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RESEARCH ARTICLE

Phylogenomic evidence reveals non-monophyly of Paepalanthoideae (Eriocaulaceae) and challenges the broad concept of *Paepalanthus*

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Abstract Eriocaulaceae is a pantropical family of approximately 1200 species across 18 genera, with its highest diversity in the Neotropical region. Traditionally, the family has been divided into two subfamilies, Eriocauloideae and Paepalanthoideae, based on floral traits. Here, we use target-capture sequencing of nuclear markers to test the monophyly of these subfamilies. Using the Angiosperms353 probe set, we analyzed data from 30 species, generating new sequences for 24 species, representing eight genera of Eriocaulaceae. Phylogenetic analyses using ASTRAL-III and a concatenated maximum likelihood approach recovered three major clades, revealing that Paepalanthoideae is non-monophyletic. *Monosperma*, a Pantepui-endemic lineage, recently elevated to generic status, emerged as the sister group to the rest of the family. Our findings suggest that the ancestral Eriocaulaceae flower had a single-whorled androecium, contrary to previous hypotheses. Additionally, this study highlights the non-monophyletic nature of the broad circumscription of *Paepalanthus*. These results challenge long-held assumptions about the evolutionary relationships within Eriocaulaceae, providing critical insight into its subfamilial and generic classification, and call for further research into the evolutionary history of the family.

Keywords Angiosperms353; floral evolution; *Monosperma*; Neotropics; phylogenomics; subfamily classification

Supporting Information may be found online in the Supporting Information section at the end of the article.

INTRODUCTION

Eriocaulaceae Martinov is a family of monocots comprising about 1200 species and 18 genera (Andrino & al., 2023), most of them more abundant in the Neotropical region, with *Eriocaulon* L. distributed pantropically (Li & al., 2023). The family has traditionally been divided into two tribes (Körnicke, 1854): Eriocauleae, characterized by diplostemonous flowers (i.e., two-whorled androecium), gynoecium with dorsal stigmas, glandular petals and many aquatic species; and Paepalantheae, characterized by isostemonous flowers (single-whorled androecium), gynoecium with dorsal nectariferous branches and commissural stigmatic branches, nonglandular petals, and mostly xerophytic species. Following the same classification proposed by Körnicke (1854), but treating them as subfamilies rather than tribes, Ruhland

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(1903) proposed the two subfamilies still currently accepted: Eriocauloideae, which includes *Eriocaulon* and the African endemic genus *Mesanthemum* Körn., and Paepalanthoideae, which encompasses 16 genera and over 800 species, mostly restricted to the Neotropical region. These subfamilies have been widely accepted in the systematic history of the family.

Stützel (1985) proposed a third subfamily, Syngonanthoideae, including *Mesanthemum* alongside *Philodice* Mart. (now a synonym of *Syngonanthus*), *Syngonanthus* Ruhland, and *Wurdackia* Moldenke (now recognized as *Rondonanthus flabelliformis* (Moldenke) Hensold & Giul.). This subfamily was proposed based on the presence of a fusioned syngonanthoid corolla observed in the pistillate flowers of these genera. Stützel (1985) also suggested that Eriocauloideae should solely comprise *Eriocaulon*, with the other two subfamilies, Syngonanthoideae and Paepalanthoideae, evolving independently from it. However, these hypotheses—both the creation of Syngonanthoideae and the independent evolution of the subfamilies—were met with skepticism. Early phylogenetic studies, such as Andrade & al. (2010), demonstrated that Syngonanthoideae was not monophyletic, leading to the rejection of Stützel's classification due to insufficient phylogenetic support. On the other hand, the monophyly of subfamilies Eriocauloideae and Paepalanthoideae has not been rigorously tested. Phylogenetic evidence available thus far, composed mostly of few plastid regions, supports the subfamilies as monophyletic (Andrade & al., 2010; Larridon & al., 2019; Liang & al., 2019; Andrino & al., 2023; Li & al., 2023).

Prior to the latest classification of the family (Andrino & al., 2023), all studies followed the taxonomic framework established by Ruhland (1903), with genera primarily distinguished by floral characters. Among these characters, the most distinguishing feature was the differentiation between diplostemonous and isostemonous flowers, as well as glandular and non-glandular petals, which were used to support the distinction between subfamilies Eriocauloideae and Paepalanthoideae (Körnicke, 1854; Ruhland, 1903). Another frequently cited distinction relates to the gynoecium structure: in Eriocauloideae, stigmas are described as dorsally positioned and associated with the dorsal carpellary bundle, while in Paepalanthoideae, the stigmatic branches are in a commissural position, arising from the elevation and fusion of adjacent carpel margins (Stützel, 1990; Rosa & Scatena, 2007).

In light of phylogenetic evidence, proposals to revise the generic classification of the Eriocaulaceae have been published. *Comanthera* L.B.Sm., previously considered synonymous with *Syngonanthus*, was re-established (Parra & al., 2010). *Blastocaulon* Ruhland was synonymized with *Paepalanthus* Mart. (Andrade & al., 2011), and *Philodice* with *Syngonanthus* (Giulietti & al., 2009). Notable revisions to the classification, particularly within Paepalanthoideae, have arisen due to the non-monophyletic and non-diagnosable nature of the genus *Paepalanthus*, as no formal proposal addressing its circumscription had been made until recently (Mabberley, 2017; Andrino & al., 2023). *Paepalanthus* has been the most significant taxonomic challenge in the family for decades: a highly heterogeneous, non-monophyletic, speciose group that has only very recently been extensively taxonomically sampled in phylogenies (Andrino & al., 2021).

In light of the results of the first phylogenetic studies by Andrade & al. (2010), Mabberley (2017) and Christenhusz & al. (2018) proposed the synonymization of three genera, *Tonina* Aubl., *Lachnocaulon* Kunth, and *Actinocephalus* (Körn.) Sano, with *Paepalanthus*. This proposition was made despite *Tonina* having priority under the *International Code of Nomenclature for algae, fungi, and plants* (*Shenzhen Code*, *ICN*; Turland & al., 2018), and was provided without a diagnosis for this *Paepalanthus* sensu lato concept. This synonymization created a sizable genus with over 500 species, without these authors providing morphological characters to support its recognition. Andrino & al. (2023) proposed a new classification for Eriocaulaceae, with a particular focus on the clade comprising *Paepalanthus* and its allied genera. Their classification prioritized the morphological diagnosability of internal clades with strong support, the geographic alignment of lineages, and the incorporation of expanded molecular, taxonomic, and geographic sampling, thereby enhancing the robustness of the dataset. Given the fluctuating support in the backbone of phylogenetic trees and the varying positions of certain groups depending on the dataset analyzed, taxonomic decisions had to be made based on well-supported shallower clades (Andrino & al., 2021, 2023). Simultaneously, phylogenies investigating Eriocauloideae had concentrated on this subfamily with few Paepalanthoideae included. Eriocauloideae always emerged as a cohesive group, but its relationships with other genera of the family have never been thoroughly investigated (Andrade & al., 2010; Li & al., 2023).

