RESEARCH ARTICLE

Species boundaries within the recently diverged South American monocot-like *Eryngium* (Apiaceae, Saniculoideae) based on biogeographic, climatic, morphological, and molecular data: *E. cerradense*, a new species from Paraguay

Patricia Esquivel Mattos, Martina Fernández & Carolina I. Calviño

INIBIOMA, Conicet-Universidad Nacional del Comahue, Centro Regional Universitario Bariloche, Quintral 1250, 8400 Bariloche, Argentina

Addresses for correspondence: *Patricia Esquivel Mattos, patriciaesquivel@comahue-conicet.gob.ar*; *Carolina I. Calviño, ccalvino@comahue-conicet.gob.ar*

DOI https://doi.org/10.1002/tax.12889

Abstract The species of *Eryngium* with a monocot-like habit are among the most taxonomically complex. They show low phenotypic and molecular differentiation due to their recent, rapid, and reticulate speciation during the Quaternary period. In this study, we evaluate the boundaries of the monocot-like *Eryngium regnellii* and a closely related entity from the grasslands in the Cerrado of Paraguay. We integrated evidence from multiple data sources to test species delimitation: we estimated the phylogenetic position of both entities within *Eryngium* based on ITS nrDNA and cpDNA sequences; we carried out univariate and multivariate morphometric analyses to test statistically significant differences and to recognize morphological diagnostic characters; we also analyzed geographic distribution, habitat, and bioclimatic variables to evaluate divergence in environmental niche, and finally, we used Bayesian coalescent-based delimitation approaches to test genetic structure. Divergence in morphology, environmental niche, and genetic structure between *E. regnellii* and the lineage from Paraguay support the hypothesis of evolutionary independence among these two lineages. Therefore, based on an integrative approach, we recognize and describe a new species from Paraguay: *Eryngium cerradense*.

Keywords Cerrado; grasslands; Quaternary; speciation; Umbelliferae

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

■ INTRODUCTION

Plant speciation and radiations were frequent and ubiquitous during the Quaternary (Kadereit & Abbott, 2021) posing big challenges for species delimitation because recently diverged species usually display low levels of morphological and genetic differentiation. Moreover, when the time separating species divergence is short (e.g., in radiations), genetic drift is unlikely to fix loci before subsequent speciation events, and the resultant gene trees may not accurately reflect the phylogenetic history because of retention and sorting of ancestral polymorphisms (Knowles & Chan, 2008). Coalescent-based approaches improve phylogenetic inference and identification of species boundaries under recent and/or rapid speciation scenarios by taking into account processes of nucleotide substitution and also incomplete lineage sorting (Knowles & Carstens, 2007; Knowles & Chan, 2008).

Nonetheless, the exclusive use of genetics to delimit species is not reliable because even the coalescent-based methods available cannot distinguish within species population structure from lineages that have become isolated due to speciation events (Sukumaran & Knowles, 2017). Therefore, an integrative approach that incorporates evidence from multiple data sources (e.g., geography, environment, morphology, and genetics) is particularly necessary to establish species boundaries in recently diverged lineages (Dayrat, 2005; de Queiroz, 2007; Wiens, 2007; Padial & al., 2010; Fujita & al., 2012; Carstens & al., 2013; Sukumaran & Knowles, 2017).

Eryngium L. (Apiaceae, Saniculoideae) is the most speciose genus of umbellifers, with ca. 250 species. Establishing species boundaries within the genus is often difficult, and this has been attributed to its recent, rapid, and reticulate evolutionary history (Calviño & al., 2008, 2010). The most complex group of species to identify and delimit are unarguably the so-called monocot-like *Eryngium* (Esquivel Mattos & Calviño, 2022). These plants resemble monocotyledons in leaf morphology and plant architecture, as they possess a rosette of sessile, generally linear or subulate and parallel-veined leaves and a well-developed cauline axis, erect and with several internodes (Calviño & al., 2008). Many monocot-like *Eryngium* are polyploids and different ploidy levels may occur within a species (Bell & Constance, 1960, 1966; Constance & al., 1971, 1976; O'Leary & al., 2004; Perthuy & al., 2010). To date, molecular phylogenies are inconclusive as to

