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Combining Angiosperms353 and Sanger data provides support for the reinstatement of the genus *Myrianthemum* (Melastomataceae)

Luo Chen[1,*], Gudrun Kadereit[1,2], Marie Claire Veranso-Libalah[1]

[1]Prinzessin Therese von Bayern-Lehrstuhl für Systematik, Biodiversität & Evolution der Pflanzen, Ludwig-Maximilians-Universität München, Menzinger Str. 67, München, 80638, Germany
[2]Staatliche Naturwissenschaftliche Sammlungen Bayerns (SNSB): Botanische Staatssammlung München, Botanischer Garten München-Nymphenburg, Menzinger Str. 65 und 67, München, 80638, Germany

[*]Corresponding author. Prinzessin Therese von Bayern-Lehrstuhl für Systematik, Biodiversität & Evolution der Pflanzen, Ludwig-Maximilians-Universität München, Menzinger Str. 67, München, 80638, Germany. E-mail: Luo.Chen@lmu.de

ABSTRACT

The increasing availability of DNA sequence data, in particular target enrichment data based on the universal Angiosperms353 probe set, but also accumulated Sanger data from previous phylogenetic studies, is facilitating the placement of taxa that are difficult to place with certainty based on morphological evidence alone. Here, we investigate phylogenetic relationships of *Medinilla mirabilis* (Melastomataceae), a species distributed in central Africa and currently classified in the mega-diverse genus *Medinilla* of tribe Sonerileae. *Medinilla mirabilis* is a twining liana with verticillate leaves when young, spherical inflorescences, 4-merous flowers, dimorphic stamens, and baccate fruits. Our results revealed that *M. mirabilis* is sister to tribe Dissochaeteae and only distantly related to *Medinilla*. We also provide new data on wood anatomical and seed morphological characters of *M. mirabilis*. The alternate inter-vessel pits in *M. mirabilis* and Dissochaeteae are consistent with the phylogenetic placement. Seeds of *M. mirabilis* are similar to those of Dissochaeteae and of *Medinilla*. Due to its unique morphology and phylogenetic position, we propose to reinstate the monospecific genus *Myrianthemum* with *Myrianthemum mirabile*. This necessitates expansion of the Southeast Asian tribe Dissochaeteae to include *Myrianthemum* as its only African member. Our study of *M. mirabile* demonstrates that the combined application of Angiosperms353 and Sanger data is a cost-effective approach to phylogenetically place enigmatic taxa.

Keywords: Africa; seed morphology; wood anatomy; phylogenetics

INTRODUCTION

Every year, ~2000 new plant species are described (Christenhusz and Byng 2016), and many are rediscovered and collected. Phylogenetic placement of these species has important implications for our understanding of their evolutionary history. The most intuitive and cost-effective way to phylogenetically place a taxon that remained unsampled is to add new data to existing sequencing data. Until recently, this has been done using Sanger sequencing data. Within Melastomataceae, phylogenetic placement using Sanger sequencing data has resulted in the descriptions of new genera [e.g. *Benna* Burgt & Ver.-Lib. (van der Burgt *et al.* 2022) and *Nothodissotis* Ver.-Lib. & G.Kadereit (Veranso-Libalah *et al.* 2019)] and new tribes [e.g. Marcetieae (Rocha *et al.* 2018), Lithobieae, and Eriocnemeae (Penneys *et al.* 2020)]. Also, *Cailliella* Jacq.-Fél., until recently only known from its type specimen, was confirmed as a member of tribe Melastomateae based on a recent collection (Veranso-Libalah *et al.* 2021). Over the last three decades, millions of Sanger sequences have been generated and used for phylogenetic studies. However, the use of only few Sanger-sequenced genes can result in stochastic errors and often does not provide enough information to achieve phylogenetic resolution (Jeffroy *et al.* 2006, Kapli *et al.* 2020).

Angiosperms353 (Johnson *et al.* 2018) is a set of target enrichment probes designed to capture 353 putatively single-copy protein-coding nuclear genes. As a universal probe set for angiosperms, Angiosperms353 is being used in 'The Plant and Fungal Trees of Life' Project (PAFTOL; www.paftol.org),

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which aims at generating target enrichment data for all genera of flowering plants (Baker *et al.* 2021a). To increase data usability, Angiosperms353 has also been included in the design of group-specific probe sets (Angiosperms353-related) (Jantzen *et al.* 2020, Ogutcen *et al.* 2021, Acha and Majure 2022). The rapid accumulation of Angiosperms353 and Angiosperms353-related data is making it possible for us to place a taxon of interest in the flowering plant tree of life using phylogenomic data, which can greatly reduce the aforementioned stochastic errors and provide more sequence information. For example, phylogenomic data for 412 families, 7514 genera, and 9404 species have been released in the Kew Tree of Life Explorer Release 2.0 (https://treeoflife.kew.org/release-history). However, although a large quantity of data has been generated, many genera (especially from the tropics) remain unsampled. Given the vast diversity of flowering plants (~14 000 genera and ~325 000 species; Antonelli *et al.* 2020), it will take time before a few taxa of interest can be placed phylogenetically at genus or even species level using Angiosperms353 data. One expedient approach to this problem is to additionally use Sanger sequencing data accumulated over the past three decades. The combination strategy of using both Angiosperms353 and Sanger sequencing data so far has only been used to investigate generic limits in Urticaceae (Wells *et al.* 2021).