Incongruences in the backbone of phylogenetic trees of Neotropical Eriocaulaceae have consistently been associated with groups occurring in the Amazon, Pantepui, or Central America (Trovó & al., 2013; Andrino & al., 2021). This inconsistency is particularly observed in species-rich or endemic groups from these regions, previously classified within *Paepalanthus* but now placed in the genus *Monosperma* (Hensold) Andrino (a Pantepui-endemic genus), *Giuliettia* Andrino (a genus rich in species in the Amazonian lowlands and Central America) and the limited samples of *Rondonanthus* Herzog. *Rondonanthus* has emerged at different positions in various analyses, always with limited support. *Monosperma*, with three species sampled in Trovó & al. (2013), emerged as sister to *Paepalanthus* s.str., and with a larger sample of 14 species in Andrino & al. (2021), as sister to the entire core Neotropical clade, which includes *Paepalanthus*, *Actinocephalus*, *Lachnocaulon* and *Tonina*, but with low support in all analyses. *Giuliettia*, sampled extensively only in Andrino & al. (2023), emerged at different positions in different analyses (Vasconcelos & al., 2020). While these groups are strongly supported phylogenetically, the relationships between them and the other genera of the family remain unresolved due to limited taxonomic sampling and phylogenetic uncertainty. This has impeded conclusive inferences regarding their placement within the family's phylogeny, which in turn affects studies on systematics, biogeography, and morphological evolution of the family.

During ongoing studies on endemic angiosperm lineages of the Pantepui region, crucial samples of endemic Eriocaulaceae were obtained. New phylogenetic hypotheses presented here, enabled by target capture sequencing focusing on understudied taxa, challenge our understanding of the family's evolution. These observations also challenge the subfamilial classification of Eriocaulaceae, revealing new perspectives on the position of *Monosperma* within the family, the monophyly of its subfamilies, and the validity of the historical classification approach of *Paepalanthus* s.l. (Mabberley, 2017; Christenhusz & al., 2018; Stützel & al., 2024). The aim of this study is to provide new phylogenomic evidence to evaluate the monophyly of the subfamilies of Eriocaulaceae and clarify classification decisions within the family, using high-throughput DNA targeted sequence capture with the universal Angiosperms353 probe set for the first time in the family.

■ MATERIALS AND METHODS

Taxon sampling. — The sampling encompassed 30 species of Eriocaulaceae, with 15 belonging to subfamily Eriocauloideae, specifically from the genus *Eriocaulon*. *Mesanthemum* was not included in the sampling. The remaining 15 species belong to Paepalanthoideae, with a primary focus on the Pantepui-endemic genus *Monosperma*, comprising eight sampled species. The total sampled species represent eight genera of the family. Additionally, six ingroup samples were sourced from the NCBI Sequence Read Archive, originally generated by the Kew Plant and Fungal Trees of Life Project (https://treeoflife.kew.org/): *Syngonanthus oblongus* (Körn.) Ruhland (ERX10415003), *Comanthera paepalophylla* (Silveira) L.R.Parra & Giul. (ERX10415000), *Rondonanthus capillaceus* (Klotzsch ex Körn.) Hensold & Giul. (ERX10415002), *Lachnocaulon anceps* (Walter) Morong (SRX1639018), *Paepalanthus falcatus* (published as *P. pedunculatus* Ruhland, nom. illeg. [Andrino & al., 2022]; ERX10415002), and *Eriocaulon petraeum* S.M.Phillips & Burgt (ERX4143627). Outgroup taxa were obtained from NCBI SRA and included Rapateaceae (*Monotrema* Körn. ERX10415009 and *Cephalostemon* R.H.Schomb. ERX10415006), Xyridaceae (*Xyris* Gronov. ex L.; ERX4819253), and Mayacaceae (*Mayaca* Aubl.; ERX4143474). Voucher information can be found in Appendix 1.

DNA extraction and library preparation. — Two methods of DNA extraction and library preparation were used here. For *Monosperma* and *Giuliettia* species, DNA extractions were carried out using modified protocols from Neubig & al. (2014). The genomic DNA (gDNA) underwent visual inspection for color and viscosity, and its total quantity was assessed using a spectrofluorimetric assay with Qubit (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts, U.S.A.) with High Sensitivity dsDNA Quantitation Kit. Samples were then analyzed using the TapeStation 4200 (Agilent, Santa Clara, California, U.S.A.) platform with a High Sensitivity D1000 tape. For samples exhibiting predominantly high molecular weight morphology, up to 80% of the gDNA was purified using a bead-based method, followed by fragmentation using an enzymatic approach. In cases of mixed morphology or inconclusive TapeStation profiles, up to 80% of available gDNA was sonicated. Samples primarily featuring low molecular weight morphology underwent no fragmentation. Subsequently, the samples were processed into dual-indexed Illumina-compatible libraries, targeting an average insert size of approximately 500 nt whenever feasible. Quantification of the indexed libraries was conducted using a spectrofluorimetric assay. Capture pools were then prepared, with each pool comprising up to 500 ng of four libraries per reaction. Each capture pool was reduced to 7 μl through vacuum centrifugation. Captures were carried out following the myBaits v.5.02 protocol utilizing the myBaits Expert Angiosperms353 probe set, including overnight hybridization and washes at 62°C. Post-capture, reactions were subjected to 12–20 cycles of amplification and quantified once more using a spectrofluorimetric assay. The captures were combined in approximately equimolar ratios. Finally, the samples were sequenced on the AVITI (Element Biosciences, San Diego, California, U.S.A.) platform using a partial high-throughput PE150 flow cell, generating a total of approximately 148 Gbp. Genomic library preparation, target enrichment, and sequencing were performed by Daicel Arbor Biosciences (Ann Arbor, Michigan, U.S.A.).

For *Eriocaulon* species and *Syngonanthus* sp., DNA isolation was performed (Jobson & al., 2017; Baleeiro, 2024) using the DNeasy Plant DNA Mini kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol. DNA extractions were sent to AGRF (The University of Queensland) for library preparation and sequencing. A probe capture set containing targets that overlap with the Angiosperms353 set was used for sequencing. The DNA libraries were prepared using the IDT xGen Prism DNA Library Prep kit. It includes an enzymatic fragmentation module, a high-efficiency ligase, and a PCR Master Mix that can generate library yields sufficient for hybridization-based enrichment or direct sequencing. Both kits have workflows compatible with high-throughput applications and have been automated on multiple liquid handlers. Finally, Custom Next Gen Sequencing Angiosperms 353 was performed in eight reactions (8 RXN Kit). A single Novaseq flow cell lane was used, and ran for 300 cycles to generate sequencing data.

