Molecular phylogeny of the tribe Danaideae (Rubiaceae: Rubioideae): Another example of out-of-Madagascar dispersal

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Abstract Extensive efforts have been made to resolve the phylogeny of the large coffee family (Rubiaceae) based on molecular data. As a result, several small tribes have been described, but the phylogenies and generic delimitations for many of these groups remain unclear. This study focuses on the small tribe Danaideae that belongs to subfamily Rubioideae and whose generic limits have not previously been addressed with molecular data. It is the sole rubiaceous tribe distributed almost entirely in the Western Indian Ocean region, with the exception of the East African Danais xanthorrhoea. The tribe consists of three genera: Danais, Payera (including the monotypic genus Coursiana), and Schismatooclada. We present the first molecular phylogenetic study of Danaideae including representatives from all three genera and using Bayesian and maximum parsimony methods and sequence data from nuclear DNA (nITS) and chloroplast DNA (petD, psbA-trnH, rpl32-trnL, rps16). Our main objectives were to rigorously test the monophyly of Danaideae as currently circumscribed and assess phylogenetic relationships within the tribe. The findings of this study shed light on the colonization history of the tribe. Our analyses reaffirm the monophyly of Danaideae and Danais but reveal the paraphyly of Payera and Schismatooclada. The close relationship between the three Danaideae genera and Coursiana is supported. However, we found very little support for the inclusion of the latter genus in Payera as proposed earlier. The tribe is resolved in two morphologically distinct major lineages, the highly supported Danais clade with lianescent habit (= Danais sensu Buchner & Puff) and the Payera–Schismatooclada clade with arborecent habit. The Malagasy and Mauritian specimens of Danais fragrans are not closely related, and we restrict D. fragrans to the Mauritian taxa and resurrect Danais lyallii Baker to accommodate the Malagasy D. fragrans. According to our analysis, Madagascar is the origin of all species of Danaideae occurring in the Comoro archipelago, East Africa, and Mauritius. The Mauritian and East African Danais each is the result of a single colonization event, while there were at least two independent colonization events to the Comoros.

Keywords biogeography; Danaideae; Danais; molecular systematics; Payera; Schismatooclada; Western Indian Ocean

Supplementary Material The alignment file is available in the Supplementary Data section of the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

