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The phylogenetic utility of chloroplast and nuclear DNA markers and the phylogeny of the Rubiaceae tribe Spermacoceae

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ABSTRACT

The phylogenetic utility of chloroplast (*atpB-rbcL*, *petD*, *rps16*, *trnL-F*) and nuclear (ETS, ITS) DNA regions was investigated for the tribe Spermacoceae of the coffee family (Rubiaceae). ITS was, despite often raised cautions of its utility at higher taxonomic levels, shown to provide the highest number of parsimony informative characters, in partitioned Bayesian analyses it yielded the fewest trees in the 95% credible set, it resolved the highest proportion of well resolved clades, and was the most accurate region as measured by the partition metric and the proportion of correctly resolved clades (well supported clades retrieved from a combined analysis regarded as "true"). For *Hedyotis*, the nuclear 5S-NTS was shown to be potentially as useful as ITS, despite its shorter sequence length. The chloroplast region being the most phylogenetically informative was the *petD* group II intron.

We also present a phylogeny of Spermacoceae based on a Bayesian analysis of the four chloroplast regions, ITS, and ETS combined. Spermacoceae are shown to be monophyletic. Clades supported by high posterior probabilities are discussed, especially in respect to the current generic classification. Notably, *Oldenlandia* is polyphyletic, the two subgenera of *Kohautia* are not sister taxa, and *Hedyotis* should be treated in a narrow sense to include only Asian species.

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1. Introduction

When inferring phylogenies from molecular data it is important to use DNA regions with an evolutionary rate suitable for the taxa under study (Soltis and Soltis, 1998). A slowly evolving region may provide too little information to recover a fully resolved phylogeny. On the other hand, regions evolving too quickly will have their phylogenetic signal masked by homoplasy due to multiple hits, i.e., aligned nucleotides are the same by chance and not by common ancestry.

In the present paper we will compare the phylogenetic utility of chloroplast and nuclear DNA sequences for resolving the phylogeny of the species-rich tribe Spermacoceae of the coffee family (Rubiaceae). The DNA regions (e.g., *rbcL*, *atpB*, 18S rDNA) utilized in the numerous studies synthesized in the high-level classifications of the Angiosperm Phylogeny Group (APG, 1998; APGII, 2003) would be of limited value in resolving the phylogeny of Spermacoceae, due to their conservative nature. For phylogenetic studies at lower taxonomic levels noncoding chloroplast regions have been used frequently and successfully. The rationale behind using noncoding regions is the assumption that they are phylogenetically more informative because they are under less functional constraints (Gielly and Taberlet, 1994). Shaw et al. (2005) investigated the relative utility of 21 noncoding chloroplast regions and divided them into three tiers. They found that five of the regions (*rpoB-trnC*, *trnD-trnT*, *trnS-trnfM*, *trnS-trnG*, *trnT-L*; tier 1) generally have enough phylogenetic signal for studies at the lower taxonomic levels. Five additional regions (*psbM-trnD*, *rpl16*, *rps16*, *trnG*, *ycf6-psbM;* tier 2) were identified as potentially useful, but less likely to provide full resolution. The remaining regions (3'*trnK-matK*, 5'*rps12-rpL20*, *matK-5'trnK*, *psbA-3'trnK*, *psbB-psbH*, *rpS4-trnT*, *trnC-ycf6*, *trnH-psbA*, *trnL*, *trnL-trnF*, *trnS-rpS4*; tier 3) were all considered to provide too little phylogenetic information to be recommended. More recently, Shaw et al. (2007) identified nine newly explored noncoding chloroplast regions (*rpl32-trnL*$^{(UAG)}$, *trnQ*$^{(UUG)}$*-5'rps16*,

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3'trnV$^{(UAC)}$*-ndhC*, *ndhF-rpl32*, *psbD-trnT*$^{(GGU)}$, *psbJ-petA*, *3'rps16–5'trnK*$^{(UUU)}$, *atpI-atpH*, and *petL-psbE*), which provided even higher levels of variation than the ones identified in their previous study (Shaw et al., 2005).

Mort et al. (2007) further investigated the utility of the regions in tier 1 and 2 and compared them to the internal transcribed spacer (ITS) region (ITS1, 5.8S gene, and ITS2; White et al., 1990; Baldwin, 1992) of the nuclear ribosomal DNA region. As implied from its use in numerous studies, ITS is a useful marker for resolving phylogenetic relationships at various taxonomic levels, in particular infrageneric. When analysing ITS caution has to be taken to avoid problems resulting from concerted evolution on the ribosomal DNA arrays. Concerted evolution may homogenize different paralogous gene copies in a genome leading to the loss of all but one of the copies, i.e., different copies may be present in different organisms by chance and consequently the gene trees and species trees will not agree (Alvarez and Wendel, 2003). Compared to the noncoding chloroplast regions tested by Shaw et al. (2005), ITS was not consistently more informative (Mort et al., 2007). Mort et al. (2007) raised the concern of how well organismal phylogeny is inferred by ITS data. They concluded that, despite incongruences with chloroplast data (as measured by the ILD test; Farris et al., 1995), including ITS data may still result in an increase in both resolution and support suggesting that a phylogenetic signal masked by homoplasy in chloroplast data could be strengthened by a combined analysis (Nixon and Carpenter, 1996; Wenzel and Siddall, 1999; Mort et al., 2007).

The studies by Shaw et al. (2005, 2007) and Mort et al. (2007) aimed at identifying molecular markers useful at lower taxonomic levels across a wide taxonomic range. We want to explore how useful different markers are for resolving the phylogeny of a large clade where the taxonomic sample is intended to infer both infrageneric and intrageneric relationships (this terminology is admittedly unfortunate since the current generic classification of Spermacoceae is known to include both para- and polyphyletic genera; Groeninckx et al., in press).

We will identify the regions with the highest number of (parsimony) informative characters or the regions providing most resolution or the highest proportion of well resolved clades. We will also compare how effective different DNA regions are in obtaining the "true phylogeny." To make these comparisons a region should provide potentially phylogenetic informative characters or produce well supported, resolved phylogenies. It must also provide accurate topologies, i.e., provide congruent results with other regions and provide data not susceptible to analytical assumptions, for example long branch attraction. Measures of accuracy often include comparing the number of bipartitions not shared between topologies. The partition metric, often called the Robinson–Foulds distance, is twice the number of internal branches that differ in the number of bipartitions between two topologies (Robinson and Foulds, 1981; Penny and Hendy, 1985; Steel and Penny, 1993). Rzhetsky and Nei (1992) adjusted the partition metric to account for multichotomies. Other methods used to measure how much deviation exists between two trees are the number of nearest-neighbor exchanges needed to transform one tree to the other (Waterman and Smith, 1978), the number of correctly resolved taxon bipartitions divided by the number of possible bipartitions (Hillis, 1995), and comparisons of geometric distances in tree space between differing topologies (Billera et al., 2001).

We have chosen Spermacoceae, a tribe of the coffee family (Rubiaceae), to investigate the utility of four chloroplast regions (*atpB-rbcL*, *rps16*, *trnL-F*, *petD*) and three nuclear regions (ITS, the external transcribed spacer, ETS, and the 5S non-transcribed spacer, 5S-NTS). Rubiaceae are currently classified in two or three subfamilies and more than 40 tribes (Bremer et al., 1995; Robbrecht and Manen, 2006; Bremer, in press). Spermacoceae with over 1000 species have a mainly pantropical distribution, but a few genera extend into temperate regions. The majority of the species are herbaceous. Four-merous flowers together with fimbriate stipules characterize the tribe, but these characters are not omnipresent. The delimitations of Spermacoceae have varied from a long recognized morphologically well-defined tribe (Spermacoceae sensu stricto, s.s.; Hooker, 1873; Bremekamp, 1952, 1966; Verdcourt, 1958; Robbrecht, 1988) to a wider interpretation stemming from molecular analyses and including the traditionally recognized tribes Hedyotideae, Knoxieae, Manettieae, and Triainolepideae (Bremer, 1996; Bremer and Manen, 2000). Here Spermacoceae are treated to comprise 60 genera and include Manettieae and most genera of Hedyotideae, but not their probable sister tribe Knoxieae, which recently is expanded to include Triainolepideae and a few genera from Hedyotideae (Andersson and Rova, 1999; Dessein, 2003; Kårehed and Bremer, 2007).

The only previous phylogeny with a global perspective of Spermacoceae (Groeninckx et al., in press) revealed a number of well supported clades. The relationships *between* these clades are less resolved. In addition, the relationships *within* some of the clades are not strongly supported. The Spermacoceae sequence data, thus, provide an interesting mixture of early branching and late branching clades, clades with apparent different rates of evolution, as well as a combination of well supported clades and unresolved taxa. The study of Groeninckx et al. (in press) used only chloroplast data (*atpB-rbcL*, *rps16*, *trnL-F*). Since their study was intended for tribal relationships, the combination of relatively slower and faster markers (cf. Shaw et al., 2005) was rational. We chose to investigate the chloroplast region, *petD* (the *petD* intron and the *petB-petD* spacer; Löhne and Borsch, 2005), which according to Löhne and Borsch (2005) has less length variation than *trnT-F*, but has about the same number of informative characters. The *petD* region is comparatively untested in Rubiaceae.

Although used less extensively than ITS, ETS seems to provide phylogenetic information at about the same magnitude as ITS (Baldwin and Markos, 1998; Musters et al., 1990). Within Rubiaceae, ETS and ITS have been utilized together at the generic level by Negrón-Ortiz and Watson (2002) for *Erithalis*, Markey and de Lange (2003) for *Coprosma*, Nepokroeff et al. (2003) for *Psychotria*, and Razafimandimbison et al. (2005) for *Neonauclea*.

5S-NTS (Cox et al., 1992; Sastri et al., 1992) has been even less used than ETS. For Rubiaceae, 5S-NTS was reported to be about twice as informative as ITS for the *Alibertia* group (Persson, 2000). It has also been used for *Randia* (Gustafsson and Persson, 2002) and most recently for inferring the phylogeny of the *Kadua* clade (Motley, in press), one of the well supported clades of Spermacoceae (Groeninckx et al., in press).

The nuclear data were added as faster evolving DNA regions, which could provide better resolution of relationships within younger taxa or taxa with slow evolutionary rates, and strengthen phylogenetic signals masked by homoplasy in the chloroplast data. We also investigated the extent the nuclear data can resolve early branching clades of Spermacoceae, where previous chloroplast studies have failed (Groeninckx et al., in press). This will determine if the nuclear data only contribute information on closely related taxa, being too homoplastic at higher levels, or if the nuclear data also are useful for resolving relationships at somewhat higher taxonomic levels. Topological comparisons of chloroplast-trees to nuclear trees will provide evidence of suitable DNA regions to use for large, species-rich, tribal and/or family lineages.

In our evaluations of the relative utility of chloroplast (*atpB-rbcL*, *rps16*, *trnL-F*, *petD*) and nuclear (ITS, ETS, 5S-NTS) DNA regions we want to investigate if the eight well supported clades discussed by Groeninckx et al. (in press; Section 4.2) remain monophyletic with the additional data, examine if other clades retrieved in both studies have increased support with the addition of data, and

determine if additional data will resolve additional monophyletic clades. In addition, we will present a total-evidence phylogeny of Spermacoceae and discuss to what extent the phylogeny agrees with the current generic classification.

2. Materials and methods

2.1. Taxon sampling and molecular data

We used the chloroplast data set of Groeninckx et al. (in press) as the basis for our sampling. Their data set included 128 species. Our chloroplast data set was enlarged by the addition of three *atpB-rbcL* sequences (*Gomphocalyx hernarioides*, *Hydrophylax maritima*, and *Pentanopsis gracilicaulis;* the latter two species were not previously sampled), two *rps16* sequences (*Oldenlandia biflora*, *Pentanopsis gracilicaulis*), four *trnL-F* sequences (*Gomphocalyx hernarioides*, *Oldenlandia rosulata*, *Pentanopsis gracilicaulis*, *Phylohydrax carnosa*), and *petD* sequences for the newly sampled *Manettia luteorubra* and 101 of the species included by Groeninckx et al. (in press; Appendix).

Before sequencing nuclear data for the entire taxon sample, a test sample was selected. The test sample included 12 species (*Arcytophyllum thymifolium*, *Dibrachionostylus kaessneri*, *Hedyotis quinquenervis*, *Kadua littoralis*, *Kohautia amatymbica*, *Kohautia virgata*, *Mitrasacmopsis quadrivalvis*, *Oldenlandia angolensis*, *Oldenlandia echinulosa*, *Pentodon pentandrus*, *Spermacoce hispida*, and *Thecorchus wauensis*) representing the major clades recovered by Groeninckx et al. (in press). The sample was used to investigate if different nuclear regions could be amplified, and if the resulting sequences could be aligned to each other. The nuclear regions investigated were ITS, ETS, and 5S-NTS. Both the ITS and ETS sequences were straightforward to amplify and possible to align. Therefore, the two regions were chosen to be sequenced for the larger sample of Spermacoceae.

In total we sampled 139 taxa for ITS and 98 for ETS (Appendix). New taxa added to the nuclear data but not present in the taxon sample of Groeninckx et al. (in press) include: two genera (*Diodella* and *Psyllocarpus*), 13 species (*Bouvardia* sp. Torres & Torino 3637 (BR), *Galianthe* sp. Persson & Gustavsson 298 (GB), *Hedyotis capitellata*, *H. effusa*, *H. megalantha*, *Kohautia longifolia*, *K.* cf. *longifolia*, *Oldenlandia* cf. *galioides, O. monanthos*, *O.* sp. C of Flora Zambesiaca, *Richardia brasiliensis*, *Spermacoce filifolia*, *S. verticillata*), and one subspecies *Oldenlandia mitrasacmoides* subsp. *nigricans*. Extra individuals for a few taxa already included are also added, either because those sequences were already available (the outgroup taxa) or, for example, to confirm the placement of a taxon (*Amphiasma luzuloides*, *Conostomium natalense*, *Dibrachionostylus kaessneri*, *Kadua cordata*, *Oldenlandia corymbosa*, *O. goreensis*, *O. herbacea* var. *goetzei*, *O. herbacea* var. *herbacea*, *O. lancifolia*, and *Pentodon pentandrus*).

To be able to compare the phylogenetic utility of the different DNA regions without taking the effect of unequal taxon sampling into account, we also prepared a reduced taxon sample with those 49 taxa that are present in all separate data sets (referred herein as the reduced Spermacoceae data set).

The 5S-NTS region was easy to amplify, all test taxa worked. The PCR (polymerase chain reaction) products resulting in the strongest bands (as measured by the amount of staining with Ethidium bromide when separated on an agarose gel) were, however, of quite different lengths. All the test taxa produced a single predominant band, although fainter bands occurred. These might be a result of unspecific annealing or due to multiple copies. The sequenced PCR products differed from 244 base pairs (bp; *Spermacoce hispida*) to 899 bp (*Kohautia obtusiloba*). Homology between the different bands was, thus, impossible to ascertain. Even the sequences of the bands of equal lengths, for which homology could be postulated, were not possible to align.

Since 5S-NTS seemed to be easy to amplify for taxa of the Spermacoceae, but was too variable to be used for the entire tribe, we wanted instead to test its usefulness for more restricted, well defined clades. For that purpose we chose *Hedyotis* s.s. (Groeninckx et al, in press). 5S-NTS was sequenced for nine *Hedyotis* species present in the ITS and ETS data sets. This matrix (the *Hedyotis* data set) was used to determine how informative and accurate the 5S-NTS region is compared to the other two nuclear data sets (see below). For the *Hedyotis* data set we made no attempts to cut out the predominant band or clone the PCR product, because the obtained sequences showed no indications of multiple copies (e.g., multiple peaks in the chromatograms).

The low copy-number nuclear marker *Tpi* (Strand et al., 1997) was also investigated. We were not able to obtain PCR products for any of the 12 test taxa. Nevertheless, the use of this and other low copy-number nuclear markers with specifically designed primers may prove to be valuable in the future for exploring clades that remain unresolved with other DNA regions.

2.2. Sequencing

DNA was extracted from fresh, silica-gel dried material or herbarium specimens using the CTAB method (Doyle and Doyle, 1987). The *petD* region was amplified with the forward primer PIpetB1365F and the reverse primer PIpetD738R (Löhne and Borsch, 2005). Polymerase chain reactions and the sequencing of the new chloroplast data were performed as described by Groeninckx et al. (in press).

Polymerase chain reactions for the nuclear data sets were run on an Eppendorf® Mastercycler® gradient (Bergman & Beving Instrument, Stockholm, Sweden). The 50-μl reactions included 5 μl reaction buffer, 5 μl $MgCl_2$, 5 μl TMACL (Chevet et al., 1995), 4 μl DNTP, 0.25 μl *Taq (5 U/μl),* 0.5 μl 5′ primer (20 μM), 0.5 μl 3′ primer (20 μM), 0.5 μl BSA 1%, and 1–2 μl of DNA templates and sterilized H_2O adding up to 50 μl. The amplifications consisted of an initial denaturation for 1 min at 95 °C, followed by 37 cycles of 1 min at 95 °C, 1 min 30 s at 50 °C, 1 min 30 s at 72 °C (usually +1 s/cycle), and a final extension phase of 7 min at 72 °C. The PCR products were purified with the MultiScreen® Separations System (Millipore, USA). The purified products were subsequently sequenced with the BigDye™ terminator cycle sequencing kit (Applied Biosystems, Stockholm, Sweden) on a GeneAmp PCR System 9700 (Applied Biosystems) and analyzed on an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). Primers used for both PCR and sequence reactions were for ITS the forward primer P17 and the reverse primer 26S-82R (Popp and Oxelman, 2001) or P25 (Oxelman, 1996). For ETS the primer pair ETS-Erit-F (Negrón-Ortiz and Watson, 2002) and 18S-E (Baldwin and Markos, 1998) was used. The 5S-NTS region was amplified using the primers PI and PII (Cox et al., 1992) and attempts to amplify Tpi were performed with primers TPIX4FN and TPIX6RN (Strand et al., 1997).

