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## Rare and enigmatic genera (*Dunnia*, *Schizocolea*, *Colletocema*), sisters to species-rich clades: Phylogeny and aspects of conservation biology in the coffee family

Catarina Rydin<sup>a,b,\*</sup>, Sylvain G. Razafimandimbison<sup>b</sup>, Birgitta Bremer<sup>b</sup>

<sup>a</sup>University of Zürich, Institute of Systematic Botany, Zollikerstrasse 107, CH-8008 Zürich, Switzerland

<sup>b</sup>Bergius Foundation, Royal Swedish Academy of Sciences and Department of Botany, Stockholm University, SE-106 91 Stockholm, Sweden

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### ABSTRACT

Despite extensive efforts, parts of the phylogeny of the angiosperm family Rubiaceae has not been resolved and consequently, character evolution, ancestral areas and divergence times of major radiations are difficult to estimate. Here, phylogenetic analyses of 149 taxa and five plastid gene regions show that three enigmatic genera are sisters to considerably species rich clades.

The rare and endangered species *Dunnia*, endemic to southern Guangdong, China, is sister to a large clade in the Spermaceae alliance; the rarely collected *Schizocolea* from western tropical Africa is sister to the Psychotriaceae alliance; and *Colletocema* from central tropical Africa is sister to remaining Rubioideae. The morphology of these taxa has been considered “puzzling”. In combination with further morphological studies, our results may help understanding the apparently confusing traits of these plants.

Phylogenetic, morphological, and geographical isolation of *Dunnia*, *Schizocolea* and *Colletocema* may indicate high genetic diversity. They are lone representatives of unique lineages and if extinct, the loss would not only mean loss of genetic diversity of a single species but of an entire lineage.

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### 1. Introduction

Despite extensive efforts there are still today unresolved dilemmas, parts of the tree of life within which evolutionary events have not been confidently reconstructed. Consequently, classification and circumscription of these groups are inadequate and issues such as character evolution, ancestral areas and divergence times of major radiations cannot be estimated. In Rubiaceae, the coffee family, there are several such unresolved questions.

Rubiaceae is one of the largest families of flowering plants; the family comprises more than 13,000 species (Govaerts et al., 2006). Distribution is worldwide, with a particularly high diversity in the tropics and subtropics. The species of Rubiaceae are easily recognized with (generally) opposite branching and phylotaxis, interpetiolar stipules and epigynous flowers.

Recent studies generally recognise three major lineages within Rubiaceae (Andersson and Rova, 1999; Bremer, 1996b; Bremer et al., 1995, 1999; Robbrecht and Manen, 2006; Rova et al., 2002), often referred to as subfamilies Rubioideae, Ixoroideae and Cinchonoideae sensu Bremer et al. (1999). Subsequent studies have

further investigated relationships within these subfamilies, for example Bremer and Manen (2000, Rubioideae), Andreasen and Bremer (2000, Ixoroideae) and Andersson and Antonelli (2005, Cinchonoideae). Two South East Asian genera, *Luculia* Sweet and *Coptosapelta* Kort., are in most studies excluded from the subfamilial clades (see e.g. Bremer et al., 1999; Robbrecht and Manen, 2006).

However, despite these extensive efforts, several questions on evolutionary relationships within Rubiaceae have remained unanswered and intrafamilial phylogeny and character evolution are still not fully comprehended. We introduce three small genera here, whose systematic affinities are uncertain or unknown and for which morphological features have been poorly known or have appeared puzzling from an evolutionary perspective.

#### 1.1. *Dunnia*

*Dunnia sinensis* Tutch. was first described in 1905 (Tutcher, 1905). The genus is monotypic; the single species is endemic to the southern Guangdong Province of China (Chen, 1999). It is seriously threatened due to human exploitation of its natural habitats along streams and hillsides at low altitudes (Chiang et al., 2002; Ge et al., 2002). Only five isolated populations remain (Chiang et al., 2002; Ge et al., 2002). It is a woody scrub with pentamerous flowers in clusters.

\* Corresponding author. Address: Bergius Foundation, Royal Swedish Academy of Sciences and Department of Botany, Stockholm University, SE-106 91 Stockholm, Sweden. Fax: +46 8 165525.

E-mail address: [Catarina.Rydin@botan.su.se](mailto:Catarina.Rydin@botan.su.se) (C. Rydin).

To our knowledge, this endangered Chinese plant has not been included in any previous phylogenetic study. We have included five specimens of *Dunnia sinensis* from five different localities in this study. The vouchers have previously been used in a study of sequence divergences between populations (Ge et al., 2002).

### 1.2. *Schizocolea*

The enigmatic rubiaceous genus *Schizocolea* Bremek. has rarely been included in molecular phylogenetic studies, due to lack of material. *Schizocolea* is endemic to West and Central tropical Africa (Govaerts et al., 2006). It is a small tree with white, pentamerous flowers. The genus was originally described as monotypic, comprising only *Schizocolea linderi* (Hutch. and Dalziel) Bremek. (Bremekamp, 1950), distributed in Sierra Leone, Liberia and the Ivory Coast. Petit (1962) described an additional species, *Schizocolea ochreatea* E.M.A. Petit, restricted to Congo and Gabon.

We have used material of *Schizocolea linderi* from a voucher collected by J. G. Adam in Liberia in 1964 and sequenced the gene regions included in this study, in order to further investigate the systematic position of this species.

### 1.3. *Colletoecema*

*Colletoecema* E.M.A. Petit is a monotypic genus of trees or scrubs with pentamerous flowers in axillary clusters (Petit, 1963). The single species, *C. dewevrei* (De Wild.) E.M.A. Petit, is endemic to the West and Central regions of tropical Africa (Govaerts et al., 2006). Classification has mainly been based on embryological and endosperm characters, and has differed between authors (see e.g. de Wildeman, 1904; Petit, 1963). Robbrecht (1993) considered the position of *Colletoecema* uncertain due to lack of data.

Piesschaert et al. (2000a) made a thorough study of the morphology and anatomy of *Colletoecema* and used this information in combination with *rps16* intron data to investigate its phylogenetic position. Their molecular analysis weakly supported a sister relationships between *Colletoecema* and *Ophiorrhiza* L. In Robbrecht and Manen (2006), analysis of *atpB-rbcL* spacer data resolved *Colletoecema* as sister to remaining species in Rubioideae.

We test these hypotheses on the phylogenetic position of *Colletoecema*, using a larger set of gene regions and species.

### 1.4. Focus of this study

Here we use a data set comprising 149 terminals and 9630 characters from five plastid gene regions to address the systematic positions of *Dunnia*, *Schizocolea* and *Colletoecema*. Sequences of the rarely collected and poorly known *Schizocolea* are here published for the first time. *Dunnia* has been investigated for intra-specific variation but has never been included in a phylogenetic study.

*Dunnia* has been shown to be highly endangered (Chiang et al., 2002; Ge et al., 2002). The status of *Schizocolea* and *Colletoecema* are not known but all three genera are endemic to restricted geographic areas. The connection between phylogeny and conservation biology is discussed.

## 2. Material and methods

### 2.1. Selection of species and laboratory procedures

To cover the diversity in Rubiaceae relatively well, and thus be able to place taxa with unknown affinity, we selected 149 taxa for this study (Table 1). The taxa represent the major clades within Rubiaceae, with special emphasis on Rubioideae. We included 85

specimens from Rubioideae, 26 species of Ixoroideae, 11 species of Cinchonoideae, and in addition 19 specimens from genera outside of the three subfamilies. Eight outgroup taxa from the sister group of Rubiaceae (the other families within Gentianales, Backlund et al., 2000) were selected.

We utilized information from five chloroplast regions (*rbcl*, *ndhF*, the *trnT-L-F* region, the *rps16* intron, *atpB-rbcL* spacer). We used information from GenBank when available but we also produced 31 new sequences from *Colletoecema*, *Dunnia* and *Schizocolea*. GenBank accession numbers are given in Table 1. DNA was extracted, amplified and sequenced using standard procedures previously described (Kårehed and Bremer, 2007). For *ndhF*, *trnT-L-F* and *atpB-rbcL* spacer, new primers were designed for this study (Table 2). For *rbcl* and *rps16* primers from previous studies were used (Table 2). Sequence fragments were assembled using the Staden package (Staden, 1996).

### 2.2. Alignment and phylogenetic reconstruction

Sequences were aligned using software Se-Al v.2.0 (Rambaut, 1996). Insertion/deletion events were inferred by eye. Gaps were treated as missing data in the alignment and were added as binomial characters (absent or present) at the end of the matrix.

We analysed each gene separately, including and excluding information from indels. Further, all matrices were analysed with two approaches: Bayesian inference and parsimony. Each specific analysis was run twice.

Bayesian analyses were performed in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). For each single gene data set, the best performing evolutionary models were identified under three different model selection criteria: Akaike information criterion (AIC) (Akaike, 1973), AICc (a second order AIC, necessary for small samples) and the Bayesian information criterion (BIC) (Schwartz, 1978). For a review of these criteria, see Burnham and Andersson (2002). We performed these calculations in software MrAIC ver. 1.4.3 (Nylander, 2004). Indels were treated as a morphological partition.

For single gene analyses, the best performing model under the AICc criterion was selected (Table 1), one million generations were run, with a sample frequency of 1000 and four parallel chains. A flat Dirichlet prior probability (all values are 1.0) was selected for the substitution rates (revmatpr) and the nucleotide frequencies (statefreqpr). The prior probability for the shape parameter of the gamma distribution of rate variation (shapepr) was uniformly distributed in the interval (0.1, 50.0). For analyses using a gamma distribution with a proportion of invariable sites, we specified a prior probability for this proportion (pinvarpr), uniformly distributed on the interval (0.0, 1.0).