INTRODUCTION

Over the past 11 years many small tribes (e.g., Danaideae, Bremer & Manen, 2000; Hymenodictyceae, Razafimandimbison & Bremer, 2001; Mitchellaeae, Razafimandimbison & al., 2008; Aleisanthieae, Mouly & al., 2009) in the coffee family (Rubiaceae) have been described based on molecular phylogenetic studies. These are tribes that accommodate relatively small problematic genera traditionally associated with different tribes and/or subfamilies. This study focuses on the Madagascar-centered tribe Danaideae, which belongs to the species-rich Spermacoceae alliance of subfamily Rubioideae (Bremer & Manen, 2000; Robbrecht & Manen, 2006; Rydin & al., 2009). Danaideae as delimited by Bremer & Manen (2000) consists of three genera: Danais Comm. ex Vent. (31 spp., The Plant List, 2010; Govaerts & al., 2011), Payera Baill. (10 spp., Buchner & Puff, 1993), and Schismatooclada Baker (19 spp., Buchner & Puff, 1993). The tribe is distributed almost entirely in the Western Indian Ocean region, but has its center of species diversity in Madagascar. Both Payera and Schismatooclada are endemic to Madagascar (Schatz, 2000), while Danais is mostly Malagasy, with one endemic species each in East Africa (D. xanthorrhoea (K. Schum.) Bremek.), Mauritius (D. sulcata Pers.), Rodrigues (D. corymbosa Balf. f.), and the Comoros (D. comorenisi Drake). Danais humblotii Homolle occurs both in Madagascar and the Comores, while Danais fragrans (Lam.) Pers. is found in Madagascar and the Mascarenes. Close relationships between Danais, Payera, Schismatooclada, and the Malagasy monotypic genus Coursiana Homolle (Homolle, 1942) were postulated by J.-F. Leroy (a former curator at P herbarium). However, Leroy never published his findings, but instead made notes on herbarium sheets at the Paris herbarium; these notes showed his intention to merge these genera in Danais. The genera included in Danaideae today were traditionally associated with different tribes in subfamilies Cinchonoideae and Rubioideae (see Buchner & Puff, 1993 for an overview of the taxonomic history of the four genera). Coursiana was described by Homolle (1942), although it was Cavaco (1968) who validly described it with the species C. homolleana Cavaco. Homolle’s genus was initially placed in subtribe Cinchoninae of tribe Cinchoneae, a position accepted by Robbrecht...
(1988). However, this taxonomic decision was rejected by Bremekamp (1952, 1966), Verdcourt (1958), and Andersson & Persson (1991), who suggested a transfer to tribe Hedyotidae (now synonymous with tribe Spermacoceae). The morphological study by Buchner & Puff (1993) confirmed the close affinities between Coussarea, Danais, Payera, and Schismatoclada. The authors presented a combination of morphological characters that hold these genera together as a group, which was named the Danais-Schismatoclada-Payera genus complex, merging Coussarea in Payera. However, no potential morphological synapomorphy for the group was proposed. A distinct character separating the genera is growth habit; the members of Danais are lianescent, while both Payera and Schismatoclada are arborescent. Other characters suggested are the dehiscence of the capsular fruits: capsular fruits in Danais and Payera have loculicidal dehiscence, whereas those of Schismatoclada are septicidal (Buchner & Puff, 1993); aestivation of corolla lobes (Danais and Schismatoclada valvate-reduplicate but valvate in Payera, Buchner & Puff, 1993). Another character often used for separating Payera from Schismatoclada is the presence of conspicuous bracts subtending the inflorescence in Payera (Schatz, 2001). Buchner & Puff (1993) proposed new circumscriptions of Payera and Schismatoclada based mainly on the type of fruit dehiscence and aestivation of corolla lobes; accordingly, they transferred several species of Schismatoclada to Payera and merged the Malagasy monotypic genus Coussarea in Payera.

The monophyly of the genera Danais (sensu Puff & Buchner, 1994), Payera and Schismatoclada (both sensu Buchner & Puff, 1993) has not been tested thoroughly, as Payera has never been included in any molecular phylogenetic study. In Bremer & Manen (2000), the sampled Danais and Schismatoclada formed a monophyletic group; this sister-group relationship was further confirmed by Rydin & al. (2009b), who investigated five and four species of Danais and Schismatoclada, respectively. Therefore, the phylogenetic relationships between the three genera have not yet been investigated. The present study is the first molecular study to investigate all three genera.

In their revision of Danais, Puff & Buchner (1994) recognized an informal group of closely related species, the “microcarpa” group, which consists of D. microcarpa Baker, D. verticillata Baker, and D. rhamnifolia Baker and is characterized by mainly terminal inflorescences, small flowers, small fruits, and small seeds. The East African species D. xanthorrhoea was postulated to be closely related to D. rhamnifolia by Bremekamp (1966). Puff & Buchner (1993) also suggested close relationships between D. coronata (Pers.) Steud., D. volubilis Baker, and D. sulcata (hereafter called the “coronata” group), which is characterized by having axillary inflorescences, elongated calyx lobes, large fruits, and large seeds. The monophyly of these two groups has yet to be assessed with molecular data.

The main objectives of this investigation were to phylogenetically study the tribe Danaideae to test: (1) the monophyly of the tribe Danaideae, (2) its generic limits, and (3) the relationships between the species of the tribe. Hopefully, the findings of this study should shed light on the colonization history of Danaideae in the Western Indian Ocean region.