2.3. Phylogenetic analyses

All alignments were made by eye. Simple indel coding (Simmons and Ochoterena, 2000) of insertion/deletion (indel) events was performed with the computer program SeqState (Müller, 2005). The indels were subsequently included in the analyses.

Bayesian analyses to estimate the phylogeny of the entire Spermacoceae data set and the reduced Spermacoceae data set were done for all regions independently, the combined four chloroplast regions, the combined nuclear ITS and ETS regions, and a total evidence analysis. For the *Hedyotis* taxon sample the ITS, ETS, and 5S-NTS data were analyzed both separately and combined.

The choice of nucleotide-substitution models for the Bayesian analyses of the molecular data was determined based on the corrected Akaike criterion (Burnham and Anderson, 2002) calculated using the computer program MrAIC (Nylander, 2004) in conjunction with the program PhyML (Guindon and Gascuel, 2003). The general time reversible model with a gamma distribution of substitution rates (GTR + G) was chosen for all the DNA regions of the Spermacoceae data set, except ETS. For ETS the model (GTR + I + G) includes a proportion of invariant sites. In the reduced Spermacoceae data set the GTR + G model was used for all regions. The *Hedyotis* data set was analysed under the GTR + G model for ETS and 5S-NTS, and the HKY model (with different substitution rates for transitions and transversions) was used for ITS. The insertion/deletion data for all data sets were analyzed under the standard discrete (morphology) model. In the combined analysis the data set was partitioned and the partitions were unlinked and, consequently, had their own set of parameters. The Bayesian analyses were performed using the computer program MRBAYES (v3.1.2; Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). For the Spermacoceae data set the Markov chain was run for 5,000,000 generations with three additional "heated" chains (Metropolis-coupled Markov chain Monte Carlo; Huelsenbeck et al., 2001). Trees were sampled every 100th generation. Two separate runs were run for all data sets. When the average standard deviation of split frequencies between the separate runs was <0.05, it was taken as an indication that the Markov Chains had converged on the stationary distribution. The first 500,000 generations were, consequently, discarded as a burn-in period for all analyses except for the combined nuclear data and for the combined chloroplast and nuclear data, for which a burn-in period of 1,000,000 generations was used. The reduced Spermacoceae and the *Hedyotis* data sets were run for 1,000,000 generations with a burn-in period of 100,000 generations and every 100th tree was sampled.

2.4. Comparison of phylogenetic utility of the seven DNA regions

In order to investigate the phylogenetic utility of the seven DNA regions we compared the information content, i.e., how much of the information that could be contributed to sequence length, the number of trees in the 95% credible set of trees, the proportion of well resolved nodes, and the phylogenetic accuracy of the respective regions. The number of parsimony informative characters excluding and including indels are shown in Table 1 and Fig. 1(a–c). Linear regressions of the number of parsimony informative characters against the aligned length of the data matrices are shown in Fig. 1(d–f). We also present the 95% credible set of trees (the smallest set of trees whose cumulative posterior probability sum to 0.95; Felsenstein, 1968; Table 1, Fig. 1g–i). The 95% credible set gives an indication of the precision of the maximum posterior probability estimate of the phylogeny (the most probable tree). A DNA region which produces a 95% credible set including few trees would indicate that the region better discriminates between conflicting topologies than another region which produces a larger 95% credible set. The size of the 95% credible set could, thus, be seen as an approximation of the strength of the phylogenetic signal in a data set (with more phylogenetically informative data the likelihood function can update the prior distribution more strongly).

In Table 2 and Fig. 2(a–c) the proportion of clades resolved with PP $\geq$0.95 divided by the number of possible bipartitions (i.e., number of bipartitions in a fully dichotomous tree, which equals the number of terminal taxa minus three) are shown. We choose to compare only clades with PP $\geq$0.95, since these clades could be considered "true" given the data and the models used in the Bayesian analyses (Huelsenbeck et al., 2002).

As a measure of phylogenetic accuracy we used the partition metric (PM; Penny and Hendy, 1985) modified to account for multichotomies (Rzhetsky and Nei, 1992). The partition metric ranges from zero (identical phylogenies) to twice the number of possible clades resolved (number of taxa minus three). We only compared phylogenetic accuracy for the reduced Spermacoceae data set and the *Hedyotis* data set. Table 2 and Fig. 2(d and e) show the partition metric for the phylogenetic tree from the Bayesian analyses of each of the DNA regions compared to the phylogenetic tree from the Bayesian analysis of the combined data set, the latter taken as a reasonable estimation of the "true" phylogeny. When calculating the partition metric only nodes with PP $\geq$0.95 were considered. The calculations were made using the function dist.topo in the R package APE (Paradis et al., 2004; R Development Core Team, 2007). As another approximation of accuracy, we calculated the proportion of the number of clades with PP $\geq$0.95 in the phylogenetic tree from the separate analysis of a given marker, which also has a PP $\geq$0.95 in the tree from the combined analysis, divided by the total number of clades with PP $\geq$0.95 in the combined tree (i.e., "correctly" resolved clades assuming the combined tree to be the "true" tree; Table 2; Fig. 2f and g).

Table 1
Number of taxa, aligned length of the data matrices, number of parsimony informative characters, and the number of trees in the 95% credible set for the different data sets and DNA regions. N, number of taxa; Al. l., aligned length; Pars. inf. – indels, number of parsimony informative characters excluding indel characters; Pars. inf. indels, number of parsimony informative indel characters,% Pars. inf., percentage of the total number of aligned characters that are parsimony informative.

Data set	*N*	Al. l. (bp)	Pars. inf. - indels	Pars. inf. indels	% Pars. inf.	No. of trees in 95% cred. set
Spermacoceae						
atpB-rbcL	103	1,366	142	46	13.8%	85,420
rps16	109	715	153	26	25.0%	85,373
trnL-F	115	1,003	138	62	19.9%	85,486
petD	102	1,799	276	90	20.3%	79,697
ITS	139	2,173	452	308	35.0%	60,098
ETS	98	1,055	329	100	40.7%	66,519
Reduced Spermacoceae						
atpB-rbcL	49	1,366	71	19	6.5%	17,001
rps16	49	715	102	10	15.7%	17,020
trnL-F	49	1,003	88	17	10.5%	17,066
petD	49	1,799	163	42	11.4%	14,812
ITS	49	2,173	294	181	21.9%	7,670
ETS	49	1,055	253	76	31.2%	12,410
Hedyotis						
ITS	9	424	33	1	8.0%	3
ETS	9	799	27	9	4.5%	19
5S-NTS	9	334	55	14	20.7%	9

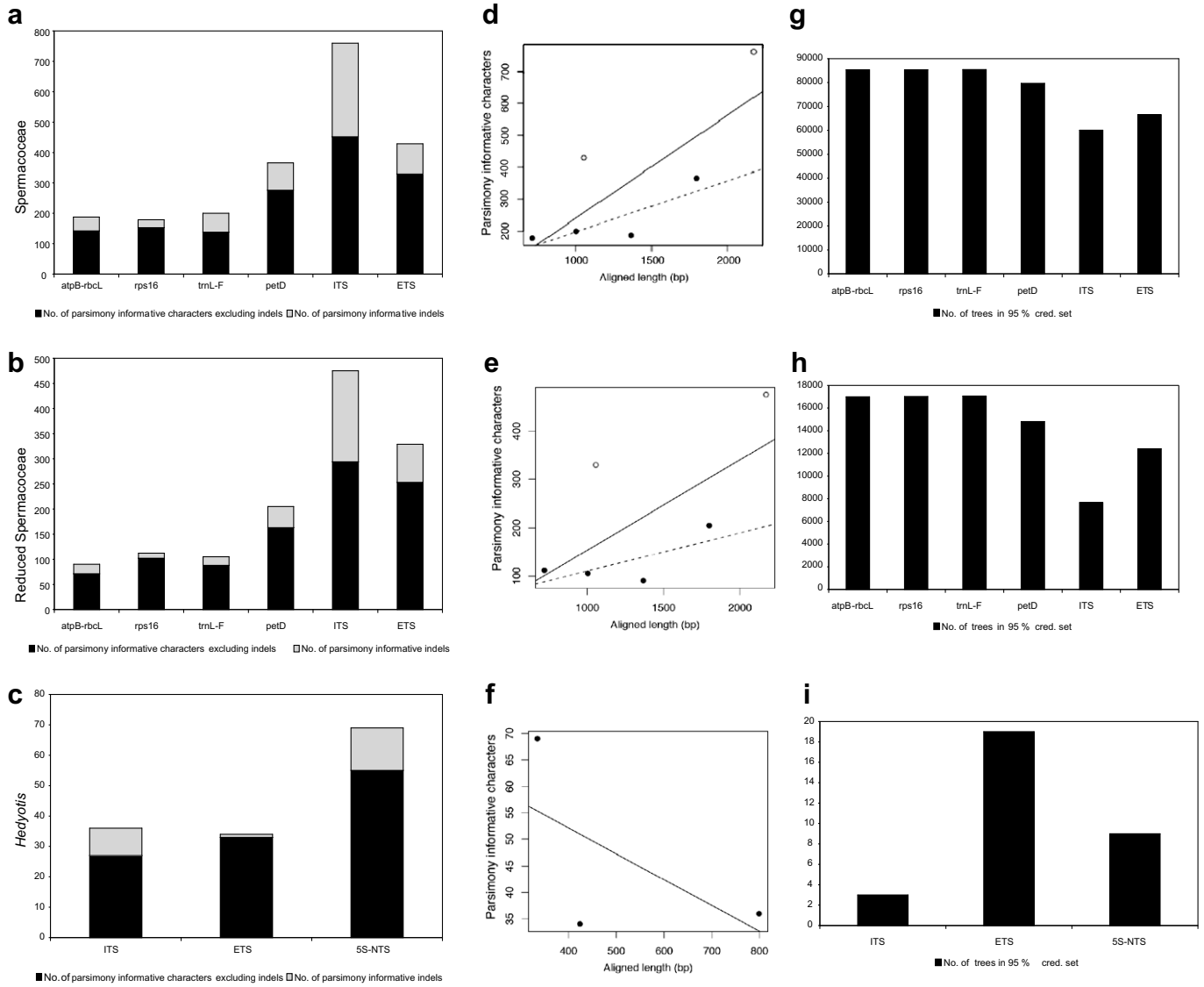

Fig. 1. (a–c) Number of parsimony informative characters in: (a) each of the six regions included in the analysis of the Spermacoceae data set, (b) the three nuclear regions included in the analysis of the reduced Spermacoceae data set, (c) the three nuclear regions included in the analysis of the *Hedyotis* data set. (d–f) Linear regression of number of parsimony informative characters (including indels) against aligned length (bp) of: (d) the separate DNA regions of the Spermacoceae data set (black circles = chloroplast regions, white circles = nuclear regions, dashed line = regression line of the chloroplast data, $r^2 = 0.70$, black line = regression line of all data combined, $r^2 = 0.61$), (e) the separate DNA regions of the reduced Spermacoceae data set (black circles = chloroplast regions, white circles = nuclear regions, dashed line = regression line of the chloroplast data, $r^2 = 0.43$, black line = regression line of all data combined, $r^2 = 0.51$), (f) the *Hedyotis* data set ($r^2 = 0.37$). (g–i) Number of trees in the 95% credible set for: (g) the Spermacoceae data set, (h) the reduced Spermacoceae data set, (i) the *Hedyotis* data set.

3. Results

3.1. Phylogenetic utility

In the analysis of the Spermacoceae data the nuclear regions provide most information (Table 1, Fig. 1a). ITS is the most informative region and provides 1.8 times as many parsimony informative characters as ETS and is more than twice as informative as *petD*, the most informative chloroplast region. The *petD* region is in turn about twice as informative as the other chloroplast regions.

Linear regressions of the number of parsimony informative characters (including indels) against the lengths of the separate matrices for both the chloroplast data separately ($r^2 = 0.70$, $p = 0.161$) and in combination with the nuclear data ($r^2 = 0.61$, $p = 0.067$) are shown in Fig. 1d. When the nuclear data are added, less of the information content can be explained by just sequence length, i.e., the nuclear data are indicated to be more informative than the chloroplast data for a given sequence length.

A similar pattern is retrieved when comparing the data from the reduced Spermacoceae data set (Table 1, Fig. 1b). ITS provides 1.4 times as many parsimony informative characters as ETS and 2.3 times as many as *petD*, the latter region being about twice as informative as the other chloroplast data. The linear regressions of parsimony informative characters relative to aligned length of the chloroplast and combined data for the reduced Spermacoceae data set are shown in Fig. 1e. The regressions indicate for this data set, i.e., with reduced taxon sampling and no missing data, that the length of the sequences explains less of the information content ($r^2 = 0.50$, $p = 0.289$ for the chloroplast and $r^2 = 0.43$, $p = 0.155$ for the combined data), than for the entire Spermacoceae data set.

For the *Hedyotis* data ETS and ITS provide about the same number of informative characters, but the proportion of informative in-

Table 2
Proportion of clades with posterior probability ≥ 0.95 (PP ≥ 0.95) divided by the number of possible bipartitions for the different data sets and DNA regions, the partition metric (PM), and proportion of correctly resolved clades for the reduced Spermacoceae and the *Hedyotis* data sets.

Data set	Clades with PP ≥ 0.95/ possible bipartitions	PM (PP ≥ 0.95)	Correctly resolved clades
Spermacoceae			
atpB-rbcL	36.0%	n.a.	n.a.
rps16	45.3%	n.a.	n.a.
trnL-F	39.3%	n.a.	n.a.
petD	61.6%	n.a.	n.a.
ITS	65.7%	n.a.	n.a.
ETS	63.2%	n.a.	n.a.
Reduced Spermacoceae			
atpB-rbcL	37.0%	21	44.7%
rps16	47.8%	20	55.3%
trnL-F	41.3%	19	52.6%
petD	63.0%	15	71.1%
ITS	69.6%	12	81.6%
ETS	58.7%	14	71.1%
Hedyotis			
ITS	83.3%	2	40.0%
ETS	50.0%	4	80.0%
5S-NTS	66.7%	1	80.0%

dels is larger for ITS. The 5S-NTS region provides the most parsimony informative characters (Table 1, Fig. 1c). It is about twice as informative as the other two nuclear regions. A linear regression of parsimony informative characters against the length of the matrices is shown in Fig. 1f ($r^2 = 0.37$, $p = 0.582$). Since the shortest marker is the most informative, there is a negative correlation between parsimony informative characters and sequence length for this data set.

For all data sets it seems that there is a variation in the information content among the DNA regions, more variability than can be explained by sequence length alone. In particular, the nuclear data are more informative than the chloroplast regions and the 5S-NTS is the most informative nuclear region despite its short length.

Examination of DNA region variability using the number of trees in the 95% credible sets (Table 1, Fig. 1g–i) from the separate analyses of the Spermacoceae and the reduced Spermacoceae data sets give nearly the same picture as the number of parsimony informative characters: ITS had the fewest trees, followed by ETS and *petD*. The other three chloroplast regions had about the same number of trees. For the *Hedyotis* data set the ITS data, not the 5S-NTS, had the fewest number of trees in the 95% credible set.

Additionally, the number of well resolved nodes in the resulting phylogenies can be an indication of the phylogenetic utility of a DNA region. Here we consider a node to be well resolved if the posterior probability of that node is ≥0.95. To account for the different sized taxon samples in the Spermacoceae data set, we compared the number of nodes with a posterior probability ≥0.95 divided by the maximum number of internal nodes in an unrooted, fully dichotomous tree (Table 2; Fig. 2). For the Spermacoceae data, the ITS region resolved the highest proportion of well resolved nodes (66%; 70% for the reduced data set). The *petD* region performed comparatively better in this evaluation and resolved almost as many nodes as ETS (62% and 63%, respectively). In the reduced Spermacoceae data set, *petD* resolved more nodes than ETS (63% vs. 59%). The other three chloroplast regions as predicted retrieved less nodes (36% for *atpB-rbcL* to 45% for *rps16*; 37% to 48% for the reduced data set). For the *Hedyotis* data set ITS resolved the highest number of nodes (83%) in spite of the fact that the 5S-NTS provided the highest number of informative characters.

The most accurate region for the reduced Spermacoceae data set (Table 2; Fig. 2d) was ITS (PM = 12) followed by ETS (PM = 14), and the *petD* region (PM = 15). For the *Hedyotis* data set (Table 2; Fig. 2e), 5S-NTS was more accurate than ITS (PM = 1 vs. PM = 2, respectively), which was in turn more accurate than ETS (PM = 4). When comparing the number of correctly resolved clades, the same picture emerges (Table 2; Fig. 2f and g). ITS correctly resolved the highest proportion of nodes for the reduced Spermacoceae data set and is followed by ETS and *petD*, which performed equally well. For the *Hedyotis* data set both ITS and 5S-NTS resolved the same proportion of the clades supported in the combined analysis.

