

Phylogeny of *Hedyotis* L. (Rubiaceae: Spermaceae): Redefining a complex Asian-Pacific assemblage

Niklas Wikström,¹ Suman Neupane,² Jesper Kårehed,³ Timothy J. Motley² & Birgitta Bremer¹

¹ Bergius Foundation, The Royal Swedish Academy of Sciences and Department of Botany, Stockholm University, 10691 Stockholm, Sweden

² Department of Biological Sciences, Old Dominion University, Norfolk, Virginia 23529-0266, U.S.A.

³ The Linnaean Gardens of Uppsala, Uppsala University, Villavägen 8, 752 36 Uppsala, Sweden

Author for correspondence: Niklas Wikström, niklas.wikstrom@bergianska.se

Abstract The genus *Hedyotis* (Rubiaceae: Spermaceae) has long served as a repository for tropical herbaceous species that do not fit readily into other genera. Circumscribed broadly the genus becomes a highly heterogeneous assembly, but relationships of *Hedyotis* have been difficult to resolve and it has proven very difficult to circumscribe the genus in a more narrow sense. Here we present Bayesian phylogenetic analyses of *Hedyotis* using plastid (*rps16*, *petD*) and nuclear (ITS, ETS) sequence data to resolve monophyletic lineages, to test former taxonomic hypotheses, and to revise the taxa within a well-supported evolutionary framework. Four hundred and sixty-seven sequences representing 129 accessions, never previously included in any phylogenetic analyses, are newly reported. *Hedyotis*, as previously circumscribed, is polyphyletic, but all investigated species, except for *Hedyotis coronaria*, are resolved in one of three well-supported monophyletic groups. The largest clade includes all investigated species of *Hedyotis* from the Indian subcontinent as well as three groups of species with primarily Chinese distributions. The type species of *Hedyotis* (*H. fruticosa*) is resolved with the Indian subcontinent species and following previous suggestions this group is referred to as *Hedyotis* s.str. Species currently recognized under the generic names *Metabolos* and *Pleiocraterium* are resolved in *Hedyotis* s.str. The second-largest group comprises a series of smaller, but well-supported, clades including the *Leptopetalum* clade, the genus *Kadua*, an unnamed group distributed in Asia and the Pacific, and a large Asian group referred to here as the *Exallage/Dimetia* clade. The third group includes a few SE Asian *Hedyotis*, as well as all investigated species of the genus *Neanotis*. *Hedyotis coronaria* is not closely related to other species from Asia and is resolved with *Spermaceae hispida*. The analyses indicate that diplophragmous capsules and “fruticosa-type” seeds occur outside of *Hedyotis* s.str., and several species suggested to have these features are resolved in the *Exallage/Dimetia* clade. Species suggested to have indehiscent capsules, a feature used by Bremekamp to characterize the genus *Exallage*, are also resolved in both the *Exallage/Dimetia* clade and in *Hedyotis* s.str., but a close examination indicates that the capsules are not truly indehiscent in the *Hedyotis* s.str. species. One species of *Metabolos* and one species of *Pleiocraterium* are given new species names, and one species of *Pleiocraterium* is transferred to *Hedyotis* and three species of *Hedyotis* are transferred to *Neanotis*.

Keywords Asia; *Hedyotis*; phylogenetic analysis; Rubiaceae; Spermaceae

Supplementary Material The Electronic Supplement (Table S1) and the alignment file are available in the Supplementary Data section of the online version of this article (<http://www.ingentaconnect.com/content/iapt/tax>).

Submitted: 20 June 2012; revision received; 18 Nov. 2012; accepted: 28 Jan. 2013

■ INTRODUCTION

In its broadest circumscription (Fosberg, 1943; Fosberg & Sachet, 1991), the genus *Hedyotis* L. includes 500 to 600 species, making it one of the largest genera of the coffee family (Rubiaceae). However, the genus has long served as a repository for tropical herbaceous species with multiovulate locules that do not fit readily into other genera, and circumscribed this broadly, *Hedyotis* became a highly heterogeneous group. Consequently, a large number of segregate genera have been recognized, often based on species confined to particular geographic regions. Most African species, for example, have generally been treated as *Oldenlandia* L., but Bremekamp (1952) recognized an additional 20 segregate genera in this group.

North American species have most often been treated as *Houstonia* L. (Terrell, 1975, 1991, 1996, 2001b) and South American species as *Arcytophyllum* Willd. ex Schult. & Schult. f. (Mena, 1990). Additional small genera such as *Carterella* Terrell, *Stenaria* (Raf.) Terrell and *Stenotis* Terrell have been recognized for species from southern U.S.A. and Mexico (Terrell, 1991, 2001a, c), and the generic name *Kadua* Cham. & Schldtl. was recently resurrected for Pacific, mainly Hawaiian, species (Terrell & al., 2005).

Phylogenetic analyses (Bremer, 1996; Andersson & Rova, 1999; Bremer & Manen, 2000; Dessein & al., 2005) have placed *Hedyotis*, and other genera of the former tribe Hedyotideae, together with herbaceous taxa from the tribe Spermaceae. This is a tribe that has been treated both as a rather

small (Bremekamp, 1952, 1966; Verdcourt, 1958; Robbrecht, 1988, 1993) or as a very large group (Bremer, 1996; Bremer & Manen, 2000), and sometimes including the tribes Hedyotideae, Knoxieae, Manettieae, and Triainolepideae. Following the results from the most recent phylogenetic analyses (Dessein, 2003; Kårehed & Bremer, 2007; Kårehed & al., 2008; Groeninckx & al., 2009) and the most recent classification of the family (Bremer & Eriksson, 2009), Spermaceae are here treated as a tribe of ca. 1000 species and 60 genera, including Manettieae and most genera of Hedyotideae, but excluding Knoxieae and Triainolepideae (Bremer & Eriksson, 2009).

Using plastid DNA data and combined plastid and nuclear rDNA data Groeninckx & al. (2009) and Kårehed & al. (2008) recently analyzed the phylogenetic relationships of Spermaceae. Contrary to previous analyses they adopted a global perspective on Spermaceae and their analyses included a broad sampling of taxa covering most of the geographic and taxonomic diversity of the group. While their analyses found a large number of well-supported groups within Spermaceae, they (Kårehed & al., 2008; Groeninckx & al., 2009) also highlighted considerable problems with our contemporary taxonomy. Many of the smaller segregate genera, confined to restricted geographic regions, were supported as monophyletic, but at the same time their analyses indicated problems with some of the larger, and less easily characterized, genera such as *Oldenlandia* and *Hedyotis*. *Oldenlandia* in particular was problematic and representatives included in their analyses showed a broad range of unexpected relationships (Kårehed & al., 2008; Groeninckx & al., 2009).

There was no support in the analyses by Kårehed & al. (2008) and Groeninckx & al. (2009) for circumscribing *Hedyotis* in a way to also include American and Polynesian taxa (Merrill & Metcalf, 1942; Fosberg, 1943; Fosberg & Sachet, 1991; Dutta & Deb, 2004). *Hedyotis fruticosa* L., the type species of the genus from Sri Lanka, was grouped with strong support with a few Asian and Micronesian species, and the authors argued that the generic name *Hedyotis* probably should be restricted to this smaller group (Kårehed & al., 2008; Groeninckx & al., 2009). It is possible that this group of Asian and Micronesian species corresponds to a group of species recognized already by Wight & Arnott (1834: 405–418) as *Hedyotis* sect. *Diplophragma* Wight & Arn. Surveying Asian and Pacific species of the genera *Hedyotis* and *Exallage* Bremek., a large number of species from Sri Lanka, China, and Micronesia were associated with this group by Terrell & Robinson (2003), and this possibility was also discussed briefly by Groeninckx & al. (2009). The sampling in the two studies by Kårehed & al. (2008) and Groeninckx & al. (2009) was however limited and resolving the taxonomic problems in this group required a more exhaustive sampling. *Pleiocraterium* Bremek., for example, was not included in their analyses and may be closely related to other Asian species of *Hedyotis* (Groeninckx & al., 2009). The genera *Exallage* and *Leptopetalum* Hook. & Arn. were also absent in their analyses (Kårehed & al., 2008; Groeninckx & al., 2009). *Exallage* was originally described by Bremekamp (1952), and includes several species from Asia that have been suggested to belong to *Hedyotis* (Ridsdale, 1998). *Leptopetalum* has also

been associated with *Hedyotis*, at least in a broad sense (see Fosberg & Sachet, 1991, for a discussion). Other unsampled groups included the genera *Neanotis* W.H. Lewis (ca. 30 spp.) and *Metabolos* Blume (1 or 2 spp.).

Building on the analyses by Kårehed & al. (2008) and Groeninckx & al. (2009) we initiated a phylogenetic analysis focusing on the Asian and Micronesian species referred to as *Hedyotis* s.str. in their studies. Our primary aims are to better circumscribe the genus *Hedyotis*, and to identify some of the relationships within this group.

■ MATERIALS AND METHODS

Taxon sampling. — Taxa were sampled with two primary objectives: (1) to include as many species as possible from *Hedyotis* s.str. (Kårehed & al., 2008; Groeninckx & al., 2009); and (2) to include as many taxa as possible from the tribe Spermaceae that have been suggested to belong to *Hedyotis*, but that have not been included in any previous phylogenetic analysis, or have not in some other way been shown to be distantly related to *Hedyotis*.

In total 203 accessions were included. The backbone of the datasets from Groeninckx & al. (2009) and Kårehed & al. (2008) remain, but the number of taxa from groups not in the focus of the present analyses was significantly reduced. Sixty-two accessions from the analyses by Kårehed & al. (2008), twelve accessions from the analyses by Guo & al. (2011) and 129 accessions not previously included in any phylogenetic analysis were selected. Comprehensive information on species names, voucher information, and references is given for included accessions in Table S1 (Electr. Suppl.).

Following the first description of *Hedyotis* by Linnaeus (1753), there have been continuous disagreements over the delimitation of the genus, particularly with respect to *Oldenlandia*. The two genera have often been treated as congeneric, and to make things worse, they have variously been treated as either *Hedyotis* (Wight & Arnott, 1834; Fosberg, 1943; Fosberg & Sachet, 1991; Fosberg & al., 1993) or *Oldenlandia* (Hiern, 1877; Schumann, 1891). Consequently, species described under one generic name (i.e., *Hedyotis*) almost certainly have a synonym under the other (*Oldenlandia*) and by what name an individual species should be referred to is an almost arbitrary choice. In order to be consistent, and without any preconceived ideas of what is right or wrong, we have chosen to apply the generic names *Hedyotis* and *Oldenlandia* to individual taxa following Govaerts & al. (2011).

DNA extraction, amplification, and sequencing. — Total genomic DNA was extracted from silica-dried material and/or herbarium specimens using a standard cetyl trimethyl ammonium bromide (CTAB) protocol (Doyle & Doyle, 1987), combined with the QIAquick PCR cleaning kit (Qiagen, Hilden, Germany) following the protocol specified by the manufacturer. Amplification and sequencing of the plastid regions *rps16* and *petD*, and of the internal and external transcribed spacers (ITS, ETS) of the nuclear rDNA were carried out using primers listed in Table 1. Amplification reactions were performed

Table 1. Primers used for amplification and sequencing of new sequences in this study.

