

# Molecular systematics and incongruent gene trees of Urophylleae (Rubiaceae)

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**Abstract** The phylogeny of the Pan-tropical tribe Urophylleae is poorly understood. Earlier phylogenetic work has identified major evolutionary lineages within the group, each geographically restricted to a single continent, but relationships among taxa within these lineages are so far largely unresolved. This study uses parsimony and Bayesian phylogenetic analyses of chloroplast DNA (cpDNA) and nuclear ribosomal DNA (nrDNA) to resolve phylogenetic relationships within Urophylleae. The results show that there are conflicts between cpDNA and nrDNA regarding species-level relationships within *Pauridiantha*, *Urophyllum*, and *Amphidasya*, which provides evidence of a complex genetic history. Different types of tests are used to explore the magnitude of the incongruence and locate the exact nodes in the two gene trees that are in conflict. This approach makes it possible to use the topology from the combined analysis, despite the separate datasets being strongly incongruent, since areas of the tree that are free from conflict can be identified. Based on the results presented in this study, Urophylleae is indicated to consist of *Temnopteryx*, *Raritebe*, *Amphidasya*, *Urophyllum* s.l., *Pauridiantha* s.l., and *Pentaloncha*. Several genera are shown to be nested inside *Pauridiantha*, two of which have already been included in *Pauridiantha* based on other data (*Pamplathantha*, *Commitheca*), and two which are included in this genus here (*Poecilocalyx*, *Stelechantha*). Likewise, three genera are shown to be ingroups in *Urophyllum* (*Pravinaria*, *Maschalocorymbus*, *Pleiocarpidia*), and are therefore subsumed under this genus. New combinations under *Pauridiantha* are proposed for three species in *Poecilocalyx* and three species in *Stelechantha*, as well as new combinations under *Urophyllum* for two species in *Pravinaria* and 17 species in *Pleiocarpidia*. For one of the latter species a new name is presented to avoid homonymy.

**Keywords** DNA sequence data; incongruence; phylogeny; Rubiaceae; taxonomy; Urophylleae

## ■ INTRODUCTION

Urophylleae Bremek. ex Verdc. are a tribe in the coffee family (Rubiaceae) including about 240 species (Govaerts & al., 2009), which are usually woody and have indehiscent, often fleshy, fruits with many seeds. The group is distributed mainly in the Palaeotropics but a couple of smaller genera occur in the Neotropics. It belongs in Rubioideae Bremek. ex Verdc. and is probably the sister group of Ophiorrhizeae Bremek. ex Verdc. (Rydin & al., 2009). The taxonomic history of the group is dominated by the revisionary work of Bremekamp (1940, 1941, 1952, 1966) and was reviewed by Smedmark & al. (2008). Currently, 20 genera are recognised in Urophylleae (Table 1). Bremekamp (1940, 1941, 1952, 1966) concluded that the two large genera *Urophyllum* Jack ex Wall. and *Pauridiantha* Hook. f. are restricted to Asia and Africa, respectively. In the conviction that large genera are undesirable, he also described a number of small satellite genera including one or a few species, assumed to be closely related to each of these two genera (Bremekamp, 1940, 1941). Later, African and Asian genera were classified in different tribes (Bremekamp, 1966). The African taxa, characterized by axillary inflorescences, bisexual heterostylous flowers, and usually bilocular ovaries with false septa in the upper part of the locules, were placed in Pauridiantheae Bremek., while the Asian taxa, with axillary inflorescences, dioecious flowers, plurilocular ovaries, and spreading

stigmata, were placed in Urophylleae. More recently, only one large tribe, Urophylleae, also comprising one or two Neotropical genera, has been recognised (Bremer & Manen, 2000; Robbrecht & Manen, 2006; Ntore, 2008). From a phylogenetic perspective, the fact that there are clear diagnostic features for the tribes Pauridiantheae and Urophylleae, as well as for each of the segregate genera, but not for either of *Pauridiantha* s.str. or *Urophyllum* s.str., leads to the suspicion that *Pauridiantha* and *Urophyllum* could be paraphyletic. Of Bremekamp's segregate genera, *Pravinaria* Bremek., with single-flowered, axillary inflorescences, and *Maschalocorymbus* Bremek., with a ring of hairs in the corolla throat and trichotomously corymbose inflorescences, have been shown by molecular phylogenetic methods to be nested inside *Urophyllum* (Smedmark & al., 2008). *Pamplathantha* Bremek., with terminal inflorescences, and *Commitheca* Bremek., with four carpels and thickened radial walls of the exotesta, have both been included in *Pauridiantha* based on morphology (Hallé, 1966; Ntore & al., 2003). However, for the other nine genera described by Bremekamp the relationships to *Urophyllum* and *Pauridiantha* are still not known, mainly because of the difficulty in obtaining plant material. Two of them, the West African genera *Poecilocalyx* Bremek., characterized by its enlarged calyx lobes and four- or five-locular ovaries, and the cauliflorous *Stelechantha* Bremek., were both included in a previous phylogenetic study (Smedmark & al., 2008) but the results were inconclusive. In

**Table 1.** Recognised genera in Urophyllaeae with the number of species (Govaerts & al., 2011), geographic distribution, and diagnostic characters of each genus.