### MATERIALS AND METHODS

**Taxon sampling.** — A total of 66 accessions were investigated (Appendix), of which 51 were from Danaideae, 7 from the Spermacoceae alliance, and 7 from the Psychotrieae alliance (see Appendix). The sampled Danaideae were represented by 19 accessions of Danais, 17 of Payera (Coussarea included), and 15 of Schismatoclada. Coussarea hydangeifolia from tribe Coussareae of subfamily Rubioidae was chosen as outgroup to root the trees. Our sampling covered the entire geographic range of the three Danaideae genera, with the exception of D. corymbosa from Rodrigues. This latter species appears to have gone extinct (C. Baider, pers. comm.). The species including the types of Danais (D. fragrans) and Schismatoclada (S. psychotrioides Baker) were included in the analyses. However, the type species of Payera (P. conspicua) was not investigated due to lack of sequenceable material. Several specimens included in our study could not be identified at species level despite using the available keys for the three genera (Cavaco, 1964; Puff & Buchner, 1994) and checking against the protologues. Some of these species are potentially new to science.

**DNA extraction, amplification, and sequencing.** — Total DNA was extracted from dried leaf material preserved in silica gel (Chase & Hills, 1991). DNA extraction and amplification were made according to protocols outlined in Kärrehed & Bremer (2007), except for the amplification of petD (Kärrehed & al., 2008). Sequence data from one nuclear DNA (nrITS) and four chloroplast (petD, psbA-trnH, rpl32-trnLtrnUAG, rps16) regions were utilized. The primers used in this study are summarized in Table 1. Sequences were assembled using Staden programs v.1.5 preGAP and GAP4 (Staden, 1996). Alignments were made manually in the Se-Al v.2.0a11 program (Rambaut, 2002). The criteria outlined in Oxelman & al. (1997) were applied in case of insertion or deletion events in the alignment.

**Phylogenetic analyses.** — Each dataset was analyzed separately to detect any strongly supported topological conflict. No hard incongruences were found and the datasets were combined. Both maximum parsimony (PAUP* v.4.0B10, Swofford, 2003) and Bayesian analyses (MrBayes v.3.1, Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) were performed for the separate as well as the combined datasets. For the parsimony analyses we utilized the following settings: heuristic search, tree bisection-reconnection branch swapping, 1000 random sequence addition replicates and a maximum limit of 10 trees saved per replicate. The same settings were used for calculating bootstrap support except for the use of 3 instead of 1000 random sequence addition replicates. In the Bayesian analysis the best model under the corrected Akaike information criterion, AICc (Akaike, 1973; Sugiyama, 1978) was chosen using the program MrAIC v.1.4.2 (Nylander, 2004). Each analysis included two parallel runs of four MCMC chains over 50 × 10⁶ generations, with every 1000 generation sampled. The standard deviation of split frequencies between the two runs was below 0.01 and the potential scale-reducing factor (PSRF) was 1 for all parameters. The first 25% of the sampled trees were discarded as burn-in. The datasets were analyzed divided in partitions to enable each region to be assigned its best
fitting evolutionary model. In one analysis each gene region was considered as an individual partition and the partitions were unlinked. In another the datasets were divided in two partitions, the chloroplast regions were regarded as one and nrITS the other. The same procedures were carried out for three additional analyses, to detect any effect that the taxon sampling might have on the monophyly of the tribe and its generic delimitations, using a different taxon sampling: the same ingroup accessions from Danaideae and the (seven) Psychotrieae alliance; the same ingroup taxa from Danaideae and the (seven) Spermacoceae alliance; the same ingroup taxa from Danaideae and the (nine) Psychotrieae alliance and tribe Knoxieae.

### RESULTS

The parsimony and Bayesian analyses based on the combined chloroplast and nuclear datasets (divided in two partitions) generated almost identical phylogenetic trees with several resolved nodes (Fig. 1). The monophyly of Danaideae sensu Bremer & Manen (2000) is strongly supported (posterior probability PP = 1 and bootstrap support BS = 100%).