Region	Primer	Primer sequence from the 5' end	Reference
<i>rps16</i>	rps16_F	GTG GTA GAA AGC AAC GTG CGA CTT	Oxelman & al. (1997)
	rps16_R2	TCG GGA TCG AAC ATC AAT TGC AAC	Oxelman & al. (1997)
<i>petD</i>	PlpetB1365F	TTGACYCGTTTTTATAGTTTAC	Löhne & Borsch (2005)
	PlpetD738R	AATTTAGCYCTTAATACAGG	Löhne & Borsch (2005)
ITS	ITS_P17	CTA CCG ATT GAA TGG TCC GGT GAA	Popp & Oxelman (2001)
	ITS_P25	GGG TAG TCC CGC CTG ACC TG	Popp & Oxelman (2001)
	26S_82R	TCC CGG TTC GCT CGC CGT TAC TA	Popp & Oxelman (2001)
ETS	18S-E	GCA GGA TCA ACC AGG TAG CA	Baldwin & Markos (1998)
	18S-ETS	ACT TAC ACA TGC ATG GCT TAA TCT	Baldwin & Markos (1998)
	HedETS-Erit	TGG WTA GCA CGG TTT GGT TGG A	newly designed

using Phusion enzyme (Finnzymes, Espoo, Finland) in 20 μ l reactions; template DNA 1 μ l, 5 \times Phusion HF Buffer 4 μ l, 10 mM dNTP 0.5 μ l each, 10 μ M forward and reverse primers 0.5 μ l each, Phusion enzyme 0.2 μ l. The thermal cycling profile was set to 98°C 2 min (98°C 15 s, 60°C 15 s, 72°C 30 s) \times 45, 72°C 7 min. PCR products were cleaned using the MultiScreen Separations System (Millipore, Billerica, Massachusetts, U.S.A.), sequenced with the BigDye terminator cycle sequencing kit using the amplification primers, and analyzed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, U.S.A.).

Sequence assembly, editing and phylogenetic analyses.

— Sequences were edited and assembled using the Staden Package (Staden, 1996; Staden & al., 2000) and Seaview v.4.3.1 (Gouy & al., 2010). All four regions were aligned using the program MUSCLE v.3.8.31 (Edgar, 2004) with default settings. Before sequences were submitted to MUSCLE for alignment they were sorted by size using the program USEARCH v.5.2.32 (Edgar, 2010).

Markov chain Monte Carlo (MCMC) methods (Larget & Simon, 1999) within a Bayesian framework were used to approximate the posterior distribution of trees using MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). In the MCMC the data was partitioned into 3 partitions (*rps16*, *petD*, rDNA) and each partition was allowed partition-specific parameters (Ronquist & Huelsenbeck, 2003; Nylander & al., 2004). The nucleotide substitution model for each partition was selected based on a corrected Akaike information criterion (AICc) as calculated using MrAIC v.1.4.4 and PHYML v.2.4 (Guindon & Gascuel, 2003; Nylander, 2004). The GTR+ Γ model was selected for the plastid partitions *rps16* and *petD* whereas GTR+I+ Γ was selected for the rDNA partition.

Bayesian analyses were run for 20 million generations with four MCMC chains in two independent and parallel runs. Following a “burn-in” phase of 10 million generations, we sampled trees and parameters every 2000th generation leaving a final posterior distribution of 5000 trees and parameter estimates in each run. Convergence of the two runs was confirmed (standard deviation of split frequencies below 0.01 during the last 10 milj. generations) and the two posterior distributions of trees and parameters were pooled and used to calculate Bayesian posterior probabilities.

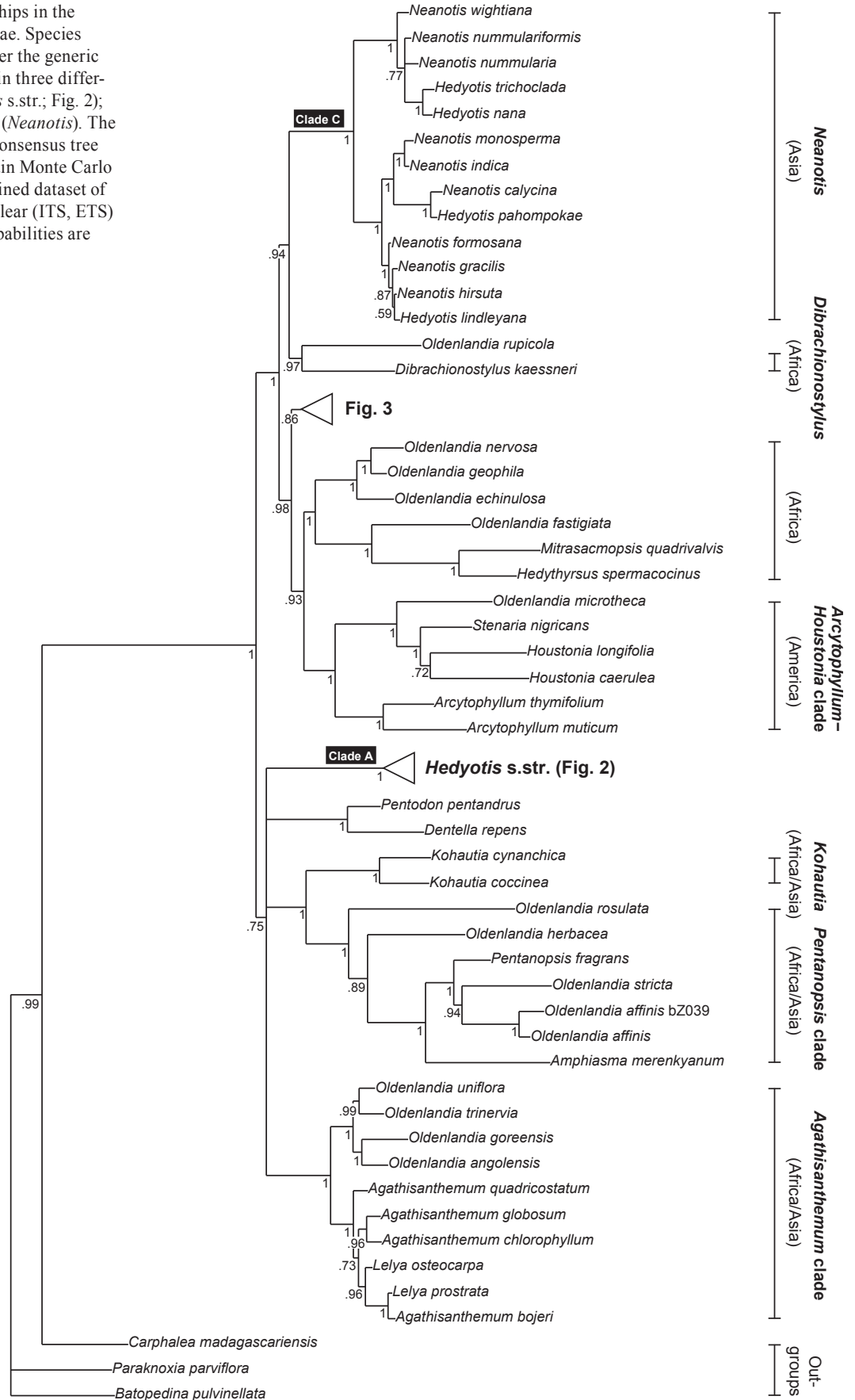
RESULTS

Sequence data from the plastid regions *rps16* and *petD* and the nuclear regions ITS and ETS were successfully generated for 129 accessions for which sequence data have not previously been reported. In total 125 sequences of *rps16*, 106 sequences of *petD*, 117 sequences of ITS, and 119 sequences of ETS were newly generated. Sequences are deposited at the EMBL Nucleotide Sequence Database and their EMBL accession numbers are reported in Table S1 (Electr. Suppl.). The compiled dataset used in the phylogenetic analyses comprised 203 taxa and 4308 characters and is available in the Supplementary Data section of the online version of this article.

Phylogenetic relationships indicated by the MCMC analyses are summarized as a 50% majority-rule consensus tree in Figures 1 to 3, and posterior probability values greater than 0.50 are reported below each node. Nodes with posterior probability values equal to or greater than 0.95 (the node appears in at least 95% of the sampled trees) are considered well-supported (Alfaro & al., 2003). A large number of monophyletic groups and relationships in Spermaceae are identified and supported by the phylogenetic analyses. Some correspond to those already identified by previous analyses, but others are new and identified for the first time in the analyses presented here. Groups identified and supported are referred to by the names indicated in Figures 1 to 3. A summary of groups identified (and possible subgroups), their habit, fruit and seed morphology, and distribution is also given in Table 2.

The genus *Hedyotis* as previously circumscribed is polyphyletic. All investigated species recognized under *Hedyotis* by Govaerts & al. (2011), except for *Hedyotis coronaria* (Kurz) Craib that resolves with *Spermaceae hispida* L. (Spermaceae clade; Fig. 3), are resolved in one of three monophyletic clades (clades A–C; Figs. 1–3; Table 2), all with Asian or Asian-Pacific distributions. The first clade (clade A; Figs. 1, 2) is well-supported by the analyses (BPP = 1.00) and includes the majority of *Hedyotis* investigated and a large number of species currently recognized under the generic name *Oldenlandia* by Govaerts & al. (2011). We will from here on refer to this group as *Hedyotis* s.str. Included in *Hedyotis* s.str. are all Indian subcontinent species of *Hedyotis* that were investigated (including the type species *H. fruticosa*), as well as the majority

Fig. 1. Phylogenetic relationships in the Rubiaceae tribe Spermacoceae. Species traditionally recognized under the generic name *Hedyotis* are resolved in three different clades: clade A (*Hedyotis* s.str.; Fig. 2); clade B (Fig. 3); and clade C (*Neanotis*). The tree is a 50% majority-rule consensus tree from a Bayesian Markov chain Monte Carlo (MCMC) analysis of a combined dataset of plastid (*rps16*, *petD*) and nuclear (ITS, ETS) data. Bayesian posterior probabilities are indicated at the nodes.