Data cleaning and targeted loci assembly, filtering, and alignment. — The demultiplexed raw reads from sequencing were subjected to quality filtering and trimming using the default settings of the "clean" function in Captus v1.3.2 (Ortiz & al., 2023), where average PHRED quality score threshold of 13 for trailing reads and 16 for entire reads were applied. The "assemble" function was used to assemble paired reads into contigs. Then, the "extract" function was used to capture only the reference sequence set of nuclear proteins contained in the "Mega353" file (McLay & al., 2021) from the assemblies. The "align" function aligned the extracted loci using MAFFT's automatic selection based on the amount of data from complete gene sequences (exons + introns) with flanking upstream and downstream base pairs using the "GF" argument. Paralogs were filtered using "naive" option. All final alignments used in phylogenetic analyses are available at https://doi.org/10.5281/zenodo.15232458.

Phylogenetic reconstruction and divergence time. — Four phylogenetic trees were generated in this study. We generated a maximum likelihood (ML) tree derived from the analysis of the concatenated dataset, implemented in IQ-TREE v.2 (Minh & al., 2020). This analysis was conducted as a partitioned analysis, using different character substitution models for each alignment, which were calculated with ModelTest within the IQ-TREE search. The analysis was carried out with 1000 replicates of ultrafast bootstrap (Hoang & al., 2018) to assess branch support. A multispecies coalescent tree was generated using ASTRAL-III v.5.7.1 (Zhang & al., 2018). For this analysis, we selected the best-fitting model for each gene supercontig alignment using ModelFinder Plus v.1.5.4 (Kalyaanamoorthy & al., 2017) and conducted 1000 replicates of ultrafast bootstrap in IQ-TREE v.2 for each gene alignment, from which we inferred the individual gene trees. The 345 gene trees obtained from individual loci were then used to infer a species tree using ASTRAL-III. Support values were calculated as bootstrap values for the concatenated analysis and local posterior probabilities for the multispecies coalescent analysis. To test a potential impact of sampling a larger proportion of *Monosperma* and *Eriocaulon* species in relation to the rest of the family, we ran separate concatenated and coalescence analyses with a paired down alignment, containing one representative of each genus, following the same procedures above.

For time calibration, we applied secondary calibrations, matching the ages of major clades with dates recovered in a recent angiosperm-wide phylogenetic study based on the same probe set (Zuntini & al., 2024). We included four crown-node calibration points: root (125.1 Ma), Rapateaceae crown (76.4 Ma), Mayacaceae-Eriocaulaceae split (119.7 Ma), and core Eriocaulaceae crown (74.7 Ma). The backbone was time-calibrated using the penalized likelihood method implemented in TreePL v.1.0 (Smith & O'Meara, 2012), with a sequence length of 888,031. The full calibration file can be found at https://github.com/carol-siniscalchi/eriocs.

Ancestral state reconstruction. — Ancestral states for androecium morphology (single-whorled versus two-whorled) were estimated using a Markov model for marginal reconstruction. To ensure reliable parameter estimation and minimize the risk of local maxima, the fitting process employed 100 random starts with different initial parameters. We compared two models: one with equal transition rates between states (equal rates, ER) and another with varying rates between states (all rates different, ARD). Based on the best-fitting model, determined by Akaike's information criterion corrected for small sample sizes (AICc) and Akaike weights (AICw), the node probabilities were inferred and mapped throughout the tree. All analyses were performed using the corHMM v.2.8 (Beaulieu & al., 2017) and phytools v.2.4-4 packages (Revell, 2024) in the R statistical software (R Core Team, 2023).

■ RESULTS

For the new sequences generated in this study (i.e., excluding those obtained from SRA), we recovered on average 1,083,071 reads per accession (range: 40,278–41,015,127), with the percentage of on-target reads varying from 7.8% to 98.9%. Of the 353 genes targeted by the Angiosperms353 probe set, we retrieved on average 297.5 (range: 79–353). Both ASTRAL and concatenated analyses were performed on 345 gene alignments. All statistics concerning sequence recovery are presented in suppl. Table S1 (with data on vouchers and DNA accession numbers).

Eriocaulaceae backbone. — The phylogenetic analysis recovered Eriocaulaceae as a highly supported monophyletic group (Fig. 1). We recovered two major lineages in Eriocaulaceae, one composed of *Monosperma*, and the other of the remaining taxa (core Eriocaulaceae clade). This result was recovered with high support in both the concatenated and coalescent analyses (bootstrap support [BS] 100%, local posterior probability [LPP] 0.92). The second larger clade contains the majority of the family's diversity, including the pantropical genus *Eriocaulon*, recovered also with full support (BS 100, LPP 1) and sister to a clade containing all other Neotropical genera, with full support (BS 100%, LPP 1). This Neotropical clade is split into two subclades, one containing *Syngonanthus*, *Comanthera*, and *Rondonanthus* (BS 100%, LPP 1) and another with *Giuliettia*, *Lachnocaulon*, and *Paepalanthus* (BS 100%, LPP 1). *Paepalanthus* was recovered as sister to a *Giuliettia-Lachnocaulon* clade.

Timing of diversification. — The first split in Eriocaulaceae occurred in the Early Cretaceous, near the origin of the family, approximately 115 Ma (Fig. 2). The *Monosperma* crown clade experienced early diversification in the Early Miocene, ca. 17 Ma. The core Eriocaulaceae clade originated in the Late Cretaceous, around 75 Ma. This clade subsequently gave rise to the large genus *Eriocaulon* around the same time (stem node ca. 75 Ma), although the genus itself underwent more recent diversification in the Miocene (crown node ca. 32 Ma). The Neotropical clade sister to *Eriocaulon* shows earlier divergence, with a crown age of ca. 62 Ma, in the Paleocene.

Ancestral state reconstruction. — The equal rates (ER) model was the best-fitted model (suppl. Table S1, AICw = 0.75) and the one used for estimating node probabilities of androecium morphology throughout the tree. The single-whorled androecium morphology is ancestral in Eriocaulaceae (97% probability), with two-whorled androecium appearing in *Eriocaulon* (Figs. 3, 4). The two-whorled androecium flower morphology also appears in the outgroup.

■ DISCUSSION

Our study is the first to utilize high-throughput target capture sequencing in Eriocaulaceae. The topologies we found reject the monophyly of Paepalanthoideae, as well as the broad taxonomic circumscriptions of *Paepalanthus* (Mabberley, 2017; Christenhusz & al., 2018; Stützel & al., 2024). On the other hand, our analyses confirm the monophyly of *Monosperma* and for the first time reveal its position as sister to the rest of the family, suggesting a South American origin of Eriocaulaceae. Furthermore, our results indicate that the ancestral flower of Eriocaulaceae likely possessed a single whorl of stamens, and the presence of two whorls is a derived characteristic within the family (Fig. 2). Due to the absence of *Mesanthemum* in our sampling, the monophyly of Eriocauloideae could not be fully assessed. Nevertheless, our data support the monophyly of the genus *Eriocaulon*.