The tribe was resolved in two well-supported major lineages: one corresponding to the genus Danais (PP = 1, BS = 91%) and the other to the Payera-Schismatocladia clade (PP = 1, BS = 89%).

The Danais clade was resolved into three subclades: a subclade containing a single specimen Danais sp. 1 (subclade A), and two subclades with moderate support: the “microcarpa” (subclade B, PP = 0.89), and the “coronata” group (subclade C, PP = 0.84). Subclade A was sister to the remaining Danais, and the “microcarpa” and “coronata” groups are sister to each other. Moreover, the Malagasy and Mauritian accessions of D. fragrans did not form a clade; the Mauritian D. fragrans (= D. fragrans 1) and D. sulcata were resolved as sisters, and this Mauritian group was sister to a Malagasy Danais group (D. coronata to D. fragrans 2 & 3). Within the “microcarpa” group the Comorian D. comorensis and the East African D. xanthorrhoea were sisters, and this clade was deeply nested within the otherwise Malagasy group.

The Payera-Schismatocladia clade was resolved into three subclades (D–F): a group of arborescent Payera-Schismatocladia (including P. homolleana = Coursiana homolleana) which received low support in the parsimony analysis but was supported in the Bayesian analysis (PP = 1 BS = 67%; subclade D); a well-supported group of small species (<50 cm tall) of Payera (PP = 1, BS = 95%; subclade E); and a highly supported, arborescent Payera-Schismatocladia group (PP = 1, BS = 91%; subclade F). In sum, Payera and Schismatocladia are mutually paraphyletic (Fig. 1). The additional analyses using different sets of taxa (only representatives of the Psychotrieae alliance, only representatives of the Spermacoceae or Psychotrieae alliance together with taxa of tribes Knoxieae and Spermacoceae) further supported the monophyly of Danaideae, the Danais clade, subclade E, and subclade F (results not presented). The support for the subclades, particular those of Payera and Schismatocladia, differs slightly from the results presented here. The main difference was the collapse of the Payera-Schismatocladia clade and subclade D, while subclades E and F were still strongly supported but had a different, weakly supported position. Table 2 summarizes the tree data and statistics from the analyses.

### DISCUSSION

**Monophyly of Danaideae sensu Bremer & Manen (2000).** — The monophyly of Danaideae sensu Bremer & Manen (2000) was demonstrated by Rydin & al. (2009b), however, their study included a limited sample of Danais and Schismatocladia and

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**Table 2.** Data description of the markers and datasets used. Number of informative characters, number of variable characters and best evolutionary model are shown for the different regions.

<table>
<thead>
<tr>
<th></th>
<th>nrITS</th>
<th>petD</th>
<th>psbA-trnH</th>
<th>rpl32-trnL(^ {UGA})</th>
<th>rps16</th>
<th>Combined</th>
</tr>
</thead>
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<tr>
<td>Total number of accessions in matrix</td>
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<td>64</td>
<td>66</td>
<td>60</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td>Total number of characters in matrix</td>
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<td>936</td>
<td>513</td>
<td>1092</td>
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<td>4346</td>
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<tr>
<td>Number of variable characters</td>
<td>117</td>
<td>236</td>
<td>109</td>
<td>332</td>
<td>290</td>
<td>1148</td>
</tr>
<tr>
<td>Number of parsimony-informative characters</td>
<td>234</td>
<td>204</td>
<td>231</td>
<td>224</td>
<td>152</td>
<td>1045</td>
</tr>
<tr>
<td>Employed evolutionary model (AICc weights)</td>
<td>GTRG</td>
<td>HKYG</td>
<td>HKYG</td>
<td>GTRG</td>
<td>GTRG</td>
<td>—</td>
</tr>
<tr>
<td>% informative characters</td>
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<td>22</td>
<td>45</td>
<td>20</td>
<td>12</td>
<td>24</td>
</tr>
</tbody>
</table>
Fig. 1. Phylogenetic tree of tribe Danaideae generated from the Bayesian inference analysis of the combined chloroplast, petD, psbA-trnH, rps16 and rpl32-trnL(UAG), and nuclear nrITS sequence data. Posterior probabilities are shown above and bootstrap values below branches. ANT = Anthospermeae, ARG = Argostemmateae, KNO = Knoxieae, MIT = Mitchelleae, MOR = Morindeae, PRI = Prismatomerideae, PSY = Psychotrieae, SCH = Schizocoleae, SPE = Spermacoceae.
no members of Payera were included. The present study is the first to focus on Danaideae and include all three recognized genera (Danais, Payera, Schismatoclada) in a molecular phylogenetic analysis of Rubiaceae. The monophyly of tribe Danaideae as circumscribed by Bremer & Manen (2000) is strongly supported by our analyses. This is consistent with Buchner & Puff (1993) who named the group Danais-Schismatoclada-Payera genus complex. The tribe seems to have no obvious morphological synapomorphy but can be characterized by a combination of characters: woody growth habit, many-flowered thyrsoid inflorescences, heterodistylos flowers, and two-locular ovaries containing many ovules in each locale (Buchner & Puff, 1993; Bremer & Manen, 2000).

