Botanical Journal of the Linnean Society, 2024, **XX**, 1–17
https://doi.org/10.1093/botlinnean/boae025
Advance access publication 6 May 2024
Original Article

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Molecular phylogenetics of *Distephanus* supports the recognition of a new tribe, Distephaneae (Asteraceae)

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ABSTRACT

Distephanus Cass. comprises 43 distinctive species of shrubs and small trees that have been placed historically within the ironweed tribe, Vernonieae (Asteraceae). Using the most expansive sampling of *Distephanus* to date, this study aims to test the monophyly of this genus and facilitate its classification. Molecular phylogenetic analyses were conducted using four molecular markers from the nuclear and plastid genomes. These data also supported divergence dating analyses that were performed to understand the timing of diversification events within *Distephanus* and other related genera as well as ancestral area reconstruction analyses to infer the biogegraphic history of species diversity in this group. Results from this study indicate that, as currently circumscribed, Vernonieae is not monophyletic and that *Distephanus* is, in fact, sister to a clade that comprises Vernonieae and another tribe, Moquinieae, which only includes two species restricted to Brazil. On the basis of these findings, *Distephanus* is classified in a new tribe that we describe here, Distephaneae. This new tribe comprises 41 species of *Distephanus* that are easily distinguished from Moquinieae and Vernonieae based on the presence of florets with yellow corollas and trinervate leaves.

Keywords: Africa; Asteraceae; ITS; Madagascar; plastid loci; Vernonieae

INTRODUCTION

With nearly 1500 species, the 'ironweed tribe', Vernonieae, is among the largest tribes in the sunflower family (Asteraceae) (Susanna *et al.* 2020, Keeley *et al.* 2021). A priority for advancing Asteraceae systematics is the necessary revision of this tribe, which was largely instigated by the reduction of the widely distributed species-rich genus *Vernonia* Schreb. (~1200 spp.) to a North American lineage of 20 spp. (see Robinson 1999a, b, Robinson and Funk 2018). Although most Western Hemisphere species formerly recognized in the broad concept of *Vernonia* have been transferred to other segregate genera (Robinson 1999a), nearly 200 species from the Eastern Hemisphere remain in *Vernonia* and are awaiting re-circumscription.

Taxonomic revision of Vernonieae in the Eastern Hemisphere has proceeded thanks largely to regional treatments during the latter half of the 20th century (e.g. tropical east Africa, Jeffrey 1988 and Jeffrey and Beentje 2000, and Flora Zambesiaca, Pope 1992) and the past two decades (e.g. Southern Africa, Robinson *et al.* 2016, and Thailand, Bunwong *et al.* 2014). Together with these regional studies, some recent treatments of segregate genera have helped advance the taxonomy of Vernonieae in the Eastern Hemisphere (see Robinson 1990, 1999b, 2005, 2009a, 2009b, 2012, Robinson and Funk 2011, 2018, Robinson and Skvarla 2006, 2007, 2009a, 2009b, 2009c, 2010a, 2010b, 2011, 2013, Robinson *et al.* 2008, 2014; and 2016). Prior to these studies, only a few addressed genus-level taxonomic revisions, including *Baccharoides* Moench (Isawumi *et al.* 1996), *Distephanus* Cass. (Robinson and Khan 1986), and *Phyllocephalum* Blume (Kirkman 1981). Among these, the treatment of *Distephanus* is notable because the genus is distinguished by remarkable morphological features, a complicated taxonomic history, and has consistently occupied an important phylogenetic position in relation to the remaining species of Vernonieae.

Distephanus Cass. comprises 43 distinctive species that have historically been placed within Vernonieae. Although long recognized in synonymy with *Vernonia* (Bentham and Hooker 1873,

Received 1 November 2023; revised 21 February 2024; accepted 7 April 2024

Humbert 1960, Jones 1981), *Distephanus* was resurrected by Robinson and Kahn (1986) on the basis of trinervately veined leaves and yellow florets (Fig. 1). These features are not particularly uncommon in Asteraceae, but are extraordinarily rare in Vernonieae (outside of *Distephanus*, trinervate sub-involucral bracts are found in *Hololepis* DC., and pale yellow florets in some populations of *Crystallopollen jelfiae* (S.Moore) J.C.Manning and a unique population of *Chresta curumbensis* (Philipson) H.Rob. (Moreira and Teles 2014, Loeuille *et al.* 2019). Since the early 1990s, both morphological and molecular phylogenetic studies have consistently placed *Distephanus* as sister to (or part of a clade that is sister to) all other species of Vernonieae (Keeley and Turner 1990, Keeley and Jansen 1994, Keeley *et al.* 2007, Keeley and Robinson 2009).

More recently, the phylogenetic placement of *Distephanus* within the tribe Vernonieae has come into question. Comparative studies by Funk and Chan (2009) indicated that *Distephanus* is not correctly placed in Vernonieae, but rather is sister to a clade that comprises both Vernonieae and a much smaller tribe, Moquinieae. This relationship has been confirmed in subsequent studies (Mandel *et al.* 2019, Siniscalchi *et al.* 2019, Keeley *et al.* 2021), but these studies included very few taxa of Eastern Hemisphere Vernonieae and a question has remained whether this is an accurate representation of evolutionary relationships or an artefact of limited taxon sampling. If *Distephanus* is, indeed, sister to a clade that includes both Moquinieae and Vernonieae, it would suggest a need to elevate *Distephanus* from recognition as a subtribe (Distephaninae) in Vernonieae to its own tribe entirely (Distephaneae) or to place Moquinieae into synonymy of Vernonieae. The tribe Moquinieae is composed of two monotypic genera (*Moquinia* DC. and *Pseudostifftia* H.Rob.), endemic to Brazil. The tribal position of its members has been controversial (Mutisieae, Cabrera 1977, Vernonieae, Robinson 1979, Gamerro 1990) but Robinson (1994) placed them in a separate tribe. They differ from Vernonieae by their thickened scabrid style (vs. with long sweeping hairs) and pollen grains with *baculae* not directly positioned under the spines (Robinson and Skvarla 2007, 2009). Hence, establishing the correct position of *Distephanus* is important not only for resolving its classification within Asteraceae, in particular within the subfamily Vernonioideae, but also could clarify the delimitation and taxonomic status of Vernonieae and Moquinieae.

Biogeographic history

The species that belong in *Distephanus* occupy a broad geographical area, extending from West Africa, throughout tropical and subtropical Africa and Madagascar, Mauritius, Socotra, and the Yunnan province in southern China (Fig. 2). The centre of diversity is Madagascar, where 32 (~75%) of the 43 species are endemic. The broadly disjunct distribution of species diversity in *Distephanus*—particularly between southern China and continental Africa and Madagascar—is remarkable and it remains unclear which features may have led to such a distribution. The oldest ages that have been estimated in recent fossil-calibrated divergence dating analyses of Asteraceae and Vernonieae suggest that the origin of this tribe was far too recent (e.g. 24 and 53 Mya for the stem age of the tribe in Mandel *et al.* 2019 and Keeley *et al.* 2021, respectively) for the distribution of species diversity in *Distephanus*—especially in continental Africa and Madagascar—to be explained by any possible vicariant origin (see Yoder and Nowak 2006, Vences *et al.* 2009).

In ruling out a vicariant origin for most disjunct species in *Distephanus*, we instead consider two possible explanations for the current distribution of species in this genus. First, the geographic area once occupied by the ancestors of species currently recognized in *Distephanus* was once much greater and potentially continuous throughout its modern range, but the distribution subsequently contracted significantly, with extant species persisting in refugia that correspond to their current distribution. Second, the geographic range of species currently recognized in *Distephanus* may have expanded as a result of long-distance dispersal, sometimes covering very long distances (e.g. dispersal between southern China and tropical Africa or islands in the western Indian Ocean). Implementing a calibrated divergence dating analysis may help to explain the current geographic range of species in this genus and the corresponding biogeographic events that underly their distribution. Although once considered rare among the flora of Madagascar, recent studies have revealed a much closer biogeographic history between continental Africa and Madagascar, with the diversification of some groups explained by multiple instances of interchange between these two landmasses (see Gostel *et al.* 2016, 2017, Linan *et al.* 2019, Larridon *et al.* 2021, Kimball *et al.* 2023). The current study will help explain the biogeographic history of one of the most species-rich genera, *Distephanus*, in tribe Vernonieae from the Eastern Hemisphere.

In this paper, we include the most exhaustive sampling to date of the genus *Distephanus* as well as samples of Vernonieae from the Eastern Hemisphere. The primary goals of this work are to test the monophyly of *Distephanus* and its phylogenetic placement in subfamily Vernonioideae (*sensu* Susanna *et al.* 2020), and to specifically test the phylogenetic position of *Distephanus* in relation to the tribes Moquinieae and Vernonieae. We also carried out divergence dating and ancestral area reconstruction analyses to understand the temporal and biogeographic context of diversification of species in *Distephanus*.

METHODS

Taxon sampling

In this study, 99 vouchers, including material from 55 specimens representing 23 distinct species of *Distephanus*, were sampled. Additionally, 32 specimens were sampled from other 'core' Vernonieae taxa and 12 outgroups were included from the tribes Arctotideae (three spp.), Eremothamneae (one spp.), Liabeae (five spp.), and Moquinieae (three accessions).