Genus	Species	Geographic distribution	Diagnostic morphological characters
<i>Amphidasya</i> Standl. in Publ. Field Mus. Nat. Hist., Bot. Ser. 11: 180. 1936	13	Central & Southern Tropical America	Inflorescences terminal or pseudoaxillary, flowers bisexual homostylous, and ovaries bilocular.
<i>Antherostele</i> Bremek. in J. Arnold Arbor. 21: 26. 1940	4	Philippines	Leaves with acaridomatia, corolla with clavate hairs on the inside, and anthers linear, syngenesious.
<i>Crobylanthe</i> Bremek. in Recueil Trav. Bot. Néerl. 37: 191. 1940	1	Borneo	Hairs in corolla throat inserted on two scales at the base of each lobe.
<i>Didymopogon</i> Bremek. in Recueil Trav. Bot. Néerl. 37: 180. 1940	1	Sumatera	Hairs in corolla tube forming two rings, one in the throat and one at the base.
<i>Lepidostoma</i> Bremek. in Recueil Trav. Bot. Néerl. 37: 190. 1940	1	Sumatera	Hairs in corolla throat inserted on a scale at the base of each lobe.
<i>Leucolophus</i> Bremek. in Recueil Trav. Bot. Néerl. 37: 187. 1940.	3	Western Malesia	Hairs in corolla throat forming a ring and stipules glabrous inside.
<i>Maschalocorymbus</i> Bremek. in Recueil Trav. Bot. Néerl. 37: 181. 1940	4	Vietnam to Malesia	Inflorescences trichotomously corymbose and hairs in corolla throat forming a ring.
<i>Pauridiantha</i> Hook. f. in Bentham & Hooker, Gen. Pl. 2: 69. 1873	38	Tropical Africa	Inflorescences axillary, flowers bisexual heterostylous, and ovaries with two locules and false septa in the upper part.
<i>Pentaloncha</i> Hook. f. in Bentham & Hooker, Gen. Pl. 2: 73. 1873	2	W-Central Tropical Africa	Leaves anisophyllous, calyx lobes petaloid, flowers bisexual heterostylous, and ovaries bilocular.
<i>Pleiocarpidia</i> K. Schum. in Engler & Prantl, Nat. Pflanzenfam., Nachtr. 1: 314. 1897	28	Myanmar to Malesia	Inflorescences trichotomously corymbose or paniculate, hairs in corolla throat moniliform from base, and stigma peltate.
<i>Poecilocalyx</i> Bremek. in Bot. Jahrb. Syst. 71: 220. 1940	4	W & W-Central Tropical Africa	Calyx lobes enlarged and ovaries with four or five locules with false septa in the upper part.
<i>Pravinaria</i> Bremek. in Recueil Trav. Bot. Néerl. 37: 184. 1940	2	Borneo	Inflorescences single-flowered, axillary with one involucl.
<i>Praravinia</i> Korth., Verh. Nat. Gesch. Ned. Bezitt., Bot.: 189. 1842	49	Malesia	Inflorescences with two involucl, corolla with more numerous segments than the calyx, and corolla throat densely covered with stiff, white hairs.
<i>Raritebe</i> Wernham in J. Bot. 55: 336. 1917	1	Costa Rica to Peru	Inflorescences terminal, flowers bisexual and either hetero- or homostylous, and ovaries bilocular.
<i>Rhaphidura</i> Bremek. in Recueil Trav. Bot. Néerl. 37: 186. 1940	1	Borneo	Hairs in corolla throat forming a ring and stipules adpressed pubescent inside.
<i>Rhipidantha</i> Bremek. in Bot. Jahrb. Syst. 71: 222. 1940	1	Tanzania	Ovaries four- or five-locular with false septa in the upper part.
<i>Stelechantha</i> Bremek. in Bot. Jahrb. Syst. 71: 218. 1940	4	W & W-Central Tropical Africa	Cauliflorous, flowers bisexual heterostylous, and ovaries two-locular with false septa in the upper part.
<i>Stichianthus</i> Valetton in Bull. Jard. Bot. Buitenzorg, ser. 3, 2: 349. 1920	1	Borneo	Cauliflorous, inflorescences uniflowered and arranged in a row extending between internodes.
<i>Temnopteryx</i> Hook. f. in Bentham & Hooker, Gen. Pl. 2: 72. 1873	1	Cameroon to Gabon	Calyx lobes petaloid, flowers dioecious, and ovaries plurilocular.
<i>Urophyllum</i> Jack ex Wall. in Roxburgh, Fl. Ind. 2: 184. 1824	85	Tropical & Sub-tropical Asia	Hairs in the corolla throat sparsely and irregularly constricted near the top and style branches erect or ascending, acute, or obtuse.

that study, the only included species of *Poecilocalyx* was shown to be the sister of a *Stelechantha* species, and they were found in an unresolved position within the large Palaeotropical clade. Based on morphology, the distinction of *Poecilocalyx* and *Stelechantha* from *Pauridiantha* has been questioned (Ntore & al., 2010). The other seven genera described by Bremekamp have not yet been included in any phylogenetic study and so far nothing is known for certain about their relationships. These genera are: *Antherostele* Bremek., with acaridomata on the leaves; *Crobylanthe* Bremek., with distinctive hairs in the corolla throat inserted on two scales at the base of each corolla lobe; *Didymopogon* Bremek., with a ring of hairs at the base of the corolla tube; *Lepidostoma* Bremek., with hairs in the corolla throat inserted on a scale at the base of each corolla lobe; *Leucolophus* Bremek., with hairs in the corolla throat inserted on a ring and stipules that are glabrous inside; *Rhaphidura* Bremek., with hairs in the corolla throat inserted on a ring and stipules that are pubescent inside; and *Rhipidantha* Bremek., with four- or five-locular ovaries. Another small genus that has been classified in Urophylleae and whose phylogenetic affinity is still unknown is the Asian *Stichianthus* Valeton, diagnosed by having uniflorous inflorescences that cover the internodes.

The first phylogenetic study of Urophylleae (Smedmark & al., 2008) showed that it consists of a large Palaeotropical clade, including taxa placed in groups that are sometimes recognized as tribes Pauridiantheae and Urophylleae (Bremekamp, 1966), and a smaller Neotropical clade, consisting of the genera *Amphidasya* Standl. and *Raritebe* Wernham. Morphological characters shared by this Neotropical clade are usually terminal inflorescences, bisexual and usually homostylous flowers, and bilocular ovaries (Kirkbride, 1979; Taylor & Clark, 2001). This previous phylogenetic study also indicated, albeit with poor support, that the monotypic African *Temnopteryx* Hook. f., with petaloid calyx lobes and plurilocular ovaries, is the sister of these two groups (Smedmark & al., 2008). This relationship was confirmed by a later study (Smedmark & al., 2010), which also showed that the Palaeotropical clade is divided into an African and an Asian clade and resolved five other lineages within Urophylleae that correspond to recognised genera (*Amphidasya*, *Raritebe*, *Urophyllum*, *Pauridiantha*, *Pentaloncha* Hook. f.). These previous studies have, however, not managed to resolve relationships within the Palaeotropical clade and many questions are still unanswered.

