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RESEARCH ARTICLE

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Phylogenomics, historical biogeography, and diversification of leaf traits in the Malagasy-endemic genus *Uncarina* (Pedaliaceae)

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Abstract

Premise: *Uncarina* contains 14 species of woody plants endemic to Madagascar. Its occurrence across dryland biomes on the island make it an interesting system to study the diversification of the flora.

Methods: Using samples of all species and 512 nuclear loci, we reconstructed phylogenetic trees to examine species relationships and assess their monophyly. We also studied the historical biogeography of the genus and combined leaf trait data derived from SEM photography of trichomes and geometric morphometric analysis of leaf shape to better understand its diversification across dryland biomes.

Results: *Uncarina* is monophyletic, and major clades showed a clear biogeographical signal. Leaf traits also corroborated relationships among major clades. Although most species are monophyletic, at least one cryptic species exists. *Uncarina*, like many arid-adapted plant lineages in Madagascar originated in the Miocene or Pleistocene. Geographic movement has been primarily along a south–north axis, with river basins apparently acting as barriers to gene flow. The evolution of leaf traits corroborated movement from the spiny thicket to the dry forest biome.

Conclusions: As with Malagasy lemurs and other animals, riverine barriers may have been involved in the diversification of *Uncarina* and may apply more broadly to epizoochorous angiosperms of Madagascar. Leaf traits suggest either a loss of adaptations to extremely arid, high irradiance environments or a release from herbivores. As is likely needed in other Malagasy lineages, more thorough population-level sampling and specimen collecting is needed to fully understand the taxonomic and morphological diversity in the genus.

KEYWORDS

geometric morphometrics, Madagascar, Pedaliaceae, quantitative biogeography, trichome, *Uncarina*

Madagascar is well known as a biodiversity hotspot, with 82% of its ~11,500 native plant species and 310 genera endemic to the island (Phillipson et al., 2006; Callmander et al., 2011). While much of Madagascar's plant diversity is concentrated in the humid forests, dry and spiny forests harbor a concentration of neo-endemic lineages, a feature shared with other dryland biomes (Omollo et al., 2024). Apart from the long history of geographic isolation from continental regions resulting in geographic speciation, additional hypotheses have been proposed to explain the species diversity in Madagascar (Vences et al., 2009; Antonelli et al., 2023; Omollo et al., 2024). These include isolation via landscape features due to either topographic heterogeneity or riverine barriers (Pastorini et al., 2003; Liu et al., 2024), isolation on mountains (Wollenberg et al., 2008) or within watersheds (Wilmé et al., 2006) during dry periods followed by secondary contact, and/or local adaptations to the diversity of biome types present across the island ranging from humid forests along

the eastern coast to spiny thickets in the southern portion of the island (Pearson and Raxworthy, 2009; Vences et al., 2009).

Although there has been great effort in understanding the biogeographic origin of and a basic understanding of relationships among plant lineages in Madagascar (e.g., Wikström et al., 2010; Buerki et al., 2013; Federman et al., 2015; Janssens et al., 2016; Antonelli et al., 2023; Skema et al., 2023), comparatively little effort has been made toward understanding biogeographic patterns of plant groups, especially on the western side of the island—either at the genus or species level (but see Dransfield and Rakotoarinivo, 2011; Hanes et al., 2022; Karimi and Hanes, 2024). This is in stark contrast to lineages of Malagasy animals, where molecular data suggest major riverine or montane systems as lineage breakpoints (e.g., Pastorini et al., 2003; Raselimanana et al., 2009; Shi et al., 2013; Pabijan et al., 2015; Lei et al., 2017).

Apart from the Central Highlands, which runs north and south and restricts longitudinal dispersal, rivers serve as the other major geophysical barrier on the island and their east and west orientation may impact latitudinal dispersal (Vences et al., 2009). Within animal lineages, a repeatedly observed pattern of distinct southern, central, and northern clades implies a pattern of gradual dispersal and subsequent population isolation across a latitudinal gradient. Although most studies lack a phylogenetic hypothesis calibrated to absolute time, because these studies examine clades at both the species and genus level, it can be reasonably inferred that these barriers have been operative for extended periods of time.

Uncarina (Baill.) Stapf (Pedaliaceae) is a genus of small trees and shrubs endemic to Madagascar. Most species possess yellow corollas that often have red stripes down the throats (Figure 1A), although one species has white corollas [*U. leptocarpa* (Decne.) Ihlenf. & Straka] and two species have bright pink corollas [*U. abbreviata* (Baill.) Ihlenf. & Straka and *U. stellulifera* Humbert; Figure 1B]. Of the seven species whose pollination ecology has been studied, species are exclusively pollinated by bees (Gamo et al., 2006).

In his posthumous treatment for the flora of Madagascar, Humbert (1971) recognized nine species of *Uncarina*. Taxonomic efforts in the ensuing half century have added five additional species for a total of 14 accepted species (Ihlenfeldt, 2010). The majority of Pedaliaceae are restricted to mainland Africa (Ihlenfeldt, 1994), with *Uncarina* being the only genus native to Madagascar. *Uncarina* is well-nested within Pedaliaceae and is sister to *Rogeria* J. Gay (Gormley et al., 2015; Zaborsky, 2018; Zhigila and Muasya, 2023), with which it shares the derived trait of dichasia with lateral flowers not aborted (Ihlenfeldt, 2004a). As in many genera of Pedaliaceae, *Uncarina* possesses capsular fruits, which are adorned with sharp outgrowths to aid in epizoochory (Figure 1C, D; Ihlenfeldt, 2004a). The outgrowths of *Uncarina* are either long prickles with hooked tips or short prickles that can be hooked or unhooked. Although Midgley and Illing (2009) proposed that *Uncarina* fruits were dispersed as "trample-burs" by extinct elephant birds (Aepyornithiformes), the fruits are very fragile and can be easily crushed, even with bare hands (J. Zaborsky, personal observation), making it more likely that the fruits are dispersed as "sticktights." There are reports of lemurs (Lemuroidea), specifically ring-tailed lemurs (*Lemur catta*) with *Uncarina* fruits tangled in their fur (Crowley, 2010), and lemurs preferentially use *Uncarina* to scent mark (Tinsman et al., 2017), suggesting that lemur dispersal is more likely.

Uncarina is noteworthy in Pedaliaceae because all species are woody and have some degree of stem succulence, a trait that has attracted considerable interest from succulent plant enthusiasts. Apart from the pachycaul habit, *Uncarina* species show additional vegetative adaptations. Their leaves are highly variable across species in degree of lobing and their shape ranges from triangular to digitate (Figure 1E). Such variation in leaf shape can even be found within an individual throughout the growing season. In addition, leaf indumentum consists of a mixture of simple and glandular trichomes. The glandular trichomes bear a four-celled head (found only in Pedaliaceae) and release a large amount of mucilage when ruptured in the presence of water (Ihlenfeldt, 2004a). Although the purpose of this mucilage is unknown, it may act to deter herbivores. The shape of the head and the distribution of the trichomes across lamina surfaces have been used as a taxonomic character in *Uncarina* (Ihlenfeldt, 2002, 2004b; Lavranos, 2004), although no study has fully detailed the morphology of these mucilage glands across the genus.

Uncarina is confined to arid and semiarid regions of Madagascar, most commonly occurring in dry spiny forests and western dry deciduous forests. *Uncarina* species occur from the far northern tip of the island near Antsiranana, along the western extent of the island into the south, reaching as far east as Ambovombe (Figure 1F). The geographical distribution of described species varies from wide-ranging species (e.g., *U. decaryi* Humbert, *U. peltata* Stapf) to narrow endemics, the latter especially frequent among recently described species (e.g., *U. roeoesliana* Rauh, *U. platycarpa* Lavranos), and many species have adjacent or overlapping ranges (Figure 1F).