Molecular sampling and sequencing

Whole genomic DNA was extracted from leaf tissue derived from herbarium specimens or silica-preserved samples collected in the field using the QIAGEN DNEasy Plant Mini Kit (Germantown, MD, USA). Four molecular markers commonly used in comparative phylogenetic studies at the genus or tribal level in Asteraceae were targeted for PCR amplification and sequencing and included one nuclear locus (nrITS) and three plastid loci (*ndhF*, *psbA-trnH*, and *trnL*). Primers used in this study are provided in Table 1 along with reference information and thermal cycler profiles for each locus. PCR

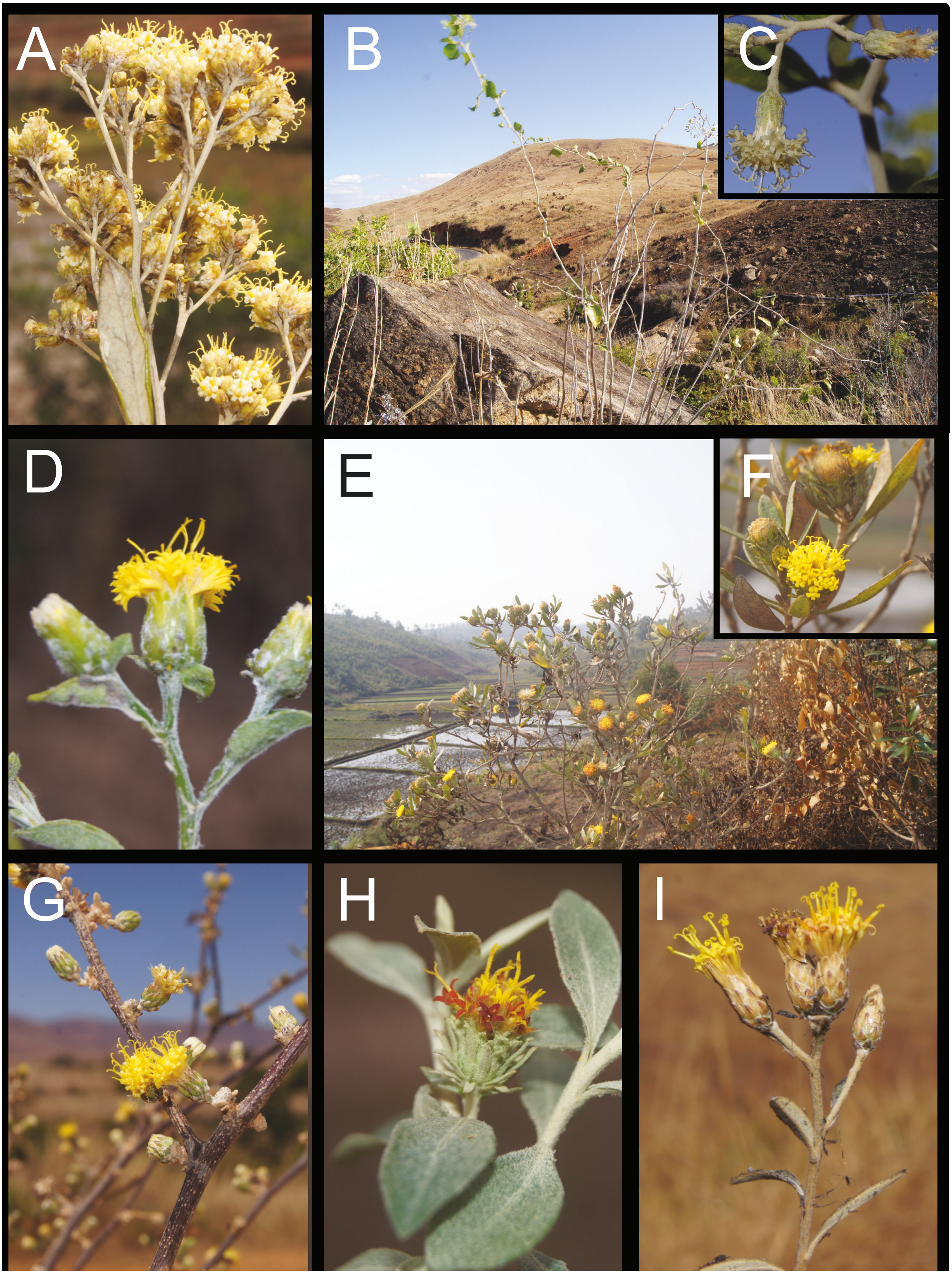

Figure 1. Habit, habitat, and general morphology of representative species of *Distephanus*. A, *D. garnerianus* (Funk 13410); B, *D. malacophytus* (Funk 13430); C, *D. malacophytus,* close up of capitulum from the same individual shown in B (Funk 13430); D, *D. rochonioides* (Funk 13381); E, *D. glutinosus* (Funk 13528); F, *D. glutinosus,* close up of capitulum from the same individual shown in E (Funk 13430); G, *D. swinglei* (Funk 13438); H, *D. trinervis* (Funk 13535); and I, *D. bara* (Funk 13428). Photograph credits: M. Gostel.

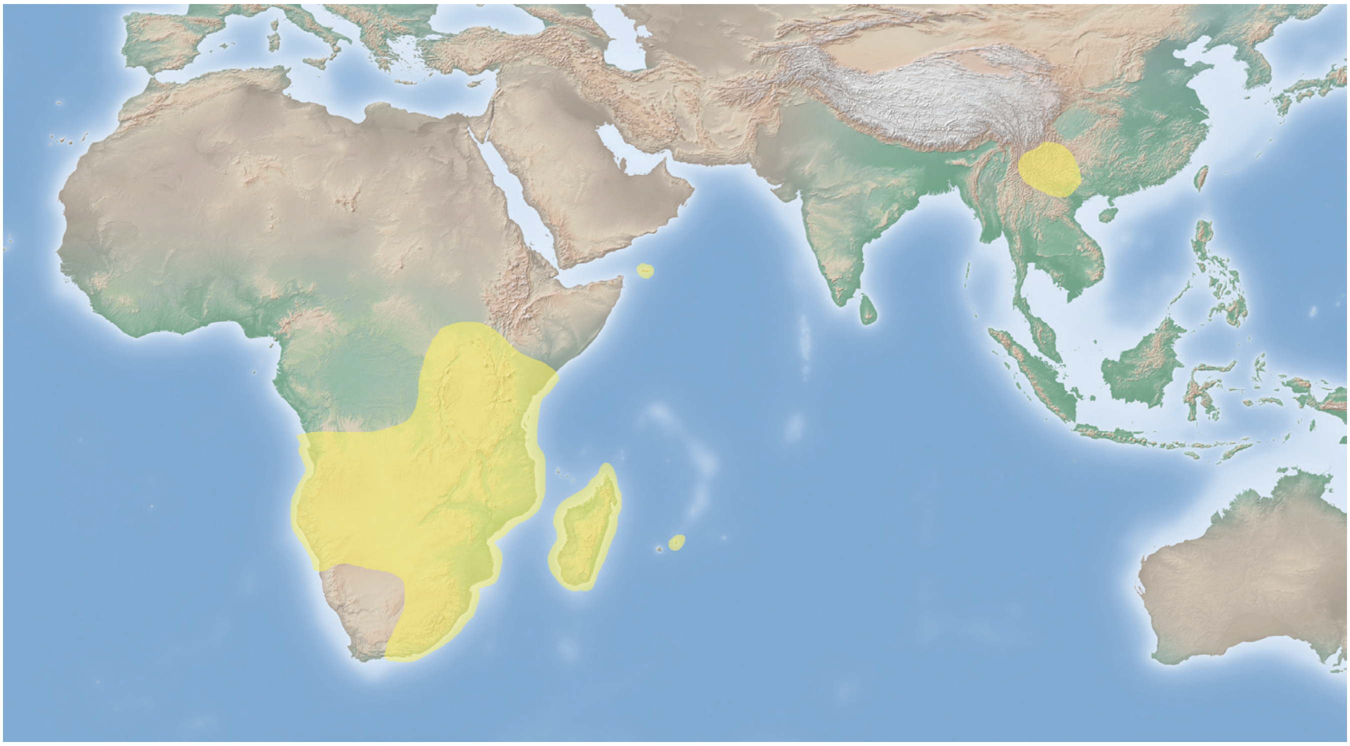

Figure 2. Distribution map showing the geographic range of species in *Distephanus*.

amplification was carried out using the FastStart™ High Fidelity PCR System, dNTPack (Sigma-Aldrich, Burlington, MA, USA). Amplified PCR products were purified prior to sequencing using ExoSAP-IT (ThermoFisher Scientific, Waltham, MA, USA) following the suggested manufacturer's protocols.

Sequence processing

Sanger sequencing was performed with the purified PCR amplicons using the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems®, Norwalk, CT, USA) and sequenced on an ABI3730xl or ABI3730 sequencer (Life Technologies, Carlsbad, CA, USA) by Psomagen, Inc. (USA) or at the Laboratories of Analytical Biology (LAB) at the National Museum of Natural History (Smithsonian Institution, Washington, D.C., USA), respectively. Chromatogram files were edited using Geneious Prime (https://www.geneious.com) and uploaded to GenBank (see Supporting Information, Appendix 1 for accession numbers). Multiple sequence alignment was performed using edited sequence data in the program MAFFT v.7 (Katoh *et al.* 2019) with minor adjustment made to trim the ends of the multiple sequence alignments.

Phylogenetic analysis

Phylogenetic reconstruction was carried out using maximum likelihood and Bayesian inference for three datasets, including the multiple sequence alignments from (i) the nrITS locus, (ii) a concatenation of all three plastid loci, and (iii) a concatenation of all four nuclear and plastid loci. Maximum likelihood (ML) analyses were run using the software IQ-TREE v.1.6.11 (Nguyen *et al.* 2015) and the best-fitting models for datasets (i) and (ii) were estimated in IQ-TREE (Kalyaanamoorthy *et al.* 2017). Except for the designation of appropriate substitution models for each locus or concatenation, ML analyses in IQ-TREE included default parameters and branch support was estimated using ultrafast bootstrapping with 1000 iterations. An alternative phylogenetic analysis was carried out on the concatenated, four-locus dataset using IQ-TREE, but using the GTR+ Γ+I model of sequence evolution, to compare with other methods of phylogeny inference that do not allow for as much parameterization of models (see the description of Bayesian inference and divergence dating analyses next). The best tree resulting from this alternative IQ-TREE run was used as a constraint tree for the divergence dating analyses.