Article history: Received: 9 May 2022 | returned for (first) revision: 2 Aug 2022 | (last) revision received: 3 Feb 2023 | accepted: 9 Feb 2023 | published online: 10 Apr 2023 | **Associate Editor:** Levent Can |

the monophyly of this striking group (Calviño & al., 2008, 2010), comprising at least two different clades (i.e., South American monocot-like and North American monocot-like clades) that are part of a polytomy together with additional South American monocot-like species, and other *Eryngium* clades (e.g., Pacific clade; Calviño & al., 2008, 2010). The monocot-like *Eryngium* clades likely originated and diversified during the Late Pliocene and Quaternary (A.L. Padin & C.I. Calviño, unpub.), and speciation has, therefore, been not only rapid and likely reticulate, but also recent.

Within South America, the greatest diversity of monocot-like *Eryngium* is found in southern Brazil, northeastern Argentina, Uruguay, and Paraguay (Turmel, 1949). As part of our ongoing studies on *Eryngium*, we found populations in grasslands from the Cerrado biome in Paraguay that were unassignable to any known *Eryngium* species. The plants within these populations have subulate basal leaves with delicate setae in the margins, ovoid capitula with no prominent involucre, and fruits with lateral scales and dorsal papillae. Wolff (1913) classified all South American monocot-like *Eryngium* with subulate basal leaves in *E.* sect. *Paniculata* H.Wolff subsect. *Eupaniculata* H.Wolff. Wolff's (1913) treatment does not reflect phylogeny (Calviño & al., 2008, 2010), but is still the best framework to identify groups of morphologically similar species (Calviño & Levin, 2019). Within *E.* subsect. *Eupaniculata*, the plants collected in the Cerrado of Paraguay clearly fall within *E.* ser. *Latifolia* H.Wolff, which includes robust perennial herbs, 1–3 m tall, with wide (1 to 6 cm), spinose or setose basal leaves. Within this series, the putative new entity from Paraguay finds greatest morphological affinity with *E. regnellii* given their similarities in type of indumentum and fruit morphology. *Eryngium regnellii*, however, differs in habitat, consistency of the leaves, and length and density of indumentum (Fig. 1). This species has a conspicuous rosette of herbaceous, flaccid leaves and it is found in northeastern Argentina, central-west, southeastern and southern Brazil, and Uruguay, on rock outcrops associated with the Brazilian and the Tandilia highlands (Mathias & al., 1972; Calviño & Martínez, 2007, 2019; Martínez & Calviño, 2019; Cardozo & al., 2021).

The aim of this study was to test if the populations found in Paraguay conform to a species distinct from *Eryngium regnellii* using an integrative approach. We first estimated the phylogenetic position of the putative new species within the genus to corroborate if it falls within the same clade of South American monocot-like *Eryngium* as *E. regnellii*. Based on these results, we analyzed evidence from geographical distribution, climate, morphology, and molecular data from

Fig. 1. Habit and habitat of: **A**, *Eryngium* sp. nov.; **B**, *Eryngium regnellii* Malme. — Photos by P. Esquivel Mattos (A), Carolina I. Calviño (B).

E. regnellii and the putative new species that could be indicative of an independent evolution of these lineages, and of speciation. We generated distribution maps based on the revision of numerous herbarium collections. We applied univariate and multivariate statistical analyses of quantitative morphological characters to find diagnostic characteristics, and of climatic, and topographic variables, to explore differences in the environmental niche. We also applied Bayesian coalescent-based approaches to detect genetic structure. The results of all these analyses are discussed to explore the boundaries of *E. regnellii* and the putative new species from Paraguay.