Given its occurrence across dryland habitats in Madagascar, *Uncarina* presents an interesting case study to examine patterns of plant dispersal and trait divergence across the island. For the present study, we sampled all known *Uncarina* taxa and used anchored hybrid enrichment to present a phylogenetic hypothesis for the genus to better understand the biogeographic history and diversification of traits in the dryland flora of Madagascar. We expected that the biogeographic history of this epizoochorus genus would match that of their putative dispersers, with riverine systems acting as isolating barriers and providing strong latitudinal structuring of clades.

MATERIALS AND METHODS

Tissue sampling, DNA extraction, sequencing, and assembly

Silica-dried leaves of all 15 recognized taxa (all 14 species plus one variety) of *Uncarina* were collected from the living

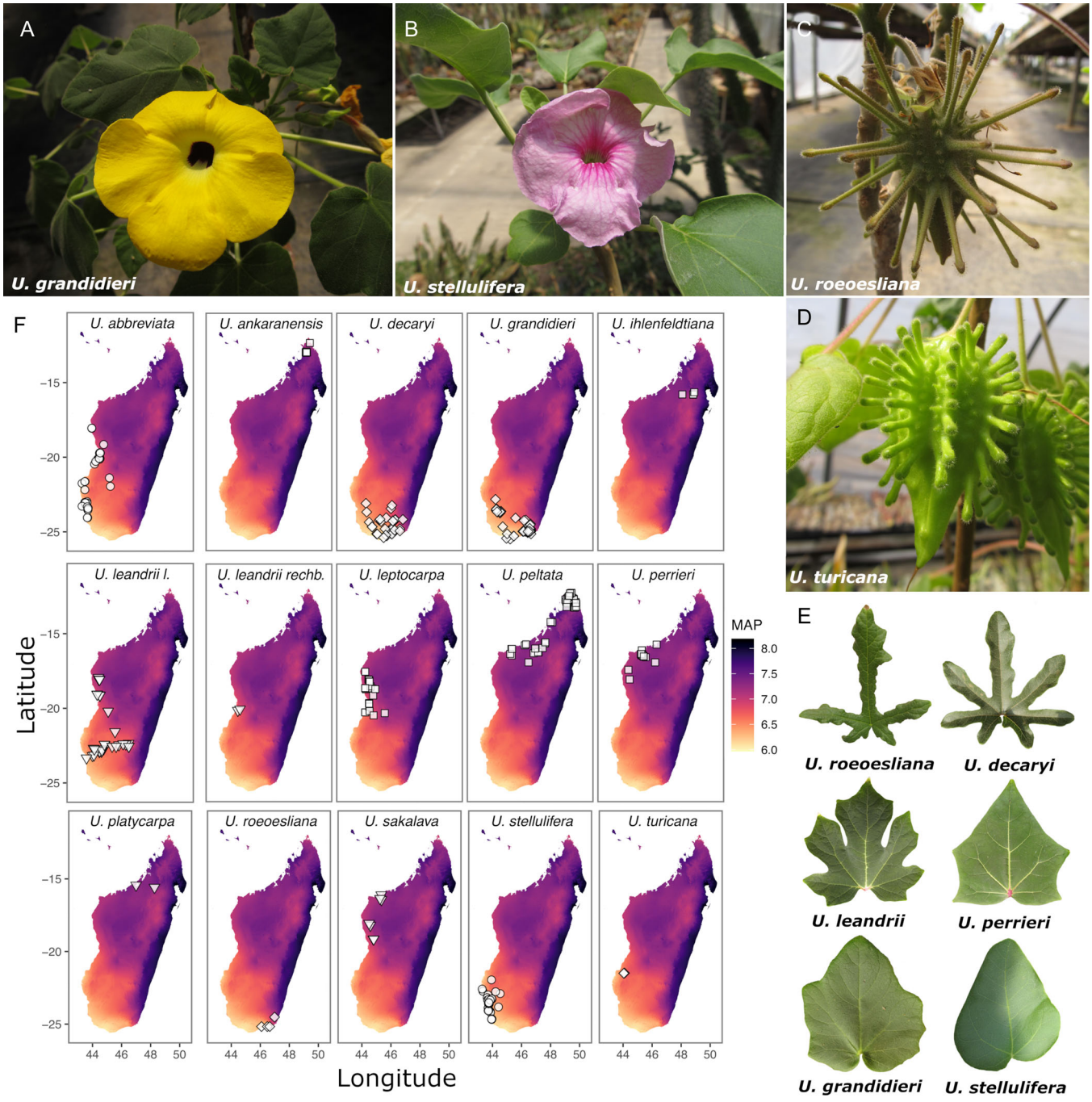

FIGURE 1 Morphological variation and geographic distribution of *Uncarina*. (A, B) Flowers showing the two major corolla colors in the genus. (C, D) Variation in capsule projections showing both long prickles with hooked tips (C) and short prickles with unhooked tips (D). (E) Variation in the shape of the leaf lamina from deeply lobed to entire. (F) Geographical distribution of the 15 accepted taxa.

collection at the Huntington Botanical Garden in San Marino, California, USA, with additional specimens obtained from the private collection of Walter Röösli (Appendix S1). Samples were only obtained from specimens positively known to have come from the wild and contained 20 accessions in total. Outgroup sampling consisted of *Sesamothamnus guerichii* (Engl.) E.A. Bruce, *Pterodiscus aurantiacus* Welw., and *Sesamum* (*Ceratotheca*) *trilobum* (Bernh.) Byng & Christenh. Total DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's specifications.

We used the anchored hybrid enrichment (AHE) method (Lemmon et al., 2012) to target 517 generic angiosperm loci (Buddenhagen et al., 2016). The library preparation, enrichment, assembly, and initial alignment of nuclear loci were performed at the Florida State University Center for Anchored Phylogenomics (www.anchoredphylogeny.com). DNA was sonicated to fragment sizes between 200–600 bp before library

preparation and indexing following a modified protocol of Meyer and Kircher (2010). Indexed samples were pooled and enriched using the Angiosperm v.1 enrichment kit (Buddenhagen et al., 2016). Sequencing was conducted on an Illumina HiSeq 2500 at the Translational Science Laboratory, College of Medicine, Florida State University, Tallahassee, Florida, USA. Raw reads have been deposited in the NCBI Sequence Read Archive (SRA) as BioProject PRJNA1214278.

Paired reads were merged before assembly using the method of Rokyta et al. (2012). Reads were mapped to the probe regions and assembled de novo as described by Prum et al. (2015) and Buddenhagen et al. (2016), with orthology determined as done by Prum et al. (2015). Sequences in each orthologous cluster were aligned using MAFFT v.7.023b (Katoh and Standley, 2013), then trimmed and masked (Prum et al., 2015). Finally, the masked alignments were inspected by eye. Regions considered obviously misaligned or likely paralogous were removed, and poorly aligned sections in each alignment were deleted.

