Molecular Phylogenetics and Evolution 211 (2025) 108405

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogenomics reveals taxonomic challenges in *Calibrachoa* (Solanaceae) and sheds light on the origins of cultivated million bells

Alice Backes [1], Leonardo T. Gonçalves [1,*], Pedro H. Pezzi, Loreta B. Freitas [*]

Department of Genetics, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

ARTICLE INFO

Keywords:
Domestication
Hybridization
Petunia
Reticulate evolution
Stimomphis

ABSTRACT

Phylogenies are essential for understanding evolutionary relationships, testing species boundaries, and defining species status. However, hybridization and incomplete lineage sorting (ILS) can obscure these relationships in recently diversified plant lineages by creating conflicting signals across the genome and reducing phylogenetic resolution. *Calibrachoa* (million bells), a Neotropical genus of ornamental plants with high ecological diversity, underwent rapid diversification during the Pleistocene, leading to extensive ILS and interspecific hybridization. In this study, we sampled multiple individuals from all recognized species, a potential new species, and a commercial cultivar of *Calibrachoa*. We used genome-wide genotyping to investigate phylogenetic relationships, species cohesion, hybridization, and the origin of one commercial cultivar. Our results confirm the division of the genus into two subgenera: *Calibrachoa* sensu stricto and *Stimomphis*. Within *Stimomphis*, we observed paraphyly and polyphyly, with low support for several phylogenetic nodes. This uncertainty likely reflects challenges in species delimitation, high ILS due to recent diversification, and ongoing hybridization. We also confirm the hybrid origin of the commercial cultivar. Our findings provide new insights into the evolutionary history of *Calibrachoa*, improving our understanding of its taxonomy and hybridization dynamics. These results illustrate the need to account for reticulate evolution and ILS when resolving phylogenies in rapidly diversifying plant groups.

1. Introduction

How species are defined significantly impacts all areas of the biological sciences that depend on species as fundamental units, affecting fields from phylogenetics and biogeography to macroevolutionary studies and conservation. Historically, plant classification relied on morphological and anatomical traits to define species; however, these can be misleading due to evolutionary convergence, where unrelated species evolve similar characteristics, or phenotypic plasticity, where the same species develops different morphological features in response to environmental factors (Miner et al., 2005). Multiple species concepts have been proposed to broaden the criteria for defining species (reviewed in De Queiroz, 2007), with the Biological Species Concept (BSC) being one of the first and most well-known. This concept delimits species based on reproductive isolation, using the inability to produce viable or fertile offspring as the primary method to distinguish between populations and species (Mayr, 1942). Despite advances, defining and delimiting species remains complex, especially in recently and rapidly diversified plant lineages that often defy traditional species criteria as not enough time has passed to achieve complete reproductive isolation, reciprocal monophyly, or clear morphological differentiation (Shaffer and Thomson, 2007). Consequently, integrative approaches that combine phylogenetic, morphological, and ecological evidence are essential for developing robust species hypotheses (Rouhan and Gaudeul, 2014).

Since their introduction in the late 20th century, molecular phylogenies have become invaluable for species delineation, particularly benefiting species concepts such as the Phylogenetic Species Concept (PSC) (Nixon and Wheeler, 1990; Wheeler and Platnick, 2000). The PSC defines a species as a group of individuals that share a common ancestor and can be distinguished by unique, derived characteristics, while molecular phylogenies serve as powerful tools to test these hypotheses. Another important concept is the Evolutionary Species Concept (ESC), which defines a species as a group of interbreeding populations that share a common evolutionary history (Simpson, 1951; Wiley, 1978). Thus, under the ESC, species are evolutionary lineages that have evolved

* Corresponding authors at: Department of Genetics, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. PoBox 15053 91501-970.
E-mail addresses: tresoldigoncalves@gmail.com (L.T. Gonçalves), loreta.freitas@ufrgs.br (L.B. Freitas).
[1] These authors have contributed equally to this work.

https://doi.org/10.1016/j.ympev.2025.108405
Received 22 January 2025; Received in revised form 24 June 2025; Accepted 26 June 2025
Available online 30 June 2025

independently from others, and molecular phylogenies can help identifying these lineages, especially when multiple populations from the same species are sampled.

Phylogenies play a key role not only in identifying evolutionary lineages but also in addressing broader evolutionary questions, such as the classification and evolution of Angiosperms (One Thousand Plant Transcriptomes Initiative, 2019; The Angiosperm Phylogeny Group, 2016; Zuntini et al., 2024), global plant biogeographical patterns (e.g., Donoghue, 2008; Maestre et al., 2021; Morris et al., 2018; Wang et al., 2025), diversification rates (e.g., Smith et al., 2011; Tietje et al., 2022), and conservation strategies (e.g., Purvis et al., 2005). Despite their significance, building phylogenies is not straightforward and presents several challenges. The sampling scheme, including the number of species sampled (Guillaume et al., 1993; Nabhan and Sarkar, 2012) and the number of individuals sampled per species (Fulton and Strobeck, 2010), along with the number (Rokas and Carroll, 2005), completeness (De La Torre-Bárcena et al., 2009), genomic location (Hu et al., 2023; Stull et al., 2020), and selective pressures (Roje, 2014) of genetic loci can impact phylogenetic accuracy (Naciri and Linder, 2015). These variables, in turn, affect how we delimit species and interpret evolutionary trends.

In addition to noise introduced by species sampling and the choice of genetic markers, biological processes can also obscure boundaries between species and create challenges for resolving evolutionary relationships, particularly in recently radiated plant groups (e.g, Kleinkopf et al., 2019; Murillo et al., 2022; You et al., 2022). Among these processes, incomplete lineage sorting (ILS) is the most extensively studied source of discordance (e.g., Liu et al., 2015; Maddison and Knowles, 2006; Yan et al., 2022), occurring when gene lineages coalesce in a manner that do not reflect the "true" species relationships (Maddison, 1997). A similar pattern of discordance arises from hybridization, in which different species interbreed and exchange genetic material—a relatively common and important evolutionary process in plant evolution (e.g., Goulet et al., 2017; Soltis and Soltis, 2009; Whitney et al., 2010). Despite the challenges, modern phylogenetic methods are starting to assess the impact of ILS and hybridization on phylogenetic trees (e.g., Folk et al., 2018; Solís-Lemus and Ané, 2016; Yu et al., 2013).

Calibrachoa (Solanaceae) is a genus native to the Neotropical region and is closely related to *Petunia* within the tribe Petunieae (Olmstead, 2013) (Fig. 1). Wild *Calibrachoa* species exhibit a subtropical distribution, with the highest species richness found in southern Brazil, Argentina, and Uruguay (Fregonezi et al., 2012; Stehmann, 1999). Based on morphological and molecular data (Fregonezi et al., 2012), the genus was divided into two subgenera: *Calibrachoa* sensu stricto, which includes *C. parviflora* and *C. pygmaea*, and *Stimomphis*, which encompasses the remaining 26 species. Species in the subgenus *Calibrachoa* exhibit distinct seed, leaf, and flower morphologies compared to those in

Fig. 1. Morphological diversity of *Calibrachoa*. (A) *C. parviflora*. (B) *C. humilis*. (C) *C. thymifolia*. (D) *C. sendtneriana*. (E) *C. caesia*. (F) *C. excellens*. (G–H) The P15 commercial *Calibrachoa* cultivar purchased for this study. Pictures A–F sourced from iNaturalist.com under a Creative Commons license. iNaturalist observation numbers and username credits, in order: 252413452 (gusgomez), 204994541 (Guillermo Menéndez, gmmv80), 185084173 (Ariadna Tripaldi, ariadnat), 34643676 (Lucas C. Wheeler, lcwheeler), 139819183 (Patricio A. Mantinian, pmantinian1951), 241927679 (Mateus Henrique Schenkel, mateus_henrique_schenkel).

subgenus *Stimomphis*, and they also display significant variation in flower morphology and reproductive strategies within the subgenus (Fregonezi et al., 2012). Conversely, species in *Stimomphis* exhibit more conserved flower morphologies, are all self-incompatible, and many species show overlapping distributions in southern South America. Moreover, all species except *C. sendtneriana* (Fig. 1D) and *C. serrulata* exhibit bee-pollination syndromes, although little is known about their effective pollinators and mating systems (John et al., 2019). The absence of geographic and pollinator barriers among species within *Stimomphis* creates a favorable context for interspecific gene flow, as has been documented for several pairs of *Petunia* species (e.g., Caballero-Villalobos et al., 2021; Giudicelli et al., 2024; Pezzi et al., 2022).

Known as "million bells", these plants are widely cultivated as ornamental plants (Fig. 1) and were commercially introduced in Japan and Europe in the 1990s (Rice, 1997), gaining popularity, particularly in Europe and North America (Murakami et al., 2004). Since then, numerous cultivars have been developed in diverse floral colors, including hues not found in wild populations (Kanaya et al., 2010). These cultivars are collectively called *Calibrachoa* × *hybrida*, which suggests a hybrid origin. However, the wild species involved in this putative hybridization remain largely unknown. Clarifying the origins of the commercial cultivar is key to understanding their genetic diversity and breeding history, having practical implications for horticulture, such as improving trait selection and hybridization strategies. Furthermore, identifying parental species can show how artificial selection has shaped phenotypic divergence from wild relatives, offering insights into the evolutionary processes underlying plant domestication (Qi et al., 2022; Raymond et al., 2018; Segatto et al., 2014).

Phylogenetic studies on the genus *Calibrachoa* utilizing Sanger sequencing methods have revealed challenges in resolving interspecies relationships, evidenced by multiple polytomies (Fregonezi et al., 2013) and low support for shallow nodes in the subgenus *Stimomphis* (Mäder and Freitas, 2019). Furthermore, earlier studies typically included only a single individual per species, making it impossible to test for species monophyly. The divergence time between *Calibrachoa* and *Petunia* was initially estimated to be around 8.5 million years ago (Mya; Särkinen et al., 2013), but recent analyses utilizing transcriptomic and genomic data have revised this estimate to 19.7 Mya (Huang et al., 2023). The latter study included only one representative of *Calibrachoa*, preventing divergence time estimation within the genus, while the former estimated the crown age of *Calibrachoa* to be approximately 3.9 Mya. Combined with evidence indicating that Pleistocene climatic cycles significantly influenced the diversification of the genus—particularly within the subgenus *Stimomphis* (Mäder et al., 2013; Mäder and Freitas, 2019)—this suggests a recent and rapid diversification in *Calibrachoa*. Such rapid diversification has hindered gene coalescence and prevented species from achieving reproductive isolation, resulting in a phylogeny characterized by short branch lengths and high discordance among gene trees (Pezzi et al., 2024), arising from both high levels of ILS and interspecific hybridization. Consequently, the phylogenetic relationships among *Calibrachoa* species, especially within the subgenus *Stimomphis*, remain largely elusive.

In this study, we applied high-throughput genotyping across multiple individuals of all known *Calibrachoa* species, representing both subgenera (*Stimomphis* and *Calibrachoa* sensu stricto) and spanning their entire native distribution, to clarify evolutionary relationships and test species cohesiveness. Specifically, we tested the hypotheses that (1) phylogenetic analyses would support the monophyly of recognized *Calibrachoa* species, (2) interspecific gene flow may contribute to phylogenetic incongruences within the genus, and (3) the commercial *Calibrachoa* cultivar has a hybrid origin involving two or more wild species. Additionally, we included an undescribed putative new species to assess its phylogenetic placement and relationships with wild taxa.