Melastomataceae, with 173 genera and ~5858 species (Ulloa Ulloa *et al.* 2022), belong to Myrtales (Maurin *et al.* 2021). Using the Angiosperms353 probe set, Maurin *et al.* (2021) provided the most comprehensive phylogeny to date for Myrtales, representing ~76% of the generic diversity of the order. In Melastomataceae, 193 species representing 116 genera and 19 tribes were included in this analysis. Most of the unsampled or under-sampled taxa of Melastomataceae are restricted mainly to the Old World. One such taxon is *Medinilla* Gaudich. ex DC., a morphologically diverse genus with 379 species in the Afrotropics, Asia, and Oceania (Liu *et al.* 2022), of which only three Southeast Asian and one Malagasy species were sampled by Maurin *et al.* (2021). *Medinilla* has been treated in either tribe Dissochaeteae (e.g. Triana, 1871 ['1872'], Van Vliet *et al.* 1981) or Miconieae (Renner 1993) based on morphological and anatomical evidence, but molecular phylogenetic analyses of a few representatives have revealed that although the monophyly of *Medinilla* and its circumscription remain to be resolved, all the sampled species belong to Sonerileae (Zhou *et al.* 2019, 2022, Kartonegoro *et al.* 2021, Penneys *et al.* 2022, Veranso-Libalah *et al.* 2022).

In tropical Africa and Madagascar, there exist 73 species of *Medinilla*, of which 70 are endemic to Madagascar, the center of diversity. Only three species occur in mainland Africa, i.e. *Medinilla engleri* Gilg, a rare species endemic to Tanzania, *Medinilla mannii* Hook. f., which is widely distributed in West-Central Africa and the Gulf of Guinea islands, and *Medinilla mirabilis* (Gilg) Jacq.-Fél. (Fig. 1) occurring in Central African countries (Fig. 2) (Veranso-Libalah *et al.* 2022). *Medinilla mirabilis* can easily be distinguished from the other two mainland African species by its growth form (twining liana), leaf arrangement (leaves verticillate in young and alternate in old parts of a plant), its spherical inflorescence composed of many cymes, and its dimorphic stamens. The other two African species are epiphytic shrubs with opposite leaves, fewer flowers, and equal to subequal stamens. *Medinilla mirabilis* was first described in 1897 as *Myrianthemum mirabile* Gilg (Engler, 1897) and later transferred to *Medinilla* by Jacques-Félix (1977) who believed that *Myrianthemum mirabile* fits into the very diverse and widespread palaeotropical genus *Medinilla*, despite its marked differences from the other two mainland African *Medinilla* species (Jacques-Félix 1977). *Myrianthemum* Gilg has since been treated as a synonym of *Medinilla*. Although widely collected within its distribution range, *M. mirabilis* has not been included in any phylogenetic study, so its tribal and generic placement remain untested.

Before the molecular era, both wood anatomical (Koek-Noorman *et al.* 1979, Van Vliet 1981, Van Vliet *et al.* 1981, Welle and Koek-Noorman 1981) and seed morphological characters (Whiffin and Tomb 1972, Michelangeli 2000) were used for the tribal and generic classification of Melastomataceae, and many aspects of the classification based on wood anatomical evidence (Van Vliet *et al.* 1981) have been supported by molecular data (Clausing and Renner 2001, Penneys *et al.* 2022). In particular, Dissochaeteae were divided into two subtribes based on wood anatomy: Medinillinae, including *Medinilla* and its alliance, and Dissochaetinae including most genera still belonging to Dissochaeteae. Seed morphological studies in Miconieae (Ocampo and Almeda 2013) and the whole family (Ocampo *et al.* 2022) indicated that seed morphology does not reflect phylogenetic relationships, although it is quite conserved in Melastomateae (Veranso-Libalah *et al.* 2020). However, as many species of Melastomataceae have not been included in wood anatomical and seed morphological studies, it remains unknown whether these characters can provide useful information for generic delimitation.

In this paper, using Angiosperms353 data and the phylogenomic framework generated by Maurin *et al.* (2021), we investigate the tribal and generic placement of *M. mirabilis*. Additional Sanger data are used to overcome the problem of insufficient taxon sampling for phylogenomic data. We use the phylogenetic placement of *M. mirabilis* to demonstrate that combining Angiosperms353 and Sanger data is a cost-effective way to investigate the phylogenetic placement of taxa. Furthermore, we provide new data on the wood anatomy and seed morphology of *M. mirabilis* and compare them with existing data.

MATERIALS AND METHODS

Taxon sampling and DNA extraction

We sampled four individuals of *M. mirabilis* for the phylogenetic study. Sample *Medinilla_mirabilis* 1928 was used for both Sanger and target enrichment sequencing, while the other three samples were only Sanger sequenced. For the target enrichment data, raw data of all 194 Melastomataceae samples generated by Maurin *et al.* (2021) were accessed from the European Nucleotide Archive (ENA) using enaBrowserTools (https://github.com/enasequence/enaBrowserTools). In addition, 10 CAP (Crypteroniaceae–Alzateaceae–-Penaeaceae) clade species were included as outgroups. For the Sanger data, we retrieved sequences of 152 taxa from GenBank covering most major clades of Melastomataceae identified by Penneys *et al.* (2022), including 31 plastomes. Dissochaeteae and Sonerileae were densely sampled. The script 'get_annotated_regions_from_gb.py' (https://