Pedaliaceae-wide supermatrix

Because previous analyses have only included one or two samples of *Uncarina*, we first tested the monophyly of *Uncarina* by assembling a supermatrix of Pedaliaceae sequences available on GenBank (Appendix S2). The supermatrix consisted of nuclear ribosomal ITS and ETS and six plastid loci (*psbA-trnH*, *rps16*, *trnL-trnF*, *rbcL*, *matK*, *ndhF*). Outgroups were Acanthaceae and Martyniaceae. We supplemented this supermatrix by mapping off-target Illumina reads to reference GenBank sequences of ITS (*U. grandidieri* (Baill.) Stapf: AY178659), ETS [*Sesamum sesamoides* (Endl.) Byng & Christenh.: KJ884303], and each plastid region based on the reference plastome of *Sesamum indicum* L. (NC016433) as described by Kriebel et al. (2019). Loci were aligned with MAFFT v.7.023b (Katoh and Standley, 2013) using default parameters, with the ends subsequently trimmed by eye. All loci were concatenated into a single matrix, which was then analyzed using IQ-TREE v.2.2.2.6 (Minh et al., 2020) under the GTR+I+G model with separate partitions for the nuclear ribosomal and plastid DNA. Branch support was assessed with 1000 ultrafast bootstrap (BS) replicates. For this and other ML analyses, we interpreted branches as receiving "strong" support only when BS ≥ 95% (Minh et al., 2013).

Uncarina phylogenies

Bifurcating trees

First, a maximum likelihood (ML) phylogenetic tree was estimated in IQ-TREE based on a concatenated analysis of all loci. For each locus, we selected the best-fitting nucleotide substitution model using the -m TEST option. Using the optimal substitution models for each locus, we inferred a species tree based on the concatenated matrix, with 1000 BS replicates with the GENESITE sampling scheme. In addition, the species tree was estimated under the coalescent model weighted ASTRAL in ASTER v.1.15.2.3 (Mirarab et al., 2014; Zhang and Mirarab, 2022), with the ML gene trees estimated in IQ-TREE as described above.

Phylogenetic networks

Intrinsic barriers to interspecific gene flow may be absent within *Uncarina*, evidenced by the ability to artificially cross species and apparent morphological intermediates in wild populations (Humbert, 1971; Ihlenfeldt, 2010). Therefore, we also inferred phylogenetic networks using the maximum pseudolikelihood method implemented in SNaQ (Solís-Lemus and Ané, 2016), which infers reticulate evolutionary histories while accounting for incomplete lineage sorting (ILS). Using alignments trimmed to include only *Uncarina* and *Pterodiscus*, Bayesian phylogenetic inference was first performed on each locus separately with MrBayes v.3.2.7 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). MrBayes analyses consisted of four linked chains with a heat of 0.2 and were run for 2,000,000 generations, with 25% discarded as burn-in. Convergence was assessed by the standard deviation of split frequencies. The resulting posterior distributions were analyzed via Bayesian concordance analysis (Ané et al., 2007; Bond and Silander, 2007) in BUCKy v.1.4.4 (Ané et al., 2007; Larget et al., 2010) using alpha = 1 and 1,000,000 generations. The concordance factor (CF) table and population tree with branch lengths in coalescent units generated by BUCKy was then used as input into SNaQ. We ran 50 independent runs of SNaQ to infer the optimal network without hybridization edges ($h = 0$). The $h = 0$ network was then used as input for network searches with a maximum of one to five hybridization edges (hmax). Network searches were increased sequentially in each case starting from the previous optimal network. The preferred number of hybridizations was determined by plotting log-pseudolikelihood against hmax (Solís-Lemus and Ané, 2016). The best network was then used as the starting network for 100 independent BS replicates (sampling from the confidence intervals for each possible quartet CF) with 20 runs per replicate, where 10 runs per replicate started with the optimal network and the other 10 started with the $h = 0$ population tree.

Dating the diversification of *Uncarina*

Because the size of the final AHE data set precluded dating using all loci, we opted for a "gene-shopping" approach (Smith et al., 2018) similar to that of Rose et al. (2024), by selecting the 30 loci complete for all samples whose gene tree topology was most similar to that of the ASTRAL species tree based on the Robinson–Foulds distance after poorly supported (BS < 75) nodes were collapsed in all trees.

Divergence times were estimated in BEAST 2 v.2.7.1 (Bouckaert et al., 2014), treating each locus as a separate partition and using the substitution model selected by IQ-TREE as described above. All trees were linked, clock models implemented an optimized relaxed clock, and a Yule tree prior was used. We used three secondary age priors from the Lamiales-wide chronogram of Rose et al. (2022). Each prior was uniformly distributed with minima and maxima based on the 95% highest posterior density (HPD) of the estimated age: the crown of Pedaliaceae (8.80–32.97 million years ago [Mya]), the most recent common ancestor (MRCA) of *Sesamum* and *Sesamothamnus* (2.59–18.85 Mya), and the MRCA of *Pterodiscus* and *Uncarina* (5.40–27.46 Mya). We also placed a prior constraining the monophyly of *U. leandrii* based on our other analyses (see results). The BEAST 2 analysis was run for 100,000,000 generations, with sampling every 100,000 generations.

Quantitative historical biogeography

Occurrence records were downloaded from the Global Biodiversity Information Facility (GBIF; GBIF.org, 2023). Records were cleaned to remove any cultivated material, specimens without coordinates, imprecisely georeferenced specimens lacking at least three decimal places in both decimal latitude and longitude, and spurious collections outside of the known range of a species. After cleaning, all records were rounded to three decimal places, and duplicates were removed. Due to uncertainty regarding the monophyly of the two varieties of *U. leandrii* (see results), we merged all occurrences of the varieties of this species.

For each species, we modeled species range using α-hull polygons, which require more than three unique localities for each species. For species with two unique localities, occurrence records were separated by less than 10 km. Thus, for species with less than three localities, we generated range polygons by taking one point at random and generating a 10 km buffer around it. For species represented by more than three records, we first fit an α-hull polygon for each species using the custom R function flexible.alpha.hull (available at https://github.com/jrosaceae/Uncarina), which is a wrapper function for the getDynamicAlphaHull function in the R package rangeBuilder v. 1.5 (Davis Rabosky et al., 2016) that is robust to failures in hull fitting by allowing up to three attempts, if necessary, using 100%, 95%, and 90% of the points. We allowed up to five discontinuous polygons and clipped polygons to remove any area over oceans.

To reconstruct the historical biogeography of *Uncarina*, we implemented rase (Quintero et al., 2015), which uses a Brownian motion model of dispersal to infer ancestral range centroids, using species polygons to integrate over uncertainty. We used the polygons generated using flexible.alpha.hull as described above as input to the rase function by converting them from latitude/longitude to the ESPG:9003 Mercator projection. Using the time-calibrated species tree and projected polygons, we ran rase for 20,000 iterations, logging parameters every 10 iterations. Stationarity was assessed using the R package coda (Plummer et al., 2015). After applying a 20% burn-in, we reconstructed ancestral centroids at five time periods intersecting major stem lineages using the rase.slice function, with 20,000 iterations and logging parameters every 10 iterations per time period.

Vegetative morphology

Trichomes

Trichome variation in *Uncarina* has been used to delimit species, yet it has not been thoroughly surveyed across the genus. Scanning electron microscopy (SEM) was utilized to examine adaxial and abaxial leaf trichomes from silica-dried, wild-collected specimens of all known taxa. Leaves from one specimen per taxon were photographed with a Quanta 200 Environmental Scanning Electron Microscope (FEI, Hillsboro, OR, USA) at 20 kV at the University of Wisconsin-Madison Newcomb Imaging Center. Specimens did not undergo any special treatment before being photographed.

Geometric morphometrics

While leaf shape in *Uncarina* is variable within and among species, its phylogenetic significance or potential adaptive value have not been discussed. Images of digitized herbarium specimens of *Rogeria* and *Uncarina* were gathered from GBIF, with a focus on specimens housed at Muséum national d'Hístoire naturelle (P) and Missouri Botanical Garden (MO). Images of well-pressed, mature leaves were converted into outlines, with the outlines filled in black with white backgrounds. To mitigate artifacts caused by artificially asymmetric leaves, we reflected one half of each leaf. Images were saved as JPEG files using GIMP v.2.8 (Solomon, 2009).