2. Material and methods

2.1. Sampling, DNA extraction, and sequencing

We collected young and healthy leaves from 30 wild *Calibrachoa* taxa, including, when possible, multiple individuals per species (Table 1). Additionally, we sampled five individuals of *Calibrachoa* sp. 1, a putative new species based on morphological traits, and five individuals from the commercial P15 cultivar of *Calibrachoa* (million bells), sold by Veiling Holambra (Santo António de Posse, SP, Brazil; https://veiling.com.br/produtos/calibrachoa-p15/). Five individuals from *Petunia integrifolia* were included to serve as the outgroup. Sampling was conducted under ICMBIO-SISBIO scientific permit number 41530–11. Plant identification was based on morphological characteristics consistent with species descriptions. Voucher specimens were deposited in the herbaria of the Universidade Federal de Minas Gerais (BHCB-UFMG; Belo Horizonte, MG, Brazil), Universidade Federal do Rio Grande do Sul (ICN-UFRGS; Porto Alegre, RS, Brazil), Universidade Regional de Blumenau (FURB; Blumenau, SC, Brazil), Universidad Nacional del Nordeste (CTES; Corrientes, Argentina), Instituto de Recursos Naturales (BAB; Buenos Aires, Argentina), and Instituto de Botânica (SP; São Paulo, SP, Brazil). Some samples were donated from individuals cultivated in-house (Data S1). Silica-dried leaves were ground in liquid nitrogen, and genomic DNA was extracted using a cetyltrimethylammonium bromide (CTAB; Sigma-Aldrich Chem. Co., St. Louis, USA) protocol (Roy et al., 1992). DNA concentration was measured with a Qubit Fluorometer (Thermo Fisher Scientific Co., Waltham, USA), and quality was assessed with a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher). Samples with 260/280 and 260/230 absorbance ratios above 1.80 were considered high quality and selected for genomic library construction. We confirmed the absence of nucleases in the samples using EcoRI (NEB—New England BioLabs Inc., Ipswich, USA). DNA libraries were prepared using DArTseq™ complexity reduction method (Cruz et al., 2013; Kilian et al., 2012) with the PstI-MseI (NEB) enzyme combination. This protocol replaces a single adaptor with two distinct adaptors. Equimolar amplification products from each sample were pooled into 96-well microtiter plates and subjected to c-Bot bridge PCR (Illumina Inc., San Diego, USA). Samples were sent to Diversity Arrays Technology Pty Ltd (DArT, Canberra, Australia) and sequencing was performed on an Illumina HiSeq2500 platform (Illumina).

2.2. Filtering and variant discovery

To prepare the dataset, we used the *process_radtags* module in Stacks v2.65 (Catchen et al., 2013) with default settings to demultiplex the reads and remove barcodes, low-quality reads, and reads containing adaptor contamination. Data quality was monitored throughout these steps with FastQC v0.11.7 (Wingett and Andrews, 2018). Additionally, cutadapt v4.9 (Martin, 2011) was used to trim the first four nucleotides from each read, as the FastQC quality assessment indicated. For individuals sequenced across multiple runs, we merged data post-filtering to create a single file per individual (Data S2).

Next, we aligned reads to the *Petunia axillaris* subsp. *axillaris* reference genome (v1.6.2; Bombarely et al., 2016), a species from a closely related genus of *Calibrachoa*, using BWA v0.7.10-r789 (Li and Durbin, 2010) with default parameters. Unmapped reads were discarded, and the resulting SAM files were sorted and converted to BAM format with Samtools v1.21 (Danecek et al., 2021; Li et al., 2009). We then combined BAM files into a single file using bamaddrg (https://github.com/ekg/bamaddrg).

Variant calling was conducted with FreeBayes v0.9.21 (Garrison and Marth, 2012), applying the following filters: mapping quality > 30, base quality > 30, and read depth > 10. We used VCFtools v0.1.16 (Danecek et al., 2011) to retain only biallelic single-nucleotide polymorphisms (SNPs) with < 30 % missing data and a minimum allele frequency of

Table 1
Monophyly assessment of *Calibrachoa* species based on phylogenetic analyses using IQ-TREE and SVDquartets. The table indicates whether the hypothesis of monophyly is supported (YES) or not (NO) by each analysis, with YES* denoting partial monophyly as discussed in the main text. For the IQ-TREE analysis, ultrafast bootstrap (UFB) support values are shown, while bootstrap (BS) support values are reported for the SVDQuartets analysis. The sample size for each taxon (n) is also provided.

Species	n	IQ-TREE Monophyly	UFB	SVDQuartets Monophyly	BS
Subgenus *Calibrachoa* Cerv.					
Calibrachoa parviflora (Juss.) D'Arcy	6	YES	100	YES	100
Calibrachoa pygmaea (R.E. Fr.) Wijsman	9	YES	100	YES	100
Subgenus *Stimomphis* (Raf.) Stehmann, Fregonezi & L.B. de Freitas					
Calibrachoa atropurpurea Stehmann & Semir	6	NO	–	NO	–
Calibrachoa caesia (Sendtn.) Wijsman	6	YES	100	YES	100
Calibrachoa cordifolia Stehmann & L.W. Aguiar	4	YES*	100	NO	–
Calibrachoa dusenii (R.E. Fr.) Stehmann & Semir	6	YES	100	YES	90
Calibrachoa eglandulata Stehmann & Semir	6	YES	100	YES*	100
Calibrachoa elegans (Miers) Stehmann & Semir	8	YES	100	YES*	100
Calibrachoa ericifolia (R.E. Fr.) Wijsman	8	YES	100	NO	–
Calibrachoa excellens (R.E. Fr.) Wijsman	6	NO	–	NO	–
Calibrachoa felipponei (Sandwith) Stehmann	3	YES	100	YES	88
Calibrachoa heterophylla (Sendtn.) Wijsman	6	YES	100	YES	87
Calibrachoa humilis (R.E. Fr.) Stehmann & Semir	8	YES*	73	NO	–
Calibrachoa irgangiana Stehmann	6	YES*	87	YES*	50
Calibrachoa linearis (Hook.) Wijsman	6	NO	–	NO	–
Calibrachoa linoides subsp. *furcata* Greppi & Stehmann	6	NO	–	YES	35
Calibrachoa linoides (Sendtn.) Wijsman subsp. *linoides*	6	NO	–	NO	–
Calibrachoa longistyla Stehmann & Greppi	1	–	–	–	–
Calibrachoa micrantha (R.E. Fr.) Stehmann & Semir	2	YES	100	YES	100
Calibrachoa missionica Stehmann & Semir	6	YES	100	YES	99
Calibrachoa ovalifolia (Miers) Stehmann & Semir	6	NO	–	NO	–
Calibrachoa paranensis (Dusén) Wijsman	5	YES	100	YES	100
Calibrachoa pubescens (Spreng.) Stehmann	5	NO	–	NO	–
Calibrachoa scabridula (C.V. Morton) Stehmann	3	YES	100	YES	93
Calibrachoa sellowiana (Sendtn.) Wijsman	6	YES*	98	YES*	62
Calibrachoa sendtneriana (R.E. Fr.) Stehmann & Semir	10	YES	100	NO	–
Calibrachoa serrulata (L.B. Sm. & Downs) Stehmann & Semir	6	YES	100	YES*	100
Calibrachoa sp. (comm. cultivar)	5	NO	–	NO	–
Calibrachoa sp. 1	6	YES*	38	NO	–
Calibrachoa spathulata (L.B. Sm. & Downs) Stehmann & Semir	6	YES*	100	YES*	100
Calibrachoa synanthera Stehmann & G. Mäder	6	YES	100	YES	96
Calibrachoa thymifolia (A. St.-Hil.) Stehmann & Semir	6	NO	–	NO	–

0.01. To remove loci under linkage disequilibrium (LD), we applied a minimum site distance of 100 bp, retaining only one SNP per read. Outlier loci were identified and removed using pcadapt v4.4.0 (Luu et al., 2017) in R, yielding a final dataset of putatively neutral loci. For specific downstream analyses, we repeated the filtering and variant discovery process on subsets of individuals (namely from Subclades I, and II; see section 3.2) to maximize the number of SNPs retained in the VCF files for these subsets.

2.3. Phylogenetic reconstruction, evolutionary relationships, and genetic structure

We applied four complementary approaches to the filtered SNP dataset to infer evolutionary relationships among *Calibrachoa* species. First, we generated a maximum likelihood phylogeny using IQ-TREE v2.3.6 (Minh et al., 2020), including only variable sites and applying ascertainment bias correction appropriate for SNP data (model GTR + ASC; Lewis, 2001). Branch support was assessed with 1,000 ultrafast bootstraps (UFB; Hoang et al., 2018) replicates. Next, we used SVDQuartets, a coalescent-based method implemented in PAUP* 4a (Swofford, 2003), which infers relationships by analyzing quartets of taxa and synthesizing them into a phylogenetic tree. We evaluated all possible quartets (evalq = all) for this analysis and conducted 100 bootstrap replicates to assess node support. As our third approach, we performed a discriminant analysis of principal components (DAPC) using the R package adegenet (Jombart and Ahmed, 2011) to identify the optimal number of genetic clusters within our *Calibrachoa* dataset. We used the *find.clusters* function to determine the clusters and *optim.a.score* to identify the optimal number of PCs to retain. This method maximizes genetic differentiation among clusters while minimizing variation within clusters. For DAPC, we also analyzed subsets of individuals (Subclades I and II; see section 3.2), allowing a clearer visualization of the distinct genetic clusters. Lastly, we used SplitsTree v6.4.11 (Huson and Bryant, 2024) to construct a phylogenetic network based on P distances, averaging ambiguous sites, with the NeighborNet method (Bryant and Moulton, 2004). This combination of tree-based (IQ-TREE, SVDQuartets), clustering (DAPC), and network-based (SplitsTree) approaches together provided a broader perspective on both hierarchical and reticulate relationships among *Calibrachoa* species.

2.4. Hybridization and gene flow inference

We assessed patterns of historical and recent hybridization within *Calibrachoa* using ABBA–BABA, f_4, and f-branch analyses (Malinsky et al., 2018; Patterson et al., 2012) implemented in Dsuite (Malinsky et al., 2021). The f-branch analysis, a heuristic approach, aids in interpreting correlated f_4-ratio results by identifying internal branches in the phylogeny where significant allele sharing suggests hybridization across a clade (Malinsky et al., 2021, 2018). We used the −Z parameter to calculate Z-scores for all f-branch values. Those with $Z < 3$ were considered not significant and were changed to 0 using a custom script (https://github.com/joanam/scripts/blob/master/removeNonsignDsuite.r), as in Fark et al. (2022). These analyses were conducted on the full dataset and Subclades I and II. As the IQ-TREE results demonstrated greater topological stability and higher support than the SVDQuartets tree (see section 3.2), we opted to use the IQ-TREE results as the initial tree for the hybridization analyses. Individuals from the same species grouped in monophyletic lineages were collapsed into a single branch.

