asian and Micronesian *Hedyotis* species, except *H. tenelliflora* Blume, are part of a strongly supported *Hedyotis* s. str. clade (JS = 100, BS = 100, PP = 1), which is sister to a clade including *Agathisanthemum* Klotzsch and its allies. This clade of Asian and Micronesian *Hedyotis* species also includes *H. fruticosa* L., the type species of the genus. Relationships within this *Hedyotis* s. str. clade remain mostly unresolved. Within the *Agathisanthemum* clade, *Agathisanthemum* is paraphyletic to *Lelya osteocarpa* Bremek. (JS = 100, BS = 99, PP = 1), both sister to a lineage of African (*Oldenlandia angolensis* K. Schum. and *O. gorensis* (DC.) Summerh.) and North American (*O. uniflora* L.) *Oldenlandia* species (JS = 100, BS = 99, PP = 1).

In the MP consensus, clade II is sister to clade III (Figs. 2A, 3A). However, this sister relationship lacks

significant jackknife and bootstrap support and is not recovered in the BI (Figs. 2B, 3B).

Within clade III (Figs. 3, 4), the earlier derived clades lack significant support values in the MP consensus (Figs. 3A, 4A) and are collapsed in the BI (Figs. 3B, 4B). Therefore, relationships between the different subclades of clade III should be interpreted with caution. In the following paragraphs, these subclades are discussed individually.

In Figure 3, the monospecific genus *Dibrachionostylus* Bremek. is sister to a clade of African *Oldenlandia* species (*O. echinulosa* K. Schum., *O. geophila* Bremek., and *O. nervosa* Hiern). However, this sister relationship lacks significant jackknife and bootstrap support (Fig. 3A) and is not supported by the BI (Fig. 3B). The sister relationship of this clade with respect to *Mitrasacmopsis* Jovet and its allies also lacks support. *Mitrasacmopsis*, another monospecific genus in the *Hedyotis*–*Oldenlandia* group, is nevertheless highly supported as sister to *Hedythyrus* Bremek. (JS = 99, BS = 97, PP = 1), and both are sister to *O. fastigiata* Bremek. (JS = 99, BS = 99, PP = 1).

The genus *Kadua* Cham. & Schltldl. (including *Oldenlandia biflora* L.) is resolved as monophyletic with moderate jackknife and bootstrap support but maximum Bayesian posterior probability (JS = 87, BS = 86, PP = 1). The Hawaiian *Kadua* species are unresolved with respect to the French Polynesian species, *K. rapensis* F. Br. The genus *Kadua* shares a most recent common ancestor with all sampled Australian taxa (*O. galioides* (F. Muell.) F. Muell., *O. mitrasacmoides* F. Muell., and *Synaptantha tillaeacea* (F. Muell.) Hook. f.), the Austro-Asian species *Hedyotis tenelliflora*, and the African species *O. lancifolia* (Schumach.) DC. (JS = 91, BS = 86, PP = 1).

The genus *Arcytophyllum* is strongly supported as monophyletic by our analysis (JS = 93, BS = 92, PP = 1). It is sister to a clade of North and Central American species of *Houstonia*, *Oldenlandia*, and *Stenaria* (Raf.) Terrell. The *Houstonia* species plus *S. nigricans* (Lam.) Terrell form one clade, although without significant support.

In Figure 4, Spermaceae s. str. is nested within the *Hedyotis*–*Oldenlandia* group. In the MP consensus (Fig. 4A), it forms a monophyletic lineage (although lacking significant jackknife support and bootstrap support), while in the BI tree (Fig. 4B), *Nesohedyotis arborea* (Roxb.) Bremek. is nested within the Spermaceae s. str. clade (although with low PP = 0.77). In both MP and BI analysis, Spermaceae s. str. has uncertain relationships with respect to *Arcytophyllum serpyllaceum* (Schltldl.) Terrell, *Bowardia*, *Manettia*, *Nesohedyotis* (Hook. f.) Bremek. (Fig. 4A), *O. tenuis* K. Schum., and *O. salzmannii*

(DC.) Benth. & Hook. f. ex B. D. Jacks. Sister to this polytomy is a clade with species of *Kohautia* subg. *Pachystigma* Bremek. and *Oldenlandia* species, including the type species *O. corymbosa* L. (JS = 99, BS = 98, PP = 1). Consequently, species of the genus *Kohautia* Cham. & Schltldl. fall in two well-supported, not closely related clades, which correspond to the two described subgenera: subgenus *Kohautia* (JS = 99, BS = 99, PP = 1) and subgenus *Pachystigma* (JS = 96, BS = 96, PP = 1).

DISCUSSION

Our analysis corroborates the monophyly of Spermaceae s.l. (Table 1), a mainly herbaceous assemblage distributed pantropically, with only a few genera penetrating into more temperate regions. The morphological variation is considerable, but the fimbriate stipules and tetramerous flowers are shared by most species. There are no clear morphological synapomorphies that separate Spermaceae s.l. from its sister tribe, the emended Knoxieae. The main differences are listed in Table 3.

Our analyses show several major evolutionary lineages within Spermaceae s.l. and allow us to evaluate the monophyly of a number of genera. Several genera that have been recognized within the *Hedyotis*–*Oldenlandia* group are supported here as monophyletic (*Amphiasma*, *Arcytophyllum*, *Dentella*, *Kadua*, and *Phylohydrax*), while others appear to be paraphyletic (e.g., *Agathisanthemum*), biphyletic (*Kohautia*), or polyphyletic (*Hedyotis* and *Oldenlandia* sensu Bremekamp). These groups are discussed in the following paragraphs.

SPERMACEAE S. STR.

In our analyses, Spermaceae s. str. is nested within the *Hedyotis*–*Oldenlandia* group, which no longer makes it possible to recognize this lineage at a tribal level. Additionally, Spermaceae s. str. as delimited by Robbrecht (1988) is not corroborated as monophyletic. Both MP and BI analyses show that it is necessary to exclude *Gomphocalyx* and *Phylohydrax* for Spermaceae s. str. to be monophyletic, which is in agreement with Thulin and Bremer (2004) and Dessein et al. (2005a).

In the BI analyses, *Nesohedyotis arborea*, a species previously included in Hedyotideae, is placed within Spermaceae s. str. as sister to *Emmeorhiza umbellata* (Spreng.) K. Schum., but lacking significant posterior probability (PP = 0.67). This position of *Nesohedyotis* within Spermaceae s. str. was not recovered in the MP analysis. Because no morphological characters can be found to support *Nesohed-*

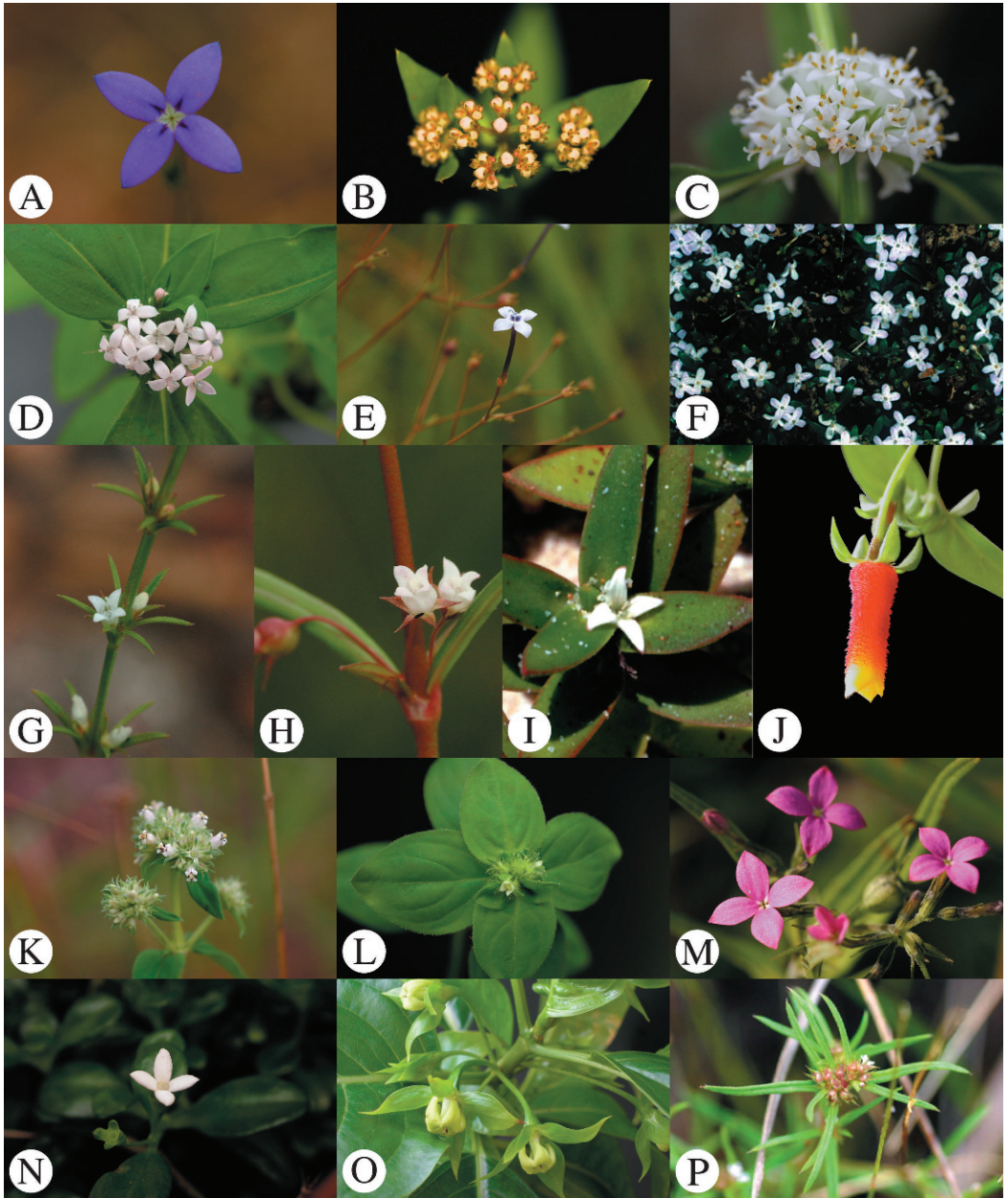


Figure 1. Floral diversity among species of Spermacoceae. —A. *Kohautia microcala* Bremek. —B. *Hedythyrus spermacocinus* (K. Schum.) Bremek. —C. *Mitracarpus frigidus* (Willd. ex Roem. & Schult.) K. Schum. —D. *Spermacoce debilis* Benth. —E. *Oldenlandia herbacea* (L.) Roxb. —F. *Gomphocalyx herniarioides* Baker. —G. *Manostachya ternifolia* E. S. Martins. —H. *Oldenlandia lancifolia* (Schumach.) DC. —I. *Phylohydrax madagascariensis* (Willd. ex Roem. & Schult.) Puff. —J. *Manettia luteorubra* (Vell.) Benth. —K. *Agathisanthemum globosum* (Hochst. ex A. Rich.) Klotzsch. —L. *Oldenlandia goreensis* (DC.) Summerh. —M. *Kohautia coccinea* Royle. —N. *Oldenlandia biflora* L. —O. *Kadua acuminata* Cham. & Schltdl. —P. *Oldenlandia robinsonii* Pit.

yotis as part of Spermacoceae s. str., we suggest that the difference between the MP and BI analysis could be the result of data sampling artifacts (only *rps16* was sequenced for *N. arborea*), which probably affected the BI more than the MP analysis.