We quantified leaf shape using elliptical Fourier analysis (eFa) using Momocs v.1.3.2 (Bonhomme et al., 2014). Reflected outlines were imported into R where they were centered, scaled, and landmarked to avoid unwanted twisting of the specimens during the eFa. To ensure that enough harmonics were included in the eFa, we checked harmonic power with the function calibrate_harmonicpower_efourier, which selected 12 harmonics. Fourier coefficients were subjected to a principal component analysis (PCA) to see which were most important in describing leaf shape. Collections of *U. leandrii* were treated as described in the methods for assessing historical biogeography.

Ancestral habitat reconstruction

To tie potential shifts in geography and traits with shifts in habitat type, we reconstructed ancestral habitat type in

Uncarina. Using the cleaned coordinates, we extracted biome from a shapefile of the World Wildlife Fund terrestrial ecoregions (Olson et al., 2001). Ancestral states were reconstructed by fitting a Markov-k (Mk) model using maximum likelihood as implemented in the corHMM function in the R package corHMM v. 1.22 (Beaulieu et al., 2022). We reconstructed traits using an equal rate (ER), symmetric rates (SYM), and all rates different (ARD) model of trait evolution. The best-fitting model was selected based on corrected Akaike information criterion (AICc) scores.

RESULTS

Monophyly of *Uncarina*

Our Pedaliaceae-wide supermatrix contained 49 terminals and 9414 aligned base pairs. The phylogenetic tree based on concatenated data recapitulated the major familial relationships found in previous studies (Appendix S3). *Uncarina* was recovered as monophyletic (BS = 99) and sister to *Rogeria* (BS = 99). While these eight loci supported some sister relationships (ultrafast BS ≥ 95), 56% of internal branches in *Uncarina* had ultrafast BS < 95.

Relationships within *Uncarina*

We recovered 512 of the 517 targeted nuclear loci (99%), for a total of 364,334 aligned base pairs. The topologies of the concatenated, ASTRAL, and BUCKy analyses were all identical (Figure 2; Appendix S4). Support for relationships in the first two analyses was mostly high, and although BUCKy inferred low CFs for some relationships, only a few had overlapping confidence intervals, indicating alternative topologies were not supported.

The pink-flowered species ("pink" clade), *U. abbreviata* and *U. stellulifera*, were resolved as sister to the rest of the

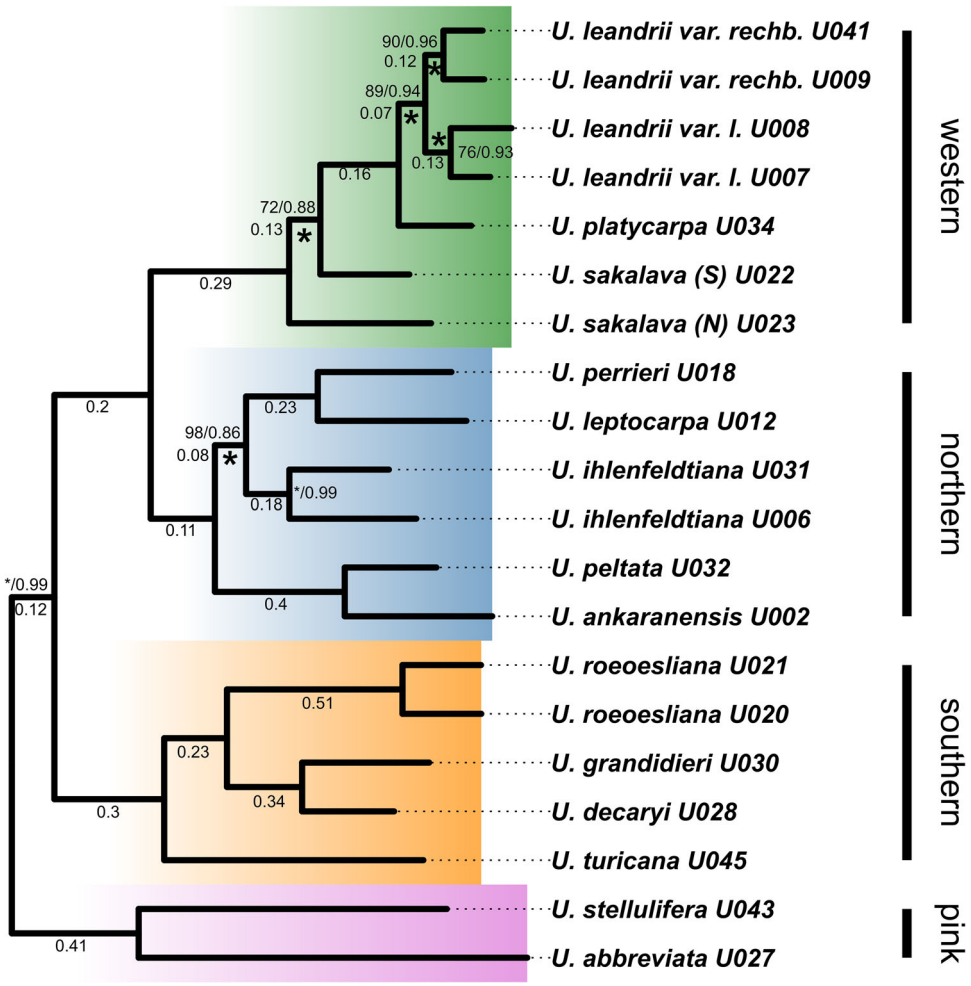

FIGURE 2 Phylogenetic tree of *Uncarina*. Maximum likelihood tree of the concatenated matrix with major clades colored and annotated to the right of the tree (*rech*. = *rechbergeri*). Branch lengths are in substitutions per site. Numbers above or to the right of branches are maximum likelihood support based on 1000 ultrafast bootstraps, followed by ASTRAL local posterior probabilities. Unnumbered branches receive maximal support in both analyses. Asterisks (*) denote full support in one analysis. Numbers below branches are Bayesian concordance factors. For those branches with a bold asterisk above or below them, conflicting topologies cannot be rejected.

genus (BS = 100, ASTRAL local posterior probability [LPP] = 1.0, CF = 0.41). The remainder of the genus formed three clades that are biogeographically coherent and whose monophyly received maximal support (BS = 100 and LPP = 1.0): a clade of largely southern species ("southern" clade; CF = 0.30), a clade of northern species ("northern" clade, CF = 0.29), and a clade of west-central species ("western" clade, CF = 0.11), with the latter two sister to each other (BS = 100, LPP = 1.0, CF = 0.20).

Within the southern clade, the more northerly *U. turicana* was sister to the remainder of the clade (BS = 100, LPP = 1.0, CF = 0.23). Relationships within the western clade were the least certain, with the most salient points being first: that *U. sakalava* was not monophyletic, with a sample from the northern part of the range (sample U23) sister to the rest of the western clade (BS = 72, LPP = 0.88, CF = 0.13). The alternative plausible topology yielded from BUCKy was a reversed placement of the *U. sakalava* samples, rather than a monophyletic *U. sakalava*. Second, although *U. leandrii* and the two varieties were monophyletic, none were strongly so, and alternative topologies were possible (*U. leandrii*: BS = 89, LPP = 0.94, CF = 0.07; *U. leandrii* var. *leandrii*: BS = 76, LPP = 0.94, CF = 0.13; *U. leandrii* var. *rechbergeri*: BS = 90, LPP = 0.97, CF = 0.12).