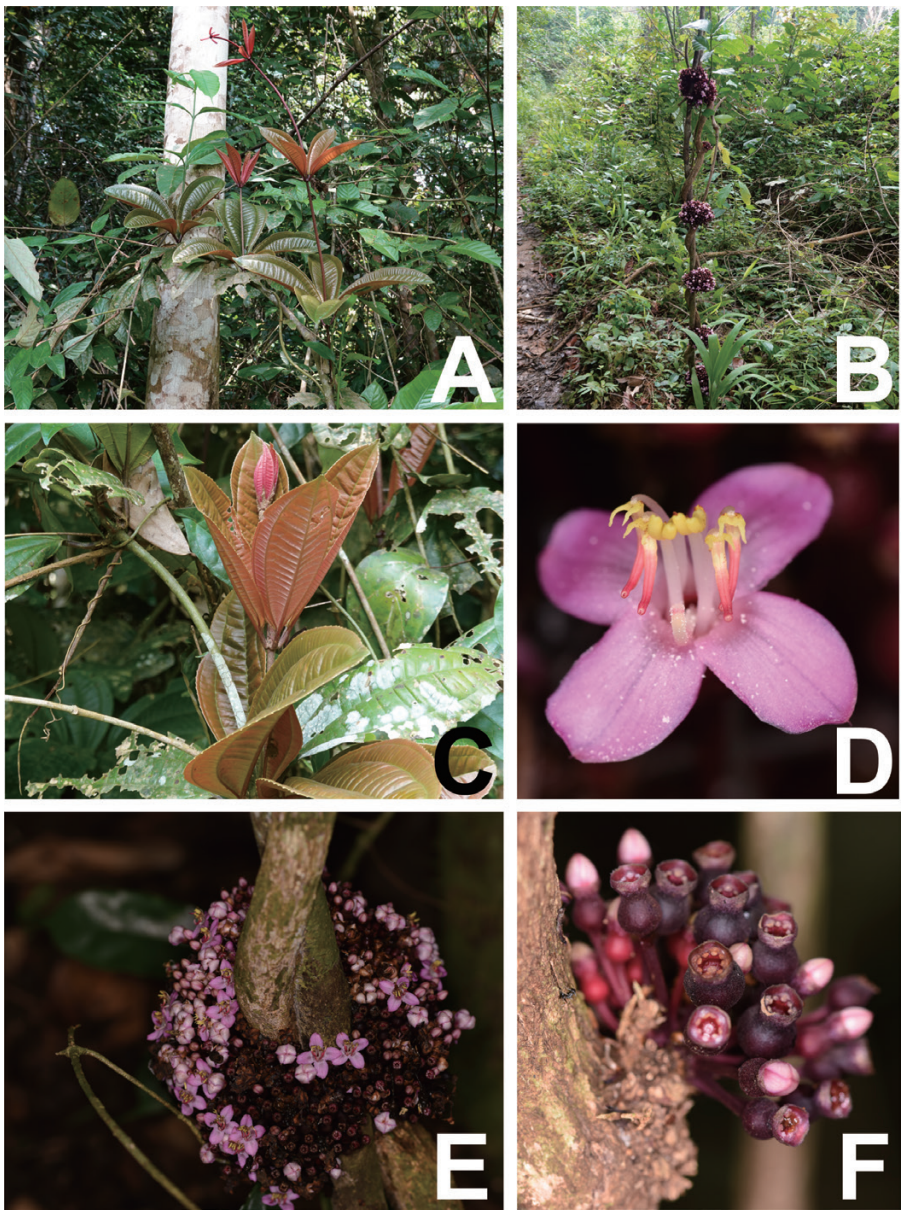

Figure 1. Photographs of *Medinilla mirabilis* in its natural habitat. A, young twigs with verticillate leaves. B, mature stem coiling around the stem of a tree. C, young leaves with clearly dentate leaf margins. D, close-up image of a single flower. E, spherical inflorescence composed of many cymes. F, fruits. All photos by Luo Chen.

github.com/Kinggerm/GetOrganelle/blob/master/Utilities/get_annotated_regions_from_gb.py) was used to extract plastid regions of interest from these 31 plastomes. In total, the target enrichment data included 205 samples, while the Sanger data included 156 samples. Detailed information on the taxa sampled, accession numbers, and vouchers can be found in Supplementary Table S1.

Total genomic DNA was extracted from silica-dried leaves or herbarium specimens using a modified CTAB protocol (Doyle and Doyle 1987, Majure *et al.* 2019). DNA concentration was measured with a Qubit 4 fluorometer.

Sequence assembly of target enrichment data

The extracted DNA of *Medinilla_mirabile* 1928 was sent to Rapid Genomics (Gainesville, FL, USA) for library preparation, target enrichment, and next-generation sequencing. Melastomataceae-specific probes developed by Jantzen *et al.* (2020) were used to capture the targeted 384 loci, of which 266 are from the Angiosperms353 project.

Raw sequences were trimmed with Trimmomatic v.0.39 (Bolger *et al.* 2014). We recovered the target loci from both paired and unpaired trimmed sequences using HybPiper (Johnson *et al.* 2016). As it has been demonstrated that the mega353 target file can improve locus recovery (McLay *et al.* 2021), the target file we used for recovery was a tailored mega353 file containing transcriptome sequences from Myrtales species and the original default353 file. Since using a target file containing protein sequences for mapping yields better results than using a target file containing nucleotide sequences, we used DIAMOND for mapping (see https://