New perspectives on Eriocaulaceae. — With the exception of *Monosperma*, the positioning of most genera remains consistent with previous studies (Andrade & al., 2010; Giulietti & al., 2012; Andrino & al., 2021). *Comanthera* and *Syngonanthus* form a well-supported clade, as does the grouping

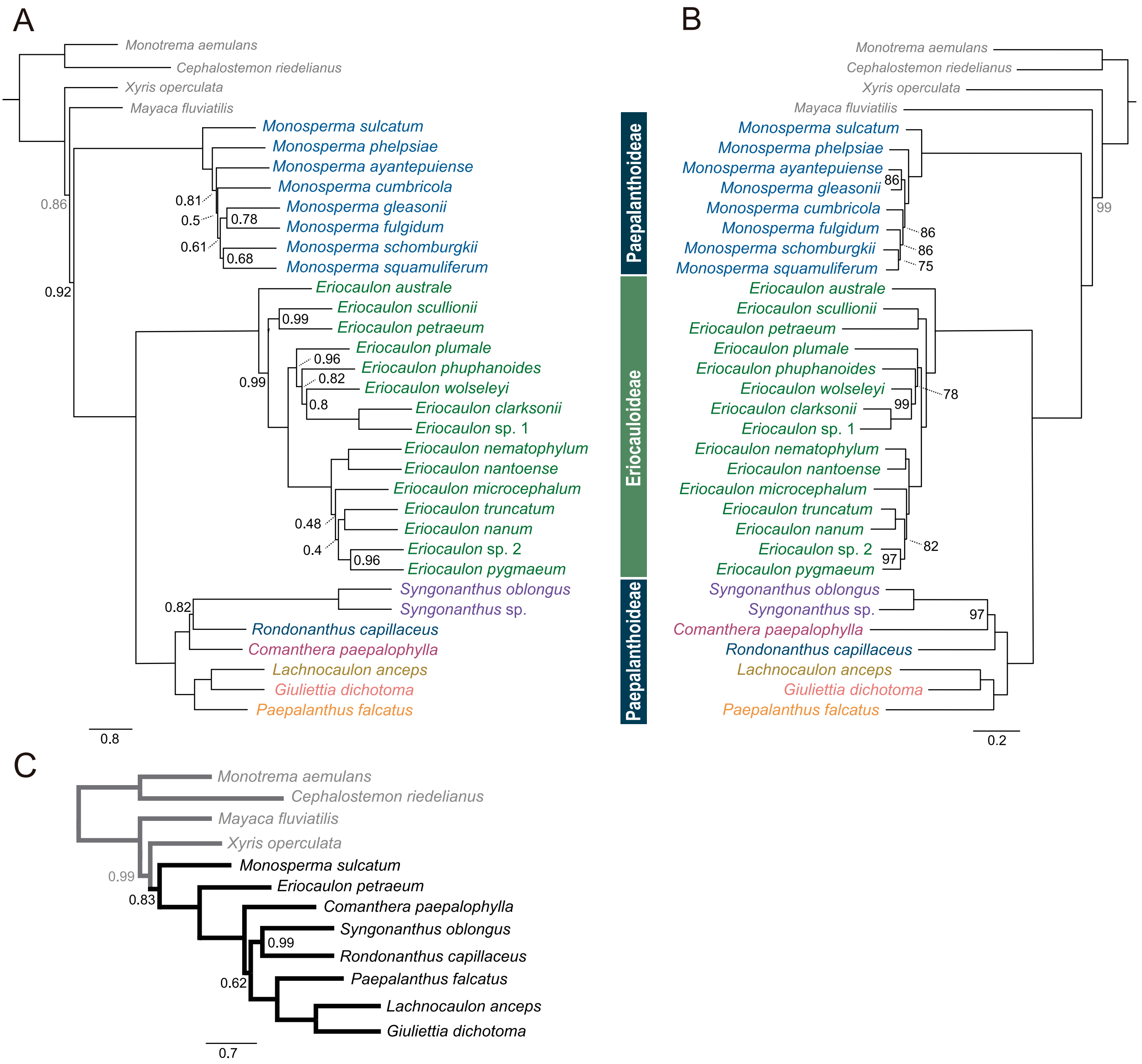

Fig. 1. Phylogenetic trees of Eriocaulaceae: **A**, Coalescent species tree based on ASTRAL analysis; **B**, Concatenated tree based on maximum likelihood (ML) analysis; **C**, Reduced tree with one representative species per genus. — The central bar represents the subfamilies (Paepalanthoideae, Eriocauloideae). Numbers at nodes represent branch support values: Bayesian posterior probability for A & C; bootstrap for B. Nodes without values indicate maximum support (BS/LPP = 100%/1, not shown).

of *Paepalanthus*, *Lachnocaulon*, and *Giuliettia*. The main novelty lies in *Giuliettia*'s shift in position, now sister to *Lachnocaulon*, contrasting with a previous analysis in which *Giuliettia* was placed as sister to *Paepalanthus* (Andrino & al., 2023). The placement of *Rondonanthus capillaceus* as sister to *Syngonanthus* and *Comanthera* differs from earlier findings (Andrade & al., 2010, Trovó & al., 2013; Andrino & al., 2023), in which it was recovered as sister to the entire subfamily Paepalanthoideae. However, its poor representation in phylogenetic analyses limits broader conclusions, and its uncertain placement underscores the need for increased sampling to clarify its evolutionary relationships.

Our study, although with a non-exhaustive sampling, was able to shed light on the generic relationships within Eriocaulaceae, revealing novel evidence that challenges previous knowledge. Notably, we identified the non-monophyly of subfamily Paepalanthoideae, a result that contradicts the longstanding infrafamilial classification of Eriocaulaceae, based on morphological evidence (Körnicke, 1854; Ruhland, 1903). This unexpected finding prompts a re-evaluation of the evolutionary history and taxonomic delineation within Eriocaulaceae, changing our previous understanding of the family's origins and morphological evolution.