The main aim of this study is to resolve relationships within the Palaeotropical clade and elucidate generic delimitation within this group. Of special interest is the question whether the two large genera *Pauridiantha* and *Urophyllum* are monophyletic, or if other smaller genera are nested inside them, which has been suggested by previous phylogenetic studies (Smedmark & al., 2008; Sonké & al., 2008). We also want to test the conclusions based on morphology that *Pamphlethantha* (included in the present study under the synonym *Pauridiantha viridiflora*) and *Commitheca* (included under the synonym *Pauridiantha liebrechtsiana*) are ingroups in *Pauridiantha* (Hallé, 1966; Ntore & al., 2003).

Previous phylogenetic studies including more than one or two representatives from Urophylleae have been based on

cpDNA data alone (Robbrecht & Manen, 2006; Smedmark & al., 2008; Sonké & al., 2008). In this study we use nrDNA data (ITS, ETS) to obtain an independent phylogenetic estimate that can be compared with one based on maternally inherited cpDNA (*rps16*, *trnTF* region). It is not uncommon that datasets from these two different genomes for the same set of organisms support different phylogenetic trees (Rieseberg & Soltis, 1991). Incongruent gene trees may be caused by evolutionary processes on the level of genes, such as recombination between genes, confusion of orthology and paralogy or concerted evolution, or on the level of organisms, such as hybridization, convergent evolution or lineage sorting (Wendel & Doyle, 2000). Therefore, the two datasets will be compared using different types of tests that measure phylogenetic incongruence in order to discriminate between stochastic differences between trees and differences caused by different underlying evolutionary histories. If trees are found to differ significantly we will try to determine what specific nodes are in conflict.

## ■ MATERIALS AND METHODS

**Material.** — Fifty-six Urophylleae species were selected to represent as many as possible of the genera in Urophylleae. Compared to a previous phylogenetic study of relationships within the group (Smedmark & al., 2008), new sequence accessions for twelve species of *Pauridiantha*, three species of *Poecilocalyx* Bremek. (including the type species *P. schumannii*), two species of *Stelechantha* Bremek. (including the type species *S. cauliflora*), and the type species of *Pleiocarpidia* K. Schum. (*P. enneandra*) have been included. The sample was limited by the access to well-preserved herbarium material from which DNA could be extracted and amplified. The missing genera (*Antherostele*, *Crobylanthe*, *Didymopogon*, *Lepidostoma*, *Leucolophus*, *Rhaphidura*, *Rhipidantha*, *Stichianthus*) are nearly all monotypic, and all but one are of Asian provenance (Table 1). Type species were included for all genera except *Praravinia*. Fifteen outgroup species were also selected to obtain a good representation from the rest of Rubiaceae, especially from taxa in Rubioideae that have been shown to be closely related to Urophylleae (Bremer & Eriksson, 2009). For most species, leaf material was sampled from herbarium specimens but in some cases silica-gel-dried material was used. Voucher specimens are listed in Smedmark & al. (2010).

**Molecular methods and dataset construction.** — Extraction of genomic DNA, amplification and sequencing of the *rps16* intron, the *trnTF* region, the internal transcribed spacer (ITS) region, and the external transcribed spacer (ETS) followed standard laboratory procedures described in detail by Smedmark & al. (2010). Sequences were assembled and edited using the Staden package (Staden & al., 1998), and sequence alignment was performed by eye, in the sequence alignment editor Se-Al (Rambaut, 1996).

**Model selection and phylogenetic analyses.** — Evolutionary models for each DNA region selected based on the Akaike information criterion (AIC; Akaike, 1973), which was calculated with MrAIC (Nylander, 2004), are specified



in Smedmark & al. (2010). In the analyses of cpDNA (*trnTF* region, *rps16*) and nrDNA (ITS, ETS), mixed models incorporating the selected models for each individual region were used. In the Bayesian inference (BI) analysis of all data in a combined dataset, the two partitions, cpDNA and nrDNA, were both allowed to evolve according to the GTR+ $\Gamma$  model. Bayesian phylogenetic analyses were performed in MrBayes (v.3.2, Huelsenbeck & Ronquist, 2000; Ronquist & Huelsenbeck, 2003). In the BI analyses of the separate datasets the Markov chain was run for four million generations, and in the combined analyses it was run for 10 million generations, sampling every 1000 generations. The Markov chain was assumed to have reached convergence when plots of the overall likelihood, as well as individual parameters of the model, were fluctuating around stable values. At this point the average standard deviation of split frequencies for two analyses run in parallel was less than 0.01 and the potential scale reduction factor was 1.00 for all parameters. Phylogenetic hypotheses sampled during the first 1,000,000 generations were discarded as “burn-in” and the remaining trees from the two parallel analyses were used to construct a majority-rule consensus tree and calculate posterior probabilities (PPs) of clades. A maximum parsimony (MP) bootstrap analysis (Felsenstein, 1985) with 10,000 pseudoreplicates was performed with PAUP\* (Swofford, 2002). Each pseudoreplicate was analysed with heuristic search, creating ten start trees by random addition and improving these by tree bisection–reconnection (TBR) branch swapping. One tree was saved for each bootstrap replicate and used to construct a majority-rule consensus tree and calculate bootstrap proportions (BPs). All phylogenetic analyses were repeated three times to make sure that independent runs converged on similar topologies containing the same nodes with PPs  $\geq 0.95$  or BPs  $\geq 70$ . Trees were rooted on *Luculia* Sweet because this taxon has been shown to be a basal lineage in Rubiaceae (Bremer, 1996).

**Tests of topological incongruence.** — Three different approaches to incongruence testing were used to determine whether there is significant disagreement between the cpDNA- and nrDNA datasets. The first was parsimony-based incongruence-length difference test (ILD, Mickevich & Farris, 1981; Farris & al., 1994) implemented in PAUP\* (Swofford, 2002). In this analysis, 1000 pseudoreplicates were performed and each of these were analysed with heuristic search, creating ten start trees by random addition and improving these by tree bisection–reconnection (TBR) branch swapping.