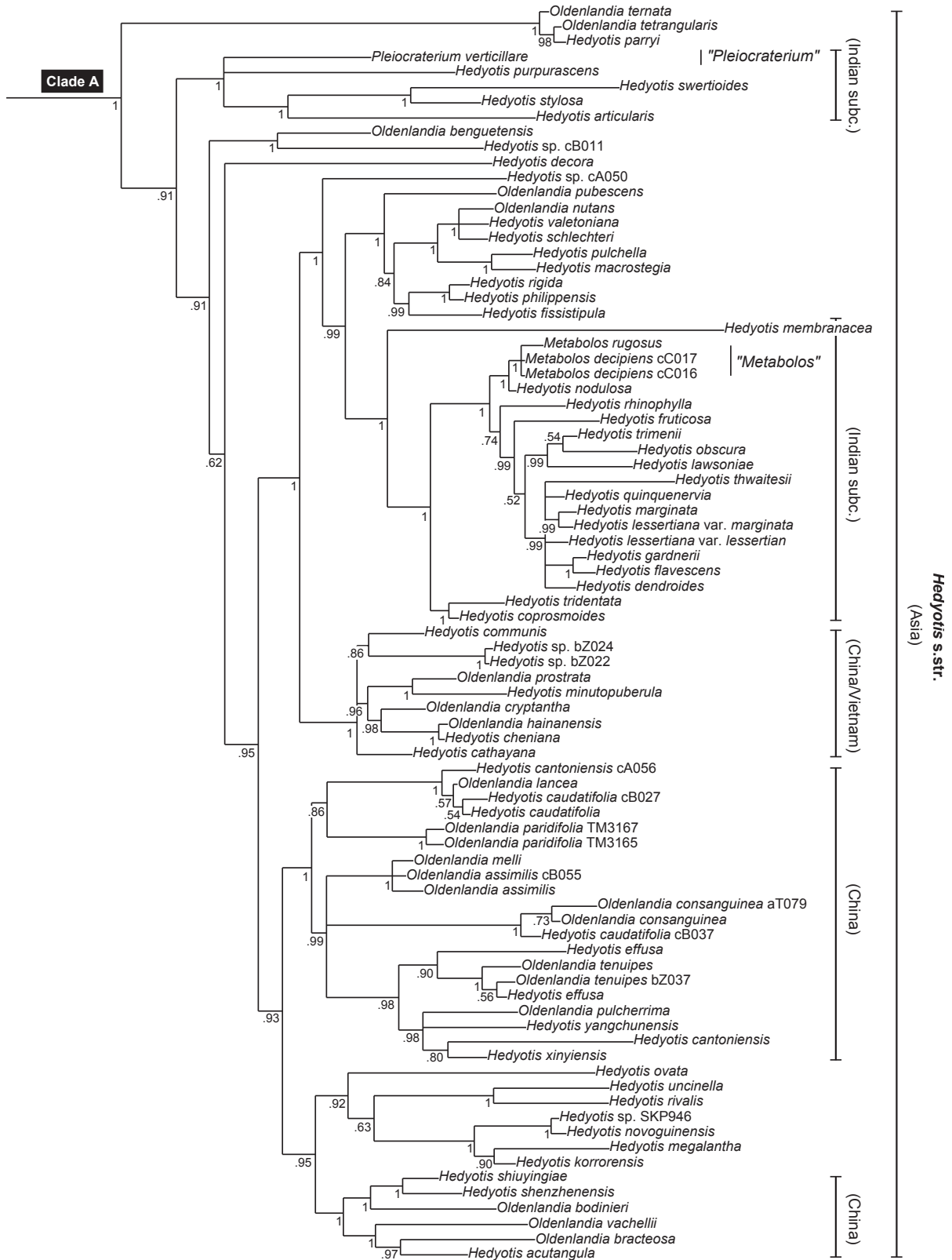


Fig. 2. Phylogenetic relationships of *Hedyotis* s.str. The two small genera *Metabolos* (2 spp.) and *Pleio craterium* (4 spp.) are both resolved well inside *Hedyotis* s.str. The results also indicate biogeographical patterns in *Hedyotis* s.str. Species from the Indian subcontinent are resolved in two different groups, and three different groups with primarily Chinese distributions are indicated. The tree is a 50% majority-rule consensus tree from a Bayesian Markov chain Monte Carlo (MCMC) analysis of a combined dataset of plastid (*rps16*, *petD*) and nuclear (ITS, ETS) data. Bayesian posterior probabilities are indicated at the nodes.

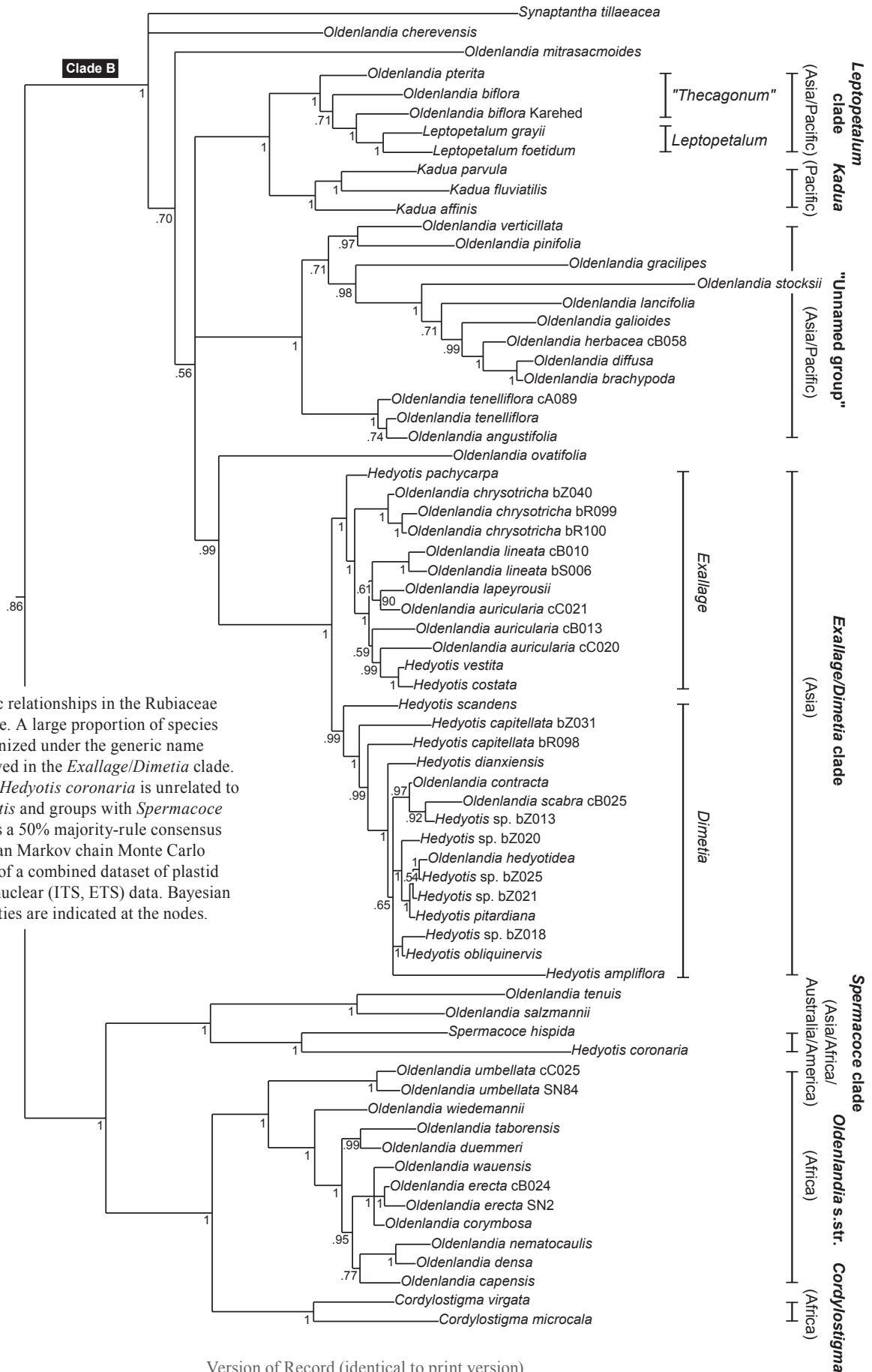


Fig. 3. Phylogenetic relationships in the Rubiaceae tribe Spermaceae. A large proportion of species traditionally recognized under the generic name *Hedyotis* are resolved in the *Exallage*/*Dimetia* clade. The Asian species *Hedyotis coronaria* is unrelated to other Asian *Hedyotis* and groups with *Spermaceoce hispida*. The tree is a 50% majority-rule consensus tree from a Bayesian Markov chain Monte Carlo (MCMC) analysis of a combined dataset of plastid (*rps16*, *petD*) and nuclear (ITS, ETS) data. Bayesian posterior probabilities are indicated at the nodes.

of *Hedyotis* species from temperate and tropical Asia. Species from the Indian subcontinent are resolved in two separate groups. The first consists of four species (*Hedyotis purpurascens* Hook. f., *Hedyotis swertioides* Hook. f., *Hedyotis stylosa* R. Br. ex G. Don, *Hedyotis articularis* R. Br. ex G. Don) and the only representative of the genus *Pleiocraterium* (BPP = 1.00) included in the study. The second group includes the remaining Indian subcontinent species of *Hedyotis*, but also two species of the genus *Metabolos* (BPP = 1.00).

Three separate clades with primarily Chinese distributions are also identified in *Hedyotis* s.str. One clade includes seven Chinese species (*H. communis*–*H. cathayana*; Fig. 2) and two unidentified accessions from Vietnam (*Hedyotis* sp. bZ024 and *Hedyotis* sp. bZ022). Together they form a well-supported

group (BPP = 1.00). The second and largest clade includes 20 accessions (*H. cantoniensis* cA056–*H. xinyiensis*; BPP = 1.00; Fig. 2), representing at least 13 different species. The last clade includes six species (*Hedyotis shiuyingiae*–*Hedyotis acutangula*; BPP = 1.00; Fig. 2). All three of these groups also include species that Govaerts & al. (2011) currently recognizes under the generic name *Oldenlandia*.

Species placed in *Hedyotis* by Govaerts & al. (2011) are also resolved in a second large group with an Asian and Pacific distribution (clade B; Fig. 3). This lineage comprises three well-supported and monophyletic clades resolved in a trichotomy. The first is the *Leptopetalum* clade + the genus *Kadua* (BPP = 1.00), the second is an unnamed group distributed in Asia and the Pacific (BPP = 1.00), and the third is an Asian group (BPP

Table 2. A summary of the three clades (A–C) in which *Hedyotis* species are resolved. The summary indicates groups identified by the analyses and that are indicated on Figures 1 to 3, subgroups that are discussed in the text and their habit, fruit and seed morphology, and distribution.

Clade	Groups identified	Subgroups discussed	Habit and other morphology	Fruit dehiscence	Seed	Distribution	
Clade A (Fig. 2)	<i>Hedyotis</i> s.str.		Suffrutescent herb to shrub or rarely small tree	Capsules apex not protruding beyond calyx lobes; septicidal dehiscence usually followed by a partial apical loculicidal dehiscence, usually resulting in two semi-split valves	Dorsiventrally compressed, with a conspicuous hilum	Sri Lanka, India, SE China, Indo-China, Malesia, Papuaasia, NW Pacific	
			<i>Leptopetalum</i>	Slender, glabrous subshrubs	Capsules subglobose, firm and thick-walled; dehiscence loculicidal from apex	Slightly compressed into irregular polyhedrons with rounded angles and corners	Pacific
			<i>Leptopetalum</i> clade	“ <i>Thecagonum</i> ” (see text)	Small annual or perennial herbs	Capsules subglobose to winged with somewhat compressed walls; thin and fragile or firm and thick-walled; dehiscence loculicidal from top	Globose or sub-globose with deeply pitted exotesta
Clade B (Fig. 3)	<i>Kadua</i>		Shrubby to small trees, corolla salverform, fleshy, long tubed, and appendaged	All the taxa in subg. <i>Kadua</i> , except sect. <i>Oceanica</i> , have capsules with initial loculicidal dehiscence from the apex followed by septicidal dehiscence at maturity; in subg. <i>Gouldia</i> and subg. <i>Kadua</i> sect. <i>Oceanica</i> the fruits are fleshy and indehiscent	Various: fan-shaped, ovoid, flat with broad wing or brick-like	Hawaiian Islands and French Polynesia	
			<i>Exallage/Dimetia</i> clade	<i>Exallage</i>	Suffrutescent herb with axial inflorescences	Capsules indehiscent	Trigonous (oldenlandioid)
			<i>Dimetia</i>	Herbs or shrubs, lianescent, climbing and scandent	Capsule apex protruding beyond calyx lobes; dehiscence loculicidally from apex followed by partial septicidal dehiscence	Dorsiventrally compressed, with an apical centric hilum and sometimes winged margin	
Clade C (Fig. 1)	<i>Neanotis</i>		Annual herbs, pluriaperturate pollen	Capsule subglobose; dehiscence loculicidally from apex	Cymbiform to shallowly cup-shaped	Tropical Asia, Malesia, Papuaasia and Pacific	

The information was compiled using Fosberg & Sachet (1991), Terrell & Robinson (2003, 2007), Terrell & al. (2005), Dutta & Deb (2004), Chen & Taylor (2011), Neupane & al. (2009) and from our personal observations.