Bayesian inference was carried out using MrBayes v.3.2.7 (Ronquist and Huelsenbeck 2013) in the CIPRES Science Gateway (Miller *et al.* 2010). Because MrBayes is unable to accommodate the best-fitting models identified by ModelFinder in IQ-TREE, a partitioned analysis using the GTR+Γ+I model of sequence evolution was implemented for the concatenated dataset with all four loci. To compare results from IQ-TREE and MrBayes using the same model of sequence evolution, a partitioned analysis was also run in IQ-TREE with the GTR+Γ+I model, keeping all other IQ-TREE parameters consistent with our other ML analyses. For Bayesian analysis, two searches were carried out in MrBayes for each database using four chains (one cold) for 10 000 000 generations, sampling every 1000 generations. Output files were inspected using the software Tracer v.1.7.2 (Rambaut *et al.* 2018) to analyse convergence and identify the number of generations to discard as burn-in (25%).

Divergence dating

Divergence dating analyses were conducted using BEAST v.1.10.4 (Suchard *et al.* 2018). Parameters were specified in .xml files using the software BEAUti v.1.10.4, which is part of the

BEAST package. Divergence dating analyses in BEAST were performed using the concatenated matrix of all four sequenced loci and using as topological constraint the best tree that resulted from the ML analyses implemented in IQ-TREE from the partitioned, four-locus, concatenated dataset using the GTR+Γ+I model of sequence evolution. Because there are currently no known fossils of Vernonieae available for calibration, we used secondary calibration points derived from a recent family-wide study (Mandel *et al.* 2019). Four calibration nodes were selected and included: (i) 30 Mya (including the stem) for the split between the clade comprising *Distephanus* + Moquinieae + Verno nieae and the Liabeae; (ii) 20 Mya for the tribe Liabeae (crown); (iii) 9 Mya for the maximum age of *Distephanus populifolius*, a species endemic to Mauritius; and (iv) 8 Mya for the tribe Moquinieae (crown). Each of the four calibration points were sampled from a normal prior distribution with a mean equal to the calibration age and a 10% standard deviation. Other analysis parameters included a relaxed, uncorrelated lognormally distributed clock that was sampled from the GTR+Γ+I model of sequence evolution. Sampling included 100 million generations (logged every 10 000 generations). Convergence of parameters was assessed using log output files in the program Tracer v.1.7 (Rambaut *et al.* 2018) and confirmed by very high effective sample size (ESS) values (generally > 1000); only one parameter (ucld.mean) had an ESS <200 (196). Of the trees, 25% were discarded as burn-in, a value that maximized the ESS values without discarding too much data. A maximum clade credibility tree was generated using TreeAnnotator (which is also part of the BEAST package) to combine the output trees (discarding the burn-in) using the output tree files from BEAST. Mean node ages were reported, overlaid on bars that correspond to the 95% highest posterior density (95% HPD).

Table 1. Information for each locus sequenced in this study. Thermal cycler profile column indicates details (temperature in Celsius and time in seconds) for the initial denaturation, followed by the cycling profile for each of cycle, followed by the final extension. Superscripts next to primer names indicate references for the primer sequence.

Locus	Primer name (reference)	Primer sequence	Aligned length	Thermal cycler profile	Number of sequences	Parsimony informative characters
nrITS	ITS5A[a]	GGAAGGAGAAGTCGTAACAAGG	675	95° (120), [95° (45), 54° (45), 72° (120)] × 40, 72° (240)	94	390 (57.8%)
	ITS4[b]	TCCTCCGCTTATTGATATGC				
ndhF	ndhF1603[c]	CCTYATGAATCGGACAATACTATGC	758	95° (180), [94° (45), 48° (45), 72° (120)] × 37, 72° (240)	60	57 (7.5%)
	ndhF + 607[c]	ACCAAGTTCAATGYTAGCGAGATTAGTC				
psbA-trnH	psbA3f[d]	GTTATGCATGAACGTAATGCTC	665	95° (120), [95° (45), 54° (45), 72° (120)] × 40, 72° (240)	69	70 (10.5%)
	trnHF[e]	CGCGCATGGTGGATTCACAATCC				
trnL	trnL-Fc[f]	CGAAATCGGTAGACGCTACG	868	95° (180), [94° (45), 54° (45), 72° (120)] × 37, 72° (240)	62	37 (4.3%)
	trnL-Ff[f]	ATTTGAACTGGTGACACGAG				

[a]Downie and Katz-Downie 1996, [b]White *et al.* 1990, [c]Jansen 1992, [d]Sang *et al.* 1997, [e]Tate and Simpson 2003, [f]Taberlet *et al.* 1991.

Ancestral area reconstruction

Ancestral area reconstruction was performed using the software package BioGeoBEARS (v.1.1.3, Matzke 2013, 2014) using the time-calibrated trees that resulted from our divergence dating analysis in BEAST. Ancestral area reconstruction was carried out using six models, including DEC (Ree and Smith 2008), DIVALIKE, and BAYAREALIKE as well as each of these models with an additional parameter (+ *j*), which corresponds to founder-event speciation. We defined five areas of distribution that correspond to broad areas of distribution for taxa included in our ingroup as continental Africa, Asia, Madagascar, Mauritius, and the Americas. Among the six models that we implemented for this dataset, the 'best' model was selected using the Akaike information criterion.

RESULTS

Sequencing and phylogenetic analysis

Sequence data were recovered from each of the 99 vouchers included in this study, producing a total of 285 sequences. All sequences were uploaded to GenBank (accession numbers provided in Supporting Information, Appendix 1) and details about individual sequenced loci, their alignments, and statistics are provided in Table 1. The percentage of parsimony informative characters for each sequence alignment ranged from 57.8% (nrITS) to just 4.3% (*trnL-F*); generally the plastid loci were much less variable than the nrITS locus (see Supporting Information, Fig. S1). Results from comparative phylogenetic analyses for each of the three datasets used in this study are well-resolved and largely congruent, with some notable exceptions. Not surprisingly, phylogenetic reconstruction using sequence data from the least variable dataset (Supporting Information, Fig. S2, concatenation of three plastid loci) recovered the fewest number of clades and indicated low branch support values for many of them.

Among the three datasets analysed in this study, the most well-resolved phylogeny was recovered from the four-locus concatenation that included nrITS and three plastid loci (Fig. 3). This phylogeny is nearly fully resolved, with strong branch support for most clades (~80% greater than 80% maximum likelihood bootstrap support or 'MLBS'). The single-locus nrITS dataset recovered the second-most well-resolved phylogeny (Supporting Information, Fig. S1), which—similar to the four-locus concatenation dataset—was also nearly fully resolved, but overall had lower branch support values. Results from the alternative analysis carried out using IQ-TREE with the GTR+ Γ+I model of sequence evolution is provided in Supporting Information, Figure S4.

Despite the low sequence variation and limited resolution provided by the plastid data (Supporting Information, Fig. S2), several key clades were recovered, including a clade that comprises Vernonieae + one accession from Moquinieae (*Moquinia*) as well as a well-supported clade that corresponds to *Distephanus* (Supporting Information, Fig. S2, 84% MLBS). This dataset only included sequence data for two species in Moquinieae (*Pseudostifftia kingii* H.Rob. and *Moquinia racemosa* DC.) and together these only generated three plastid sequences (*ndhF* only for *M. racemosa* and *ndhF* + *trnL* for *P. kingii*). Despite not recovering tribe Moquinieae as monophyletic in the plastid dataset, most species of *Distephanus* that were sampled with multiple accessions were recovered as species-specific subclades (nine of 12, 75%) in the plastid dataset and all such taxa (100%) in the four-locus concatenated dataset, respectively. For the purpose of discussion and interpretation of results from phylogenetic analyses as well as subsequent studies (e.g. divergence dating), this study will emphasize results from the four-locus concatenation (Fig. 3).

Results from the phylogenetic analysis of all three datasets used in this study recover similar overall topologies (Fig. 3, Supporting Information, Figs S1–S3). Notably, none of the phylogenetic results recover Vernonieae (as currently circumscribed including *Distephanus* and Moquinieae as a separate tribe) as a monophyletic group. However, all three analyses recover, with strong branch support, a clade that is identified as 'core Vernonieae' (100, 100, and 68% MLBS, henceforth and 1.0 posterior probability or 'PP', henceforth; Figs 3, Supporting Information, Figs S1–S3, respectively) and includes all species of Vernonieae sampled in this study, except for most of those in the genus *Distephanus*. Sister to 'core Vernonieae' is the small tribe Moquinieae, which only includes two monotypic genera, *Moquinia* and *Pseudostifftia* (100, 100% MLBS, and 1.0 PP; Fig. 3, Supporting Information, Figs S1 and S3, respectively). Although not recovered within core Vernonieae, the genus *Distephanus* is sister to the clade comprising Moquinieae + core Vernonieae in all analyses (99, 100, and 75% MLBS, and 1.0 PP; Fig. 3, Supporting Information, Figs S1–S3, respectively). In the plastid analyses, Moquinieae does not form a clade; however, only two accessions were included (*M. racemosa* and *P. kingii*) and, as stated before, only one (*ndhF*) and two (*ndhF* and *trnL-F*) plastid loci were sequenced from each, respectively.