3. Results

3.1. Data quality and SNP calling

Our DArTseq analysis yielded a dataset comprising 190 individuals and 268,670,412 reads. Per-individual read counts ranged from 277,749

to 1,908,960, averaging 514,799 reads per sample (Data S2). Initial SNP calling identified 736,080 loci, of which 716,545 were removed due to failure to meet sample-based criteria (see section 2.2). This filtering resulted in 19,535 variant sites. Using PCAdapt, we identified and removed 1,495 outlier loci, yielding a final dataset of 18,040 putatively neutral SNPs for subsequent analyses.

3.2. Evolutionary relationships and genetic structure

Phylogenetic analyses recovered *Calibrachoa* as a robustly supported monophyletic group (Fig. 2; UFB = 100; BS = 100). Both currently recognized subgenera were also recovered as monophyletic: *Calibrachoa* sensu stricto, consisting of *C. pygmaea* and *C. parviflora* (UFB = 100; BS = 76), and *Stimomphis*, comprising the remaining *Calibrachoa* species (UFB = 100; BS = 100). However, within *Stimomphis*, shallow nodes exhibited low support (Fig. 2, Figs. S1 and S2) and branches were extremely short, especially in the tree recovered with SVDQuartets (Figs. S1 and S2). These results were consistent with the clustering patterns recovered with DAPC, where *Calibrachoa* sensu stricto formed a well-defined cluster. In contrast, *Stimomphis* showed considerable overlap and limited genetic differentiation in the scatterplot (Fig. 3A and Fig. S3). For the DPAC analysis with all individuals, the *find.clusters* function indicated that the lowest BIC value corresponded to 11 clusters. We retained 66 principal components and two discriminant components, accounting for 67 % of the variance. For the DAPC analysis of Subclade I (Fig. 3B and Fig. S4), we retained five principal components and three discriminant components, accounting for 25 % of the variance. This revealed four groups that largely aligned with species delimitations. In

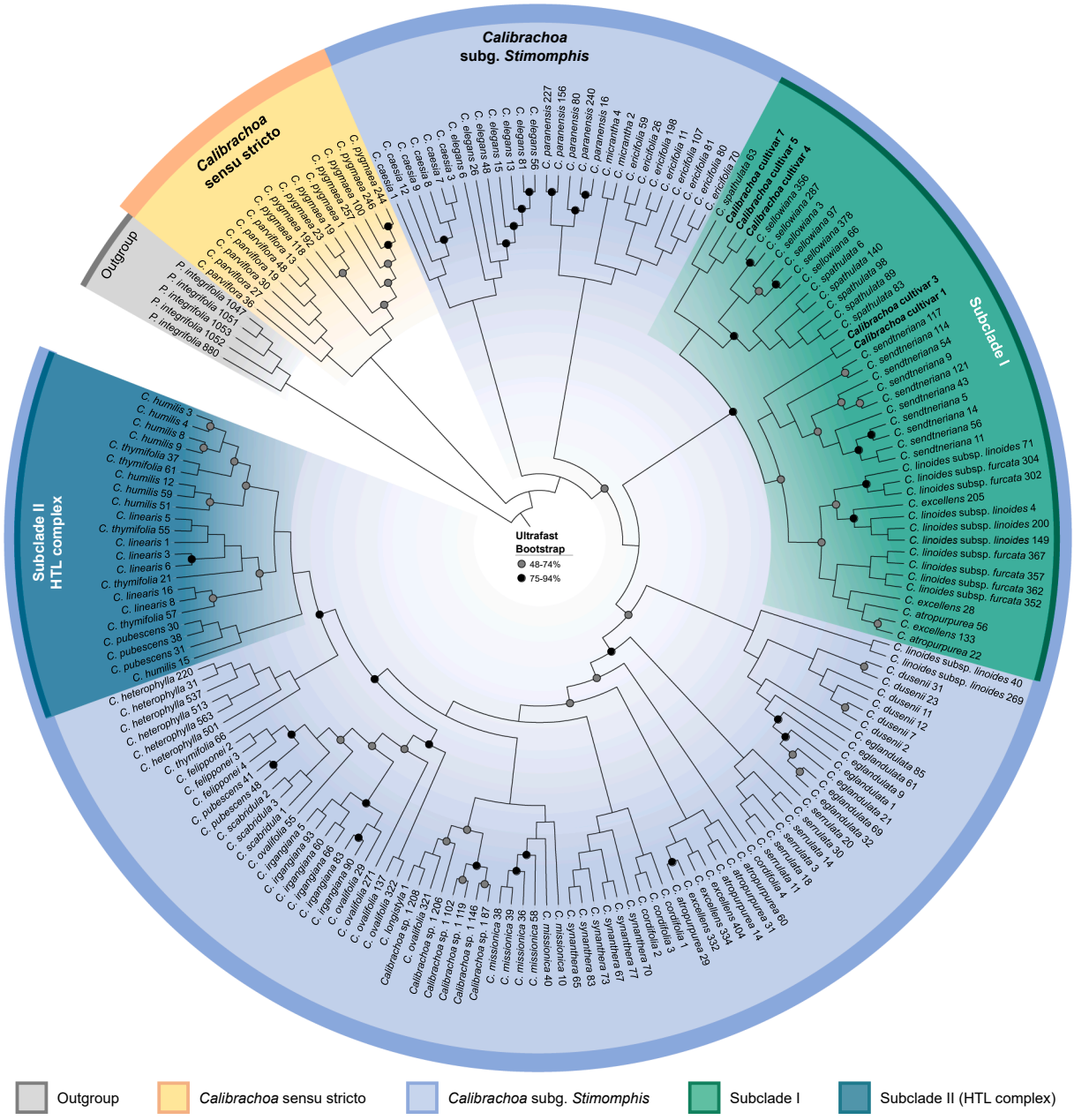

Fig. 2. Phylogenetic tree of *Calibrachoa* inferred from the SNP dataset using IQ-TREE. Ultrafast bootstrap support values are indicated at the nodes: nodes without circles indicate high support (≥95 %), black circles represent moderate support (75–94 %), and gray circles represent low support (48–74 %). Major clades are color-coded: yellow represents *Calibrachoa* sensu stricto, blue represents *Stimomphis*, and gray represents the outgroup (*Petunia integrifolia*). Subclade I and Subclade II (HTL complex), for which hybridization tests were conducted, are also highlighted. Branch lengths are not drawn to scale to improve visualization; the full tree with scaled branch lengths is available in Fig. S1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

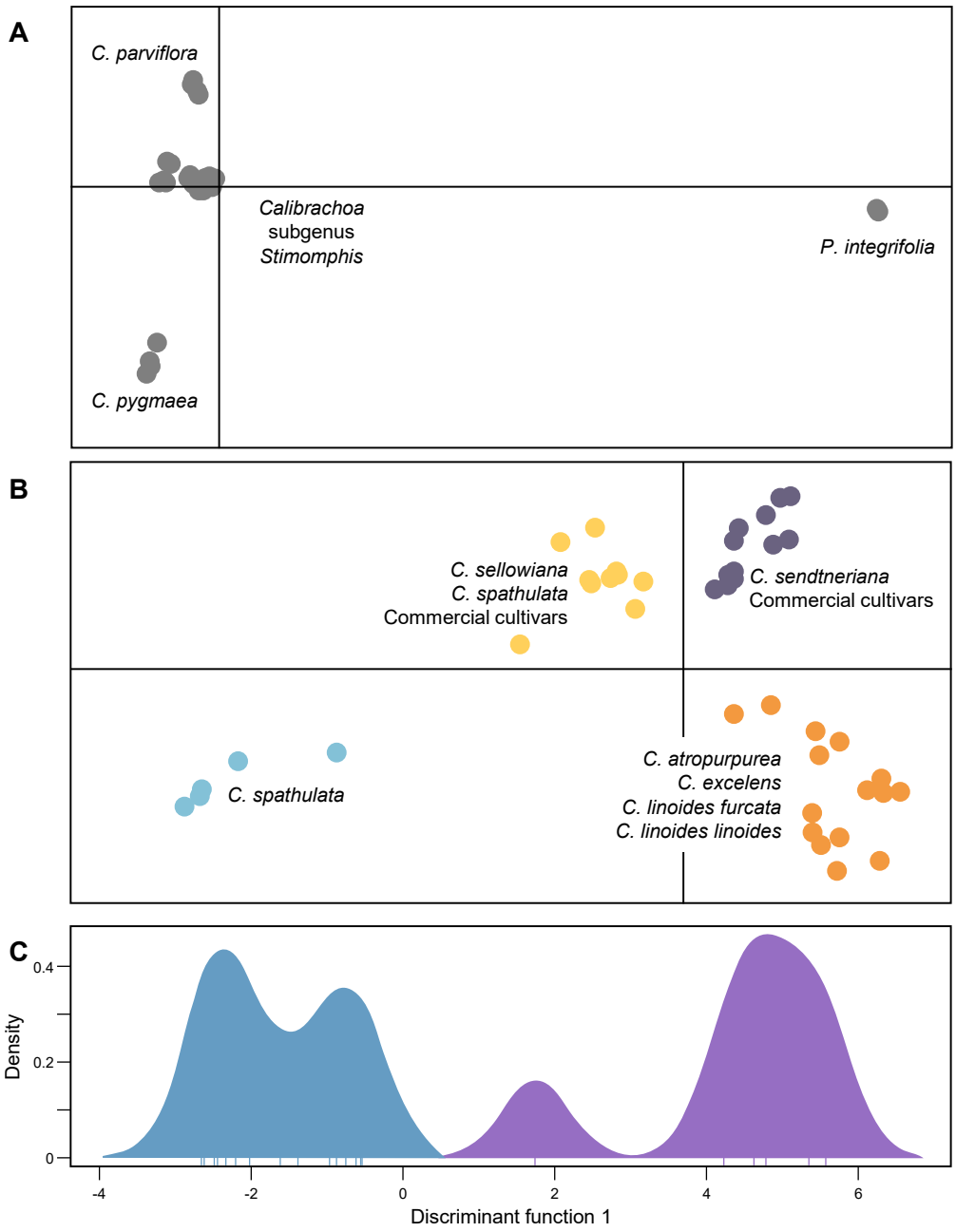

Fig. 3. DAPC analysis results, illustrating genetic differentiation across *Calibrachoa* lineages. (A) DAPC results considering all sampled individuals. The list of species and individuals belonging to each cluster is available in Data S3. (B) DAPC for Subclade I, which includes commercial cultivars and wild relatives. (C) Density plot of the first discriminant component for Subclade II, showing overlapping distributions among closely related species due to weak genetic differentiation.

contrast, little differentiation among species was observed for Subclade II (Fig. 3C and Fig. S5), where we retained three principal components and two discriminant components, also accounting for 25 % of the variance. The network constructed with all individuals using SplitsTree divided them into the subgenus *Calibrachoa*, the subgenus *Stimomphis*, and a mixed group containing species from both subgenera (Fig. S6), with limited phylogenetic signal to solve reticulation events within the genus.