■ MATERIALS AND METHODS

Taxon sampling and data. — Morphological and/or geographical data were obtained from herbarium specimens from BCRU, CTES, G, K, LP, MVFA, MVJB, NY, P, S, SI, UC and US and from specimens collected in the field (Appendix 1). These specimens represent the morphological and geographic variation of *Eryngium regnellii* and the putative new species and include the lectotype and other original material of *E. regnellii* (Calviño & Martínez, 2019). A total of 16 quantitative morphological characters (12 vegetative, 4 reproductive; Table 1) were measured in all available specimens of *E. regnellii* (N = 30) and of the putative new species (N = 6). All observations and measurements were made from dry material or from photographs. All individuals from each specimen were measured, when available. Leaf measurements were made on the basal leaves. Leaf indumentum was measured at the middle of each third of the lamina and at its base. All measurements of reproductive characters were taken on the terminal capitule of first- or second-order branches of the distal cyme of the main axis. In some cases, it was not possible to evaluate all of the character states because the material was either incomplete, the specimens were immature, or because it was not possible to make measurements from photographs. These missing values represent 17% of the total data, and for the multivariate analyses were replaced by mean-substitutions (i.e., by the mean of the available cases for the variable; El-Masri & Fox-Wasylyshyn, 2006). Morphological terminology follows the Systematics Association Committee for Descriptive Terminology (1962), Ellis & al. (2009), and Beentje (2010).

Bioclimatic and topographic data were obtained from Worldclim 2.1 database, including altitude and 19 biologically meaningful climatic variables derived from monthly temperature and rainfall values with a spatial resolution of 2.5 arcminutes (Table 2) (Fick & Hijmans, 2017). Geographic coordinates (latitude, longitude) were obtained directly from GPS data from the labels of the herbarium specimens, or the localities were georeferenced using the Google Earth Pro v.7.3.2 program (available at https://www.google.com/intl/en-419/earth/). All specimens that presented uncertain localities were excluded. As a result, 26 of the 36 available collections were included in the statistical studies related to the environment. Bioclimatic and topographic variables from each specimen's location were extracted using QGIS v.3.16.6-Hannover (GIS Development Team, 2021). Soil type was obtained from the labels of the herbarium specimens and from our own observations in the field.

For the molecular analyses, a total of 116 accessions of Saniculoideae were examined for cpDNA (*trnQ-rps16*, *rps16* intron, *rps16-trnK*^{UUU} 5′exon) and/or nrDNA ITS sequence variation. These accessions included 89 species of *Eryngium*, 10 species of *Sanicula*, and the monotypic *Petagnaea*. DNA sequences for four of these accessions were obtained specifically for this study and are reported here for the first time (Appendix 2); data for the remaining accessions were obtained from previous studies on Saniculoideae and *Eryngium* (Calviño & Downie, 2007; Calviño & al., 2008). To conduct coalescent-based species delimitation analyses, we also generated *trnG*^{GCC}*-trnS*^{GCU}, and/or *rpL32-trnL*^{UAG} (cpDNA) sequences for the accessions of *E. regnellii* and the putative new species (Appendix 2). Leaf material for DNA extraction was obtained from herbarium specimens or field collections. Total genomic DNA was obtained from about 20 mg of dried leaf tissue using Purelink Plant Total DNA Purification Kit (Invitrogen, Carlsbad, California, U.S.A.). The regions were PCR-amplified using specific primers and reactions according to Calviño & al. (2006, 2008) and Shaw & al. (2007). All sequencing was done using an ABI (Applied Biosystems, Foster City, California, United States) 3730XL high-throughput DNA capillary sequencer at Macrogen (Seoul, Korea). All cpDNA and ITS sequences obtained have been submitted to GenBank (Appendix 2).

Phylogenetic analyses. — New DNA sequences were edited, assembled and aligned manually with the *trnQ-trnK* and ITS data matrices used in Calviño & al. (2008). The resulting data matrix (available at http://hdl.handle.net/11336/190593) was analyzed using maximum parsimony (MP), Bayesian inference (BI), and maximum likelihood (ML) methods using the settings described in Calviño & al. (2008). All phylogenetic trees were rooted with *Petagnaea gussonei*, which is the sister genus of *Eryngium* plus *Sanicula* (Calviño & Downie, 2007).

Geographical analyses. — The geographic distributions of *Eryngium regnellii* and the putative new species were mapped using QGIS v.3.16.6-Hannover (GIS Development Team, 2021) to evaluate if the lineages occupy different biogeographic areas.