With the deeper nodes unresolved or only weakly supported, the relationships within Spermacoceae s. str. remain unclear and should be the subject of further phylogenetic studies including more taxa and/or characters. Nevertheless, our analyses corroborate

Table 2. Characteristics of each data matrix and the corresponding tree statistics.

	No. of taxa	No. of char.	No. of PI char.	No. of PI indels	No. of MPT	MPT length	CI	RI
<i>atpB-rbcL</i>	100	1237	175	31	1949	399	0.55	0.84
<i>rps16</i>	105	705	191	20	1351	525	0.56	0.82
<i>trnL-trnF</i>	107	1053	184	29	343	423	0.62	0.88
Combined	128	2995	550	80	4782	1385	0.56	0.84

Char, characters; CI, consistency index (Kluge & Farris, 1969); MPT, most parsimonious tree(s); PI, potentially informative; RI, retention index (Farris, 1989).

the monophyly of most of the commonly accepted genera within Spermaceae s. str., notably *Crusea* Cham. & Schtdl., *Mitracarpus* Zucc. ex Schult. & Schult. f., and *Richardia* L., although these were sampled only with a few species. In contrast, the two *Galianthe* Griseb. sampled species are paraphyletic to *Diodia spicata* Miq., a species that was recently excluded from *Diodia* s. str. and transferred to *Borreria*. If the position of *D. spicata* is confirmed by further phylogenetic studies, the generic circumscription of *Galianthe* should be widened to include at least this species. Dessein (2003) already showed that palynological data (7-zonocolporate pollen, long ectocolpi, double reticulum) support a close relation between *D. spicata* and *Galianthe*. *Diodia* L. as traditionally delimited, including species referred to *Diodella* Small by Bacigalupo and Cabral (1999), is not supported as monophyletic. Also, *Spermaceae* s.l., including *Borreria*, is not supported as monophyletic.

BOUWARDIA AND MANETTIA

Manettia is strongly supported as monophyletic (JS = 100, BS = 100, PP = 1), whereas support for *Bouwardia* is moderate (JS = 85, BS = 87, PP = 0.99). In accordance with Andersson et al. (2002), *Arcytophyllum serpyllaceum* is corroborated as sister to *Bouwardia*. This strongly supported relationship (JS = 99, BS = 99, PP = 1), in combination with the fact that the remaining *Arcytophyllum* species are strongly supported as a monophyletic and distinct lineage (see below), suggests that at least *A. serpyllaceum* should be included within *Bouwardia*. Although *Bouwardia* is generally considered as a genus of shrubs only, it comprises both subshrubs and perennial herbs (Blackwell, 1968), which makes it possible to fit in *A. serpyllaceum*. *Arcytophyllum serpyllaceum* is similar to *Bouwardia* and different from other *Arcytophyllum* species in many respects. First, the stipule margin of *A. serpyllaceum* is not dentate or fimbriate, as in most *Arcytophyllum* species (Mena, 1990), but consists of a basal sheath and a trullate mucro as in most *Bouwardia* species (Blackwell, 1968). Second, where-

as the seeds of *Arcytophyllum* are more or less cymbiform (Mena, 1990), those of *A. serpyllaceum* are discoid with a central hilum as in *Bouwardia* (Andersson et al., 2002). The major difference between seeds of *A. serpyllaceum* and *Bouwardia* is that *Bouwardia* seeds are winged, whereas those of *A. serpyllaceum* are not.

ARCYTOPHYLLUM-HOUSTONIA CLADE

Previous studies based on plastid DNA sequences have shown *Arcytophyllum* to be monophyletic and closely related to the North American *Houstonia* (Andersson & Rova, 1999; Andersson et al., 2002). Our analyses support the monophyly of the Neotropical genus *Arcytophyllum* (JS = 93, BS = 92, PP = 1) only if *A. serpyllaceum* is excluded from the genus (see above). Sister to *Arcytophyllum* is a group of North and Central American species presently classified in the genera *Houstonia*, *Oldenlandia*, and *Stenaria*. By having its closest relatives in North America rather than in South America, *Arcytophyllum* may be one of the few examples within Rubiaceae that has reached the Andes by a southern migration (Andersson et al., 2002). From this perspective, Mesoamerican species like *O. microtheca* (Cham. & Schtdl.) DC. may represent remnants of stepping-stone populations.

The *Arcytophyllum-Houstonia* clade as defined by our results is thus restricted to the New World. Seeds of *Arcytophyllum* and *Houstonia* are generally more or less cymbiform. Our results thus support Schumann's (1891) grouping of genera with cymbiform seeds. So far, *Neanotis* W. H. Lewis has not been sequenced, but if seed shape is indeed a good phylogenetic marker, *Neanotis* could be the closest non-American relative of the *Arcytophyllum-Houstonia* clade (Lewis, 1966).

There has been much discussion about the recognition of *Houstonia* at the generic level. In a recent molecular study based on ITS and *trnL* intron data (Church, 2003), *Houstonia* appeared to be paraphyletic with respect to the North American genus *Stenaria*. Therefore, Church (2003) suggested that *Houstonia* and *Stenaria* are better treated as a single genus. As currently circumscribed (Terrell, 1996), the genus *Houstonia* is composed of 20 species

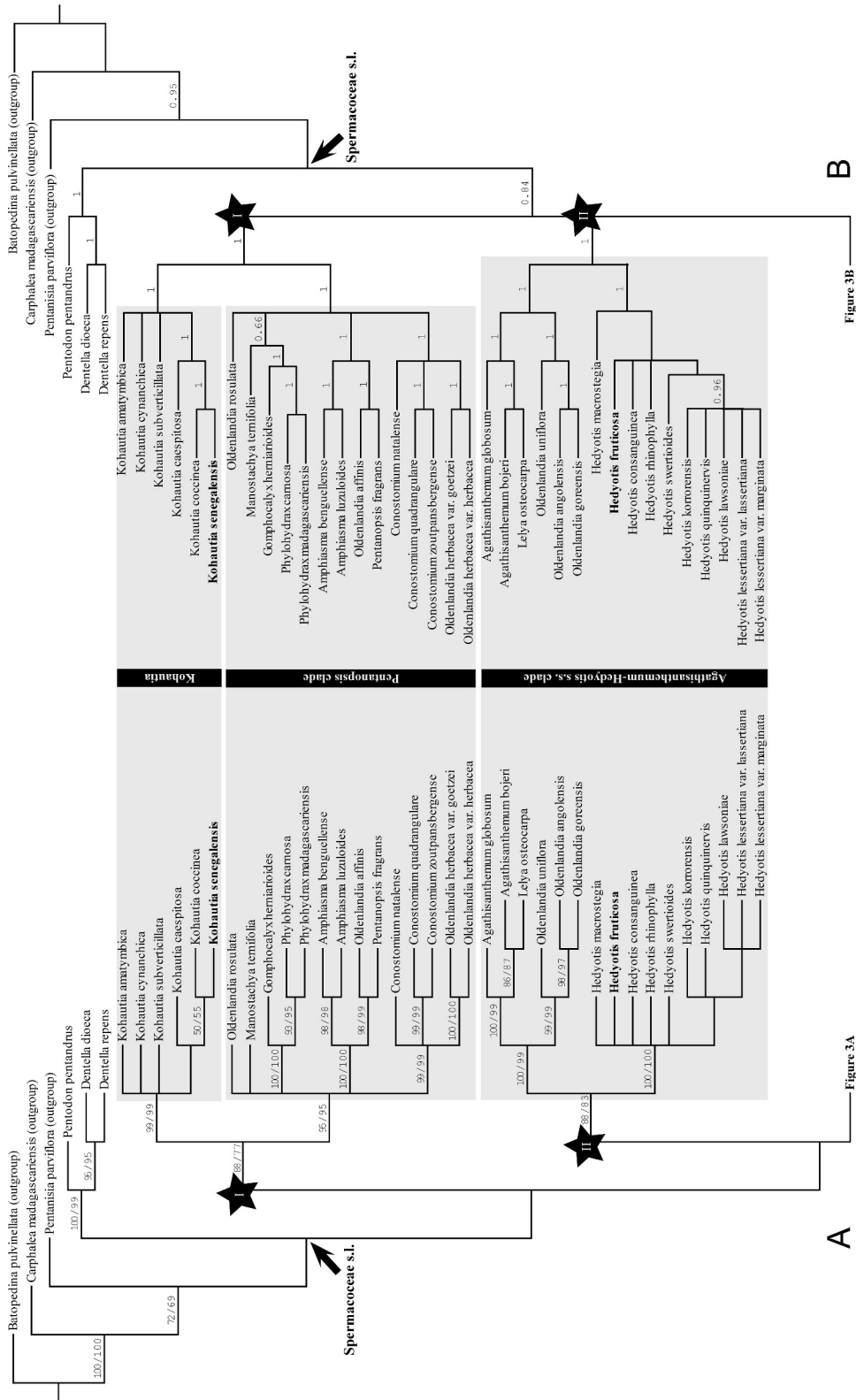


Figure 2. —A. Part one of the strict consensus tree of the 4782 MPTs from the combined analysis including *atpB-rbcL*, *rps16*, and *trnL-trnF* sequences ($L = 1385$, consistency index [CI] = 0.56, retention index [RI] = 0.84). Jackknife (left) and bootstrap (right) values (> 50) are indicated above branches. —B. Part one of the Bayesian tree based on combined *atpB-rbcL*, *rps16*, and *trnL-trnF* data. Posterior probabilities are indicated above branches. The tribe Spermaceae s.l. starts on Figure 2 (as indicated by an arrow) and continues over to Figures 3 and 4. Stars with Roman numerals I to III are assigned to the three reasonably supported clades within Spermaceae s.l.