For combined analyses, model selection and settings were selected in the same way as for single gene analyses (see above), but in order to ensure that the analyses represented an adequate sample of the posterior distribution, three million generations were run. Indels constituted a separate morphological partition as before, and partitions were unlinked so that each partition was allowed to have its own set of parameters.

Parsimony analyses were performed for single gene data sets, as well as for the combined data set, in Paup\* version 4.0b10 for Unix (Swofford, 1998). Most parsimonious trees were calculated using the heuristic search option, 500 random sequence additions and tree bisection reconnection branch swapping. Support values were obtained by using bootstrap in Paup\*, performing 1000 bootstrap replicates, each with 10 random sequence additions. A majority rule consensus tree was produced from the resulting trees, in which nodes with a bootstrap support <50% were collapsed.

**Table 1**  
List of investigated taxa, voucher information, classification and accession numbers

Taxon	Voucher (of previously unpublished sequences)	Classification	<i>rbcl</i>	<i>rps16</i>	<i>ndhF</i>	<i>atpB-rbcl</i> spacer	<i>trnT/F</i>
<i>Aidia micrantha</i> (K.Schum.) Bullock ex F.White		Ixo-Gardenieae	Z68844 <sup>19</sup>	AF200974 <sup>29</sup>	—	—	AF201028 <sup>29</sup>
<i>Alberta magna</i> E.Mey.	Tonkin 200 (UPS) <sup>36</sup>	Ixo-Albertheae	Y18708 <sup>17</sup>	EU145491 <sup>†</sup>	AJ236282 <sup>16</sup>	—	AJ620118 <sup>47</sup>
<i>Alstonia</i>		APOCYNACEAE	X91760 <sup>7</sup>	AJ431032 <sup>4</sup>	AJ011982 <sup>5</sup>	DQ359161 <sup>6</sup>	AJ430907 <sup>4</sup>
<i>Amphidasya ambigua</i> (Standl.) Standl.	Clark & Watt 736 (UPS)	Rub-Urophyllaeae	Y11844 <sup>14</sup>	AF129271 <sup>24</sup>	—	EU145337 <sup>†</sup>	EU145576 <sup>†</sup>
<i>Anthocleista</i>		GENTIANACEAE	L14389 <sup>8</sup>	—	AJ235829 <sup>9</sup>	DQ131695 <sup>6</sup>	AJ490190 <sup>44</sup>
<i>Anthospermum herbaceum</i> L.f.	Bremer, 3093 (UPS)	Rub-Anthospermeae	X83623 <sup>1</sup>	EU145496 <sup>†</sup>	AJ236284 <sup>16</sup>	AJ234028 <sup>2</sup>	EU145544 <sup>†</sup>
<i>Arcytophyllum aristatum</i> Standl.		Rub-Spermacoaceae	AJ288595 <sup>2</sup>	AF333348 <sup>20</sup>	—	—	AF333349 <sup>20</sup>
<i>Argostemma hookeri</i> King	Malaysia, Wanntorp s.n. (S)	Rub-Argostemmataeae	Z68788 <sup>21</sup>	EU145497 <sup>†</sup>	EU145419 <sup>†</sup>	AJ234032 <sup>2</sup>	EU145545 <sup>†</sup>
<i>Batopedina pulvinellata</i> Robbr.		Rub-Knoxieae	AJ288596 <sup>2</sup>	AM266813 <sup>37</sup>	—	—	AM266902 <sup>37</sup>
<i>Bertiera guianensis</i> Aubl.		Ixo-Bertiereae	AJ224845 <sup>17</sup>	AF200983 <sup>29</sup>	—	—	AF152670 <sup>12</sup>
<i>Bouvardia glaberrima</i> Engelm. (accepted name <i>Bouvardia ternifolia</i> (Cav.) Schltld.)		Rub-Spermacoaceae	X83626 <sup>1</sup>	AF002758 <sup>11</sup>	—	X76478 <sup>41</sup>	DQ359165 <sup>6</sup>
<i>Calycophyllum candidissimum</i> (Vahl) DC.		Ixo-Condamineae	X83627 <sup>1</sup>	AF004030 <sup>11</sup>	AJ236285 <sup>16</sup>	DQ131708 <sup>6</sup>	AF152646 <sup>12</sup>
<i>Carphalea glaucescens</i> (Hiern) Verdc. (accepted name <i>Dirichletia glaucescens</i> Hiern)		Rub-Knoxieae	Z68789 <sup>21</sup>	AM266817 <sup>37</sup>	AJ236287 <sup>16</sup>	—	AM266906 <sup>37</sup>
<i>Catesbaea spinosa</i> L.		Cinch-Chiococceae	X83628 <sup>1</sup>	AF004032 <sup>11</sup>	AM117343 <sup>35</sup>	—	AF152706 <sup>12</sup>
<i>Cephalanthus occidentalis</i> L.		Cinch-Naucleeae	X83629 <sup>1</sup>	AF004033 <sup>11</sup>	AJ236288 <sup>16</sup>	DQ131710 <sup>6</sup>	AF152692 <sup>12</sup>
<i>Chiococca alba</i> (L.) Hitchc.		Cinch-Chiococceae	L14394 <sup>8</sup>	AF004034 <sup>11</sup>	AJ130835 <sup>16</sup>	DQ131711 <sup>6</sup>	AY763813 <sup>13</sup>
<i>Cinchona pubescens</i> Vahl		Cinch-Chiococceae	X83630 <sup>1</sup>	AF004035 <sup>11</sup>	AJ235843 <sup>9</sup>	AJ233990 <sup>2</sup>	AJ346963 <sup>3</sup>
<i>Coccocypselum condalia</i> Pers.	Pirani & Bremer 4891 (SPF)	Rub-Coussareae	AM117217 <sup>35</sup>	EU145499 <sup>†</sup>	EU145420 <sup>†</sup>	EU145324 <sup>†</sup>	EU145547 <sup>†</sup>
<i>Coccocypselum hirsutum</i> Bartl. ex DC.	CT 908, Bremer 2700 (S)	Rub-Coussareae	X87145 <sup>22</sup>	EU145500 <sup>†</sup>	EU145421 <sup>†</sup>	EU145325 <sup>†</sup>	EU145548 <sup>†</sup>
<i>Coffea arabica</i> L.		Ixo-Coffeae	X83631 <sup>1</sup>	AF004038 <sup>11</sup>	AJ236290 <sup>16</sup>	X70364 <sup>40</sup>	DQ153845 <sup>46</sup>
<i>Colletocema dewevrei</i> (De Wild.) E.M.A.Petit	S Lisowski 47195 (K)	Rub-Colletocemaeae <sup>***</sup>	EU145457 <sup>**</sup>	AF129272 <sup>24</sup>	EU145409 <sup>**</sup>	DQ131713 <sup>6</sup>	EU145532 <sup>**</sup>
<i>Condaminea corymbosa</i> (Ruiz & Pav.) DC.		Ixo-Condamineae	Y18713 <sup>16</sup>	AF004039 <sup>11</sup>	AJ236291 <sup>16</sup>	—	AF102406 <sup>43</sup>
<i>Coptosapelta diffusa</i> (Champ.) Steenis (specimen 1)	Bartholomew et al 847 (AAU)	—Coptosapelteae	EU145452 <sup>†</sup>	EU145482 <sup>†</sup>	EU145403 <sup>†</sup>	EU145315 <sup>†</sup>	EU145527 <sup>†</sup>
<i>Coptosapelta diffusa</i> (Champ.) Steenis (specimen 2)	Steward et al. 594 (S)	—Coptosapelteae	EU145453 <sup>†</sup>	EU145483 <sup>†</sup>	EU145404 <sup>†</sup>	AJ233987 <sup>2</sup>	DQ359166 <sup>6</sup>
<i>Coptosapelta flavescens</i> Korth. (specimen 1)	Puff 950720-1/2 (WU)	—Coptosapelteae	Y18714 <sup>16</sup>	EU145484 <sup>†</sup>	AJ236292 <sup>16</sup>	EU145316 <sup>†</sup>	AM117354 <sup>35</sup>
<i>Coptosapelta flavescens</i> Korth. (specimen 2)	Gardette et al. EG1716 (K)	—Coptosapelteae	EU145454 <sup>†</sup>	EU145485 <sup>†</sup>	EU145405 <sup>†</sup>	EU145317 <sup>†</sup>	EU145528 <sup>†</sup>
<i>Coptosapelta flavescens</i> Korth. (specimen 3)	Larsen et al 31147 (AAU)	—Coptosapelteae	—	EU145488 <sup>†</sup>	EU145408 <sup>†</sup>	—	EU145531 <sup>†</sup>