= 1.00), from here on referred to as the *Exallage/Dimetia* clade. The *Leptopetalum* clade includes Asian and Pacific species of *Oldenlandia*, sometimes recognized under the generic name *Thecagonum* Babu (see Discussion), as well as two representatives of the genus *Leptopetalum*. The *Exallage/Dimetia* clade comprises a number of Asian species recognized either under *Hedyotis* or *Oldenlandia* by Govaerts & al. (2011). Sister to the *Exallage/Dimetia* clade is the Asian species *Oldenlandia ovatifolia* (Fig. 3).

The third, and last group (clade C; Fig. 1) includes *Hedyotis trichoclada* Merr. & L.M. Perry and *H. nana* Merr. & L.M. Perry from New Guinea, *H. pahompokae* Fukuoka from Thailand, and *H. lindleyana* Hook. ex Wight & Arn. from Asia and they are resolved with representatives of the genus *Neanotis* (BPP = 1.0; Fig. 1). We will from here on refer to this group as *Neanotis* (see Nomenclatural changes outside *Hedyotis* s.str. below). Sister to *Neanotis* is an African group including the monotypic *Dibrachionostylus* Bremek. and *Oldenlandia rupicola* (Sond.) Kuntze.

The analyses also provide continued support for groups previously identified by Kårehed & al. (2008) and Groeninckx & al. (2009). These groups include the *Arcytophyllum-Houstonia* clade, *Kohautia* Cham. & Schltdl., the *Pentanopsis* clade, the *Agathisanthemum* clade (Fig. 1), *Cordylostigma* Groeninckx & Dessein and *Oldenlandia* s.str. (Fig. 3).

DISCUSSION

In their phylogenetic analyses of the tribe Spermacoceae both Kårehed & al. (2008) and Groeninckx & al. (2009) retrieved and discussed a number of large and well-supported clades: *Kohautia*, the *Pentanopsis* clade, the *Agathisanthemum-Hedyotis* s.str. clade, *Kadua*, the *Arcytophyllum-Houstonia* clade, *Oldenlandia* s.str., “*Pachystigma*”, and the *Spermacoce* clade. With one exception, these groups are also retrieved and supported in the present analyses. Following Groeninckx & al., (2010c) *Pachystigma* is here referred to by the generic name *Cordylostigma*. The only group not retrieved in the present study is the *Agathisanthemum-Hedyotis* s.str. clade, a clade that comprised two main components, *Hedyotis* s.str. and a group of two African genera, *Agathisanthemum* Klotzsch and *Lelya* Bremek., and three species of *Oldenlandia*. We will refer to this latter assemblage as the *Agathisanthemum* clade (Fig. 1). Both *Hedyotis* s.str. (Fig. 2) and the *Agathisanthemum* clade are retrieved and well-supported in our analyses, but there is no support for their sister-group relationship as indicated by Kårehed & al. (2008) and Groeninckx & al. (2009). One reason behind this lack of support is that two plastid regions, the *trnL-F* intergenic spacer and the *atpB-rbcL* intergenic spacer, were not included in the present analyses. Reanalysis of the dataset from Kårehed & al. (2008), excluding these two plastid regions, resulted in relationships consistent with those obtained here (i.e., with no support for the *Agathisanthemum-Hedyotis* s.str. clade).

Three out of four species of *Oldenlandia* that resolve in the *Agathisanthemum* clade (*O. trinervia* Retz., *O. goreensis* (DC.)

Summerh., *O. angolensis* K. Schum.), are from *Oldenlandia* subg. *Anotidopsis* (Hook. f.) K. Schum., and this relationship was seen and discussed previously by Kårehed & al. (2008) and Groeninckx & al. (2009). They debated if the three species of *Oldenlandia* they resolved with *Agathisanthemum* and *Lelya* should be transferred to a new genus or treated as members of *Agathisanthemum*, and if this treatment should apply only to the three species or to the entire subgenus. Our analyses added *O. trinervia* from *O.* subg. *Anotidopsis* that also came out in the *Agathisanthemum* clade, but at the same time our results clearly indicate that the subgenus is polyphyletic. Further complicating this issue is that other species included in our analyses from *O.* subg. *Anotidopsis*, such as *Neanotis hirsuta*, and *Hedyotis lindleyana*, (Schumann, 1891), are resolved in the genus *Neanotis* (see below).

Much uncertainty reported in the analyses by Kårehed & al. (2008) and Groeninckx & al. (2009) remains. In Kårehed & al. (2008), for example, Spermacoceae was resolved in a basal dichotomy, with a well-supported clade (their clade A), comprising *Kohautia* and the *Pentanopsis* clade, sister to a poorly supported group (their clade B) including remaining taxa. Our analyses continue to support a sister relationship between *Kohautia* and the *Pentanopsis* clade (PP 1.00), but clade B (sensu Kårehed & al., 2008) is not retrieved at all in our analyses. Our analyses resolve Spermacoceae in a basal dichotomy with a well-supported group (BPP = 1.00) that corresponds to clade D (sensu Kårehed & al., 2008), and this group is sister to a poorly supported group (BPP = 0.75) comprising four subgroups: (i) *Hedyotis* s.str. (clade A; BPP = 1.00); (ii) *Kohautia* + the *Pentanopsis* clade (BPP = 1.00); (iii) the *Agathisanthemum* clade (BPP = 1.00); and (iv) *Pentodon* Hochst. + *Dentella* Forst. (BPP = 1.00). The second group, *Kohautia* + the *Pentanopsis* clade, corresponds to clade A (sensu Kårehed & al., 2008). Although these differences are substantial, they all concern relationships that were poorly supported in the analyses by Kårehed & al. (2008) and Groeninckx & al. (2009), and that remain poorly supported in the present analyses. Additional data is required before these relationships can be resolved.

***Hedyotis* s.str.** — *Hedyotis*, as previously circumscribed, is resolved as polyphyletic. All investigated species recognized under *Hedyotis* by Govaerts & al. (2011), except for *Hedyotis coronaria*, are grouped in one of three monophyletic groups (clades A–C; Figs. 1–3). With few exceptions these groups only include species from Asia and the Pacific. The first of these groups (clade A; Figs. 1, 2) includes the majority of the species in *Hedyotis* investigated, and more or less corresponds to a group that has been recognized for a long time and diagnosed by fruit characteristics. Originally the group was described by Wight & Arnott (1834) who characterized the capsules as having an “apex more or less 4-lobed and slightly produced beyond the calyx-tube, at length septicial, splitting to the base into two somewhat bony cocci”. They included seven species in this group, all from the Indian peninsula (Wight & Arnott, 1834). The key feature of the capsules, separating them from those in other *Hedyotis*, is that they dehisce along the septum (septicial dehiscence) and separate into two distinct valves or cocci (Terrell & Robinson, 2003; Neupane & al., 2009). Wight

& Arnott (1834) named the type of dehiscence diplophragmous and described the group as *Hedyotis* sect. *Diplophragma*. Following the initial description, the group has consistently been recognized by later workers, although at various taxonomic levels. Bentham & Hooker (1873) included 24 species in their *H.* sect. *Diplophragma* and also divided the group further based on features of the stipules. Hooker (1880), in his *Flora of British India*, included 30 species under *H.* sect. *Diplophragma*. Schumann (1891), also recognized the group, but included it under a broadly defined *Oldenlandia* as *O.* sect. *Diplophragma*. More recently the group has been elevated and recognized under *Hedyotis* at the rank of subgenus, as *H.* subg. *Diplophragma* (Wight & Arn.) Fosberg (Fosberg, 1943), or *H.* subg. *Hedyotis* (Terrell & Robinson, 2003), or at the rank of genus, *Diplophragma* (Wight & Arn.) Meisner (Devi & Raju, 1990). The name *Diplophragma*, irrespective of rank, can not be maintained under *Hedyotis*, and this was noted by Fosberg & Sachet (1991). *Hedyotis auricularia* L., the species originally proposed as lectotype for *Hedyotis* (Chamisso & Schlechtendal, 1829; Hitchcock, 1929), was for good reason dismissed by Bremekamp (1939, 1952) who instead suggested *H. fruticosa* as the type species. Bremekamp's suggestion was adopted by Jarvis (1992), who proposed *H. fruticosa* as the conserved type of *Hedyotis*. Their proposal was subsequently approved (Barrie, 2006) and a nomenclatural consequence of this is that section (or subgenus) *Diplophragma*, if including *H. fruticosa*, will have to be referred to as section (or subgenus) *Hedyotis*. Terrell & Robinson (2003) accepted this consequence and included section *Diplophragma* and subgenus *Diplophragma* as synonyms under *Hedyotis* subg. *Hedyotis*. Kårehed & al. (2008) and Groeninckx & al. (2009) took this one step further and suggested that the generic name of *Hedyotis* should be restricted to this smaller group. Following their suggestion we continue to refer to this group as *Hedyotis* s.str.

In addition to having diplophragmous capsules the group has been characterized as having “fruticosa-type” seeds. The name for this type of seeds was originally coined by Terrell & Robinson (2003) and refers to seeds that are dorsiventrally flattened, lenticular, and with an irregularly narrow and wing-like margin, resembling those in *H. fruticosa*. Surveying Asian and Pacific *Hedyotis*, Terrell & Robinson (2003) found that most of the Sri Lankan and Micronesian species have both diplophragmous capsules and fruticosa-type seeds, and they included these species under *Hedyotis* subg. *Hedyotis*. Also, a large proportion of species recognized under *Hedyotis* in the *Flora of China* have been associated to this group, either by explicitly describing the capsules as having septicidal dehiscence (Chen & Taylor, 2011), or by including the species in *Hedyotis* sect. *Diplophragma* (Lo & al., 1999).

Our analyses provide strong support for the monophyly of *Hedyotis* s.str. (clade A; BPP = 1.00). However, species characterized as having indehiscent fruits such as *Oldenlandia paridifolia* (Dunn) Chun, *O. prostrata* (Blume) Kuntze, *O. cryptantha* (Dunn) Chun, *Hedyotis philippensis* (Willd. ex Spreng.) Merr. ex C.B. Rob., and *H. rigida* (Blume) Walp., and that previously have been associated to *O. auricularia* (L.) F. Muell. (see the *Exallage/Dimetia* clade below), are here resolved in

Hedyotis s.str. Upon close examination of the fruits in these species we note that they are not truly indehiscent, as seen among species in the *Exallage/Dimetia* clade, but retain sutures that with mechanical pressure open septicidally. All *Hedyotis* s.str. species may therefore still satisfy the diplophragmous character. A complication though is that some species, characterized to have diplophragmous capsules (i.e., *Hedyotis scandens* Roxb., *H. capitellata* Wall. ex G. Don, *H. ampliflora* Hance, *H. dianxiensis* W.C. Ko, *Oldenlandia hedyotideae* (DC.) Hand.-Mazz.), show close relationships to species with truly indehiscent fruits, and resolve in the *Exallage/Dimetia* clade (Fig. 3). Most of these species were also explicitly stated to have “fruticosa type” of seeds by Terrell & Robinson (2003), indicating a complication also in the seed character. Species, with diplophragmous capsules, and that group in the *Exallage/Dimetia* clade, were all included in *Hedyotis* sect. *Dimetia* Wight & Arn. and not in *Hedyotis* sect. *Diplophragma* by Lo & al. (1999). *Hedyotis* sect. *Dimetia* has been characterized as having “tumescentes”, a swollen capsular top, protruding beyond the calyx (Terrell & Robinson, 2003). Perhaps this feature can be used to help discriminate between species with diplophragmous capsules that group in *Hedyotis* s.str., and those that group in the *Exallage/Dimetia* clade.