Generic delimitations in Danaideae. — Danais as delimited by Buchner & Puff (1993) is highly supported as monophyletic. Its lianescent habit is probably a morphological synapomorphy for the genus. Our findings do not support the idea of recognizing a broadly circumscribed Danais, which would include Payera and Schismatoclada. We reject this taxonomic suggestion because it would make Danais sensu lato highly heterogenous morphologically. In contrast, neither Payera nor Schismatoclada (both sensu Buchner & Puff, 1993) is supported as monophyletic in our analyses. This implies that the characters currently used for circumscribing these genera, namely dehiscence of capsular fruit, corolla aestivation, and presence of conspicuous bracts subtending the inflorescence, are homoplasious; as a result, they cannot be used for recognizing these genera. Our analyses confirm the close relationships between Coursiana, Payera, and Schismatoclada postulated by Leroy (unpub. data) and Buchner & Puff (1993). However, we do not support the taxonomic decision made by Buchner & Puff (1993) to merge the Malagasy genus Coursiana in Payera, which is paraphyletic with respect to Schismatoclada, as Coursiana is nested in subclade D of the Payera-Schismatoclada clade.

The present analyses demonstrate that the generic status of Payera and Schismatoclada as delimited by Buchner & Puff (1993) is untenable. There are at least two possible alternative classifications. One is to recognize a broadly delimited Payera (= the Payera-Schismatoclada clade), which would include all species of Schismatoclada and Coursiana. The second scenario is to recognize the three subclades (D–F) of the Payera-Schismatoclada clade at generic level. We favor the first scenario, as the Payera-Schismatoclada clade is highly supported in our analyses (PP = 1; BS = 89%, Fig. 1), and more importantly can easily be distinguished from its sister Danais by its arborescent habit. However, we refrain from making such a decision here because no specimen of the type of Payera (P. conspicua) was investigated in our study. The second scenario is not an attractive solution because there seems to be no obvious morphological synapomorphy or combination of characters for distinguishing subclade D from subclade F. In addition, we do not know the phylogenetic position of P. conspicua in the Payera-Schismatoclada clade (Fig. 1).

Phylogenetic relationships within the major lineages. — Our phylogenetic analyses resolve Danaideae in two major lineages: the Danais clade and the Payera-Schismatoclada clade. The Danais clade (= Danais) is resolved in three subclades (A–C); subclade A is resolved as sister to the rest of Danais. The two latter subclades are sisters and correspond to the two informal groups, “microcarpa” and “coronata”, of Danais; however, these groups seem to be much larger according to our analyses. The “microcarpa” and “coronata” groups each contains Danais species occurring outside of Madagascar. The Comorian D. comorensis and the East African D. xanthorrhoea belong in the “microcarpa” group, while the Mauritian D. sulcata and the Malagasy-Mauritian D. fragans are members of the “coronata” group. In the “microcarpa” group, the close relationships between D. microcarpa and D. rhamnifolia postulated by Puff & Buchner (1994) and between D. rhamnifolia and D. xanthorrhoea are consistent with our findings. The suggestion made by Puff & Buchner (1994) that D. comorensis could be a variant of the Malagasy-Comorian D. humboldtii is not supported by our analyses, which instead suggest that D. comorensis should be retained at species level.