In the plastid results (Supporting Information, Fig. S2), *Pseudostifftia* is sister to a clade that includes core Vernonieae, *Moquinia*, *Distephanus*, and another tribe, Liabeae (included as an outgroup). The position of *Pseudostifftia* in this tree may be spurious and result from missing data—particularly when one considers the two loci that generated sequence data were the least variable of all four loci included in this study, with just 4.3% and 7.5% parsimony informative characters for *trnL-F* and *ndhF*, respectively. Branch support along the backbone of the plastid phylogeny is correspondingly lower than those recovered in the other analyses, including just 74% MLBS for core Vernonieae + *Moquinia racemosa*; 84% for *Distephanus*; 75% for the clade that comprises Distephanus and Moquinieae + Vernonieae (the 'DMV clade'); and 75% for the DMV + Liabeae clade.

The well-supported clade corresponding to *Distephanus* that is recovered in the results of each analysed dataset in this study includes 21 of the 23 species of *Distephanus* that were sampled (100, 93, and 84% MLBS, and 1.0 PP; Fig. 3, Supporting Information, Figs S1–S3, respectively). Two other species of *Distephanus* (*D. angulifolius* (DC.) H.Rob. & B.Kahn and *D. biafrae* (Oliv. & Hiern) H.Rob.) are nested deeply within the core Vernonieae (Fig. 3, Supporting Information, Figs S1–S3). This indicates that *Distephanus*, as currently circumscribed, is not monophyletic. Among the 23 species of *Distephanus* included in this study, 14 (>60%) were represented by more than one accession and among these, nearly 80% formed species-specific subclades (only *D. biafrae*, *D. malacophytus* (Baker) H.Rob. & B.Kahn, and *D. subluteus* (S.Elliot) H.Rob. & B.Kahn did not).

Although the phylogeny of *Distephanus* is not fully resolved, a few clades are well-supported. First, the species *D. plumosus* (O.Hoffm.) Mesfin from eastern tropical Africa appears to be sister to all other species in the genus with moderate branch support (81 and 77% MLBS; Fig. 3, Supporting Information, Figs S1 and S2), but this position is not strongly supported in all analyses and a very short branch separates it from another species, *D. henryi* (Dunn) H.Rob., from the southern province of Yunnan in China, which is then placed as sister to all remaining species in the genus (81 and 77% MLBS; Fig. 3 and Supporting Information, Fig. S2, respectively). Results from Bayesian inference (Supporting Information, Fig. S3) place these two species in a polytomy with a large clade that corresponds to the rest of the genus (1.0 PP). Second, two species-rich and well-supported clades are recovered within *Distephanus* that merit further discussion. Clade A (87 and 78% MLBS, and 0.99 PP' Fig. 3, Supporting Information, Figs S2 and S3, respectively) comprises 13 species, but surprisingly is not recovered in the analysis of the ITS locus (Supporting Information, Fig. S1). A second well-supported clade, Clade B (100 and 98% MLBS, and 1.0 PP; Fig. 3, Supporting Information, Figs S2 and S3, respectively),

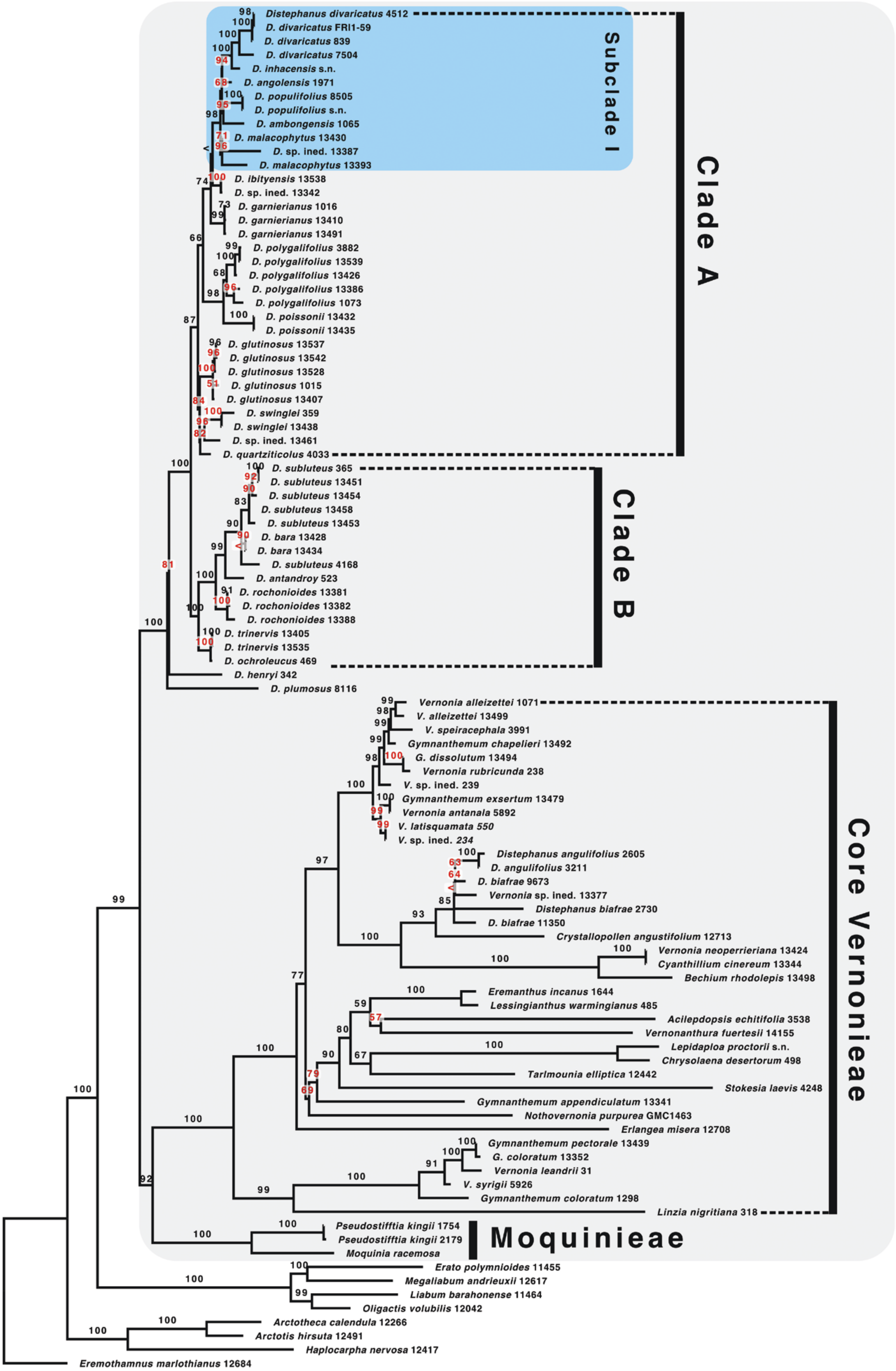

Figure 3. Results of ML analysis of the partitioned, four-locus concatenated dataset. Values above branches indicate ML bootstrap values. Clade names used in the text are indicated with brackets to the right of branch tip labels. The blue shaded box corresponds to subclade 1, referred to in the text. Support values in red have been provided for visibility in portions of the tree where black text was difficult to distinguish.

includes six species, but is similarly not recovered in the ITS analysis.

There are four primary sources of conflict between the phylogenetic results produced from the four-locus concatenation and plastid datasets and the nrITS analysis. First, whereas five accessions of *D. glutinosus* that form a well-supported subclade (100% MLBS and 1.0 PP; Fig. 3 and Supporting Information, Fig. S3) within Clade A and a poorly supported (62% MLBS) subclade in Supporting Information, Figure S2, these accessions form a well-supported subclade in Clade B in the ITS analysis (100% MLBS, Supporting Information, Fig. S1) that is sister to *D. trinervis* Bojer ex DC. (96% MLBS, Supporting Information, Fig. S1). Second, another well-supported subclade (98, 100, and 90% MLBS, and 0.94 PP; Fig. 3, Supporting Information, Figs S1–S3, respectively) that comprises two species (*D. poissonii* (Humbert) V.A.Funk & H.Rob. and *D. polygalifolius* (Less.) H.Rob. & B.Kahn) is nested within Clade A in results from the four-locus and plastid analyses (Fig. 3 and Supporting Information, Fig. S2), but is sister to all other species of Clade B in the ITS analyses (96% MLBS, Supporting Information, Fig. S1). Third, three other accessions that are placed in Clade A in Figure 3 and Figures S2 and S3 (in the Supporting Information) are also placed in Clade B in the results from ITS sequence analysis (*D.* sp. 13451, *D. swinglei* (Humbert) H.Rob. & B.Kahn 359, and *D. quartziticolus* (Humbert) V.A.Funk & H.Rob. 4033; Supporting Information, Fig. S1). Last, all accessions representing *D. garnerianus* (Klatt) H.Rob. & B.Kahn belong to Clade A in all analyses; however, in the 4-locus concatenated dataset this species is sister to a subclade we have identified as 'Subclade I' (74% MLBS and 0.97 PP, Fig. 3 and Supporting Information, Fig. S3) and in results from the plastid dataset accessions representing this species do not form a clade, but instead are part of a larger, poorly resolved clade (Supporting Information, Fig. S2, 94% MLBS) that is sister to 'Subclade I' (Supporting Information, Fig. S2); however, results from the ITS analyses recover all accessions of this species within Subclade I (Supporting Information, Fig. S1). A tanglegram (Fig. 4) was generated to highlight the differences between the results of phylogenetic analysis of the nuclear and plastid datasets, summarized before.

Divergence dating

Results from the divergence dating analysis suggest that *Distephanus* shared a most recent common ancestor (MRCA) with the clade comprising Moquinieae and core Vernonieae during the late Oligocene or early Miocene, ~22.6 Mya (19.3–26 Mya 95% HPD, Fig. 5). This analysis also suggests that the MRCA of core Vernonieae and Moquinieae also diverged shortly after the split with *Distephanus*, ~21.7 Mya (18.1–25.1 Mya, 95% HPD, Fig. 5). The crown age for *Distephanus* dates to the Miocene, approximately 18 Mya (13.2–22.9 Mya, 95% HPD, Fig. 5). Crown ages for Clades A and B, as well as Subclade I are estimated at 12.3 (8.8–16.1 Mya, Fig. 5), 10.5 (5.9–15.2 Mya, Fig. 5), and 8.5 (6.9–12.3 Mya, Fig. 5).