<i>Coptosapelta montana</i> Korth. ex Valetton	Clemens & Clemens 40864 (K)	—Coptosapelteae	EU145451 <sup>†</sup>	EU145481 <sup>†</sup>	EU145402 <sup>†</sup>	EU145314 <sup>†</sup>	EU145526 <sup>†</sup>
<i>Coptosapelta tomentosa</i> Valetton ex K.Heyne (specimen 1) (accepted name <i>Coptosapelta flavescens</i> Korth.)	Beusekom & Charoenpol 1741 (AAU)	—Coptosapelteae	EU145455 <sup>†</sup>	EU145486 <sup>†</sup>	EU145406 <sup>†</sup>	EU145318 <sup>†</sup>	EU145529 <sup>†</sup>
<i>Coptosapelta tomentosa</i> Valetton ex K.Heyne (specimen 2) (accepted name <i>Coptosapelta flavescens</i> Korth.)	Beusekom & Charoenpol 1933 (AAU)	—Coptosapelteae	EU145456 <sup>†</sup>	EU145487 <sup>†</sup>	EU145407 <sup>†</sup>	EU145319 <sup>†</sup>	EU145530 <sup>†</sup>
<i>Coussarea hydrangeifolia</i> (Benth.) Benth. & Hook.f. ex Müll.Arg.	Fuentes 5504 (GB)	Rub-Coussareae	EU145460 <sup>†</sup>	EU145501 <sup>†</sup>	EU145422 <sup>†</sup>	EU145326 <sup>†</sup>	EU145549 <sup>†</sup>
<i>Coussarea macrophylla</i> (Mart.) Müll.Arg.		Rub-Coussareae	Y11847 <sup>14</sup>	AF004040 <sup>11</sup>	—	—	AF152612 <sup>12</sup> (C. sp)
<i>CreMASpora triflora</i> (Thonn.) K.Schum.		Ixo-Cremasporaeae	Z68856 <sup>19</sup>	AF200990 <sup>29</sup>	—	DQ131718 <sup>6</sup>	AF201040 <sup>29</sup>
<i>Cremocarpon lantzii</i> Bremek.	Razafimandimbison 517 (UPS)	Rub-Psychotriaceae	AM117222 <sup>35</sup>	AM117296 <sup>35</sup>	—	—	AM117356 <sup>35</sup>
<i>Cruckshanksia hymenodon</i> Hook. & Arn.	Rodríguez 10 (K)	Rub-Coussareae	AJ288599 <sup>2</sup>	EU145502 <sup>†</sup>	—	AJ234004 <sup>2</sup>	EU145550 <sup>†</sup>
<i>Cubanola domingensis</i> (Britton) Aiello		Cinch-Chiococceae	X83632 <sup>1</sup>	AF004044 <sup>11</sup>	AM117345 <sup>35</sup>	DQ131720 <sup>6</sup>	AF152701 <sup>12</sup>
<i>Damnacanthus indicus</i> C.F.Gaertn.		Rub-Morindeae	Z68793 <sup>21</sup>	AF331647 <sup>20</sup>	—	AJ234015 <sup>2</sup>	—
<i>Danais xanthorrhoea</i> (K.Schum.) Bremek.	Bremer 3079 (UPS)	Rub-Danaideae	Z68794 <sup>21</sup>	AM117297 <sup>35</sup>	AJ236293 <sup>16</sup>	AJ234019 <sup>2</sup>	DQ662138 <sup>32</sup>
<i>Declieuxia cordigera</i> Mart. & Zucc. ex Schult. & Schult.f.	Pirani & Bremer 4893 (SPF)	Rub-Coussareae	AM117224 <sup>35</sup>	AM117298 <sup>35</sup>	EU145423 <sup>†</sup>	EU145327 <sup>†</sup>	EU145551 <sup>†</sup>
<i>Declieuxia fruticosa</i> (Willd. ex Roem. & Schult.) Kuntze	B. Hammel 20875 (MO, CR)	Rub-Coussareae	AJ002177 <sup>23</sup>	EU145503 <sup>†</sup>	—	DQ131721 <sup>6</sup>	EU145552 <sup>†</sup>
<i>Dentella repens</i> (L.) J.R.Forst. & G.Forst.		Rub-Spermacoaceae	—	AF333370 <sup>20</sup>	—	—	AF381540 <sup>49</sup>
<i>Dibrachionostylus kaessneri</i> (S.Moore) Bremek.	Strid 2564 (UPS)	Rub-Spermacoaceae	AJ616211 <sup>28</sup>	AF002761 <sup>11</sup>	—	—	EU145574 <sup>†</sup>
<i>Didymaea alsinoides</i> (Cham. & Schltld.) Standl.	Keller 1901 (CAS)	Rub-Rubieae	Z68795 <sup>21</sup>	—	—	AJ234036 <sup>2</sup>	EU145570 <sup>†</sup>
<i>Diplospora polysperma</i> Valetton	Ridsdale IV.E.130 (L)	Ixo-Coffeae	AJ286703 <sup>18</sup>	AM117301 <sup>35</sup>	—	—	EU145538 <sup>†</sup>
<i>Dunnia sinensis</i> Tutcher (Specimen 1)	Yangchun 10, Ge et al. 2002	Rub-Dunnieae <sup>***</sup>	EU145467 <sup>**</sup>	EU145515 <sup>**</sup>	EU145442 <sup>**</sup>	EU145339 <sup>**</sup>	EU145583 <sup>**</sup>
<i>Dunnia sinensis</i> Tutcher (Specimen 2)	Taishan 10, Ge et al. 2002	Rub-Dunnieae <sup>***</sup>	EU145468 <sup>**</sup>	EU145516 <sup>**</sup>	EU145443 <sup>**</sup>	EU145340 <sup>**</sup>	EU145584 <sup>**</sup>
<i>Dunnia sinensis</i> Tutcher (Specimen 3)	Zhuhai 12, Ge et al. 2002	Rub-Dunnieae <sup>***</sup>	EU145469 <sup>**</sup>	EU145517 <sup>**</sup>	EU145444 <sup>**</sup>	EU145341 <sup>**</sup>	EU145585 <sup>**</sup>
<i>Dunnia sinensis</i> Tutcher (Specimen 4)	Longmen 12, Ge et al. 2002	Rub-Dunnieae <sup>***</sup>	EU145470 <sup>**</sup>	EU145518 <sup>**</sup>	EU145445 <sup>**</sup>	EU145342 <sup>**</sup>	EU145586 <sup>**</sup>
<i>Dunnia sinensis</i> Tutcher (Specimen 5)	Xinhui 16, Ge et al. 2002	Rub-Dunnieae <sup>***</sup>	EU145471 <sup>**</sup>	EU145519 <sup>**</sup>	EU145446 <sup>**</sup>	EU145343 <sup>**</sup>	EU145587 <sup>**</sup>
<i>Emmenopterys henryi</i> Oliv.		Ixo-Condamineae	Y18715 <sup>16</sup>	AM117302 <sup>35</sup>	AJ236294 <sup>16</sup>	DQ131728 <sup>6</sup>	AF152637 <sup>12</sup>
<i>Ernodea littoralis</i> Sw.		Rub-Spermacoaceae	AJ288601 <sup>2</sup>	AF002763 <sup>11</sup>	—	AJ234025 <sup>2</sup>	—
<i>Faramaea multiflora</i> A.Rich.	Bremer et al. 3331 (UPS)	Rub-Coussareae	Z68796 <sup>21</sup>	AF004048 <sup>11</sup>	EU145424 <sup>†</sup>	EU145328 <sup>†</sup>	AF102422 <sup>43</sup>
<i>Ferdinandusa speciosa</i> Pohl	Malme 2442 (UPS)	Ixo-Condamineae	AM117226 <sup>35</sup>	AM117304 <sup>35</sup>	EU145412 <sup>†</sup>	DQ131735 <sup>6</sup>	EU145534 <sup>†</sup>
<i>Feretia aeruginescens</i> Stapf	Bremer 3137 (UPS)	Rub-Dunnieae <sup>***</sup>	Z68857 <sup>19</sup>	AM117305 <sup>35</sup>	—	—	EU145539 <sup>†</sup>
<i>Fernelia buxifolia</i> Lam.	de Block s.n. (BR)	Ixo-Octotropideae	AJ286704 <sup>18</sup>	AM117306 <sup>35</sup>	—	DQ131736 <sup>6</sup>	EU145540 <sup>†</sup>
<i>Galium album</i> Mill.		Rub-Rubieae	X81090 <sup>27</sup>	AF004050 <sup>11</sup>	—	X76459 <sup>41</sup>	—
<i>Gelsemium</i>		GELSEMIACEAE	L14397 <sup>8</sup>	AJ431033 <sup>4</sup>	AJ011984 <sup>5</sup>	AJ233985 <sup>2</sup>	AF102428 <sup>43</sup>
<i>Gentiana</i>		GENTIANACEAE	L14398 <sup>8</sup>	AJ431034 <sup>4</sup>	L36400 <sup>38</sup>	DQ398604 <sup>39</sup>	X77893 <sup>45</sup>
<i>Geophila obvallata</i> Didr.	Q Luke 9037 (FR)	Rub-Psychotriaceae	AM117228 <sup>35</sup>	AF369845 <sup>26</sup>	—	—	EU145569 <sup>†</sup>
<i>Guettarda uruguensis</i> Cham. & Schltld.	X5-127, Gillis 9575 (FTG)	Cinch-Guettardeae	X83638 <sup>1</sup>	EU145489 <sup>†</sup>	AJ236297 <sup>16</sup>	DQ131739 <sup>6</sup>	EU145533 <sup>†</sup>