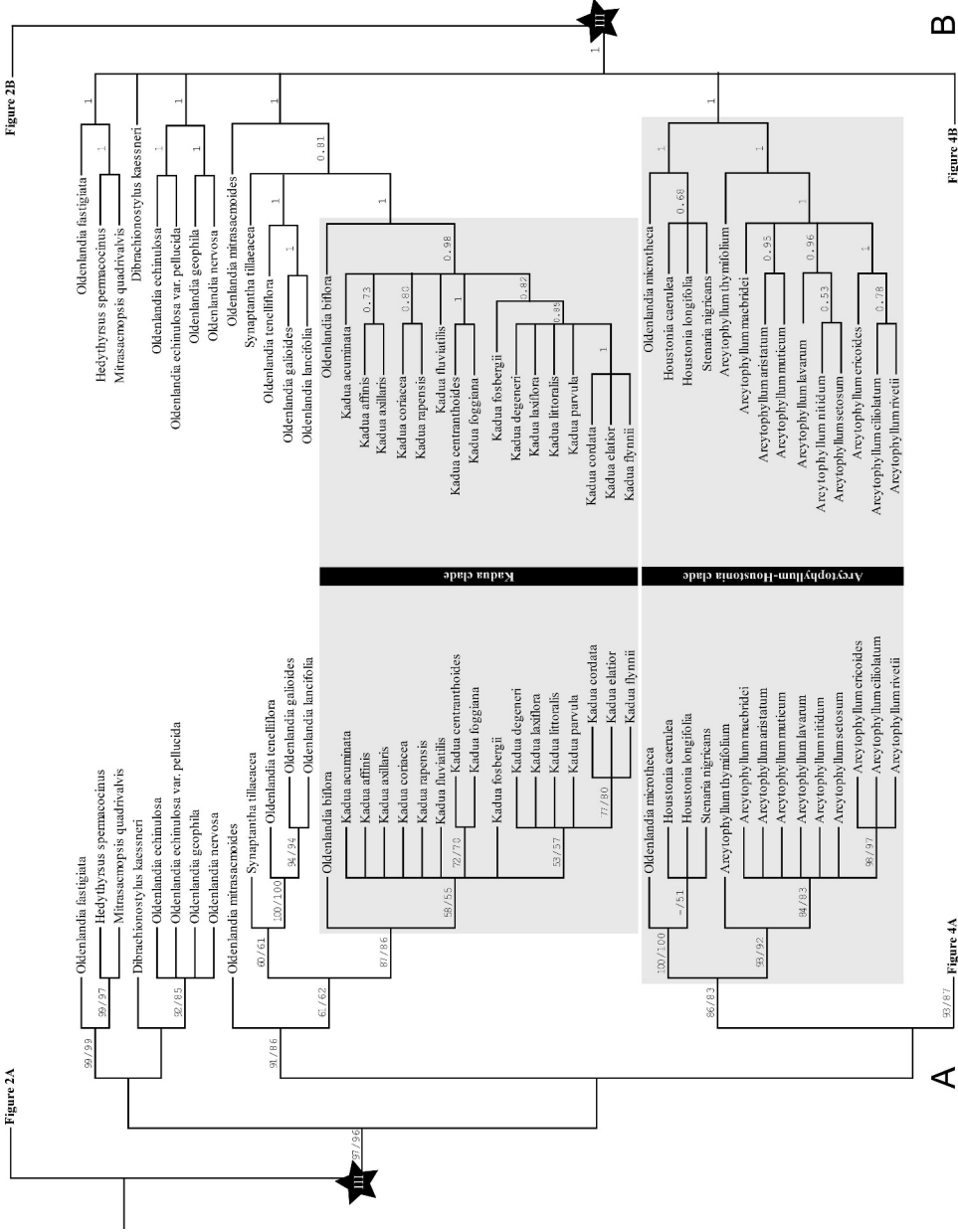


Figure 3. —A. Part two of the strict consensus tree of the 4782 MPITs from the combined analysis including *atpB-bcL*, *rps16*, and *trnL-trnF* sequences ($L = 1385$, $CI = 0.56$, $RI = 0.84$). Jackknife (left) and bootstrap (right) values (> 50) are indicated above branches. —B. Part two of the Bayesian tree based on combined *atpB-bcL*, *rps16*, and *trnL-trnF* data. Posterior probabilities are indicated above branches. Roman numeral III is assigned to the deeper internal node with reasonable support. The tribe Spermaceae s.l. starts on Figure 2 and continues over to Figures 3 and 4. Stars with Roman numerals I to III are assigned to the three reasonably supported clades within Spermaceae s.l.

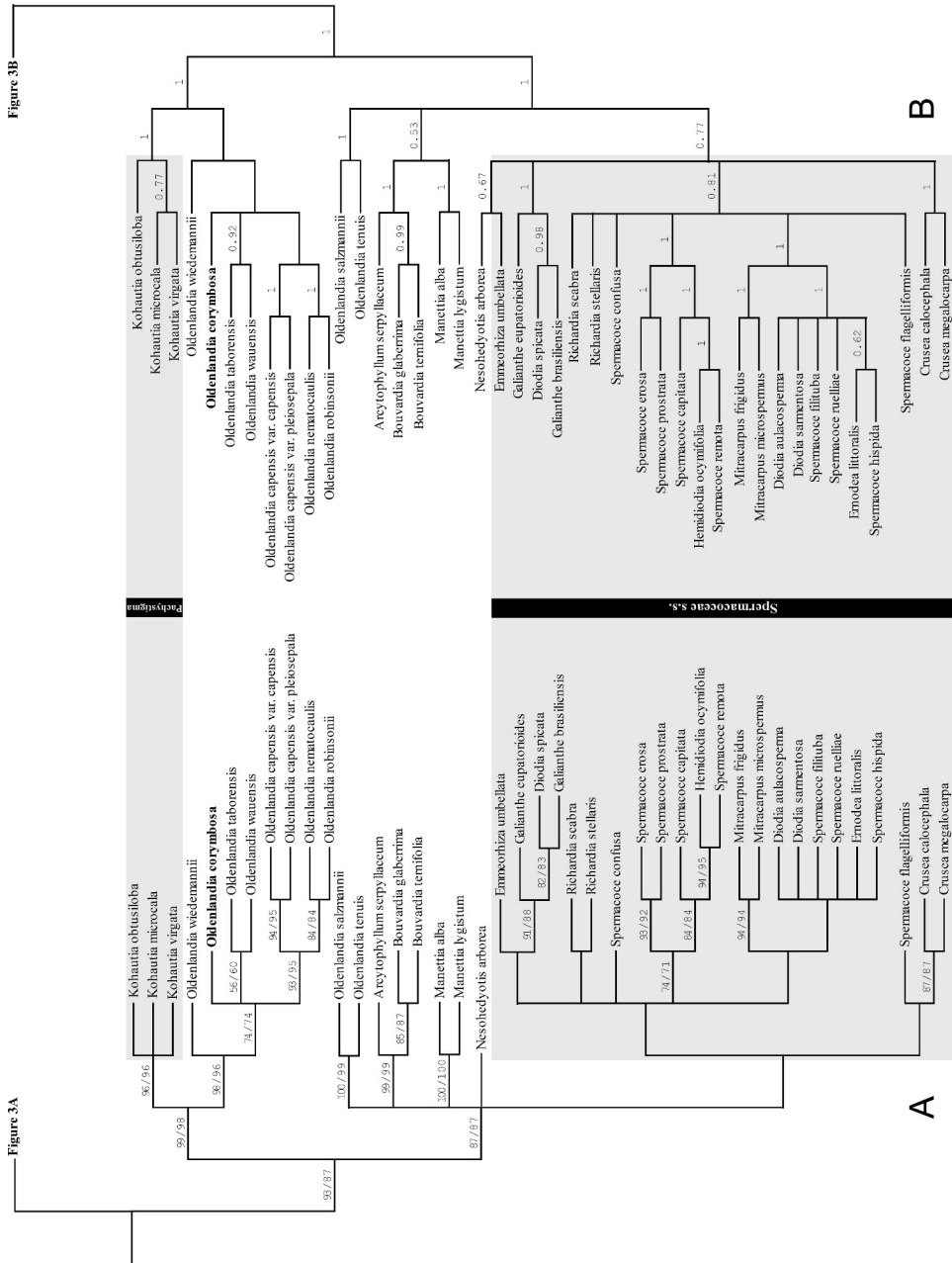


Figure 4. —A. Part three of the strict consensus tree of the 4782 MPTs from the combined analysis including *atpB-rbcL*, *rps16*, and *trnL-trnF* sequences ($L = 1385$, $CI = 0.56$, $RI = 0.84$). Jackknife (left) and bootstrap (right) values (> 50) are indicated above branches. —B. Part three of the Bayesian tree based on combined *atpB-rbcL*, *rps16*, and *trnL-trnF* data. Posterior probabilities are indicated above branches. The tribe Spermaceae s.l. starts on Figure 2 and continues over to Figures 3 and 4. Representatives of the former tribe Spermaceae s. str. are shown in this portion of the strict consensus tree and the Bayesian tree.

Table 3. Major morphological differences between Knoxieae and Spermacoceae s.l.

	Knoxieae s.l.	Spermacoceae s.l.
Merosity	often 5-merous or derived from the 5-merous state	often 4-merous, rarely 5-merous
Inflorescence	terminal (including pseudoaxillary)	terminal or axillary
Calyx lobes	often 1 or more calyx lobes enlarged	rarely enlarged calyx lobes
Pollen	bireticulum not yet reported	bireticulum common, often associated with heterostyly
Exotesta	ITW often slightly thickened	ITW without thickenings
Distribution	paleotropical, centered in Madagascar and continental Africa	pantropical, with a few taxa reaching outside the tropics

ITW, inner tangential wall.

restricted to North America. The genus contains both annual and perennial herbs with either heterostylous or homostylous flowers, crateriform seeds, and colpate pollen. Chromosome numbers are variable among species of the genus with $x = 6, 7, 8,$ or 11 . *Stenaria*, a genus only recently described (Terrell, 2001a), includes five species previously included in the North American *Hedyotis*. The genus contains only perennial, heterostylous herbs. Due to our incomplete sampling of these two genera, and given that *Houstonia* forms a polytomy with *Stenaria*, our results are not conclusive with respect to whether it is best to recognize *Stenaria* or consider it part of a more broadly delimited *Houstonia*. A more extensive sampling should focus further on this question.