Interestingly, phylogenetic studies on Eriocaulaceae have mostly avoided questioning the monophyly of the subfamilies, mostly due to a lack of comparable sampling of members of each subfamily in the same study. Phylogenies focusing on *Eriocaulon* have mostly included few outgroups from

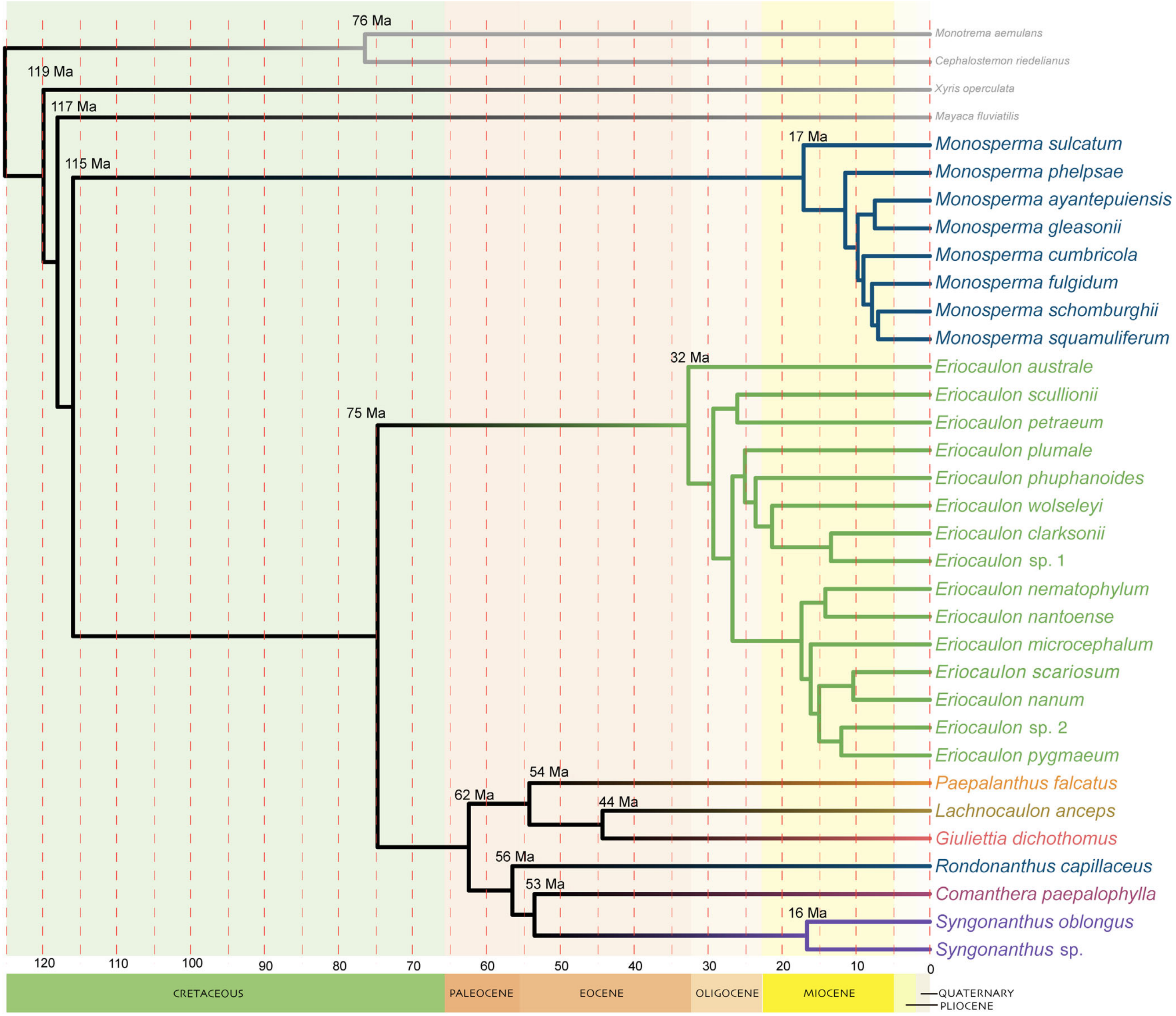

Fig. 2. Time-calibrated maximum likelihood phylogenetic tree inferred from the concatenated dataset of Eriocaulaceae. The numbers above nodes indicate the age of each major clade, in million years (Ma). The colors on the branches represent different genera within Eriocaulaceae, showing diversification over geological time, from the Cretaceous to the present. The timeline at the base of the figure highlights the geological eras and periods.

Paepalanthoideae (Larridon & al., 2019; Li & al., 2023), while studies focusing on Paepalanthoideae usually have sampled just a handful of Eriocauloideae (e.g., Andrade & al., 2010). The separation of subfamilies into two clades consistently emerges in these studies, despite the unbalanced sampling. Our study is the first to present a balanced sampling of both subfamilial lineages, considering that species of *Monosperma* were included under the assumption they were part of Paepalanthoideae; indeed, previous studies that sampled *Monosperma* recovered it within Paepalanthoideae (Trovó & al., 2013; Andrino & al., 2021), further reinforcing this assumption. This raises questions about the position of other small, morphologically and geographically anomalous groups found in Paepalanthoideae, which should be the target of further phylogenomic sampling.

One additional factor that should be taken into account is the different sets of markers used in the past phylogenetic studies of Eriocaulaceae and the present work. Past studies have used two to four plastid markers and one to two nuclear markers (Andrade & al., 2010; Trovó & al., 2013; Liang & al., 2019; Andrino & al., 2023). Studies that sampled more Paepalanthoideae species used nuclear ribosomal ITS and ETS, which are known for having multiple copies in the genome. Despite its wide phylogenetic use and utility, paralogy can play a role on the topologies obtained with multiple-copy markers, such as clades reflecting the duplication event of a certain copy of the marker and not truly representing species relationships (e.g., Bailey & al., 2003). Chloroplast markers, although being easy to use and generally not presenting orthology issues, tend to be conserved and show little variation, thus having lower phylogenetic utility. On the other hand, we for the first time use target-capture sequencing in Eriocaulaceae, generating hundreds of low-copy nuclear markers.

The Angiosperm353 probe set was developed to work across flowering plants and reduce the number of potentially paralogous markers (Johnson & al., 2019). The usually small number of markers that are recovered with multiple copies can be further filtered with tree-based methods (e.g., the Yang