3.2. Phylogenetic analyses

Spermacoceae are monophyletic in all analyses and all independent and combined analyses retrieved a number of well supported clades (Fig. 3). The separate analyses were naturally less resolved than the combined. The topologies from the separate analyses, even if less resolved, agree with the topology of the combined analysis for all clades discussed here. No well supported relationships in any of the separate analyses are contradicted by the other data. The separate analyses of the Spermacoceae data will, thus, not be discussed in detail. Neither will the phylogenies resulting from the analyses of the reduced Spermacoceae data set, since these were merely performed to enable us to compare the number of parsimony informative characters and to calculate the partition metric on congruent data sets. We regard the combined analysis as the best estimate of the phylogeny (total evidence) of Spermacoceae and, if not otherwise indicated, it is the result on which we will focus our discussions (Fig. 3). We also present both the combined and separate analyses of the *Hedyotis* data set (Fig. 4), because the 5S-NTS was not included in the combined analysis of the Spermacoceae data set.

Adding the *petD* data to the previously published chloroplast data (Groeninckx et al., in press) increased the proportion of well resolved nodes from 52% to 56%. The addition of nuclear data further increased the proportion to 70%. In fact the nuclear data by themselves resolved 67% of the possible nodes. The combined analyses showed a marked decrease in the number of trees in the 95% credible set compared to the separate analysis (33,152 for the Spermacoceae data set compared to 60,098 for ITS). In the reduced Spermacoceae data the combined analyses yielded 553 credible trees as compared to 7670 for ITS. The combined *Hedyotis* data set produced three trees as did the ITS analysis. Apart from the difference in resolution between the DNA regions, we do not see any bias for any of the regions towards better resolving either early or late branching taxa.

4. Discussion

4.1. Phylogenetic utility

Inferring the phylogeny of a group naturally implies finding one or several gene regions which are preferably single copy and are informative. In a phylogenetic study the actual number of potentially informative characters are of interest, i.e., a longer but less variable region is of more interest than a highly variable but short region, which despite a high information content per sequenced base pair may not provide enough characters to resolve the phylogeny. The 21 noncoding chloroplast regions studied by Shaw et al. (2005) varied in the extent (22–83%) that length per se could explain the variation in number of potentially informative characters. Mort et al. (2007) found a much lower correlation between length and information content (5.2% for chloroplast data and 0.9% for chloroplast plus ITS data), but since they included only rapidly evolving regions this result is not unanticipated. The linear regres-

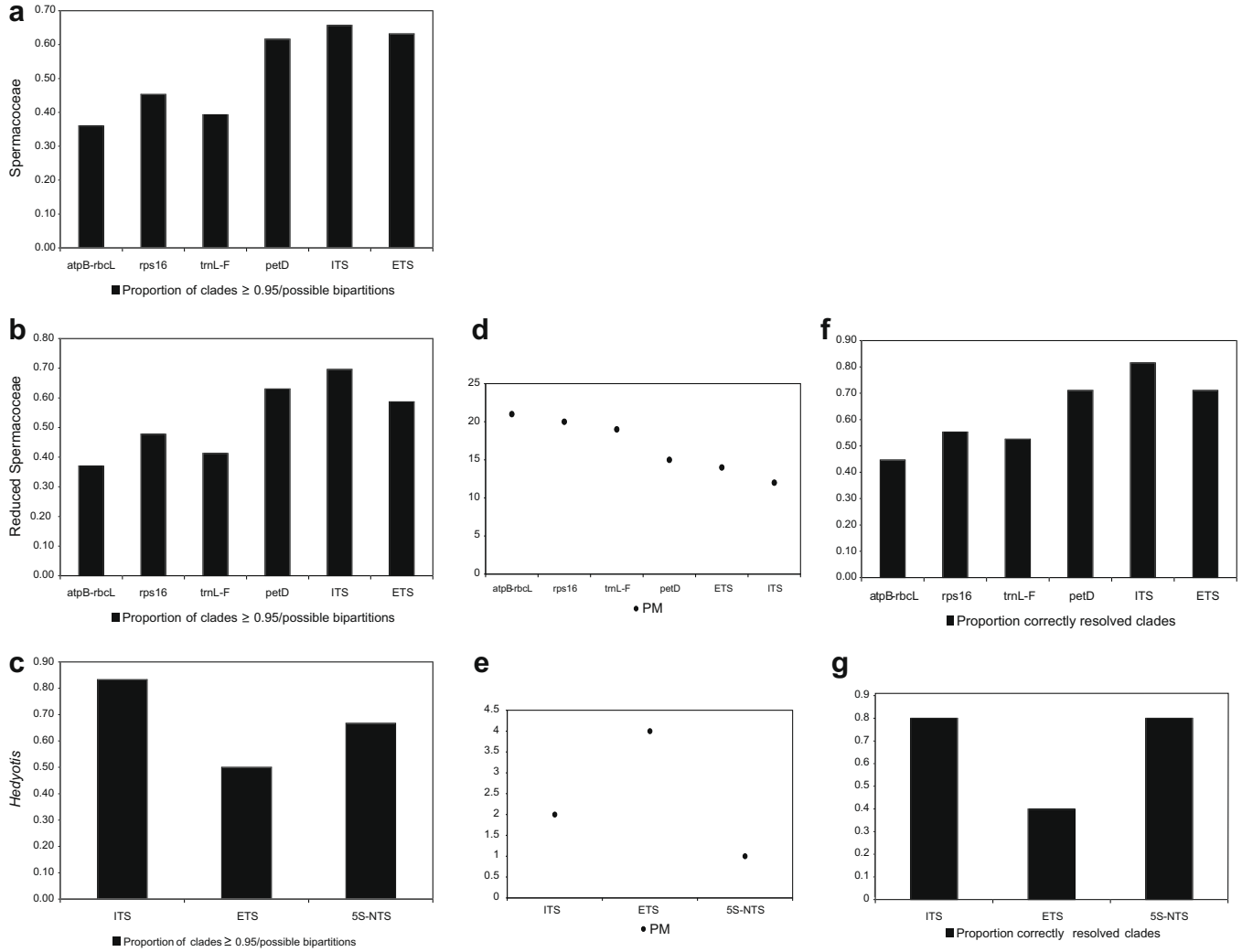

Fig. 2. (a–c) Proportion of the possible number of clades with a PP ≥ 0.95 for: (a) the Spermacoceae data set, (b) the reduced Spermacoceae data set, and (c) the *Hedyotis* data set. (d and e) The partition metric (PH-85) for: (d) the reduced Spermacoceae data set, (e) the reduced *Hedyotis* data set. (f and g) Proportion of correctly resolved clades in: (f) the reduced Spermacoceae data set, (g) the *Hedyotis* data set.

sion of the chloroplast regions of our Spermacoceae data (Fig. 1) showed that more than two-third of the parsimony informative variation could be explained by the length of the sequences ($r^2 = 0.70$, $p = 0.161$). With the inclusion of the nuclear data the correlation decreased (Fig. 1d; $r^2 = 0.61$, $p = 0.067$). The greater number of informative characters provided by the nuclear data are, thus, indicative of the nuclear data being more informative than the chloroplast data and not merely an effect of sequence length. For the nuclear regions examined using the *Hedyotis* data set, where the shortest region provided the highest number of informative characters, the linear regression naturally shows a negative correlation (Fig. 1f; $r^2 = 0.37$, $p = 0.582$). These results indicate that nuclear data generally can be expected to provide more information than chloroplast data (Fig. 1). The highly variable chloroplast regions identified by Shaw et al. (2005, 2007) should be further explored, especially in cases where gene trees are suspected to differ from the species tree (Alvarez and Wendel, 2003). However, for Spermacoceae the chloroplast and nuclear phylogenies are essentially identical (only the position of taxa with low posterior probabilities differ, and this is here interpreted as a lack of phylogenetic signal and not due to incongruent data sets; see Section 4.2. for details of the Spermacoceae phylogeny). When investigating more rapidly evolving chloroplast regions with ITS, Mort et al. (2007) did not find ITS to be either universally the most informative region or the region providing the highest clade support, although it generally was one of the best choices for resolving their low taxonomic level phylogenies.

It is noteworthy that the nuclear data not only resolve nodes between closely related taxa, but also support some of the early branching events in Spermacoceae. Finding relatively fast evolving DNA regions useful also at higher taxonomic levels (less homoplastic than is often assumed) has also been shown previously in the Asterids (Bremer et al., 2002), Rubiaceae (Motley et al., 2005), and basal angiosperms (Müller et al., 2006).

In order to obtain a well supported phylogeny of a group of organisms it is, however, not enough to analyze a high number of potentially phylogenetic informative characters. The included characters should naturally reflect our best estimate of the true phylogeny.

According to our results, the regions providing the most information are in fact also the most accurate. The proportion of well resolved nodes is generally also a good approximation, but with two exceptions. Apparently, the number of parsimony informative characters and the number of trees in the 95% credible set actually give a valuable insight into the accuracy of a DNA region, i.e., the

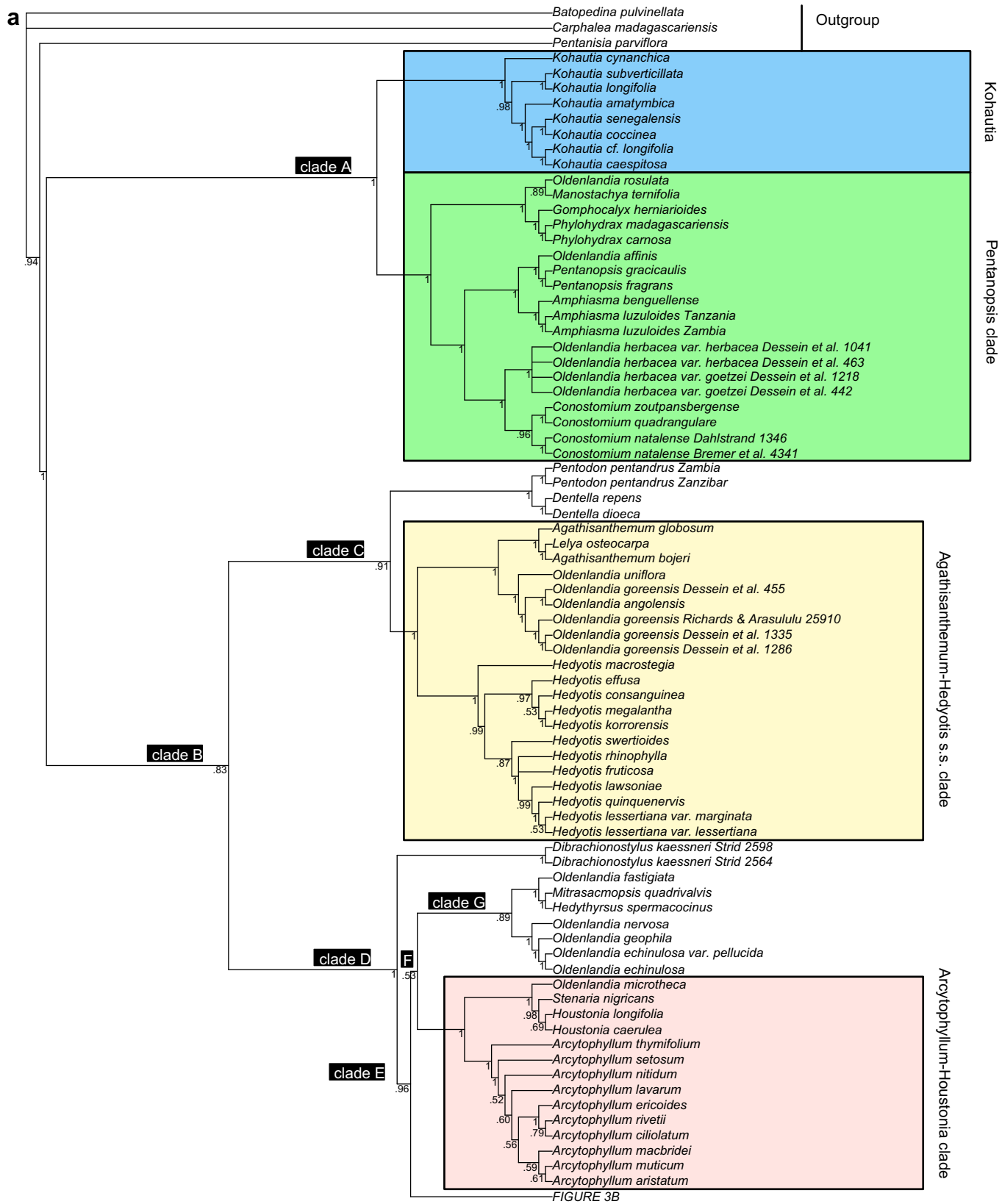

Fig. 3. (a and b) Phylogenetic tree of the combined analysis with posterior probabilities indicated below the branches.

more potentially phylogenetic informative characters a region provides the better one would expect it to estimate the correct species phylogeny.

For data sets such as our Spermacoceae tribe data, with a wide range of taxa representing different taxonomic levels, it seems that one does not have to be too concerned for nuclear data to be either

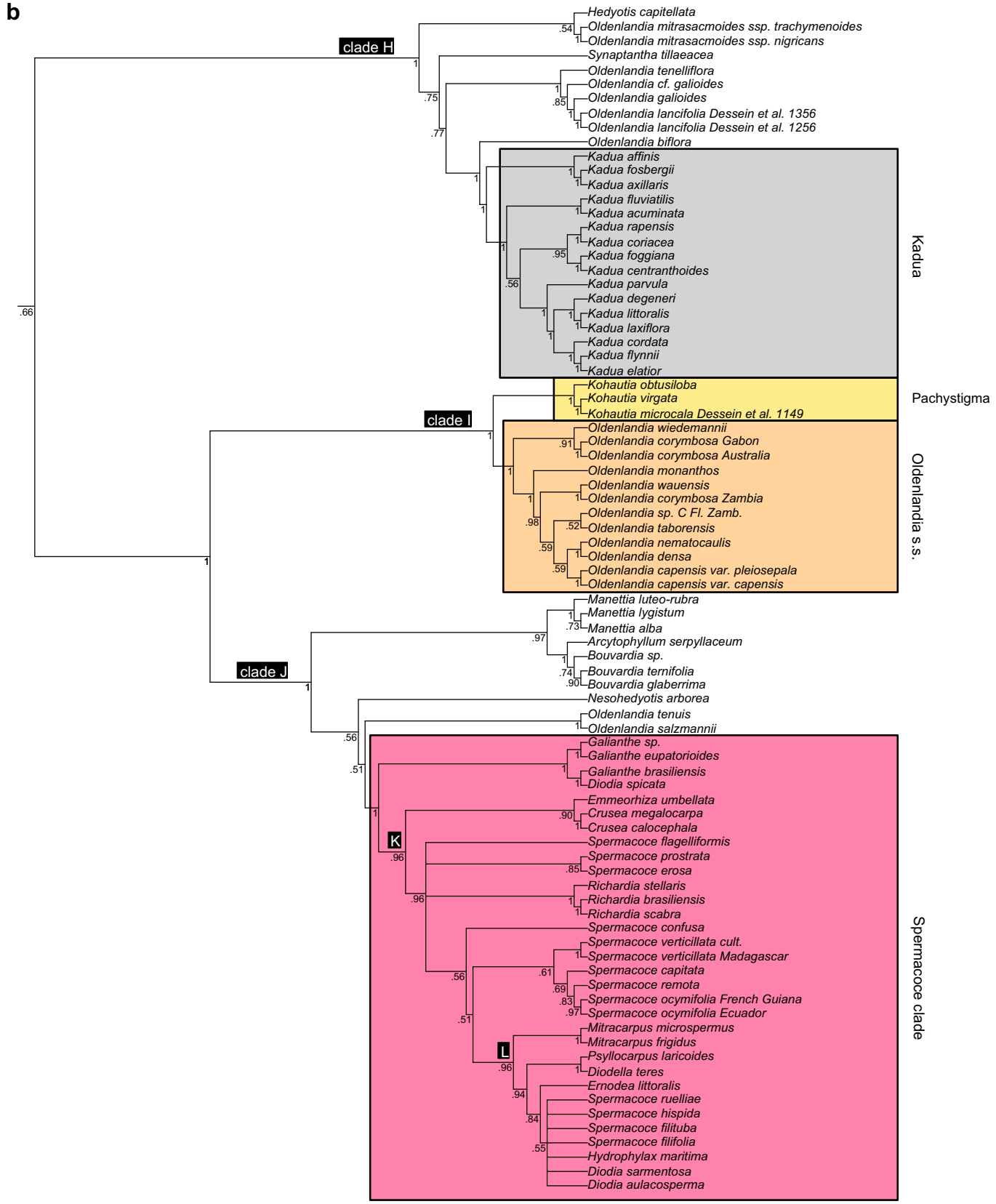

Fig. 3 (*continued*)

too homoplastic to provide a phylogenetic signal or to be indicative of other gene trees not congruent with the chloroplast tree. Both ITS and ETS, turn out to be the most useful regions. Perhaps with a denser sample within some of the smaller well-defined clades caution must be taken to caveats of nuclear data in general, and ITS and ETS in particular (see, e.g., Alvarez and Wendel, 2003).