Of the 30 taxonomic units analyzed within *Stimomphis*—including 27 nominal species, one species with two subspecies, one commercial cultivar, and a putative new species—14 were recovered as monophyletic in the tree generated by IQ-TREE (Fig. 2; Table 1). In contrast, the tree inferred with SVDQuartets showed lower support values, with only 10 species recovered as monophyletic (Table 1). Several species displayed partial monophyly: in *C. cordifolia* and *C. sellowiana*, most individuals formed distinct clades with a single outlier placed outside each main clade, whereas in *C. irgangiana* and *C. spathulata*, conspecific individuals grouped into clades that also included individuals from other species. The commercial *Calibrachoa* cultivar appeared in two clades (nested within Subclade I; Fig. 2), supporting their hypothesized hybrid origin: three individuals grouped with one individual of *C. spathulata* as a clade sister to *C. sellowiana*, while two others grouped with *C. sendtneriana*. Evidence of polyphyly was apparent in several taxa, such as *C. ovalifolia*, which appeared in multiple locations within the phylogeny. Furthermore, a clade comprising *C. humilis*, *C. thymifolia*, *C. linearis* (hereafter, we refer to this clade as the HTL complex), and *C. pubescens* lacked reciprocal monophyly (Subclade II; Fig. 2), suggesting the presence of a species complex within this group. The phylogeny also strongly suggested that *C. excellens* and *C. atropurpurea* do not represent distinct, well-defined lineages as currently circumscribed.

Instead, individuals from these two species were distributed across multiple lineages, with these lineages not aligning with current species boundaries. Lastly, the putative new species *C.* sp1 was not recovered as a monophyletic group; instead, it grouped with the single individual of *C. longistyla* in our dataset (long1) and one individual of *C. ovalifolia* (oval321), albeit with low support.

Based on the observed patterns, we identified two subclades in the IQ-TREE phylogeny for targeted hybridization tests (Fig. 2). Subclade I included all individuals of the commercial cultivar, and we applied the hybridization tests to detect excess allele sharing supporting their hybrid origin. Subclade II comprised the HTL complex and three individuals identified as *C. pubescens*.

3.3. Hybridization tests

Hybridization analyses across all *Calibrachoa* lineages suggest that the paraphyly observed in the phylogenetic trees may be partly attributed to interspecific gene flow (Fig. S7), particularly involving Subclade II, where we found evidence of hybridization between one individual of *C. thymifolia* and *C. humilis* (Fig. 4). Moreover, the *f*-branch analysis for Subclade I suggested that the commercial P15 cultivar sampled is a hybrid between *C. sendtneriana* and *C. sellowiana*, with a potential contribution from *C. spathulata* (Fig. 4). We also found that commercial cultivar lineages recovered in distinct clades show an excess of allele sharing (Fig. 4), further suggesting hybridization events. Additionally, several individuals with atypical phylogenetic positions—distant from the main clade of the species they were identified as—are likely hybrids, as revealed by the *f*-branch tests (Fig. 4). For example, thym66 appears to be a hybrid of *C. heterophylla* and Subclade II, whereas thym57 appears to be a hybrid between the HTL complex and *C. ovalifolia* (specifically oval55).

4. Discussion

Understanding evolutionary relationships in recently diversified plant lineages remains a persistent challenge in systematics, particularly when hybridization, ILS, and rapid speciation obscure species boundaries. In this study, we leveraged genome-wide genotyping to investigate phylogenetic relationships, species cohesiveness, and interspecific gene flow of *Calibrachoa*, a Neotropical genus of ornamental and ecologically diverse plants. By sampling multiple individuals across all described species, as well as a putative new species and commercial cultivars, we addressed critical gaps in previous phylogenetic studies by generating genome-wide data for tree inference and by explicitly testing for species monophyly. Our results highlight the limitations of traditional species concepts, such as BSC and PSC, when applied to rapidly radiating groups. Instead, the ESC, which defines species as independently evolving lineages, provides a more flexible framework for reconciling the complex patterns of paraphyly, polyphyly, and hybridization observed here. Moreover, we found that the sampled commercial cultivars of *Calibrachoa* are a hybrid of at least two wild species from subgenus *Stimomphis* (*C. sendtneriana* and *C. sellowiana*), providing novel insights about their domestication.

4.1. Phylogenetic relationships

The phylogeny of *Calibrachoa* revealed a complex evolutionary history, characterized by deep divergences between subgenera, consistent with previous studies based on Sanger markers (Fregonezi et al., 2013; Mäder and Freitas, 2019) and transcriptomic data (Pezzi et al., 2024; Wheeler et al., 2022), and challenging patterns of diversification within *Stimomphis*. The clear phylogenetic separation between *Calibrachoa* sensu stricto and the remaining taxa highlights its distinct evolutionary trajectory (Figs. S1 and S2). This aligns with biological traits, such as their annual life cycle (in contrast to the perennial habit of *Stimomphis*) (Stehmann, 1999) and unique morphoanatomy, including differences in the calyx lobes and the endodermis surrounding the vascular bundles (Fregonezi et al., 2012). Within *Stimomphis*, however, phylogenetic relationships proved complicated and less stable, characterized by short branches with little support in the phylogenetic trees (Figs. S1 and S2)

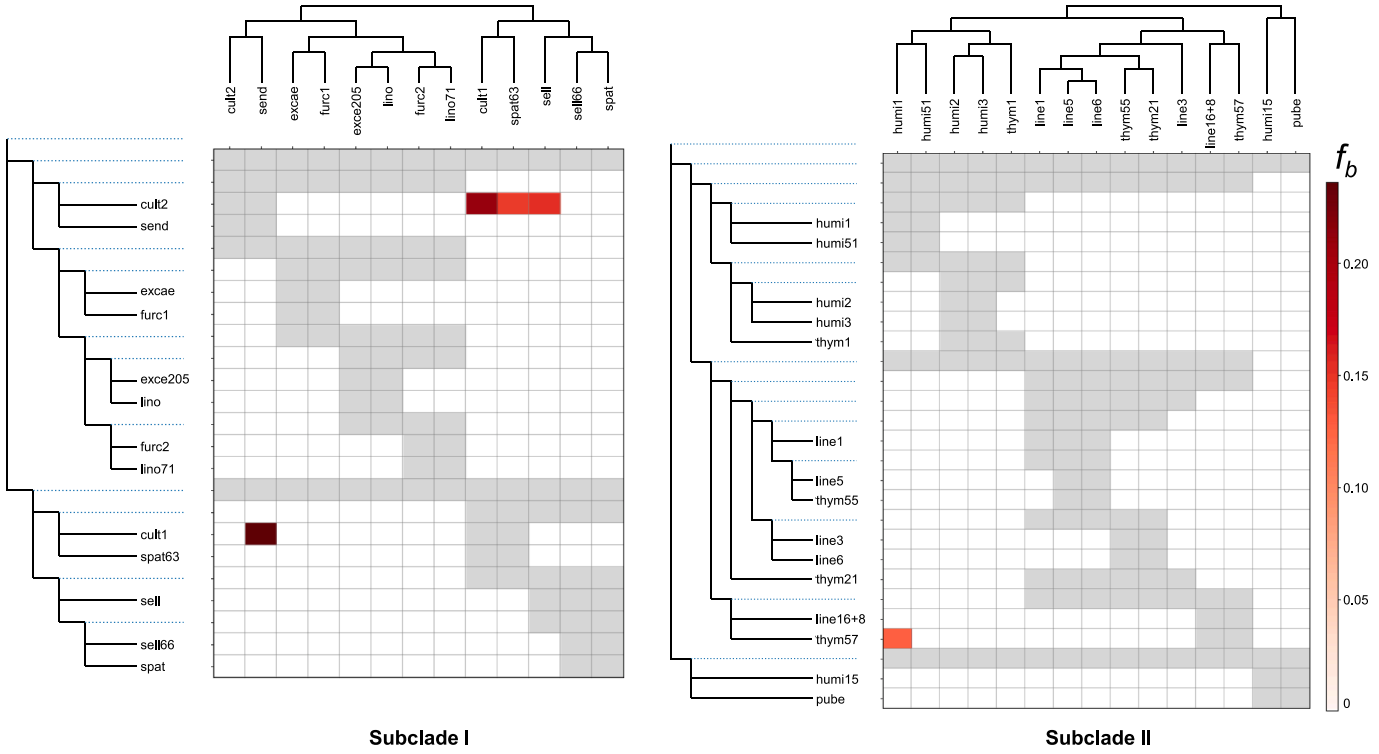

Fig. 4. Summary of Dsuite *f*-branch statistics for Subclade I (left) and Subclade II (right) using the maximum likelihood tree as the starting tree. Dashed lines represent the common ancestor of all subsequent species. Darker colors on the heatmap indicate excessive allele sharing.

and a lack of resolution in the network (Fig. S6). Previous studies suggested subdividing *Stimomphis* into four main clades, which broadly correspond to the geographical distribution of its species (Mäder and Freitas, 2019). However, our results did not support these subdivisions, as none of the previously proposed clades were recovered. This discrepancy may stem from methodological limitations in prior studies, such as reliance on a small number of genetic markers or limited sampling (e.g., single representatives per species). The multiple instances of species paraphyly and polyphyly observed in our study support the need to reassess *Stimomphis* taxonomy and species delimitation.

The subgenus *Stimomphis* rapidly diversified into multiple species during the climatic oscillations of the Pleistocene (Mäder et al., 2013; Mäder and Freitas, 2019). This rapid radiation led to high levels of ILS, as previously described in the genus (Pezzi et al., 2024), and partially explains the low support for nodes within the subgenus. A complex case involves the phylogenetic placement of *C. elegans*. This species, located at the northernmost limit of the subgenus distribution in the state of Minas Gerais (Backes et al., 2019), southeastern Brazil, was recovered as the sister species of *C. caesia*, which occurs in southern Brazil, the biodiversity hotspot for *Calibrachoa*. Together, these species formed a clade sister to all remaining *Stimomphis* species (Fig. 2), a result consistent with maximum likelihood analyses by Pezzi et al. (2024), though not corroborated by their quartet-based analysis. Although *Calibrachoa* species have limited seed dispersal ability (Stehmann, 1999), where seeds typically fall close to the mother plant, it is possible that rare long-distance dispersal events occurred and led to vicariant speciation, as seen in other plants (Escudero et al., 2009; Rodrigues et al., 2020). Alternatively, speciation might also be explained by peripatric or parapatric divergence processes, followed by the extinction of intermediate populations. Future studies integrating finer-scale population genomic data, ecological niche modeling, and fossil-calibrated phylogenies would help distinguish between these mechanisms by clarifying patterns of historical connectivity, demographic changes, and potential environmental barriers.

4.2. Blurry species boundaries due to rapid speciation and interspecific hybridization

At the species level, our analyses identified multiple instances of paraphyly and polyphyly, underscoring ongoing challenges in delimiting species within *Calibrachoa* and highlighting the need for taxonomic revisions. For instance, *C. linoides* subsp. *linoides* and *C. linoides* subsp. *furcata* are currently treated as subspecies, but they were consistently recovered in independent lineages, suggesting they are not valid subspecies and instead represent multiple lineages. *Calibrachoa linoides* subsp. *linoides* appears at least as three independent lineages and *C. linoides* subsp. *furcata* as two independent lineages (Fig. 2). Similarly, *C. pubescens* and *C. ovalifolia* were recovered as polyphyletic. While *C. pubescens* appears in two different clades (two individuals grouped with *C. felipponei* and the other three with the HTL complex), individuals of *C. ovalifolia* appeared throughout the phylogeny, and conspecific individuals seldom were recovered with reciprocal monophyly. At first glance, the phylogenetic positioning of *C. ovalifolia* could be seen as the result of extensive hybridization, but the lack of excessive allele sharing in our *f*-branch test (Fig. S7) implied that it may represent multiple different species. Further complicating its phylogenetic placement, *C. ovalifolia* was recovered external to major clades (oval271, oval55) or nested within otherwise monophyletic species clades (oval29 among *C. irgangiana*; oval321 among *C.* sp1), but with low support at most nodes (Fig. 2). These findings reflect the taxonomic complexity of *Calibrachoa*, where low morphological differentiation among species presents challenges for their delimitation.