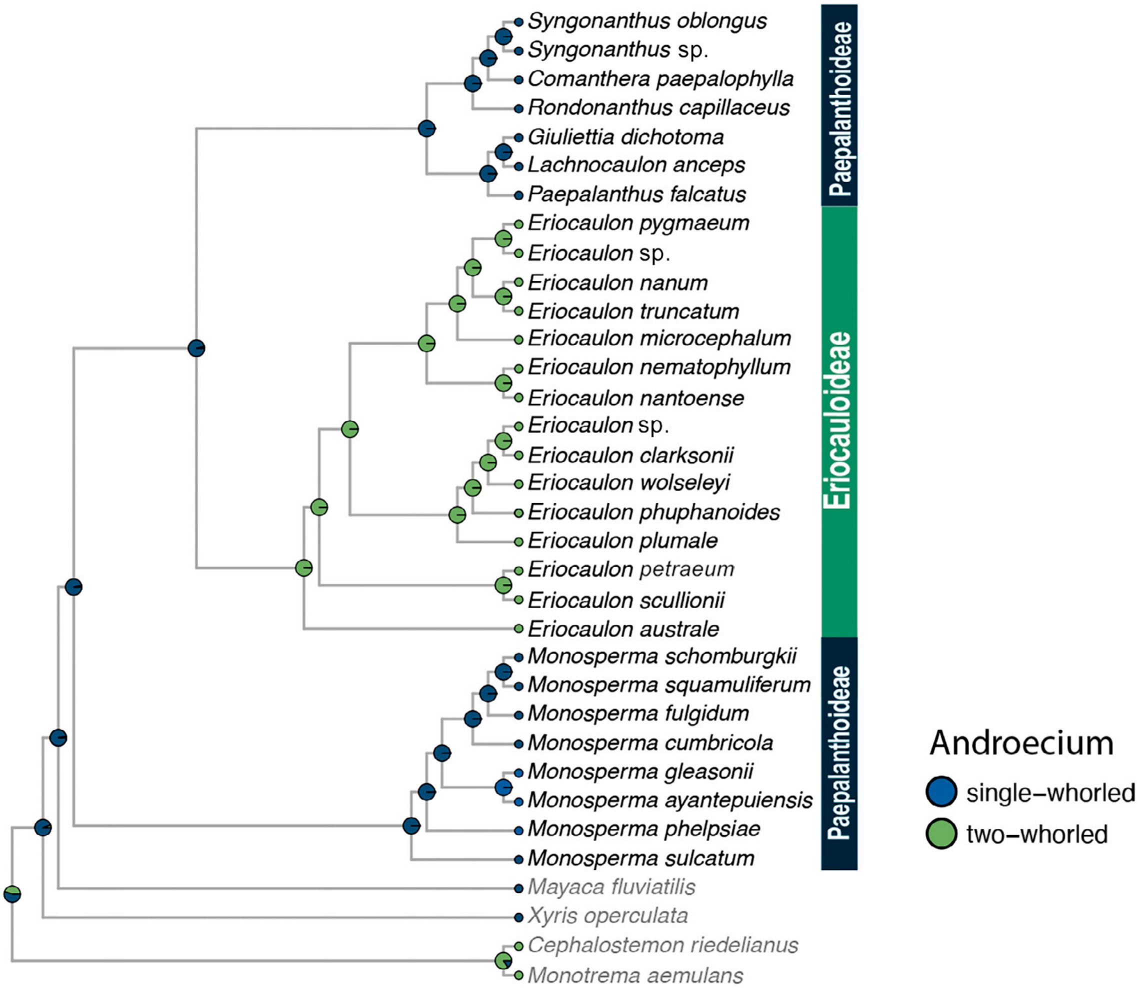

Fig. 3. Graphical representation of the ancestral state reconstruction for androecium morphology across Eriocaulaceae and outgroups. The reconstruction shows the ancestral states at each node, with green circles representing a two-whorled androecium and blue circles representing a single-whorled androecium.

Fig. 4. A, Pantepui, the region around Pico da Neblina, home to the endemic genus *Monosperma*. **B–D**, Eriocaulaceae flowers with a single-whorled androecium, illustrating the ancestral trait: **B**, *Monosperma sulcatum* (Hensold) Andrino; **C**, *Coracoralina acanthophylla* (Ruhland) Andrino with dimerous flowers; **D**, *Paepalanthus lanatus* Silveira with trimerous flowers. **E** & **F**, Flowers with a two-whorled androecium, a derived characteristic within the family: **E**, *Eriocaulon tuberiferum* A.R.Kulk. & Desai, from India; **F**, *Eriocaulon sellowianum* Kunth, from Brazil. — Photos: A–D & F: Rafael Barbosa-Silva; E: Gaurang Tawde.

& Smith, 2014 pipeline). In our study, due to the small number of genes with putative paralogs found (1 to 6, none in *Monosperma*), we used the "naive" option in Captus, which retains the copy that is more similar to the reference locus as the putative ortholog. The higher number of nuclear markers, which increased the number of informative sites in orders of magnitude in comparison with past studies, enhances our confidence that we obtained a phylogenetic tree that most likely represents the actual evolutionary history of the species, and not of the genes used. The increased use of target capture sequencing for phylogenies has almost universally led to challenging evolutionary relationships from previous studies, in different levels of the tree of life such as: subfamily relationships in Asteraceae (Mandel & al., 2019), the position of Gnetales as sister to Pinales (Ran & al., 2018), and infrageneric positions in the iconic Andean bromeliad *Puya* (Aguirre-Santoro & al., 2024). With the increased use of target capture and other high-throughput sequencing methods in phylogenetic studies with narrower taxonomic focus, changes in known evolutionary relationships should not be a surprise.

Not only does the non-monophyly of Paepalanthoideae challenge previous familiar classifications, but the data presented here also undermines the discussion about the taxonomy of the problematic genus *Paepalanthus*, which has sparked heated debates (Stützel & al., 2024). The new data presented here reveals that *Paepalanthus* as circumscribed by Mabberley (2017), is not monophyletic. However, our present results indicate a broader challenge: not only does *Paepalanthus*, in the broad sense adopted by Mabberley (2017) and Christenhusz & al. (2018), include other genera, but the *Monosperma* lineage, previously included in *Paepalanthus*, emerges not only outside of *Paepalanthus* (in this broader concept) but of the whole subfamily. Since *Monosperma* exhibits all the morphological traits that have been used to characterize the broad concept of *Paepalanthus* (Stützel & al., 2024), its exclusion renders *Paepalanthus* sensu Stützel & al. (2024) inherently inconsistent and unsupported by phylogenetic and morphological evidence. This makes the adoption of Mabberley's (2017) and Christenhusz & al.'s (2018) circumscription clearly baseless, while positioning the classification proposed by Andrino & al. (2023) as both more coherent and easily applicable, as it proposes generic names (e.g., the name *Monosperma*) for supported clades identified in the phylogeny of Andrino & al. (2021).

There are two differing perspectives regarding the classification of Eriocaulaceae. One approach supports adopting a broad clade, *Paepalanthus* s.l. (Mabberley, 2017; Christenhusz & al., 2018; Stützel & al., 2024), while we advocate for splitting the smaller lineages into distinct genera (Andrino & al. 2021, 2023). The primary justification for maintaining Paepalanthus s.l., according to Stützel & al. (2024), was the supposed presence of diagnostic morphological traits that could define this broad circumscription. However, these same features—such as isostemonous staminate flowers, pistillate flowers with free petals (rarely reduced to lobes or absent), scalelike staminodes, and a gynoecium with a short style, with nectariferous and stigmatic branches separating at the same level—are also found in *Monosperma*, which falls outside *Paepalanthus* s.l. This undermines the rationale for recognizing *Paepalanthus* s.l. as a single, morphologically diagnosable entity. One could argue for the re-recircumscription of *Paepalanthus* s.l. by excluding *Monosperma*, but this approach would still lack exclusive morphological characters and would necessitate a definition based purely by molecular data—contradicting the premise of Stützel & al. (2024) that *Paepalanthus* s.l. is morphologically defensible.