Climatic analyses. — To visualize how specimens grouped according to their climatic characteristics and to infer if *Eryngium regnellii* and the putative new species have different climatic niches, a principal component analysis (PCA) was performed with the 19 bioclimatic variables obtained from Worldclim database. Contributions of the bioclimatic variables to the PCA axes were interpreted as significant when $\geq|0.6|$. Correlations between climate and geographic latitude, longitude, and altitude were also explored by treating the geographic variables as illustrative (i.e., not participating in the

ordination) in the PCA of climatic data. Univariate analyses were also performed to identify the bioclimatic variables that differed between the groups found in the PCA of climatic data. For each bioclimatic variable, one-way analyses of variance (F-ANOVA) with post-hoc tests for homogeneous groups (Tukey's HSD) were performed for those variables with normal distribution. For variables without normal distribution, non-parametric Kruskal-Wallis (H-KW) was used, and multiple comparisons were performed by the method of Dunn. In both, Bonferroni corrections for multiple comparisons were included (Rice, 1989).

Before conducting all multivariate analyses, measurements were standardized. All statistical analyses were performed using the software SPAD v.5.5 (2012) and/or STATISTICA v.7.0 (StatSoft, 2014).

Morphometric analyses. — In order to evaluate differences in morphology between *Eryngium regnellii* and the putative new species, for each morphometric variable, one-way

Table 1. Morphometric variables and differences between *Eryngium regnellii* and the putative new species (*Eryngium* sp. nov.).

Morphometric variables	*E. regnellii*	*E.* sp. nov.	F-ANOVA	H-KW
1. Basal leaf (B.l.) length (cm)	71.68 ± 4.42 (38–119)	78.75 ± 6.33 (65–90)	0.27	
2. B.l. sheath length (cm)	4.94 ± 0.29 (3–9)	5.83 ± 0.31 (5–7)	1.92	
3. B.l. sheath width (cm)	1.36 ± 0.06 (1–2)	1.98 ± 0.27 (1.4–3)		5.73*
4. B.l. blade length (cm)	65.71 ± 4.30 (33–112)	72.5 ± 6.43 (59–84)	0.25	
5. B.l. blade width (cm)	1.07 ± 0.05 (0.8–1.7)	1.56 ± 0.1 (1.2 –1.8)		8.10*
6. Setae length at the base of B.l. blade (mm)	8.29 ± 0.74 (3–18)	n/a		
7. Setae length at basal third of B.l. blade (mm)	6.32 ± 0.62 (2–15)	n/a		
8. Distance between setae at basal third of B.l. blade (mm)	8.97 ± 1.22 (2–19)	n/a		
9. Setae length at middle third of B.l. blade (mm)	4.78 ± 0.40 (1.5–11)	2.17 ± 0.21 (1.5–3)		11.83*
10. Distance between setae at middle third of B.l. blade (mm)	8.78 ± 1.31 (3–34)	2.58 ± 0.27 (1.5–3)		14.09*
11. Setae length at distal third of B.l. blade (mm)	3.72 ± 0.30 (2–8)	2.4 ± 0.24 (2–3)		5.52*
12. Distance between setae at distal third of B.l. blade (mm)	5.54 ± 0.45 (2.5–11)	4 ± 0.45 (3–5)		3.11
13. Capitulum length (mm)	9.86 ± 0.26 (8–12)	8.5 ± 0.29 (8–9)		3.81*
14. Capitulum width (mm)	9.11 ± 0.39 (7–12)	6.25 ± 0.25 (6–7)		6.50*
15. Peduncle length (cm)	2.01 ± 0.21 (0.7–5)	1.17 ± 0.12 (1–1.5)		3.40
16. Involucral bract length (mm)	3.79 ± 0.17 (2.8–5)	3 ± 0 (3–3)		3.72

For each species, the mean and standard error of each vegetative (1–12) and reproductive (13–16) variable are indicated with ranges in parentheses. Results of ANOVA (F-ANOVA) or Kruskal-Wallis (H-KW) test with asterisks denote significant differences between species ($P < 0.05$). n/a = not applicable.

Table 2. Bioclimatic and topographic variables and differences between northern *Eryngium regnellii*, southern *E. regnellii* and the putative new species (*Eryngium* sp. nov.).