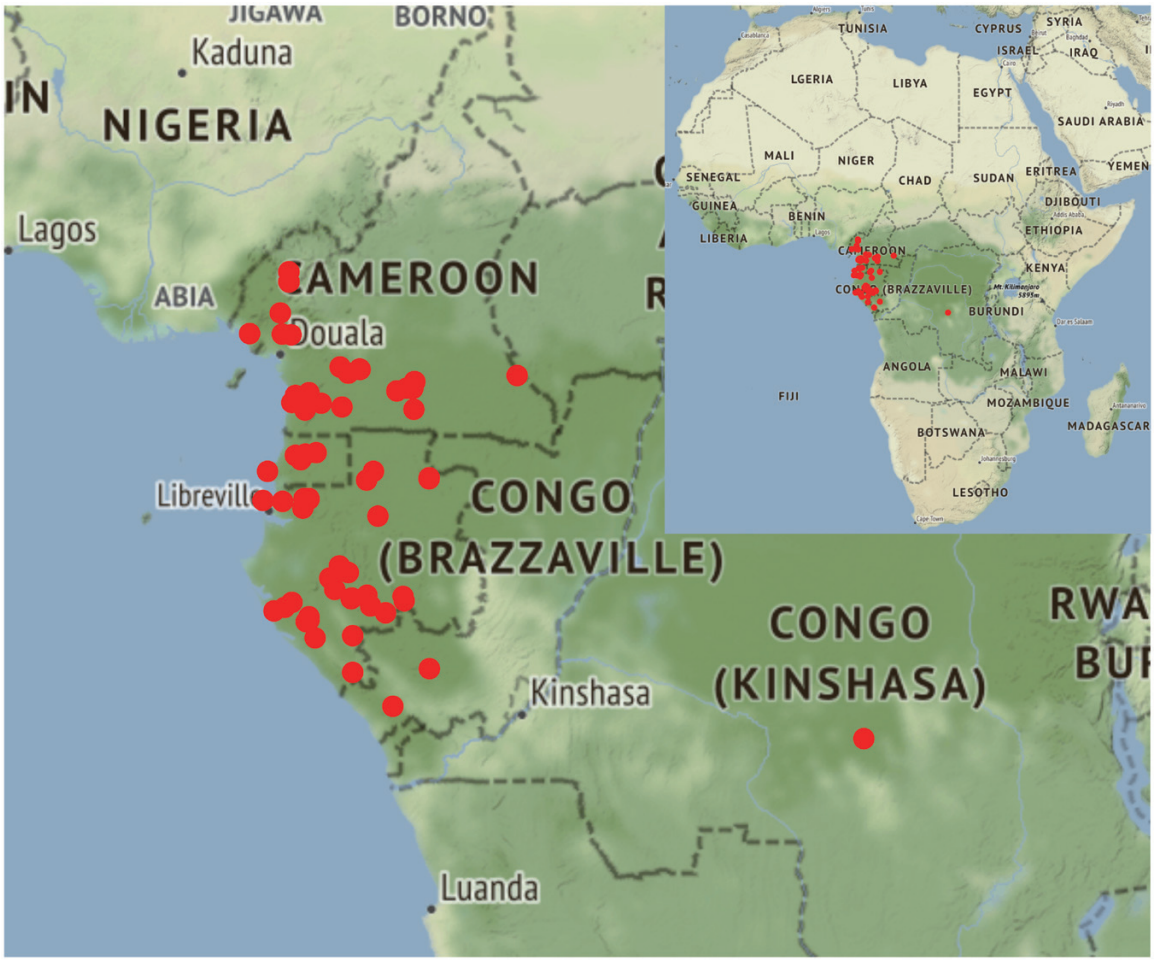

Figure 2. Geographical distribution of *Medinilla mirabilis*.

github.com/mossmatters/HybPiper/wiki/ for detailed explanation). Before mapping, the tailored mega353 file was filtered using a script provided by the authors of HybPiper (https://github.com/mossmatters/HybPiper/files/8933179/get_inframe_targetfile.py.gz) to rewrite the sequences in multiples of three and remove sequences that have stop codons in any forward frame. We ran the HybPiper command 'hybpiper paralog_retriever' to retrieve both the main sequence and putative long paralogues for orthologue inference.

Retrieved loci were first aligned using MAFFT v.7.453 (Katoh and Standley 2013) with the algorithm L-ENS-I and the function 'reversing complete sequences' (--adjustdirectionaccurately). The alignments were trimmed with Phyutility v.2.7.1 (Smith and Dunn 2008) to remove sites missing 90% data. We discarded short sequences (< 50 bp) and gappy sequences (missing 90% sites) before generating single-gene trees, for which IQ-Tree v.2.1.3 (Minh *et al.* 2020) was used with 1000 ultrafast bootstrap replicates. Raw single-gene trees were first trimmed by masking the monophyletic and paraphyletic tips that belong to the same taxon following Yang and Smith (2014) and Morales-Briones *et al.* (2021). TreeShrink v.1.3.7 (Mai and Mirarab 2018) was later used to remove outlier long branches with default settings.

We employed DISCO (Willson *et al.* 2021) to decompose the single-gene trees for species tree inference. DISCO takes the Newick format gene trees as input, roots, and labels the internal nodes as either duplication or speciation events using ASTRAL-Pro's method (Zhang *et al.* 2020), and decomposes the gene trees with its decomposition algorithm. We ran DISCO with default settings except for using the option -m 20 for filtering trees with fewer than 20 taxa.

Sequence assembly of Sanger data

Two nuclear (ITS and ETS) and four plastid loci (*psbK-psbI*, *ndhF*, *rbcL*, and *rpl16*) were amplified with the primers used by Kartonegoro *et al.* (2021). Only ITS-241r, instead of the reverse primer ITS2-MEL R, was used for amplifying ITS2. We followed the PCR protocols used by Veranso-Libalah *et al.* (2017) and Kartonegoro *et al.* (2021) with minor modifications. Details of the PCR amplification are given in Table 1. Sequencing was performed either at the Institute of Systematics, Biodiversity and Evolution of Plants or the sequencing service of the Faculty of Biology, LMU Munich, Germany. Sequencher v.5.1 (Gene Codes Corp., Ann Arbor, MI, USA) was used to manually edit and assemble the raw sequencing data. MAFFT was used for sequence alignment with the '--adjustdirection' option used.

Phylogenetic analyses

For the target enrichment data, after decomposition, both coalescent and concatenation methods were used to estimate the species tree. For the coalescent analysis, ASTRAL v.5.7.7 (Zhang