Ancestral area reconstruction

Results from our ancestral area reconstruction using BioGeoBEARS supported three models as having nearly identical Akaike information criterion values: DEC + j, DIVALIKE + *j*, and BAYAREALIKE + *j* and on visual inspection of the ancestral area reconstruction results there were no differences regarding the likely ancestral area for *Distephanus* nor any clades or subclades within the genus. Results from the ancestral area reconstruction implementing the DEC + *j* model are provided in Figure 6. All three models supported continental Africa as the most likely ancestral area for *Distephanus*. The ancestral area for the MRCA of *D. henryi* and the rest of *Distephanus* was most likely Madagascar, but Asia cannot be ruled out. Subsequent to dispersal to and radiation in Madagascar, two additional dispersals took place, including one from Madagascar to Mauritius 6.7 Mya (4.9–8.5 Mya, 95% HPD, Fig. 6) and another from Madagascar to continental Africa 6.7 Mya (3.5–9.5 Mya, 95% HPD, Fig. 6).

DISCUSSION

This work represents the largest sample size in a phylogenetic study of *Distephanus* and Vernonieae from the Eastern Hemisphere to date. Furthermore, this work represents the first detailed molecular phylogenetic study of the distinctive and species-rich genus, *Distephanus*. Key findings of this work have important taxonomic implications that include the following: (i) Vernonieae, as currently circumscribed, is not monophyletic and (ii) a new tribe, Distephaneae, is established that comprises 41 species of the genus *Distephanus* to recover a monophyletic Vernonieae instead of sinking Moquinieae into Vernonieae. Morphological characteristics used to distinguish Distephanae from Vernonieae and Moquinieae are further clarified to include a combination of both (rather than just one of these characters) trinervate leaves and florets with yellow, pale yellow, orange, or white corollas (but not violet).

Phylogenetic results and taxonomic implications of this work

Since the genus *Distephanus* was resurrected by Robinson and Kahn (1986), there has been little doubt regarding its monophyly and distinctiveness within the tribe to which it has been ascribed, Vernonieae. Two characters in particular, yellow florets and trinervate leaves, have proven to be informative for the recognition of the 43 known species in this genus, making it one of the most species-rich genera of Vernonieae in the Eastern Hemisphere. The goal of this study was to leverage a combination of the largest sampling of species of Vernonieae from the Eastern Hemisphere to date as well as a comparison from four commonly used molecular markers to test the monophyly of *Distephanus*, the generic boundaries of this genus, and its position within Vernonieae. Results of comparative phylogenetic reconstruction using four molecular markers used in this study reveal two findings of broad significance for the systematics and taxonomy of *Distephanus* and the tribe Vernonieae. First, as confirmed by our analyses, following the exclusion of three species *Distephanus* is overwhelmingly supported as a monophyletic group that is sister to a clade comprising the tribes Moquinieae and Vernonieae. Second, our findings support those of other recent studies (Mandel *et al.* 2019, Siniscalchi *et al.* 2019, and Keeley *et al.* 2021) that, despite limited sample size, suggested that Vernonieae is not monophyletic as currently circumscribed, with the small South American tribe Moquinieae embedded within Vernonieae. The latter is of greatest consequence in this study because to maintain a monophyletic Vernonieae, two

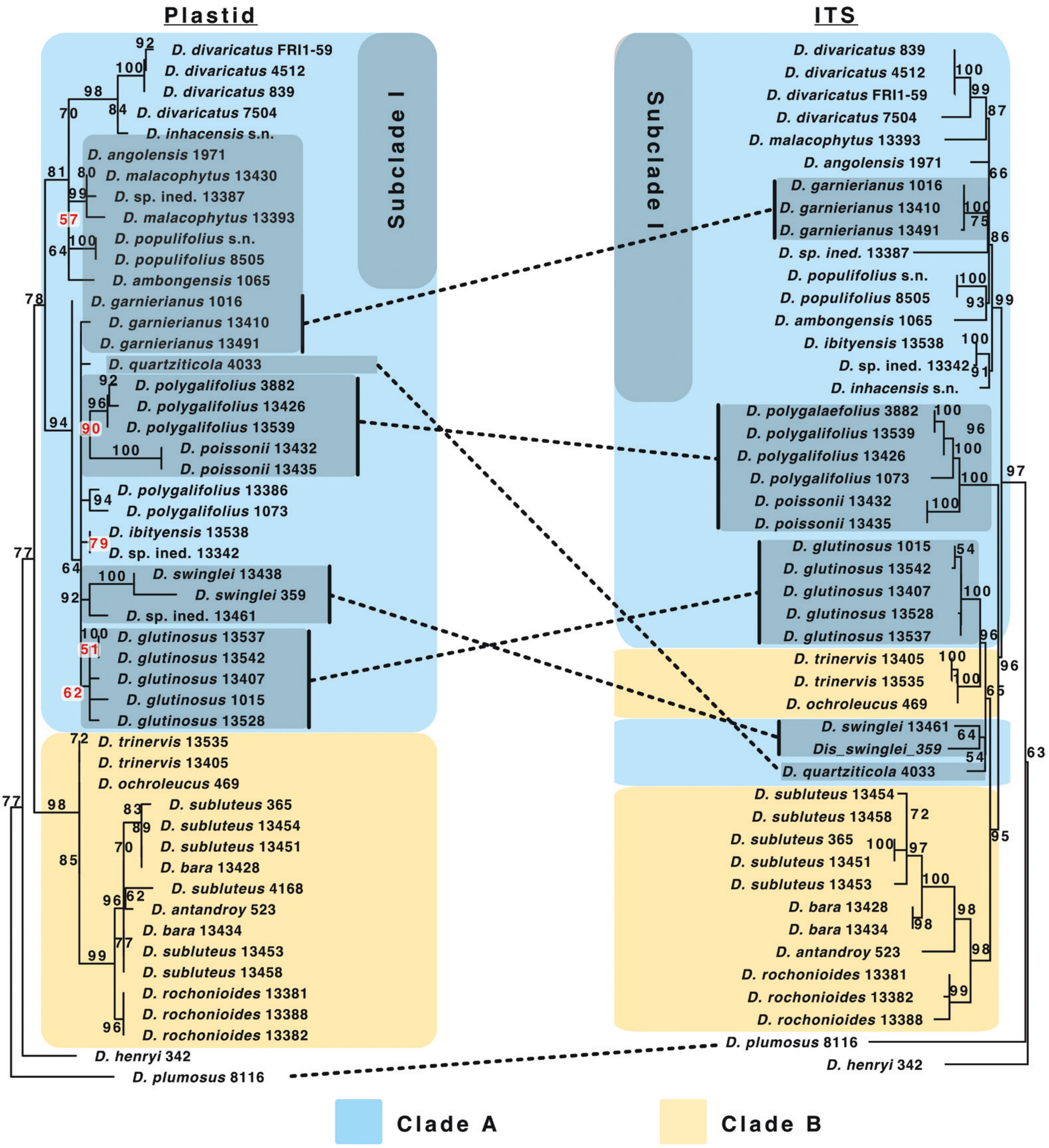

Figure 4. A mirror tree, depicting side-by-side results of phylogenetic analysis from the nuclear and plastid datasets in this study for comparison. Dashed lines correspond to clades that occupy a position incongruent between these datasets. Support values in red have been provided for visibility in portions of the tree where black text was difficult to distinguish.

alternatives are feasible. First would be to sink Moquinieae into Vernonieae, but as noted by Robinson (1994) the presence of styles with scabrid hairs (vs. long sweeping hairs) and pollen grains with columellae arranged in a distinctive pattern that seems independent of the positions of the spines (vs. centred under the spines) make this placement uncomfortable and would be disruptive for the concept of Vernonieae. The second would be to establish a new tribe, Distephaneae. This new tribe includes 41 species of *Distephanus* (see the Taxonomic Treatment section next) and, by segregating this genus from Vernonieae, we are able to resolve the matter of a paraphyletic Vernonieae.

The findings of this study are not entirely novel, however, the large sample size that was used allowed us to overcome challenges that resulted from small sample sizes in previous studies