Sister to the *Houstonia*–*Stenaria* clade is *Oldenlandia microtheca*. The prevailing basic chromosome number in *Oldenlandia* is $n = 9$, which occurs in the type species *O. corymbosa* and in many of the species native to North America, Asia, Africa, and Australia (Lewis, 1965), but not in *O. microtheca*, which is exceptional in having a chromosome number $n = 11$. The same chromosome number is found in *Oldenlandiopsis* Terrell & W. H. Lewis (Terrell, 1991), not included in this study, and in some *Houstonia* species (e.g., *H. rubra* Cav.). Until now, *Oldenlandia microtheca* and *Oldenlandiopsis* were never considered to be closely related to *Houstonia* because of the lack of morphological similarities (Lewis, 1965; Terrell, 1991).

Oldenlandiopsis contains only one species, *O. callitrichoides* (Griseb.) Terrell & W. H. Lewis, previously included in *Oldenlandia*. This small-leaved, small-flowered, creeping herb is native to the West Indies and southern Mexico. Based on its chromosome number and its distribution, a position of *Oldenlandiopsis* in the *Arcytophyllum*–*Houstonia* clade close to *Oldenlandia microtheca* seems quite likely. However, seeds of *Oldenlandiopsis* are non-crateriform and pollen are 8-colpate with a lalongate, slightly crassimarginate endoaperture (Terrell & Lewis, 1990). These types of seeds and pollen

are unusual within the *Arcytophyllum*–*Houstonia* clade. Plurizonocolpate pollen grains are also exceptional within the rest of the *Hedyotis*–*Oldenlandia* group, where the aperture number rarely exceeds five. The Asian genus *Neanotis* (Lewis, 1966), the Malagasy endemic *Gomphocalyx* (Dessein et al., 2005a), the Afro-Madagascan *Phylohydrax* (Puff, 1986), and the West Indian monotypic genus *Lucya* DC. (Terrell & Lewis, 1990) are notable exceptions within the *Hedyotis*–*Oldenlandia* group in having plurizonocolpate pollen grains. Both *Gomphocalyx* and *Phylohydrax* belong to the *Pentanopsis* clade (see below). With no molecular sequence data available for *Lucya*, *Neanotis*, and *Oldenlandiopsis*, it would be premature to hypothesize a close relationship between any of these taxa and the *Arcytophyllum*–*Houstonia* clade or the *Pentanopsis* clade. Nevertheless, considering their distribution, the Caribbean-Mexican genera *Lucya* and *Oldenlandiopsis* are more likely to fall in the *Arcytophyllum*–*Houstonia* clade, whereas the Asian genus *Neanotis* is more likely to have its closest relatives within the *Pentanopsis* clade.

Two closely related genera from Baja California, *Stenotis* Terrell (Terrell, 2001b) and *Carterella* Terrell (Terrell, 1987), may also belong to the *Arcytophyllum*–*Houstonia* clade. Like the Mesoamerican species *Oldenlandia microtheca*, they may represent remnants of stepping-stone populations. The monospecific genus *Carterella* was described based on *Bouvardia alexandrae* A. M. Carter. It resembles *Bouvardia* in having unusually long corolla tubes, but differs from *Bouvardia* in having wingless seeds and chromosome number $n = 13$. The genus *Stenotis*, on the other hand, includes seven former *Hedyotis* species endemic to the Baja California peninsula (Terrell, 2001b). These heterostylous, annual or perennial herbs also have chromosome number $x = 13$. According to Terrell (1987, 2001b), *Carterella* and *Stenotis* have their closest relatives among the Baja California species of *Houstonia* (*H. mucronata* group sensu Terrell et al., 1986).

KADUA

Our results support the resurrection of the genus *Kadua* for the Polynesian Hedyotideae (Hawaiian Islands and French Polynesia: Terrell et al., 2005). This taxonomic change was previously suggested by unpublished molecular data (Motley et al., 1998; Motley, 2003) and by morphological studies of the seed anatomy of the Hawaiian species (Terrell et al., 2005). The genus *Kadua* was treated as a distinct genus until Fosberg's (1943) revision of the group. He included the genus within a broadly delimited *Hedyotis*, except for the fleshy-fruited species, which he treated as *Gouldia* A. Gray (Fosberg, 1937). *Kadua* species can, however, easily be distinguished from other *Hedyotis* species by their salverform, fleshy corollas with appendaged lobes, and by their either tardy, often incomplete septicidal dehiscent capsules or indehiscent drupaceous fruits (Terrell et al., 2005). The genus *Kadua* currently comprises 28 species; all are indigenous to the Pacific Islands with the majority (21 species) occurring on the Hawaiian Islands (Terrell et al., 2005). Seeds of these Hawaiian *Kadua* species fall into four groups, described by Terrell et al. (2005). Based on the chloroplast data alone, the relationships within the genus *Kadua* remain mostly unresolved. Only section *Wiegmannia* Meyen, W. L. Wagner & Lorence (represented in our sampling by *K. cordata* Cham. & Schldtl., *K. degeneri* (Fosberg) W. L. Wagner & Lorence, *K. elatior* (H. Mann) W. L. Wagner & Lorence, *K. flynnii* (W. L. Wagner & Lorence) W. L. Wagner & Lorence, *K. laxiflora* H. Mann, *K. littoralis* Hillebr., and *K. parvula* A. Gray) and section *Gouldiopsis* (Fosberg) W. L. Wagner & Lorence (represented in our sampling by *Kadua centranthoides* Hook. & Arn. and *K. foggiana* (Fosberg) W. L. Wagner & Lorence) were recovered. A broader sampling including more *Kadua* species and more molecular markers is needed to discuss molecular evolution in the light of the seed morphological observations of Terrell et al. (2005).

Oldenlandia biflora is sister to the *Kadua* clade. Its distribution from (sub)tropical Asia to the western Pacific is consistent with the sister relationship to the Polynesian *Kadua* clade. Our results show that *O. biflora* can no longer be included within the genus *Oldenlandia*, but it is necessary to await further studies before transferring it to *Kadua* or describing a new genus. So far, we have not found morphological characters to support the transfer.

HEDYOTIS S. STR.

It seems appropriate to restrict the name *Hedyotis* to the Asian and Micronesian species of the genus,

which includes the type species *H. fruticosa* (Sri Lanka). Several authors already considered the genus *Hedyotis* to be a distinct Asian taxon (Bremekamp, 1952; Hallé, 1966; Terrell, 1975, 1991; Andersson et al., 1999). *Hedyotis fruticosa* and its Asian relatives are not closely related to the American species of *Hedyotis* (*Houstonia* lineage) or to the Polynesian species (*Kadua*). The Asian and Micronesian *Hedyotis* species (*Hedyotis* s. str.) differ from the American and Polynesian ones in their combination of a robust (sometimes shrubby) habit, small beaked and diplophragmous capsules, dorsiventrally compressed seeds with the hilum on a conspicuous central ridge (Terrell & Robinson, 2003), and a high chromosome number (Kiehn, 1986). Our results clearly demonstrate that a broad concept of *Hedyotis*, merging several genera (*Hedyotis* s. str., *Houstonia*, *Kadua*, *Kohautia*, *Oldenlandia*, etc.), as was proposed by several researchers (Fosberg, 1943; Merrill & Metcalf, 1946; Rogers, 1987; Wagner et al., 1989; Fosberg & Sachet, 1991; Dutta & Deb, 2004), is no longer supported. If this is confirmed with further sampling, all North American species now called *Hedyotis* would require new combinations under other generic names.

Pleio craterium Bremek. (not included in this study) is probably related to the *Hedyotis* s. str. clade. The genus was described by Bremekamp in 1939, including four species distributed in India, Sri Lanka, and Sumatra. The generic name refers to the numerous cups that are formed by the connate leaf bases. The type species of the genus, *P. verticillare* (Wall. ex Wight & Arn.) Bremek., was previously included in *Hedyotis*. However, the genus differs from other *Hedyotis* s. str. species in having distinctly beaked capsules and parallel-nerved, quaternate leaves. The internodes remain very short, as a result of which the leaf whorls are clustered in rosettes. It will be necessary to wait, however, until molecular data of *Pleio craterium* become available before a close relation of the genus to the Asian *Hedyotis* species is confirmed.

AGATHISANTHEMUM CLADE (CLADE II)

The African genus *Agathisanthemum* is not supported as monophyletic by our analyses. The monotypic African genus *Lelya* Bremek. is nested within *Agathisanthemum*, making it paraphyletic as currently circumscribed and suggesting that *Lelya* should be reduced to *Agathisanthemum*. This proposal is supported by several palynological characters. Scheltens (1998) showed that *Agathisanthemum* and *Lelya* share the same pollen type, characterized by a distinct endocolpus or endocingulum, a mesoporus surrounded by a costa at the inside of the grain (described as a compound ora by Lewis, 1965), and a microreticulate

sexine with granules on the muri facing the lumina (bireticulum).

A group of African *Oldenlandia* species is sister to *Agathisanthemum*. Two of the three *Oldenlandia* species, *O. angolensis* and *O. goreensis*, belong to *Oldenlandia* subg. *Anotidopsis* (Hook. f.) K. Schum. This subgenus, as described by Bremekamp (1952), includes three other putative species of which only *O. cephalotes* (Hochst.) Kuntze (not included in our sampling) is currently recognized. Subgenus *Anotidopsis* is distributed in Asia, Australia, and Africa and is characterized by distinctly beaked capsules. The New World taxon *O. uniflora* is sister to *O. angolensis* and *O. goreensis*. More detailed (molecular as well as morphological) studies within the *Agathisanthemum* clade are needed to evaluate if the three *Oldenlandia* species, *O. angolensis*, *O. goreensis*, and *O. uniflora*, or the entire *Oldenlandia* subg. *Anotidopsis*, are to be transferred to a new genus or if these species are better treated as members of the genus *Agathisanthemum*.

The Asian *Hedyotis* species are sister to the *Agathisanthemum*–*Oldenlandia* clade. This relationship is not unexpected as Bremekamp (1952) already suggested a close relationship between *Agathisanthemum* and the Asian *Hedyotis* species (i.e., *Hedyotis* sect. *Diplophragma*) based on a similar type of dehiscence of the capsules.

PENTANOPSIS CLADE

Our sampling resulted in a broader concept of the *Pentanopsis* clade than proposed by Thulin and Bremer (2004). They included *Amphiasma*, *Conostomium* (Stapf) Cufod., *Manostachya* Bremek., *Pentanopsis*, and *Phylohydrax*.

Oldenlandia affinis was not included in the study of Thulin and Bremer (2004), but it was shown to be closely related to the African genus *Amphiasma* by Andersson and Rova (1999) and Dessein et al. (2005a). *Amphiasma*, *O. affinis*, and *Pentanopsis* share sessile linear leaves, indistinctly beaked capsules, non-mucilaginous seeds and nonpunctate testa cells (Bremekamp, 1952). However, a detailed study is needed to find more unambiguous morphological characters to support a relation among the three taxa.