Table 1. PCR primers used in this study

Locus	Primer	Primer sequence, 5′→3′	Source
ITS	ITS1-MEL F	GGAGAAGTCGTAACAAGGTTTC	Veranso-Libalah *et al.* (2017)
	ITS1-MEL R	CTTGCGTTCAAAGAATTGATGG	
ITS	ITS2-MEL F	CGGCTCTTGCATCGATGAAG	Veranso-Libalah *et al.* (2017)
	ITS-241r	CAGTGCCTCGTGGTGCGACA	Michelangeli *et al.* (2004)
ETS	ETS NY320 F	AGACAAGCATATGACTACTGGCA	Kartonegoro *et al.* (2021)
	ETS 1428 Mel Spec R	ACGTGTCGCGTCTAGCAGGCT	
psbK-psbI	psbK F	TTAGCCTTTGTTTGGCAAG	Reginato *et al.* (2010)
	PsbI R	AGAGTTTGAGAGTAAGCAT	
ndhF	ndhF-972 F	GTCTCAATTGGGTTATATGATG	Olmstead and Sweere (1994)
	ndhF-1603 R	GCATAGTATTGTCCGATTCATRAGG	
ndhF	ndhF-1318 F	GGATTAACYGCATTTTATATGTTTCG	Olmstead and Sweere (1994)
	ndhF-1955 R	CGATTATATGACCAATCATATA	
rbcL	rbcL-1 F	ATGTCACCACAAACRGAGACTAAAGC	de Groot *et al.* (2011)
	rbcL-1361 R	TCAGGACTCCACTTACTAGCTTCACG	
rpl16	rpl16-71 F	GCTATGCTTAGTGTGTGACTCGTTG	Jordan *et al.* (1996)
	rpl16-1516 R	CCCTTCATTCTTCCTCTATGTTG	Kelchner and Clark (1997)

et al. 2018) was used with the decomposed gene trees as input. For the concatenation analysis, we concatenated the gene alignments that correspond to the decomposed gene trees using the script ca_disco.py (https://github.com/JSdoubleL/DISCO/blob/master/ca_disco.py) and then inferred the maximum-likelihood (ML) phylogeny using IQ-Tree as described above.

For the Sanger data, we generated three concatenated alignments using FASconCAT-G v.1.04 (Kück and Longo 2014) for phylogenetic inference: (i) an alignment containing 154 chloroplast sequences, (ii) an alignment comprising 141 nuclear sequences, and (iii) an alignment including 156 both nuclear and chloroplast sequences. The ML analysis was performed as described above.

All phylogenetic trees were visualized using ITOL v.6.6 (Letunic and Bork 2021).

Distribution map

We accessed all available distribution records of *M. mirabilis* from GBIF [GBIF.org (1 January 2023) GBIF Occurrence Download https://doi.org/10.15468/dl.febs8q] and further used herbarium specimens from BRLU, BR, and P. Information regarding all records used are listed in Supplementary Table S2. Visualization was performed using ggmap v.3.0.1 (Kahle and Wickham 2013) in R Statistical Software v.4.2.2 (R Core Team 2022).

Wood anatomy and seed morphology

The wood sample analyzed was collected from specimen *Medinilla mirabilis* 35_21. Following Van Vliet (1981), we studied those three characters that were used to categorize Dissochaeteae, i.e. arrangement of inter-vessel pits, vessel element diameter, and ray width. Following Jansen *et al.* (1998), the dry wood sample was first softened by soaking in a mixture of glycerin/water (1:10 volume ratio) for 2 days. Tangential sections 25–30 μm thick were cut with a sledge microtome (Microm, Heidelberg, Germany), mounted on slides, and subsequently embedded in ROTI Histokitt (Carl Roth, Karlsruhe, Germany). The embedded slides were observed under a light microscope (Leica DM750, Wetzlar, Germany) equipped with Leica LAS X software. Vessel element diameter data were obtained from 12 measurements (Supplementary Table S3).

Seed morphological character examination was performed using a scanning electron microscope (Leo 438VP, Carl Zeiss AG, Oberkochen, Germany). Seeds were collected from the specimen *Medinilla mirabilis* 35_21. Following Ocampo *et al.* (2022), seed morphology was described using 14 characters (Supplementary Table S3).

RESULTS

Phylogenetic placement of *Medinilla mirabilis*

Medinilla mirabilis is recovered in all analyses as sister to Dissochaeteae *s.s.* recently circumscribed by Kartonegoro *et al.* (2021). The species is neither part of Miconieae nor of Sonerileae and does not group with the other species of *Medinilla s.s.* which are part of Sonerileae.

In the Angiosperms353 data, five species of tribe Dissochaeteae belonging to *Dissochaeta* and *Diplectria* were sampled, and *M. mirabilis* is strongly supported as sister to these species. The clade comprising *M. mirabilis* and Dissochaeteae is most closely related to the South American tribe Pyramieae. These results are consistent between the concatenation (Supplementary Fig. S1) and coalescent analyses (Fig. 3).

In the three Sanger data sets (Fig. 3, Supplementary Figs S2, S3), Dissochaeteae are well represented with all six genera and ~50% of its species sampled. Phylogenetic relationships recovered among analyses from different data sets are mostly consistent. In all three analyses, the four accessions of *M. mirabilis* sampled form a clade that is highly supported as sister to a monophyletic group comprising all Dissochaeteae species.

Wood anatomy and seed morphology

Inter-vessel pits in *M. mirabilis* are alternate in spite of the presence of some elongated pits (Fig. 4). Vessel element diameter