There is no support for a close relationship between the Malagasy D. coronata and the Mauritian D. sulcata as suggested by Puff & Buchner (1994). The Mauritian D. sulcata and D. fragans form a clade, which is sister to the rest of the “coronata” group. The Mauritian and Malagasy accessions of D. fragans are not closely related to each other; this implies that D. fragans should be restricted to material from Mauritius, where the type specimen of the species was collected. The Malagasy D. fragans should be given a new species name. There are two potential names based on Malagasy specimens that are currently considered synonyms of D. fragans sensu Puff & Buchner (1994): D. iyallii Baker (Baker, 1887) and D. obovata Drake (Drake, 1899). The former has priority over the latter.

The Payera-Schismatoclada clade is resolved in three subclades: a poorly supported subclade consisting of P. decaryi, P. homolleiana (= Coursiana) and Schismatoclada sp. (subclade D or the Coursiana group); a highly supported subclade of all sampled small Payera species (subclade E); and a highly supported large subclade containing the remaining species of Payera and Schismatoclada sequenced (subclade F). The relationships between these groups are unresolved in our analyses. The Coursiana (subclade D) and the large Payera-Schismatoclada (subclade F) groups are mostly shrubs or small trees, while the members of subclade E are typically no more than 50 cm tall. Most of the Schismatoclada species belong to subclade F, within which S. bracteata is resolved as sister to the remaining species. Finally, all sampled specimens of the type of Schismatoclada, S. psychotrioides, group together.

Biogeographical remarks. — Tribe Danaideae as presently defined by Bremer & Manen (2000) comprises about 60 species and is the largest rubiaceous group distributed almost entirely in the Western Indian Ocean region, with the exception of one species, Danais xanthorrhoea, in East Africa. Madagascar is the center of species diversity with ca. 98% of the species. All early diverging members of the Danais clade are Malagasy endemics suggesting a Malagasy origin of the genus. All sampled non-Malagasy species of Danais (the East African D. xanthorrhoea, the Comorian D. comorensis, the Mauritian D. fragans and D. sulcata) are well nested in
the otherwise Malagasy clade. The Mauritian *D. fragrans* and *D. sulcata* form a clade, implying that these species are the result of a single colonization event from Madagascar. *Danais corymbosa* from Rodrigues was not investigated in this study, so we do not know whether this species is a result of a dispersal event from Madagascar or Mauritius. The sister-group relationship between the East African *D. xanthorrhoea* and the Comorian *D. comorensis* indicates that they could be the result of a single dispersal event from Madagascar to the Comoro archipelago followed by a second dispersal to East Africa from the Comoros or vice versa (a single dispersal event to East Africa from Madagascar followed by a second dispersal to the Comoros from East Africa). Alternatively, two separate dispersal events from Madagascar, from the same or closely related ancestors, to East Africa and the Comoro archipelago may have occurred. A proper biogeographic analysis using a much larger sample of *Danais* is needed to test these scenarios. *Danais humboldtii* occurs on both Madagascar and the Comoro archipelago and is not closely related to *D. comorensis*, which indicates an independent dispersal event to the Comoro Islands from Madagascar. In sum, this study reaffirms that Madagascar is the main source of the majority of Rubiaceae found on neighboring islands (Wikström et al., 2010), and tribe Danai-deae provides another example of out-of-Madagascar dispersal.

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LITERATURE CITED


Appendix. GenBank accession numbers and origin of samples used in this study.