The best-fitting phylogenetic network suggested by SNaQ was $h = 3$ (Appendices S5–S8). However, $h = 1$ was the most biologically realistic network since additional networks seem implausible. For $h = 2$, there was a reticulation event between the ancestor of the western clade excluding northern *U. sakalava* into only one accession of *U. ihlenfeldtiana* (sample U006), and for $h = 3$, there was a reticulation event between *Pterodiscus* and the pink clade of *Uncarina* (Appendix S8). Therefore, the most probable network recovered was the single reticulation event (estimated at approximately 21% of the genome) from stem lineage of *U. turicana* to the stem lineage of *U. decaryi* + *U. grandidieri*.

Divergence times and historical biogeography

The topology of the BEAST 2 chronogram was identical to all other topologies except *U. leandrii* var. *leandrii* was not recovered as monophyletic. The crown age of *Uncarina* was estimated to date to the Late Miocene or Early Pleistocene at 2.64 Mya (95% HPD = 1.71–5.16 My). Crown ages of major clades were 1.77 My for the pink clade (95% HPD = 1.14–3.50 My), 1.38 My for the southern clade (95% HPD = 0.89–2.76 My), 0.99 My for the northern clade (95% HPD = 0.64–2.01 My), and 0.50 Ma for the western clade (95% HPD = 0.30–1.01 My).

The rase analysis estimated a preponderance of latitudinal dispersal within *Uncarina*, with a mean latitudinal dispersal rate (σ^2) of 6.54 (95% HPD: 5.34–7.85), compared to a longitudinal dispersal rate of 2.22 (95% HPD: 1.64–2.91). *Uncarina* originated in west-central Madagascar, followed by northward movement in the ancestor of the northern + western clades ~2.00 Mya (Figure 3). The pink and southern clades were in close geographic proximity for their entire history, although the southern clade began moving to the southeast at least 1.20 Mya. Since at least 0.90 Mya, soon after its divergence from the northern clade, the ancestors of the western clade moved slightly farther south. By contrast, the northern clade appeared to have a much more dynamic geographic history in the northern half of Madagascar, with northward movement toward the tip of the island in the ancestor of *U. ankaranensis* + *U. peltata* and southern movement into west-central Madagascar in the ancestor of *U. leptocarpa* + *U. perrieri*.

Trait evolution

Trichome morphology

Adaxial and abaxial leaf surfaces varied greatly among species in terms of trichome type, distribution, and density. Representative SEM micrographs for all species are depicted in Appendix S9. Both members of the pink clade, *U. abbreviata* and *U. stellulifera*, bore stellate mucilage glands on both leaf surfaces, with the dense covering of hairs on the abaxial surfaces giving a white appearance. By contrast, species of the southern clade varied greatly in indument type, from a dense indument of strictly glandular hairs on the abaxial surface of *U. decaryi*, to a combination of glandular and simple trichomes on the abaxial surfaces of *U. grandidieri* and *U. turicana*, with simple trichomes restricted to the veins in the latter, to long-stalked mucilage glands in *U. roeoesliana*. Within the western clade (*U. leandrii*, *U. platycarpa*, and *U. sakalava*), species all had a dense concentration of mucilage glands on the abaxial veins, with long, simple hairs of the adaxial surfaces of both *U. leandrii* and *U. platycarpa*. By contrast, *U. sakalava* (sample "U23") bore dense glandular trichomes on the adaxial leaf surface. Finally, species of the northern clade showed various reductions in trichome glandularity and density. Although the abaxial surface of all species other than *U. ihlenfeldtiana* showed scattered glandular trichomes, the adaxial surfaces were either sparsely glandular (*U. leptocarpa*, *U. perrieri*), or covered in hairs which are either simple (*U. ihlenfeldtiana*) or bore a one-celled glandular head (*U. ankaranensis*, *U. peltata*).

Leaf shape

We obtained 183 leaf outlines (171 *Uncarina*, 12 *Rogeria*; mean = 10.17 images/species ± 7.53 SD). The eFa and subsequent PCA suggested that 38.2% of the variation in leaf shape was captured in the first principal component (PC), which captured leaf lobing from deeply trifid (low scores) to unlobed (high scores) (Figure 4). In the other components that captured >5% of the variation, PC2 captured 29.4% and described relative leaf length from broad lateral lobes similar in shape to the terminal

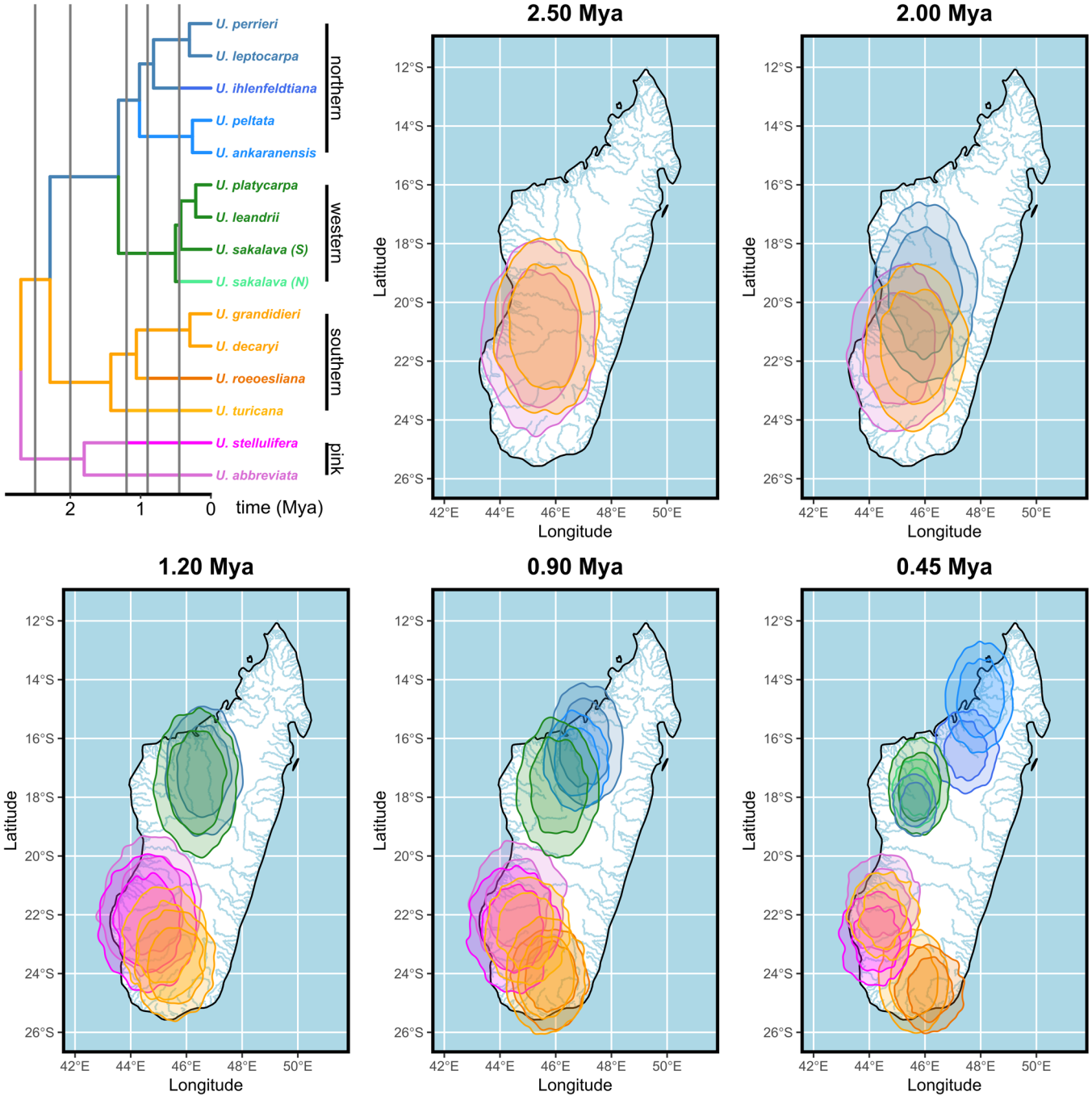

FIGURE 3 Quantitative historical biogeography of *Uncarina*. The chronogram of *Uncarina* at the top left was sectioned at five time periods to reconstruct ancestral centroids for major stem lineages. Contours represent the 50% and 95% confidence intervals for ancestral centroid reconstructions and are shaded by stem lineage.