The second approach was Bayesian incongruence testing (Irestedt & al., 2004) using Bayes factors (Kass & Raftery, 1995). How to calculate Bayes factors using the output from MrBayes (v.3.2, Huelsenbeck & Ronquist, 2000; Ronquist & Huelsenbeck, 2003) was described by Nylander & al. (2004). Two MrBayes analyses, differing only in whether both partitions had their own topology parameter ( $M_1$ ) or were assumed to have evolved on the same topology ( $M_0$ ), were run with the settings described above for the combined Bayesian phylogenetic analysis. Then the marginal likelihood of each model was used to calculate the Bayes factor and evaluate the support in favour of one model ( $M_1$ ) as opposed to the other ( $M_0$ ). A value for the twice the logarithm of the Bayes

factor  $> 10$  has been suggested as strong evidence against  $M_0$  (Kass & Raftery, 1995).

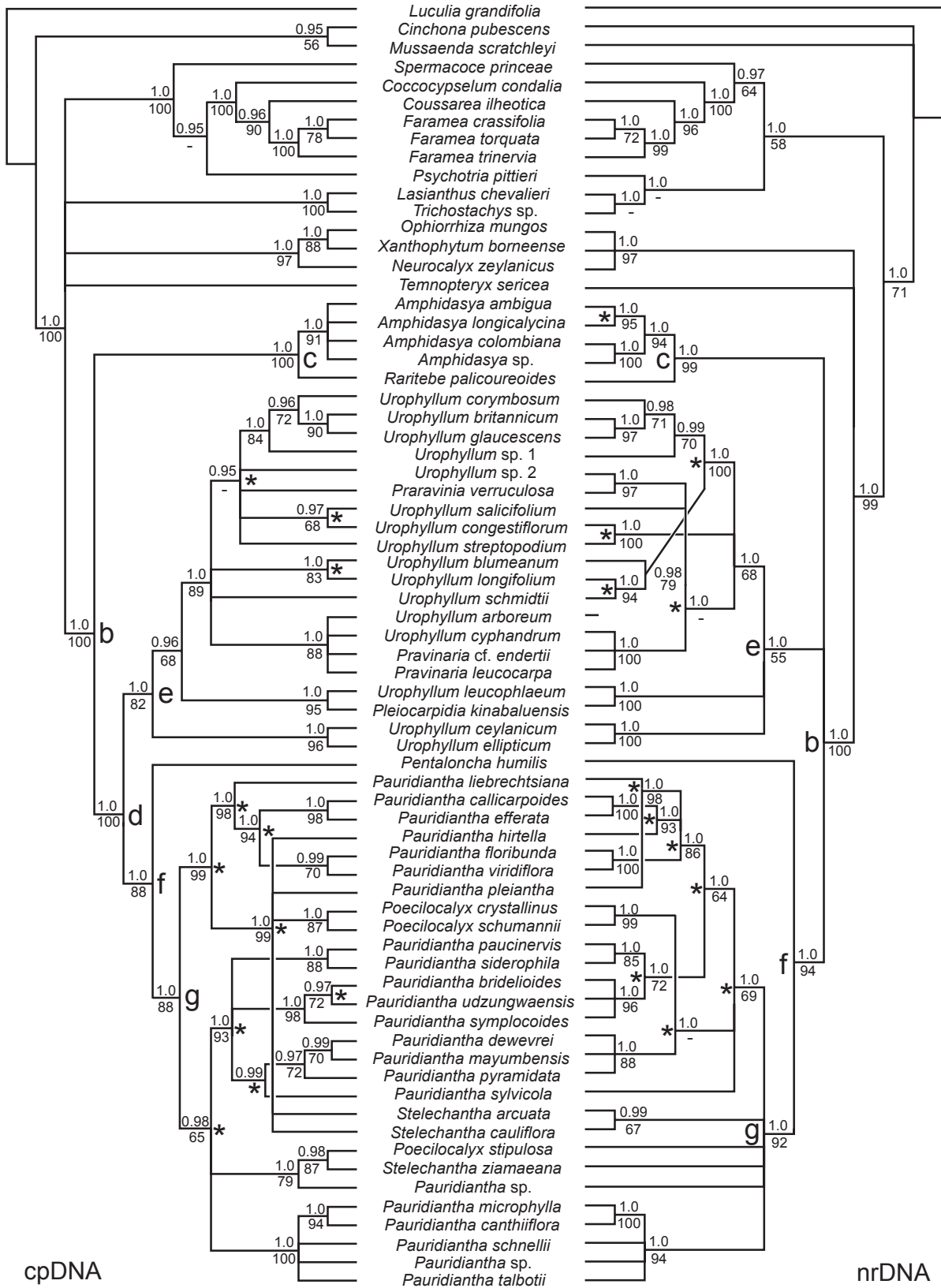
The program NodeScan (Eriksson, 2008) was used to determine which specific nodes in the cpDNA and nrDNA trees were in conflict with each other. This program counts the number of trees in a Bayesian tree sample where a certain node is present. Many nodes that are present in a large proportion of trees based on one dataset but few or none of the trees based on another dataset gives a strong indication of incongruence.