Bioclimatic variables	Northern *E. regnellii*	Southern *E. regnellii*	*E.* sp. nov.	F-ANOVA	H-KW
BIO1. Annual mean temperature (°C)	16.86 ± 0.70 a (13.23–23.81)	20.74 ± 0.48 b (19.31–23.04)	21.93 ± 0.18 b (21.45–22.21)		15.66*
BIO2. Mean diurnal range	11.00 ± 0.36 (8.69–13.26)	11.49 ± 0.20 (10.84–12.15)	11.77 ± 0.07 (11.65–11.95)	1.00	
BIO3. Isothermality	49.42 ± 1.11 a (43.55–55.40)	67.59 ± 0.36 b (66.99–69.96)	57.36 ± 0.27 ab (57.03–58.18)		20.35*
BIO4. Temperature seasonality	419.34 ± 18.84 a (294.58–539.86)	156.70 ± 17.46 c (92.79–210.17)	325.39 ± 3.46 b (316.98–333.95)	49.34*	
BIO5. Max. temperature of warmest month	28.56 ± 0.63 a (22.77–31.53)	28.03 ± 0.44 a (26.75–30.24)	31.36 ± 0.24 b (30.65–31.62)	4.26*	
BIO6. Min. temperature of coldest month	6.21 ± 0.73 a (1.22–9.96)	11.04 ± 0.76 b (8.63–14.33)	10.86 ± 0.2 b (10.38–11.2)	12.96*	
BIO7. Temperature annual range	22.34 ± 0.76 a (17.74–28)	17.00 ± 0.34 b (15.91–18.12)	20.5 ± 0.13 a (20.27–20.88)	14.97*	
BIO8. Mean temperature of wettest quarter	17.23 ± 1.01 a (12.11–23.62)	21.91 ± 0.29 b (20.99–23.27)	24.02 ± 0.19 b (23.51–24.3)		15.19*
BIO9. Mean temperature of driest quarter	15.52 ± 1.49 (7.23–22.27)	18.67 ± 0.68 (16.59–21.83)	17.86 ± 0.17 (17.47–18.14)		0.99
BIO10. Mean temperature of warmest quarter	21.96 ± 0.62 a (17.48–25.42)	22.17 ± 0.3 4 a (21.36–23.95)	25.52 ± 0.21 b (24.92–25.81)	6.16*	
BIO11. Mean temperature of coldest quarter	11.76 ± 0.81 a (7.23–16.11)	18.46 ± 0.71 b (16.30–21.78)	17.86 ± 0.17 b (17.47–18.14)	21.03*	
BIO12. Annual precipitation (mm)	1341.42 ± 83.55 (814–1731)	1604.12 ± 19.69 (1527–1649)	1615.5 ± 19.35 (1560–1650)		5.37
BIO13. Precipitation of wettest month	139.21 ± 8.44 a (98–193)	281.00 ± 4.76 b (253–290)	191.5 ± 0.96 ab (190–194)		19.48*
BIO14. Precipitation of driest month	82.00 ± 7.19 a (31–112)	15.87 ± 3.50 b (4–26)	82.25 ± 2.25 a (77–88)		16.36*
BIO15. Precipitation seasonality	17.51 ± 2.47 a (6.1–37.42)	76.61 ± 1.74 b (72.29–85.12)	25.53 ± 0.69 a (24.01–27.36)		16.96*
BIO16. Precipitation of wettest quarter	391.00 ± 21.06 a (277–487)	782.12 ± 12.42 b (725–822)	539.5 ± 9.5 ab (514–560)		20.35*
BIO17. Precipitation of driest quarter	270.00 ± 22.67 a (96–346)	56 ± 11.74 b (17–89)	271.75 ± 2.25 a (265–274)		16.70*
BIO18. Precipitation of warmest quarter	354.28 ± 22.13 a (241–487)	578.87 ± 51.72 b (422–716)	470.25 ± 14.65 ab (427–492)		12.68*
BIO19. Precipitation of coldest quarter	299.79 ± 27.59 a (96–403)	77.25 ± 12.96 b (33–116)	271.75 ± 2.2 a (265–274)		15.58*
Altitude (m)	532.50 ± 79.65 a (83–1312)	995.50 ± 52.91 b (770–1185)	279.25 ± 42.52 a (222–402)		11.93*