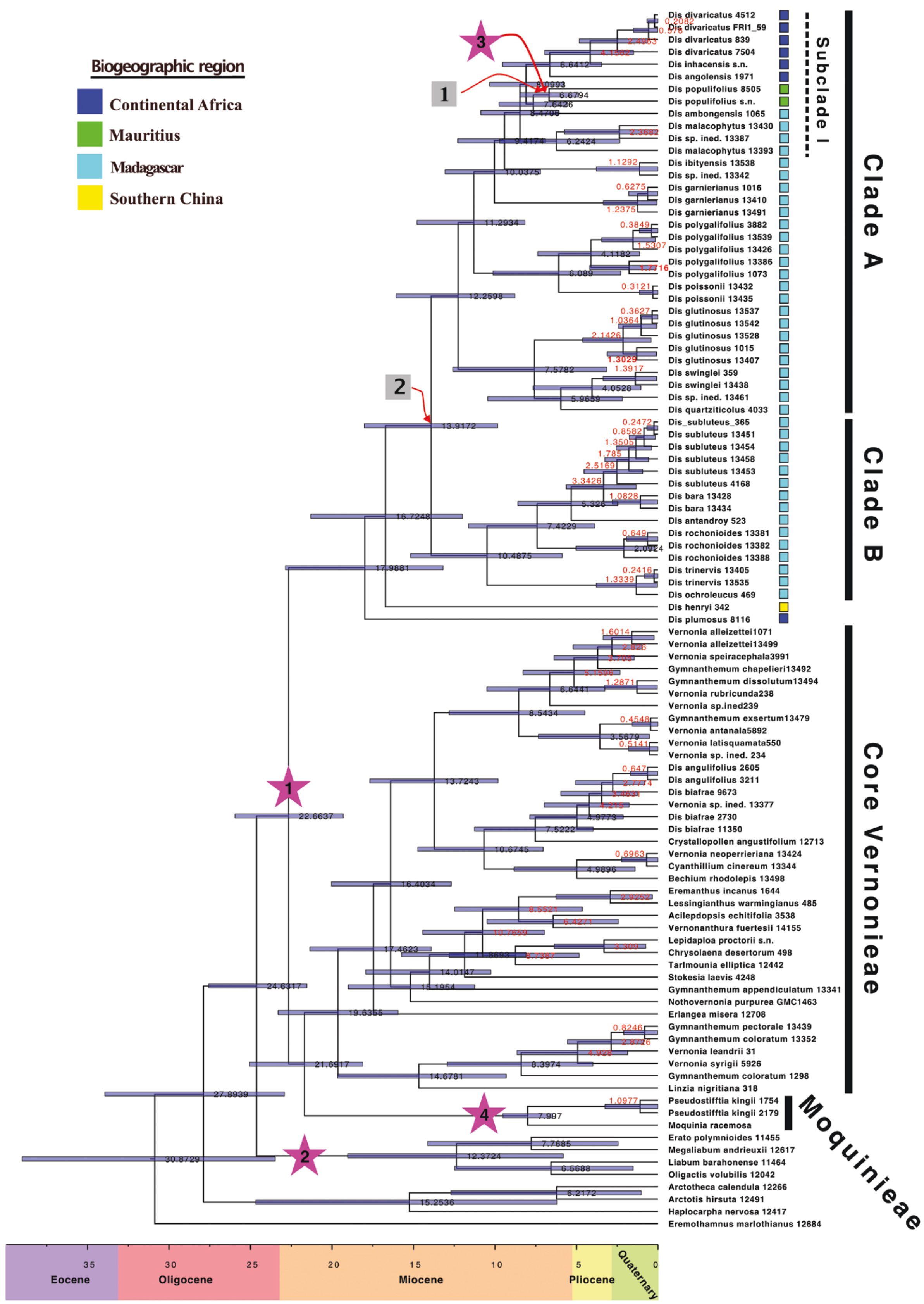

Figure 5. Results of the divergence dating analysis carried out using BEAST. Pink stars with a number 1–4 correspond to calibration points and grey squares with numbers 1 and 2 correspond to nodes discussed in the text. A geological time scale is provided below the chronogram for reference.

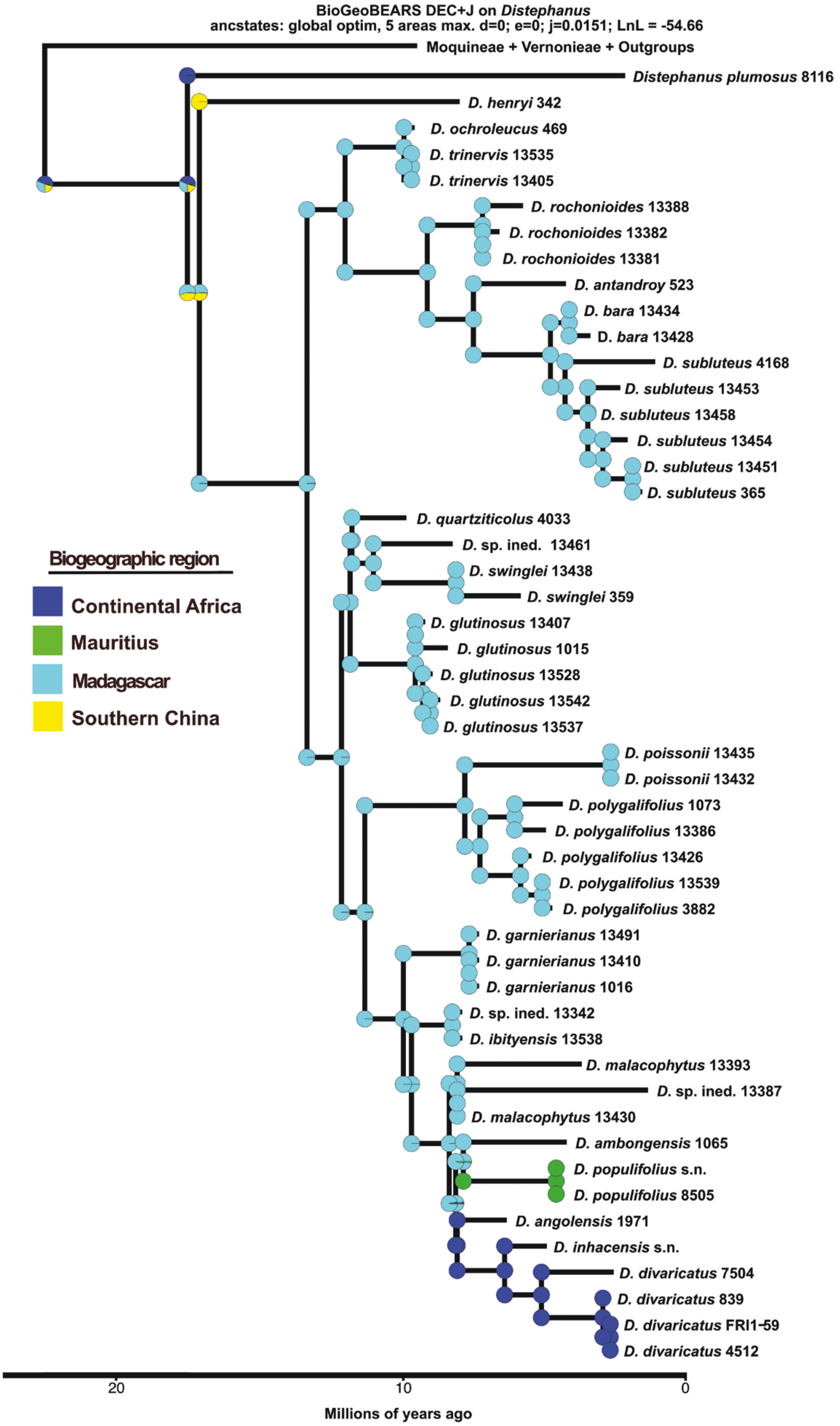

Figure 6. Results of ancestral area reconstruction performed using BioGeoBEARS. The full figure has been truncated to highlight results for the ingroup, *Distephanus*.

that found similar phylogenetic results (see Mandel *et al.* 2019, Siniscalchi *et al.* 2019, and Keeley *et al.* 2021). For example, despite findings in Keeley *et al.* (2021) that suggested the position of *Distephanus* in Vernonieae was problematic, they only included eight species in their study and a larger sample size was suggested before making taxonomic decisions based on their results. Keeley *et al.* (2021) also noted some conflict between their nuclear and plastid loci: in particular, their ITS data suggested that Moquinieae was nested within *Distephanus* (but not in the plastid analyses). They also indicated that when one particular taxon (*D. ambongensis* (Humbert) H.Rob.) was excluded from ITS analyses, *Moquinia* was recovered as sister to the 'core Vernonieae' clade and not nested within *Distephanus*. Our results do not find such a discrepancy and instead, Moquinieae is consistently placed as sister to Vernonieae in all analyses, with one small exception. We only recovered plastid sequence data from two of the three accessions from Moquinieae in this study (*Moquinia racemosa* and *Pseudostifftia kingii*), however, they do not form a clade in plastid-only analyses. Instead, in the results from the plastid dataset *Pseudostifftia* falls outside of the clade formed by *Distephanus* + Moquinieae + Vernonieae (the DMV clade) and rather it is sister to a weakly supported clade (75% MLBS, Supporting Information, Fig. S2) that includes the DMV clade and Liabeae. This topology is not recovered in the results from any other dataset, and it is likely the result of a lack of data, as the single accession from *Moquinia racemosa* included in this study is missing sequence data from two of the three plastid loci (*psbA-trnH* and *trnL-F*) and *Pseudostifftia* is missing sequence data from the *psbA-trnH* locus. We hypothesize the position of *Pseudostifftia* in the plastid analyses is spurious and results from missing data; however, we cannot entirely rule out alternative explanations for this position, such as chloroplast capture as a result of hybridization, but this is unlikely due the vast geographic distance that separates Moquinieae in South America from early-diverging taxa in Vernonieae from Africa as well as the estimated timing of the origin and radiation of these groups.

Among other studies that found the position of *Distephanus* problematic, Siniscalchi *et al.* (2019) found support for a clade formed by *D. ambongensis* and *M. racemosa*; albeit with low branch support and the authors acknowledged this could have been biased by limited outgroup sampling (just one accession from Liabeae). In this study, we generated sequence data from the exact same accession of *D. ambongensis* (van Ee 1065, see Supporting Information, Appendix 1) and find that it is placed (with strong branch support, Fig. 3, Supporting Information, Figs S1–S3) as sister to the type species of the genus (*D. populifolius*) in all analyses. It is likely the clade formed by this accession and *M. racemosa* resulted, as the authors suggested (Siniscalchi *et al.* 2019), from sampling bias and/or long branch attraction.