In the past, *Gomphocalyx* (a monospecific genus endemic to Madagascar) and *Phylohydrax* (a genus described in 1986 by Puff to accommodate the East African and Madagascan *Hydrophylax* L. f. species) were both included in Spermaceae s. str. based on their uniovulate ovaries and plurizonocolporate pollen grains (Robbrecht, 1988). However, recent molecular studies excluded both genera from Spermaceae s. str. and suggested that they are closely related to one another and to the *Pentanopsis* clade (Dessein, 2003;

Thulin & Bremer, 2004; Dessein et al., 2005a). The close relationship between *Gomphocalyx* and *Phylohydrax* is supported by our results and by several morphological characters (amphistomatic leaves, plurizonocolporate pollen, indehiscent fruits, and seeds with a weak, pale exotesta) as shown by Dessein et al. (2005a). Almost all taxa in the *Hedyotis*–*Oldenlandia* group have multiovulate ovaries, and the number of pollen apertures rarely exceeds five. The presence of uniovulate ovaries and plurizonocolporate pollen were the main reasons why *Gomphocalyx* and *Phylohydrax* were previously included in Spermaceae s. str., where it is more common than in the rest of the Spermaceae s.l. tribe, in which 3-colporate pollen predominates (Dessein et al., 2002, 2005b; Dessein, 2003). As mentioned above, the Asian genus *Neanotis* is a notable exception in having plurizonocolporate pollen grains. The genus also shows a trend toward reduction in the number of seeds per locule. In mature fruits, only one or two seeds are present. However, with no molecular sequence data available for the genus it would be premature to hypothesize a close relationship between *Neanotis*, *Gomphocalyx*, and *Phylohydrax*. A few authors (Capuron, 1973; Pies-schaert, 2001) also proposed a close relationship between *Gomphocalyx* and *Lathraeocarpa* Bremek., another endemic to Madagascar. Although *Lathraeocarpa* is not a trailing herb like *Gomphocalyx* but a (sub)shrub, the two taxa share a calyx with eight lobes, uniovulate ovaries, and plurizonocolporate pollen. The last two characters also support a close relationship between *Phylohydrax* and *Lathraeocarpa*. However, several morphological characters distinguish *Lathraeocarpa* from *Gomphocalyx*, some of which might even point to an affinity with *Triainolepis* Hook. f. First, the (sub)shrubby habit of *Lathraeocarpa* is much more similar to the shrubby habit of *Triainolepis* than to the herbaceous habit of *Gomphocalyx*. Second, the pyrene of *L. decaryi* Bremek. is surrounded by eight strands of thin-walled cells, a condition very similar to that observed in some *Triainolepis* species (Bremekamp, 1957; Pies-schaert, 2001). Likewise, *Lathraeocarpa* and *Triainolepis* have a plurilocular ovary and fleshy fruits, whereas *Gomphocalyx* has a bilocular ovary and dry fruits, which has prompted some authors (Kårehed & Bremer, 2007) to tentatively include *Lathraeocarpa* in the emended tribe Knoxieae rather than in Spermaceae s.l. However, we will have to wait until molecular data become available to assess the taxonomic position of *Lathraeocarpa* with more certainty (Dessein et al., 2005a).

Species of *Conostomium* form a strongly supported clade (JS = 99, BS = 99, PP = 1) together with *Oldenlandia herbacea*. The type of the genus *Conostomium*, *C. natalense* (Hochst.) Bremek., is unre-

solved with respect to the other species of *Conostomium* and to *O. herbacea*. Both *Conostomium* and *O. herbacea* have seeds with coarsely granulate testa cells (Bremekamp, 1952; Dessein, 2003) and pollen that is larger than that of most other genera within the *Hedyotis*–*Oldenlandia* group (Bremekamp, 1952; Scheltens, 1998). These characters, however, are homoplasious because granulate testa cells and large pollen grains also occur elsewhere in the *Hedyotis*–*Oldenlandia* group. We observed granulate testa cells in *Kohautia* subg. *Pachystigma*, *O. corymbosa*, and *O. nematocaulis* Bremek., whereas large pollen grains are characteristic of *Amphiasma*, *Gomphocalyx*, and *Phyllohydrax*. The most striking feature of *Conostomium* pollen, namely the short ectocolpi (Scheltens, 1998; Dessein et al., 2005a), is not found in *O. herbacea* or in most other members of the *Pentanopsis* clade, but it is reported for *Gomphocalyx* and *Phyllohydrax* (Dessein et al., 2005a).

The last additional species falling in the *Pentanopsis* clade is *Oldenlandia rosulata*, an African species named after its basal rosulate leaves. The relationship of *O. rosulata* to other members of the *Pentanopsis* clade remains unclear.

Despite the strong support for the *Pentanopsis* clade (JS = 95, BS = 95, PP = 1) in our molecular analyses, the group is not easily morphologically characterized. The only unifying feature for the clade would be what Thulin and Bremer (2004) called basal placentation. Nevertheless, the placentation is not truly basal, but rather axile with the placenta or ovule attached near the base of the septum. Our observations show that this kind of placentation is also found outside the *Pentanopsis* clade. Moreover, the basal placentation character state is only vaguely defined, and more detailed placentation studies within Spermaceae s.l. are needed before further conclusions can be drawn about the phylogenetic value of this character.

MONOSPECIFIC GENERA WITHIN THE *HEDYOTIS*–*OLDENLANDIA* GROUP

Besides the genus *Gomphocalyx* of the *Pentanopsis* clade, the *Hedyotis*–*Oldenlandia* group comprises several other monospecific genera. These monospecific genera often have several peculiar characters, making it very difficult to discuss their relationship with other Spermaceae.

In our sampling, for example, the Afro-Madagascan genus *Mitrasacmopsis* has seeds with undulating radial exotesta cell walls, distinctly stalked placentas with ovules positioned on the periphery of the placental tissue, pollen grains with a double reticulum, and fruits with a conspicuous beak (Groeninckx et al., 2007). Our molecular results suggest a close

relationship of this monospecific genus to *Hedythyrus* and *Oldenlandia fastigiata*. Our own observations have identified similar placentation types within these taxa. Moreover, *Hedythyrus* and *Mitrasacmopsis* have the same type of capsule dehiscence (loculicidal followed by septicidal dehiscence), seeds with testa cells that show the same undulating radial walls, and pollen with a double reticulum (Groeninckx, 2005).

The monospecific genus *Dibrachionostylus* is sister to a clade of African *Oldenlandia* species. The genus was separated from *Oldenlandia* largely on the basis of its capsule dehiscence (both loculicidal and septicidal vs. only loculicidal in *Oldenlandia*). Bremekamp (1952) closely associated *Dibrachionostylus* with *Agathisanthemum* because of their similar fruit dehiscence. However, *Dibrachionostylus* differs markedly from *Agathisanthemum* in the pollen aperture morphology (Lewis, 1965). As mentioned above, *Agathisanthemum* has a distinct ectocolpus, an endocolpus or endocingulum, and a mesoporus surrounded by a costa at the inside of the grain (Lewis, 1965). Pollen grains of *Dibrachionostylus* are also 3-colporate but do not have a costa on the inside (Lewis, 1965). The apertures of *Dibrachionostylus* pollen are, therefore, more similar to those of *Amphiasma*, *Oldenlandia*, and *Pentodon* (Lewis, 1965).

Nesohedyotis is another monospecific genus previously included in the Hedyotideae. Its only species, *N. arborea*, shows a superficial resemblance to the East African genus *Hedythyrus*; specimens of both taxa turn black when dried, and their leaf shape and inflorescence structure are similar (Bremekamp, 1952). However, our results show that *Nesohedyotis* is more closely related to the former tribes Spermaceae s. str. and Manettieae than to members of the *Hedyotis*–*Oldenlandia* group. *Nesohedyotis* has unisexual flowers, which are unusual among Spermaceae, and, in contrast to *Hedythyrus*, its fruits open by a single loculicidal split. Although it is one of the more common endemic species on St. Helena, its small population size and small geographical distribution make *Nesohedyotis* Endangered (EN) according to IUCN Red List criteria (IUCN, 2001).

According to Verdcourt (1976), the monospecific Tanzanian *Pseudonesohedyotis* Tennant, which is not included in our sampling, is closely related to *Nesohedyotis* and *Hedythyrus*. *Pseudonesohedyotis* has indeed the same leaf shape and inflorescence structure as the latter two taxa. In habit and distribution, however, it resembles *Hedythyrus* more than *Nesohedyotis*. Both *Pseudonesohedyotis* and *Hedythyrus* are (sub)shrubs, whereas *Nesohedyotis* is a small tree. Moreover, *Pseudonesohedyotis* differs from *Nesohedyotis* in having hermaphroditic flowers. Again, it is necessary to wait until molecular data become available to discuss the taxonomic position of *Pseudonesohedyotis* with more confidence.

Based on the presence of an apparently superior ovary, Jovet (1941) originally placed *Mitrasacmopsis* and *Astiella* Jovet, another monospecific genus of the *Hedyotis–Oldenlandia* group endemic to Madagascar (not included in this study), within Loganiaceae–Spigeliaceae. Members of Rubiaceae are generally characterized by the presence of an inferior ovary. Groeninckx et al. (2007) demonstrated that flowers of *Mitrasacmopsis* are initially epigynous with inferior ovaries. Expansion of the upper part of the ovary in fruiting stage results in a change in the ovary position of *Mitrasacmopsis* from basically inferior to secondarily semi-inferior. The same kind of fruit development also most likely occurs in *Astiella*. In her morphological study of the Rubioideae, Hayden (1968) stated that some genera of Spermaceae s. str. have semi-inferior fruits. According to Robbrecht (1988), this statement is based on the strong expansion of the top of the nectary disc in the fruiting stage. However, we have not observed semi-inferior ovaries within Spermaceae s. str. Nevertheless, within Spermaceae s.l. several other taxa, apart from *Mitrasacmopsis* and *Astiella*, are characterized by the presence of a beak at fruit stage (*Conostomium* spp., *Hedythyrus* spp., *Kohautia* spp., *Oldenlandia* spp.). These beaks are not remnants of the nectary disc and probably originate in a similar way as in *Mitrasacmopsis*. However, the ovaries of these species do not undergo a remarkable reverse in shape in the fruiting stage as observed in *Mitrasacmopsis* and *Astiella*. Based on their fruit shape, *Mitrasacmopsis* and *Astiella* seem closely related. However, Jovet (1941) also suggested a close relationship between *Astiella* and the Asian *Anotis* DC. species, presently classified in the genus *Neanotis* (Lewis, 1966). *Astiella* differs from both genera in having only two calyx lobes, a character that so far has not been observed within the *Hedyotis–Oldenlandia* group, and uniovulate locules. Molecular sequence data of *Astiella* will allow us to discover the taxonomic position of the genus in the future.