Table 1 (continued)

Taxon	Voucher (of previously unpublished sequences)	Classification	<i>rbcl</i>	<i>rps16</i>	<i>ndhF</i>	<i>atpB-rbcl</i> spacer	<i>trnT/F</i>
<i>Gynochthodes coriacea</i> Blume		Rub-Morindeae?	AJ288603 <sup>2</sup>	AM117311 <sup>35</sup>	—	—	AJ847407 <sup>42</sup>
<i>Hedyotis fruticosa</i> L.		Rub-Spermacoaceae	Z68799 <sup>21</sup>	—	—	AJ234026 <sup>2</sup>	AF381539 <sup>49</sup>
<i>Hillia triflora</i> (Oerst.) C.M.Taylor		Cinch-Hillieae	X83642 <sup>1</sup>	AM117315 <sup>35</sup>	AJ236298 <sup>16</sup>	AJ233993 <sup>2</sup>	AM117362 <sup>35</sup>
<i>Houstonia caerulea</i> L.		Rub-Spermacoaceae	AJ288604 <sup>2</sup>	AF333379 <sup>20</sup>	—	—	AF381524 <sup>49</sup>
<i>Hydnophytum formicarum</i> Jack		Rub-Psychotriaceae	X83645 <sup>1</sup>	AF001339 <sup>11</sup>	—	X76480 <sup>41</sup>	—
<i>Hymenodictyon floribundum</i> (Hochst. & Steud.) Rob.	Puff 861109-3/1 (WU)	Cinch-Hymenodictyeae	AJ347015 <sup>3</sup>	AF004058 <sup>11</sup>	EU145411 <sup>+</sup>	DQ131742 <sup>6</sup>	AY538454 <sup>15</sup>
<i>Ixora coccinea</i> L.		Ixo-Ixoreae	X83646 <sup>1</sup>	AM117321 <sup>35</sup>	AJ236299 <sup>16</sup>	—	AJ620117 <sup>47</sup>
<i>Kohautia caespitosa</i> Schnizl.	Bremer et al. 42566B (UPS)	Rub-Spermacoaceae	Z68800 <sup>21</sup>	AM117324 <sup>35</sup>	—	—	EU145573 <sup>+</sup>
<i>Kopsia</i>		APOCYNACEAE	X91763 <sup>8</sup>	—	AJ235824 <sup>9</sup>	—	AM295091 <sup>10</sup>
<i>Kraussia floribunda</i> Harv.		Ixo-Octotropideae	Z68858 <sup>19</sup>	AM117325 <sup>35</sup>	—	DQ131746 <sup>6</sup>	AM117368 <sup>35</sup>
<i>Lasianthus kilimandscharicus</i> K.Schum.	H. Lantz 119 (UPS)	Rub-Lasiantheae	AM117237 <sup>35</sup>	AM117327 <sup>35</sup>	EU145426 <sup>+</sup>	EU145330 <sup>+</sup>	DQ662147 <sup>32</sup>
<i>Lasianthus lanceolatus</i> (Griseb.) Urb.	Taylor 11719 (MO)	Rub-Lasiantheae	AM117238 <sup>35</sup>	AF004062 <sup>11</sup>	—	EU145331 <sup>+</sup>	EU145554 <sup>+</sup>
<i>Lasianthus pedunculatus</i> E.A.Bruce	Andreasen 71 (UPS)	Rub-Lasiantheae	Z68802 <sup>21</sup>	EU145504 <sup>+</sup>	EU145427 <sup>+</sup>	AJ234003 <sup>2</sup>	EU145555 <sup>+</sup>
<i>Lasianthus strigosus</i> Wight	Bremer & Bremer 3902 (UPS)	Rub-Lasiantheae	AM117239 <sup>35</sup>	EU145505 <sup>+</sup>	EU145428 <sup>+</sup>	—	EU145556 <sup>+</sup>
<i>Lerchea bracteata</i> Valetton	Axelius 343 (S)	Rub-Ophiorrhizeae	AJ288610 <sup>2</sup>	EU145508 <sup>+</sup>	EU145433 <sup>+</sup>	AJ233997 <sup>2</sup>	EU145561 <sup>+</sup>
<i>Luculia grandifolia</i> Ghose	Bremer 2713 (S)	—Luculieae <sup>***</sup>	X83648 <sup>1</sup>	AM900593 <sup>60</sup>	AM117346 <sup>35</sup>	AJ233986 <sup>2</sup>	AJ346929 <sup>3</sup>
<i>Luculia gratissima</i> (Wall.) Sweet	Cult in Univ. Conn. Storres 870064 (no voucher)	—Luculieae <sup>***</sup>	AM117243 <sup>35</sup>	AJ431036 <sup>4</sup>	AJ011987 <sup>5</sup>	EU145308 <sup>+</sup>	AJ430911 <sup>4</sup>
<i>Luculia intermedia</i> Hutch.	Howick et al. HOMC1524 (K)	—Luculieae <sup>***</sup>	—	EU145473 <sup>+</sup>	EU145396 <sup>+</sup>	—	EU145520 <sup>+</sup>
<i>Luculia pinceana</i> Hook.	NN Thin et al. 3061 (AAU)	—Luculieae <sup>***</sup>	EU145447 <sup>+</sup>	EU145472 <sup>+</sup>	EU145395 <sup>+</sup>	DQ131749 <sup>6</sup>	AM117371 <sup>3</sup>
<i>Manostachya ternifolia</i> E.S.Martins	Bamps & Martins 4410 (UPS)	Rub-Spermacoaceae	AJ616213 <sup>28</sup>	AM117328 <sup>35</sup>	—	—	EU145572 <sup>+</sup>
<i>Margaritopsis acujfolia</i> C.Wright (accepted name <i>Margaritopsis nudiflora</i> (Griseb.) K. Schum.)	Ekman 10248 (UPS)	Rub-Psychotriaceae	AM117247 <sup>35</sup>	AF001340 <sup>11</sup>	—	—	EU145568 <sup>+</sup>
<i>Maschalocorymbus corymbosus</i> (Blume) Bremek.	Ridsdale 2471 (L)	Rub-Urophyllaeae	AJ288611 <sup>2</sup>	AM900611 <sup>60</sup>	—	—	EU145577 <sup>+</sup>
<i>Mitchella repens</i> L.		Rub-Morindeae	Z68805 <sup>21</sup>	AF001441 <sup>11</sup>	—	—	—
<i>Mitrasacmopsis quadrivalvis</i> Jovet	Kayombo et al (UPS)	Rub-Spermacoaceae	AJ616214 <sup>28</sup>	AM117329 <sup>35</sup>	EU145439 <sup>+</sup>	EU145336 <sup>+</sup>	EU145575 <sup>+</sup>
<i>Morinda citrifolia</i> L.		Rub-Morindeae	AJ318448 <sup>25</sup>	AJ320078 <sup>25</sup>	AJ236300 <sup>16</sup>	AJ234013 <sup>2</sup>	AF152616 <sup>12</sup>
<i>Mostuea</i>		GELSEMIACEAE	L14404 <sup>8</sup>	—	AJ235828 <sup>9</sup>	DQ131697 <sup>6</sup>	—
<i>Mussaenda erythrophylla</i> Schumach. & Thonn.	Gillis 10838 (FTG)	Ixo-Mussaendeae	X83652 <sup>1</sup>	EU145493 <sup>+</sup>	AJ130836 <sup>16</sup>	DQ131754 <sup>6</sup>	EU145535 <sup>+</sup>
<i>Mycetia malayana</i> (G.Don) Craib		Rub-Argostemmateae	Z68806 <sup>21</sup>	AF002771 <sup>11</sup>	—	AJ234033 <sup>2</sup>	AF152622 <sup>12</sup>
<i>Nauclea orientalis</i> (L.) L.	c.f. Novotny et al. (2002)	Cinch-Naucleaeae	X83653 <sup>1</sup>	AJ320080 <sup>25</sup>	EU145410 <sup>+</sup>	EU145320 <sup>+</sup>	AJ346958 <sup>3</sup>
<i>Nertera granadensis</i> (Mutis ex L.f.) Druce (accepted name <i>Coprosma granadensis</i> Mutis ex L.f.)		Rub-Anthospermeae	X83654 <sup>1</sup>	AF002741 <sup>11</sup>	—	—	AF152623 <sup>12</sup>
<i>Neurocalyx championii</i> Benth. ex Thwaites	Thor 601 (S)	Rub-Ophiorrhizeae	EU145463 <sup>+</sup>	EU145509 <sup>+</sup>	EU145435 <sup>+</sup>	—	EU145563 <sup>+</sup>
<i>Neurocalyx zeylanicus</i> Hook.	B & K Bremer 937 (S)	Rub-Ophiorrhizeae	Z68807 <sup>21</sup>	AM900594 <sup>60</sup>	EU145434 <sup>+</sup>	AJ233995 <sup>2</sup>	EU145562 <sup>+</sup>
<i>Normandia neocaledonica</i> Hook.f.	Munzinger 532 (MO)	Rub-Anthospermeae	AM117250 <sup>35</sup>	AF257931 <sup>31</sup>	—	—	EU145543 <sup>+</sup>
<i>Oldenlandia corymbosa</i> L.		Rub-Spermacoaceae	X83655 <sup>1</sup>	AF333381 <sup>20</sup>	AJ130837 <sup>16</sup>	—	AF381537 <sup>49</sup>
<i>Ophiorrhiza elmeri</i> Merr.	Kjeldsen & Poulsen 233 (AAU)	Rub-Ophiorrhizeae	EU145464 <sup>+</sup>	EU145510 <sup>+</sup>	EU145436 <sup>+</sup>	—	EU145564 <sup>+</sup>
<i>Ophiorrhiza mungos</i> L.	Bremer 3301 (UPS)	Rub-Ophiorrhizeae	X83656 <sup>1</sup>	AF004064 <sup>11</sup>	AJ130838 <sup>16</sup>	—	DQ662151 <sup>6</sup>
<i>Oreopolus glacialis</i> (Poepp.) Ricardi		Rub-Coussareeae	AJ288612 <sup>2</sup>	AF004042 <sup>11</sup>	—	—	—
<i>Paederia foetida</i> L.		Rub-Paederieae	AF332373 <sup>20</sup>	AF004065 <sup>11</sup>	—	AJ234006 <sup>2</sup>	AF152619 <sup>12</sup>
<i>Palicourea crocea</i> (Sw.) Schult		Rub-Psychotriaceae	AM117253 <sup>35</sup>	AF147510 <sup>33</sup>	—	—	—
<i>Palicourea guianensis</i> Aubl.		Rub-Psychotriaceae	—	AF001345 <sup>11</sup>	—	—	AF152615 <sup>12</sup>
<i>Parapentas silvatica</i> (K.Schum.) Bremek.		Rub-Knoxieae	X83657 <sup>1</sup>	AM266849 <sup>37</sup>	—	AJ234021 <sup>2</sup>	AM266937 <sup>37</sup>
<i>Pauridiantha paucineris</i> (Hiern) Bremek.	Bremer 3090 (UPS)	Rub-Urophyllaeae	Z68811 <sup>21</sup>	AM900600 <sup>60</sup>	AJ236302 <sup>16</sup>	AJ233998 <sup>2</sup>	EU145578 <sup>+</sup>
<i>Pauridiantha symplocoides</i> (S.Moore) Bremek.	H. Lantz 123 (UPS)	Rub-Urophyllaeae	AY538502 <sup>15</sup>	AF004068 <sup>11</sup>	EU145440 <sup>+</sup>	EU145338 <sup>+</sup>	AF102467 <sup>43</sup>
<i>Pentas lanceolata</i> (Forssk.) Deflers		Rub-Knoxieae	X83659 <sup>1</sup>	AM266875 <sup>37</sup>	AJ236304 <sup>16</sup>	X76479 <sup>41</sup>	AM266963 <sup>37</sup>
<i>Pentodon pentandrus</i> (Schumach. & Thonn.) Vatke, Oesterr.		Rub-Spermacoaceae	X83660 <sup>1</sup>	AF003612 <sup>11</sup>	—	AJ234024 <sup>2</sup>	—
<i>Pouchetia gillettii</i> De Wild. (accepted name <i>Pouchetia baumanniana</i> Büttner)	Kiehn HBV sub RR-81-31 (WU)	Ixo-Octotropideae	Z68859 <sup>19</sup>	AM117336 <sup>35</sup>	—	—	EU145541 <sup>+</sup>
<i>Praravinia suberosa</i> (Merr.) Bremek.	Sabah: Ridsdale no voucher	Rub-Urophyllaeae	AJ288616 <sup>2</sup>	EU145514 <sup>+</sup>	—	—	EU145579 <sup>+</sup>
<i>Pravinaria leucocarpa</i> Bremek.	Beaman 7950 (S)	Rub-Urophyllaeae	AJ288617 <sup>2</sup>	AM900613 <sup>60</sup>	EU145441 <sup>+</sup>	AJ234001 <sup>2</sup>	EU145580 <sup>+</sup>
<i>Psychotria kirkii</i> Hiern		Rub-Psychotriaceae	X83663 <sup>1</sup>	AF410728 <sup>34</sup>	AJ236307 <sup>16</sup>	X76481 <sup>41</sup>	AY538469 <sup>15</sup>
<i>Psychotria pittieri</i> Standl.		Rub-Psychotriaceae	—	AF002746 <sup>11</sup>	—	—	AF152614 <sup>12</sup>
<i>Psychotria poeppigiana</i> Müll.Arg.		Rub-Psychotriaceae	Z68818 <sup>21</sup>	AF002748 <sup>11</sup>	—	AJ234018 <sup>2</sup>	—
<i>Pyrostria hystrix</i> (Bremek.) Bridson	Bremer 3791 (UPS)	Ixo-Vanguerieae	AM117262 <sup>35</sup>	AM117338 <sup>35</sup>	EU145418 <sup>+</sup>	—	AJ620168 <sup>47</sup>
<i>Retiniphyllum pilosum</i> (Spruce ex Benth.) Müll.Arg.	Wurdack & Adderley 43270 (S)	Ixo-Retiniphyllaeae	AF331654 <sup>20</sup>	AF004076 <sup>11</sup>	—	—	EU145536 <sup>+</sup>
<i>Rhachicallis americana</i> (Jacq.) Hitchc.		Cinch-Rondeletieae	X83664 <sup>1</sup>	AF004073 <sup>11</sup>	—	—	AF152747 <sup>12</sup>
<i>Rondeletia odorata</i> Jacq.	Bremer & Andreasen 3504 (UPS)	Cinch-Rondeletieae	Y11857 <sup>14</sup>	EU145490 <sup>+</sup>	AJ235845 <sup>9</sup>	EU145321 <sup>+</sup>	AF152741 <sup>12</sup>
<i>Rubia tinctorum</i> L.		Rub-Rubieae	X83666 <sup>1</sup>	—	DQ359167 <sup>6</sup>	X76465 <sup>41</sup>	—
<i>Sabicea aspera</i> Aubl.	Andersson et al. 1941 (NY)	Ixo-Sabiceaeae	AY538508 <sup>15</sup>	AF004079 <sup>11</sup>	EU145416 <sup>+</sup>	—	AY538475 <sup>15</sup>
<i>Sabicea diversifolia</i> Pers.	Bremer et al. 4018-B18 (UPS)	Ixo-Sabiceaeae	EU145459 <sup>+</sup>	EU145494 <sup>+</sup>	EU145415 <sup>+</sup>	DQ131781 <sup>6</sup>	AJ847396 <sup>42</sup>
<i>Saldinia</i> A.Rich. ex DC. (specimen 1)	Bremer & al 4038-BB38 (UPS)	Rub-Lasiantheae	AM117269 <sup>35</sup>	AF129275 <sup>24</sup>	EU145429 <sup>+</sup>	EU145332 <sup>+</sup>	EU145557 <sup>+</sup>
<i>Saldinia</i> A.Rich. ex DC. (specimen 2)	Kårehed et al. 257 (UPS)	Rub-Lasiantheae	EU145461 <sup>+</sup>	EU145506 <sup>+</sup>	EU145430 <sup>+</sup>	EU145333 <sup>+</sup>	EU145558 <sup>+</sup>
<i>Schismatoclada</i> sp. Baker	Razafimandimbison & Ravelonarivo 373 (MO)	Rub-Danaideae	AM117271 <sup>35</sup>	AM117341 <sup>35</sup>	EU145425 <sup>+</sup>	EU145329 <sup>+</sup>	EU145553 <sup>+</sup>
<i>Schizocolea linderi</i> (Hutch. & Dalziel) Bremek.	Adam 20116 (UPS)	Rub-Schizocoleae <sup>***</sup>	AM117272 <sup>35</sup>	EU145498 <sup>+</sup>	—	EU145323 <sup>+</sup>	EU145546 <sup>+</sup>
<i>Schradera</i> sp. K.Krause		Rub-Schradereae	Y11859 <sup>14</sup>	AF003617 <sup>11</sup>	—	AJ234014 <sup>2</sup>	AF152613 <sup>12</sup>