lobe (low scores) to a narrow terminal lobe with short lateral lobing (high scores). PC3 captured 9.7% and described the amount of lobing from deeply 5-lobed (low scores) to deeply trilobed (high scores). Finally, PC4 captured 7.6% and described the position of deep lobing from the distal portion of the leaf (low scores) to the proximal portion (high scores) (Appendix S10).

Apart from the southern clade, all other major clades of *Uncarina* were well structured in morphospace, with both species in the pink clade bearing similarly shaped, unlobed, ovate leaves, the northern clade bearing unlobed or shallowly trilobed, obovate leaves (except for some deeply lobed leaves of *U. ihlenfeldtiana*), and the western clade consistently bearing deeply lobed leaves with well-developed lateral lobes. Leaves of most *Rogeria* were ovate and unlobed or shallowly lobed, with only Namibian *R. bigibbosa* Engl. bearing deeply lobed leaves. The southern clade of *Uncarina* encompassed nearly the entire range of leaf morphospace,

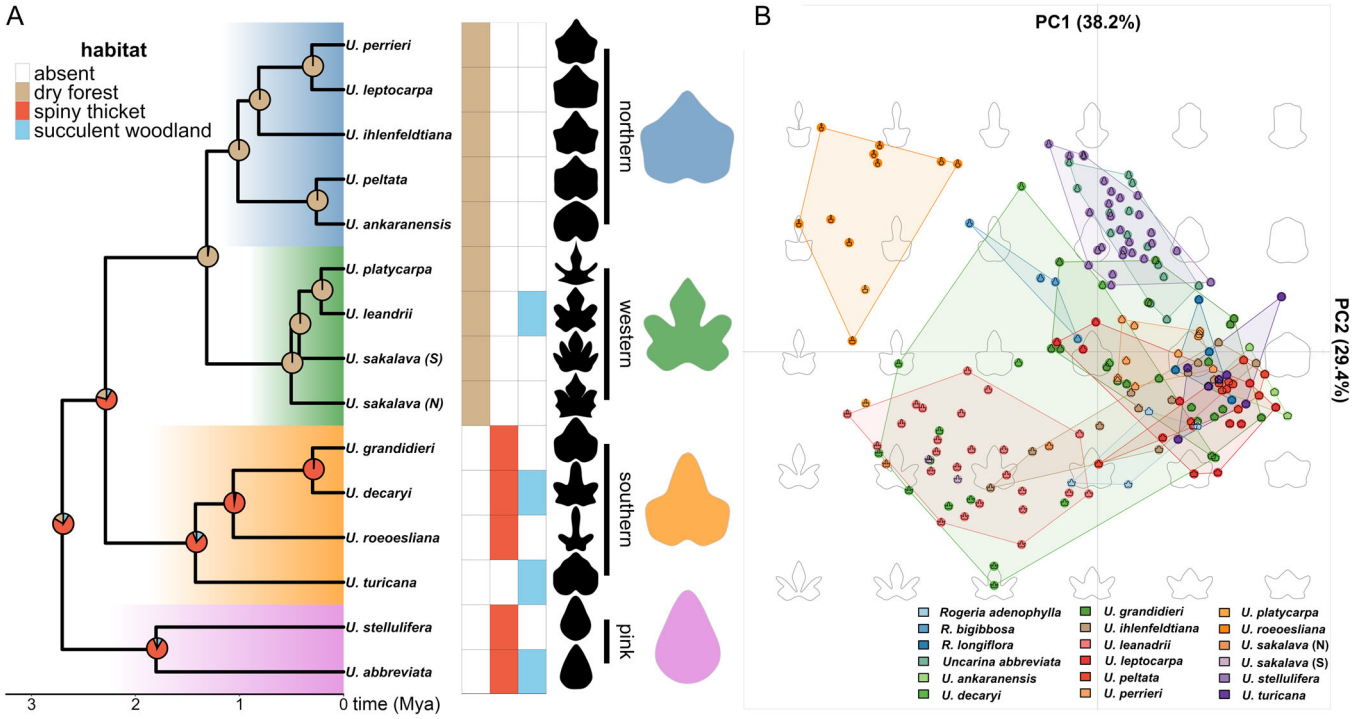

FIGURE 4 Geometric morphometrics and evolution of leaf shape in *Uncarina*. (A) Ancestral state reconstruction of habitat type and the evolution of leaf shape. Colored tip boxes depict the presence or absence of each species in one of three dryland biome types; node pies show the probabilities of ancestors occupying those habitats. Black leaf outlines to the right of tip labels show mean leaf shape for each species; colored shapes to the right of clade labels show mean leaf shape for each clade. (B) Ordination of leaf shapes for *Rogeria* and *Uncarina*. Points represent individual leaf outlines, with point colors and convex hulls delimiting species. Background shapes show theoretical leaf shapes in the morphospace; shapes for points show the actual shape of each sample.

with *U. roeoesliana* occupying the unique area of morphospace described by leaves with narrow terminal lobes and well-developed lateral lobes. *Uncarina decaryi* occupied a remarkably wide range of leaf morphospace, containing samples of both deeply and shallowly lobed leaves.

Ancestral habitats

The ER model was the best fit for the data. *Uncarina* arose within the spiny thicket biome, with a major shift to the dry forest within the MRCA of the northern and western clades (Figure 4). There were four independent shifts to the succulent woodland biome: three times from the spiny thicket biome and once from the dry forest biome (*U. leandrii*).

DISCUSSION

Nuclear genes provide a well-supported phylogenetic hypothesis

Because prior studies only included one or two samples of *Uncarina*, it was unclear whether the genus was monophyletic (Refulio-Rodriguez and Olmstead, 2014; Gormley et al., 2015; Rose et al., 2022; Zhigila and Muasya, 2023). By combining off-target reads with available data from Sanger sequencing, we demonstrated that the genus is indeed monophyletic. *Uncarina* is supported as morphologically distinct in Pedaliaceae by the presence of flowers borne in unreduced cymes (as in *Rogeria*), absence of extrafloral nectaries, fruits dehiscing both loculicidally and septicidally, and an anther "block" formed by the stamen filaments, which bees must penetrate with their proboscis to access nectar (Ihlenfeldt, 2010).

Regarding relationships within *Uncarina*, our nuclear data provided a largely well-supported phylogenetic hypothesis for *Uncarina* that appeared generally robust across methodologies, although some nodes were not robustly supported by the underlying CFs (Figure 2).

Major clades were geographically and morphologically cohesive, with both members of the pink clade united by their corolla color, mucilage glands with a stellate head, entire or very shallowly lobed, triangular to ovate leaves, and the occurrence of false septa in the fruits.