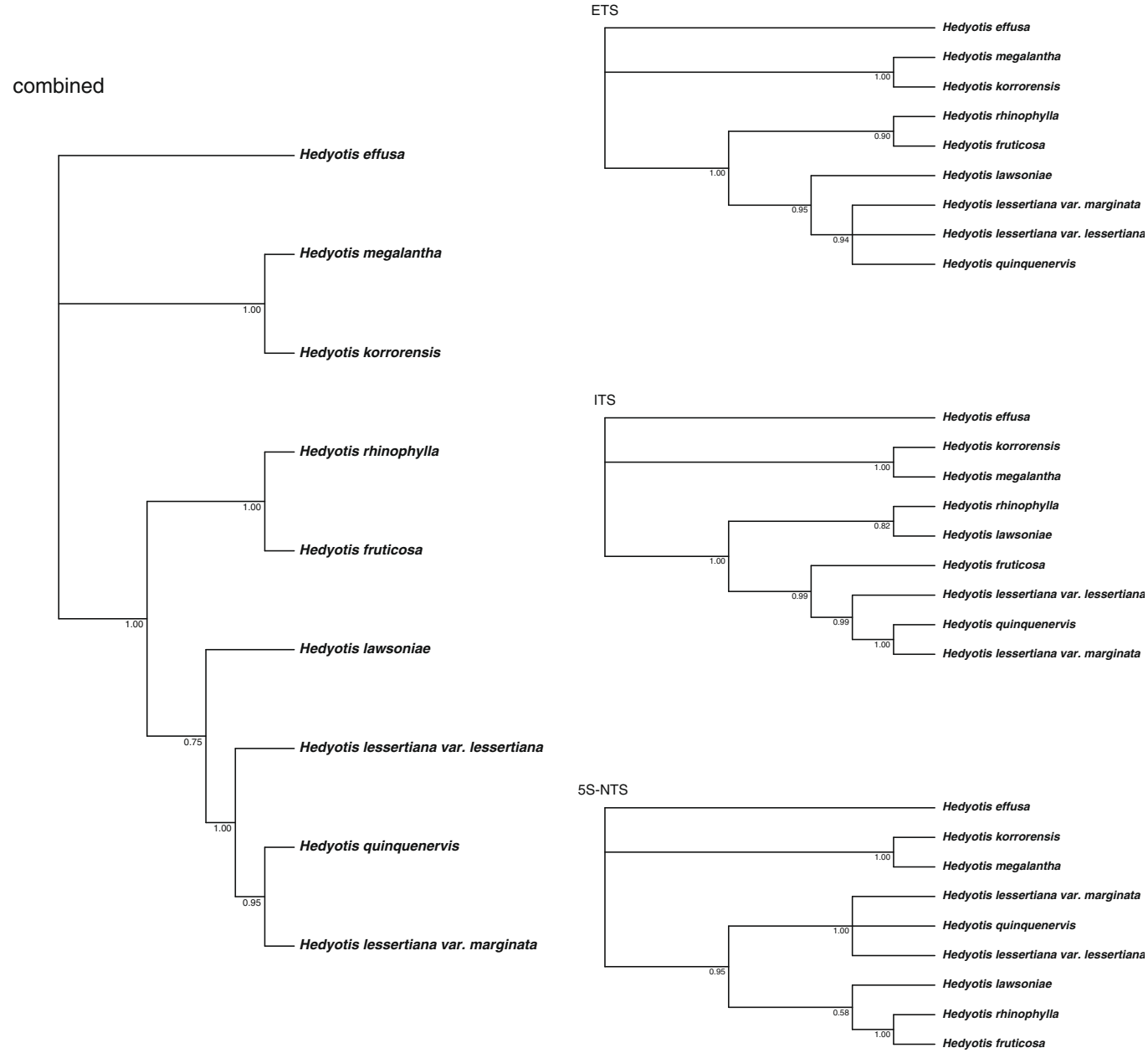

Fig. 4. Phylogenetic trees from the combined and separate analyses of the *Hedyotis* data set with posterior probabilities indicated below the branches.

For example hybridization events between closely related taxa might become an important issue to account for when studying lower level relationships of particular clades. The highly variable 5S-NTS region seems, however, promising for such studies. This region is very informative, but should preferably best be used in combination with some of the fast evolving chloroplast regions recently identified (Shaw et al. 2005, 2007; Mort et al., 2007) to ascertain that the information it provides does infer the species phylogeny.

4.2. Phylogeny of Spermacoceae

Our analysis combining nuclear and chloroplast data (Fig. 3) agrees strongly with the chloroplast phylogeny presented by Groeninckx et al. (in press). For example, Spermacoceae are monophyletic, *Hedyotis* and *Oldenlandia* are polyphyletic, and *Kohautia* is biphyletic. Some genera are paraphyletic (e.g., *Agathisanthemum* and *Spermacoce*) and others are monophyletic (e.g., *Dentella* and *Kadua*) in both studies. Groeninckx et al. (in press) specifically discussed eight well supported clades: *Kohautia*, the *Pentanopsis* clade, the *Agathisanthemum–Hedyotis* s.s. clade, *Kadua*, the *Arcytophyllum-Houstonia* clade, *Oldenlandia* s.s., *Pachystigma*, and Spermacoceae s.s. These clades are also retrieved in this study. We will refer to Spermacoceae s.s. as the *Spermacoce* clade, since we do not want to imply that the clade could be recognized at tribal level. If such a tribe is acknowledged several other clades would also have to be recognized as tribes and we currently do not have the appropriate knowledge to delimit all such taxa. Furthermore, we also question the value of a split of Spermacoceae into smaller entities, especially considering that many taxa outside the *Spermacoce* clade often have been treated as congeneric (see below).

Our analyses provide several new insights into the phylogeny of Spermacoceae. Spermacoceae in the combined analysis are resolved as a basal dichotomy. Clade A (PP 1.00) constitutes *Kohautia* + the *Pentanopsis* clade and includes *Conostomium*, which is retrieved as monophyletic in contrast to previous results of

Groeninckx et al. (in press). Clade B was not recovered in the study by Groeninckx et al. (in press), but it is not supported (PP 0.83). Clade B constitutes two clades, clades C (PP 0.91) and D (PP 1.00). Clade C contains two subclades, *Pentodon* + *Dentella* and their sister group, the *Agathisanthemum–Hedyotis* s.s. clade. This sister relationship is a novel finding. In the analyses by Groeninckx et al. (in press) *Pentodon* + *Dentella* were resolved as the possible first branching clade of the entire tribe. Clade D constitutes the remaining taxa of Spermacoceae. The *Spermacoce* clade is for the first time retrieved as a well supported monophyletic clade. The relationships within Spermacoceae will be dealt with in following detailed discussions of each major clade.

4.2.1. Kohautia

Kohautia has 36 species distributed from Africa via the Arabian Peninsula to India and Australia (Govaerts et al., 2008; number of species and distribution for genera are in the following taken from this work, if not otherwise stated). It is well characterized by its flowers, which always have both anthers and stigmas included (Bremekamp, 1952). The stigmas are usually situated well below the anthers, but occasionally they are nearly equal in height. This flower type is so rare in Rubiaceae that the generic status of *Kohautia* never has been questioned. Groeninckx et al. (in press), nevertheless, showed that the genus was not monophyletic. Their result is confirmed by our analyses. The *Kohautia* species form two well supported clades corresponding to the two subgenera of *Kohautia*, i.e., *Kohautia* and *Pachystigma*. Subgenus *Kohautia* is the sister group (PP 1.00) of the so-called *Pentanopsis* clade and subgenus *Pachystigma* is well supported as the sister group to *Oldenlandia* s.s. (PP = 1.00; clade I).

The subgenera *Kohautia* and *Pachystigma* are easily distinguished by their stigmas. The former has a style ending with two filiform stigmas and the latter has a single ovoid or cylindrical stigma (Bremekamp, 1952). Indications from chromosomal and palynological data (Lewis, 1965a) as well as from seed shape (Mantell, 1985) support the division into two separate clades. *Pachystigma* is restricted to Africa and Madagascar, while *Kohautia* extends to Tropical Asia and Australia. The two subgenera should be treated as separate genera. Alternatively, the nine species of *Pachystigma* could be included in *Oldenlandia* s.s., but considering the distinct flower type we suggest the recognition of a new genus.

4.2.2. The Pentanopsis clade

The *Pentanopsis* clade as identified by previous chloroplast studies (Thulin and Bremer, 2004; Dessein et al., 2005; Groeninckx et al., in press) is also supported by our results (PP 1.00). It is an Afro-Madagascan clade. In addition to *Pentanopsis* it consists of *Amphiasma*, *Conostomium*, *Gomphocalyx*, *Manostachya*, *Phylohydrax*, and three species of *Oldenlandia*.

Pentanopsis itself consists of two species distributed from Ethiopia to Northern Kenya. *Pentanopsis gracilicaulis* was recently transferred from *Amphiasma*. It agrees with *Pentanopsis fragrans* in having larger flowers than the species of *Amphiasma*, persistent, more or less woody stipules, and four- versus three-colporate pollen grains (Thulin and Bremer, 2004).

Amphiasma, here represented by two of its seven species, is distributed from southern Tanzania to Namibia. Our data together with morphological support (e.g., tubular stipular sheaths, very short corolla tube with a hairy throat, flattened seeds, and three-colporate pollen; Bremekamp, 1952) suggest that *Amphiasma* is monophyletic (PP 1.00). *Oldenlandia affinis* has been shown to be closely related to *Amphiasma* by Andersson and Rova (1999). *Pentanopsis* was not included in that study and *Oldenlandia affinis* is in fact well supported as the sister group to *Pentanopsis* (PP 1.00; this study; Groeninckx et al., in press). A detailed morphological study should address whether additional *Oldenlandia* species also belong to this clade and if there are morphological characters shared by *Oldenlandia affinis*, *Pentanopsis*, and *Amphiasma* other than general characters such as sessile, linear leaves, only indistinctly beaked capsules, and seeds with non-punctate testa cells (Bremekamp, 1952).

Conostomium is a genus of five species distributed from Ethiopia to South Africa. It was initially described based on capsules that are loculicidally dehiscent at the apex. This capsule type is, however, also observed in other genera of Spermacoceae. *Conostomium* is further characterized by its seeds with granulate testa cells and characteristic pollen grains (Bremekamp, 1952). Other studies (Lewis, 1965a) have shown that the distinctiveness of the pollen grains was overemphasized.

Conostomium was not supported as monophyletic by Groeninckx et al. (in press); *Oldenlandia herbacea* was nested within it. In the present analyses we sequenced additional specimens (another specimen of *Conostomium natalense*, *Oldenlandia herbacea* var. *goetzei*, and *O. herbacea* var. *herbacea*, i.e. two of the three varieties) in order to retest these results. Our data support *Conostomium* as monophyletic (PP 0.96) with *Oldenlandia herbacea* as its sister group (PP 1.00).

Oldenlandia herbacea is similar to *Conostomium* in having coarsely granulate testa cells (Bremekamp, 1952; Dessein, 2003) and comparatively large pollen grains (Bremekamp, 1952; Scheltens, 1998). Bremekamp (1952) placed *Oldenlandia herbacea* in the subgenus *Euoldenlandia* (the subgeneric classification of Bremekamp, 1952, only includes African species, but non-African species are sometimes referred to). He mentioned that together with *O. pumila* it differs from the other species of the subgenus by having long-pedicellate flowers and granulate testa cells. *Oldenlandia pumila* is distributed from India to Java (and is mentioned as introduced elsewhere; Tanzania and Jamaica according to Bremekamp, 1952). Bremekamp (1952) suggested, consequently, that *Oldenlandia herbacea*, which is widespread in Africa and present in India and on Sri Lanka, originated in Asia and spread into Africa. Considering that it is nested within the African *Pentanopsis* clade, this biogeographic hypothesis seems reversed. Since *Oldenlandia herbacea* is related to *Conostomium* and not to *Oldenlandia* s.s., it should be transferred out of *Oldenlandia*.

Phylohydrax with two species and the monotypic *Gomphocalyx* are sister taxa (PP 1.00). They were formerly included in the *Spermacoce* clade (Robbrecht, 1988) based on their habit, uniovulate ovary locules, and pluri-colporate pollen grains. Their placement in the *Pentanopsis* clade is, however, supported both by their distribution (East Africa and Madagascar) and by several morphological characters (e.g., heterostylous flowers, filiform stigma lobes, placenta attached at the base of the septum; Thulin and Bremer, 2004; Dessein et al., 2005).

Manostachya and *Oldenlandia rosulata* (PP 0.89) also belong to the *Pentanopsis* clade as the sister group (PP 1.00) to *Phylohydrax* and *Gomphocalyx*. *Oldenlandia rosulata* occurs in Tropical and Southern Africa and is closely related to *Oldenlandia microcalyx* from Cameroon and Angola, the only other member of *Oldenlandia* subgenus *Trichopodium* (Bremekamp, 1952). Since these two species of *Oldenlandia* cannot be kept in *Oldenlandia* a new genus should possibly be erected.

The three species of *Manostachya* from Central and Eastern Tropical Africa are characterized by their testa cells with thick outer walls and a network of ridges on the inside and by large pollen grains (Bremekamp, 1952). *Manostachya* notably has a basic chromosome number of $x = 11$, while most other African Spermacoceae have $x = 9$ (Lewis, 1965a). *Stephanococcus* (not investigated here due to lack of material) with a single winding species from Cameroon, Gabon, and D.R. Congo has been considered an isolated genus, but was suggested as the closest relative of *Manostachya* based on similarities in the axillary flower clusters, stipular sheath,

and in the flattened seeds (Bremekamp, 1952). It might consequently also belong to the *Pentanopsis* clade.

4.2.3. Clade C

The *Pentodon* and *Dentella* clade (PP 1.00) is the sister (PP 0.91) to the *Agathisanthemum–Hedyotis* s.s. clade (PP 1.00). This sister relationship is not found in the chloroplast analyses. Groeninckx et al. (in press) reported *Pentodon* + *Dentella* as the first branching clade of Spermacoceae (BS < 50%, PP 0.84). According to our chloroplast data the two genera are indicated as the sister group to clade D, albeit with very low posterior probability (PP 0.56).

Because *Pentodon* and *Dentella* are supported as sister taxa in all independent analyses (PP 1.00), a second individual of *Pentodon pentandrus* was sequenced for the nuclear data. It reconfirms the position and identity of the first individual. *Pentodon* has two African species, one of which is extending to the Arabian Peninsula and Madagascar and is also introduced to the New World. *Pentodon* is atypical among the Spermacoceae in having five-merous flowers and peltate placentas with a bilobed apex (Bremekamp, 1952). *Dentella* with its eight species (sometimes only one or two recognized; e.g., Ridsdale, 1998) has a wide distribution from Tropical and Subtropical Asia to the Southwest Pacific. It shares with *Pentodon* the character five-merous flowers.

4.2.4. The Agathisanthemum–Hedyotis s.s. clade

Agathisanthemum forms a well supported clade (PP 1.00) together with *Lelya* and three species of *Oldenlandia* (*O. angolensis*, *O. goreensis*, *O. uniflora*). This clade is sister to *Hedyotis* s.s. (PP 1.00). This result supports Bremekamp's (1952, p. 5) notion that *Agathisanthemum* is "in aspect not unlike the Indian *Hedyotis fruticosa* L and its nearest allies."

The four species of the African *Agathisanthemum* are characterized by capsules which split into two mericarps. This capsule structure is similar to *Hedyotis* s.s., but *Agathisanthemum* differs from *Hedyotis* s.s. by having more numerous, smaller, angular seeds and shorter stigmas (Bremekamp, 1952). Verdcourt (1976) suggested that *Agathisanthemum* possibly would be better classified as a section of *Hedyotis*.

Agathisanthemum is, however, not monophyletic (Groeninckx et al., in press; this study). The monotypic *Lelya* falls within *Agathisanthemum*. Both genera have the same pollen aperture type (Lewis, 1965a). *Lelya* differs from all other Spermacoceae in having a thick-walled capsule with a solid beak. This unique capsule was the reason for the generic status for *Lelya*, which otherwise resembles *Oldenlandia* (Bremekamp, 1952). In habit it specifically resembles *Oldenlandia goreensis* (Bremekamp, 1952), one of the three *Oldenlandia* species which constitute the sister group (PP 1.00) to *Agathisanthemum* + *Lelya* (PP 1.00). Two of the three *Oldenlandia* species are classified in *Oldenlandia* subgenus *Anotidopsis* (*O. angolensis*, *O. goreensis*). This subgenus includes other African species and Asian species that are characterized by distinctly beaked capsules and sheathing stipules with a bifid or bipartite lobe on each side of the stem (Bremekamp, 1952). The New World taxon *Oldenlandia uniflora* (central and eastern North America, Caribbean, Brazil, Paraguay, N. Argentina), is the sister to *O. angolensis* and *O. goreensis* (PP 1.00). More detailed studies with extended sampling are needed to evaluate if a new genus should be described to encompass all or part of subgenus *Anotidopsis* and *O. uniflora* or if these species are better treated as members of *Agathisanthemum*.

Hedyotis has traditionally been treated either in a narrow sense (*Hedyotis* s.s.; Bremekamp, 1952; Hallé, 1966; Terrell, 1975, 1991, 2001c; Andersson et al., 2002) restricted to tropical and subtropical Asia to the north-western Pacific or has been given a wide circumscription including also American and Polynesian taxa (e.g., Fosberg, 1943; Fosberg and Sachet, 1991; Merrill and Metcalf, 1942; Hsienshui et al., 1999; Dutta and Deb, 2004). *Hedyotis* s.s. is a well supported clade (PP 1.00). There is no support for a *Hedyotis* s.l. (Andersson et al., 2002; Groeninckx et al., in press). All of the North American species should, thus, be recognized as *Houstonia* or other segregate genera (Terrell, 1991, 2001a,b) and the Polynesian taxa are to be treated as *Kadua* (Terrell et al., 2005), as done here and by Govaerts et al. (2008). One of the sampled species currently accepted as *Hedyotis* (Govaerts et al., 2008) do not group with the *Hedyotis* s.s. clade (*Hedyotis capitellata*; clade H in Fig. 3b). On the other hand, two Asian species of *Oldenlandia*, *O. consanguinea* and *O. effusa*, belong to *Hedyotis* s.s. and consequently the names *Hedyotis consanguinea* Hance and *H. effusa* Hance should be used for the two species.