Another convoluted case involves the HTL complex, which forms a highly supported clade (Fig. 2) despite morphological variation. Within this group, the presence of white-flowered individuals (*C. humilis*; Fig. 1B) alongside purple-flowered individuals (*C. thymifolia* and *C. linearis*; Fig. 1C) suggests a degree of floral plasticity potentially linked to ecological adaptation. Backes et al. (2023) correlated vegetative trait variation in *C. linearis* and *C. thymifolia* with ecological niches, implying phenotypic plasticity as a driver of local adaptation. Our findings extend this idea, highlighting the potential role of reproductive traits, such as flower color, in shaping adaptation within this species complex. Abiotic factors, such as soil nitrogen, may influence floral plasticity, affecting insect visitation and reproductive success (Majetic et al., 2017; reviewed in Narbona et al., 2021). In some plants, increased soil nitrogen alters anthocyanin synthesis, a key determinant of floral pigmentation (Rausher, 2008). While this mechanism has been observed in many taxa, it does not appear to influence flower color in the closely related *Petunia* (Majetic et al., 2017). In *Nicotiana mutabilis*, another Solanaceae species, color transitions from white to pink during flower senescence have been linked to chalcone synthase upregulation (Macnish et al., 2010). Furthermore, in the tribe Petunieae, to which *Calibrachoa* belongs, floral pigmentation is regulated by MYB anthocyanin activators (Wheeler et al., 2022). This suggests that changes in flower color in *Calibrachoa* could arise from shifts in gene expression rather than substitutions in coding regions, enabling rapid and independent color changes. Future studies are needed to confirm whether this mechanism operates in *Calibrachoa* and, more specifically, in the HTL complex.

The phylogeny also revealed that *C. excellens* and *C. atropurpurea* do not form distinct, monophyletic groups (Fig. 2). Instead, individuals from both taxa are intermingled within the same clades, suggesting that these species represent multiple lineages, but not the way they are presently circumscribed. Since we did not find evidence for hybridization between these lineages in the *f*-branch test (Fig. S7), this pattern may reflect a cryptic diversity within and between these taxa or operational biases such as misidentification. Originally, *C. atropurpurea* was interpreted as a putative subspecies of *C. excellens* (Fregonezi et al., 2013, 2012; Mäder and Freitas, 2019; Stehmann, 1999), but further morphological examination circumscribed it as a separate species (Stehmann et al., 2022). The results we recovered here reflect the difficulty in identifying in the field these species that represent independent evolutionary lineages but show strong morphological resemblance (Stehmann, 1999; Stehmann et al., 2022).

We also included a putative undescribed species, *C.* sp. 1, to evaluate its phylogenetic placement and potential relationships with other wild taxa. It was initially thought to represent a new evolutionary lineage due to its morphological differentiation compared to other species in the subgenus (Mäder and Freitas, 2019). The clade containing *C.* sp. 1 showed low support, suggesting some uncertainty regarding its distinctiveness or evolutionary history. As in Mäder and Freitas (2019), *C.* sp. 1 was recovered as phylogenetically related to *C. missionica*. Interestingly, this clade also includes individuals of *C. longistyla* and *C. ovalifolia*, with *C.* sp. 1 nested among them. These results raise the possibility that *C.* sp. 1 may not represent a distinct species, but rather could belong to *C. longistyla*, indicating a potential lineage within this species.

It is well established that closely related species, particularly those in rapidly radiating groups, exhibit a higher propensity for hybridization (Abbott et al., 2013; Gourbière and Mallet, 2010). Hybridization has already been documented in *Calibrachoa* (Pezzi et al., 2024). However, unlike *Petunia,* where studies focusing on microevolutionary processes have demonstrated the extent of historical and ongoing hybridization (e.g., Caballero-Villalobos et al., 2021; Giudicelli et al., 2024; Pezzi et al., 2022), microevolutionary and population genetics studies in *Calibrachoa* remain scarce. Evidence of hybridization was revealed in our *f*-branch tests (Fig. 4 and Fig. S7), where we identified hybrid individuals, such as thym66, grouping with *C. heterophylla* but exhibiting clear genetic signals of admixture with the HTL clade. Another individual identified as *C. thymifolia*, thym57, was grouped with *C. pubescens* and showed excess allele sharing with the HTL clade. Such hybrids highlight the dynamic evolutionary processes within *Calibrachoa,* where interspecific gene

flow blurs species boundaries and adds to phylogenetic instability.

4.3. Novel insights into the origin of commercial cultivars

Although *Calibrachoa* is widely popular in horticulture, the genetic origins of its commercial cultivars and the wild species involved in their development have remained unclear. Our results provide novel insights into this question, supporting the original hypothesis that the commercial cultivar resulted from interspecific hybridization between species within the *Stimomphis* subgenus (Kanaya et al., 2010). The cultivated individuals were present in two distinct clades, each with high support, indicating hybrid origins involving at least *C. sendtneriana* and *C. sellowiana* (Fig. 2). Since *C. sendtneriana* has red flowers and is primarily hummingbird-pollinated—traits that deviate from the white and purple flowers and bee-pollination typical of most *Calibrachoa* species—it is plausible that hybridization involving this species was artificially facilitated during cultivar development. While this reasoning aligns with patterns seen in closely related genera such as *Petunia* (Bombarely et al., 2016; Segatto et al., 2014), we cannot entirely dismiss the possibility that natural hybridization plays a role in this process.

The results from *f*-branch analyses further support the hypothesis of a single domestication event, evidenced by the excess allele sharing among cultivated individuals in distinct clades (Fig. 4). However, these results may also reflect ongoing admixture between cultivated and wild populations, as the commercial cultivar we sequenced was purchased in a city located within the native range of the genus. Although this cultivar was obtained from flower shops and not sampled from naturalized populations, we cannot discard the possibility that some genetic introgression from local wild species has occurred historically or during recent cultivation. Repeated admixture among cultivated plants and wild populations could obscure a more precise delineation between single versus multiple domestication events, as observed in other ornamental species (Altman et al., 2022; Zang et al., 2023). Including a broader sample of cultivars from regions outside the native range of *Calibrachoa* would provide additional resolution into their evolutionary history and clarify the extent of human-mediated gene flow.

In addition to genetic analyses, the morphological variation observed in cultivated individuals warrants further investigation. For instance, the yellow flowers presented by the cultivar we sampled (Fig. 1G–H) represent a notable divergence from the purple and white flowers characteristic of most wild *Calibrachoa* species, as well as the red flowers of *C. sendtneriana*, one of their parental species. This variation likely arises from intensive artificial selection, as has been documented in *Petunia* (Chen et al., 2007). Since a few loci with large effects control flower color in related genera (Esfeld et al., 2018; but see Berardi et al., 2021), similar mechanisms may operate in *Calibrachoa*. The colors found in commercial cultivars are absent in wild species, despite several wild species displaying dark rings or yellow patches. This suggests that regulation beyond differentiated gene expression still needs to be elucidated.

Finally, the potential for admixture or gene introgression from domesticated cultivars into wild populations underscores a critical evolutionary dynamic between horticultural practices and natural ecosystems (Purugganan, 2019). Such gene flow could influence local adaptation, alter ecological interactions, and impact genetic diversity within *Calibrachoa*. Investigating these processes will provide valuable insights into how domestication and cultivation practices affect the evolutionary trajectories of both cultivated and wild species, offering a deeper understanding of the interplay between human activity and plant evolution.

5. Conclusions

This study provides the first comprehensive phylogeny for *Calibrachoa*, encompassing all currently recognized taxa with multiple individuals and genomic data, while including a putative new species and commercial cultivars. Previous research has highlighted the challenges in resolving species relationships within the genus, owing to its recent diversification, high levels of ILS, and extensive hybridization, and our study encountered similar difficulties. Despite a robust dataset and extensive sampling, we found low support for some nodes and observed several cases of paraphyly and polyphyly. These findings suggest that specific genomic regions may play a critical role in speciation and diversification within the genus (i.e., speciation genes) that could be further investigated once whole-genome sequences are available for multiple species. Furthermore, our results confirm that hybridization is a significant evolutionary force in the group and that the commercial cultivar resulted from the hybridization between *C. sendtneariana* and *C. sellowiana*. Despite these challenges, our study makes significant contributions to the systematics of the genus, flagging taxa that require further investigation to clarify their taxonomic status.

CRediT authorship contribution statement

Alice Backes: Investigation, Methodology, Writing – review & editing. **Leonardo T. Gonçalves:** Data curation, Formal Analysis, Methodology, Software, Writing – original draft, Writing – review & editing. **Pedro H. Pezzi:** Formal Analysis, Methodology, Software, Writing – original draft, Writing – review & editing. **Loreta B. Freitas:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Acknowledgments

This work was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), CAPES (Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), and PPGBM-UFRGS (Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul). AB was supported by a CAPES Ph.D. scholarship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ympev.2025.108405.

References

Abbott, R., Albach, D., Ansell, S., Arntzen, J.W., Baird, S.J.E., Bierne, N., Boughman, J., Brelsford, A., Buerkle, C.A., Buggs, R., Butlin, R.K., Dieckmann, U., Eroukhmanoff, F., Grill, A., Cahan, S.H., Hermansen, J.S., Hewitt, G., Hudson, A.G., Jiggins, C., Jones, J., Keller, B., Marczewski, T., Mallet, J., Martinez-Rodriguez, P., Möst, M., Mullen, S., Nichols, R., Nolte, A.W., Parisod, C., Pfennig, K., Rice, A.M., Ritchie, M.G., Seifert, B., Smadja, C.M., Stelkens, R., Szymura, J.M., Väinölä, R., Wolf, J.B.W., Zinner, D., 2013. Hybridization and speciation. J. Evol. Biol. 26, 229–246. https://doi.org/10.1111/j.1420-9101.2012.02599.x.

Altman, A., Shennan, S., Odling-Smee, J., 2022. Ornamental plant domestication by aesthetics-driven human cultural niche construction. Trends Plant Sci. 27, 124–138. https://doi.org/10.1016/j.tplants.2021.09.004.

Backes, A., Mäder, G., Turchetto, C., Segatto, A.L., Fregonezi, J.N., Bonatto, S.L., Freitas, L.B., 2019. How diverse can rare species be on the margins of genera distribution? AoB Plants 11, plz037. https://doi.org/10.1093/aobpla/plz037.

Backes, A., Pezzi, P.H., Gonçalves, L.T., Greppi, J.A., Freitas, L.B., 2023. Integrating morphology, niche modelling, and molecular data to disentangle taxonomic challenges in a species complex of *Calibrachoa* (Solanaceae). Plant Ecol. Divers. 16, 165–177. https://doi.org/10.1080/17550874.2023.2274842.

Berardi, A.E., Esfeld, K., Jäggi, L., Mandel, T., Cannarozzi, G.M., Kuhlemeier, C., 2021. Complex evolution of novel red floral color in *Petunia*. Plant Cell 33, 2273–2295. https://doi.org/10.1093/plcell/koab114.