Species, country, area, voucher information, nTRTS, petD, rps16-trnl-trnL
Anthericum herbarum L., Tanzania, Eastern So Morogoro, Bremer 3093 (UPS), EU145355, JQ729862*, JQ729926*, EU145496*, JQ729750*. Argo-
stemma hookeri King, Malaysia, -, Wan-toorp s.n. (S), EU145356, JQ729863*, JQ729927*, EU145497*, JQ729751*. Coelospermum fragrans (Montrouz.) Baill.
Guillem, New Caledonia, -, Johannsen 87 (S), AM945194*, JQ729857*, JQ729920*, AF001438*, JQ729745*. Coussarea hydrgofolia (Benth.) Benth.
-, -, ATV 13238*, -, JQ729747*, JQ729924*, Danais comorensis Drake, Comoros Islands, Moly 687 (P), JQ729803*, JQ729864*, JQ729869*, FJ695263*,
JQ729753*. Danais fragrans (Lam.) Pers., 1, Mauritius, Brise Fer, Razafimandimison & al. 815 (S), JQ729807*, JQ729868*, JQ729932*, JQ729793*,
JQ729756*. Danais fragrans 2, Madagascar, -, Kårehed & al. 291 (UPS), JQ729806*, JQ729867*, JQ729931*, JQ729962*, JQ729755*. Danais fragrans 3, Madagascar,
5316 (S), JQ729808*, JQ729869*, JQ729933*, JQ729964*, JQ729757*. Danais microcarpa Baker, Madagascar, -, Kårehed & al. 290 (UPS), JQ729809*,
JQ729783*, JQ729934*, JQ729965*, JQ729758*. Danais pubescens Baker, Madagascar, -, Kårehed & al. 312 (UPS), JQ729810*, JQ729971*,
-, Eriksson Ti032 (S), JQ729812*, JQ729874*, JQ729938*, JQ729699*, JQ729762*. Danais sp. 3, Madagascar, -, Razafimandimison & al. 658 (S),
JQ729813*, JQ729841*, JQ729941*, JQ729706*, JQ729763*. Danais sp. 4, Madagascar, -, Razafimandimison & Raveloniarivo 631 (S), JQ729814*, JQ729878*,
JQ729946*, JQ729706*, JQ729769*. Danais xanthorrhiza (K. Schum.) Bremer., Tanzania, Eastern So Morogoro, Bremer 2079 (UPS), EU145364*, JQ729875*,
JQ729710*, JQ729470*, JQ729741*. Payera biannulata (Humbert) Buchner & Pfiff 1, Madagascar, Antsiranana, Bremer & al. 5340 (S), JQ729833*, JQ729896*,
JQ729958*, JQ729970*, JQ729784*. Payera boedroenia (Humbert) Buchner & Pfiff 1, Madagascar, Antsiranana, Bremer & al. 5340 (S), JQ729833*,
JQ729896*, JQ729958*, JQ729970*, JQ729784*. Payera boedroenia 2, Madagascar, -, Razafimandimison & Raveloniarivo 631 (S), JQ729844*,
JQ729907*, JQ729971*, JQ729795*. Payera coriacea (Humbert) Buchner & Pfiff 1, Madagascar, -, Malcomber 2775 (MO), JQ729820*, JQ729883*,
JQ729947*, JQ729707*, JQ729771*. Payera coriacea 2, Madagascar, -, Razafimandimison & Raveloniarivo 623 (S), JQ729846*, JQ729909*, JQ729971*.
Appendix. Continued.

JQ729734*, JQ729797*. Payera coriacea 3, ... & Taylor (unpub.); 12, Andersson (unpub.); 13, Paul & al. (2008); 14, Bremer & Eriksson (2009).

Appendix. Continued.

JQ729801*. 4083-B83.

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Razafimandimbison & Ravelonarivo 584 (S), JQ729825*, JQ729888*, JQ729952*, JQ729712*, JQ729776*.