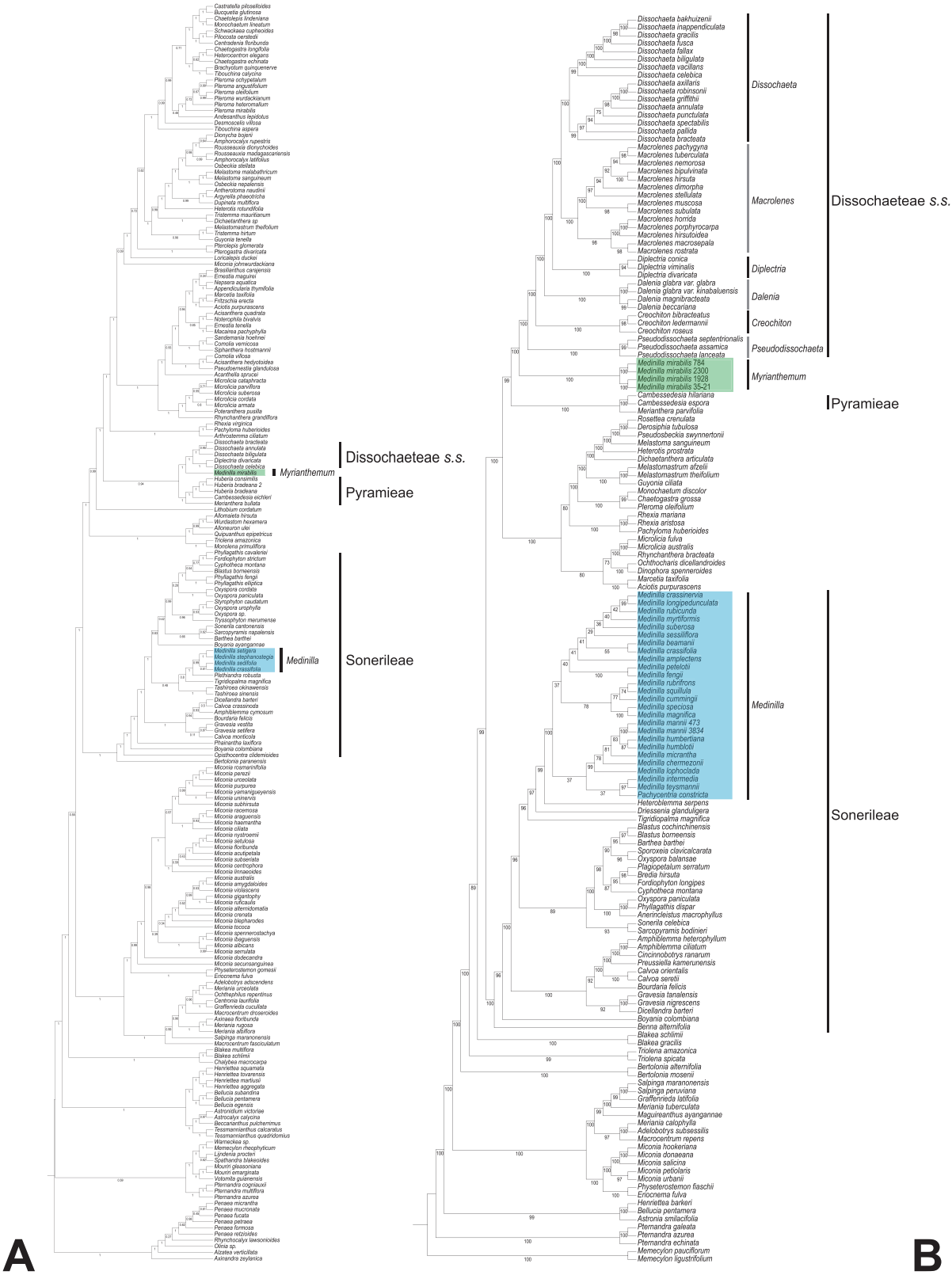

Figure 3. Phylogenetic trees inferred from target enrichment data using the coalescent method (A) and Sanger data using both nuclear and chloroplast sequences (B).

varies from 18.6 to 76.9 μm (Supplementary Table S3). Only uniseriate and biseriate rays were found (Fig. 4).

Seeds are numerous, ovoid, and angled. The lateral symmetrical plane and antiraphal symmetrical plane are both ovate. An appendage is absent. An expansion at the base of the seed is not evident. Multicellular sculpturing is absent, individual cells are elongated, anticlinal boundaries are undulate, and periclinal walls are flat to convex (Supplementary Table S3; Fig. 5).

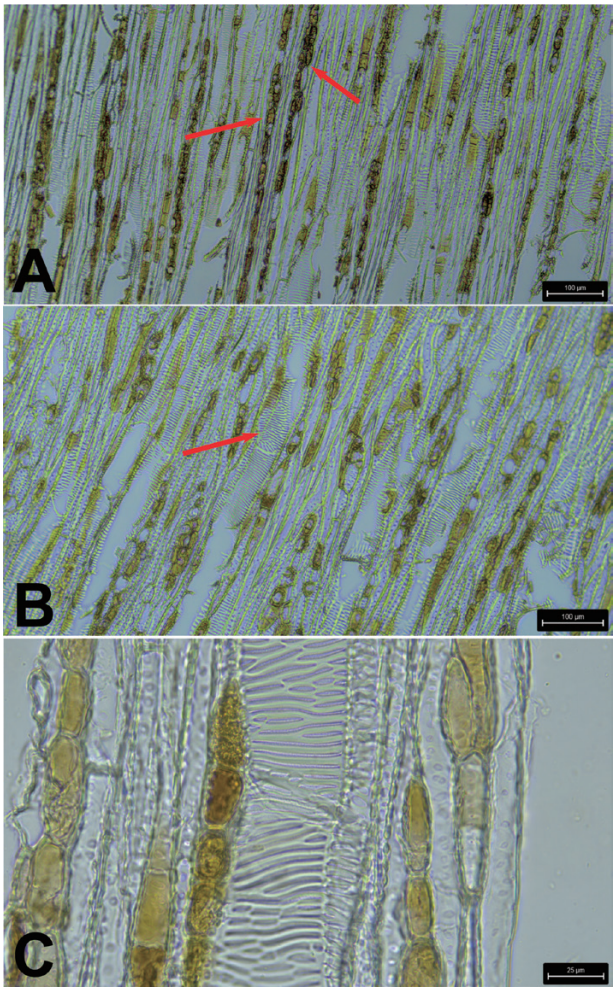

Figure 4. Tangential sections of *Medinilla mirabilis* wood. A (×10), rays (indicated by arrows) are uniseriate or biseriate. B (×10), inter-vessel pits (indicated by arrows) are alternate. Note that infrequently some pits are elongated, forming a scalariform or reticulate arrangement. C (×40), close-up image of a vessel element.