Bombarely, A., Moser, M., Amrad, A., Bao, M., Bapaume, L., Barry, C.S., Bliek, M., Boersma, M.R., Borghi, L., Bruggmann, R., Bucher, M., D'Agostino, N., Davies, K., Druege, U., Dudareva, N., Egea-Cortines, M., Delledonne, M., Fernandez-Pozo, N., Franken, P., Grandont, L., Heslop-Harrison, J.S., Hintzsche, J., Johns, M., Koes, R., Lv, X., Lyons, E., Malla, D., Martinoia, E., Mattson, N.S., Morel, P., Mueller, L.A., Muhlemann, J., Nouri, E., Passeri, V., Pezzotti, M., Qi, Q., Reinhardt, D., Rich, M., Richert-Pöggeler, K.R., Robbins, T.P., Schatz, M.C., Schranz, M.E., Schuurink, R.C., Schwarzacher, T., Spelt, K., Tang, H., Urbanus, S.L., Vandenbussche, M., Vijverberg, K., Villarino, G.H., Warner, R.M., Weiss, J., Yue, Z., Zethof, J., Quattrocchio, F., Sims, T.L., Kuhlemeier, C., 2016. Insight into the evolution of the

Solanaceae from the parental genomes of *Petunia hybrida*. Nat. Plants 2, 16074. https://doi.org/10.1038/nplants.2016.74.
Bryant, D., Moulton, V., 2004. Neighbor-net: an agglomerative method for the construction of phylogenetic networks. Mol. Biol. Evol. 21, 255–265. https://doi.org/10.1093/molbev/msh018.
Caballero-Villalobos, L., Silva-Arias, G.A., Turchetto, C., Giudicelli, G.C., Petzold, E., Bombarely, A., Freitas, L.B., 2021. Neutral and adaptive genomic variation in hybrid zones of two ecologically diverged *Petunia* species (Solanaceae). Bot. J. Linn 196, 100–122. https://doi.org/10.1093/botlinnean/boaa091.
Catchen, J., Hohenlohe, P.A., Bassham, S., Amores, A., Cresko, W.A., 2013. Stacks: an analysis tool set for population genomics. Mol. Ecol. 22, 3124–3140. https://doi.org/10.1111/mec.12354.
Chen, S., Matsubara, K., Kokubun, H., Kodama, H., Watanabe, H., Marchesi, E., Ando, T., 2007. Reconstructing Historical events that Occurred in the *Petunia* Hf1 Gene, which Governs Anthocyanin Biosynthesis, and Effects of Artificial selection by Breeding. Breed. Sci. 57, 203–211. https://doi.org/10.1270/jsbbs.57.203.
Cruz, V.M.V., Kilian, A., Dierig, D.A., 2013. Development of DArT Marker Platforms and Genetic Diversity Assessment of the U.S. Collection of the New Oilseed Crop Lesquerella and Related Species. PLoS One 8, e64062. https://doi.org/10.1371/journal.pone.0064062.
Danecek, P., Auton, A., Abecasis, G., Albers, C.A., Banks, E., DePristo, M.A., Handsaker, R.E., Lunter, G., Marth, G.T., Sherry, S.T., McVean, G., Durbin, R., 1000 Genomes Project Analysis Group, 2011. The variant call format and VCFtools. Bioinformatics 27, 2156–2158. https://doi.org/10.1093/bioinformatics/btr330.
Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O., Whitwham, A., Keane, T., McCarthy, S.A., Davies, R.M., Li, H., 2021. Twelve years of SAMtools and BCFtools. GigaScience 10, giab008. https://doi.org/10.1093/gigascience/giab008.
De La Torre-Bárcena, J.E., Kolokotronis, S.-O., Lee, E.K., Stevenson, D.W., Brenner, E.D., Katari, M.S., Coruzzi, G.M., DeSalle, R., 2009. The Impact of Outgroup Choice and Missing Data on Major seed Plant Phylogenetics using Genome-Wide EST Data. PLoS One 4, e5764.
De Queiroz, K., 2007. Species Concepts and Species Delimitation. Syst. Biol. 56, 879–886. https://doi.org/10.1080/10635150701701083.
Donoghue, M.J., 2008. A phylogenetic perspective on the distribution of plant diversity. Proc. Natl. Acad. Sci. U.S.A. 105, 11549–11555. https://doi.org/10.1073/pnas.0801962105.
Escudero, M., Valcárcel, V., Vargas, P., Luceño, M., 2009. Significance of ecological vicariance and long-distance dispersal in the diversification of *Carex* sect. *Spirostachyae* (Cyperaceae). Am. J. Bot. 96, 2100–2114. https://doi.org/10.3732/ajb.0900134.
Esfeld, K., Berardi, A.E., Moser, M., Bossolini, E., Freitas, L., Kuhlemeier, C., 2018. Pseudogenization and Resurrection of a Speciation Gene. Curr. Biol. 28, 3776–3786. e7. https://doi.org/10.1016/j.cub.2018.10.019.
Fark, S.N., Gerber, S., Alonzo, S.H., Kindsvater, H.K., Meier, J.I., Seehausen, O., 2022. Multispecies colour polymorphisms associated with contrasting microhabitats in two Mediterranean wrasse radiations. J. Evol. Biol. 35, 633–647. https://doi.org/10.1111/jeb.13999.
Folk, R.A., Soltis, P.S., Soltis, D.E., Guralnick, R., 2018. New prospects in the detection and comparative analysis of hybridization in the tree of life. American J of Botany 105, 364–375. https://doi.org/10.1002/ajb2.1018.
Fregonezi, J.N., Freitas, L.B., Bonatto, S.L., Semir, J., Stehmann, J.R., 2012. Infrageneric classification of *Calibrachoa* (Solanaceae) based on morphological and molecular evidence. Taxon 61, 120–130. https://doi.org/10.1002/tax.611009.
Fregonezi, J.N., Turchetto, C., Bonatto, S.L., Freitas, L.B., 2013. Biogeographical history and diversification of *Petunia* and *Calibrachoa* (Solanaceae) in the Neotropical Pampas grassland: Species diversification in the Pampas. Bot. J. Linn. Soc. 171, 140–153. https://doi.org/10.1111/j.1095-8339.2012.01292.x.
Fulton, T.L., Strobeck, C., 2010. Multiple markers and multiple individuals refine true seal phylogeny and bring molecules and morphology back in line. Proc. R. Soc. B 277, 1065–1070. https://doi.org/10.1098/rspb.2009.1783.
Garrison, E., Marth, G., 2012. Haplotype-Based Variant Detection from Short-Read Sequencing. https://doi.org/10.48550/ARXIV.1207.3907.
Giudicelli, G.C., Pezzi, P.H., Guzmán-Rodriguez, S., Turchetto, C., Bombarely, A., Freitas, L.B., 2024. Historical and ongoing hybridisation in Southern south American grassland species. Sci. Rep. 14, 27989. https://doi.org/10.1038/s41598-024-79584-9.
Goulet, B.E., Roda, F., Hopkins, R., 2017. Hybridization in Plants: Old ideas. New Techniques. Plant Physiol. 173, 65–78. https://doi.org/10.1104/pp.16.01340.
Gourbière, S., Mallet, J., 2010. Are Species real? the Shape of the Species Boundary with Exponential failure, Reinforcement, and the "Missing Snowball.". Evol. 64, 1–24. https://doi.org/10.1111/j.1558-5646.2009.00844.x.
Guillaume, L., Hervé, P., Vân, L.H.L., Hervé, L.G., 1993. Species Sampling has a Major Impact on Phylogenetic Inference. Mol. Phylogenet. Evol. 2, 205–224. https://doi.org/10.1006/mpev.1993.1021.
Hoang, D.T., Chernomor, O., Von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: improving the Ultrafast Bootstrap Approximation. Mol. Biol. Evol. 35, 518–522. https://doi.org/10.1093/molbev/msx281.
Hu, H., Sun, P., Yang, Y., Ma, J., Liu, J., 2023. Genome-scale angiosperm phylogenies based on nuclear, plastome, and mitochondrial datasets. J. Integr. Plant Biol. 65, 1479–1489. https://doi.org/10.1111/jipb.13455.
Huang, J., Xu, W., Zhai, J., Hu, Y., Guo, J., Zhang, C., Zhao, Y., Zhang, L., Martine, C., Ma, H., Huang, C.-H., 2023. Nuclear phylogeny and insights into whole-genome duplications and reproductive development of Solanaceae plants. Plant Commun. 4, 100595. https://doi.org/10.1016/j.xplc.2023.100595.
Huson, D.H., Bryant, D., 2024. The SplitsTree App: interactive analysis and visualization using phylogenetic trees and networks. Nat. Methods 21, 1773–1774. https://doi.org/10.1038/s41592-024-02406-3.
John, A.L.D.W., Mäder, G., Fregonezi, J.N., Freitas, L.B., 2019. Genetic diversity and population structure of naturally rare *Calibrachoa* species with small distribution in southern Brazil. Genet. Mol. Biol. 42, 108–119. https://doi.org/10.1590/1678-4685-gmb-2017-0314.
Jombart, T., Ahmed, I., 2011. *adegenet 1.3-1*: new tools for the analysis of genome-wide SNP data. Bioinformatics 27, 3070–3071. https://doi.org/10.1093/bioinformatics/btr521.
Kanaya, T., Kokubun, H., Watanabe, H., Hashimoto, G., Marchesi, E., Bullrich, L., Ando, T., 2010. Flowering ability of commercial *Calibrachoa* cultivars as compared to that of natural species. Sci. Hortic. 126, 276–283. https://doi.org/10.1016/j.scienta.2010.07.013.
Kilian, A., Wenzl, P., Huttner, E., Carling, J., Xia, L., Blois, H., Caig, V., Heller-Uszynska, K., Jaccoud, D., Hopper, C., Aschenbrenner-Kilian, M., Evers, M., Peng, K., Cayla, C., Hok, P., Uszynski, G., 2012. Diversity Arrays Technology: A Generic Genome Profiling Technology on Open Platforms, in: Pompanon, F., Bonin, A. (Eds.), Data Production and Analysis in Population Genomics, Methods in Molecular Biology. Humana Press, Totowa, NJ, pp. 67–89. Doi: 10.1007/978-1-61779-870-2_5.
Kleinkopf, J.A., Roberts, W.R., Wagner, W.L., Roalson, E.H., 2019. Diversification of hawaiian *Cyrtandra* (Gesneriaceae) under the influence of incomplete lineage sorting and hybridization. J. Syst. Evol. 57, 561–578. https://doi.org/10.1111/jse.12519.
Lewis, P.O., 2001. A Likelihood Approach to estimating Phylogeny from Discrete Morphological Character Data. Syst. Biol. 50, 913–925. https://doi.org/10.1080/106351501753462876.
Li, H., Durbin, R., 2010. Fast and accurate long-read alignment with Burrows–Wheeler transform. Bioinformatics 26, 589–595. https://doi.org/10.1093/bioinformatics/btp698.
Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., 1000 Genome Project Data Processing Subgroup, 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics 25, 2078–2079. https://doi.org/10.1093/bioinformatics/btp352.
Liu, L., Xi, Z., Davis, C.C., 2015. Coalescent Methods are Robust to the Simultaneous Effects of Long Branches and Incomplete Lineage sorting. Mol. Biol. Evol. 32, 791–805. https://doi.org/10.1093/molbev/msu331.
Luu, K., Bazin, E., Blum, M.G.B., 2017. *pcadapt*: an R package to perform genome scans for selection based on principal component analysis. Mol. Ecol. Res. 17, 67–77. https://doi.org/10.1111/1755-0998.12592.
Macnish, A.J., Jiang, C.-Z., Negre-Zakharov, F., Reid, M.S., 2010. Physiological and molecular changes during opening and senescence of *Nicotiana mutabilis* flowers. Plant Sci. 179, 267–272. https://doi.org/10.1016/j.plantsci.2010.05.011.
Maddison, W.P., 1997. Gene Trees in Species Trees. Syst. Biol. 46, 523–536. https://doi.org/10.1093/sysbio/46.3.523.
Maddison, W.P., Knowles, L.L., 2006. Inferring Phylogeny despite Incomplete Lineage sorting. Syst. Biol. 55, 21–30. https://doi.org/10.1080/10635150500354928.
Mäder, G., Fregonezi, J.N., Lorenz-Lemke, A.P., Bonatto, S.L., Freitas, L.B., 2013. Geological and climatic changes in quaternary shaped the evolutionary history of *Calibrachoa heterophylla*, an endemic South-Atlantic species of petunia. BMC Evol. Biol. 13, 178. https://doi.org/10.1186/1471-2148-13-178.
Mäder, G., Freitas, L.B., 2019. Biogeographical, ecological, and phylogenetic analyses clarifying the evolutionary history of *Calibrachoa* in South American grasslands. Mol. Phylogenet. Evol. 141, 106614. https://doi.org/10.1016/j.ympev.2019.106614.
Maestre, F.T., Benito, B.M., Berdugo, M., Concostrina-Zubiri, L., Delgado-Baquerizo, M., Eldridge, D.J., Guirado, E., Gross, N., Kéfi, S., Le Bagousse-Pinguet, Y., Ochoa-Hueso, R., Soliveres, S., 2021. Biogeography of global drylands. New Phytol. 231, 540–558. https://doi.org/10.1111/nph.17395.
Majetic, C.J., Fetters, A.M., Beck, O.M., Stachnik, E.F., Beam, K.M., 2017. *Petunia* floral trait plasticity in response to soil nitrogen content and subsequent impacts on insect visitation. Flora 232, 183–193. https://doi.org/10.1016/j.flora.2016.08.002.
Malinsky, M., Matschiner, M., Svardal, H., 2021. Dsuite - Fast *D*-statistics and related admixture evidence from VCF files. Mol. Ecol. Res. 21, 584–595. https://doi.org/10.1111/1755-0998.13265.
Malinsky, M., Svardal, H., Tyers, A.M., Miska, E.A., Genner, M.J., Turner, G.F., Durbin, R., 2018. Whole-genome sequences of Malawi cichlids reveal multiple radiations interconnected by gene flow. Nat. Ecol. Evol. 2, 1940–1955. https://doi.org/10.1038/s41559-018-0717-x.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. Embnet J. 17, 10. https://doi.org/10.14806/ej.17.1.200.
Mayr, E., 1942. Systematics and the Origin of Species. Columbia University Press, New York, NY, USA.
Miner, B.G., Sultan, S.E., Morgan, S.G., Padilla, D.K., Relyea, R.A., 2005. Ecological consequences of phenotypic plasticity. Trends Ecol. Evol. 20, 685–692. https://doi.org/10.1016/j.tree.2005.08.002.
Minh, B.Q., Hahn, M.W., Lanfear, R., 2020. New Methods to Calculate Concordance Factors for Phylogenomic Datasets. Mol. Biol. Evol. 37, 2727–2733. https://doi.org/10.1093/molbev/msaa106.
Morris, J.L., Puttick, M.N., Clark, J.W., Edwards, D., Kenrick, P., Pressel, S., Wellman, C. H., Yang, Z., Schneider, H., Donoghue, P.C.J., 2018. The timescale of early land plant evolution. Proc. Natl. Acad. Sci. U.S.A. 115. https://doi.org/10.1073/pnas.1719588115.
Murakami, Y., Fukui, Y., Watanabe, H., Kokubun, H., Toya, Y., Ando, T., 2004. Floral coloration and pigmentation in *Calibrachoa* cultivars. J. Hortic. Sci. Biotechnol. 79, 47–53. https://doi.org/10.1080/14620316.2004.11511735.
Murillo-A., J., Valencia-D., J., Orozco, C.I., Parra-O., C., Neubig, K.M., 2022. Incomplete lineage sorting and reticulate evolution mask species relationships in Brunelliaceae,