Last, another result from this study that has taxonomic implications is the clarification of generic boundaries within *Distephanus*. Among the 23 species of *Distephanus* included in this study are two from continental Africa (*D. angulifolius* and *D. biafrae*) that led Robinson (2009b) and others to question the significance of corolla colour and leaf venation in delimiting this genus. *Distephanus angulifolius* has 3–5-veined leaves with pale mauve to creamy white corollas (Pope 1992) and *D. biafrae* has pinnately veined leaves and reddish to purplish corollas (Robinson 2009b). The placement of these two species within 'core Vernonieae' in our results helps to clarify these doubts: *Distephanus* is recognized only by the combination of both corolla colour and trinervate leaves; the rare occurrence of species with trinervate leaves, but reddish or violet corollas, as seen in *D. angulifolius*, may simply be homoplastic. Pale mauve florets have also been reported for another species, *D. inhacensis* (G.V.Pope) R.G.C.Boon & Glen (Pope 1988). However, field observations of white to cream florets with yellow anthers (Hilliard 1994, Boon and Glen 2013) have contradicted this statement. Our results show that *D. inhacensis* is deeply nested within *Distephanus*. Thus, *D. angulifolius* and *D. biafrae* are placed in the excluded taxa (see Taxonomic Treatment). Ongoing studies will help to clarify into which genus these two species belong.

Diversity within the newly established tribe, Distephaneae

Distephaneae are easily distinguished from the two tribes that they are most closely related to (Moquinieae and Vernonieae); however, despite strong phylogenetic resolution within the genus, few patterns of morphological evolution are clarified. Two clades are well-supported within *Distephanus*, Clade A includes at least 14 species sampled in this study and Clade B includes at least six species sampled in this study. Species in Clade A are morphologically diverse and include a variety of habits (i.e. small shrubs, scandent shrubs, and small trees). This clade is also widespread and the species that comprise it occupy a geographic range extending from Mauritius, Madagascar, and further to Angola and Namibia in western tropical Africa. Species in Clade B are exclusively shrubs and entirely restricted to Madagascar. Species in these clades can be distinguished on the basis of capitulescence arrangement, which is oligocephalous (rarely solitary) and often in large paniculiform capitulescences in Clade A, and almost exclusively monocephalous (rarely oligocephalous) in Clade B.

Noteworthy among species that belong to Clade A is a scandent habit that appears to be synapomorphic in subclade 1 (but lost in *D. populifolius*). Species with this scandent habit have been described variously as scandent shrubs, climbers, lianas, or vines (e.g. Humbert 1960), but on the basis of our field observation they are slender, erect, scandent shrubs that begin to scramble on adjacent vegetation while growing. Regardless, the evolution of this trait appears to have triggered a rapid radiation and range expansion in the genus. In Madagascar alone, six endemic species of *Distephanus* share this scandent habit (although only two are included in this study) and most species of *Distephanus* sampled in this study that occur outside of Madagascar also share this habit (except *D. henryi* and *D. plumosus*). The tendency toward scandent shrubs might have been facilitated by the elongation and expansion of capitulescences in this subclade, which are notably large, loose, and in some cases have been described as having a 'zig-zag' appearance (Humbert 1960, see Fig. 1). The 'zig-zag' arrangement of these large, loose, and sprawling capitulescences may help species in this subclade to climb and clamber over the tops of adjacent vegetation. The role that this trait has played in the diversification and range expansion of *Distephanus* should be a focus of future studies, ideally with a comprehensive sampling of all species that share it. Other than the scandent habit and expansion of the capitulescence, the only other characteristic that helps to distinguish this subclade is the presence of long acuminate (sometimes reflexed) phyllary apices.

Compared with Clade A, Clade B is seemingly less morphologically diverse, but characterized by smaller shrubs (never climbing, never trees) with often solitary capitula (very rarely arranged in oligocephalous cymes). Both vegetative and reproductive organs of species in Clade B are often covered in a dense, but short pubescence, often white and sometimes referred to as 'cottony' (e.g. *D. rochonioides* (Humbert) H.Rob. & B.Kahn and *D. subluteus* (Scott Elliot) H.Rob. & B.Kahn).

More exhaustive taxonomic sampling might help improve phylogenetic resolution within *Distephanus*, particularly along the backbone of Clade A, which is the least well-resolved (perhaps due to conflict between the nuclear and plastid datasets). Conflict between results from the nuclear and plastid datasets led to four key differences in the topology and include the position of a subclade comprising five accessions of *D. glutinosus*; a subclade comprising two species (*D. poissonii* and *D. polygalifolius*), the position of two species (*D. quartziticolus* and *D. swinglei*), and the position of *D. henryi* and *D. plumosus* relative to the rest of the genus. Although there is no direct evidence of interspecific hybridization within this genus in the literature, Humbert (1960) noted the possibility that *D. polytricholepis* is a hybrid between *D. garnerianus* and *D. malacophytus* and, interestingly, we do note the position of *D. garnerianus* in the phylogenetic analysis of the plastid dataset varies compared with those of the nuclear ITS dataset, potentially indicating hybrid ancestry. Considering the propensity for gregariousness (multiple species occupying overlapping geographic space) among species of *Distephanus*, hybridization should not be ruled out as a possible explanation for phylogenetic conflict observed here.

The age of Distephaneae and subsequent radiation

Results of fossil-calibrated divergence dating in Mandel *et al.* (2019) suggested ages for the stem leading to *Distephanus* + Moquinieae + Vernonieae of 30.5 Mya; however, results from Keeley *et al.* (2021) indicate much older ages for most of the clades including this one. This latter study recovered a stem age for the clade that comprises *Distephanus* + Moquinieae + Vern onieae of 53 (43–63 Mya 95% HPD)—nearly two times older than the age recovered by Mandel *et al.* (2019). Our results from divergence dating were similar to (although slightly younger) the ages estimated by Mandel *et al.* (2019) for the split of *Distephanus* from the Moquinieae + core Vernonieae in the late Oligocene or early Miocene (22.7 Mya, 19.3–26 Mya, 95% HPD, Fig. 5); however, our estimates for the crown age of *Distephanus* in the mid- to Late Miocene (18 Mya, 13.2–22.9 Mya, 95% HPD, Fig. 5) are more similar to those of Keeley *et al.* (2021), who suggested 15.13 Mya (7.5–27 Mya, 95% HPD), compared with Mandel *et al.* (2019) which estimated 24 Mya. Importantly, the divergence dating analysis of this study significantly narrows the range in node age for *Distephanus* from the previous study by Keeley *et al.* (2021) from nearly 20 to <10 Mya. The present study also sheds light on the timing of diversification and range expansion in *Distephanus*, with dispersal from continental Africa to Madagascar taking place ~13.9 Mya (9.8–18 Mya, 95% HPD, Fig. 5, node 1) and subsequent, more widespread dispersal from Madagascar back to continental Africa ~6.6 Mya (3.5–9.5 Mya, 95% HPD, Fig. 5) and to Mauritius 6.7 Mya (4.9–8.5 Mya, 95% HPD, Fig. 5, node 2).

These dates are consistent with estimates from other recent studies in Asteraceae, such as Tarchonantheae (Kimball *et al.* 2023), another tribe in the same family that is restricted to continental Africa, Madagascar, and the Mascarenes and underwent significant diversification during the Miocene. Recent studies in other angiosperm groups have inferred similar dates (Late Miocene) for radiation to and throughout Madagascar, such as *Commiphora* (Burseraceae, Gostel *et al.*, 2016), Cyperaceae (Larridon *et al.* 2021), and *Diospyros* (Ebenaceae, Linan *et al.*, 2019), among others. A recent review (Génin *et al.* 2022) suggested that discontinuous and ephemeral land bridges in the Mozambique Channel during the Late Miocene could have led to bird-mediated dispersal and an increase in the arrival of angiosperms to Madagascar during this time.

Biogeography

Results of this work suggest that *Distephanus* likely originated in continental Africa near the mid-Miocene (~18 Mya, Figs 5, 6), as indicated by the results of ancestral area reconstruction using BioGeoBEARS; however, the short branch (and comparatively low branch support) that separates *D. plumosus* and *D. henryi*—which is one of only two species restricted to the Yunnan province of southern China—could be interpreted to suggest these may be surviving members of a lineage that was historically more diverse and occupied a more widespread ancestral area. Although results of the ancestral area reconstruction indicate continental Africa as most likely for the origin of *Distephanus*, the results do not rule out the possibility of an ancestral area in either Asia or Madagascar. If this were the case, these species might currently occupy historical refugia that correspond with a much broader ancestral geographic range throughout the Eastern Hemisphere tropics.

Subsequently, results from the ancestral area reconstruction indicate that a single dispersal event most probably took place from continental Africa to Madagascar during the mid-Miocene, followed by another dispersal event from Madagascar to Asia, as suggested by the most likely ancestral area for the MRCA of *D. henryi* and all remaining species of *Distephanus*, but these analyses only slightly favoured Madagascar as the most likely ancestral area for this clade. Two more independent dispersal events took place during the Late Miocene from Madagascar to Mauritius and from Madagascar to continental Africa. Surprisingly, our results indicate these dispersals took place at nearly the same time. This pattern of range expansion is especially notable, considering it is quite uncommon in the biogeographic literature for taxa to disperse to Madagascar, from continental Africa and then back-disperse to continental Africa again, however, see recent studies by Kimball *et al.* (2023) and Linan *et al.* (2019) that suggest that dispersal among the flora of continental Africa and islands in the Indian Ocean Basin might be more frequent than expected based on documented occurrences in the biogeographic literature. This pattern would also be consistent with the hypothesis of Génin *et al.* (2022), who point to data from Masters *et al.* (2021) and others suggesting a series of disconnected, ephemeral, land bridges existed during the Late Miocene that could have facilitated bird-mediated dispersal of angiosperms from continental Africa to Madagascar. The biogeographic origins of the highly diverse and endemic biota of Madagascar, one of the world's hottest biodiversity hotspots, remains one of the major mysteries in global biodiversity science and evidence from the present study provides yet another example that biogeographic connections between Madagascar and other landmasses have been more common than often thought.