DISCUSSION

Placement of Medinilla mirabilis and its implication

Our phylogenetic analyses using both the Sanger data and Angiosperms353 data showed clearly that *M. mirabilis* is phylogenetically distinct and not closely related to *Medinilla*. Our results do not support Jacques-Félix's (1977) treatment of the species, which he transferred from *Myrianthemum* to *Medinilla*. *Medinilla mirabilis* is most closely related to the Southeast Asian endemic tribe Dissochaeteae (Fig. 3, Supplementary Figs S1–S3).

In terms of wood anatomy, the arrangement of inter-vessel pits in *Medinilla* is distinctly scalariform with narrow rays and narrow vessel elements (see Van Vliet 1981: plate 3) instead of being mostly alternate and infrequently scalariform or reticulate as in *M. mirabilis* (Fig. 4). Overall the inter-vessel pit arrangement in *M. mirabilis* is quite similar to that of Dissochaeteae (= subtribe Dissochaetinae; see Van Vliet 1981: plate 8). The narrow rays and vessel element diameters of *M. mirabilis* are different from those found in Dissochaeteae, where rays can be up to seven cells wide and vessel element diameters are wide (Kartonegoro *et al.* 2022, Kartonegoro *et al.* 2021). However, not all genera of Dissochaeteae have multiseriate rays. Only uniseriate and biseriate rays as seen in *M. mirabilis* have also been found in *Creochiton* and *Pseudodissochaeta* (Van Vliet 1981). In general, lianas tend to have large vessel elements for efficient water and nutrient transport (Carlquist 1985, Ewers and Fisher 1991, Angyalossy *et al.* 2012). The widest vessel element observed in *M. mirabilis* was only 76.9 μm, which is narrower than in some genera of Dissochaeteae [e.g. (58–) 96–215 (–283) μm in *Dissochaeta*]. However, vessel element diameters in *Pseudodissochaeta* were up to 84 μm (Van Vliet 1981), which is similar to those of *M. mirabilis*.

In terms of seed morphology, the seeds of *M. mirabilis* are quite similar to those of Dissochaeteae in almost all characters examined. However, the seeds of *Medinilla* also have similar characters (Supplementary Table S2, Fig. 5; Ocampo *et al.* 2022). As stated above, phylogeny and seed morphology often do not fit well in Melastomataceae (Ocampo *et al.* 2022).

Based on the most recent delimitation of Dissochaeteae, it is a Southeast Asian tribe with six genera (i.e. *Creochiton*, *Dalenia*, *Diplectria*, *Dissochaeta*, *Macrolenes*, and *Pseudodissochaeta*) and ~90 species (Kartonegoro *et al.* 2022). Morphologically, they are mostly woody climbers or creepers, have interpetiolar outgrowths, thyrsoid inflorescences, 4-merous flowers with dimorphic or isomorphic stamens, and baccate fruits. Anatomically, inter-vessel pits are arranged in an alternate pattern, vessel elements are comparatively wide, and multiseriate rays are present (Van Vliet 1981, Maxwell 1983, Kartonegoro *et al.* 2021, 2022). As a liana with interpetiolar outgrowths, eight dimorphic stamens, baccate fruits, and alternate inter-vessel pits, *M. mirabilis* fits well into Dissochaeteae. It can be differentiated from other Dissochaeteae by its verticillate leaf arrangement when young and its spherical inflorescences composed of many cymes (Diels *et al.* 1898).

Based on the morphological and molecular evidence provided, we propose that the genus *Myrianthemum* be reinstated to include *Myrianthemum mirabile* as its sole species (hereafter *M. mirabile* is used to replace *M. mirabilis*). The species is restricted to central African forests and has been found in Cameroon, Gabon, Congo, Equatorial Guinea, and Democratic Republic of Congo (Fig. 2). A variety of the species, *M. mirabile* var. *dentata* nom. nudum (Rendle *et al.* 1913), has been reported from Oban, southern Nigeria, which is the only collection known from Nigeria. It is uncertain whether this specimen was indeed collected in Nigeria because continuous forest covers southwest Cameroon and southeast Nigeria. Specimens of *M. mirabile* with dentate leaf margins seem to be quite frequent and have been collected in different countries (e.g. Fig. 1C). The isotype in Paris also has dentate leaf margins (MNHN-P-P04807525). This shows that *M. mirabile* can have entire or dentate leaves.

Irrespective of its unique morphology and geographical distribution, we advocate inclusion of *M. mirabile* in an expanded Dissochaeteae (Fig. 3). Tribe Dissochaeteae, currently endemic to Southeast Asia, would then include one African taxon, *M. mirabile*. In Melastomataceae, only six of the 23 major clades are distributed disjunctly across major biogeographical realms (Reginato *et al.* 2022). An expanded Dissochaeteae belongs among such clades with a disjunct distribution. Since the Neotropical tribe Pyramieae is sister to Dissochaeteae, the