4.2.5. Clade D

The first branching taxon of clade D (PP 1.00) is the Kenyan *Dibrachionostylus*. It is rather distinct within Spermacoceae in having a style divided into two branches. Bremekamp (1952) considered the monotypic *Dibrachionostylus* as a close relative of *Agathisanthemum* and he identified additional differences (e.g., corolla tube glabrous inside, entirely glabrous style, and non-punctate testa cells). Verdcourt (1976) suggested affinities also to *Hedythyrsus*.

Clade D constitutes, apart from *Dribrachionostylus*, a clade (clade E; PP 0.96) with two lineages: clade F (PP 0.53) and the remaning taxa of clade D (Fig. 3b; PP 0.66). Clade F contains *Mitrasacmopsis* + *Hedythyrsus* + four species of *Oldenlandia* (clade G; PP 0.89) and the *Arcytophyllum–Houstonia* clade (PP 1.00).

4.2.6. Clade G

The monotypic *Mitrasacmopsis* from Central and Eastern Tropical Africa and Madagascar was originally placed in Loganiaceae because of its semi-inferior to superior ovary (Jovet, 1941). Bremekamp (1952) described a new genus *Diotocranus* within Hedyotideae, which was later reduced to the synonymy of *Mitrasacmopsis*. According to Bremekamp (1952), *Mitrasacmopsis* is morphologically similar to *Hedythyrsus* in the type of capsule dehiscence (loculicidal followed by septicidal dehiscence), the small number of seeds per capsule, and the strongly undulating walls of the testa cells. They also share beaked capsules (although the beak is much more pronounced and the capsule base is bilobed in *Mitrasacmopsis*) and a similar placentation type (distinctly stalked placentas with the ovules at a peripheral position of the placenta; Groeninckx et al., 2007, in press).

Hedythyrsus includes two species with a similar distribution to *Mitrasacmopsis*, but is absent from Madagascar. According to our data *Hedythyrsus* is sister to *Mitrasacmopsis* (PP 1.00). The monotypic genera *Pseudonesohedyotis* and *Nesohedyotis* are allied to *Hedythyrsus* according to Verdcourt (1976) and, consequently, they probably also belong to this clade. The *rps16* sequence of *Nesohedyotis* (Andersson and Rova, 1999) does according to our analysis, however, belong to clade J (Fig. 3b). *Nesohedyotis* with unisexual flowers is unusual among Spermacoceae. It is endemic to St. Helena. There is, thus, some biogeographical indication that its position in the largely American clade J might be correct. The hypothesis that the Tanzanian *Pseudonesohedyotis* with hermaphroditic flowers also belongs to clade J seems less likely from a biogeographical and morphological standpoint and it has more likely affinities to *Hedythyrsus*.

The sister group to *Mitrasacmopsis* and *Hedythyrsus* is *Oldenlandia fastigiata* (PP 1.00), which is distributed from Ethiopia to Mozambique. It has a similar placentation type as the other two genera (Groeninckx et al., in press), but has testa cells with straight walls (Bremekamp, 1952). *Oldenlandia fastigiata* is classified in subgenus *Oldenlandia* (Bremekamp, 1952), a classification that is unsupported phylogenetically.

The sister group (PP 0.89) of *Mitrasacmopsis*, *Hedythyrsus*, and *Oldenlandia fastigiata* is a clade (PP 1.00) of three other species of

Oldenlandia: *O. echinulosa* from Tropical Africa, *O. geophila* from Zambia, and *O. nervosa* distributed from West Central Tropical Africa to Angola. Clade G, thus, seems to be an entirely African clade. *Oldenlandia echinulosa* and *O. nervosa* are classified in subgenus *Hymenophyllum* and *O. geophila* in subgenus *Orophilum* (Bremekamp, 1952). Both subgenera are characterized by distinctly petiolate leaves. *Hymenophyllum* comprises annuals with rather large and thin leaves, a short, fimbriate stipular sheath, glabrous style, and testa cells with undulating walls. It differs from subgenus *Orophilum*, which includes perennial species with smaller, often leathery leaves, a stipular sheath drawn out into a triangular lobe, hirtellous style, and testa cells with straight walls (Bremekamp, 1952). The type species of subgenus *Orophilum*, *Oldenlandia monanthos*, belongs to *Oldenlandia* s.s. (Fig. 3b).

Further studies are needed to properly investigate how to classify the taxa of clade G. If the current phylogenetic relationships remain with a denser taxon sampling, three possibilities should be investigated: (1) lumping all taxa into *Mitrasacmopsis* (generic name with priority), (2) merging *Hedythyrsus* and *Oldenlandia fastigiata* into *Mitrasacmopsis* and erecting a new genus for the other species of *Oldenlandia*, or (3) keeping *Mitrasacmopsis* and *Hedythyrsus* as distinct genera and erecting two new genera to encompass *Oldenlandia fastigiata* and the other species of *Oldenlandia*, respectively.

4.2.7. The Arcytophyllum–Houstonia clade

The *Arcytopyllum–Houstonia* clade is well supported (PP 1.00). *Arcytophyllum* with 16 species (excluding *A. serpyllaceum*; see below) distributed from Mexico to western South America is well supported as monophyletic (PP 1.00; Andersson et al. 2002). The sister group of *Arcytophyllum* is a clade (PP 1.00) comprising *Houstonia* (PP 0.69) + *Stenaria nigricans* (PP 0.98), and one species of *Oldenlandia* (PP 1.00): *O. microtheca* from Mexico. This clade as well as the entire *Arcytophyllum–Houstonia* clade seems to be restricted to the New World. That *Oldenlandia microtheca* belongs to the clade seems reasonable considering both its distribution and the fact that it has a basic chromosome number of $x = 11$, which is in contrast to most species of *Oldenlandia* (Lewis, 1965a), and agrees with several counts made for species of *Houstonia* (Lewis, 1962, 1965b). The relationships within *Houstonia*, which consists of 30 North and Central American species, has recently been closer studied by Church (2003).

Stenaria contains five species distributed from Central and Eastern USA to Mexico and Bahamas. It was previously included in *Hedyotis*, but was elevated to generic status by Terrell (2001a). In the study by Church (2003), *Stenaria* was nested within *Houstonia* and, consequently, suggested to be merged with *Houstonia*. Two closely related genera from Baja California, *Stenotis* (Terrell, 2001b) with seven species and the monotypic *Carterella* (Terrell, 1987; possibly congeneric with *Stenotis*; Church, 2003), most likely also belong to the *Arcytophyllum–Houstonia* clade.

4.2.8. Clade H; Asian, Australian, and Pacific Spermacoceae

Clade H (Fig. 3b) is a well supported clade (PP 1.00), but its basal branches are poorly supported. *Hedyotis capitellata,* distributed from North Eastern India (Assam) to the Philippines, and the two Australian species *Oldenlandia mitrasacmoides* and *Synaptantha* belong here, but their relationships to the other taxa of clade H are uncertain.

Synaptantha with two species from Australia might be the sister (PP 0.75) to the remaining taxa of clade H, the majority of which have an Australian-Asian-Pacific distribution. *Synaptantha* has long been recognised as a separate genus (Hooker, 1873). It differs from other Spermacoceae by having corolla with the lobes slightly connate, stamens with filaments attached to both the ovary and the corolla, and semi-inferior ovaries (Halford, 1992). *Synaptantha* does not only have a distinct morphology, it also has many autapomorphies in the ITS tree (one of the longest branches in the ITS tree; not present in the ETS data). The sequence does, however, not seem to represent a pseudogene since 5.8S is conserved; in a pseudogene one would expect also 5.8S to have an elevated substitution rate (Bailey et al., 2003). *Synaptantha* also seems to have an elevated rate of evolution in the chloroplast regions. In the *trnL-F* analysis it has the longest branch. It also has long branches in the other three chloroplast regions, but in these regions the rate is not as conspicuously elevated as for ITS and *trnL-F*.

The remaining taxa of clade H (PP 0.77) include a clade with three *Oldenlandia* species (*O. galioides*, *O. lancifolia, O. tenelliflora* [syn. *Scleromitrion tenelliflorum; Hedyotis tenelliflora*]; PP 1.00), *Kadua* (PP 1.00), and its sister species *O. biflora* (PP 1.00).

Both *Oldenlandia galioides*, which is distributed from New Guinea to the South West Pacific, and *O. tenelliflora*, from Tropical and Subtropical Asia to Northern Queensland, have obconic seeds (in contrast to, e.g., *O. mitrasacmoides*, which has scutelliform seeds; Halford, 1992). *Oldenlandia lancifolia* has more angulate seeds (Bremekamp, 1952) and is widespread in Africa and naturalized in Tropical South America. Bremekamp (1952) placed it in the subgenus *Aneurum* together with the North American *Oldenlandia boscii* and the Asian *O. diffusa*. One or several new genera should better be recognised to acknowledge the early branching members of clade H, since the species clearly do not belong to either *Hedyotis* s.s. or *Oldenlandia* s.s.

Oldenlandia biflora is the sister group to *Kadua* (PP 1.00) as in the chloroplast study by Groeninckx et al. (in press). It is distributed from tropical and subtropical Asia to the West Pacific. That the sister group to the Polynesian *Kadua* would have such a distribution seems reasonable. *Oldenlandia biflora* should be transferred from *Oldenlandia*, but whether there is morphological support to include it in *Kadua* will have to await further studies.

Kadua with presently 28 species (Terrell et al., 2005; Govaerts et al., 2008) is monophyletic (PP 1.00). This supports the conclusion from previous molecular and seed anatomical studies that the Polynesian taxa formerly included in Hedyotideae (as *Hedyotis*, *Gouldia*, or *Wiegmania*) should be regarded as the separate genus *Kadua* (Motley, 2003; Terrell et al., 2005). Motley (in press) has studied *Kadua* with a broader sampling and found that *Kadua* is monophyletic and subclades for the most part reflect former classifications. This study also showed that the Hawaiian *Kadua* species are paraphyletic with respect to the French Polynesian species and provide strong evidence for migration of a plant lineage out of the archipelago. The nested position of *K. rapensis* in the *Kadua* clade in this study supports this finding.

4.2.9. Clade I, the true Oldenlandia and Pachystigma

Oldenlandia is clearly polyphyletic. The clade including the type species, *O. corymbosa*, is here referred to as *Oldenlandia* s.s. (PP 1.00). All species of *Oldenlandia* not belonging to this clade should be transferred to other genera or assigned to new genera. Some suggestions are already made above, but we refrain from making the formal taxonomic changes pending studies with better sampling. The monotypic genus *Thecorchus* distributed from Ethiopia to Senegal should, however, no longer be recognized (Kårehed and Bremer, 2007; Groeninckx et al, in press). It is nested within *Oldenlandia* s.s. and the use of the name *Thecorchus wauensis* (Schweinf. ex Hiern) Bremek. should be abandoned in favor of its basionym *Oldenlandia wauensis* Schweinf. ex Hiern.

As mentioned above, the sister group (PP 1.00) of *Oldenlandia* s.s. is *Kohautia* subgenus *Pachystigma* (PP 1.00), which should be recognized as a distinct genus.

4.2.10. Clade J and the origin of the Spermacoce clade

Clade J (PP 1.00) is the sister group (PP 1.00) of clade I. It is almost entirely restricted to the New World. All taxa of clade J, ex-

cept *Nesohedyotis* from St. Helena, are from the New World. A South American origin of the *Spermacoce* clade as suggested by Dessein (2003) seems very probable, since the *Spermacoce* clade is a predominantly New World taxon. Only *Diodia* s.l., *Hydrophylax*, and *Spermacoce* s.l. (pantropical) extend into other tropical regions.

Manettia (PP 1.00) and *Bouvardia* (PP 0.74) are sister genera (PP 0.97). If *Arcytophyllum serpyllaceum* (*rps16* data only) is included in *Bouvardia* (PP 1.00) as suggested by Andersson et al. (2002) they appear monophyletic. *Manettia* with 124 species from Tropical America has been recognised as a separate tribe, Manettieae (Bremekamp, 1934). Based on its winged seeds, Manettieae were previously placed in the subfamily Cinchonoideae (Schumann, 1891). *Manettia* was later moved to the Rubioideae and Hedyotideae because of the presence of raphides (Bremekamp, 1966). Robbrecht (1988) included both genera in Cinchoneae, but because of the shared presence of raphides the Hedyotideae were suggested as a possible alternative placement. *Manettia* is morphologically similar to *Bouvardia* and Bremer (1996) found the two genera to be sister taxa in Rubioideae based on molecular data. *Bouvardia* comprises 41 species from Southern USA to Central America. *Manettia* differs from *Bouvardia* mainly in its viney habit and hard endosperm.

Nesohedyotis (*rps16* data only) and two *Oldenlandia* species (*O. salzmanii* + *O. tenuis*; PP 1.00) belong to clade J, but their position as successive sister taxa to the *Spermacoce* clade is very weekly supported.

4.2.11. The Spermacoce clade

The *Spermacoce* clade, although long recognized as a separate tribe (Spermacoceae; Berchtold and Presl, 1820; Hooker, 1873; Bremekamp, 1952, 1966; Verdcourt, 1958; Robbrecht, 1988, 1994), was not recovered as a well supported monophyletic group in the chloroplast study by Groeninckx et al. (in press). However, with the addition of nuclear data, the *Spermacoce* clade is well supported (PP 1.00), provided that *Gomphocalyx* and *Phylohydrax* are excluded. Both genera belong to the *Pentanopsis* clade (Thulin and Bremer, 2004; Dessein et al., 2005; see above).

The *Spermacoce* clade differs from the rest of the tribe in having ovaries with a single ovule per locule attached near the middle of the septum and often pluriaperturate pollen grains, in contrast to few to many ovules per locule and often tricolporate pollen grains. The genera of the *Spermacoce* clade are often recognized based on the type of fruit dehiscence. A detailed overview of the *Spermacoce* clade is given by Dessein (2003).

Galianthe (PP 1.00; including *Diodia spicata*) is the first branching taxon of the *Spermacoce* clade. It constitutes 45 species from Mexico to South America and is characteristic among Spermacoceae s.s. in having terminal lax inflorescences, heterostylous flowers, and a stigma with two distinct lobes (Cabral, 1991). *Diodia spicata* (treated under the name *Spermacoce spicata* (Miq.) in ed. by Govaerts et al., 2008) makes *Galianthe* paraphyletic. *Diodia spicata* differs by having terminal spicate inflorescences with isostylous flowers and fruits with two cocci separating from the base. Nevertheless, since it approaches *Galianthe* with its two-armed stigma and 7-zonocolporate pollen with relatively long colpi and double reticulum, it seems reasonable to include *Diodia spicata* in *Galianthe* (Dessein, 2003).

The Central American *Crusea*, here represented by two of 14 species, is monophyletic (PP 1.00). *Emmeorhiza*, a South American monotypic genus, is the sister to *Crusea* (PP 0.90). This relationship was not found by Groeninckx et al. (in press). The position of *Emmeorhiza* was not well supported in their study. In the strict consensus tree of the parsimony analysis it was the sister to *Galianthe* + *Diodia spicata* (JK and BS <50%) and in the Bayesian inference it was the sister to *Nesohedyotis arborea* (PP 0.67). The addition of *petD* data to the other chloroplast data places *Emmeorhiza* in the same larger clade as in the combined analysis (clade K; PP 0.96), but as the first branching taxon (PP 0.96) and not as the sister to *Crusea*.

Emmeorhiza is somewhat similar in habit to the genus *Denscantia*. Both genera are climbers with thyrsoid-like inflorescences with isostylous flowers (Dessein, 2003). *Denscantia* (first published as *Scandentia*, but later changed to *Denscantia*; Cabral and Bacigalupo, 2001a,b) was segregated from the probably closely related *Galianthe*. *Denscantia* has isostylous flowers, lateral fusion of the stipular bases, and pollen with multiple endoapertures whereas *Galianthe* often has heterostylous flowers, stipules fused only with the leaf base or the petiole, and pollen with a simple endocingulum (Cabral and Bacigalupo, 2001a; Dessein, 2003). Further study is needed to determine if *Denscantia* belong to the *Crusea* + *Emmeorhiza* clade or if the morphological resemblance to *Emmeorhiza* is due to convergence.

The genus *Spermacoce* is not monophyletic in the present study. Several smaller genera are intermingled with *Spermacoce* species.

Richardia (PP 1.00) has 16 species from Tropical and Subtropical America and is naturalized elsewhere. It is a well defined genus characterized by mainly 3–4-carpellate ovaries and schizocarpous fruits splitting into indehiscent mericarps (Dessein, 2003).

Spermacoce ocymifolia has been treated as *Hemidiodia ocymifolia* in a genus of its own (Schumann, 1888) characterized by its fruits. They consist of two indehiscent mericarps. At maturity the mericarps are only partially joined by their bases. *Hemidiodia* was sunk into *Borreria* (a genus previously recognized to encompass American *Spermacoce*) because, apart from the fruit type, they are morphologically similar (Bacigalupo and Cabral, 1996). Our data show *Spermacoce ocymifolia* as sister to *S. remota* (PP 0.83).

Mitracarpus (PP 1.00; represented by two out of the 49 species) also belongs to the *Spermacoce* clade as the first branching taxon of a well supported subclade (clade L; PP 0.96). *Mitracarpus* is from Tropical America and is naturalized elsewhere. The genus is distinguished by its cirumscissile opening of the fruits and in having two large and two small calyx lobes and seeds with an X-shaped ventral groove (Dessein, 2003).

Diodella teres and *Psyllocarpus laricoides*, two species only represented by nuclear data, are each others closest relatives (PP 1.00) and the second branching clade (PP 0.94) of clade L. If their sister group relationship is due only to the present taxon sampling or a true relationship will have to await further studies. No obvious morphological characters are shared by the two genera. *Psyllocarpus* with its nine Brazilian species is distinct from the other genera of the *Spermacoce* clade by having a capsule strongly compressed parallel to the septum.