(continued on next page)

Table 1 (continued)

Taxon	Voucher (of previously unpublished sequences)	Classification	<i>rbcl</i>	<i>rps16</i>	<i>ndhF</i>	<i>atpB-rbcL</i> spacer	<i>trnT/F</i>
<i>Sherardia arvensis</i> L.	K. Andreasen 345 (SBT)	Rub-Rubieae	X81106 <sup>27</sup>	AF004082 <sup>11</sup>	—	X76458 <sup>41</sup>	EU145571 <sup>*</sup>
<i>Sipanea biflora</i> (L.f.) Cham. & Schltdl.	Rova et al. 2005 (S)	Ixo-Sipaneeae	AY538509 <sup>15</sup>	AF004085 <sup>11</sup>	EU145413 <sup>*</sup>	DQ131788 <sup>6</sup>	AF152675 <sup>12</sup>
<i>Sipanea hispida</i> Benth. ex Wernham	Irwin et al. 34756 (UPS)	Ixo-Sipaneeae	EU145458 <sup>*</sup>	EU145492 <sup>*</sup>	EU145414 <sup>*</sup>	EU145322 <sup>*</sup>	AY555107 <sup>48</sup>
<i>Sipanea pratensis</i> Aubl.		Ixo-Sipaneeae	—	AF243022 <sup>30</sup>	—	—	AF152677 <sup>12</sup>
<i>Spermacoce laevis</i> Lam.		Rub-Spermacoceae	Z68823 <sup>21</sup>	—	AJ236309 <sup>16</sup>	—	—
<i>Spigelia anthelmia</i> L.		LOGANIACEAE	Y11863 <sup>14</sup>	AF004093 <sup>11</sup>	AJ235840 <sup>9</sup>	—	—
<i>Spiradiclis bifida</i> Kurz	J. B. H. 55 (S)	Rub-Ophiorrhizeae	EU145465 <sup>*</sup>	EU145511 <sup>*</sup>	EU145437 <sup>*</sup>	—	EU145565 <sup>*</sup>
<i>Strychnos</i> L.		LOGANIACEAE	L14410 <sup>8</sup>	AF004094 <sup>11</sup>	AJ235841 <sup>9</sup>	DQ131691 <sup>6</sup>	AF102484 <sup>43</sup>
<i>Theorchus wauensis</i> (Schweinf. ex Hiern) Bremek.		Rub-Spermacoceae	AM117282 <sup>35</sup>	AM266901 <sup>37</sup>	—	—	AM266987 <sup>37</sup>
<i>Theligonum cynocrambe</i> L.		Rub-Theligoneae	X83668 <sup>1</sup>	AF004087 <sup>11</sup>	—	X81680 <sup>40</sup>	AF152621 <sup>12</sup>
<i>Tricalysia cryptocalyx</i> Baker		Ixo-Coffeae	Z68854 <sup>19</sup>	AF004088 <sup>11</sup>	—	—	AF152669 <sup>12</sup>
<i>Trichostachys aurea</i> Hiern	Andersson & Nilsson 2304 (GB)	Rub-Lasiantheae	EU145462 <sup>*</sup>	EU145507 <sup>*</sup>	EU145431 <sup>*</sup>	EU145334 <sup>*</sup>	EU145559 <sup>*</sup>
<i>Trichostachys</i> Hook.f. (hybrid species)	B. Sonké 1725 (UPS)	Rub-Lasiantheae	AJ288626 <sup>2</sup>	AM900595 <sup>60</sup>	EU145432 <sup>*</sup>	DQ131792 <sup>6</sup>	EU145560 <sup>*</sup>
Unknown Rubiaceae	Ridsdale 2470 (L)		AM117198 <sup>35</sup>	EU145477 <sup>*</sup>	EU145400 <sup>*</sup>	—	AJ847408 <sup>42</sup>
Unknown Rubiaceae	Bremer 1731 (UPS)		AM117199 <sup>35</sup>	EU145478 <sup>*</sup>	—	EU145312 <sup>*</sup>	EU145524 <sup>*</sup>
Unknown Rubiaceae	KH Kjeldsen 54 (AAU)		—	EU145480 <sup>*</sup>	—	—	EU145525 <sup>*</sup>
Unknown Rubiaceae	AD Poulsen 52 (AAU)		EU145449 <sup>*</sup>	EU145475 <sup>*</sup>	EU145398 <sup>*</sup>	EU145310 <sup>*</sup>	EU145522 <sup>*</sup>
Unknown Rubiaceae	Klackenberg & Lundin 541 (S)		EU145448 <sup>*</sup>	EU145474 <sup>*</sup>	EU145397 <sup>*</sup>	EU145309 <sup>*</sup>	EU145521 <sup>*</sup>
Unknown Rubiaceae	Larsen 45665 (AAU)		EU145450 <sup>*</sup>	EU145476 <sup>*</sup>	EU145399 <sup>*</sup>	EU145311 <sup>*</sup>	EU145523 <sup>*</sup>
Unknown Rubiaceae	Puff 990826-1/1 (WU)		AM117200 <sup>35</sup>	EU145479 <sup>*</sup>	EU145401 <sup>*</sup>	EU145313 <sup>*</sup>	—
<i>Urophyllum arboreum</i> (Reinw. ex Blume) Korth.	Boeea 7887 (S)	Rub-Urophyllaeae	—	AM900617 <sup>60</sup>	—	DQ131793 <sup>6</sup>	EU145582 <sup>*</sup>
<i>Urophyllum ellipticum</i> (Wight) Thwaites	Lundqvist 11085 (UPS)	Rub-Urophyllaeae	AJ288627 <sup>2</sup>	AM900619 <sup>60</sup>	—	AJ234002 <sup>2</sup>	EU145581 <sup>*</sup>
<i>Vangueria madagascariensis</i> J.F.Gmel.	Bremer 3077 (UPS)	Ixo-Vanguerieae	X83670 <sup>1</sup>	—	AJ130840 <sup>16</sup>	—	EU145542 <sup>*</sup>
<i>Viretaria major</i> (K.Schum.) Verdc.	Reekmans 10916 (UPS)	Ixo-Sabiceae	Y11861 <sup>14</sup>	EU145495 <sup>*</sup>	EU145417 <sup>*</sup>	AJ233989 <sup>2</sup>	EU145537 <sup>*</sup>
<i>Xanthophytum borneense</i> (Valeton) Axelius	Axelius 316 (S)	Rub-Ophiorrhizeae	EU145466 <sup>*</sup>	EU145513 <sup>*</sup>	EU145438 <sup>*</sup>	EU145335 <sup>*</sup>	EU145567 <sup>*</sup>
<i>Xanthophytum capitellatum</i> Ridl.	Ridsdale 2473 (L)	Rub-Ophiorrhizeae	AJ288628 <sup>2</sup>	EU145512 <sup>*</sup>	—	AJ233996 <sup>2</sup>	EU145566 <sup>*</sup>
Total number of taxa			141	141	91	97	135
Total number of characters in matrix			1402	1602 + 23	2243 + 7	1098 + 18	3219 + 18
Number of variable characters			527	1029	1172	605	1837
Number of informative characters			404	648	856	395	1145
Best fitting model; AiCc weights			GTRIG	GTRG	GTRG	GTRG	GTRIG
Second best fitting model; AiCc weights			SYMIG	GTRIG	GTRIG	GTRIG	GTRIG
Conflicts between Bayesian and parsimony analyses			No	No	No	No	No
Conflicts between indels-no indels			—	No	No	No	No
Single gene bootstrap analysis (bootstrap replicates/random sequence additions in each replicate)			1000/10	1000/10	1000/10	1000/10	1000/10
Single gene Bayesian analysis (number of generations run, evolutionary model employed)			1 million, GTRIG	1 million, GTRG	1 million, GTRG	1 million, GTRG	1 million, GTRIG