## ■ RESULTS

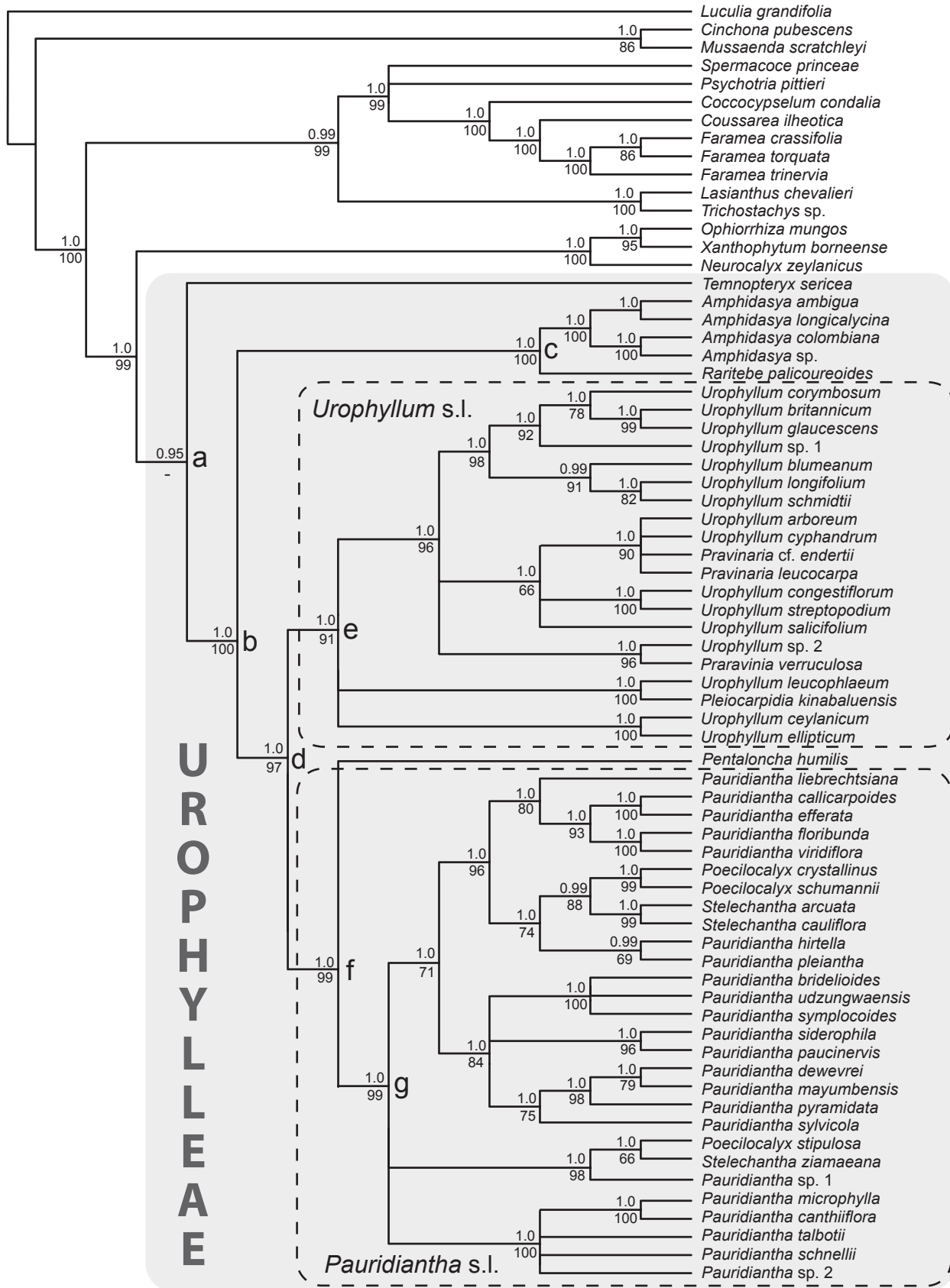
**Data.** — All EMBL accession numbers of DNA sequences are presented in Smedmark & al. (2010: table 1). The characteristics of the matrices from the different molecular markers are presented in Smedmark & al. (2010: table 2).

**Phylogenetic relationships.** — Trees from the separate analyses of cpDNA (*trnTF*, *rps16*) and nrDNA (ITS, ETS) agree on the relationships among major lineages within Urophyllaeae (Fig. 1). The support for *Temnopteryx* being the sister of the rest of Urophyllaeae is very low in the separate analyses (cpDNA PP = 0.33, BP = 29; nrDNA PP = 0.91, BP = 19). The two Neotropical genera *Amphidasya* (type species *A. ambigua*) and *Raritebe* (type species *R. palicoureoides*) are sisters (Fig. 1, node c). *Urophyllum* is indicated to be paraphyletic by both datasets (Fig. 1, node e). All included species of *Urophyllum*, including the type species *U. arboreum*, form a group that is strongly supported by BI but less by MP. Chloroplast DNA as well as nrDNA show that at least three accepted genera are supported to be ingroups in *Urophyllum*. *Maschalocorymbus* (type species *M. corymbosus* = *Urophyllum corymbosum*), *Pravinaria* (type species *P. leucocarpa*) and two more genera, *Praravinia* Korth. and *Pleiocarpidia*, for which the type species were not included in the analyses, are all nested inside *Urophyllum*. For *Pleiocarpidia* we did, however, obtain material of the type species (*P. enneandra*) at a late stage, and phylogenetic analysis of ITS sequences shows that this species is also nested inside *Urophyllum*. It is the sister of *Pleiocarpidia kinabaluensis* (PP 1.0, results not shown). *Pentaloncha* (type species *P. humilis*) and a paraphyletic *Pauridiantha* (type species *P. canthiiflora*) together form a large African clade (Fig. 1, node f). According to both datasets, *Commitheca* (type species *C. liebrechtsiana* = *Pauridiantha liebrechtsiana*), *Pamplethantha* (type species *P. viridiflora* = *Pauridiantha viridiflora*), *Poecilocalyx* (type species *P. schumannii*), and *Stelechantha* (type species *S. cauliflora*) are all nested inside *Pauridiantha* (Fig. 1, node g).

In the BI analysis of the combined dataset (Fig. 2) *Temnopteryx* is the sister of the rest of *Urophyllaeae* (node a, PP = 0.95), but this relationship receives minimal support in the MP analysis (BP = 18). The *Temnopteryx* branch is very long in proportion to those of other species in Urophyllaeae (not shown). The low support by MP in this study could therefore be a long-branch attraction phenomenon where *Temnopteryx* is incorrectly assumed to be more closely related to taxa outside Urophyllaeae that also have long branches. In an analysis by Sonké & al. (2008) *Temnopteryx* is not found to be the sister



**Fig. 1.** 95% majority-rule trees of Urophyllaeae from the Bayesian phylogenetic analyses of cpDNA (left) and nrDNA (right) with Bayesian PPs above branches and parsimony BPs below. Nodes that are present in more than 95% of the trees from the posterior distribution based on one dataset, but less than 2% based on the other dataset are marked with asterisks. Lower-case letters in the trees indicate clades that are mentioned in the text.