Govaerts et al. (2008) accepted 30 species in *Diodia*. In contrast, Bacigalupo and Cabral (1999) only recognized five species in the genus. These authors transferred 16 species provisionally to the genus *Diodella*. Nine of these are now formally published under *Diodella*. *Diodella* is distributed from Central USA to Tropical America and is also present in Africa. It is characterized by fruits dehiscing into two, indehiscent, one-seeded mericarps. Dessein (2003) suggests that species assigned to *Diodella* fall into two groups: one group is related to *Diodella teres*, the type species, the other one to *Diodella sarmentosa*. This is confirmed by our analysis as *Diodella sarmentosa* forms a clade (PP 0.84) with *Diodia aulacosperma*, *Ernodea*, *Hydrophylax*, *Spermacoce hispida*, *S. ruelliae*, *S. filifolia*, and *S. fillituba*.

The African *Diodia aulacosperma* (Socotra, S. Somalia to E. Tanzania (including Zanzibar) is not regarded as a member of a restricted *Diodia* (Bacigalupo and Cabral, 1999; Dessein, 2003). *Ernodea*, a genus with nine species distributed from Florida south to Central America and in the Caribbean Islands, shares some seed and pollen morphological characters with *Diodella sarmentosa* (Dessein, 2003), but are in habit and fruit morphology quite different. *Diodella sarmentosa* has dry fruits splitting into two mericarps, while *Ernodea* has drupaceous fruits, a rare condition in Spermacoceae. *Hydrophylax maritima*, the sole species in the genus, is a sea shore plant from India, Sri Lanka, and Thailand. Dessein (2003) pointed to the strong similarity in habit between *Hydrophylax*

and the West African *Diodia vaginalis* (another of the species probably to be excluded from *Diodia*) and species of *Diodia* s.s. However, pollen grains are very different in these taxa.

Further studies, especially with a larger sampling of *Spermacoce* species, will reveal if *Spermacoce* should be split or if several of the other genera of the *Spermacoce* clade should be merged.

Note added in proof

The DNA here sequenced as *Spermacoce filifolia* was extracted from *Spermacoce flagelliformis*, De Block et al. 794 (BR). The accession numbers AM939538 and AM933010 consequently refer to sequence data from the latter species.

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Appendix

List of taxa used in the phylogenetic analyses with voucher information (geographic origin, collector, collector number, herbarium), and accession numbers. New taxa/specimens not included in by Groeninckx et al. (in press) are indicated in bold. Missing sequences are marked with. Literature citations for previous published sequences: (1)=Andersson and Rova 1999, (2)=Andersson et al. 2002, (3)=Dessein et al. 2005.

Taxon	Voucher information	*atpB-rbcL*	*rps16*	*trnL-trnF*	*petD*	ITS	ETS	5S-NTS
***Agathisanthemum* Klotzsch**								
A. bojeri Klotzsch	Zambia: Dessein et al. 671 (BR)	EU542917	EU543018	EU543077	EU557678	AM939424	/	/
A. globosum (Hochst. ex A. Rich.) Klotzsch	Zambia: Dessein et al. 201 (BR)	EU542918	EU543019	EU543078	EU557679	AM939425	/	/
***Amphiasma* Bremek.**								
A. benguellense (Hiern) Bremek.	Angola: Kers 3350 (S)	EU542919	AF002753(1)	EU543079	EU557680	AM939426	AM932918	/
A. luzuloides (K. Schum.) Bremek.	Zambia: Dessein et al. 1167 (BR)	EU542920	EU543020	EU543080	EU557681	AM939428	AM932919	/
	Tanzania: Iversen et al. 87694 (UPS)	/	/	/	/	**AM939427**	**AM932920**	/
***Arcytophyllum* Willd. ex Schult. & Schult. f.**								
A. aristatum Standl.	Ecuador: Hekker & Hekking 10335 (GB)	/	AF333348(2)	AF333349(2)	/	/	/	/
A. ciliolatum Standl.	Unknown: Oligaard et al. 58395 (NY)	/	AF333350(2)	AF333351(2)	/	/	/	/
A. ericoides (Willd. ex Roem. & Schult.) Standl.	Unknown: Edwin et al. 3624 (S)	/	AF333352(2)	AF333353(2)	/	/	/	/
A. lavarum K. Schum.	Unknown: Cronquist 8827 (NY)	/	AF333354(2)	AF333355(2)	/	/	/	/
A. macbridei Standl.	Unknown: Wurdack 1073 (NY)	/	AF333356(2)	AF333357(2)	/	/	/	/
A. muticum (Wedd.) Standl.	Colombia: Andersson et al. 2195 (GB)	EU542921	AF002754(1)	EU543081	EU557682	AM939429	/	/
A. nitidum (Kunth) Schltdl.	Unknown: Pipoly et al. 6467 (GB)	/	AF333359(2)	/	/	/	/	/
A. rivetii Danguy & Cherm.	Ecuador: Harling & Andersson 22232 (GB)	EU542922	AF333362(2)	AF333363(2)	/	AM939430	/	/
A. serpyllaceum (Schltdl.) Terrell	Mexico: Stafford et al. 203 (MO)	/	AF333364(2)	/	/	/	/	/
A. setosum (Ruiz & Pav.) Schltdl.	Unknown: Andersson et al. 2196 (GB)	/	AF002755(1)	AF333365(2)	/	/	/	/

(*continued on next page*)

Appendix Table 1 (*continued*)

Taxon	Voucher information	*atpB-rbcL*	*rps16*	*trnL-trnF*	*petD*	ITS	ETS	5S-NTS
A. thymifolium (Ruiz & Pav.) Standl.	Ecuador: Ståhl 4481 (GB)	EU542923	AF333366[2]	EU543082	EU557683	AM939431	AM932921	/
***Batopedina* Verdc. (outgroup)**								
B. pulvinellata Robbrecht	Zambia: Dessein et al. 264 (BR)	EU542924	EU543021	EU543083	EU557684	/	/	/
	D.R. Congo: Malaisse 7695 (UPS)	/	/	/	/	**AM266989**	/	/
***Bouvardia* Salisb.**								
B. glaberrima Engelm.	Cult.: Forbes s.n. (S)	EU542925	EU543022	EU543084	EU557685	AM939432	AM932922	/
B. ternifolia (Cav.) Schltdl.	Cult.: S2928 (BR)	/	AF002758[1]	/	/	/	/	/
	Mexico: Spencer et al. 363 (NY)	/	/	s.n.	/		/	/
***B.* sp.**	**Mexico: Torres & Torino 3637 (BR)**	/	/	/	/	**AM939433**	**AM932923**	/
***Carphalea* Juss.** (outgroup)								
C. madagascariensis Lam.	Madagascar: De Block et al. 578 (BR)	EU542926	EU543023	/	EU557686	/	/	/
	Madagascar: Razafimandimbison 524 (UPS)	/	/	/	/	**AM266995**	/	/
***Conostomium* (Stapf) Cufod.**								
C. natalense (Hochst.) Bremek.	South Africa: Dahlstrand 1346 (GB)	EU542927	AF002760[1]	EU543085	EU557687	AM939435	AM932925	/
	South Africa: Bremer et al. 4341 (UPS)	/	/	/	/	**AM939434**	**AM932924**	/
C. quadrangulare (Rendle) Cufod.	Ethiopia: Puff & Kelbessa 821222 2/2 (UPS)	EU542928	EU543024	EU543086	EU557688	AM939436	AM932926	/
C. zoutpansbergense (Bremek.) Bremek.	South Africa: Bremer et al. 4331 (UPS)	EU542929	/	EU543087	EU557689	AM939437	AM932927	/
***Crusea* Cham. & Schltdl.**								
C. calocephala DC.	Guatemala: Gustafsson et al. 215 (GB)	EU542930	/	EU543088	EU557690	AM939438	AM932928	/
C. megalocarpa (A. Gray) S. Watson	Mexico: Pringle 3852 (S)	EU542931	EU543025	EU543089	EU557691	AM939439	AM932929	/
***Dentella* J. R. Forst & G. Forst.**								
***D. dioeca* Airy Shaw**	Australia: Harwood 1559 (BR)	/	/	EU543090	EU557692	/	/	/
D. repens (L.) J. R. Forst. & G. Forst.	Australia: Andersson 2262 (GB)	EU542932	AF333370[2]	EU543091	EU557693	AM939440	AM932930	/
***Dibrachionostylus* Bremek.**								
D. kaessneri (S. Moore) Bremek.	Kenya: Strid 2598 (GB)	EU542933	AF002761[1]	/	EU557694	AM939442	AM932932	/
	Kenya: Strid 2564 (UPS)	/	/	/	/	**AM939441**	**AM932931**	/
***Diodella* Small**								
D. sarmentosa Sw.	French Guiana: Anderson et al. 2071 (GB)	/	AF002762[1]	/	/	/	/	/

Appendix Table 1 (*continued*)

Taxon	Voucher information	*atpB-rbcL*	*rps16*	*trnL-trnF*	*petD*	ITS	ETS	5S-NTS
***D. teres* (Walter) Small**	**Madagascar: De Block et al. 793 (BR)**	/	/	/	/	**AM939443**	**AM932933**	/
***Diodia* L. s.l.**								
D. aulacosperma K. Schum.	Kenya: Luke 9029 (UPS)	EU542934	EU543026	EU543092	EU557695	AM939444	AM932934	/
D. spicata Miq. (*Spermaoce spicata* (Miq.) in ed.; Govaerts et al., 2008)	French Guiana: Anderson et al. 1961 (GB)	EU542935	EU543027	EU543093	EU557696	AM939535	AM933008	/
***Emmeorhiza* Pohl ex Endl.**								
E. umbellata (Spreng.) K. Schum.	Trinidad: Hummel s.n. (GB)	EU542936	AY764289(3)	EU543094	EU557697	AM939445	AM932935	/
***Ernodea* Sw.**								
E. littoralis Sw.	Cuba: Rova et al. 2286 (GB)	EU542937	AF002763(1)	EU543095	EU557698	AM939446	AM932936	/
***Galianthe* Grieseb.**								
G. brasiliensis (Spreng.) E. L. Cabral & Bacigalupo	Argentina: Vanni & Radovancick 996 (GB)	EU542938	AY764290(3)	EU543096	EU557699	AM939447	AM932937	/
G. eupatorioides (Cham. & Schltdl.) Cabral	Argentina: Schinini & Cristobal 9811 (GB)	EU542939	EU543028	EU543097	EU557700	AM939448	AM932938	/
G. sp.	**Bolivia: Persson & Gustavsson 298 (GB)**	**/**	**/**	**/**	/	**AM939449**	**AM932939**	/
***Gomphocalyx* Baker**								
G. herniarioides Baker	Madagascar: De Block et al. 569 (BR)	EU542940	AY764291(3)	EU567466	EU567461	/	/	/
***Hedyotis* L.**								
***H. capitellata* Wall.**	**Burma: Meebold 17373 (S)**	/	/	/	/	**AM939452**	/	/
H. consanguinea Hance (syn. *Oldenandia consanguinea* (Hance) Kuntze)	Hong Kong: Shiu Ying Hu 10821 (S)	EU542941	/	/	EU557701	AM939450	/	AM931939
***H. effusa* Hance (syn. *Oldenlandia effusa* (Hance) Kuntze)**	**China: Tsang 21044 (S)**	/	/	/	/	**AM939491**	**AM932940**	**AM931935**
H. fruticosa L.	Sri Lanka: Larsson & Pyddoke 22 (S)	EU542942	/	EU543098	EU557702	AM939453	AM932941	AM931929
H. korrorensis (Valeton) Hosok.	The Caroline Islands: Fosberg 47697 (S)	EU542943	/	EU543099	EU557703	AM939454	AM932942	AM931937
H. lawsoniae Wight	Sri Lanka: Wambeek & Wanntorp 2996 (S)	EU542944	/	/	EU557704	AM939455	AM932943	AM931931
H. lessertiana var. *lessertiana* Thwaites	Sri Lanka: Klackenberg 413 (S)	EU542945	EU543029	EU543100	EU557705	AM939466	AM932944	AM931932
H. lessertiana var. *marginata* Thwaites & Trimen	Sri Lanka: Fagerlind 3668 (S)	EU542946	EU543030	EU543101	EU557706	AM939456	AM932945	AM931934
H. macrostegia Stapf.	Sabah:Wallander 6 (GB)	EU542947	AF002767(1)	EU543102	/	AM942768	/	/

(*continued on next page*)

Appendix Table 1 (*continued*)

Taxon	Voucher information	*atpB-rbcL*	*rps16*	*trnL-trnF*	*petD*	ITS	ETS	5S-NTS
***H. megalantha* Merr.**	**Marianas (Guam): Andersson 07 (S)**	/	/	/	/	**AM939457**	**AM932946**	**AM931936**
H. quinquenervis Thwaites	Sri Lanka: Bremer et al. 163 (S)	EU542948	/	EU543103	EU557707	AM939458	AM932947	AM931933
H. rhinophylla Thwaites ex Trimen	Sri Lanka: Fagerlind 5082 (S)	EU542949	/	EU543104	EU557708	AM939459	AM932948	AM931930
H. swertioides Hook. f.	South India: Klackenberg & Lundin 03 (S)	EU542950	EU543031	EU543105	EU557709	AM939460	/	AM931938
***Hedythyrsus* Bremek.**								
H. spermacocinus (K. Schum.) Bremek.	Zambia: Dessein et al. 1017 (BR)	EU542951	EU543032	EU543107	EU557711	AM939461	AM932950	/
***Houstonia* L.**								
H. caerulea L.	USA: Vincent & Lammers s.n. (GB)	EU542953	AF333379[(2)]	EU543109	EU557713	AM939464	/	/
H. longifolia Gaertn.	USA: Yatskievych 96-49 (MO)	EU542954	AF002766[(1)]	/	EU567462	AM939465	/	/
	USA: Weigend 9963 (NY)	/	/	s.n.	/	/	/	/
***Hydrophylax* L. f.**								
***H. maritima* L. f.**	**Sri Lanka: Lundqvist 8945 (UPS)**	**EU567457**	/	/	/	/	/	/
***Kadua* Cham. & Schltdl.**								
K. acuminata Cham. & Schltdl.	Hawaii: cult. at BR	EU542955	/	EU543110	EU557714	AM939467	AM932952	/
K. affinis Cham. & Schltdl.	Hawaii HI: Motley 1733 (NY)	/	s.n.	s.n.	/	AM942769	/	/
K. axillaris (Wawra) W. L. Wagner & Lorence	Hawaii: Harrison-Gagne s.n. (GB)	/	AF002765[(1)]	/	/	/	/	/
	Maui HI: Motley 1724 (NY)	/	s.n.	s.n.	/	AM942770	/	/
K. centranthoides Hook. & Arn.	Hawaii: Skottsberg 6788 (S)	EU542956	EU543033	EU543111	EU557715	AM939468	/	/
K. cordata Cham. & Schltdl.	Cult.: Lorence 8021 (PTBG)	EU542957	AF333376[(2)]	EU543112	EU557716		/	/
	Hawaii HI: Fagerlind 6863 (S)	/	/	/	/	**AM939469**	/	/
K. coriacea (J. E. Smith) W. L. Wagner & Lorence	Hawaii HI: Motley 1703 (NY)	/	s.n.	s.n.	/	AM942771	/	/
K. degeneri (Fosberg) W. L. Wagner & Lorence	Cult.: Wood 5062 (PTGB)	EU542958	AF333371[(2)]	EU543113	EU557717	AM939470	AM932953	/
K. elatior (H. Mann) W. L. Wagner & Lorence	Kauai HI: Wagner 6350 (BISH)	/	s.n.	s.n.	/	AM942772	/	/
K. fluviatilis C. N. Forbes	Oahu HI: Motley 1747 (NY)	/	s.n.	s.n.	/	AM942773	/	/
K. flynnii (W. L. Wagner & Lorence) W. L. Wagner & Lorence	Kauai HI: Perlman 15631 (BISH)	/	s.n.	s.n.	/	AM942774	/	/
K. foggiana (Fosberg) W. L. Wagner & Lorence	Hawaii: Sparre 27 (S)	EU542959	/	EU543114	EU557718	AM939471	/	/

Appendix Table 1 (*continued*)