Notes. Classification: Subfamily abbreviation-Tribe. For outgroup taxa, only the Family name is given.

<sup>\*</sup>Previously unpublished sequence, presented in Rydin et al. (submitted). <sup>\*\*</sup>Sequences published in this study. <sup>\*\*\*</sup>New classification proposed in Rydin et al. (submitted for publication).

Published sequences: 1: (Bremer et al., 1995). 2: (Bremer and Manen, 2000). 3: (Razafimandimbison and Bremer, 2002). 4: (Bremer et al., 2002). 5: (Oxelman et al., 1999). 6: Manen, J.-F. (Genbank unpublished). 7: (Sennblad and Bremer, 1996). 8: (Olmstead et al., 1993). 9: (Backlund et al., 2000). 10: M.E. Endress et al. (GenBank unpublished). 11: (Andersson and Rova, 1999). 12: (Rova et al., 2002). 13: (Motley et al., 2005). 14: (Bremer et al., 1998). 15: (Andersson and Antonelli, 2005). 16: (Bremer et al., 1999). 17: (Andreasen et al., 1999). 18: (Andreasen and Bremer, 2000). 19: (Andreasen and Bremer, 1996). 20: Andersson, L. (Genbank unpublished). 21: (Bremer, 1996b). 22: (Bremer, 1996a). 23: (Nepokroeff et al., 1999). 24: (Piesschaert et al., 2000a). 25: (Novotny et al., 2002). 26: (Andersson, 2001). 27: (Manen and Natali, 1995). 28: (Thulin and Bremer, 2004). 29: (Persson, 2000). 30: Rova, J.H.E. (GenBank unpublished). 31: Anderson, C.L. et al. (GenBank unpublished). 32: Backlund, M. (Genbank unpublished). 33: Andersson, L. & Taylor, C. (GenBank unpublished). 34: (Andersson, 2002). 35: B. Bremer (in prep.). 36: A. Mouly (unpublished). 37: (Kårehed and Bremer, 2007). 38: (Olmstead and Reeves, 1995). 39: X.L. Zhang et al. (GenBank unpublished). 40: (Natali et al., 1995). 41: (Manen et al., 1994). 42: (Alejandro et al., 2005). 43: (Struwe et al., 1998). 44: (Yuan et al., 2003). 45: (Gielly and Taberlet, 1996). 46: O. Maurin et al. (GenBank unpublished). 47: (Lantz and Bremer, 2004). 48: (Delprete and Cortes-B, 2004). 49: (Church, 2003). 50: (Lantz et al., 2002). 51: (Gould and Jansen, 1999). 52: (Nakamura et al., 2006). 53: P. Ding et al. (GenBank unpublished). 54: J. Yokoyama et al. (Genbank unpublished). 55: A.D. Proujansky and D.L.Stern (Genbank unpublished). 56: C.W. Dick and E. Bermingham (Genbank unpublished). 57: D. Wolff and S. Liede-Schumann (GenBank unpublished). 58: (Church and Taylor, 2005). 59: C.I. Yuan (GenBank unpublished). 60: (Smedmark et al., 2008).

### 3. Results

#### 3.1. Data and alignment

The aligned, complete data set includes 149 terminals, 9630 characters (*rbcl*: 1402 characters, *rps16*: 1602 characters, *ndhF*: 2243 characters, *atpB-rbcL* spacer: 1098 characters, *trnT-F*: 3219 characters, and indels: 66 characters, see also Table 1). The data

set comprised 5228 variable characters, of which 3449 were phylogenetically informative. Numbers of variable and informative characters for single gene analyses are given in Table 1.

#### 3.2. Model choice and analyses

For each single gene analysis, the best performing model according to the corrected Akaike information criterion (AICc

**Table 2**  
Primers used in this study

DNA region	Primer names	Sequence 5'–3'/Reference
<i>rbcl</i>	5'F, 3'R and 427F	(Bremer et al., 2002)
<i>rbcl</i>	Z895R	Zurawski, DNAX Research institute
<i>rps16</i>	F and 2R	(Oxelman et al., 1997)
<i>ndhF</i>	2F	atg gaa cag aca tat caa tac gg <sup>a</sup>
<i>ndhF</i>	1000R <sup>b</sup>	cct aga gct agc atc ata taa ccc <sup>a</sup>
<i>ndhF</i>	720F <sup>b</sup>	gca caa ttt ccc ctt cat gta tgg <sup>a</sup>
<i>ndhF</i>	1700R <sup>b</sup>	agt att atc cga ttc ata agg at <sup>a</sup>
<i>ndhF</i>	1320F <sup>b</sup>	ggg att aac ygc att tta tat gtt tgc <sup>a</sup>
<i>ndhF</i>	2280R <sup>b</sup>	aag aaa aga taa gaa gag atg cg <sup>a</sup>
<i>atpB-rbcl</i> spacer	rbcl5'R <sup>c</sup>	ctc ttt aac acc akc ytt gaa tcc <sup>a</sup>
<i>atpB-rbcl</i> spacer	atpB5'R <sup>d</sup>	ccg atg att tgg aca ata cg <sup>a</sup>
<i>trnT-F</i>	A1	(Bremer et al., 2002)
<i>trnT-F</i>	940R	gat tyt atc att tcy gtv tmy gc <sup>a</sup>
<i>trnT-F</i>	820F	gaa tcg ayt stc caa gta ttc <sup>a</sup>
<i>trnT-F</i>	IR	(Bremer et al., 2002)
<i>trnT-F</i>	1250F	atggcgaattgtagacgc <sup>a</sup>
<i>trnT-F</i>	D	(Taberlet et al., 1991)
<i>trnT-F</i>	1880F	tcaaaygattcactcatagtc <sup>a</sup>
<i>trnT-F</i>	2670R	gattttcagtcctctgcttacc <sup>a</sup>

<sup>a</sup> Designed by C. Rydin, previously unpublished.

<sup>b</sup> Primer name reflects direction and approximate position in *ndhF* sequence *Ophiorrhiza mungos* AJ130838.

<sup>c</sup> Primer positioned in the 5' end of *rbcl*.

<sup>d</sup> Primer positioned in the 5' end of *atpB*.