For each species, the mean and standard error of each bioclimatic (BIO1–BIO19) and the altitude variable are indicated with ranges in parentheses. Results of ANOVA (F-ANOVA) or Kruskal-Wallis (H-KW) test with asterisks denote significant differences between the groups ($P < 0.05$). For each variable, different letters indicate significant differences between means.

analyses of variance (F-ANOVA) were performed for those variables with normal distribution. For variables without normal distribution, non-parametric Kruskal-Wallis (H-KW) was used. To graphically explore variation, we constructed boxplots featuring medians, 25th and 75th percentiles, and error bars with 10th and 90th percentiles. The basal third of the basal leaves of the putative new species are inermous, therefore indumentum characteristics for this portion of the leaves (characters 6–8; Table 1) were not analyzed with univariate methods. To visualize how specimens are grouped according to their morphological affinities and to identify the characters that best define different groups (i.e., putative different species based on morphology), PCA was performed with all specimens and considered all morphological characters. Contributions of the variables (correlation values) to each principal component (PC) were interpreted as significant when $\geq|0.6|$. Graphs were constructed with axes corresponding to the most informative PCs. To evaluate normality in each variable, the Shapiro-Wilk test (Shapiro & Wilk, 1965) was used.

In addition, a discriminant analysis (DA) of quantitative morphological variables was used to discriminate between the two groups defined a priori (i.e., *Eryngium regnellii* and the putative new species). Quantitative characters that were not normally distributed were log10 transformed before the analysis. Traits that strongly correlated with each other, as determined by a Pearson's correlation coefficient $r > |0.9|$, were identified, and one of the traits of each correlating pair was then excluded from the DA analysis. Mahalanobis distances between the centroids of the two groups were calculated to establish a measure of morphological affinity.

Coalescent-based species delimitation analyses. — In order to evaluate competing models of species delimitation based on molecular evidence, we conducted Bayesian phylogenetics and phylogeography (BPP) A11 analyses as implemented in BPP v.4.4 (Yang & Rannala, 2014; Yang, 2015). This method calculates the posterior probabilities of competing models of species delimitation and species phylogeny, accounting for incomplete lineage sorting due to ancestral polymorphism and gene tree vs. species tree discordance (Yang & Rannala, 2010, 2014; Rannala & Yang, 2013). For the BPP analyses, we included the five DNA accessions of *Eryngium regnellii* and the putative new species (Appendix 2) from the total evidence data matrix (i.e., all cpDNA and nDNA data), without indels. The DNA accessions of *Eryngium* from Paraguay were assigned to a different lineage than those of *E. regnellii* (splitter model) to compare this species delimitation hypothesis against a hypothesis where all accessions belong to *E. regnellii* (lumper model). A11 analyses were conducted assigning equal probabilities for the rooted trees with labeled histories (speciesmodelprior = 1; Yang & Rannala, 2014). Because prior choice of effective population size (θ) and root height of the species tree ($\tau 0$) can affect the posterior probabilities of the models (Zhang & al., 2011), we used different prior distributions for these two parameters in order to test their influence in our results. The BPP program uses an inverse gamma distribution ($IG(\alpha, \beta)$) for the priors of parameters θ and $\tau 0$, therefore we set $\alpha = 3$ as a diffuse prior and adjusted β to cover alternative scenarios for these parameters (θ = IG(3, 0.001), IG(3, 0.01), IG(3, 0.02); $\tau 0$ = IG(3, 0.06), IG(3, 0.04), IG(3, 0.01)). Consequently, we analyzed a total of nine different combinations of priors that cover small and large ancestral population sizes with shallow and deep divergences among species, considering pairwise sequence divergence and divergence times among monocot-like *Eryngium*. Mutation rates were estimated, allowing them to vary according to a Dirichlet distribution. Ploidy level for the chloroplast and nuclear loci was set to 0.5 and 1, respectively. Each Markov chain Monte Carlo (MCMC) analysis was run for 200,000 generations for most combinations of priors of parameters θ and $\tau 0$, sampling every 5 generations, and discarding 10% of the posterior sample as the burn-in. For $\tau 0$ = IG(3, 0.06), and θ = IG(3, 0.01) and IG(3, 0.02), MCMC was run for 1 million generations to reach convergence. MCMC convergence was assessed by comparing the consistency of the results between three independent runs with different starting trees for each combination of θ and $\tau 0$ (Yang, 2015; Flouri & al., 2018). Posterior probabilities of the number of species delimited and of each species delimited ≥ 0.95 were considered strong support for a speciation event based on molecular evidence (Leaché & Fujita, 2010).