Taxon	Voucher information	*atpB-rbcL*	*rps16*	*trnL-trnF*	*petD*	ITS	ETS	5S-NTS
K. fosbergii (W. L. Wagner & D. R. Herbst) W. L. Wagner & Lorence	Oahu HI: Motley 1677 (NY)	/	s.n.	s.n.	/	AM942775	/	/
K. laxiflora H. Mann	Molokai HI: Perlman 6677 (BISH)	/	s.n.	s.n.	/	AM942776	/	/
K. littoralis Hillebr.	Cult. at WU: Kiehn & Luegmayr 920823-2/1	EU542960	s.n.	EU543115	EU557719	AM939472	AM932954	/
K. parvula A. Gray	Cult.: Perlman 12783 (GB)	EU542961	AF333375[(2)]	EU543116	EU557720	AM939473	AM932955	/
K. rapensis F. Br.	Rapa Is.French Polynesia: Perlman 17953 (NY)	/	s.n.	s.n.	/	/	/	/
***Kohautia* Cham. & Schltdl.**								
K. amatymbica Eckl. & Zeyh.	South Africa: Bremer et al. 4307 (UPS)	EU542962	EU543035	EU543117	EU557721	AM939484	AM932956	/
K. caespitosa Schnizl.	Zambia: Dessein et al. 432 (BR)	EU542963	EU543036	EU543118	EU557722	AM939474	AM932957	/
K. coccinea Royle	Zambia: Dessein et al. 751 (BR)	EU542964	EU543037	EU543119	EU557723	AM939476	AM932959	/
K. cynanchica DC.	Zambia: Dessein et al. 469 (BR)	EU542965	EU543038	EU543120	EU557724	AM939477	AM932960	/
***K. longifolia* Klotsch**	**Zambia: Dessein et al. 462 (BR)**	/	/	/	/	**AM939478**	**AM932961**	/
***K.* cf. *longifolia* Klotsch**	**Zambia: Dessein et al. 790 (BR)**	/	/	/	/	**AM939475**	**AM932958**	/
K. microcala Bremek.	Zambia: Dessein et al. 1149 (BR)	EU542966	EU543039	EU543121	EU557725	AM939479	AM932962	/
	Zambia: Dessein et al. 1321 (BR)	/	/	/	/	AM939480	AM932963	/
K. obtusiloba Schnizl.	Kenya: Luke 9035 (UPS)	EU542967	EU543040	EU543122	EU557726	AM939481	/	/
K. senegalensis Cham. & Schltdl.	Burkina Faso: Madsen 5940 (NY)	/	/	s.n.	/	/	/	/
K. subverticillata (K. Schum.) D. Mantell	Zambia: Dessein et al. 432 (BR)	EU542968	EU543041	EU543123	EU557727	AM939482	AM932964	/
K. virgata (Willd.) Bremek.	Madagascar: De Block et al. 539 (BR)	EU542969	/	EU543124	EU557728	AM939483	AM932965	/
***Lelya* Bremek.**								
L. osteocarpa Bremek.	Tanzania: Gereau 2513 (BR)	EU542970	/	EU543125	EU557729	AM939485	/	/
***Manettia* Mutis ex L.**								
M. alba (Aubl.) Wernh.	French Guiana: Andersson et al. 1917 (GB)	EU542971	AF002768[(1)]	/	/	AM939486	AM932966	/
***M. luteorubra* (Vell.) Benth.**	**Unknown: Bremer 2716 (UPS); cult. at Stockholm University**	/	/	/	**EU567463**	/	/	/
M. lygistum (L.) Sw.	Colombia: Andersson et al. 2128 (GB)	EU542972	AF002769[(1)]	EU543126	EU557730	AM939487	AM932967	/
***Manostachya* Bremek.**								
M. ternifolia E. Sampaio Martins	Zambia: Dessein et al. 265 (BR)	EU542973	EU543042	EU543127	EU557731	/	AM932968	/

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Appendix Table 1 (*continued*)

Taxon	Voucher information	*atpB-rbcL*	*rps16*	*trnL-trnF*	*petD*	ITS	ETS	5S-NTS
***Mitracarpus* Zucc. ex Schult. & Schult. f.**								
M. frigidus (Willd. ex Roem. & Schult.) K. Schum.	French Guiana: Andersson et al. 1995 (GB)	EU542974	AF002770[(1)]	EU543128	EU567464	AM939488	/	/
M. microspermus K. Schum.	Guiana: Jansen-Jacobs et al. 4785 (GB)	EU542975	EU543044	/	EU557732	AM939489	AM932969	/
***Mitrasacmopsis* Jovet**								
M. quadrivalvis Jovet	Zambia: Dessein et al. 1273 (BR)	EU542976	EU543045	EU543129	EU557733	AM939490	AM932970	/
***Nesohedyotis* (Hook. f.) Bremek.**								
N. arborea (Roxb.) Bremek.	Cult.: Chase 2915 (K)	/	AF003607[(1)]	/	/	/	/	/
***Oldenlandia* L.**								
O. affinis (Roem. & Schult.) DC.	Zambia: Dessein et al. 627 (BR)	EU542977	EU543046	EU543130	EU557734	AM939492	AM932971	/
O. angolensis K. Schum.	Zambia: Dessein et al. 932 (BR)	EU542978	EU543047	EU543131	EU557735	AM939493	AM932972	/
O. biflora (L.) Lam.	Unknown: cult. at BR	EU542979	EU567459	EU543132	EU557736	AM939494	AM932973	/
O. capensis L. f. var. *capensis*	Zambia: Dessein et al. 843 (BR)	EU542980	EU543048	EU543133	EU557737	AM939496	AM932974	/
O. capensis L. f. var. *pleiosepala* Bremek.	Tanzania: Kayombe et al. s.n. (BR)	EU542981	EU543049	EU543134	EU557738	AM939497	AM932975	/
O. corymbosa L.	Zambia: Dessein et al. 487 (BR)	EU542982	EU543050	EU543135	EU557739	AM939502	AM932979	/
	Australia: Andersson 2260 (GB)	/	/	/	/	**AM939500**	**AM932977**	/
	Gabon: Andersson & Nilsson 2263 (GB)	/	/	/	/	**AM939501**	**AM932978**	/
O. densa in ed. (*O. robinsonii* Verdc., nom. illeg.)	Zambia: Dessein et al. 346 (BR)	/	EU543061	EU543147	EU557751	AM939503	AM932980	/
O. echinulosa K. Schum.	Zambia: Dessein et al. 928 (BR)	EU542983	EU543051	EU543136	EU557740	AM939504	AM932981	/
O. echinulosa K. Schum. var. *pellucida* (Hiern) Verdc.	Tanzania: Kayombo & Kahemela 1993 (BR)	EU542984	/	EU543137	EU557741	AM939505	AM932982	/
O. fastigiata Bremek.	Zambia: Dessein et al. 1019 (BR)	EU542985	EU543052	EU543138	EU557742	AM939506	AM932983	/
O. galioides (F. Muell.) F. Muell.	Australia: Harwood 1511 (BR)	EU542986	EU543053	EU543139	EU557743	AM939507	/	/
***O.* cf. *galioides* (F. Muell.) F. Muell.**	**Australia: Harwood 1519 (BR)**	/	/	/	/	**AM939498**	/	/
O. geophila Bremek.	Zambia: Dessein et al. 935 (BR)	EU542987	EU543054	EU543140	EU557744	AM939508	/	/
O. goreensis (DC.) Summerh.	Zambia: Dessein et al. 1286 (BR)	EU542988	EU543055	EU543141	EU557745	AM939510	AM932985	/
	Zambia: Dessein et al. 455 (BR)	/	/	/	/	**AM939509**	**AM932984**	/
	Zambia: Dessein et al. 1335 (BR)	/	/	/	/	**AM939511**	/	/
	Tanzania: Richards & Arasululu 25910 (BR)	/	/	/	/	**AM939495**	/	/

Appendix Table 1 (*continued*)

Taxon	Voucher information	*atpB-rbcL*	*rps16*	*trnL-trnF*	*petD*	ITS	ETS	5S-NTS
O. herbacea (L.) Roxb. var. *goetzei* (DC.) Summerh.	Zambia: Dessein et al. 442 (BR)	EU542989	EU543056	EU543142	EU557746	AM939550	AM932986	/
	Zambia: Dessein et al. 1218 (BR)	/	/	/	/	**AM939551**	**AM932987**	/
O. herbacea (L.) Roxb. var. *herbacea*	Zambia: Dessein et al. 463 (BR)	EU542990	EU543057	EU543143	EU557747	AM939552	AM932988	/
	Zambia: Dessein et al. 1041 (BR)	/	/	/	/	**AM939553**	**AM932989**	/
O. lancifolia (Schumach.) DC.	Zambia: Dessein et al. 1356 (BR)	EU542991	EU543058	EU543144	/	AM939512	AM932990	/
***O. lancifolia* (Schumach.) DC.**	**Zambia: Dessein et al. 1256 (BR)**	/	/	/	/	**AM939499**	**AM932976**	/
O. microtheca (Cham. & Schltdl.) DC.	Mexico: Frödeström & Hultén 681 (S)	EU542992	EU543059	EU543145	EU557749	AM939513	AM932991	/
***O. mitrasacmoides* (F. Muell.) F. Muell. subsp. *nigricans* Halford**	**Australia: Harwood 1520 (BR)**	/	/	/	/	**AM939514**	**AM932993**	/
O. mitrasacmoides (F. Muell.) F. Muell. subsp. *trachymenoides* (F. Muell.) Halford	Australia: Harwood 1516 (BR)	EU542993	/	EU543146	EU557750	AM939515	AM932992	/
***O. monanthos* (Hochst. ex A. Rich.) Hiern**	**Ethiopia: Friis et al. 276 (BR)**	/	/	/	/	**AM939516**	/	/
O. nematocaulis Bremek.	Zambia: Dessein et al. 924 (BR)	EU542994	EU543060	/	/	AM939517	AM932994	/
O. nervosa Hiern	Gabon: Andersson & Nilsson 2326 (GB)	/	AF333382[(2)]	/	XX999999	AM939518	AM932995	/
O. rosulata K. Schum.	Zambia: Dessein et al. 1197 (BR)	/	EU543043	EU567467	EU567465	AM939519	/	/
O. salzmannii (DC.) Benth. & Hook. f. ex B. D. Jacks.	Brazil: Harley 15514 (UPS)	EU542995	AY764294[(3)]	EU543148	EU557752	AM939520	AM932996	/
***O.* sp. *C* Fl. Zamb.**	**Zambia: Dessein et al. 716 (BR)**	/	/	/	/	**AM939549**	**AM932997**	/
O. taborensis Bremek.	Tanzania: Bidgood et al. 4015 (BR)	EU542996	/	EU543149	EU557753	AM939522	/	/
O. tenelliflora (Blume) Kuntze	Unknown: cult. at BR	EU542997	EU543062	EU543106	EU557710	AM939451	AM932949	/
O. tenuis K. Schum.	Guyana: Jansen-Jacobs et al. 41 (UPS)	EU542998	AY764293[(3)]	/	EU557754	AM939523	/	/
O. uniflora L.	USA: Godfrey 57268 (GB)	EU542999	AY764295[(3)]	EU543150	EU557755	AM939524	/	/
O. wauensis Schweinf. ex Hiern (syn. *Thecorchus wauensis* (Schweinf. ex Hiern) Bremek.)	Ethiopia: Friis et al. 2560 (UPS)	EU543017	EU543076	EU543168	EU557774	AM939548	AM933018	/
O. wiedemannii K. Schum.	Kenya: Luke & Luke 8362 (UPS)	EU543000	EU543063	EU543151	EU557756	AM939525	AM933001	/
***Pentanisia* (*Paraknoxia*) Bremek.** (outgroup)								
P. parviflora (Stapf ex Verdc.) Verdc. ex Bremek.	Zambia: Dessein et al. 678 (BR)	EU543001	EU543064	EU543152	EU557757	/	/	/

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Appendix Table 1 (*continued*)

Taxon	Voucher information	*atpB-rbcL*	*rps16*	*trnL-trnF*	*petD*	ITS	ETS	5S-NTS
	Kenya: Verdcourt 2454 (S)	/	/	/	/	**AM267020**	/	/
***Pentanopsis* Rendle**								
P. fragrans Rendle	Ethiopia: Gilbert et al. 7458 (UPS)	/	EU543065	EU543153	EU557758	AM939526	AM933002	/
***P. gracilicaulis* (Verdc.) Thulin & B. Bremer**	**Somalia: Thulin et al. 10512 (UPS)**	**EU567458**	**EU567460**	**EU567468**	/	/	/	/
***Pentodon* Hochst.**								
P. pentandrus (K. Schum. & Thonn.) Vatke	Zambia: Dessein et al. 598 (BR)	EU543002	EU543066	EU543154	EU557759	AM939528	AM933003	/
	Zanzibar: Sundström 2 (GB)	/	/	/	/	**AM939527**	**AM933004**	/
***Phylohydrax* Puff**								
P. carnosa (Hochst.) Puff	South Africa: Bremer 3783 (UPS)	EU543003	EU543067	XX999999	EU557760	AM939529	/	/
P. madagascariensis (Willd. ex Roem. & Schult.) Puff	Madagascar: De Block et al. 640 (BR)	EU543004	AY764292[3]	EU543155	EU557761	AM939530	/	/
***Psyllocarpus* Mart. & Zucc.**								
***P. laricoides* Mart. & Zucc.**	Brazil: Andersson et al. 35750 (UPS)	/	/	/	/	AM939531	AM933005	/
***Richardia* L.**								
***R. brasiliensis* Gomes**	**Madagascar: De Block et al. 904 (BR)**	/	/	/	/	**AM939533**	**AM933007**	/
R. scabra L.	Colombia: Andersson et al. 2073 (GB)	EU543005	AF003614[1]	EU543156	EU557762	AM939532	AM933006	/
R. stellaris L.	Australia: Egeröd 85343 (GB)	EU543006	EU543068	EU543157	EU557763	AM939534	/	/
***Spermacoce* L.**								
S. capitata Ruiz & Pav.	French Guiana: Andersson 1908 (GB)	EU543007	EU543069	EU543158	EU557764	AM939536	/	/
S. confusa Rendle ex Gillis	Colombia: Andersson et al. 2074 (GB)	/	AF003619[1]	/	/	/	/	/
S. erosa Harwood	Australia: Harwood 1148 (BR)	EU543008	EU543070	EU543159	EU557765	AM939537	AM933009	/
***S. filifolia* (Schumach. & Thonn.) J.-P. Lebrun & Stork**	**Zambia: Dessein et al. 881 (BR)**	/	/	/	/	**AM939538**	**AM933010**	/
S. filituba (K. Schum.) Verdc.	Kenya: Luke 9022 (UPS)	EU543009	EU543071	EU543160	EU557766	AM939539	AM933011	/
S. flagelliformis Poir.	Madagascar: De Block et al. 794 (BR)	EU543010	EU543072	EU543161	EU557767	/	/	/
S. hispida L.	Sri Lanka: Wanntorp et al. 2667 (S)	EU543011	EU543073	EU543162	EU557768	AM939540	AM933017	/
S. ocymifolia Willd. ex Roem. & Schult. (*Hemidiodia ocymifolia* (Willd. ex Roem. & Schult.) K. Schum.)	French Guiana: Andersson et al. 2040 (GB)	EU542952	/	EU543108	EU557712	AM939463	/	/

Appendix Table 1 (*continued*)

Taxon	Voucher information	*atpB-rbcL*	*rps16*	*trnL-trnF*	*petD*	ITS	ETS	5S-NTS
	Ecuador: Bremer 3340 (UPS)	/	/	/	/	**AM939462**	**AM932951**	/
S. prostrata Aubl.	Colombia: Andersson et al. 2078 (GB)	EU543012	/	EU543163	EU557769	AM939541	AM933012	/
S. remota (Lam.) Bacigalupo & Cabral	French Guiana: Andersson et al. 2016 (GB)	EU543013	/	EU543164	EU557770	AM939542	AM933013	/
S. ruelliae DC.	Gabon: Andersson & Nilsson 2296 (GB)	EU543014	EU543074	EU543165	EU557771	AM939543	AM933014	/
***S. verticillata* L.**	**Madagascar: De Block et al. 632 (BR)**	/	/	/	/	**AM939544**	**AM933015**	/
	cult. BR; no voucher	/	/	/	/	**AM939545**	**AM933016**	/
***Stenaria* (Raf.) Terrell**								
S. nigricans (Lam.) Terrell	USA: Yatskievych 96-92 (MO)	EU543015	AF333373[(2)]	EU543166	EU557772	AM939546	/	/
***Synaptantha* Hook. f.**								
S. tillaeacea (F. Muell.) Hook. f.	Australia: Lazarides & Palmer 272 (K)	EU543016	EU543075	EU543167	EU557773	AM939547	/	/

References

Alvarez, I., Wendel, J.F., 2003. Ribosomal ITS sequences and plant phylogenetic inference. Mol. Phylogenet. Evol. 29, 417.

Andersson, L., Rova, J.H.E., 1999. The *rps16* intron and the phylogeny of the Rubioideae (Rubiaceae). Pl. Syst. Evol. 214, 161–186.

Andersson, L., Rova, J.H.E., Guarin, F.A., 2002. Relationships, circumscription, and biogeography of *Arcytophyllum* (Rubiaceae) based on evidence from cpDNA. Brittonia 54, 40–49.

APG, 1998. An ordinal classification for the families of the flowering plants. Ann. MO Bot. Gard. 85, 531–553.

APG, 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Bot. J. Linn. Soc. 141, 399–436.

Bacigalupo, N.M., Cabral, E.L., 1996. Infrageneric classification of *Borreria* (Rubiaceae-Spermacoceae) on the basis of American species. Op. Bot. Belg. 7, 294–308.

Bacigalupo, N.M., Cabral, E.L., 1999. Révision de las especies Americanas del género *Diodia* (Rubiaceae, Spermacoceae). Darwiniana 37, 153–165.

Bailey, C.D., Carr, T.G., Harris, S.A., Hughes, C.E., 2003. Characterization of angiosperm nrDNA polymorphism, paralogy, and pseudogenes. Mol. Phylogenet. Evol. 29, 435–455.

Baldwin, B.G., 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. Mol. Phylogenet. Evol. 1, 3–16.

Baldwin, B.G., Markos, S., 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: congruence of ETS and ITS trees of *Calycadenia* (Compositae). Mol. Phylogenet. Evol. 10, 449–463.

Berchtold, F., Presl, J.S., 1820. O přirozenosti Rostlin. Krala Wiljma Endersa, Prague.

Billera, L.J., Holmes, S.P., Vogtmann, K., 2001. Geometry of the space of phylogenetic trees. Adv. Appl. Math. 27, 733–767.

Bremekamp, C.E.B., 1934. Notes on the Rubiaceae of Surinam. Recueil Trav. Bot. Néerl. 31, 248–308.