an Andean family with rapid, recent diversification. Am. J. Bot. 109, 1139–1156. Doi: 10.1002/ajb2.16025.
Nabhan, A.R., Sarkar, I.N., 2012. The impact of taxon sampling on phylogenetic inference: a review of two decades of controversy. Brief. Bioinform. 13, 122–134. https://doi.org/10.1093/bib/bbr014.
Naciri, Y., Linder, H.P., 2015. Species delimitation and relationships: the dance of the seven veils. Taxon 64, 3–16. https://doi.org/10.12705/641.24.
Narbona, E., Arista, M., Whittall, J.B., Camargo, M.G.G., Shrestha, M., 2021. Editorial: the Role of Flower Color in Angiosperm Evolution. Front. Plant Sci. 12, 736998. https://doi.org/10.3389/fpls.2021.736998.
Nixon, K.C., Wheeler, Q.D., 1990. An Amplification of the Phylogenetic Species Concept. Cladistics 6, 211–223. https://doi.org/10.1111/j.1096-0031.1990.tb00541.x.
Olmstead, R.G., 2013. Phylogeny and biogeography in Solanaceae, Verbenaceae and Bignoniaceae: a comparison of continental and intercontinental diversification patterns. Bot. J. Linn. Soc. 171, 80–102. https://doi.org/10.1111/j.1095-8339.2012.01306.x.
One Thousand Plant Transcriptomes Initiative, 2019. One thousand plant transcriptomes and the phylogenomics of green plants. Nature 574, 679–685. https://doi.org/10.1038/s41586-019-1693-2.
Patterson, N., Moorjani, P., Luo, Y., Mallick, S., Rohland, N., Zhan, Y., Genschoreck, T., Webster, T., Reich, D., 2012. Ancient Admixture in Human history. Genetics 192, 1065–1093. https://doi.org/10.1534/genetics.112.145037.
Pezzi, P.H., Guzmán-Rodriguez, S., Giudicelli, G.C., Turchetto, C., Bombarely, A., Freitas, L.B., 2022. A convoluted tale of hybridization between two *Petunia* species from a transitional zone in South America. Perspect. Pl. Ecol. Evol. Syst. 56, 125688. https://doi.org/10.1016/j.ppees.2022.125688.
Pezzi, P.H., Wheeler, L.C., Freitas, L.B., Smith, S.D., 2024. Incomplete lineage sorting and hybridization underlie tree discordance in *Petunia* and related genera (Petunieae, Solanaceae). Mol. Phylogenet. Evol. 198, 108136. https://doi.org/10.1016/j.ympev.2024.108136.
Purugganan, M.D., 2019. Evolutionary Insights into the Nature of Plant Domestication. Curr. Biol. 29, R705–R714. https://doi.org/10.1016/j.cub.2019.05.053.
Purvis, A., Gittleman, J.L., Brooks, T.M., 2005. Phylogeny and conservation. Cambridge University Press, Cambridge, UK.
Qi, S., Twyford, A.D., Ding, J., Borrell, J.S., Wang, L., Ma, Y., Wang, N., 2022. Natural interploidy hybridization among the key taxa involved in the origin of horticultural chrysanthemums. J. Syst. Evol. 60, 1281–1290. https://doi.org/10.1111/jse.12810.
Rausher, M.D., 2008. Evolutionary Transitions in Floral Color. Int. J. Plant Sci. 169, 7–21. https://doi.org/10.1086/523358.
Raymond, O., Gouzy, J., Just, J., Badouin, H., Verdenaud, M., Lemainque, A., Vergne, P., Moja, S., Choisne, N., Pont, C., Carrère, S., Caissard, J.-C., Couloux, A., Cottret, L., Aury, J.-M., Szécsi, J., Latrasse, D., Madoui, M.-A., François, L., Fu, X., Yang, S.-H., Dubois, A., Piola, F., Larrieu, A., Perez, M., Labadie, K., Perrier, L., Govetto, B., Labrousse, Y., Villand, P., Bardoux, C., Boltz, V., Lopez-Roques, C., Heitzler, P., Vernoux, T., Vandenbussche, M., Quesneville, H., Boualem, A., Bendahmane, A., Liu, C., Le Bris, M., Salse, J., Baudino, S., Benhamed, M., Wincker, P., Bendahmane, M., 2018. The *Rosa* genome provides new insights into the domestication of modern roses. Nat. Genet. 50, 772–777. https://doi.org/10.1038/s41588-018-0110-3.
Rice, G., 1997. Petunias: a garden paradigm. Garden 122, 390–393.
Rodrigues, A.S.B., Martins, A., Garcia, C.A., Sérgio, C., Porley, R., Fontinha, S., González-Mancebo, J., Gabriel, R., Phephu, N., Van Rooy, J., Dirkse, G., Long, D., Stech, M., Patiño, J., Sim-Sim, M., 2020. Climate-driven vicariance and long-distance dispersal explain the Rand Flora pattern in the liverwort *Exormotheca pustulosa* (Marchantiophyta). Biol. J. Linn. Soc. 130, 480–496. https://doi.org/10.1093/biolinnean/blaa071.
Roje, DM., 2014. Evaluating the Effects of Non-Neutral Molecular Markers on Phylogeny Inference. PLoS One 9, e87428. https://doi.org/10.1371/journal.pone.0087428.
Rokas, A., Carroll, S.B., 2005. More Genes or more Taxa? the Relative Contribution of Gene Number and Taxon Number to Phylogenetic Accuracy. Mol. Biol. Evol. 22, 1337–1344. https://doi.org/10.1093/molbev/msi121.
Rouhan, G., Gaudeul, M., 2014. Plant Taxonomy: A Historical Perspective, Current Challenges, and Perspectives, in: Besse, P. (Ed.), Molecular Plant Taxonomy, Methods in Molecular Biology. Humana Press, Totowa, NJ, pp. 1–37. Doi: 10.1007/978-1-62703-767-9_1.
Roy, A., Frascaria, N., MacKay, J., Bousquet, J., 1992. Segregating random amplified polymorphic DNAs (RAPDs) in *Betula alleghaniensis*. Theoret. Appl. Genetics 85–85, 173–180. https://doi.org/10.1007/BF00222856.
Särkinen, T., Bohs, L., Olmstead, R.G., Knapp, S., 2013. A phylogenetic framework for evolutionary study of the nightshades (Solanaceae): a dated 1000-tip tree. BMC Evol. Biol. 13, 214. https://doi.org/10.1186/1471-2148-13-214.
Segatto, A.L.A., Ramos-Fregonezi, A.M.C., Bonatto, S.L., Freitas, L.B., 2014. Molecular insights into the purple-flowered ancestor of garden petunias. Am. J. Bot. 101, 119–127. https://doi.org/10.3732/ajb.1300223.
Shaffer, H.B., Thomson, R.C., 2007. Delimiting Species in recent Radiations. Syst. Biol. 56, 896–906. https://doi.org/10.1080/10635150701772563.
Simpson, G.G., 1951. The Species Concept. Evolution 5, 285. https://doi.org/10.2307/2405675.
Smith, S.A., Beaulieu, J.M., Stamatakis, A., Donoghue, M.J., 2011. Understanding angiosperm diversification using small and large phylogenetic trees. Am. J. Bot. 98, 404–414. https://doi.org/10.3732/ajb.1000481.
Solís-Lemus, C., Ané, C., 2016. Inferring Phylogenetic Networks with Maximum Pseudolikelihood under Incomplete Lineage sorting. PLoS Genet. 12, e1005896. https://doi.org/10.1371/journal.pgen.1005896.
Soltis, P.S., Soltis, D.E., 2009. The Role of Hybridization in Plant Speciation. Annu. Rev. Plant Biol. 60, 561–588. https://doi.org/10.1146/annurev.arplant.043008.092039.
Stehmann, J.R., 1999. Estudos taxonômicos na tribo Nicotianeae G. Don (Solanaceae): revisão de *Petunia* Jussieu, das espécies brasileiras de *Calibrachoa* La Llave & Lexarza e o estabelecimento do novo gênero *Petuniopsis* Stehmann & Semir. UNICAMP, Brazil. Ph.D. Thesis.
Stehmann, J.R., Mäder, G., Freitas, L.B., 2022. Two New Species of Purple *Calibrachoa* (Solanaceae) from Southern Brazil Grasslands Revealed by Molecular and Morphological Data. Syst. Bot. 47, 242–250. https://doi.org/10.1600/036364422X16442668423563.
Stull, G.W., Soltis, P.S., Soltis, D.E., Gitzendanner, M.A., Smith, S.A., 2020. Nuclear phylogenomic analyses of asterids conflict with plastome trees and support novel relationships among major lineages. Am. J. Bot. 107, 790–805. https://doi.org/10.1002/ajb2.1468.
Swofford, D.L., 2003. PAUP*. Phylogenetic Analysis using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts. USA.
The Angiosperm Phylogeny Group, 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Bot. J. Linn. Soc. 181, 1–20. https://doi.org/10.1111/boj.12385.
Tietje, M., Antonelli, A., Baker, W.J., Govaerts, R., Smith, S.A., Eiserhardt, W.L., 2022. Global variation in diversification rate and species richness are unlinked in plants. Proc. Natl. Acad. Sci. U.S.A. 119, e2120662119. https://doi.org/10.1073/pnas.2120662119.
Wang, Y., Luo, A., Dimitrov, D., Freckleton, R.P., Hao, Z., Wang, Z., 2025. Global biogeography and macroevolutionary patterns of angiosperm inflorescence types. New Phytol. nph.70196. https://doi.org/10.1111/nph.70196.
Wheeler, L.C., Walker, J.F., Ng, J., Deanna, R., Dunbar-Wallis, A., Backes, A., Pezzi, P.H., Palchetti, M.V., Robertson, H.M., Monaghan, A., Freitas, L.B., Barboza, G.E., Moyroud, E., Smith, S.D., 2022. Transcription Factors Evolve Faster than their Structural Gene Targets in the Flavonoid Pigment Pathway. Mol. Biol. Evol. 39, msac044. https://doi.org/10.1093/molbev/msac044.
Wheeler, Q.D., Platnick, N.I., 2000. The phylogenetic species concept (sensu Wheeler and Platnick). In: Wheeler, Q.D., Meier, R. (Eds.), Species Concepts and Phylogenetic Theory: A Debate. Columbia University Press, New York, NY, USA., pp. 55–69
Whitney, K.D., Ahern, J.R., Campbell, L.G., Albert, L.P., King, M.S., 2010. Patterns of hybridization in plants. Perspect. Pl. Ecol. Evol. Syst. 12, 175–182. https://doi.org/10.1016/j.ppees.2010.02.002.
Wiley, E.O., 1978. The Evolutionary Species Concept Reconsidered. Syst. Zool. 27, 17. https://doi.org/10.2307/2412809.
Wingett, S.W., Andrews, S., 2018. FastQ Screen: a tool for multi-genome mapping and quality control. F1000Res 7, 1338. https://doi.org/10.12688/f1000research.15931.2.
Yan, Z., Smith, M.L., Du, P., Hahn, M.W., Nakhleh, L., 2022. Species tree Inference Methods Intended to Deal with Incomplete Lineage sorting are Robust to the Presence of Paralogs. Syst. Biol. 71, 367–381. https://doi.org/10.1093/sysbio/syab056.
You, J., Lougheed, S.C., Zhao, Y., Zhang, G., Liu, W., Lu, F., Wang, Y., Zhang, W., Yang, J., Qiong, L., Song, Z., 2022. Comparative phylogeography study reveals introgression and incomplete lineage sorting during rapid diversification of *Rhodiola*. Ann. Bot. 129, 185–200. https://doi.org/10.1093/aob/mcab133.
Yu, Y., Ristic, N., Nakhleh, L., 2013. Fast algorithms and heuristics for phylogenomics under ILS and hybridization. BMC Bioinf. 14, S6. https://doi.org/10.1186/1471-2105-14-S15-S6.
Zang, F., Ma, Y., Wu, Q., Tu, X., Xie, X., Huang, P., Tong, B., Zheng, Y., Zang, D., 2023. Resequencing of *Rosa rugosa* accessions revealed the history of population dynamics, breed origin, and domestication pathways. BMC Plant Biol. 23, 235. https://doi.org/10.1186/s12870-023-04244-5.
Zuntini, A.R., Carruthers, T., Maurin, O., Bailey, P.C., Leempoel, K., Brewer, G.E., Epitawalage, N., Françoso, E., Gallego-Paramo, B., McGinnie, C., Negrão, R., Roy, S. R., Simpson, L., Toledo Romero, E., Barber, V.M.A., Botigué, L., Clarkson, J.J., Cowan, R.S., Dodsworth, S., Johnson, M.G., Kim, J.T., Pokorny, L., Wickett, N.J., Antar, G.M., DeBolt, L., Gutierrez, K., Hendriks, K.P., Hoewener, A., Hu, A.-Q., Joyce, E.M., Kikuchi, I.A.B.S., Larridon, I., Larson, D.A., De Lírio, E.J., Liu, J.-X., Malakasi, P., Przelomska, N.A.S., Shah, T., Viruel, J., Allnutt, T.R., Ameka, G.K., Andrew, R.L., Appelhans, M.S., Arista, M., Ariza, M.J., Arroyo, J., Arthan, W., Bachelier, J.B., Bailey, C.D., Barnes, H.F., Barrett, M.D., Barrett, R.L., Bayer, R.J., Bayly, M.J., Biffin, E., Biggs, N., Birch, J.L., Bogarín, D., Borosova, R., Bowles, A.M. C., Boyce, P.C., Bramley, G.L.C., Briggs, M., Broadhurst, L., Brown, G.K., Bruhl, J.J., Bruneau, A., Buerki, S., Burns, E., Byrne, M., Cable, S., Calladine, A., Callmander, M. W., Cano, Á., Cantrill, D.J., Cardinal-McTeague, W.M., Carlsen, M.M., Carruthers, A. J.A., De Castro Mateo, A., Chase, M.W., Chatrou, L.W., Cheek, M., Chen, S., Christenhusz, M.J.M., Christin, P.-A., Clements, M.A., Coffey, S.C., Conran, J.G., Cornejo, X., Couvreur, T.L.P., Cowie, I.D., Csiba, L., Darbyshire, I., Davidse, G., Davies, N.M.J., Davis, A.P., Van Dijk, K., Downie, S.R., Duretto, M.F., Duvall, M.R., Edwards, S.L., Eggli, U., Erkens, R.H.J., Escudero, M., De La Estrella, M., Fabriani, F., Fay, M.F., Ferreira, P.D.L., Ficinski, S.Z., Fowler, R.M., Frisby, S., Fu, L., Fulcher, T., Galbany-Casals, M., Gardner, E.M., German, D.A., Giaretta, A., Gibernau, M., Gillespie, L.J., González, C.C., Goyder, D.J., Graham, S.W., Grall, A., Green, L., Gunn, B.F., Gutiérrez, D.G., Hackel, J., Haevermans, T., Haigh, A., Hall, J.C., Hall, T., Harrison, M.J., Hatt, S.A., Hidalgo, O., Hodkinson, T.R., Holmes, G.D., Hopkins, H.C. F., Jackson, C.J., James, S.A., Jobson, R.W., Kadereit, G., Kahandawala, I.M., Kainulainen, K., Kato, M., Kellogg, E.A., King, G.J., Klejevskaja, B., Klitgaard, B.B., Klopper, R.R., Knapp, S., Koch, M.A., Leebens-Mack, J.H., Lens, F., Leon, C.J., Léveillé-Bourret, É., Lewis, G.P., Li, D.-Z., Li, L., Liede-Schumann, S., Livshultz, T., Lorence, D., Lu, M., Lu-Irving, P., Luber, J., Lucas, E.J., Luján, M., Lum, M., Macfarlane, T.D., Magdalena, C., Mansano, V.F., Masters, L.E., Mayo, S.J., McColl, K., McDonnell, A.J., McDougall, A.E., McLay, T.G.B., McPherson, H., Meneses, R.I., Merckx, V.S.F.T., Michelangeli, F.A., Mitchell, J.D., Monro, A.K.,