We also took a Bayes factor (BF) delimitation approach to compare between the splitter and lumper species models, based on their marginal likelihood estimates (MLE). First, for each species delimitation model, we set a StarBeast2 v.0.15.13 (Ogilvie & al., 2017) analysis using BEAUTi as implemented in BEAST2 v.2.6 (Bouckaert & al., 2014). We included the five DNA accessions of *Eryngium regnellii* and the putative new species (Appendix 2) from the total evidence data matrix, and as an outgroup, we added the DNA sequences of *E. pandanifolium* from Calviño & al. (2008: DNA accessions: 2327, 2487, 2488). In the splitter model, *E. regnellii* and the putative new species were set as distinct species, whereas in the lumper model all these accessions were considered as part of the same entity. The ploidy level for the chloroplast and nuclear loci was set to 1 and 2, respectively (as these are hermaphrodite plants). Because the population size is not a parameter of interest for our study, we used an analytical population size integration method to improve convergence rates (Ogilvie & al., 2017). To select the nucleotide substitution models for each partition, we used jModelTest2 v.2.1.6 (Darriba & al., 2012). We set the species tree relaxed clock with the uncorrelated lognormal model and clock rates were estimated. We used a Yule model as the tree prior. For population mean and speciation rate priors, we set a gamma distribution with higher upper bounds of 10 and 10,000, respectively. The two scripts generated in BEAUTi were used to calculate MLE for each species delimitation model. We used the path sampling method (PS; Lartillot & Philippe, 2006) implemented in the Model-selection package in BEAST2, with 200 steps of 1 million generations each, $\alpha = 0.3$, and a burn-in of 10%. Finally, we calculated BFs to compare and assess the support