Other monospecific genera of the *Hedyotis–Oldenlandia* group are *Carterella*, *Dolichometra* K. Schum., *Lelya*, *Leptoscela* Hook. f., *Lucya*, *Phyllocrater* Wernham, *Polyura* Hook. f., *Stephanococcus* Bremek., and *Oldenlandiopsis*. The genera *Carterella*, *Lelya*, *Lucya*, and *Oldenlandiopsis* were already discussed in previous sections. To date, the taxonomic position of most of these monospecific genera remains controversial because molecular data are lacking.

KOHAUTIA

Kohautia is a genus of 31 species (Mantell, 1985) distributed from the Indian subcontinent through Pakistan, Iran, the Arabian Peninsula, Sinai, eastern

Egypt, and throughout most of Africa south of the Sahara (including Socotra, Cape Verde, and Madagascar). The genus can easily be distinguished from other representatives of the *Hedyotis–Oldenlandia* group by its unique flower morphology. The anthers and stigma are always included, with the stigma held well below the anthers or occasionally just touching them. This monomorphic short-styled condition is, with the exception of a few individuals of *Conostomium*, unique among the African members of the former tribe Hedyotideae. For this reason, *Kohautia* has always been considered a distinct genus (Bremekamp, 1952; Mantell, 1985). Our molecular results, however, show that the two subgenera of *Kohautia* are not sister clades. Subgenus *Kohautia* is sister to the *Pentanopsis* clade, whereas subgenus *Pachystigma* is sister to an *Oldenlandia* clade containing the type species *O. corymbosa*.

Despite the unifying floral architecture, there are numerous morphological differences between the two subgenera (Lewis, 1965; Mantell, 1985). The number of stigmatic lobes is the most striking diagnostic character that allows identification of the subgenera even in the field. Members of subgenus *Kohautia* have styles with two thin filiform stigma lobes, whereas *Pachystigma* is characterized by the presence of only a single, ovoid to cylindrical stigma lobe. Seeds are also different in the two subgenera: subgenus *Kohautia* seeds are angular-conic to subconic in shape with 5- or 6-angled testa cells, whereas in subgenus *Pachystigma* the seeds are rounded with wavy and punctate testa cells. Pollen of *Kohautia* can also be divided into two easily recognizable groups coinciding with the two subgenera (Lewis, 1965). Other differences between the two subgenera are found in floral architecture and chromosome number. Based on these differences, Mantell (1985) hypothesized that the two subgenera may have diverged and developed independently of one another fairly early on and she even tentatively proposed the elevation of the two subgenera to generic rank. At that time, Mantell decided to maintain a widely defined genus *Kohautia*, mainly for practical reasons. However, our molecular data now clearly support the recognition of two genera. Sampling within the genus still needs to be improved before proposing new generic circumscriptions.

OLDENLANDIA

Govaerts et al. (2006) currently accept 76 *Oldenlandia* species from Africa, 155 from Asia and Australia, 23 from America, and eight from the Pacific Islands. However, as documented in previous molecular studies (Bremer, 1996; Andersson & Rova, 1999;

Bremer & Manen, 2000), *Oldenlandia* is shown to be polyphyletic.

Bremekamp (1952) divided the 61 species that he recognized from Africa into 16 subgenera. Our results do not support the majority of these subgenera. Only the subgenus *Hymenophyllum* Bremek. (*Oldenlandia echinulosa* and *O. nervosa*) and subgenus *Anotidopsis* (*O. angolensis* and *O. gorenensis*) are corroborated.

The type species, *Oldenlandia corymbosa*, is sister to a clade with the African species *O. capensis* L. f., *O. robinsonii* Pit., *O. nematocaulis*, *O. taborensis* Bremek., and *O. wauensis* Schweinf. ex Hiern. The last species, *O. wauensis*, was segregated by Bremekamp (1952) in a new genus *Thecorchus* Bremek., which he proposed to be allied with *Otomeria* of the tribe Knoxiaceae because of its distinctly elongated capsules and equal number of tetramerous and pentamerous flowers. However, Kårehed and Bremer (2007) showed that *Thecorchus* is not related to *Otomeria* but is close to *Oldenlandia*. Our results, which place *Thecorchus* in a clade comprised of the type species of *Oldenlandia*, support the transfer of *T. wauensis* (Schweinf. ex Hiern) Bremek. back into *Oldenlandia*. The type species *O. corymbosa* and *O. capensis* belong to Bremekamp's (1952) subgenus *Oldenlandia* K. Schum. Besides these two species, subgenus *Oldenlandia* also includes *O. fastigiata* and *O. herbacea*. These species are apparently not related to *O. corymbosa* and its allies. *Oldenlandia fastigiata* is sister to *Hedythysus* and *Mitrasacmopsis*, whereas *O. herbacea* in the *Pentanopsis* clade is sister to a paraphyletic *Conostomium*. Bremekamp (1952) already pointed out that *O. herbacea* differs from the rest of the subgenus by the coarsely granulated walls of the testa cells, the rather large flowers, and the slender corolla tube.

The Australian species of *Oldenlandia*, *O. mitrasacmoides* and *O. galioides*, sampled here belong to a clade comprising the Australian *Synaptantha tillaeacea*, the Austro-Asian *O. tenelliflora*, the African species *O. lancifolia*, and the *Kadua* species (including *O. biflora*). *Oldenlandia mitrasacmoides* is sister to the rest of the clade. *Synaptantha tillaeacea* is sister to a clade with *Oldenlandia tenelliflora*, *O. galioides*, and *O. lancifolia*. *Synaptantha* Hook. f. may be distinguished from the other genera in the clade by its slightly connate corolla lobes, stamens with filaments attached to both the corolla and the ovary, depressed obconic or ovoid seeds, and half-inferior ovaries (Halford, 1992). In his review of Australian *Oldenlandia*, Halford (1992) distinguished five groups mostly based on seed morphology. *Oldenlandia galioides* and *O. tenelliflora* are placed together in his group one, which is characterized by obconic seeds that are slightly laterally compressed and

obtriangular in outline. *Oldenlandia mitrasacmoides* belongs to his group two, which is characterized by scutelliform seeds that are oblong or broadly elliptic in outline, with the hilum situated on a conspicuous central ridge. The African species *O. lancifolia* has seeds similar in shape to those of its sister *O. galioides* (Dessein, 1998).

Not all American *Oldenlandia* species included in our sampling are placed within the *Arcytophyllum*–*Houstonia* clade (see discussion above). The remaining South American species of *Oldenlandia*, *O. salzmannii* and *O. tenuis*, form a clade sister to the former tribes Spermaceae s. str. and Manettieae. Terrell (1990) already reported that *O. salzmannii* is clearly distinct from *Houstonia* and *Oldenlandia*. In contrast to other *Oldenlandia* species, *O. salzmannii* does not have the typical oldenlandioid seeds or base number of chromosomes ($n = 15$ instead of 9). Moreover, it shares some unusual characters with *Oldenlandiopsis*: stipules are minute, not more than 0.5 mm long (*Oldenlandia* stipules are often 2–3 mm long); few stiff hairs occur on the leaves (*Oldenlandia* species usually have smaller, softer hairs); and it has a creeping habit (which is rare in *Oldenlandia*, the usual habit being erect to spreading or prostrate). It would be very informative to include *Oldenlandiopsis* in future studies to investigate its relationship to either *O. microtheca* (see discussion of the *Arcytophyllum*–*Houstonia* clade above) or *O. salzmannii*.

FUTURE RESEARCH PLANS AND CONCLUSIONS

Although our analyses found well-supported clades within Spermaceae s.l., many relationships within and between these clades still remain unresolved. Furthermore, many relationships detected here are contradictory to previous taxonomic treatments and await morphological backup. This study was a multi-partner collaboration resulting in a framework for future Spermaceae research. Further studies will focus on obtaining additional DNA markers (i.e., nuclear DNA data) to provide better resolution within the tribe. Besides improving the character sampling, we also need to balance the taxon sampling by including more Asian and American taxa. In addition, concerted studies will focus on the morphological characterization of monophyletic groups within Spermaceae. This requires a morphological investigation across taxa to find character support for the many new phylogenetic relationships detected.

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Appendix 1. List of taxa used in the phylogenetic analyses with voucher information (geographic origin, collector, collector number, herbarium), accession numbers, and literature citations from previously published sequences for the three plastid markers *atpB-rbcL*, *rps16* intron, and *trnL-trnF*: ⁽¹⁾ Andersson & Rova, 1999; ⁽²⁾ Andersson et al., 2002; ⁽³⁾ Dessein et al., 2005a. New sequences are marked with an asterisk. Missing sequences are marked with a dash.