**Fig. 2.** 95% majority-rule tree of Urophyllaeae from the Bayesian phylogenetic analysis of the combined dataset of cpDNA and nrDNA data with Bayesian PPs above branches and parsimony BPs below. Nodes discussed in the text are indicated by lower-case letters.

of the remainder of Urophyllaeae, but only separated from this group by one poorly supported node (PP = 0.76, BP < 50). The topology reported in the present study does not change if *Colletoeccema dewevrei*, which has been shown to be one of the early branches in Rubioideae (Rydin & al., 2008), is included in the analysis (results not shown), and neither does the support for *Temnopteryx* as the sister of other Urophyllaeae.

The Neotropical clade (Fig. 2, node c) and the large Palaeotropical clade (Fig. 2, node d) are sister groups, a topology that is also supported by cpDNA alone, while nrDNA does not resolve relationships among these clades with good support (Fig. 1).

**Tests of incongruence.** — The ILD-test showed that there is significant incongruence between the two partitions; cp- and nrDNA ( $P = 0.001$ ), and the Bayes factor test also indicated that the two partitions are strongly incongruent. The marginal likelihood of the model in which each partition was allowed to evolve according to its own topology ( $M_1$ ) was higher (−32,500) than that of the model ( $M_0$ ) with a common topology parameter (−32,644). Twice the log of the Bayes factor was 288, which is considered as very strong evidence against  $M_0$  (Kass & Raftery, 1995).

The comparison of node support between the two datasets showed that 23 nodes within Urophyllaeae (marked with asterisks in Fig. 1) are supported by PPs  $\geq 0.95$  by one of the datasets, but  $\leq 0.02$  by the other dataset. This incongruence shows that there is considerable conflict between the datasets. The largest number of incongruent nodes are found within *Pauridiantha*, but conflicts are also present in several places within *Urophyllum*, and in one node in *Amphidasya*.

## ■ DISCUSSION

**Incongruent gene trees.** — Both the ILD-test and the Bayesian incongruence test show that there is substantial incongruence between the cpDNA and nrDNA datasets. By comparing the support for individual nodes in the trees from the separate analyses we found that the conflicts concern species-level relationships, primarily within *Pauridiantha* and *Urophyllum* (Fig. 1). The largest number of strongly supported instances of incongruence are found within *Pauridiantha* s.l. (15), compared to *Urophyllum* s.l. (7), and *Amphidasya* (1). The opinion that it is inadvisable to combine data when there is conflict between individual datasets has frequently been asserted in systematics (Bull & al., 1993; Miyamoto & Fitch, 1995). In this study we have chosen to do both separate and combined analyses of our two datasets. The separate analyses provide information about where in the phylogenetic tree the incongruent nodes are located and the combined analysis provides resolution and support in parts of the tree that are unaffected by the conflicts. This strategy makes it possible to use the phylogenetic hypothesis from the combined analysis, despite the incongruence between the separate datasets, since parts of the topology that are free from conflict can be identified. Thus, the conclusions drawn in the present study that are based on the phylogenetic tree from the combined analysis only pertain to the relationships among major lineages where there is no conflict between

the individual datasets. We also analysed a dataset that had been scaled down, removing all species that were involved in the conflicts, and this did not change the resolution of major relationships (results not shown). The tree from the combined analysis (Fig. 2) should, however, not be regarded as an accurate representation of species-level relationships within genera.

The present study cannot identify the cause of the incongruence between the cpDNA and nrDNA topologies. One possible explanation for the conflicts could be hybridisation, in which case the incongruent trees represent the different histories of cp- and nrDNA. Another possible cause is intra-individual polymorphism of nrDNA, which may arise through incomplete concerted evolution, and can cause paralogy problems or incomplete lineage sorting of nrDNA (Guggisberg & al., 2009). Polyploid species, especially if they are recent, may have different paralogs that could confound phylogenetic results, unless PCR products are cloned and a more in-depth study of gene phylogeny carried out. Little is known about ploidy levels in Urophyllaeae, but *Urophyllum ellipticum* and *U. ceylanicum* are known to be hexaploid while *Pauridiantha paucinervis* is diploid (Kiehn, 1995). A more extensive study of ITS and ETS variation involving cloning, or, preferably, an entirely different approach based on nuclear low-copy genes, would be possible ways of finding out why the gene trees differ and resolving the species-level phylogeny within *Pauridiantha* s.l., *Urophyllum* s.l., and *Amphidasya*. Although the analysis of nrDNA in addition to cpDNA, which is the type of data that has been used in previous studies including several representatives from Urophyllaeae (Robbrecht & Manen, 2006; Smedmark & al., 2008; Sonké & al., 2008), gave results that are difficult to interpret, the conflicts illuminate the fact that a phylogeny based on cpDNA alone may not be a correct representation of phylogenetic relationships.

**Phylogeny and taxonomy.** — This study shows that the Palaeotropical clade is divided into the Asian *Urophyllum* s.l., which has dioecious flowers, plurilocular ovaries, and spreading stigmata (Bremekamp, 1966), and an African clade consisting of *Pauridiantha* s.l. and *Pentaloncha*, which both have bisexual heterostylous flowers, and bi- or plurilocular ovaries with false septa in the upper part of the locules (Bremekamp, 1966). The two large genera *Urophyllum* and *Pauridiantha* are both shown to be paraphyletic. *Urophyllum* includes *Pravinaria*, *Maschalocorymbus* (here treated as *Urophyllum corymbosum*), and *Pleiocarpidia* (Fig. 2, node e). For *Praravinia*, on the other hand, which is represented by *P. verruculosa*, we have not been able to include the type species and can only conclude that our results give an indication that this genus probably is an ingroup in *Urophyllum* as well. *Pauridiantha* is shown to include *Poecilocalyx* and *Stelechantha* (Fig. 2, node g). Likewise, the genera *Commitheca* (treated as *Pauridiantha liebrechtsiana* in the present study) and *Pamplethantha* (treated as *Pauridiantha viridiflora*) are also shown to be ingroups in *Pauridiantha* (Fig. 2, node g), confirming the conclusions based on morphological data (Hallé, 1966; Ntore & al., 2003; Ntore, 2008). The type species for all of these four small genera are represented in the analyses and the phylogenetic support for the paraphyly of *Pauridiantha* is strongly based on both cpDNA and nrDNA.