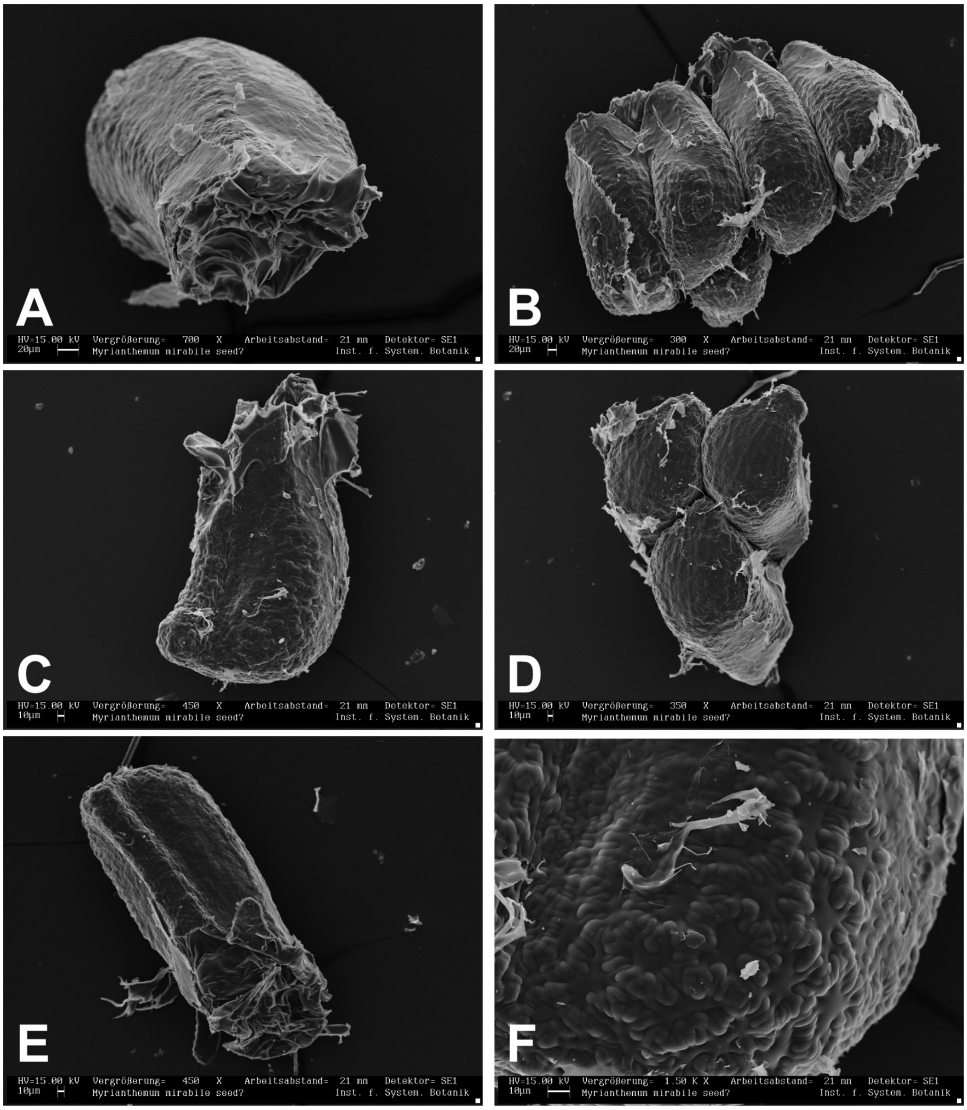

Figure 5. Scanning electron micrographs of *Medinilla mirabilis* seeds.

inclusion of *M. mirabile* may imply that this may be another example of dispersal from the Neotropics to Africa and then to Southeast Asia (Reginato *et al.* 2022).

Combining Sanger and Angiosperms353 data is cost-effective to place a taxon of interest on the flowering plant tree of life

Despite the challenges of missing data and gene duplication, Angiosperms353 has proven to be an effective tool for elucidating phylogenetic relationships among angiosperms at different levels and in different groups (Baker *et al.* 2021b, McDonnell *et al.* 2021). However, until Angiosperms353 instead of Sanger data is used as a general approach for DNA barcoding, many taxa will remain insufficiently sampled. Sanger data provide a potential solution for filling this sampling gap. Our study has shown that using Sanger data, all genera of Dissochaeteae were well represented, which allowed us to confidently conclude that *M. mirabile* is sister to Dissochaeteae. This would not have been possible with only phylogenomic data because several genera were unsampled. Our strategy can be used in any group for which Angiosperms353 or Angiosperms353-related data are available (Antonelli *et al.* 2021, Clarkson *et al.* 2021, Maurin *et al.* 2021, Haigh *et al.* 2023).

TAXONOMIC TREATMENT

Myrianthemum Gilg in Engler & Prantl, Nat. Pflanzenfam. Nachtr.: 266 (1897)

Type : *Myrianthemum mirabile* Gilg.

Notes : *Myrianthemum* is monospecific.

Myrianthemum mirabile Gilg in Engler & Prantl, Nat. Pflanzenfam. Nachtr.: 266 (1897); Mon. Afr. Pfl. 2: 33 (1898); ≡ *Medinilla mirabile* (Gilg) Jacq.-Fél., Adansonia sér. 2, 17(1): 78 (1977).

Type : Soyaux 361, Sibange farm, Munda region, Gabon (Holotype: B delet.; Isotype: P barcode P04807525!).

Specimens examined : CAMEROON. South Region: Ngovayang Massif, Ngovayang Village, *Droissart et al. 1928* (BRLU BRLU0000695). GABON. Ogooué-Maritime:

Moukalaba-Doudou National Park, ~50 km S of Mandji, *Sosef 2300* (BR BR0000019009421); Woleu-Ntem: 0.5 km E of Tchimbélé, *Wieringa 784* (BR BR0000019009445).

Notes : *Myrianthemum mirabile* was mentioned in the description of *Myrianthemum* (*descriptio generico-specifica*) and was therefore validly published in 1897. The species itself was later described in 1898. *Myrianthemum mirabile* occurs in Cameroon, Gabon, Congo, Equatorial Guinea, and Democratic Republic of Congo. Only one specimen (BM 014630982!) was collected in Oban, southern Nigeria. As this is the only collection known from Nigeria, it is somewhat uncertain if it was indeed collected in Nigeria because continuous forest covers southwest Cameroon and southeast Nigeria and all other specimens have been collected on the Cameroon side.

SUPPLEMENTARY DATA

Supplementary data are available at *Botanical Journal of the Linnean Society* online.

Figure S1. ML tree inferred from concatenated target enrichment data.

Figure S2. ML tree inferred from Sanger chloroplast data.

Figure S3. ML tree inferred from Sanger nuclear data.

Table S1. Accession numbers for downloaded and newly generated sequences, along with voucher information.

Table S2. Distribution records of *Medinilla mirabilis*.

Table S3. Vessel element diameter and seed morphological characters of *Medinilla mirabilis*.

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CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY

The raw target enrichment reads and Sanger sequences are archived in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (PRJNA961328) and GenBank (accession numbers available in Supplementary Table S1), respectively.

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