Although Stützel & al. (2024) advocate for this broad circumscription of *Paepalanthus* to ensure nomenclatural stability and avoid encumbering herbarium collections with moving specimens, the new evidence presented here should reinforce the need to keep advancing the nomenclature of Eriocaulaceae as new discoveries emerge, instead of holding on to an outdated classification. Alternatively, with the classification system of Andrino & al. (2023) established, efforts should shift towards generating genomic and other complementary data to deepen our understanding of evolutionary relationships and broader patterns in Eriocaulaceae, rather than revisiting resolved taxonomic debates.

Timing of diversification and flower evolution in Eriocaulaceae. — Our time calibration results show slight discrepancies compared to previous studies, which have generally estimated more recent divergence times. For example, Janssen & Bremer (2004) estimated the crown node of Eriocaulaceae at 58 Ma and the stem node at 105 Ma. In contrast, our data indicate that recent lineages of Eriocaulaceae originated in the Cretaceous, rather than the Paleogene (Fig. 2), as previously suggested by Larridon & al. (2019). Additionally, the genus *Eriocaulon* shows more recent divergence times for both stem and crown nodes compared to plastome-based estimates provided by Li & al. (2023). These new findings refine our understanding of the evolutionary timeline of the family, pushing the origin of key lineages further back in time.

Few phylogenetic studies have sampled endemic lineages from the Pantepui region (Liu & Smith, 2021), and this is similarly true for Eriocaulaceae. Consequently, a gap remains in our understanding of the divergence ages of these endemic lineages and the events that led to their isolation atop these mountains. Some lineages within Eriocaulaceae, such as *Paepalanthus* from the Brazilian Shield, have suffered fast speciation through rapid and recent radiations (Vasconcelos & al., 2020), despite the ancient age of the Espinhaço Mountains. In contrast, in the Pantepui, the divergence of *Monosperma* from other Eriocaulaceae is considerably older (115 Ma), and the long branch recovered along with a crown age estimated for the Miocene, draws attention to what might have occurred during this time. The Pantepui has been recognized as a center of paleoendemism for both Eriocaulaceae and all Poales (Elliott & al., 2024). Given that Poales is a rich and important group in the Pantepui flora (Riina & al., 2019), it would be beneficial for future research to evaluate extinction and diversification rates to enhance our understanding of the evolutionary trajectories of these Pantepui lineages.

In Eriocaulaceae, anatomical and developmental studies have aimed to clarify the floral homologies and evolutionary patterns of the androecium, with a particular focus on distinguishing the subfamilies Eriocauloideae and Paepalanthoideae. Both subfamilies possess an inner androecial whorl; however, the outer whorl is found exclusively in Eriocauloideae (Rosa & Scatena, 2003; Silva & al., 2021), resulting in diplostemonous flowers in this group. In Paepalanthoideae, the structures initially interpreted as outer staminodes in staminate flowers (Rosa & Scatena, 2003, 2007; Silva & al., 2021) were later reinterpreted as late-developing petals (Silva & al., 2016). The outer androecial whorl is present only in Eriocauloideae (Rosa & Scatena, 2003, 2007; Silva & al., 2021). Previous hypotheses concerning floral evolution (Silva & al., 2016, 2021), based on phylogenetic data that recovered Paepalanthoideae as monophyletic and sister to Eriocauloideae, suggested that the outer whorl had been lost in Paepalanthoideae, with the two-whorled androecium representing the ancestral condition. Also, the delayed development of the outer whorl observed in *Mesanthemum* and some species of *Eriocaulon* (subfam. Eriocauloideae) was initially interpreted as an ancestral state that led to the loss of this whorl in Paepalanthoideae (Silva & al., 2021). The new interpretation, based on the data presented here, raises the possibility that the two-whorled androecium seen in Eriocauloideae represents a derived trait, while the ancestral flower of Eriocaulaceae may have had only a single whorl of stamens. Although further sampling and a deeper understanding of relationships both within Eriocaulaceae and among its related families are necessary, these findings suggest that the two-whorled androecium may have evolved more recently in the family's history than previously proposed. If this hypothesis holds, it would imply that the ancestral flower, dating back approximately 115 million years, had a single whorl of stamens, with a second whorl potentially emerging within the Eriocauloideae clade around 75 million years ago. Nevertheless, additional data are required to further test this scenario and refine our understanding of floral evolution in Eriocaulaceae.

These findings challenge previous assumptions about floral evolution in Eriocaulaceae and underscore the need for empirical data to refine evolutionary models. The reversal of previous understandings raises new questions and calls for more complex analyses under this fresh perspective, advancing our knowledge and opening new avenues for future research to address unresolved issues.

The genus *Monosperma* as the sister group to the entire family. — Species of *Monosperma* have been sparsely sampled in previous studies, where they emerged as sister to one of two large clades of Paepalanthoideae (e.g., Andrino & al., 2021, with moderate support, BS 72%). While its position outside of both subfamilies in our study is surprising, it further shows the value of sampling "anomalous" (Panero & Funk, 2008) taxa in phylogeny, as they can represent extant remnants of once diverse, usually older, clades that suffered increased extinction. The placement of *Monosperma* as sister to the rest of Eriocaulaceae is an intriguing result with important implications for the evolution and diversification within this family.

Monosperma was initially designated as a subgenus of *Paepalanthus* (*Paepalanthus* subg. *Monosperma* Hensold [Hensold, 1991]). It was characterized by morphological traits such as single-seeded indehiscent fruits, which are notably distinct from the typical three-locular capsules found in most other genera of Eriocaulaceae. The fruits of *Monosperma* are consistently described as achenes, a unique fruit type within the family. Although recent studies suggest that *Coracoralina* Andrino & Sano may also produce indehiscent fruits (Andrino & al., 2023), these fruits are not classified as achenes. Although the exact type of fruit in *Coracoralina* is still under investigation, it differs from the achenes of *Monosperma* in both morphology and development. *Monosperma* was recovered as monophyletic in both phylogenetic analyses in which it was sampled, albeit with low support for its relative position to sister groups (Trovó & al., 2013; Andrino & al., 2021). Despite this distinctive morphological feature of the fruits, the presence of isostemonous flowers and non-glandular petals in *Monosperma* led to its placement in Paepalanthoideae. Our new findings suggest a fresh perspective is needed on the morphological characteristics used for taxonomy in the family, advocating for the investigation of other traits that may hold significant phylogenetic signal and previously overlooked due to the strong association of the two subfamilies for centuries.