Since it was recognised that the taxa that are the focus of the present study do not belong in Mussaendeae, where they were placed in earlier classifications (Bentham & Hooker, 1873; Schumann, 1891; Hallé, 1966), they have most often been classified in the tribe Urophyllaeae (Bremekamp, 1952; Verdcourt, 1958; Bremer & Manen, 2000; Robbrecht & Manen, 2006; Ntore, 2008) and sometimes in the two tribes Urophyllaeae and Pauridiantheae (Bremekamp, 1966; Robbrecht, 1988). Based on the results in this study, it seems most rational to follow the traditional wide circumscription of Urophyllaeae. If instead the two smaller tribes Urophyllaeae and Pauridiantheae (Fig. 2, nodes e and f) sensu Bremekamp (1966) were recognised, this would require the description of two new tribes, one including only *Temnopteryx*, and the other *Amphidasya* and *Raritebe*.

In conclusion, this study shows that several recognised genera simply are apomorphic derivatives of *Urophyllum* and *Pauridiantha* with their most recent ancestors within these genera. Therefore *Pravinaria*, *Maschalocorymbus*, and *Pleiocarpidia* have to be included in *Urophyllum*, and *Poecilocalyx* and *Stelechantha* in *Pauridiantha*. In some cases relevant synonyms already exist, for example for all species of *Maschalocorymbus*. However, for three species in *Poecilocalyx* and three species in *Stelechantha* formal recombinations under *Pauridiantha* need to be made. Also, two species in *Pravinaria* and 17 in *Pleiocarpidia* need new combinations under *Urophyllum*. For one species, *Pleiocarpidia capitata*, a new species name is needed since it would otherwise become a later homonym of *Urophyllum capitatum* Valetton. We name this species *Urophyllum clemensiorum* in honour of Joseph and Mary S. Clemens who collected the type specimen in Borneo. Together they did extensive collecting, especially in Southeast Asia, in the late 19th and early 20th centuries. Not only did they collect many Rubiaceae specimens but also vascular plants in general.

## ■ TAXONOMIC AND NOMENCLATURAL CHANGES

- Pauridiantha* Hook. f. in Bentham & Hooker, Gen. Pl. 2: 69. 1873 – Type: *P. canthiiflora* J.D. Hooker  
 = *Commitheca* Bremek. in Bot. Jahrb. Syst. 71: 219. 1940 – Type: *C. liebrechtsiana* (De Wild. & T. Durand) Bremek.  
 = *Pamplethantha* Bremek. in Bot. Jahrb. Syst. 71: 217. 1940 – Type: *P. viridiflora* (Schweinf. ex Hiern) Bremek.  
 = *Poecilocalyx* Bremek. in Bot. Jahrb. Syst. 71: 220. 1940, **syn. nov.** – Type: *P. schumannii* Bremek.  
 = *Stelechantha* Bremek. in Bot. Jahrb. Syst. 71: 218. 1940, **syn. nov.** – Type: *S. cauliflora* (R.D. Good) Bremek.
- Pauridiantha crystallina* (N. Hallé) Smedmark & B. Bremer, **comb. nov.** ≡ *Poecilocalyx crystallinus* N. Hallé in Fl. Gabon 12: 232. 1966 – Type: Abanga, Chantier, C.E.T.A., Gabon, *N. Hallé 2267* (holotype, P 00219633, image!; isotype, K 000172970, image!).

- Pauridiantha setiflora* (R.D. Good) Smedmark & B. Bremer, **comb. nov.** ≡ *Urophyllum setiflorum* R.D. Good in J. Bot.

64 (Suppl. 2): 6. 1926 ≡ *Poecilocalyx setiflorus* (R.D. Good) Bremek. in Bot. Jahrb. Syst. 71: 221. 1940 – Type: Port. Kongo, Mayumbe, N'Zanza, *Grossweiler 7861* (holotype, K 000172971, image!).

- Pauridiantha schumannii* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Poecilocalyx schumannii* Bremek. in Bot. Jahrb. Syst. 71: 221. 1940 – Type: Bipinde, Kamerun, *Zenker 1773* (holotype, B; isotype, P 00219674, image!).

- Pauridiantha arcuata* (S.E. Dawson) Smedmark & B. Bremer, **comb. nov.** ≡ *Stelechantha arcuata* S.E. Dawson in Kew Bull. 57: 398. 2002 – Type: Bimbia-Bonadikomba, Cameroon, *J.I. Wheatley 298* (holotype, K 000008069, K000008070, images!).

- Pauridiantha cauliflora* (R.D. Good) Smedmark & B. Bremer, **comb. nov.** ≡ *Urophyllum cauliflorum* R.D. Good in J. Bot. 61: 87. 1923 ≡ *Stelechantha cauliflora* (R.D. Good) Bremek. in Bot. Jahrb. Syst. 71: 219. 1940 – Type: Between the rivers Beize and Luali, Angola, *Gossweiler 7661* (holotype, BM; isotype, P 00539375, image!).

- Pauridiantha makakana* (N. Hallé) Smedmark & B. Bremer, **comb. nov.** ≡ *Stelechantha makakana* N. Hallé in Adansonia, n.s., 4: 236. 1964 – Type: Makak, Cameroun, *H. Jacques-Félix 2302* (holotype, P 00219700, image!; isotype, P 00219702, image!).

- Urophyllum* Jack ex Wall. in Roxburgh, Fl. Ind. 2: 184. 1824 – Type: *U. arboreum* (Reinw. ex Blume) Korth.  
 = *Pravinaria* Bremek. in Recueil Trav. Bot. Néerl. 37: 184. 1940, **syn. nov.** – Type: *P. leucocarpa* Bremek.  
 = *Maschalocorymbus* Bremek. in Recueil Trav. Bot. Néerl. 37: 181. 1940, **syn. nov.** – Type: *M. corymbosus* (Blume) Bremek.  
 = *Pleiocarpidia* K. Schum. in Engler & Prantl, Nat. Pflanzenfam., Nachtr. 1: 314. 1897, **syn. nov.** – Type: *P. enneandra* (Wight) K. Schum.