The analyses indicate the presence of biogeographic patterns within *Hedyotis* s.str. (Fig. 2). Species from the Indian subcontinent are resolved in two different groups, both well-supported (BPP = 1.00). The first and smallest group, the one including “*Pleiocraterium*”, is placed as an early-diverging group in *Hedyotis* s.str., but relationships in this part of the tree are poorly supported. The second group is the largest of the Indian subcontinent groups and is placed sister to a group variously distributed in China, Indochina, Malesia, and Papuaia. Three distinct and well-supported groups with primarily Chinese distributions are also retrieved in our analyses.

“*Metabolos*” and “*Pleiocraterium*”. — Included in *Hedyotis* s.str. are species currently recognized under the generic names *Metabolos* and *Pleiocraterium*. *Metabolos* is a small Asiatic genus whose relationship to other Rubiaceae has been difficult to understand. Hooker (1873), for example, initially associated *Metabolos* with *Lasianthus* Jack (tribe Lasiantheae; Bremer & Manen, 2000), but a few years later emended this view and included the genus in Hedyotideae (Hooker, 1880). Schumann (1891) also found similarities with *Lasianthus* and included *Metabolos* in Psychotriaceae, whereas Bremekamp (1939), like Hooker (1880), suggested a placement in Hedyotideae. More recently Puff & Igersheim (1994) surveyed the character states and taxonomic position of *Metabolos*. They acknowledged a striking habitual similarity between *Metabolos* and species of *Hedyotis*, yet they found no support for associating *Metabolos* with the tribe Hedyotideae (Puff & Igersheim, 1994). Based on what they perceived as congruent character states in inflorescence arrangement, ovary structure and placement, fruit structure and anatomy, and pollen morphology, they agreed with Schumann (1891) and argued for a close relationship to *Lasianthus* (Psychotriaceae). Piesschaert & al. (2001) on the other hand, considered a relationship to Hedyotideae a possibility based on palynological data. They

looked at photographs of *Metabolos* pollen, as reported by Puff & Igersheim (1994), and found the pollen to show a complex reticulum, consisting of a psilate suprareticulum and spinulate infrareticulum, a type of reticulum that they associated with Spermaceae sensu Bremer (1996).

The results presented here unequivocally resolve this issue and clearly place *Metabolos* well inside *Hedyotis* s.str. and as sister to *Hedyotis nodulosa* Arn., a species from Sri Lanka to which it bears a great resemblance (Puff & Igersheim, 1994). Our analyses included three different accessions of *Metabolos*, two from Sri Lanka representing *M. decipiens* (Thwaites) Ridsdale, and one from Java, Indonesia representing *M. rugosus* Blume, but whether or not the Sri Lankan and the Javanese *Metabolos* truly represent two different species has been debated (Puff & Igersheim, 1994). Our analyses indicate considerable sequence divergence between the two Sri Lankan accessions and the accession from Indonesia (Fig. 2) providing some support for maintaining two species. Govaerts & al. (2011) accept two additional names, *Metabolos angustifolius* DC. and *Metabolos macrophyllus* Zoll. & Moritz, but both were excluded from *Metabolos* already by Hochreutiner (1934) and this was followed by Bremekamp (1952) who included both species under the generic name *Exallage*.

Piesschaert & al. (2001) investigated morphological and anatomical features in the small Neotropical genus *Pagameopsis* (Standl.) Steyerm. and found considerable similarities between *Pagameopsis* and *Metabolos*. They argued that *Metabolos* should be included in future phylogenetic (e.g., molecular) analyses as a possible relative of *Pagameopsis* (Piesschaert & al., 2001). Given that our analyses placed *Metabolos* well inside *Hedyotis* s.str. we will include *Pagameopsis* in future analyses of *Hedyotis* to further evaluate these putative similarities.

The genus *Pleiocraterium* was described by Bremekamp (1939) and included four species, and all are currently recognized under *Pleiocraterium* by Govaerts & al. (2011). *Pleiocraterium verticillare* (Wall. ex Wight & Arn.) Bremek. from India, the type species, was originally described by Wight & Arnott (1834) as *Hedyotis verticillaris* Wight & Arn. They, as well as Hooker (1880), included *H. verticillaris* in *H. sect. Diplophragma*. Bremekamp (1939) considered this position anomalous, and listed a series of features separating this species from others in *H. sect. Diplophragma*. Features listed include the presence of an ovary protruding distinctly beyond the insertion of the calyx, presence of axillary, and not terminal, inflorescences, and the occurrence of strikingly different vegetative characters in *H. verticillaris* (Bremekamp, 1939). Because none of these characters occur in species that Bremekamp associated with *H. sect. Diplophragma*, he considered the creation of a separate genus fully justified. Based on the numerous cups that are formed by the connate leaf bases in *H. verticillaris*, he named the genus *Pleiocraterium*. One additional species, *P. plantaginifolium* (Arn.) Bremek. from Sri Lanka, had previously been described under *Hedyotis* and was transferred to *Pleiocraterium* by Bremekamp (1939), but he also described two additional species, *P. sumatranum* Bremek. and *P. gentianifolium* Bremek., both restricted to Sumatra.

Our analyses clearly place *Pleiocraterium* in *Hedyotis* s.str., and the species included in our analyses, *P. verticillare*, groups with other species from India including *H. purpurascens*, *H. articularis*, *H. swertioides*, and *H. stylosa* (Fig. 2). This result is not entirely surprising and although *Pleiocraterium* was not included in their analyses, Groeninckx & al. (2009) discussed this possibility. Bremekamp (1939) found the distribution of *Pleiocraterium* peculiar and compared it to that of *Metabolos*, the only genus in Rubiaceae he could think of with a similar distribution. The relationships of *Metabolos* indicate that the group possibly originated in Sri Lanka (Indian subcontinent) and subsequently spread to SE Asia, and although no SE Asian representatives of *Pleiocraterium* were included in our analyses, a corresponding pattern is hypothesized also for this group.

"Hedyotis" resolved outside of *Hedyotis* s.str. — Species previously included in *Hedyotis* are resolved in two additional clades (clades B and C), both quite large and not closely related to *Hedyotis* s.str. Species in these clades, with few exceptions, are distributed in Asia and the Pacific.

Clade B. — The largest clade (clade B; Fig. 3) comprises a series of smaller and well-supported monophyletic groups including: the *Leptopetalum* clade (BPP = 1.00); the genus *Kadua* (BPP = 1.00); an unnamed group including Asian, Pacific, and one African species of *Oldenlandia* (BPP = 1.00); and a large Asian group that we refer to as the *Exallage/Dimetia* clade (BPP = 1.00). Exceptions to the Asian-Pacific pattern of distribution are found in clade B and include *Synaptantha tillacea* (F. Muell.) Hook. f. and *Oldenlandia mitrasacmoides* (F. Muell.) F. Muell., both from Australia, and *Oldenlandia lancifolia* (Schumach.) DC. from Africa.

The *Leptopetalum* clade. — The genus *Leptopetalum* was described in 1838 by W.J. Hooker and G.A. Walker-Arnott in their *Botany of Captain Beechey's Voyage* (Hooker & Walker-Arnott, 1841). Their description was based on a specimen collected from the Bonin Islands, but the specimen was incorrectly supposed to have been collected in Mexico and the species they described was given the rather misleading name *Leptopetalum mexicanum* Hook. & Arn. Hooker (1873) and Schumann (1891), in their general treatments of Rubiaceae, recognized the group, but included the name *Leptopetalum* under a more broadly defined *Hedyotis* (Hooker, 1873) or *Oldenlandia* (Schumann, 1891). More recently, and while preparing a treatment of the family Rubiaceae for the "Flora of Micronesia", Fosberg & Sachet (1991) reviewed the taxonomic history of *Leptopetalum* and undertook an evaluation of its generic status. Following this evaluation, they recognized *Leptopetalum* at the subgeneric level and included it under a broadly defined *Hedyotis* (Fosberg & Sachet, 1991).

Six species were included in their *Hedyotis* subg. *Leptopetalum* and the group was characterized as woody, with large salverform, funnellform, or campanulate corollas, with stamens inserted on the corolla tube or near its base, and distributed in the south, central and western Pacific (Fosberg & Sachet, 1991). The species that they included correspond, with one exception, to those currently recognized under *Leptopetalum* by Govaerts & al. (2011).

Recent phylogenetic analyses by Kårehed & al. (2008) and Groeninckx & al. (2009) indicated a close relationship between *Oldenlandia biflora* L. and the genus *Kadua*, a genus that Terrell & al. (2005) recently resurrected for Pacific species previously included in *Hedyotis*. Their results clearly showed that *O. biflora* needed to be transferred from *Oldenlandia* s.str., but they considered it premature to include it in a more broadly circumscribed *Kadua*. Our analyses provide support for their hesitation to recircumscribe *Kadua*, and instead group *O. biflora* with representatives of the genus *Leptopetalum*. Our results also define an additional member of this lineage, *O. pterita* (Blume) Miq., and these species form a well-supported group (PP 1.00) that is resolved sister to the genus *Kadua*. *Kadua* could of course still be recircumscribed to include *O. biflora*, but this would require that species currently recognized under *Leptopetalum* also be included.

The two species, *O. biflora* and *O. pterita* have previously been considered closely related (Fosberg & Sachet, 1991) and both were included under the generic name *Thecagonum* Babu by Babu (1969) together with *O. ovatifolia* (Cav.) DC. and one additional *Oldenlandia* species not included in our analyses (*O. parishii* Hook. f.). Investigating seed and capsule morphology in different genera in Hedyotideae Terrell & Robinson (2007) found support for this grouping, and they added one additional species (*Oldenlandia strigulosa* DC.) to the group. However, based on considerable differences in its seed morphology, they excluded *O. ovatifolia*. Our results lend support for this exclusion, and there is no support for a close relationship between *O. ovatifolia* and remaining species included under *Thecagonum* by Terrell & Robinson (2007). The analyses place *O. ovatifolia* in a very isolated position, as sister to the *Exallage/Dimetia* clade (Fig. 3).

Additional analyses should be undertaken before taxonomic decisions are made in the *Leptopetalum* clade. Morphologically, the small and herbaceous species that have been recognized under *Thecagonum* are quite distinct, and they have never before been associated with the genus *Leptopetalum*. Recircumscribing *Leptopetalum* to also include these species is for this reason not necessarily a good option and would make *Leptopetalum* more problematic to diagnose. An alternative solution would be to recognize two genera, *Leptopetalum* and *Thecagonum*, but at present there is no support for a monophyletic *Thecagonum* (Fig. 3). In fact, the species *Oldenlandia biflora* is resolved as paraphyletic indicating that there are taxonomic problems in this species. These problems are not entirely unexpected and Fosberg & Sachet (1991) discussed at length the extensive amount of morphological variation seen in this species across its geographic distribution.