The placement of *U. turicana* as sister to the southern clade was consistent with its range in southwestern Madagascar, peripatric to the other species in the clade (Figure 1), as well as its morphology where it is florally like *U. decaryi* but vegetatively like *U. grandidieri*. Whether these trait similarities are a result of symplesiomorphies or trait flow associated with the detected reticulation event is uncertain and may be difficult to distinguish, given its phylogenetic position. The three remaining species of the southern clade

(*U. decaryi*, *U. grandidieri*, *U. roeoesliana*) have long been hypothesized to be closely related. All have glandular hairs with heads that are slightly stellate and fruits that are extremely similar, so much so that Ihlenfeldt (2002) claimed they cannot be told apart without information on leaf morphology or habit. *Uncarina decaryi* is highly variable in leaf shape, varying from slightly to moderately lobed, trilobate, or nearly digitate with five large, obtuse lobes (Figure 4). Given our sampling of only a single exemplar of this species, population-level sampling of *U. decaryi* and its sister species, *U. grandidieri*, is warranted to better understand any genetic or geographic signal to variation in leaf shape and/or if *U. decaryi* is too broadly circumscribed.

The northern clade included five species that are primarily distributed in northern and northwestern Madagascar (Figure 1) and sister species are morphologically cohesive. *Uncarina ankaranensis* and *U. peltata* are similar in seed structure and upward curvature of the corolla tube, while the sister species *U. leptocarpa* and *U. perrieri* have been hypothesized to be closely related based on the presence of pentagonal leaves with dark green adaxial surfaces, sparse glandular trichomes on both surfaces, and prominent white to reddish veins (Ihlenfeldt, 2002).

Relationships among species within the western clade were particularly difficult to resolve (Figure 2), yet the difficulty in resolving their relationships does not appear to be due to hybridization or introgression. Rather, cladogenesis within the western clade seems so rapid and recent that this data set may not contain enough phylogenetic information about the branching order in that part of the tree.

When more than one accession was available, we recovered strong support for the monophyly of each species, except for *U. sakalava* and possibly also *U. leandrii*. Although we lacked population-level sampling, each lineage of *U. sakalava* seems to correspond to the two areas of its disjunct distribution: in northwestern Madagascar and farther south, near Bekopaka and Ambinda in Melaky Region (Figure 1F). Non-monophyly of *U. sakalava* was not surprising, as its taxonomic circumscription has long been problematic. In his description of the species, Humbert (1962) expressed concerns that one of his paratypes (*Perrier 15,083* [P]), might represent a different species. It was later described as *U. ihlenfeldtiana* (Lavranos, 2004). Although we did not thoroughly examine material of *U. sakalava*, the leaves of sample U22, from the southern portion of the range of *U. sakalava*, ~105 km south of where the holotype was collected, matched the protologue better than the northern accession of *U. sakalava* (sample U23), collected near a paratype (*Perrier 8,456* [P]; Humbert [1962]). Sample U22 had leaves with adaxial surfaces covered with mucilage glands and numerous simple hairs and abaxial surfaces had a sparser indumentum with mucilage glands borne primarily along the veins (Appendix S9), while sample U23 had leaves with an adaxial indumentum almost exclusively of mucilage glands and an abaxial surface densely covered in mucilage glands, rather than confined to the veins (see Figure 2C of Lavranos [2004]). Whether sample U23 is the same taxon as *Perrier 8,456* is unknown because that specimen consists of just a leaf and fruit. However, Humbert's (1962) concept of the species was certainly too broad since it corresponds to at least three lineages (*U. ihlenfeldtiana* and two *U. sakalava*). The non-monophyly and disjunct geographic pattern of *U. sakalava* mirrors that of a Malagasy baobab species, *Adansonia za* Baill. (Karimi et al., 2020). More collections and population-level genetic sampling of *U. sakalava* are needed to better clarify the taxonomy.

The diversification of *Uncarina* tracks riverine corridors

Given the extant distribution of its closest relatives, the MRCA of *Uncarina* arrived from southern Africa (Zaborsky, 2018; Zhigila and Muasya, 2023) sometime after 9.32 Mya. This relatively recent stem age, as well as a much more recent crown age of *Uncarina* at ~2.64 Mya, clearly places the genus as one of the many neo-endemic, dry-adapted plant lineages in Madagascar (Yoder and Nowak, 2006; Janssen et al., 2008; Buerki et al., 2013; Hackel et al., 2018; Antonelli et al., 2023; Omollo et al., 2024). The Late Miocene or Early Pleistocene origin of the genus and Pleistocene diversification of its major clades coincides with a global occurrence of declining temperatures, increasing rainfall seasonality, and the expansion of C_4 grasslands, suggesting that, as with other arid-adapted (and succulent) Malagasy lineages, a drying climate facilitated the diversification of *Uncarina* (Zachos et al., 2001; Bobe, 2006; Edwards et al., 2010; Arakaki et al., 2011; Bellstedt et al., 2012; Buerki et al., 2013; Bissinger et al., 2014; Hackel et al., 2018; Antonelli et al., 2023; Tiley et al., 2023).

The Pleistocene spread of *Uncarina* out of spiny thickets or succulent woodlands in the southwestern part of the island, with subsequent movement into the dry deciduous forests present in the western and northern portions of the island, would have been possible since all major present-day biome types on the island were already established (Buerki et al., 2013). While our biogeographic reconstruction was uncertain as to whether this movement occurred along the western coast of the island or through the Central Highlands (Figure 3), the former may be more plausible, given that lemurs, the possible dispersers of *Uncarina* fruits, are absent from the Central Highlands. However, the distribution of vegetation types has likely been dynamic through time (Burney, 1993; Virah-Sawmy et al., 2010). Even if movement occurred through the Central Highlands, the clear north–south structuring of *Uncarina* clades nevertheless strongly suggests that one or more processes acting along this geographic axis, in the form of either refugia or dispersal barriers, have long shaped the history of the genus, especially in the northern and western clades.

Indeed, the deep split between the southern and northern + western clade closely matches the location of the present-day Tsiribihina River, while the region currently

bisected by the Betsiboka River matches closely with the geographic split between the northern and western clades (Figure 3). In the western case, if the river acts as a barrier, it is clearly not an impenetrable one because the northern clade subsequently dispersed southward as indicated by the inferred range of the MRCA of *U. leptocarpa* and *U. perrieri*. In contrast to the wide-ranging species in southern and western Madagascar, species of *Uncarina* in the northern part of the range of the genus are generally range-restricted endemics. Reproductive isolation arising from specialization on soil types or habitat destruction have been hypothesized to be drivers of high endemism in *Uncarina* in northern Madagascar (Ihlenfeldt, 2002, 2010), yet human-induced habitat destruction does not seem to be a plausible driver considering that the estimated divergence times of species distributed in this part of the island are on the scale of tens to hundreds of thousand years ago. We expect that this strong lineage structuring or speciation with limited morphological divergence is also present within widespread northern species, such as *U. peltata* (Figure 1), especially given that the most recently described species have come from northern populations (Lavranos, 2004; Ihlenfeldt, 2004b). Several other plant groups share similar patterns with species divergence appearing to correspond to large riverine systems (Andriamihaja et al., 2021; Hanes et al., 2022; Karimi et al. 2022; Liu et al., 2024).