Akaike, 1973) was selected (all three criteria indicated the same best performing model for all matrices, however). For the *rbcl*, and *trnT-F* data sets, the general time reversible model (GTR, Tavare, 1986) with gamma distributed rates (Yang, 1993) and a proportion of invariable sites was selected. For the *rps16*, *ndhF* and the spacer between *rbcl* and *atpB*, the general time reversible model (GTR, Tavare, 1986) with gamma distributed rates (Yang, 1993) was selected (Table 1). We found no conflicts within each region between analyses including or excluding gap coding (see also Table 1). The position of a few taxa varied between single gene data sets and supported deviations, relevant for this study, are presented below.

For the combined data set, the general time reversible model (GTR, Tavare, 1986) with gamma distributed rates (Yang, 1993) was selected for the chloroplast data partition. Values of average standard deviation of split frequencies and chain swap information in Bayesian analyses indicated no cause of concern. We therefore consider the results robust estimates of phylogeny, given the selected model and the underlying data.

Fig. 1 presents results from the Bootstrap analysis of the combined 5 chloroplast gene data set. The sizes of the grey boxes reflect the amount of species representation within respective clade. We have mapped Bayesian posterior probabilities of 50% or more, on this bootstrap tree.

### 3.3. Phylogeny—the combined data set

Subfamily Rubioideae was well supported (100% Bayesian posterior probability/100% bootstrap support). *Colletocema* was sister to remaining Rubioideae with high support (100/99). The next diverging clade consisted of Urophyllaeae and Ophiorrhizeae (–/66). Lasiantheae was the next diverging group, followed by Coussareeae, which was sister to a clade comprising the Psychotriaceae alliance+*Schizocolea* and the Spermaceae alliance (100/75).

The Psychotriaceae alliance (100/92) was here represented by 15 species. *Schizocolea linderi* was highly supported as sister to the Psychotriaceae alliance (100/100 for *Schizocolea* + Psychotriaceae alliance).

The Spermaceae alliance (100/100) comprised three major clades: The first was here represented by Anthospermeae, Argo-

stemmateae, Paederieae, Rubieae, Theligoneae and *Dunnia sinensis* (100/97). *Dunnia* was sister to remaining species (–/61). The second clade comprises Danaideae (100/100), and the third clade consists of Knoxieae and Spermaceae (100/100).

The dataset contained enough information to resolve intraspecific relationships within *Dunnia sinensis* (not shown). The samples from the Taishan population and the Zhuhai populations form a clade (100/100) and the samples from the Xinhui population and the Longmen population are sisters (100/98).

### 3.4. Phylogeny—single gene data sets

With a few exceptions, single gene analyses produced no conflicting topologies compared to those obtained from the combined data set, but trees from single gene analyses were sometimes partly collapsed. We arbitrarily decided that differences with a Bayesian posterior probability higher than 80%, and/or a bootstrap support higher than 50% can be considered “supported” and such differences are presented here.

#### 3.4.1. *rbcl*

The results from the Bayesian analysis of the *rbcl* data resolved Ophiorrhizeae and Urophyllaeae as paraphyletic assemblage (“grade”) within Rubioideae (95/– for Rubioideae except Ophiorrhizeae). *Colletocema* was sister to Urophyllaeae (80/–). Neither of these results was supported in the bootstrap analysis of the *rbcl* data.

#### 3.4.2. *ndhF*

In the Bayesian analysis of *ndhF*, *Colletocema* was sister to Lasiantheae (90/–). This relationship was not supported in the corresponding bootstrap analysis.

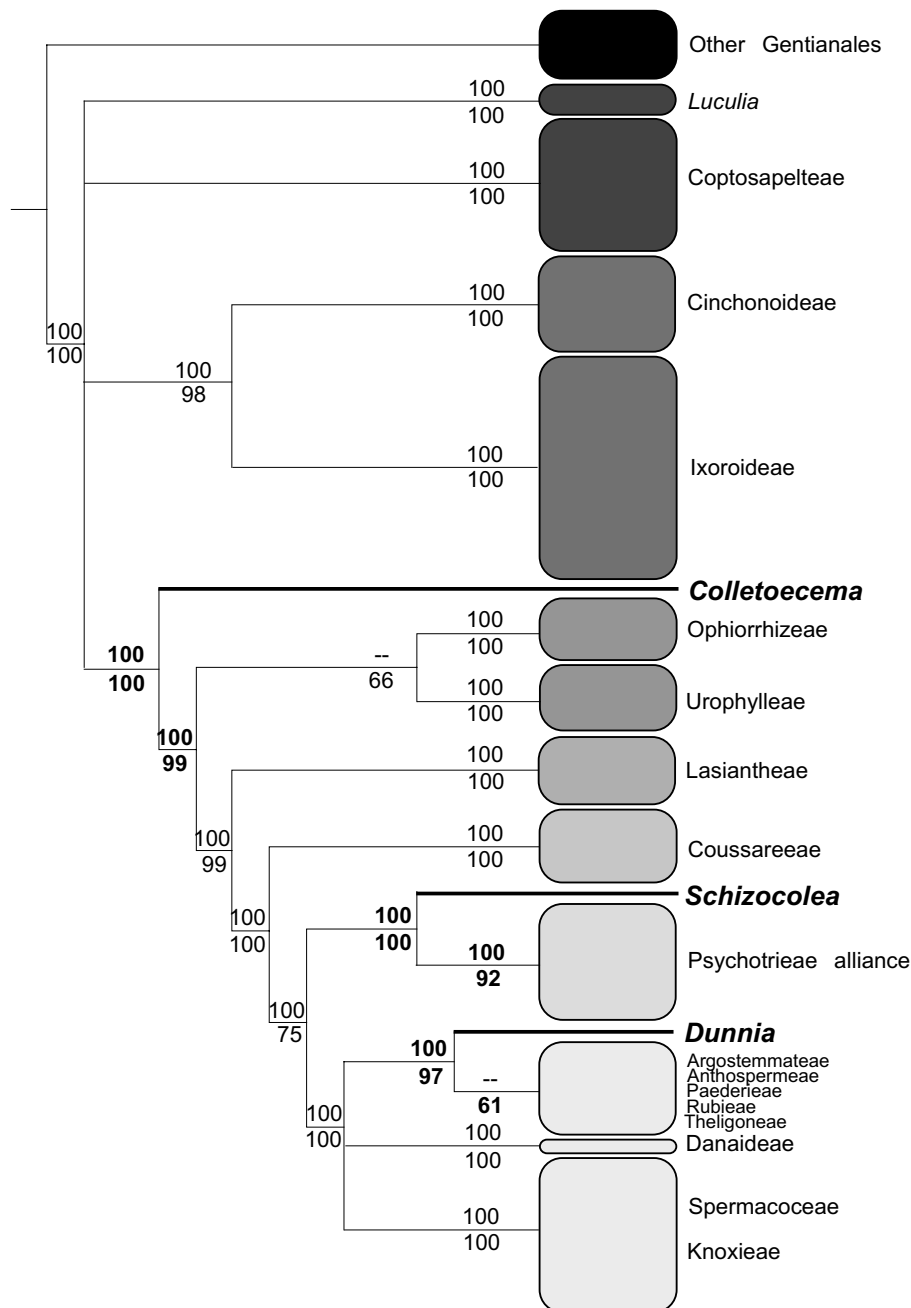
## 4. Discussion

### 4.1. General implications

The aim of this project was to investigate relationships of three rare and/or enigmatic genera (*Dunnia*, *Schizocolea*, *Colletocema*). In order to further address these issues, we sampled a large data set including taxa from all major clades of Rubiaceae, and information from five molecular markers. The complete data set comprises 149 terminals and nearly 10,000 characters. The project has thus had potential to address a number of previously unresolved relationships and conflicting results throughout the family. These results will be further discussed elsewhere (Rydin et al., submitted for publication).

*Dunnia*, *Schizocolea* and *Colletocema* are here shown to be unique sister species to considerably species-rich clades; they each represent unique evolutionary lineages. One thing they have in common is that they, based on morphology, have been considered difficult to place systematically. Throughout the years, they have often been hypothesised to belong to various clades of Rubiaceae (*Schizocolea* and *Dunnia* have rarely been investigated because there are very few collections to work with). To place species for which morphological characters have been considered confusing, using molecular data, is usually an important start for better understanding of homology and evolutionary significance of their traits. In a recent study of major clades in Asteraceae (Panero and Funk, 2008), an approach similar to ours was employed. The authors sampled genera identified by morphological studies as anomalous, and found (as we do here) that such species represented novel clades (Panero and Funk, 2008).

Even if the phylogenetic positions of *Dunnia*, *Schizocolea* and *Colletocema* are now resolved, there are many unanswered



**Fig. 1.** Phylogeny of Rubiaceae, in overview, showing the systematic positions of *Dunnia*, *Schizocolea*, *Colletocema*. The sizes of the grey boxes reflect the number of species included in respective clade. The phylogeny is based on 149 terminals and 9630 characters from the chloroplast regions *rbcl*, *rps16* intron, *ndhF*, *atpB-rbcL* spacer, and *trnT-L-F*. The topology was estimated using bootstrap in Paup. Bayesian posterior probabilities are given above branches, bootstrap values (under parsimony) below branches. Detailed relationships within groups (grey boxes) will be presented elsewhere (Rydin et al., submitted).

questions left about morphology and character evolution of these plants.

#### 4.2. *Dunnia*

*Dunnia sinensis* is with strong support included in the Spermaceae alliance. It is resolved as sister to a clade within the Spermaceae alliance comprising Anthospermeae, Argostemmateae, Paederieae, Rubieae, Theligoneae. The closest relatives of the rare and endangered *Dunnia* have previously been unknown. Interestingly, there was enough intraspecific variation in the chloroplast loci used here, to resolve relationships between populations of *Dunnia* (not shown). When including nrITS (Rydin et al., submitted

for publication) further resolution among populations was obtained; the western Yangchun population was sister to remaining populations. This is consistent with indications from Ge et al. (2002), who report that the highest amount of haplotype diversity was between the western population and the other populations.