■ CONCLUSION

This study provides critical insights that challenge longstanding knowledge about the Eriocaulaceae family, particularly concerning its subfamilial relationships. By presenting novel evidence, it reshapes our understanding of these deep taxonomic divisions, offering a fresh perspective on a classification system that has remained largely unchanged for centuries. These findings underscore the importance of evidence-based inquiry and lay the groundwork for further advancements in research methodology. While our results offer significant taxonomic and biogeographic insights, we also recognize the limitations in fully resolving the complex relationships within the family. Future investigations, utilizing advanced methodologies such as the Angiosperms353 probe set and phylogenomic approaches based on multispecies coalescence, hold promise for a more comprehensive exploration. Expanding taxonomic sampling across the genera of Eriocaulaceae will be crucial in strengthening the phylogenetic framework and addressing unresolved nodes, such as the placement of unsampled genera. This study corroborates the classification proposed by Andrino & al. (2023) and highlights the value of collaborative, rigorous scientific research in advancing our understanding of complex evolutionary questions.

AUTHOR CONTRIBUTIONS

COA, RGBS, and CS coordinated the study. COA, RGBS, PCB, RWJ, and CS contributed to sampling of specimens and RGBS and CS led the phylogenomic and biogeographical analyses. RGBS, CMS and PCB wrote the Methods section. RM conducted the Ancestral State Reconstruction analysis. RGBS led production of the figures. COA wrote the first draft of the manuscript with contributions of all authors. All authors contributed to general topics in the introduction and discussion and edited the final manuscript. Laboratory work was carried out by RGBS, LD and PCB; RWJ, PLV, RGBS and COA acquired funding for the project.

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Appendix 1. List of accessions included in the phylogenetic analysis for this study, presented as follows: species name, country, state or region, collector and number (herbarium code), and GenBank accession number. Asterisks (*) indicate sequence data newly generated in this study.

Comanthera paepalophylla (Silveira) L.R.Parra & Giul., Brazil, Bahia, *Ganev 922* (K), ERX10415000; ***Eriocaulon australe*** R.Br., Australia, Queensland, *Baleeiro 546* (BRI), SAMN48030160*; ***Eriocaulon clarksonii*** G.J.Leach, Australia, Queensland, *McDonald 19830* (BRI), SAMN48030161*; ***Eriocaulon microcephalum*** Kunth, Ecuador, *Laegaard 51867* (AAU), SAMN48030162*; ***Eriocaulon nantoense*** Hayata, China, Hong Kong, *Fisher FS 7555* (KFBG), SAMN48030163*; ***Eriocaulon nanum*** R.Br., Australia, New South Wales, *Wilson 3870* (BRI), SAMN48030164*; ***Eriocaulon nematophyllum*** Leach, Australia, Northern Territory, *Kerrigan 342* (DNA), SAMN48030170*; ***Eriocaulon petraeum*** S.M.Phillips & Burgt, Sierra Leone, *Burgt 1372* (K), ERX4143627; ***Eriocaulon phuphanoides*** Praj. & J.Parn, Laos, *Gale 936* (HNL), SAMN48030165*; ***Eriocaulon plumale*** N.E.Br., Guinea Conakry, *Magui 194* (BP), SAMN48030166*; ***Eriocaulon pygmaeum*** G.J.Leach, Australia, Queensland, *Booth 3267* (BRI), SAMN48030167*; ***Eriocaulon scariosum*** Sm., Australia, New South Wales, *Jobson 3829* (NSW), SAMN48030169*; ***Eriocaulon scullionii*** G.J.Leach, Australia, Northern Territory, *Cowie 11640* (DNA), SAMN48030180*; ***Eriocaulon* sp. 1**, Australia, Queensland, *Jobson 3455* (BRI), SAMN48030159*; ***Eriocaulon* sp. 2**, Australia, Western Australia, *Baleeiro 416* (BRI), SAMN48030168*; ***Eriocaulon wolseleyi*** G.J.Leach, Australia, Queensland, *McDonald 19392* (BRI), SAMN48030171*; ***Giuliettia dichotoma*** (Klotzsch ex Körn.) Andrino & Sano, Venezuela, Bolívar, *Maguire 33729* (NY), SAMN48030181*; ***Lachnocaulon anceps*** (Walter) Morong, U.S.A., SRX1639018; ***Monosperma auyantepuiense*** (Moldenke) Andrino, Venezuela, Bolívar, *Vareschi 4892* (NY), SAMN48030172*; ***Monosperma cumbricola*** (Moldenke) Andrino, Venezuela, Bolívar, *Wurdack 34238* (NY), SAMN48030173*; ***Monosperma fulgidum*** (Moldenke) Andrino, Venezuela, Bolívar, *Liesner 23339* (NY), SAMN48030174*; ***Monosperma gleasonii*** (Moldenke) Andrino, Venezuela, Amazonas, *Fernandez-Basil 8085* (NY), SAMN48030175*; ***Monosperma phelpsiae*** (Moldenke) Andrino, Venezuela, Amazonas, *Steyermark 124310* (NY), SAMN48030176*; ***Monosperma schomburgkii*** (Klotzsch ex Körn.) Andrino, Venezuela, Bolívar, *Huber 10040* (NY), SAMN48030177*; ***Monosperma squamuliferum*** (Moldenke) Andrino, Venezuela, Bolívar, *Steyermark 132004* (NY), SAMN48030178*; ***Monosperma sulcatum*** (Hensold) Andrino, Venezuela, Amazonas, *Liesner 21940* (NY), SAMN48030179*; ***Paepalanthus falcatus*** (Bong.) Körn., Brazil, Minas Gerais, *Hatschbach 52923* (K), ERX10415002; ***Rondonanthus capillaceus*** (Körn.) Hensold & Giul., Guyana, Cuyuni-Mazaruni, *Wurdack 5691* (K), ERX10415002; ***Syngonanthus oblongus*** Ruhland, Brazil, Mato Grosso, *Sasaki 2416* (K), ERX10415003; ***Syngonanthus* sp.**, Bolivia, Santa Cruz, *Nee 49735* (CANB), SAMN48030182*; ***Cephalostemon riedelianus*** Körn., Brazil, Minas Gerais, *Arbo 4729* (K), ERX10415006; ***Mayaca fluviatili***s Aubl., Brazil, *Faria 97837* (K), ERX4143474; ***Monotrema aemulans*** Körn., Guyana, *Jansen-Jacobs 1429* (K), ERX10415009; ***Xyris operculata*** Labill., Australia, *Chase 18964* (K), ERX4819253*.