The “unnamed group” from Asia and the Pacific. — Sister to the *Leptopetalum* clade and *Kadua* is a heterogenic group that is difficult to characterize. The group includes not only species that never before have been considered closely related, but also species commonly associated to other groups such as the *Exallage/Dimetia* clade or *Hedyotis* s.str. For example, *Oldenlandia diffusa* (Willd.) Roxb. and *O. brachypoda* DC. have been considered closely related to *O. corymbosa* L. and *O. erecta* (Manilal & Sivar.) R.R. Mill (Sivarajan & Biju, 1990; Chen

& Taylor, 2011), but our analyses clearly indicate that they are not. Furthermore, *Oldenlandia verticillata* L. from Asia has often been associated to *O. auricularia* (Fosberg & Sachet, 1991), but our analyses group *O. verticillata* with *O. pinifolia* (Wall. ex G. Don) Kuntze, and not together with *O. auricularia* (in the *Exallage/Dimetia* clade). *Oldenlandia verticillata*, together with *O. pinifolia*, is sister to six (possibly seven) species of *Oldenlandia* (*O. gracilipes* Craib, *O. stocksii* Hook. f., *O. lancifolia* (Schumach.) DC., *O. galioides* (F. Muell.) F. Muell., *O. diffusa*, *O. brachypoda*). The seventh species, *Oldenlandia herbacea* (L.) Roxb. (CB058), is a Chinese accession, determined as *O. herbacea*, which is resolved in this group. However, the accession does not group with *O. herbacea* from Africa (in the *Pentanopsis* clade), and clearly represents some other species. Chen & Taylor (2011), in their treatment of *Hedyotis* for the *Flora of China*, pointed out that they had seen very few specimens of this taxon from China, or anywhere else east of Sri Lanka. They also noted that the description of the species, given in the Chinese version of the *Flora of China* by Lo & al. (1999), indicated features not in agreement with specimens of *H. herbacea*, but that are applicable to the similar species *H. brachypoda* and *H. diffusa* (Chen & Taylor, 2011). Our results are completely consistent with their observations and group the Chinese *O. herbacea* with *O. brachypoda* and *O. diffusa*. The possibility that *O. herbacea* does not occur in China has to be considered, and Chinese specimens of this taxon should be carefully reexamined.

The *Exallage/Dimetia* clade. — The generic name *Exallage* was introduced by Bremekamp (1952) in his work on African *Oldenlandia*. Following a discussion concerning the typification of the genus *Hedyotis*, he concluded that *Hedyotis auricularia*, a species primarily found in Asia, needed to be transferred to another genus. He dismissed the possibility of including it under some generic name already in use, and described the genus *Exallage*, with *Exallage auricularia* (L.) Bremek. as the type species. Although primarily focused on African taxa, he provided a preliminary list of an additional 23 non-African species in this genus (Bremekamp, 1952). Ridsdale (1998), however, considered the genus ill-defined and argued that Bremekamp made his combinations without properly examining the taxa, and that many belong to *Hedyotis* s.str.

Surveying Asian and Pacific species of *Hedyotis* and *Exallage*, Terrell & Robinson (2003) summarized the infrageneric classification and species groups recognized by Ridsdale (1998) and Lo & al. (1999), and after having examined a large number of species and specimens from the herbarium at the Smithsonian Institution (US), they characterized *Hedyotis* and *Exallage* with respect to their fruit and seed morphology. *Exallage* was characterized as: large perennial herbs; with glomerulate inflorescences; with small globose indehiscent fruits; and with seeds similar to those found in *Oldenlandia* subg. *Oldenlandia* (with oldenlandioid seeds). Contrary to Bremekamp (1952) they included *Exallage* as a subgenus under *Oldenlandia*, and stressed that indehiscent fruits seemed to be the main defining character of the group, a feature not found in other *Oldenlandia* (Terrell & Robinson, 2003).

Our analyses found strong support for a group of species (the *Exallage/Dimetia* clade), associated with *Oldenlandia*

(*Hedyotis auricularia* and that do not group with neither *Hedyotis* s.str. nor *Oldenlandia* s.str. (Fig. 3). Corresponding relationships were indicated already by Kårehed & al. (2008) who resolved *H. capitellata* outside of *Hedyotis* s.str. and in association with several species of *Oldenlandia* (their clade H). The entire group can, however, not be unequivocally characterized as having indehiscent fruits. Species such as *Hedyotis scandens*, *H. capitellata*, *H. ampliflora*, *H. dianxiensis*, and *Oldenlandia hedyotideae* have all been characterized as having diplophragmous capsules (Terrell & Robinson, 2003; Chen & Taylor, 2011). These capsular species are resolved as a monophyletic group (“*Dimetia*” Fig. 3; BPP = 0.99), but despite having diplophragmous capsules they were not included in *H. sect. Diplophragma* by Lo & al. (1999), who classified them under *H. sect. Dimetia*. Sister to this group is a well-supported group (“*Exallage*” Fig. 3; BPP = 1.00), characterized by having indehiscent fruits, and with the exception of *Oldenlandia chrysotricha* (Palib.) Chun, all species in this group have previously been included under the generic name of *Exallage*.

There are some problems in the *Exallage/Dimetia* clade that involve the determination of specimens included in our analyses, and the synonymy of species by Govaerts & al. (2011). For example, Govaerts & al. (2011) include *H. rigida* (Blume) Walp. under the accepted name *Oldenlandia cristata* (Willd. ex Roem. & Schult.) ined. together with *Hedyotis vestita* R. Br. ex G. Don, *Hedyotis costata* (Roxb.) Kurz, and a large number of additional names, and Chen & Taylor (2011) include *H. philippensis* (Willd. ex Spreng.) Merr. ex C.B. Rob. as a synonym under *H. prostrata* Blume. Our analyses do place specimens determined as *Hedyotis vestita* R. Br. ex G. Don and *Hedyotis costata* (Roxb.) Kurz together, as implied by the synonymy. They are resolved inside the *Exallage/Dimetia* clade, as suggested by Terrell & Robinson (2003), but they are not closely related to *H. rigida* (Blume) Walp.. Also, the specimen determined as *H. philippensis* (Willd. ex Spreng.) Merr. ex C.B. Rob., as well as the one determined as *H. prostrata* Blume (determined by C. Taylor), are both placed inside *Hedyotis* s.str., but they are not sister species, as would be expected. Instead, *H. philippensis* (Willd. ex Spreng.) Merr. ex C.B. Rob. is grouped together with *H. rigida* (Blume) Walp.

Clade C (*Neanotis*). — The second clade, in which species previously included in *Hedyotis* are resolved, is clade C (Fig. 1). Four species of *Hedyotis*, *H. trichoclada* and *H. nana* from New Guinea, *H. pahompokae* from Thailand, and *H. lindleyana* from Asia, are resolved together with all included representatives of the Asian genus *Neanotis*. Following the new combinations made (see Nomenclatural changes outside *Hedyotis* s.str. below) we will refer to this clade as the genus *Neanotis*.

The generic name *Neanotis* originates from Lewis (1966) and was adopted as a substitute name for non-American taxa previously included under the generic name *Anotis* DC. Reviewing the taxonomic history of *Anotis* DC., Lewis (1966) noted that taxonomists in the 19th and 20th centuries also included Asian species from the tribes Hedyotideae (Hooker, 1873, 1880) and Oldenlandieae (Schumann, 1891) under this name. Species included were characterized as herbaceous and with few and peltate, cymbiform seeds. Going back to the

original description of *Anotis*, Lewis noted that all species of *Anotis* (sensu Candolle, 1830) were native to the New World and concluded that there was no justification for an inclusion of species from Asia. Following the taxonomists of the 19th and 20th centuries, who had recognized the Asian *Anotis* as distinct from *Hedyotis* or *Oldenlandia*, he transferred and included the Asian species under the new name *Neanotis*.

Approximately 30 species, distributed in temperate and tropical Asia, are recognized in the genus by Govaerts & al. (2011). They have cymbiform seeds (Schumann, 1891; Lewis, 1966), similar to those found in the genus *Houstonia* (Terrell & Robinson, 2007), and the possibility of *Neanotis* being the closest non-American relative of the *Arcytophyllum-Houstonia* clade was discussed briefly by Groeninckx & al. (2009).

Our analyses provide strong support for monophyly of *Neanotis* (including the former *Hedyotis* species), but do not indicate a close relationship of this group to the *Arcytophyllum-Houstonia* clade. Sister to *Neanotis* is a group including two African species, *Dibrachionostylus kaessneri* (S. Moore) Bremek. and *Oldenlandia rupicola* (Sond.) Kuntze (see below for a discussion of *Dibrachionostylus*), and together they are sister to a large group in which the *Arcytophyllum-Houstonia* clade is nested. However, the relationship to this large group, in which the *Arcytophyllum-Houstonia* clade is nested, is not well-supported (BPP = 0.94). Sister to the *Arcytophyllum-Houstonia* clade is a group comprising two small African genera, *Mitrasacmopsis* Jovet (2 spp.) and *Hedythyrus* Bremek. (1 sp.), and four African species of *Oldenlandia* (*O. fastigiata* Bremek., *O. echinulosa* K. Schum., *O. geophila* Bremek., *O. nervosa* Hiern). This last group corresponds to clade G sensu Kårehed & al. (2008) and relationships in this group, as well as in the *Arcytophyllum-Houstonia* clade were discussed exhaustively by them.

Hedyotis pahompokae was described by Fukuoka (1969) based on collections from Thailand, and is grouped with strong support within *Neanotis* (Fig. 1). Fukuoka (1970), in his “Contributions to the Flora of Southeast Asia”, associated *H. pahompokae* with *Hedyotis nalampooni* Fukuoka and *Oldenlandia krewanhensis* Pierre ex Pit. It is possible that all three species should be transferred to *Neanotis*, but the relationships of *H. nelampooni* and *H. krewanhensis* should be confirmed in analyses before such a transfer is completed. Grouped within *Neanotis* are also two species of *Hedyotis* from New Guinea, *H. nana* and *H. trichoclada*, both described by Merrill & Perry (1945), who considered them closely allied and readily distinguished from other species of *Hedyotis* by their minute leaves and prostrate habit. While discussing *H. trichoclada*, they commented that “although the habit of this plant suggests *Anotis*, it has all the characters of *Hedyotis*”. Contrary to this view, our analyses strongly support a grouping within *Neanotis*. *Hedyotis lindleyana* is indicated as a heterotypic synonym to *Neanotis hirsuta* by Govaerts & al. (2011), and our analyses also group *H. lindleyana* (accession collected in Japan) with *N. hirsuta* from Nepal.

Lewis (1966) characterized *Neanotis* palynologically as having brevicolporate pollen with (5)6–12 apertures, and saw a distinct line of demarcation in the pollen morphology of

Neanotis on the one hand and *Hedyotis* and *Oldenlandia* on the other. This indicates that pollen morphology could potentially be used to characterize the *Neanotis* clade, and species of *Hedyotis* that are resolved in the *Neanotis* clade should be investigated for their pollen morphology in order to evaluate this possibility.