Moore, M.J., Mueller, T.L., Mummenhoff, K., Munzinger, J., Muriel, P., Murphy, D. J., Nargar, K., Nauheimer, L., Nge, F.J., Nyffeler, R., Orejuela, A., Ortiz, E.M., Palazzesi, L., Peixoto, A.L., Pell, S.K., Pellicer, J., Penneys, D.S., Perez-Escobar, O.A., Persson, C., Pignal, M., Pillon, Y., Pirani, J.R., Plunkett, G.M., Powell, R.F., Prance, G.T., Puglisi, C., Qin, M., Rabeler, R.K., Rees, P.E.J., Renner, M., Roalson, E. H., Rodda, M., Rogers, Z.S., Rokni, S., Rutishauser, R., De Salas, M.F., Schaefer, H., Schley, R.J., Schmidt-Lebuhn, A., Shapcott, A., Al-Shehbaz, I., Shepherd, K.A., Simmons, M.P., Simões, A.O., Simões, A.R.G., Siros, M., Smidt, E.C., Smith, J.F., Snow, N., Soltis, D.E., Soltis, P.S., Soreng, R.J., Sothers, C.A., Starr, J.R., Stevens, P. F., Straub, S.C.K., Struwe, L., Taylor, J.M., Telford, I.R.H., Thornhill, A.H., Tooth, I., Trias-Blasi, A., Udovicic, F., Utteridge, T.M.A., Del Valle, J.C., Verboom, G.A., Vonow, H.P., Vorontsova, M.S., De Vos, J.M., Al-Wattar, N., Waycott, M., Welker, C. A.D., White, A.J., Wieringa, J.J., Williamson, L.T., Wilson, T.C., Wong, S.Y., Woods, L.A., Woods, R., Worboys, S., Xanthos, M., Yang, Y., Zhang, Y.-X., Zhou, M.-Y., Zmarzty, S., Zuloaga, F.O., Antonelli, A., Bellot, S., Crayn, D.M., Grace, O.M., Kersey, P.J., Leitch, I.J., Sauquet, H., Smith, S.A., Eiserhardt, W.L., Forest, F., Baker, W.J., 2024. Phylogenomics and the rise of the angiosperms. Nature 629, 843–850. https://doi.org/10.1038/s41586-024-07324-0.