–, Homolle, clada concinna JQ729855*, –, AF331652.

–, JQ729713*, JQ729777*.

–, –, –, –, –, –, JQ729718*, JQ729782*.

–, –, –, –, –, –, JQ729719*, JQ729724*, Psychotria kirkii Hier., –, –, Bremer 3102 (UPS), AM945214*, JQ729854*, AM945327*, FI208657*.

Schismatoclada bracteata Homolle ex Cavaco, Madagascar, –, Randriafazaka 173 (P), JQ729835*, JQ729898*, JQ729722*, JQ729786*.

Schismatoclada concinna Baker, Madagascar, –, Kårehed & al. 301 (UPS), JQ729836*, JQ729899*, JQ729723*, JQ729787*.

Schismatoclada farahimpensis Homolle, Madagascar, –, Gautier 2373 (P), JQ729837*, JQ729900*, JQ729962*, JQ729724*, JQ729788*.

Schismatoclada hamberti Kårehed & al. 283 (UPS), JQ729839*, JQ729901*, JQ729963*, JQ729725*, JQ729789*.

Schismatoclada marojejensis Kårehed & al. 283, Madagascar, Antsiranana, Bremer & al. 5309 (S), JQ729839*, JQ729902*, JQ729964*, JQ729726*, JQ729790*.

Schismatoclada psychotrioides Baker 1, Madagascar, –, Bremer & al. 4083-B83 (UPS), JQ729840*, JQ729903*, JQ729965*, JQ729727*, JQ729791*.

Schismatoclada psychotrioides 2, Madagascar, Kårehed & al. 283 (UPS), JQ729941*, JQ729904*, JQ729966*, JQ729728*, JQ729792*.

Schismatoclada psychotrioides 3, Madagascar, –, Philipsson 1608 (S), JQ729842*, JQ729905*, JQ729967*, JQ729729*, JQ729793*.

Schismatoclada sp. Baker 1, Madagascar, –, Razanatsima 728 (TAN), JQ729850*, JQ729913*, JQ729974*, JQ729737*, JQ729801*.

Schismatoclada sp. 2, Madagascar, –, Razanatsima & al. 600 (S), JQ729845*, JQ729908*, JQ729970*, JQ729732*, JQ729796*.

Schismatoclada sp. 3, Madagascar, –, Razanatsima & al. 375 (MO), JQ729849*, JQ729912*, –, JQ729736*, JQ729860*.

Schismatoclada sp. 4, Madagascar, Antsiranana, Bremer & al. 5298 (S), JQ729830*, JQ729893*, JQ729955*, JQ729717*, JQ729781*.

Schismatoclada sp. 5, Madagascar, Antsiranana, Bremer & al. 5275 (S), JQ729828*, JQ729891*, JQ729953*, JQ729715*, JQ729779*.

Schismatoclada sp. 6, Madagascar, Antsiranana, Bremer & al. 5311 (S), JQ729831*, JQ729894*, JQ729956*, JQ729718*, JQ729782*.

Schismatoclada sp. 7, Madagascar, Antsiranana, Bremer & al. 5314 (S), JQ729832*, JQ729895*, JQ729957*, JQ729719*, JQ729783*.

Schizocolea linderi (Hutch. & Dalziel) Bremek., Liberia, –, Adam 789 (P), AM945197*, JQ729853*, JQ729916*, AM945309*, JQ729740*.

* New sequences generated for this study. 1, Rydin & al. (2008, 2009a, b); 2, Razafimandimbison & al. (2008); 3, Andersson & Rova (1999, unpub.); 4, Ding & al. (unpub.); 5, Kårehed & Bremer (2007); 6, Malcomber (2002); 7, Novotny & al. (2002); 8, Kårehed & al. (2008); 9, Guo & al. (2011); 10, Chen & al. (2010); 11, Andersson & Taylor (unpub.); 12, Andersson (unpub.); 13, Paul & al. (2008); 14, Bremer & Eriksson (2009).