Like *Schizocolea*, the morphology of *Dunnia* has not been investigated in a modern cladistic framework and further studies are needed. It has heterostylous flowers (Ge et al., 2002), like many other plants in Rubioideae, but whether it has other typical characters of the Rubioideae (e.g. raphids, valvate corolla aestivation) is currently not known. Further, some of the available information on this plant is not correct. The inflorescences of *Dunnia* are subtended by a few showy, petaloid structures. Tutcher (1905) de-

scribed them as bract-like lobes near the inflorescence but they are often referred to as enlarged calyx lobes, thus of tepaloid origin (see e.g. Chen, 1999; Ge et al., 2002). In Rubiaceae, similar structures are usually derived from the calyx but new investigations (C. Taylor, personal communication) show that they are in fact modified bracts in *Dunnia* (Chen and Taylor, unpublished manuscript). Enlarged, showy bracts are found also in Hymenodictyeae (Razafimandimbison and Bremer, 2006). The genetic origin behind the bract derived showy structures in *Dunnia* and Hymenodictyeae (in contrast to the calyx derived petaloid structures in other rubiaceous plants) is yet to be discovered.

#### 4.3. *Schizocolea*

Bremekamp (1950) placed *Schizocolea* in the tribe Coussareeae based on valvate aestivation of the corolla lobes, bilocular ovaries with solitary ovules and one-seeded berries. Some of these characters occur, however, in several large groups within Rubiaceae and Bremekamp considered the position of his new genus “somewhat puzzling”. He stated that its pentamerous flowers and long, narrow and fimbriate stipules differ from those of the species in Coussareeae (Bremekamp, 1950).

The uncertainty on the evolutionary origin of morphological traits in *Schizocolea*, indicated by Bremekamp, has never been further investigated. Bremer and Manen (2000) reported that unpublished *rbcl* data contradicted the inclusion of *Schizocolea* in Coussareeae. This would be reasonable from a biogeographical point of view because the species of Coussareeae are distributed only in the New World whereas *Schizocolea* is restricted to West and Central regions of tropical Africa. But the indication was never confirmed by published data and Bremer and Manen (2000) did not specify an alternative hypothesis. As pointed out by Piesschaert et al. (2000b) there are few collections of *Schizocolea*, and morphology and phylogeny of the genus is poorly understood.

Here, *Schizocolea* is highly supported as sister to remaining species in the Psychotrieae alliance, as represented here, a result confirmed by further studies (Razafimandimbison et al., 2008).

#### 4.4. *Colletocema*

Deep divergences in Rubioideae have been a problematical part of the Rubiaceae phylogeny. Previous studies have presented conflicting and often poorly supported results. Robbrecht and Manen (2006) sequenced the *atpB-rbcl* spacer for *Colletocema* and found that it was sister to remaining Rubioideae. However, no support values were reported and *rps16* data used by Piesschaert et al. (2000a) indicated a (poorly supported) relationship between *Colletocema* and *Ophiorrhiza*.

Conflicting, poorly supported, signals on the evolutionary origin of *Colletocema* is evident also from our data. In single gene analyses, *Colletocema* was sister to Lasiantheae in the *ndhF* analysis (this study), to Urophyllaeae in the *rbcl* tree (this study), and to Urophyllaeae–Ophiorrhizeae based on nrITS (Rydin et al. submitted). However, all these results typically received only around 80% Bayesian posterior probability and no bootstrap support. In all our combined analyses (Fig. 1), as well as in single gene analyses of *rps16*, *trnT-F* and the *atpB-rbcl* spacer (not shown), *Colletocema* was highly supported as sister to remaining Rubioideae. The result found by Piesschaert et al. (2000a), based on the *rps16* intron, is thus not reproduced in *rps16* analyses with a larger sampling of species. We confirm with high support the result indicated in Robbrecht and Manen (2006).

Piesschaert et al. (2000a) further made a thorough study of the morphology of *Colletocema*. They found, however, that anatomical and morphological data provided little information on relationships, and they concluded that *Colletocema* exhibits a unique com-

bination of morphological characters not found elsewhere in Rubioideae. The presence of raphides is a synapomorphy for Rubioideae (but occur also in some groups in Cinchonoideae). Raphides are present in parts of the plants (Petit, 1963), but less conspicuous than in most species of Rubioideae, and are easily overlooked (Piesschaert et al., 2000a). All these findings, the morphological “isolation”, the less conspicuous presence of raphides, seem to be in accordance with its position as sister lineage to remaining species in the subfamily.

#### 4.5. Aspects of conservation biology

One general implication of our results concerns conservation biology. We show that there are several clades in Rubiaceae, within which one or a few rare, geographically isolated and sometimes endangered species are sister(s) to a significantly species-rich clade. This is true for example for *Colletocema dewevrei*, a single species endemic to the West and Central regions of tropical Africa. It is sister to subfamily Rubioideae, which consists of about 7475 species (estimated from Govaerts et al., 2006). *Schizocolea* comprise two species from Central and Western Africa, and they are sister to the Psychotrieae alliance, which comprises more than 3000 species (estimated from Govaerts et al., 2006). *Dunnia sinensis* is a single, seriously threatened species, sister to a clade within the Spermaceae alliance, which totally consists of a little over 1500 species (estimated from Govaerts et al., 2006).

An early strategy for conserving biodiversity was to identify threatened, species-rich geographic regions with high range of endemism (“biodiversity hot-spots”), and take action to preserve areas of interest (Myers, 1988, 1990). An alternative approach is to focus on the evolutionary history of plants and plant groups. It has been argued that “basal lineages”, (i.e. a species-poor clade, sister to a species-rich clade), are valuable and vulnerable and should receive particular attention in biodiversity assessments (Stiassny and de Pinna, 1994). Quantitative methods to estimate phylogenetic diversity and provide explicit measures of the biodiversity value of clades have been presented (e.g. Humphries and Williams, 1994; Williams et al., 1991).

*Dunnia*, *Schizocolea* and *Colletocema* are obvious examples of species with a potentially high phylogenetic diversity value. *Dunnia* has further been investigated for intraspecific genetic variation (Chiang et al., 2002; Ge et al., 2002) and for most of the investigated loci, the authors found relatively small amounts of genetic diversity within each remaining population of *Dunnia*, but surprisingly large variation between populations (Chiang et al., 2002; Ge et al., 2002). The authors suggest that neutral evolution, i.e. elimination of alleles from populations due to genetic drift, is a possible explanation, even though time seems to have been limited for such processes.

The large variation between populations of *Dunnia* is reflected also in our study. This pattern of low within-population variation but substantial genetic variation between populations would fit that of a species, which populations have been separated for a long time (Schaal et al., 1998). The current distribution of *Dunnia* is clearly the opposite and due to recent fragmentation and isolation of a former larger geographic distribution (Chiang et al., 2002; Ge et al., 2002). Such species generally have the opposite pattern, little variation between populations because of a recent common history (Schaal et al., 1998; Schaal and Olsen, 2000). However, it has clearly been shown that *Dunnia* has substantial genetic differences between populations (Chiang et al., 2002; Ge et al., 2002, and this study), despite a recent fragmentation and isolation of populations through human activities. A plausible explanation is that *Dunnia* has never been common and that it also with its former distribution was a rare species with low dispersal ability and little genetic exchange between populations.



Intraspecific variation has not been investigated for *Schizocolea* and *Colletoecema*. Their restricted distribution may, however, in itself be an indication of high biodiversity value. Not only are such taxa more vulnerable and at risk for extinction, but also, geographic isolation can be important for genetic diversity. For example, Shaw and Cox (2005) report that the peat moss *Sphagnum terenum* is one of the most genetically distinct species in its section, despite that phylogeny indicates that it is a relatively young species, which potentially have had limited time to accumulate mutation. It is in addition morphologically similar to its sister species. The authors conclude that its restricted distribution (in comparison with closely related taxa) suggests that in this case, geographic isolation has been more important for genetic diversity, than age (Shaw and Cox, 2005).

Aspects of conservation biology and biodiversity assessments are complex but among generally accepted concepts are to preserve representatives of unique and species-poor lineages (Humphries and Vane-Wright, 1995). For *Dunnia*, it is clearly important to protect all populations if the remaining genetic diversity of this lineage is to be preserved. The biodiversity status of the rarely collected species of *Schizocolea* and *Colletoecema* are more uncertain. What is clear from this study, is that *Dunnia*, *Schizocolea* and *Colletoecema* all represent phylogenetically and geographically isolated taxa. They are lone representatives of unique clades and if extinct, the loss would not only encompass genetic and morphological diversity of a single species but of an entire lineage.

## 5. Conclusions

To systematically place species for which morphological characters have been considered confusing, using molecular data, is often an important start for better understanding of homology and evolutionary significance of their traits. We resolve the phylogenetic position of three such species and find that they all represent single sister lineages to large and species rich clades. They are lone representatives of entire lineages, a finding that is in accordance with their “puzzling morphology”.

*Colletoecema dewevrei* is sister to remaining Rubioideae. *Colletoecema* is morphologically unique in several respects (Piesschaert et al., 2000a) and this is consistent with its isolated position in Rubioideae.

*Schizocolea*, from which sequences are here published for the first time, is highly supported as sister to remaining species in the Psychotriaceae alliance. *Schizocolea* comprises two rarely collected species from tropical Africa. They were previously classified in the neotropical tribe Coussareeae, but considered morphologically anomalous.

*Dunnia sinensis* is sister to a clade comprising Anthospermeae, Argostemmateae, Paderieae, Rubieae, Theligoneae. The closest relatives of this rare and endangered species, endemic to southern Guangdong, China, have previously been unknown.

Aspects of conservation biology and biodiversity assessments are complex issues that need to take into consideration information from a range of levels. One aspect is to preserve representatives of unique and species-poor lineages, because loss of such taxa would potentially mean substantial loss of morphological and genetic diversity.

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