Taxon	Voucher information		<i>rps16</i> intron	<i>atpB-rbcL</i>	<i>trnL-trnF</i>
Agathisanthemum Klotzsch					
<i>A. boyeri</i> Klotzsch	Zambia, Dessein et al. 671 (BR)		EU543018*	EU542917*	EU543077*
<i>A. globosum</i> (Hochst. ex A. Rich.) Klotzsch	Zambia, Dessein et al. 201 (BR)		EU543019*	EU542918*	EU543078*
Amphiasma Bremek.					
<i>A. benguelense</i> (Hiern) Bremek.	Angola, Kers 3350 (S)		AF002753 ⁽¹⁾	EU542919*	EU543079*
<i>A. luzuloides</i> (K. Schum.) Bremek.	Zambia, Dessein et al. 1167 (BR)		EU543020*	EU542920*	EU543080*
Arctophyllum Willd. ex Schult. & Schult. f.					
<i>A. aristatum</i> Standl.	Ecuador, Hehker & Hekking 10335 (GB)		AF333348 ⁽²⁾	—	AF333349 ⁽²⁾
<i>A. ciliolatum</i> Standl.	Ecuador, Øllgaard et al. 56395 (NY)		AF333350 ⁽²⁾	—	AF333351 ⁽²⁾
<i>A. ericoides</i> (Willd. ex Roem. & Schult.) Standl.	unknown, Edwin et al. 3624 (S)		AF333352 ⁽²⁾	—	AF333353 ⁽²⁾
<i>A. lanarum</i> K. Schum.	Costa Rica, Cronquist 8827 (NY)		AF333354 ⁽²⁾	—	AF333355 ⁽²⁾
<i>A. macbridei</i> Standl.	Peru, Wurdack 1073 (NY)		AF333356 ⁽²⁾	—	AF333357 ⁽²⁾
<i>A. muticum</i> (Wedd.) Standl.	Colombia, Andersson et al. 2195 (GB)		AF002754 ⁽¹⁾	EU542921*	EU543081*
<i>A. nitidum</i> (Kunth) Schltdl.	Venezuela, Pipoly et al. 6467 (GB)		AF333359 ⁽²⁾	—	—
<i>A. rivetii</i> Danguy & Cherm.	Ecuador, Harling & Andersson 22232 (GB)		AF333362 ⁽²⁾	EU542922*	AF333363 ⁽²⁾
<i>A. serpyllaceum</i> (Schltdl.) Terrell	Mexico, Stafford et al. 203 (MO)		AF333364 ⁽²⁾	—	—
<i>A. setosum</i> (Ruiz & Pav.) Schltdl.	Colombia, Andersson et al. 2196 (GB)		AF002755 ⁽¹⁾	—	AF333365 ⁽²⁾
<i>A. thymifolium</i> (Ruiz & Pav.) Standl.	Ecuador, Ståhl 4481 (GB)		AF333366 ⁽²⁾	EU542923*	EU543082*
Bowardia Salisb.					
<i>B. glaberrima</i> Engelm.	cult., Forbes s.n. (S)		EU543022*	EU542925*	EU543084*
<i>B. ternifolia</i> (Cav.) Schltdl.	unknown, Van Caekenberghe 264 (cult. at BR)		AF002758 ⁽¹⁾	—	—
	Mexico, Spencer et al. 363 (NY)		—	—	EU642537*
Conostonium (Stapp) Cufod.					
<i>C. natalense</i> (Hochst.) Bremek.	South Africa, Dahlstrand 1346 (GB)		AF002760 ⁽¹⁾	—	EU543085*
	South Africa, Bremer et al. 4341 (UPS)		—	EU542927*	—
	Ethiopia, Puff & Kelbessa 821222 (UPS)		EU543024*	EU542928*	EU543086*
	South Africa, Bremer et al. 4331 (UPS)		—	EU542929*	EU543087*
Crusea Cham. & Schltdl.					
<i>C. calceophala</i> DC.	Guatemala, Gustafsson et al. 215 (GB)		—	EU542930*	EU543088*
<i>C. megalocarpa</i> (A. Gray) S. Watson	Mexico, Pringle 3852 (S)		EU543025*	EU542931*	EU543089*
<i>Dentella J. R. Forst. & G. Forst.</i>			—	—	—
<i>D. dioeca</i> Airy Shaw	Australia, Harwood 1559 (BR)		—	—	EU543090*

Appendix 1. Continued.

Taxon	Voucher information	<i>atpB-rbcL</i>	<i>rps16</i> intron	<i>trnL-trnF</i>
<i>D. repens</i> (L.) J. R. Forst. & G. Forst.	Australia, Andersson 2262 (GB)	EU542932*	AF333370 ⁽²⁾	EU543091*
<i>Dibrachionostylus</i> Bremek.				
<i>D. kaessneri</i> (S. Moore) Bremek.	Kenya, Strid 2598 (GB)	EU542933*	AF002761 ⁽¹⁾	—
<i>Diodia</i> L. as traditionally delimited				
<i>D. aulacosperma</i> K. Schum.	Kenya, Luke 9029 (UPS)	EU542934*	EU543026*	EU543092*
<i>D. sarmentosa</i> Sw.	French Guiana, Anderson et al. 2071 (GB)	—	AF002762 ⁽¹⁾	—
<i>D. spicata</i> Miq.	French Guiana, Anderson et al. 1961 (GB)	EU542935*	EU543027*	EU543093*
<i>Emmeorhiza</i> Pohl ex Endl.				
<i>E. umbellata</i> (Spreng.) K. Schum.	Trinidad, Hummel s.n. (GB)	EU542936*	AY764289 ⁽³⁾	EU543094*
<i>Ernodea</i> Sw.				
<i>E. littoralis</i> Sw.	Cuba, Rora et al. 2286 (GB)	EU542937*	AF002763 ⁽¹⁾	EU543095*
<i>Galianthe</i> Griseb.				
<i>G. brasiliensis</i> (Spreng.) E. L. Cabral & Bacigalupo	Argentina, Vanni & Radonacich 996 (GB)	EU542938*	AY764290 ⁽³⁾	EU543096*
<i>G. eupatorioides</i> (Cham. & Schltdl.) E. L. Cabral	Argentina, Schinini & Cristobal 9811 (GB)	EU542939*	EU543028*	EU543097*
<i>Gomphocalyx</i> Baker				
<i>G. herniarioides</i> Baker	Madagascar, De Block et al. 569 (BR)	—	AY764291 ⁽³⁾	—
<i>Hedyotis</i> L.				
<i>H. consanguinea</i> Hance	Hong Kong, Shiu Ying Hu 10821 (S)	EU542941*	—	—
<i>H. frutescens</i> L.	Sri Lanka, Larsson & Pyddoke 22 (S)	EU542942*	—	EU543098*
<i>H. korrorensis</i> (Valeton) Hosok.	Caroline Islands, Fosberg 47697 (S)	EU542943*	—	EU543099*
<i>H. lausoninae</i> Wight	Sri Lanka, Wambeek & Wamitorp 2996 (S)	EU542944*	—	—
<i>H. lessertiana</i> Arn. var. <i>lassertiana</i> Thwaites	Sri Lanka, Klackenberg 413 (S)	EU542945*	EU543029*	EU543100*
<i>H. lessertiana</i> var. <i>marginata</i> Thwaites & Trimen	Sri Lanka, Fagehlind 3668 (S)	EU542946*	EU543030*	EU543101*
<i>H. macrostegia</i> Stapf	Malaysia, Sabah, Wallander 6 (GB)	EU542947*	AF002767 ⁽¹⁾	EU543102*
<i>H. quinquerius</i> Thwaites	Sri Lanka, Bremer et al. 163 (S)	EU542948*	—	EU543103*
<i>H. rhinophylla</i> Thwaites ex Trimen	Sri Lanka, Fagehlind 5082 (S)	EU542949*	—	EU543104*
<i>H. swertioides</i> Hook. f.	South India, Klackenberg & Lundin 03 (S)	EU542950*	EU543031*	EU543105*
<i>Hedythyrus</i> Bremek.				
<i>H. spermacocinus</i> (K. Schum.) Bremek.	Zambia, Dessein et al. 1017 (BR)	EU542951*	EU543032*	EU543107*
<i>Hemidiodia</i> K. Schum.				
<i>H. ocyimifolia</i> (Willd. ex Roem. & Schult.) K. Schum.	French Guiana, Andersson et al. 2040 (GB)	EU542952*	—	EU543108*
<i>Houstonia</i> L.				
<i>H. caerulea</i> L.	U.S.A., Vincent & Lammers s.n. (GB)	EU542955*	AF333379 ⁽²⁾	EU543109*
<i>H. longifolia</i> Gaertn.	U.S.A., Yaskievych 96-49 (MO)	EU542954*	AF002766 ⁽¹⁾	—
	U.S.A., Weigend 9963 (NY)	—	—	EU642536*

Appendix 1. Continued.

Taxon	Voucher information	<i>atpB-rbcL</i>	<i>rps16</i> intron	<i>trnL-trnF</i>
Kadua Cham. & Schldl.				
<i>K. acuminata</i> Cham. & Schldl.	U.S.A., Hawaii, cult. at BR	EU542955*	—	EU543110*
<i>K. affinis</i> Cham. & Schldl.	U.S.A., Hawaii, <i>Motley 1733</i> (NY)	—	EU642523*	EU642538*
<i>K. axillaris</i> (Wawra) W. L. Wagner & Lorence	U.S.A., Hawaii, <i>Harrison-Gagne s.n.</i> (GB)	—	AF002765 ⁽¹⁾	—
<i>K. centranthoides</i> Hook. & Arn.	U.S.A., Hawaii, Maui, <i>Motley 1724</i> (NY)	EU542956*	EU642524*	EU642535*
<i>K. cordata</i> Cham. & Schldl.	U.S.A., Hawaii, <i>Skottsberg 6788</i> (S)	EU542957*	EU543033*	EU543111*
<i>K. coriacea</i> (J. E. Smith) W. L. Wagner & Lorence	cult., <i>Lorence 8021</i> (PTBG)	—	AF333376 ⁽²⁾	EU543112*
<i>K. degeneri</i> (Fosberg) W. L. Wagner & Lorence	U.S.A., Hawaii, <i>Motley 1703</i> (NY)	EU542958*	EU642525*	EU642539*
<i>K. elatior</i> (H. Mann) W. L. Wagner & Lorence	cult., <i>Wood 5062</i> (PTGB)	—	AF333371 ⁽²⁾	EU543113*
<i>K. flaviatilis</i> C. N. Forbes	U.S.A., Hawaii, Kauai, <i>Wagner 6350</i> (BISH)	—	EU642526*	EU642540*
<i>K. flynnii</i> (W. L. Wagner & Lorence) W. L. Wagner & Lorence	U.S.A., Hawaii, Oahu, <i>Motley 1747</i> (NY)	—	EU642527*	EU642541*
<i>K. foggiana</i> (Fosberg) W. L. Wagner & Lorence	U.S.A., Hawaii, Kauai, <i>Pertman 15631</i> (BISH)	—	EU642528*	EU642542*
<i>K. fosbergii</i> (W. L. Wagner & D. R. Herbst) W. L. Wagner & Lorence	U.S.A., Hawaii, <i>Sparre 27</i> (S)	EU542959*	—	EU543114*
<i>K. littoralis</i> Hillebr.	U.S.A., Hawaii, Oahu, <i>Motley 1677</i> (NY)	—	EU642529*	EU642543*
<i>K. laxiflora</i> H. Mann	U.S.A., Hawaii, Molokai, <i>Pertman 6647</i> (BISH)	—	EU642530*	EU642544*
<i>K. parvula</i> A. Gray	U.S.A., Hawaii, Molokai, <i>Kiehn & Luegmayer 920823</i> (WU)	EU542960*	EU543034*	EU543115*
<i>K. repensis</i> F. Br.	cult., <i>Pertman 12783</i> (GB)	EU542961*	AF333375 ⁽²⁾	EU543116*
Kohautia Cham. & Schldl.	Rapa Island, French Polynesia, <i>Pertman 17953</i> (NY)	—	EU642531*	EU642545*
<i>K. amatymbica</i> Eckl. & Zeyh.	South Africa, <i>Bremer et al. 4307</i> (UPS)	EU542962*	EU543035*	EU543117*
<i>K. caespitosa</i> Schnizl.	Zambia, <i>Dessein et al. 432</i> (BR)	EU542963*	EU543036*	EU543118*
<i>K. coccinea</i> Royle	Zambia, <i>Dessein et al. 751</i> (BR)	EU542964*	EU543037*	EU543119*
<i>K. cynanchica</i> DC.	South Africa, <i>Dessein et al. 469</i> (BR)	EU542965*	EU543038*	EU543120*
<i>K. microcala</i> Bremek.	Zambia, <i>Dessein et al. 1149</i> (BR)	EU542966*	EU543039*	EU543121*
<i>K. obtusiloba</i> (Hiern) Bremek.	Kenya, <i>Luke 9035</i> (UPS)	EU542967*	EU543040*	EU543122*
<i>K. senegalensis</i> Cham. & Schldl.	Burkina Faso, <i>Madsen 5940</i> (NY)	—	—	EU642546*
<i>K. subverticillata</i> (K. Schum.) D. Mantell	Zambia, <i>Dessein et al. 470</i> (BR)	EU542968*	EU543041*	EU543123*
<i>K. virgata</i> (Willd.) Bremek.	Madagascar, <i>De Block et al. 539</i> (BR)	EU542969*	—	EU543124*
Lelya Bremek.				
<i>L. osteocarpa</i> Bremek.	Tanzania, <i>Gereau 2513</i> (BR)	EU542970*	—	EU543125*
Manettia Mutis ex L.				
<i>M. alba</i> (Aubl.) Wernham	French Guiana, <i>Andersson et al. 1917</i> (GB)	EU542971*	AF002768 ⁽¹⁾	—
<i>M. lygistium</i> (L.) Sw.	Colombia, <i>Andersson et al. 2128</i> (GB)	EU542972*	AF002769 ⁽¹⁾	EU543126*