Bremekamp, C.E.B., 1952. The African species of *Oldenlandia* L. sensu Hiern et K. Schumann. Verh. Kon. Ned. Akad. Wetensch., Afd. Natuurk., Sect. 2 48, 1–298.

Bremekamp, C.E.B., 1966. Remarks on the position, the delimitation and the subdivision of the Rubiaceae. Acta Bot. Neerl. 15, 1–33.

Bremer, B., Andreasen, K., Olsson, D., 1995. Subfamilial and tribal relationships in the Rubiaceae based on *rbcL* sequence data. Ann. MO Bot. Gard. 82, 383–397.

Bremer, B., 1996. Phylogenetic studies within Rubiaceae and relationships to other families based on molecular data. Op. Bot. Belg. 7, 33–50.

Bremer, B., Manen, J.-F., 2000. Phylogeny and classification of the subfamily Rubioideae (Rubiaceae). Pl. Syst. Evol. 225, 43–72.

Bremer, B., Bremer, K., Heidari, N., Erixon, P., Olmstead, R.G., Anderberg, A.A., Källersjö, M., Barkhordarian, E., 2002. Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. Mol. Phylogenet. Evol. 24, 274–301.

Bremer, B., in press. A review of molecular phylogenetic studies of Rubiaceae. Ann. MO Bot. Gard.

Burnham, K.P., Anderson, D.R., 2002. Model Selection and Multimodel Inference, A Practical Information-theoretic Approach. Springer, New York.

Cabral, E.L., 1991. Rehabilitación del género *Galianthe* (Rubiaceae). Bol. Soc. Argent. Bot. 27, 234–249.

Cabral, E.L., Bacigalupo, N.M., 2001a. *Scandentia*, nuevo género de Rubiaceae-Spermacoceae. Darwiniana 39, 29–41.

Cabral, E.L., Bacigalupo, N.M., 2001b. *Denscantia*, nuevo nombre en reemplazo de *Scandentia* (Rubiaceae-Spermacoceae. Darwiniana 39, 353.

Chevet, E., LeMaître, G., Katinka, M.D., 1995. Low concentrations of tetramethylammonium chloride increase yield and specificity of PCR. Nucleid Acids Res. 23, 3343–3344.

Church, S.A., 2003. Molecular phylogenetics of *Houstonia* (Rubiaceae): descending aneuploidy and breeding system evolution in the radiation of the lineage across North America. Mol. Phylogenet. Evol. 27, 223–238.

Cox, A.V., Bennett, M.D., Dyer, T.A., 1992. Use of the polymerase chain reaction to detect spacer size heterogeneity in plant 5S-rRNA gene clusters and to locate such clusters in wheat (*Triticum aestivum* L.). Theor. Appl. Genet. 83, 684–690.

Dessein, S., 2003. Systematic studies in the Spermacoceae (Rubiaceae). D. Phil. Thesis, Katholieke Universiteit Leuven.

Dessein, S., Andersson, L., Geuten, K., Smets, E., Robbrecht, E., 2005. *Gomphocalyx* and *Phylohydrax* (Rubiaceae): sister taxa excluded from Spermacoceae s.s., featuring a remarkable case of convergent evolution. Taxon 54, 91–107.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. Bot. Soc. Am. 19, 11–15.

Dutta, R., Deb, D.B., 2004. Taxonomic revision of *Hedyotis* L. (Rubiaceae) in Indian subcontinent. Botanical Survey of India, Kolkata, India.

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1995. Testing significance of incongruence. Cladistics 10, 315–319.

Felsenstein, J., 1968. Statistical Inference and the Estimation of Phylogenies. D. Phil. Thesis, University of Chicago, Chicago, USA.

Fosberg, F.R., 1943. The Polynesian species of *Hedyotis* (Rubiaceae). Bernice P. Bishop Mus. Bull. 147, 1–102.

Fosberg, F.R., Sachet, M.-H., 1991. Studies in Indo-Pacific Rubiaceae. Allertonia 6, 191–278.

Gielly, L., Taberlet, P., 1994. The use of chloroplast DNA to resolve plant phylogenies: noncoding versus rbcL sequences. Mol. Biol. Evol. 11, 769–777.

Govaerts, R., Ruhsam, M., Andersson, L., Robbrecht, E., Bridson, D., Davis, A., Schanzer, I., Sonké, B., 2008. World Checklist of Rubiaceae. The Board of Trustees of the Royal Botanic Gardens, Kew, Kew. Available from: <http://www.kew.org/wcsp/rubiaceae/> accessed September 2008.

Groeninckx, I., Vrijdaghs, A., Huysmans, S., Smets, E., Dessein, S., 2007. Floral ontogeny of the Afro-Madagascan genus *Mitrasacmopsis* with comments on the development of superior ovaries in Rubiaceae. Ann. Bot. 100, 41–49.

Groeninckx, I., Dessein, S., Ochoterena, H., Persson, C., Motley, T.J., Kårehed, J., Bremer, B., Huysmans, S., Smets, E., in press. Phylogeny of the herbaceous tribe Spermacoceae (Rubiaceae) based on platid DNA data. Ann. MO Bot. Gard.

Guindon, S., Gascuel, O., 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Syst. Biol. 52, 696–704.

Gustafsson, C., Persson, C., 2002. Phylogenetic relationships among species of the neotropical genus Randia (Rubiaceae, Gardenieae) inferred from molecular and morphological data. Taxon 51, 661–674.
Halford, D.A., 1992. Review of the genus *Oldenlandia* L. (Rubiaceae) and related genera in Australia. Austrobaileya 3, 683–722.
Hallé, N., 1966. Famille des Rubiacées, pt. 1. III. Hedyotidées. In: Aubréville, A. (Ed.), Flore du Gabon. Muséum National d'Histoire Naturelle, Paris, pp. 75–124.
Hillis, D.M., 1995. Approaches for assessing phylogenetic accuracy. Syst. Biol. 44, 3–16.
Hooker, J.D., 1873. Rubiaceae. In: Bentham, G., Hooker, J.D. (Eds.), Genera Plantarum. Lovell Reeve and Co., Williams and Norgate, London, pp. 7–151.
Hsienshui, L., Wancheung, K., Weichiu, C., Hsianghao, H., Hen, W., 1999. Angiospermae. Dicotyledoneae. Rubiaceae (1). In: Hsienshui, L. (Ed.), Flora Reipublicae Popularis Sinicae, Tomus 71 (1). Science Press, Beijing, pp. 1–427.
Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Huelsenbeck, J.P., Ronquist, F., Nielsen, R., Bollback, J.P., 2001. Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294, 2310–2314.
Huelsenbeck, J.P., Larget, B., Miller, R.E., Ronquist, F., 2002. Potential applications and pitfalls of Bayesian inference of phylogeny. Syst. Biol. 51, 673–688.
Jovet, P., 1941. Aux confins des Rubiacées et des Loganiacées. Notulae Systematicae 10, 39–56.
Kårehed, J., Bremer, B., 2007. The systematics of Knoxieae (Rubiaceae)—molecular data and their taxonomic consequences. Taxon 56, 1051–1076.
Lewis, W.H., 1962. Phylogenetic study of *Hedyotis* (Rubiaceae) in North America. Am. J. Bot. 49, 855–865.
Lewis, W.H., 1965a. Cytopalynological studies of African Hedyotideae (Rubiaceae). Ann. MO Bot. Gard. 52, 182–211.
Lewis, W.H., 1965b. Pollen morphology and evolution in *Hedyotis* subg. *Edrisia* (Rubiaceae). Am. J. Bot. 52, 257–264.
Löhne, C., Borsch, T., 2005. Molecular evolution and phylogenetic utility of the *petD* group II Intron: a case study in basal angiosperms. Mol. Biol. Evol. 22, 317–332.
Mantell, D.E., 1985. The Afro-South-West Asiatic Genus *Kohautia* Cham. & Schlechtd. (Rubiaceae—Rubioideae—Hedyotideae): Morphology, Anatomy, Taxonomy, Phytogeography and Evolution. D. Phil. Thesis, Universität Wien, Vienna, Austria.
Markey, A.S., de Lange, P.J., 2003. A new species of *Coprosma* Sect. *Acerosae* (Rubiaceae) endemic to the South and Stewart Islands of New Zealand. NZ J. Bot. 41, 459–473.
Merrill, E.D., Metcalf, F.P., 1942. *Hedyotis* Linnaeus versus *Oldenlandia* Linnaeus and the status of *Hedyotis lancea* Thunberg in relation to *H. consanguinea* Hance. J. Arnold Arbor. 23, 226–230.
Mort, M.E., Archibald, J.K., Randle, C.P., Levsen, N.D., O'Leary, T.R., Topalov, K., Wiegand, C.M., Crawford, D.J., 2007. Inferring phylogeny at low taxonomic levels: utility of rapidly evolving cpDNA and nuclear ITS loci. Am. J. Bot. 94, 173–183.
Motley, T.J., 2003. Phylogeny of Hawaiian and Pacific *Hedyotis* (Rubiaceae): fruit evolution and the implications for conservation and genomics. Abstracts of Annual Meeting, Mobile, Alabama, Botany 2003, pp. 88–89.
Motley, T.J., Wurdack, K.J., Delprete, P.G., 2005. Molecular systematics of the Chiococceae–Catesbaeeae complex (Rubiaceae): flower and fruit evolution and systematic implications. Am. J. Bot. 92, 316–329.
Motley, T.J., in press. Phylogeny of *Kadua* (=*Hedyotis*): origins, biogeography, and subgeneric classification. Ann. MO Bot. Gard.
Musters, W., Boon, K., Van Der Sande, C.A.F.M., Van Heerikhuizen, H., Planta, R.J., 1990. Functional analysis of transcribed spacers of yeast ribosomal DNA. EMBO J. 9, 3989.
Müller, K., 2005. SeqState: primer design and sequence statistics for phylogenetic DNA datasets. Appl. Bioinformatics 4, 65–69.
Müller, K.F., Borsch, T., Hilu, K.W., 2006. Phylogenetic utility of rapidly evolving DNA at high taxonomical levels: contrasting *matK*, *trnT-F*, and *rbcL* in basal angiosperms. Mol. Phylogenet. Evol. 41, 99–117.
Negrón-Ortiz, V., Watson, L.E., 2002. Molecular phylogeny and biogeography of Erithalis (Rubiaceae), an endemic of the Caribbean Basin. Pl. Syst. Evol. 234, 71–83.
Nepokroeff, M., Sytsma, K.J., Wagner, W.L., Zimmer, E.A., 2003. Reconstructing ancestral patterns of colonization and dispersal in the Hawaiian understory tree genus *Psychotria* (Rubiaceae): a comparison of parsimony and likelihood approaches. Syst. Biol. 52, 820–838.
Nixon, K.C., Carpenter, J.M., 1996. On simultaneous analysis. Cladistics 12, 221–241.
Nylander, J.A.A., 2004. MrAIC.pl. Program Distributed by the Author. Evolutionary Biology Centre. Uppsala University, Uppsala, Sweden.
Oxelman, B., 1996. RAPD patterns, nrDNA ITS sequences and morphological patterns in *Silene* section *Sedoideae* (Caryophyllaceae). Pl. Syst. Evol. 201, 93–116.
Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20, 289–290.
Penny, D., Hendy, M.D., 1985. The use of tree comparison metrics. Syst. Zool. 34, 75–82.
Persson, C., 2000. Phylogeny of the neotropical *Alibertia* group (Rubiaceae), with emphasis on the genus *Alibertia*, inferred from ITS and 5S ribosomal DNA sequences. Am. J. Bot. 87, 1018–1028.
Popp, M., Oxelman, B., 2001. Inferring the history of the polyploid *Silene aegaea* (Caryophyllaceae) using plastid and homoeologous nuclear DNA sequences. Mol. Phylogenet. Evol. 20, 474–481.
R Development Core Team, 2007. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Razafimandimbison, S.G., Moog, J., Lantz, H., Maschwitz, U., Bremer, B., 2005. Re-assessment of monophyly, evolution of myrmecophytism, and rapid radiation in *Neonauclea* s.s. (Rubiaceae). Mol. Phylogenet. Evol. 34, 334.
Ridsdale, C.E., 1998. Rubiaceae. In: Dassanayake, M.D. (Ed.), A revised Handbook to the Flora of Ceylon. A.A. Balkema, Rotterdam/Brookfield, pp. 141–343.
Robbrecht, E., 1988. Tropical woody Rubiaceae. Op. Bot. Belg. 1, 1–271.
Robbrecht, E., 1994. Supplement to the 1988 outline of the classification of the Rubiaceae. Index to genera. Op. Bot. Belg. 6, 173–196.
Robbrecht, E., Manen, J.-F., 2006. The major evolutionary lineages of the coffee family (Rubiaceae, angiosperms). Combined analysis (nDNA and cpDNA) to infer the position of *Coptosapelta* and *Luculia*, and supertree construction based on *rbcL*, *rps16*, *trnL-F* and *atpB-rbcL* data. Syst. Geogr. Pl. 76, 85–146.
Robinson, D.F., Foulds, L.R., 1981. Comparison of phylogenetic trees. Math. Biosci. 53, 131.
Ronquist, F., Huelsenbeck, J.P., 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19, 1572–1574.
Rzhetsky, A., Nei, M., 1992. A simple method for estimating and testing minimum-evolution trees. Mol. Biol. Evol. 9, 945–967.
Sastri, D.C., Hilu, K., Appels, R., Lagudah, E.S., Playford, J., Baum, B.R., 1992. An overview of evolution in plant 5S DNA. Pl. Syst. Evol. 183, 169–181.
Scheltens, A., 1998. Pollenmorfologische studie van de Afrikaanse Hedyotideae (Rubiaceae). D. Phil. Thesis, Katholieke Universiteit Leuven, Leuven, Belgium.
Schumann, K.M., 1888. Rubiaceae iia. In: von Martius and auct. suc., C.F.P. (Eds.), Flora Brasiliensis 6. R. Oldenburg, Munich, Wien, and Leipzig, pp. 1-124.
Schumann, K.M., 1891. Rubiaceae. In: Engler, A., Prantl, K. (Eds.), Die natürlichen Pflanzenfamilien, vol. 4 (4). Engelmann, Leipzig, pp. 1–156.
Shaw, J., Lickey, E.B., Beck, J.T., Farmer, S.B., Liu, W., Miller, J., Siripun, K.C., Winder, C.T., Schilling, E.E., Small, R.L., 2005. The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. Amer. J. Bot. 92, 142–166.
Shaw, J., Lickey, E.B., Schilling, E.E., Small, R.L., 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. Amer. J. Bot. 94, 275–288.
Simmons, M.P., Ochoterena, H., 2000. Gaps as characters in sequence-based phylogenetic analyses. Syst. Biol. 49, 369–381.
Soltis, D.E., Soltis, P.S., 1998. Choosing an approach and an appropriate gene for phylogenetic analysis. In: Soltis, D.E., Soltis, P.S., Doyle, J.J. (Eds.), Molecular Systematics of Plants II—DNA Sequencing. Kluwer Academic Publishers, Boston, Dordrecht, London, pp. 1–42.
Steel, M.A., Penny, D., 1993. Distributions of tree comparison metrics-some new results. Syst. Biol. 42, 126–141.
Strand, A.E., Leebens-Mack, J., Milligan, B.G., 1997. Nuclear DNA-based markers for plant evolutionary biology. Mol. Ecol. 6, 113–118.
Terrell, E.E., 1975. Relationships of *Hedyotis fruticosa* L. to *Houstonia* L. and *Oldenlandia* L. Phytologia 31, 418–424.
Terrell, E.E., 1987. *Carterella* (Rubiaceae), new genus from Baja California, Mexico. Brittonia 39, 248–252.
Terrell, E.E., 1991. Overview and annotated list of North American species of *Hedyotis*, *Houstonia*, *Oldenlandia* (Rubiaceae), and related genera. Phytologia 71, 212–243.
Terrell, E.E., 2001a. Taxonomy of *Stenaria* (Rubiaceae: Hedyotideae), a new genus including *Hedyotis nigricans*. Sida 19, 591–614.
Terrell, E.E., 2001b. *Stenotis* (Rubiaceae), a new segregate genus from Baja California, Mexico. Sida 19, 899–911.
Terrell, E.E., 2001c. Taxonomic review of *Houstonia acerosa* and *H. palmeri*, with notes on *Hedyotis* and *Oldenlandia* (Rubiaceae). Sida 19, 913–922.
Terrell, E.E., Robinson, H.E., Wagner, W.L., Lorence, D.H., 2005. Resurrection of genus *Kadua* for Hawaiian Hedyotidinae (Rubiaceae), with emphasis on seed and fruit characters and notes on South Pacific species. Syst. Bot. 30, 818–833.
Thulin, M., Bremer, B., 2004. Studies in the tribe Spermacoceae (Rubiaceae-Rubioideae): the circumscriptions of *Amphiasma* and *Pentanopsis* and the affinities of *Phylohydrax*. Pl. Syst. Evol. 247, 233–239.
Verdcourt, B., 1958. Remarks on the classification of the Rubiaceae. Bull. Jard. Bot. État. 28, 209–281.
Verdcourt, B., 1976. Rubiaceae (part 1). In: Polhill, R.M. (Ed.), Flora of Tropical East Africa. Crown Agents for Oversea Governments and Administrations, London, pp. 1–414.
Waterman, M.S., Smith, T.F., 1978. On the similarity of dendrograms. J. Theor. Biol. 73, 789–800.
Wenzel, J.W., Siddall, M.E., 1999. Noise. Cladistics 15, 51–64.
White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M., Gelfand, D., Sninsky, J., White, T. (Eds.), PCR protocols: a guide to methods and applications. Academic Press, San Diego, pp. 315–322.