CONCLUSION

This work represents the first of what is part of a larger effort to understand the diversity of more than 600 species from the Eastern Hemisphere that belong to the large and taxonomically complex ironweed tribe, Vernonieae. The establishment of tribe Distephaneae is consistent with results from other, recent molecular phylogenetic studies and will help guide further efforts to develop a revised, subtribal classification for the remaining genera and species of Vernonieae in the Eastern Hemisphere. Future work in *Distephanus* will benefit from expanded sampling of molecular loci using phylogenomic techniques, and will allow a better understanding of the patterns of morphological evolution as well as the complex biogeographic history that led to the widespread distribution of this genus throughout the Eastern Hemisphere.

Taxonomic treatment

Distephaneae (S.C.Keeley & H.Rob.) H.Rob. & V.A.Funk, *tribus status novum*. Type: *Distephanus* Cass., Bull. Sci. Soc. Philom. Paris 1817: 151. 1817.

≡Distephaninae S.C.Keeley & H.Rob. in Funk, Susanna, Stuessy & Bayer, Syst. Evol. Biogeogr. Asteraceae: 448. 2009.

Erect or frequently scandent to lianescent shrubs or small trees. Stems and leaves frequently covered by contorted or asymmetrically T-shaped trichomes. Leaves alternate, simple, coriaceous, trinervately veined, sessile, or often shortly petiolate. Capitula terminal, solitary, or arranged in small cymes with short peduncles or on large, paniculiform capitulescences. Capitula homogamous, discoid; involucre campanulate (rarely ovoid–obconical), phyllaries 3–8-seriate, acute to acuminate. Florets 5–100, corollas actinomorphic, funnelform, 5-lobed, lobes longer than wide, yellow, pale yellow, white, or cream, mostly glabrous; anthers with aglandular apical appendages, endothecial cells with simple, broad, non-contiguous sclerified shields, anther bases with distinctly broad and frequently sclerified tails. Pollen tricolporate, sublophate, or lophate, with continuous perforated tectum between colpi. Style base with large, abruptly distinct glabrous node, upper shaft, and outer surfaces of style branches with obtuse sweeping hairs, stigmatic papillae covering whole inner surface of branches. Cypselae cylindrical to prismatic, sometimes sub-triquetrous or quadrangular, with (5–)10(–12) ribs, glabrous or with hairs or glands; pappus biseriate or rarely uniseriate, setose to paleaceous, rarely plumose, whitish to stramineous, rarely reddish, persistent, or unfrequently caducous, outer series shorter than inner series or sometimes subequal. $N = 10$. Elemanolides.

Distribution

Sub-Saharan Africa, Madagascar, Mauritius, south-western China.

Genus

Distephanus Cass.

Distephanus Cass., Bull. Sci. Soc. Philom. Paris 1817: 151. 1817. Type: *Conyza populifolia* Lam., Encycl. 2(1): 87. 1786. (=*Distephanus populifolius* (Lam.) Cass., Bull. Sci. Soc. Philom. Paris 1817: 151. 1817). ≡*Vernonia* sect. *Distephanus* (Cass.) Benth & Hook.f., Gen. Pl. 2(1): 228. 1873. ≡*Vernonia* subsect. *Distephanus* (Cass.) S.B.Jones, Rhodora 83(833): 68. 1981.= *Gongrothamnus* Steetz in Peters, Naturw. Reise Mossambique 6 (Bot. 2): 336. 1864. Type: *Gongrothamnus divaricatus* Steetz in Peters, Naturw. Reise Mossambique 6 (Bot. 2): 342. 1864. (=*Distephanus divaricatus* (Steetz) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 499. 1986). ≡*Vernonia* subsect. *Gongrothamnus* (Steetz) S.B.Jones, Rhodora 83(833): 65. 1981. =*Newtonia* O.Hoffm. in Engler & Prantl, Nat. Pflanzenfam. 4(5): 285. 1892, nom. illeg., non Baillon. 1888. Type: *Newtonia angolensis* O.Hoffm., nom. illeg. (=*Distephanus angolensis* (O.Hoffm.) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 498. 1986). ≡*Antunesia* O.Hoffm., Bol. Soc. Brot. 10: 178. 1893. Type: *Antunesia angolensis* O.Hoffm., Bol. Soc. Brot. 10: 179. 1893 (=*Distephanus angolensis* (O.Hoffm.) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 498. 1986).

This genus comprises 41 species, most of them endemic to Madagascar.

Accepted species (41):

Distephanus ambongensis (Humbert) H.Rob., PhytoKeys 17: 26. 2012.

Distephanus angolensis (O.Hoffm.) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 498. 1986.

Distephanus anisochaetoides (Sond.) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 499. 1986.

Distephanus antandroy (Humbert) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 499. 1986.

Distephanus bakeri (Vatke) V.A.Funk & H.Rob., PhytoKeys 77: 90. 2017.

Distephanus barus (Humbert) H.Rob., PhytoKeys 17: 26. 2012.

Distephanus capuronii (Humbert) V.A.Funk & H.Rob., PhytoKeys 77: 90. 2017.

Distephanus cloiselii (S.Moore) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 499. 1986.

Distephanus divaricatus (Steetz) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 499. 1986.

Distephanus eriophyllus (Drake) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 499. 1986.

Distephanus forrestii (J.Anthony) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 499. 1986.

Distephanus garnierianus (Klatt) H.Rob. & B.Kahn, Biol. Soc. Washington 99(3): 499. 1986.

Distephanus glandulicinctus (Humbert) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 499. 1986.

Distephanus glutinosus (DC.) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 499. 1986.

Distephanus grevei (Drake) V.A.Funk & H.Rob., PhytoKeys 77: 90. 2017.

Distephanus henryi (Dunn) H.Rob., Proc. Biol. Soc. Washington 112(1): 238. 1999.

Distephanus ibityensis (Humbert) V.A.Funk & H.Rob., PhytoKeys 77: 91. 2017.

Distephanus inhacensis (G.V.Pope) R.G.C.Boon & Glen, Bothalia 43(1): 94. 2013.

Distephanus lastellei (Drake) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 499. 1986.

Distephanus madagascariensis (Less.) H.Rob. & V.A.Funk, Taxon 61(2): 452. 2012.
Distephanus mahafaly (Humbert) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 499. 1986.
Distephanus majungensis (Humbert) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 499. 1986.
Distephanus malacophytus (Baker) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 499. 1986.
Distephanus manambolensis (Humbert) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 500. 1986.
Distephanus mangokensis (Humbert) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 500. 1986.
Distephanus nummulariifolius (Klatt) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 500. 1986.
Distephanus ochroleucus (Baker) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 500. 1986.
Distephanus plumosus (O.Hoffm.) Mesfin, Compositae Newslett. 22: 11. 1992.
Distephanus poissonii (Humbert) V.A.Funk & H.Rob., PhytoKeys 77: 91. 2017.
Distephanus polygalifolius (Less.) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 500. 1986.
Distephanus polytricholepis (Baker) V.A.Funk & H.Rob., PhytoKeys 77: 91. 2017.
Distephanus populifolius (Lam.) Cass., Bull. Sci. Soc. Philom. Paris 1817: 151. 1817.
Distephanus qazmi N.Kilian & A.G.Mill., Willdenowia 30(1): 84. 2000.
Distephanus quartziticolus (Humbert) V.A.Funk & H.Rob., PhytoKeys 77: 91. 2017.
Distephanus rhodopappus (Baker) V.A.Funk & H.Rob., PhytoKeys 77: 91. 2017.
Distephanus rochonioides (Humbert) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 500. 1986.
Distephanus spiciformis (Klatt) V.A.Funk & H.Rob., PhytoKeys 77: 91. 2017.
Distephanus streptocladus (Baker) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 500. 1986.
Distephanus subluteus (Scott Elliot) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 500. 1986.
Distephanus swinglei (Humbert) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 500. 1986.
Distephanus trinervis Bojer ex DC., Prodr. 5: 75. 1836.
Excluded species (2):
Distephanus angulifolius (DC.) H.Rob. & B.Kahn, Proc. Biol. Soc. Washington 99(3): 499. 1986.
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SUPPLEMENTARY DATA

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

ACKNOWLEDGEMENTS AND FUNDING

This work was supported by the National Science Foundation (DEB-NERC#2210598) and Global Genome Initiative (grant GGI-SIBG-2016 to VA Funk and MR Gostel).

The authors thank the Missouri Botanical Garden Madagascar Research and Conservation Program and their staff for supporting fieldwork that contributed to this project, especially Dr Pete Lowry II, Richard Razakamalala, Charles Rakotovao, and Faranirina Lantoarisoa. The authors are grateful to Madagascar National Parks for supporting the fieldwork that was conducted as part of this research through Permit Number 224/16/MEEF/SG/DGF/DSAP/SCB.Re. The authors also wish to thank the Parc Botanique et Zoologique de Tsimbazaza in Madagascar (PBZT) for supporting the fieldwork that contributed to this project, especially Jacqueline Razanatsoa and the staff of the Herbarium at PBZT (TAN) for providing access to important collections of preserved material that were studied as part of this project. The authors also thank the Herbarium of the Muséum National d'Histoire Naturelle (P), Paris, France for providing access to important collections of preserved plant material for study as well as sampling of tissue that was used in the molecular studies presented in this project. In particular, the authors wish to thank Doctors Myriam Gaudeul and Florian Jabbour at the P Herbarium for their generous assistance. Laboratory work that contributed to this project was carried out at the Laboratories of Analytical Biology (LAB) at the Smithsonian Institution, National Museum of Natural History, and the George C. and Sue W. Sumner Molecular and Structural Laboratory at the Botanical Research Institute of Texas, Fort Worth Botanic Garden.

DATA AVAILABILITY

Data presented in this article are available in GenBank (accession numbers provided in Supporting Information, Appendix 1) and Dryad (DOI: 10.5061/dryad.vq83bk41c).

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