***Dibrachionostylus*.** — The genus *Dibrachionostylus* was described by Bremekamp (1952). He separated *Oldenlandia kaessneri* S. Moore from other species of *Oldenlandia* based on its capsule dehiscence and suggested a possible relationship of *O. kaessneri* to species in the genus *Agathisanthemum*. However, differences in style, corolla tube, and seed testa led Bremekamp to assign *O. kaessneri* to a segregate genus which he named *Dibrachionostylus*. Recent phylogenetic analyses have not supported a close relationship between *Dibrachionostylus* and *Agathisanthemum*. Groeninckx & al. (2009) resolved *Dibrachionostylus* as sister to a clade of African *Oldenlandia* including *O. echinulosa*, *O. geophila*, and *O. nervosa*. Corresponding relationships were also reported by Groeninckx & al. (2010a, b), although a different relationship now was indicated for *O. nervosa*. In both analyses the support for this placement of *Dibrachionostylus* was weak. Kårehed & al. (2008) resolved *Dibrachionostylus* as sister to a large clade (their clade E), but the two accessions of *D. kaessneri* were not closely associated with any other African taxon included in their analyses.

Our analyses support a relationship of *Dibrachionostylus* as sister to the African species *Oldenlandia rupicola*. Together these two species are resolved as sister to the Asian genus *Neanotis* (Fig. 1), but this relationship is not well-supported (BPP = 0.94). The relationship of *Dibrachionostylus* to *Oldenlandia rupicola* is interesting and provides the first support for associating *Dibrachionostylus* with any other African taxon. *Oldenlandia rupicola* was classified in *Oldenlandia* subg. *Orophilum* Bremek. by Bremekamp (1952), and he specifically associated *O. rupicola* with *O. greenwayi* Bremek., *O. muscosa* Bremek., and *O. tenella* Kuntze. However, none of these have been included in any phylogenetic analyses, and *O. geophila*, the only other representative of *Oldenlandia* subg. *Orophilum* included here, do not show a close relationship to *O. rupicola* (Fig. 1).

The grouping of *O. rupicola* with *Dibrachionostylus* adds further evidence to the problematic and polyphyletic nature of African *Oldenlandia*. In previous analyses (Kårehed & al., 2008; Groeninckx & al., 2009), as well as in the analyses presented here, *Oldenlandia* species from Africa are resolved in no less than five different groups. The association of *O. rupicola* to *Dibrachionostylus* and their relationships to Asian *Neanotis* adds a sixth lineage associated with African *Oldenlandia*.

■ CONCLUSIONS

The phylogenetic analyses presented resolve all investigated species of *Hedyotis*, except *H. coronaria*, in one of three clades (clades A–C; Figs. 1–3), each well-supported as a monophyletic group, each with an Asian-Pacific distribution, and each including a fair number of species recognized under

some other generic name by Govaerts & al. (2011). *Hedyotis coronaria* is unrelated to other *Hedyotis* and groups with *Spermacoce hispida*. A summary of the habit, fruit- and seed morphology, and distribution of all three clades is given in Table 2. All the major clades and sub-groups listed in Table 2 are currently being investigated further prior to formal taxonomic recognition. Many of the clades and sub-groups in the table will require generic status. In addition to *Hedyotis* s.str. and *Neanotis* defined herein, the clades representing *Dimetia*, *Exallage*, *Leptopetalum*, and “*Thecagonum*” will likely require elevation to the generic level. Based on geographic sampling gaps in the present study we were concerned that hasty revisions could lead to inaccurate classifications which have been all too recurrent in past treatments of these lineages. Presently detailed morphological and anatomical studies are being conducted on fruit, seed, and pollen and will be combined with more comprehensive and cosmopolitan sampling of species for phylogenetic analyses. These species-level investigation on our proposed generic assemblages will allow us to better define the range, composition and morphological synapomorphies delineating the monophyletic lineages. While we have been able to resolve many relationships and new monophyletic lineages, this diverse and widespread lineage of Rubiaceae still requires more investigation as many regions and taxa remain unsampled. Because this group contains species that can vary from trees to tiny herbs yet harbors cryptic species much effort will be required before a clear picture can be drawn about the evolution of the members of the *Hedyotis*-*Oldenlandia* complex.

■ NOMENCLATURAL TREATMENT

Species recognized under *Hedyotis* s.str.

The following list includes species names and their homotypic synonyms that we, based on the results presented here, recognize under *Hedyotis* s.str.

Hedyotis acutangula Champ. ex Benth. in Hooker’s J. Bot. Kew Gard. Misc. 4: 171. 1852 ≡ *Oldenlandia acutangula* (Champ. ex Benth.) Kuntze, Revis. Gen. Pl. 1: 292. 1891.

Hedyotis articularis R. Br. ex Wight & Arn., Prodr. Fl. Ind. Orient. 1: 407. 1834 ≡ *Hedyotis articularis* R. Br. ex G. Don, Gen. Hist. 3: 527. 1834.

Hedyotis assimilis Tutcher in Rep. Bot. Dept. Hong Kong 1914: 32. 1915 ≡ *Oldenlandia assimilis* (Tutcher) Chun in Sunyatsenia 1: 310. 1934.

Hedyotis benguetensis (Elmer) Elmer in Leaflet. Philipp. Bot. 3: 976. 1911 ≡ *Oldenlandia benguetensis* Elmer in Leaflet. Philipp. Bot. 1: 18. 1906.

Hedyotis bodinieri H. Lév. in Repert. Spec. Nov. Regni Veg. 11: 64. 1912 ≡ *Oldenlandia bodinieri* (H. Lév.) Chun in Sunyatsenia 1: 310. 1934.

- Hedyotis bracteosa* Hance in J. Bot. 23: 323. 1885 ≡ *Oldenlandia bracteosa* (Hance) Kuntze, Revis. Gen. Pl. 1: 292. 1891.
- Hedyotis cantoniensis* F.C. How ex W.C. Ko in J. S. China Agric. Univ. 16: 42. 1995.
- Hedyotis cathayana* W.C. Ko, Fl. Hainan. 3: 579. 1974.
- Hedyotis caudatifolia* Merr. & F.P. Metcalf in J. Arnold Arbor. 23: 228. 1942.
- Hedyotis ceylanica* (Thwaites) N. Wikström & Neupane, **nom. nov.** ≡ *Allaeophania decipiens* Thwaites, Enum. Pl. Zeyl. 147. 1859 [non *Hedyotis decipiens* (Valeton) Merr. & L.M. Perry in J. Arnold Arbor. 26: 2. 1945] ≡ *Metabolos rugosus* var. *decipiens* (Thwaites) Hochr. in Candollea 5: 280. 1934 ≡ *Metabolos decipiens* (Thwaites) Ridsdale in Blumea 41: 460. 1996 – **Lectotype (designated here)**: Sri Lanka, without date, *Thwaites CP-3093* (BR no. BR0000005575176 [image!]; isoelectotypes: GH no. GH00092309 [n.v.], NY no. NY130803 [image!]).
- Hedyotis cheniana* R.J. Wang in Novon 18: 264. 2008.
- Hedyotis communis* W.C. Ko, Fl. Hainan. 3: 579. 1974.
- Hedyotis consanguinea* Hance in Ann. Sci. Nat., Bot., ser. 4, 18: 221. 1862 ≡ *Oldenlandia consanguinea* (Hance) Kuntze, Revis. Gen. Pl. 1: 292. 1891.
- Hedyotis coprosmoides* Trimen, Syst. Cat. Fl. Pl. Ceylon: 42. 1885.
- Hedyotis cryptantha* Dunn in Bull. Misc. Inform. Kew 1912: 367. 1912 ≡ *Oldenlandia cryptantha* (Dunn) Chun in Sunyatsenia 1: 311. 1934.
- Hedyotis decora* Geddes in Bull. Misc. Inform. Kew 1931: 219. 1931.
- Hedyotis dendroides* Alston in Trimen, Handb. Fl. Ceylon 6: 147. 1931.
- Hedyotis effusa* Hance in J. Bot. 17: 11. 1879 ≡ *Oldenlandia effusa* (Hance) Kuntze, Revis. Gen. Pl. 1: 292. 1891.
- Hedyotis fissistipula* Merr. in J. Malayan Branch Roy. Asiat. Soc. 1: 38. 1923.
- Hedyotis flavescens* Thwaites, Enum. Pl. Zeyl.: 141. 1859.
- Hedyotis fruticosa* L., Sp. Pl.: 101. 1753 ≡ *Oldenlandia fruticosa* (L.) Kuntze, Revis. Gen. Pl. 1: 292. 1891.
- Hedyotis gardneri* Thwaites, Enum. Pl. Zeyl.: 142. 1859 ≡ *Oldenlandia gardneri* (Thwaites) Kuntze, Revis. Gen. Pl. 1: 292. 1891.
- Hedyotis gentianifolia* (Bremek.) N. Wikström & Neupane, **comb. nov.** ≡ *Pleiocraterium gentianifolium* Bremek. in Meded. Bot. Mus. Herb. Rijks Univ. Utrecht 56: 441. 1939 – Holotype: North Sumatra, Country of the Gajos, summit of Goh Lembuh, alt. 3000 m, 20 Feb 1937, *van Steenis 9038* (BO [n.v.]).
- Hedyotis hainanensis* (Chun) W.C. Ko, Fl. Hainan. 3: 580. 1974 ≡ *Oldenlandia hainanensis* Chun in Sunyatsenia 1: 311. 1934 ≡ *Oldenlandia oligantha* Chun in Sunyatsenia 1: 313. 1934 ≡ *Hedyotis oligantha* Merr. in Philipp. J. Sci. 23: 266. 1923, nom. illeg. [non *Hedyotis oligantha* Merr. in Philipp. J. Sci. 17: 431. 1921 ('1921')].
- Hedyotis korrorensis* (Valeton) Hosok. in Trans. Nat. Hist. Soc. Taiwan 24: 204. 1934 ≡ *Oldenlandia korrorensis* Valeton in Bot. Jahrb. Syst. 63: 294. 1930.
- Hedyotis lancea* Thunb. ex Maxim. in Bull. Acad. Imp. Sci. Saint-Petersbourg 29: 161. 1883 ≡ *Oldenlandia lancea* (Thunb. ex Maxim.) Kuntze, Revis. Gen. Pl. 1: 292. 1891.
- Hedyotis lawsoniae* Wight & Arn., Prodr. Fl. Ind. Orient. 1: 407. 1834 ≡ *Oldenlandia lawsoniae* (Wight & Arn.) Kuntze, Revis. Gen. Pl. 1: 292. 1891.
- Hedyotis lessertiana* Arn. in Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 18: 339. 1836 ≡ *Oldenlandia lessertiana* (Arn.) Kuntze, Revis. Gen. Pl. 1: 292. 1891.
- Hedyotis leuserensis* N. Wikström & Neupane, **nom. nov.** ≡ *Pleiocraterium sumatranum* Bremek. in Meded. Bot. Mus. Herb. Rijks Univ. Utrecht 56: 440. 1939 [non *Hedyotis sumatrana* Merr. in Pap. Michigan Acad. Sci. 23: 197. 1938] – Holotype: North Sumatra, Country of the Gajos, G. Losir, alt. 3250–3450 m, 1 Feb 1937, *van Steenis 8560* (BO [n.v.]; isotype: BM [image!]).
- Hedyotis macrostegia* Stapf in Trans. Linn. Soc. London, Bot. 4: 170. 1894.
- Hedyotis marginata* (Thwaites ex Trimen) Alston in Trimen, Handb. Fl. Ceylon 6: 147. 1931 ≡ *Hedyotis lessertiana* var. *marginata* Thwaites ex Trimen, Handb. Fl. Ceylon 2: 309. 1894.
- Hedyotis megalantha* Merr. in Philipp. J. Sci., C 9: 143. 1914 ≡ *Oldenlandia megalantha* (Merr.) Valeton in Bot. Jahrb. Syst. 63: 298. 1930.
- Hedyotis mellii* Tutcher in Rep. Bot. Dept. Hong Kong 1914: 32. 1915 ≡ *Oldenlandia mellii* (Tutcher) Chun in Sunyatsenia 1: 313. 1934.
- Hedyotis membranacea* Thwaites, Enum. Pl. Zeyl.: 143. 1859.