Appendix 1. Continued.

Taxon	Voucher information	<i>atpB-rbcL</i>	<i>rps16</i> intron	<i>trnL-trnF</i>
<i>Manostachya</i> Bremek.				
<i>M. ternifolia</i> E. S. Martins	Zambia, Dessein et al. 265 (BR)	EU542973*	EU543042*	EU543127*
<i>Mitracarpus</i> Zucc. ex Schult. & Schult. f.				
<i>M. frigidus</i> (Willd. ex Roem. & Schult.) K. Schum.	French Guiana, Andersson et al. 1995 (GB)	EU542974*	AF002770 ⁽¹⁾	EU543128*
<i>M. microspermus</i> K. Schum.	Guiana, Jansen-Jacobs et al. 4785 (GB)	EU542975*	EU543044*	—
<i>Mitrasacmopsis</i> Jovet				
<i>M. quadrivalvis</i> Jovet	Zambia, Dessein et al. 1273 (BR)	EU542976*	EU543045*	EU543129*
<i>Nesohedyotis</i> (Hook. f.) Bremek.				
<i>N. arborea</i> (Roxb.) Bremek.	cult., Chase 2915 (K)	—	AF003607 ⁽¹⁾	—
<i>Oldenlandia</i> L.				
<i>O. affinis</i> (Roem. & Schult.) DC.	Zambia, Dessein et al. 627 (BR)	EU542977*	EU543046*	EU543130*
<i>O. angolensis</i> K. Schum.	Zambia, Dessein et al. 932 (BR)	EU542978*	EU543047*	EU543131*
<i>O. biflora</i> L.	Japan, Van Caekenberghe 63 (cult. at BR)	EU542979*	—	EU543132*
<i>O. capensis</i> L. f. var. <i>capensis</i>	Zambia, Dessein et al. 843 (BR)	EU542980*	EU543048*	EU543133*
<i>O. capensis</i> var. <i>pleiosepala</i> Bremek.	Tanzania, Kayombo et al. s.n. (BR)	EU542981*	EU543049*	EU543134*
<i>O. corymbosa</i> L.	Zambia, Dessein et al. 487 (BR)	EU542982*	EU543050*	EU543135*
<i>O. echinulosa</i> K. Schum.	Zambia, Dessein et al. 928 (BR)	EU542983*	EU543051*	EU543136*
<i>O. fastigiata</i> Bremek.	Tanzania, Kayombo & Kahemela 1993 (BR)	EU542984*	—	EU543137*
<i>O. faginoides</i> (F. Muell.) F. Muell.	Zambia, Dessein et al. 1019 (BR)	EU542985*	EU543052*	EU543138*
<i>O. geophila</i> Bremek.	Australia, Harwood 1511 (BR)	EU542986*	EU543053*	EU543139*
<i>O. gorensis</i> (DC.) Summerrh.	Zambia, Dessein et al. 935 (BR)	EU542987*	EU543054*	EU543140*
<i>O. herbacea</i> (L.) Roxb. var. <i>goetzii</i> Bremek.	Zambia, Dessein et al. 1286 (BR)	EU542988*	EU543055*	EU543141*
<i>O. herbacea</i> (L.) Roxb. var. <i>herbacea</i>	Zambia, Dessein et al. 442 (BR)	EU542989*	EU543056*	EU543142*
<i>O. lancifolia</i> (Schumach.) DC.	Zambia, Dessein et al. 463 (BR)	EU542990*	EU543057*	EU543143*
<i>O. microtheca</i> (Cham. & Schltdl.) DC.	Mexico, Frödeström & Hultén 681 (S)	EU542991*	EU543058*	EU543144*
<i>O. mitrasacmoides</i> F. Muell.	Australia, Harwood 1516 (BR)	EU542992*	EU543059*	EU543145*
<i>O. nematocaulis</i> Bremek.	Zambia, Dessein et al. 924 (BR)	EU542993*	—	EU543146*
<i>O. nervosa</i> Hiern	Gabon, Andersson & Nilsson 2326 (GB)	—	EU543060*	—
<i>O. robinsonii</i> Pit.	Zambia, Dessein et al. 346 (BR)	—	AF333382 ⁽²⁾	—
<i>O. rosulata</i> K. Schum.	Zambia, Dessein et al. 1197 (BR)	—	EU543061*	EU543147*
<i>O. salzmannii</i> (DC.) Benth. & Hook. f. ex B. D. Jacks.	Brazil, Harley 15514 (UPS)	EU542995*	EU543043*	EU543148*
<i>O. taborensis</i> Bremek.	Tanzania, Bidgood et al. 4015 (BR)	EU542996*	—	EU543149*
<i>O. tenelliflora</i> (Blume) Kuntze	Japan, Van Caekenberghe 70 (cult. at BR)	EU542997*	EU543062*	EU543106*
<i>O. tenuis</i> K. Schum.	Guyana, Jansen-Jacobs et al. 41 (UPS)	EU542998*	AY764293 ⁽³⁾	—

Appendix 1. Continued.

Taxon	Voucher information	<i>atpB-rbcL</i>	<i>rps16</i> intron	<i>trnL-trnF</i>
<i>O. uniflora</i> L.	U.S.A., Godfrey 57268 (GB)	EU542999*	AY764295 ^(B)	EU543150*
<i>O. wauiensis</i> Schweinf. ex Hiern	Ethiopia, Friis <i>et al.</i> 2560 (UPS)	EU543017*	EU543076*	EU543168*
<i>O. wiedenmannii</i> K. Schum.	Kenya, Luke & Luke 8362 (UPS)	EU543000*	EU543063*	EU543151*
Pentanopsis Rendle				
<i>P. fragrans</i> Rendle	Ethiopia, Gilbert <i>et al.</i> 7458 (UPS)	–	EU543065*	EU543153*
Pentodon Hochst.				
<i>P. pentandrus</i> (K. Schum. & Thonn.) Vatke	Zambia, Dessein <i>et al.</i> 598 (BR)	EU543002*	EU543066*	EU543154*
Phylolobos Puff				
<i>P. carnosa</i> (Hochst.) Puff	South Africa, Bremer 3783 (UPS)	EU543003*	EU543067*	–
<i>P. madagascariensis</i> (Willd. ex Roem. & Schult.) Puff	Madagascar, De Block <i>et al.</i> 640 (BR)	EU543004*	AY764292 ^(B)	EU543155*
Richardia L.				
<i>R. scabra</i> L.	Colombia, Andersson <i>et al.</i> 2073 (GB)	EU543005*	AF003614 ⁽¹⁾	EU543156*
<i>R. stellaris</i> (Cham. & Schltdl.) Steud.	Australia, Egerod 85343 (GB)	EU543006*	EU543068*	EU543157*
Spermacoce L.				
<i>S. capitata</i> Ruiz & Pav.	French Guiana, Andersson 1908 (GB)	EU543007*	EU543069*	EU543158*
<i>S. confusa</i> Rendle ex Gillis	Colombia, Andersson <i>et al.</i> 2074 (GB)	–	AF003619 ⁽¹⁾	–
<i>S. erosa</i> Harwood	Australia, Harwood 1148 (BR)	EU543008*	EU543070*	EU543159*
<i>S. flagelliformis</i> Poir.	Madagascar, De Block <i>et al.</i> 794 (BR)	EU543010*	EU543072*	EU543161*
<i>S. filituba</i> (K. Schum.) Verde.	Kenya, Luke 9022 (UPS)	EU543009*	EU543071*	EU543160*
<i>S. hispida</i> L.	Sri Lanka, Wanninor <i>et al.</i> 2667 (S)	EU543011*	EU543073*	EU543162*
<i>S. prostrata</i> Aubl.	Colombia, Andersson <i>et al.</i> 2078 (GB)	EU543012*	–	EU543163*
<i>S. remota</i> Lam.	French Guiana, Andersson <i>et al.</i> 2016 (GB)	EU543013*	–	EU543164*
<i>S. ruelliae</i> DC.	Gabon, Andersson & Nilsson 2296 (GB)	EU543014*	EU543074*	EU543165*
Stenaria (Raf.) Terrell				
<i>S. nigricans</i> (Lam.) Terrell	U.S.A., Yatskiyech 96-92 (MO)	EU543015*	AF333373 ⁽²⁾	EU543166*
Synaptantha Hook. f.				
<i>S. tillaeacea</i> (F. Muell.) Hook. f.	Australia, Lazarides & Palmer 272 (K)	EU543016*	EU543075*	EU543167*
OUTGROUP TAXA				
Batopedina Verde.				
<i>B. pulvinellata</i> Robbr.	Zambia, Dessein <i>et al.</i> 264 (BR)	EU542924*	EU543021*	EU543083*
Carphalea Juss.				
<i>C. madagascariensis</i> Lam.	Madagascar, De Block <i>et al.</i> 578 (BR)	EU542926*	EU543023*	–
Pentanisia Harv.				
<i>P. parviflora</i> Stapf ex Verde.	Zambia, Dessein <i>et al.</i> 678 (BR)	EU543001*	EU543064*	EU543152*