Strong latitudinal structuring concordant with one or both major riverine breakpoints and fine-scale lineage partitioning in the north are also found in animal lineages, including lemurs (e.g., Pastorini et al., 2003; Shi et al., 2013; Pabijan et al., 2015; Lei et al., 2017). This pattern emerges both within a species and within clades that have diversified at the same time scale as *Uncarina*, suggesting that whatever process(es) has generated this latitudinal structuring has been operative for longer than the Last Glacial Maximum. However, the generative processes are unclear, although geophysical barriers or filtering across ecological gradients seem to be plausible explanations, and present-day patterns may not be able to discriminate among competing hypotheses (Pearson and Raxworthy, 2009; Brown et al, 2014). As we hypothesized, the concordant geographic patterns between *Uncarina* and animal lineages (especially lemurs) may be at least partially explained by epizoochory in *Uncarina*. Future studies should focus on plant lineages with different means of dispersal to determine whether this concordance between the rages of epizoochorous lineages and their dispersers is a general pattern in Madagascar's dryland biota or restricted to animals and animal-dispersed plants, but biogeographic patterns may be predominately lineage-specific (Brown et al., 2014).

Diversification of leaf traits

Trichome types and leaf shape have been used as diagnostic traits in *Uncarina* (Humbert, 1971; Ihlenfeldt, 2010). Indeed, similarities in trichome type and leaf shape define both major clades and are found in sister species. Based on the distribution of leaf traits among species, there were trends not only toward reduced density of glandular trichomes, but also from unlobed leaves to deeply lobed leaves. Deeply lobed leaves likely evolved three times (once in the western clade and twice in the southern clade). Their evolution seems unlinked to broad-scale habitat shifts because lobed leaves evolved well after the genus entered dry forests (western clade) and spiny thickets (southern clade) but may be a response to more fine-scale ecological gradients. Within the western clade, the evolution of leaf lobing appeared correlated with the evolution of abaxial mucilage glands restricted to the veins. These observations raise the general question of the possible adaptive significance of mucilage glands and/or lobed leaves.

Despite being energetically costly to produce (e.g., Hare et al., 2003), glandular trichomes may provide not only a functional role in deterring herbivores (Hanley et al., 2007), but trichomes in general may aid in thermoregulation or scatter ultraviolet light (Ehleringer and Mooney, 1978; Karabourniotis et al., 1995). Leaf trichomes were particularly dense in *Uncarina* in spiny thickets (Figure 4; Appendix 9), where solar irradiance is high. Moreover, flavonoid diversity is lower in the leaves of members of the northern and western clades compared to the pink and southern clades (Yamazaki et al., 2007). Trends in both trichome density and flavonoid composition are consistent with competing hypotheses of an adaptation to either mitigate the effects of photodamage or discourage herbivory. The herbivory hypothesis may be supported if the primary herbivores of *Uncarina* were absent or reduced in the northern part of Madagascar, where release from herbivores should select against production of costly trichomes. Candidates include extinct elephant birds (Grubb, 2003; Bond and Silander, 2007; Midgley and Illing, 2009; Hansford and Turvey, 2018, 2022).

Leaf lobing may have evolved as an adaptation to either herbivory or thermoregulation. Highly divided leaves may be better able to thermoregulate due to a thinner boundary layer (Givnish, 1979; Nicotra et al., 2011; Leigh et al., 2017). The small segments of highly divided leaves may also be advantageous in areas with high rates of herbivory because leaf segments reduce feeding efficiency, forming more-or-less "disposable" units that can be plucked by herbivores while minimizing energetic impacts (Brown and Lawton, 1991; Bond and Silander, 2007).

The independent origins of leaf lobing within *Uncarina* may serve either of the hypothesized functions, depending on the clade. Members of the southern clade already have leaves densely covered in trichomes. The relatively short lateral lobes of *U. roeoesliana* gives a linear appearance to the overall leaf shape (Figure 4). *Uncarina roeoesliana* is unique in the genus in occurring on compacted sand dunes and producing a large underground tuber. This divergent habitat preference suggests that this leaf shape has evolved to aid in thermoregulation in an exceptionally harsh environment.

As mentioned above, *U. decaryi* showed extreme variability in leaf shape. Our limited population-level sampling of *U. decaryi* makes it difficult to infer any geographic patterns to leaf lobing. However, specimens of plants with less deeply lobed leaves tend to be collected early in the growing season, while specimens with more deeply lobed leaves are from later in the growing season (e.g., *Seyrig 519* [P]), although the most deeply lobed leaves may come from the southern parts of the range of the species (e.g., *Du Puy MB472* [P]). The ontogeny of leaves, however, suggests a thermoregulatory function to lobing within *U. decaryi*.

Finally, the origin of deeply lobed leaves within the western clade, after a transition to the more mesic dry forest, seems unsurprising given its history of southward geographic movement. Leaf lobing in this clade could plausibly represent an adaptation to better thermoregulate in drier conditions after trichome density had been reduced. Alternately, they could represent an adaptation to herbivory following secondary contact at the northern part of the range of elephant birds. In all cases, thermoregulatory hypotheses can theoretically be tested in future experimental studies, while herbivory hypotheses, as with other evolutionary anachronisms, are difficult to test.

CONCLUSIONS

Given the wide geographic range of *Uncarina* within western Madagascar, the genus provides an interesting case study to understand not only the historical biogeography of lineages within the island, but also the evolution of traits following shifts among habitat types. Because the biology of *Uncarina* is so closely tied to animals, not only does its historical biogeography mirror that of animals, but the evolution of leaf traits may capture the historical distribution of herbivores.

Because such comparative studies require a good understanding of the lineages involved, this study highlights some problems plaguing the study of Malagasy plants. The often fragmentary and poorly preserved nature of *Uncarina* specimens has clearly resulted in underestimating the number of lineages in the genus and exacerbated uncertainty in the range of morphological variation within species. Thorough collecting and increased sampling of populations will provide us with a better understanding of the relationships and diversity within the genus. Careful and intensive study of other genera endemic to Madagascar is imperative considering the extent to which the island is undergoing deforestation and other kinds of habitat destruction.

AUTHOR CONTRIBUTIONS

J.G.Z. and K.J.S. conceived and undertook the project; J.G.Z. provided material support and extracted DNA; N.K. and J.P.R. analyzed data; N.K., J.P.R., and J.G.Z. wrote the original draft of the manuscript with writing contributions from K.J.S.

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DATA AVAILABILITY STATEMENT

Nucleotide alignments, phylogenetic trees, leaf images, and R scripts necessary to reproduce the analyses in this paper are deposited on GitHub: https://github.com/jrosaceae/Uncarina. Raw reads have been deposited in the NCBI Sequence Read Archive (SRA) as BioProject PRJNA1214278.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Voucher information for samples of Pedaliaceae newly sequenced in this study.

Appendix S2. Sample information for Pedaliaceae and outgroups used to construct the supermatrix in the Pedaliaceae-wide analysis.

Appendix S3. Maximum likelihood phylogenetic tree of Pedaliaceae based on the concatenated analysis of eight markers.

Appendix S4. Primary concordance tree from the BUCKy analysis. Internal branch lengths are in coalescent units.

Appendix S5. Plot of log-pseudolikelihood versus maximum number of reticulation events.

Appendix S6. Best-fitting phylogenetic network for hmax = 1, rooted on *Pterodiscus*.

Appendix S7. Best-fitting phylogenetic network for hmax = 2, rooted on *Pterodiscus*.

Appendix S8. Best-fitting phylogenetic network for hmax = 3.

Appendix S9. SEM micrographs of trichomes on the abaxial and adaxial leaf surfaces of selected species of *Uncarina*.

Appendix S10. Variation in leaf shape across each PC axis explaining >5% of the variation in leaf shape including the mean ± up to two SDs.

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