- Hedyotis minutopuberula* Merr. & F.P. Metcalf in J. Arnold Arbor. 23: 229. 1942.
- Hedyotis nodulosa* Arn. in Nova Acta Acad. Caes. Leop.-Carol. German. Nat. Cur. 18: 340. 1836.
- Hedyotis novoguineensis* Merr. & L.M. Perry in J. Arnold Arbor. 26: 4. 1945.
- Hedyotis nutans* (Valeton) P. Royen, Alpine Fl. New Guinea 4: 2718. 1983 ≡ *Oldenlandia nutans* Valeton in Gibbs, Fl. Arfak Mts.: 180. 1917.
- Hedyotis obscura* Thwaites, Enum. Pl. Zeyl.: 141. 1859 ≡ *Oldenlandia obscura* (Thwaites) Kuntze, Revis. Gen. Pl. 1: 292. 1891.
- Hedyotis ovata* Thunb. ex Maxim., Bull. Acad. Imp. Sci. Saint-Pétersbourg 29: 161. 1883 ≡ *Oldenlandia ovata* (Thunb. ex Maxim.) Kuntze, Revis. Gen. Pl. 1: 292. 1891, nom. illeg. [non *Oldenlandia ovata* S. Watson in Proc. Amer. Acad. Arts 18: 97. 1883].
- Hedyotis paridifolia* Dunn in Bull. Misc. Inform. Kew 1912: 366. 1912 ≡ *Oldenlandia paridifolia* (Dunn) Chun in Sunyatsenia 1: 313. 1934.
- Hedyotis parryi* Hance in Ann. Sci. Nat., Bot., ser. 4, 18: 221. 1862 ≡ *Oldenlandia parryi* (Hance) Kuntze, Revis. Gen. Pl. 1: 292. 1891.
- Hedyotis philippensis* (Willd. ex Spreng.) Merr. ex C.B. Rob. in Philipp. J. Sci., C 6: 222. 1911 ≡ *Spermacoce philippensis* Willd. ex Spreng., Syst. Veg. 1: 401. 1824 ≡ *Exallage philippensis* (Willd. ex Spreng.) Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48(2): 142. 1952.
- Hedyotis prostrata* Blume, Catalogus: 40. 1823 ≡ *Oldenlandia prostrata* (Blume) Kuntze, Revis. Gen. Pl. 1: 292. 1891.
- Hedyotis pubescens* (Valeton) Merr. & L.M. Perry in J. Arnold Arbor. 26: 3. 1945 ≡ *Oldenlandia pubescens* Valeton in Lorentz, Nova Guinea 8(1): 439. 1911 ≡ *Exallage pubescens* (Valeton) Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2, 48(2): 142. 1952.
- Hedyotis pulchella* Stapf in Trans. Linn. Soc. London, Bot. 4: 169. 1894.
- Hedyotis pulcherrima* Dunn in Bull. Misc. Inform. Kew, Addit. Ser. 10: 127. 1912 ≡ *Oldenlandia pulcherrima* (Dunn) Chun in Sunyatsenia 1: 314. 1934.
- Hedyotis purpurascens* Hook. f., Fl. Brit. India 3: 50. 1880.
- Hedyotis quinquenervia* Thwaites, Enum. Pl. Zeyl.: 141. 1859 ≡ *Oldenlandia quinquenervia* (Thwaites) Kuntze, Revis. Gen. Pl. 1: 292. 1891.
- Hedyotis rhinophylla* Thwaites ex Trimen in J. Bot. 23: 208. 1885.
- Hedyotis rigida* (Blume) Walp. in Ann. Bot. Syst. 2: 772. 1852 ≡ *Metabolos rigidus* Blume, Bijdr. Fl. Ned. Ind. 16: 992. 1826 ≡ *Hedyotis rigida* (Blume) Miq., Fl. Ned. Ind. 2: 181. 1857, nom. illeg. ≡ *Scleromitron rigidum* (Blume) Kurz in J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 46(2): 136. 1877 ≡ *Oldenlandia rigida* (Blume) Kuntze, Revis. Gen. Pl. 1: 292. 1891, nom. illeg. ≡ *Exallage rigida* (Blume) Bremek. in Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2 48(2): 142. 1952.
- Hedyotis rivalis* Ridl. in J. Fed. Malay States Mus. 6: 153. 1915.
- Hedyotis rugosa* (Blume) Korth. in Ned. Kruidk. Arch. 2(2): 160. 1851 ≡ *Metabolos rugosus* Blume, Bijdr. Fl. Ned. Ind. 16: 991. 1826 ≡ *Scleromitron rugosum* (Korth.) Kurz in J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 46(2): 137. 1877 ≡ *Allaeophania rugosa* (Blume) Boerl., Handl. Fl. Ned. Ind. 2(1): 124. 1891 ≡ *Oldenlandia rugosa* (Blume) Kuntze, Revis. Gen. Pl. 1: 292. 1891.
- Hedyotis schlechteri* Merr. & L.M. Perry in J. Arnold Arbor. 26: 1. 1945.
- Hedyotis shenzhenensis* Tao Chen in Edinburgh J. Bot. 64: 331. 2007.
- Hedyotis shiuyingiae* Tao Chen in Harvard Pap. Bot. 13: 283. 2008.
- Hedyotis stylosa* R. Br. ex Wight & Arn., Prodr. Fl. Ind. Orient. 1: 407. 1834 ≡ *Hedyotis stylosa* R. Br. ex G. Don, Gen. Hist. 3: 527. 1834, nom. illeg. ≡ *Oldenlandia stylosa* (R. Br. ex G. Don) Kuntze, Revis. Gen. Pl. 1: 293. 1891.
- Hedyotis swertioides* Hook. f., Fl. Brit. India 3: 51. 1880.
- Hedyotis tenuipes* Hemsl. ex F.B. Forbes & Hemsl. in J. Linn. Soc., Bot. 23: 375. 1888 ≡ *Oldenlandia tenuipes* (Hemsl. ex F.B. Forbes & Hemsl.) Kuntze, Revis. Gen. Pl. 1: 292. 1891.
- Hedyotis ternata* (Pierre ex Pit.) P.H. Hô, Cayco Vietnam 3(1): 133. 1993 ≡ *Oldenlandia ternata* Pierre ex Pit. in Lecomte, Fl. Indo-Chine 3: 122. 1922 ≡ *Hedyotis ternata* (Pierre ex Pit.) J.F. Maxwell in Nat. Hist. Bull. Siam. Soc. 50(1): 4. 2002, nom. illeg.
- Hedyotis tetragularis* (Korth.) Walp. in Ann. Bot. Syst. 2(5): 769. 1852 ≡ *Diplophragma tetragulare* Korth. in Ned. Kruidk. Arch. 2(2): 149. 1851 ≡ *Oldenlandia tetragularis* (Korth.) Merr. in Pap. Michigan Acad. Sci. 23: 193. 1938.

Hedyotis thwaitesii Hook. f., Fl. Brit. India 3: 54. 1880 ≡ *Hedyotis macrophylla* Thwaites, Enum. Pl. Zeyl.: 142. 1859, nom. illeg. [non *Hedyotis macrophylla* Wall. ex Wight & Arn., Prodr. Fl. Ind. Orient.: 408. 1834] ≡ *Oldenlandia thwaitesii* (Hook. f.) Kuntze, Revis. Gen. Pl. 1: 293. 1891.

Hedyotis tridentata Ridsdale in Blumea 41: 456. 1996.

Hedyotis trimenii Deb & Ratna Dutta in Taxon 34: 297. 1985.

Hedyotis uncinella Hook. & Arn., Bot. Beechey Voy.: 192. 1833 ≡ *Oldenlandia uncinella* (Hook. & Arn.) Kuntze, Revis. Gen. Pl. 1: 293. 1891.

Hedyotis vachellii Hook. & Arn., Bot. Beechey Voy.: 194. 1837 [non *Hedyotis vachellii* Benth., Fl. Hongk. 148. 1861, nom. illeg.] ≡ *Oldenlandia vachellii* (Hook. & Arn.) Kuntze, Revis. Gen. Pl. 1: 293. 1891.

Hedyotis valettoniana Merr. & L.M. Perry in J. Arnold Arbor. 26: 2. 1945.

Hedyotis verticillaris Wall. ex Wight & Arn., Prodr. Fl. Ind. Orient. 1: 409. 1834 ≡ *Pleiocraterium verticillare* (Wall. ex Wight & Arn.) Bremek. in Meded. Bot. Mus. Herb. Rijks Univ. Utrecht 56: 440. 1939.

Hedyotis xinyiensis X. Guo & R.J. Wang in Ann. Bot. Fenn. 48: 443. 2011.

Hedyotis yangchunensis W.C. Ko & Zhang in J. S. China Agric. Univ. 16(4): 45. 1995.

Nomenclatural changes outside *Hedyotis* s.str.

Neanotis nana (Merr. & L.M. Perry) N. Wikström & Neupane, **comb. nov.** ≡ *Hedyotis nana* Merr. & L.M. Perry in J. Arnold Arbor. 26: 6. 1945 – **Lectotype (designated here)**: British New Guinea: Murrey Pass, Wharton Range, alt. 2840 m, Aug 1933, *Brass* 4691 (A no. A00097100 [image!]; isolectotype: NY no. NY00131808 [image!]).

Neanotis pahompokae (Fukuoka) N. Wikström & Neupane, **comb. nov.** ≡ *Hedyotis pahompokae* Fukuoka in Acta Phytotax. Geobot. 24: 29. 1969 – Holotype: Thailand, Chiang Rai: Doi Pa Hom Pok, NW of Phan, alt. 1800 m, 12/9–1967, *Iwatsuki & al.* 9562 (KYO [n.v.]; isotypes: A [image!], K [image!]).

Neanotis trichoclada (Merr. & L.M. Perry) N. Wikström & Neupane, **comb. nov.** ≡ *Hedyotis trichoclada* Merr. & L.M. Perry in J. Arnold Arbor. 26: 5. 1945 – **Lectotype (designated here)**: Netherlands New Guinea: Lake Habbema, alt. 3225 m, Aug 1938, *Brass* 9197 (A no. A00097103 [image!]; isolectotype: BM no. BM000945138 [image!]).

ACKNOWLEDGMENTS

The authors thank two anonymous reviewers for their constructive criticism on an earlier version of the paper, and Anbar Khodabandeh for assistance with sequencing. This work was supported by the Ministry of Education of China through its 111 Project (B08044, CUN 985-3-3), a Conservation International grant for the Botanical Survey of Louisiade Archipelago, Milne Bay Province, Papua New Guinea, the J. Robert Stiffler Endowment at ODU, The Swedish Research Council, and the Knut and Alice Wallenberg Foundation.

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