- Urophyllum endertii* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pravinaria endertii* Bremek. in Recueil Trav. Bot. Néerl. 37: 185. 1940 – Type: West Kutei, summit of G. Kemul, Borneo, *Endert 3980* (holotype, BO).

- Urophyllum leucocarpum* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pravinaria leucocarpa* Bremek. in Recueil Trav. Bot. Néerl. 37: 185. 1940 – Type: Tenompok, Mount Kinabalu, Borneo, *J. & M.S. Clemens 28176* (holotype, L 0001040, image!).

- Urophyllum assahanicum* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia assahanica* Bremek. in Recueil Trav. Bot. Néerl. 37: 210. 1940 – Type: Vicinity of Loemban Ria, Asahan, Sumatra, *R. Si Boeea 7650* (holotype, A 00094981, image!).



*Urophyllum chlamydanthum* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia chlamydantha* Bremek. in Recueil Trav. Bot. Néerl. 37: 224. 1940 – Type: Kenepai, Gunung, Borneo, *J.G. Hallier 1749* (holotype, L 0001012, image!).

*Urophyllum clemensiorum* Smedmark & B. Bremer, **nom. nov.** ≡ *Pleiocarpidia capitata* Bremek. in Recueil Trav. Bot. Néerl. 37: 222. 1940 – Type: Tenompok, Mount Kinabalu, Borneo, *J. & M.S. Clemens 28307* (holotype, A; isotype, L 0057688, image!).

*Urophyllum coffeoides* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia coffeoides* Bremek. in Recueil Trav. Bot. Néerl. 37: 233. 1940 – Type: Kenepai, Sungai, Borneo, *J.G. Hallier 1991* (holotype, L 0001014, image!).

*Urophyllum corniculatum* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia corniculata* Bremek. in Recueil Trav. Bot. Néerl. 37: 232. 1940 – Type: Central East Borneo, West Kutei, H. Petak, *Endert 3831* (holotype, BO).

*Urophyllum deliense* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia deliensis* Bremek. in Recueil Trav. Bot. Néerl. 37: 212. 1940 – Type: Sibolangit, Sumatra, *J.A. Lörzing 5645* (holotype, U, 0006153, image!).

*Urophyllum elmeri* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia elmeri* Bremek. in Recueil Trav. Bot. Néerl. 37: 227. 1940 – Type: Tawao, Borneo, *A.D.E. Elmer 21471* (holotype, A; isotype, U, 0006154, image!).

*Urophyllum holectomium* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia holectomia* Bremek. in Recueil Trav. Bot. Néerl. 37: 221. 1940 – Type: Kapit, Upper Rejang River, Borneo, *J. & M.S. Clemens 21401* (holotype, A 00032331, image!).

*Urophyllum kinabaluense* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia kinabaluensis* Bremek. in Recueil Trav. Bot. Néerl. 37: 217. 1940 – Type: Marai Parai, Mount Kinabalu, Borneo, *J. & M.S. Clemens 32279* (holotype, A; isotype, L 0001018, image!).

*Urophyllum lanaense* (Merr.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia lanaensis* Merr. in Philipp. J. Sci. 20: 462. 1922 – Type: Mindanao, Lanao, Philippines, *M.S. Clemens 882* (location of type unknown).

*Urophyllum macrurum* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia macrura* Bremek. in Recueil Trav. Bot. Néerl. 37: 219. 1940 – Type: Penibukan, Borneo, *J. & M.S. Clemens 31384* (holotype, BO; isotype, L 0001019, image!).

*Urophyllum magnifolium* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia magnifolia* Bremek. in Recueil

Trav. Bot. Néerl. 37: 216. 1940 – Type: Kenepai, Gunung, Borneo, *J.G. Hallier 1813* (holotype, L 0001020, image!).

*Urophyllum maingayi* (King & Gamble) Smedmark & B. Bremer, **comb. nov.** ≡ *Aulacodiscus maingayi* King & Gamble in J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 72: 189. 1983 ≡ *Pleiocarpidia maingayi* (King & Gamble) Ruhsam. in Bot. J. Linn. Soc. 157: 12. 2008 – Type: Malay Peninsula, Malacca, *A.C. Maingay 938* (syntype, K 000265258, 000265259, images!), Malay Peninsula, Selangor, *Curtis 2343* (syntype), Malay Peninsula, Perak, *Scoretechini 2004* (syntype), loc. cit., *King's Collector 1086* (syntype).

*Urophyllum opacum* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia opaca* Bremek. in Recueil Trav. Bot. Néerl. 37: 225. 1940 – Type: Borneo, *Sarawak Museum 2031* (holotype, A 00032335, image!).

*Urophyllum parvistipulum* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia parvistipula* Bremek. in Recueil Trav. Bot. Néerl. 37: 220. 1940 – Type: Kenepai, Goenoeng, Borneo, *J. & M.S. Clemens 32516* (holotype, A; isotype, L 0001022, image!).

*Urophyllum rufescens* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia rufescens* Bremek. in Recueil Trav. Bot. Néerl. 37: 228. 1940 – Type: Amai Ambit, Borneo, *J.G. Hallier 3187* (holotype, L 0001030, image!).

*Urophyllum sandahanicum* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia sandahanica* Bremek. in Recueil Trav. Bot. Néerl. 37: 212. 1940 – Type: British North Borneo, *J. Agama 565* (holotype, NY; isotype, A 00032337, image!).

*Urophyllum sintangense* (Bremek.) Smedmark & B. Bremer, **comb. nov.** ≡ *Pleiocarpidia sintangensis* Bremek. in Recueil Trav. Bot. Néerl. 37: 218. 1940 Type: G. Klam en S. Djemek, Borneo, *J.G. Hallier 2542* (holotype, L 0001032, image!).

## ■ ACKNOWLEDGEMENTS

We thank the curators of the herbaria AAU, BR, GB, K, S, and UPS for the loan of herbarium material, and A and K for providing digital images of type specimens. This study was supported by grants from the Swedish Research Council and the Knut and Alice Wallenberg Foundation to B. Bremer.

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