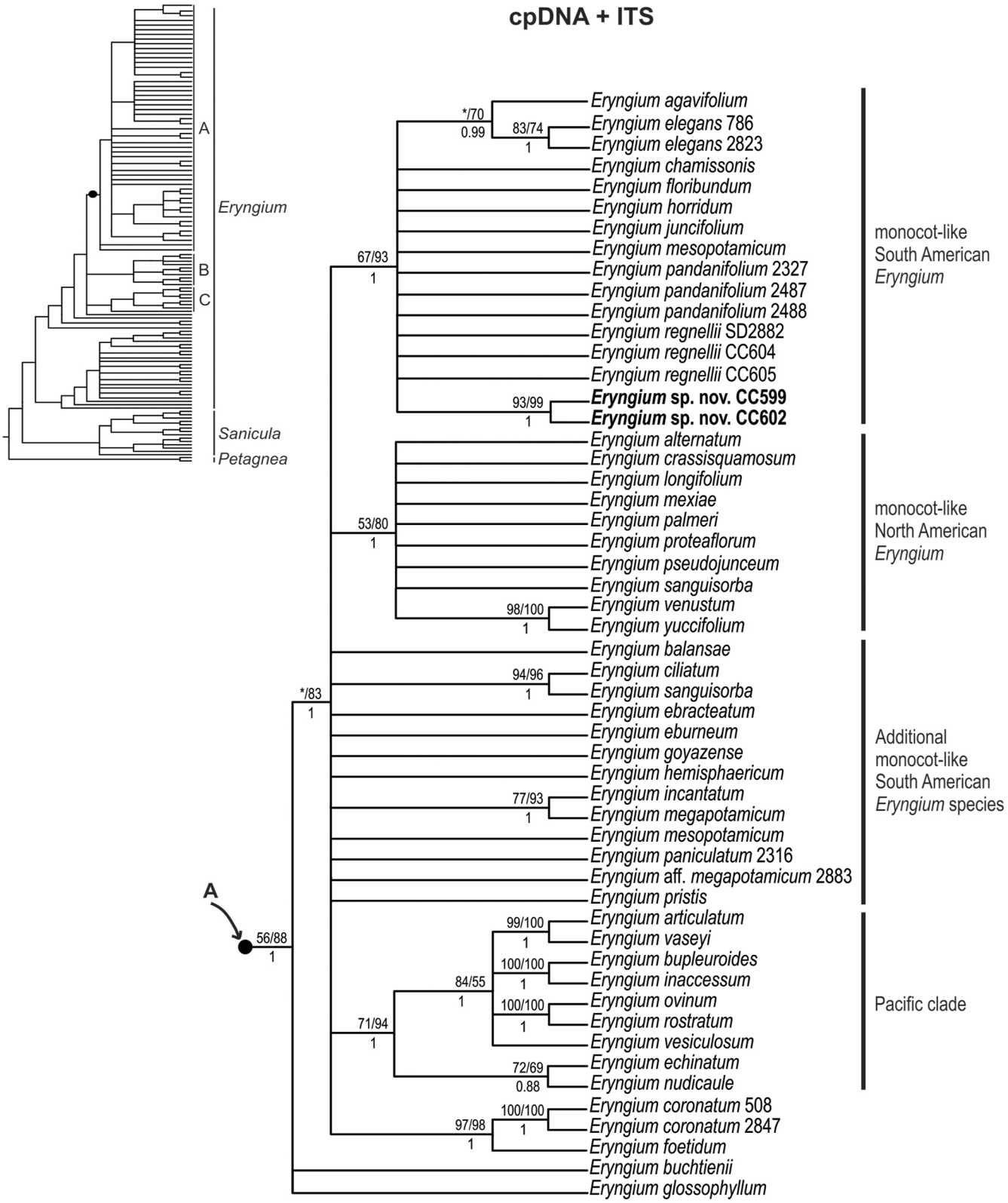
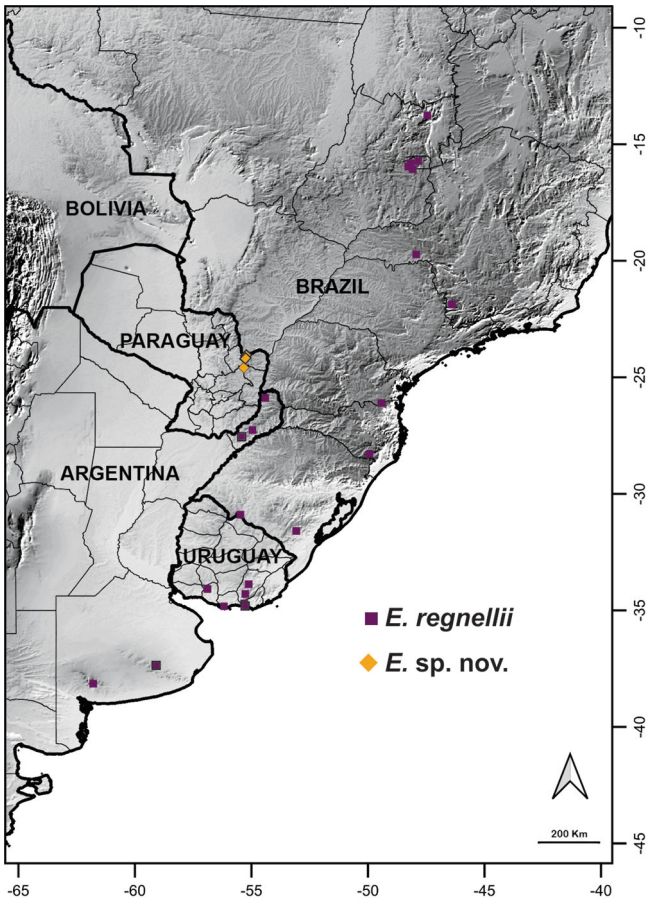


Fig. 2. Backbone of the Saniculoideae majority-rule consensus of 78,300 trees derived from Bayesian analysis of 116 non-coding cpDNA *trnQ-trnK* and nrDNA ITS sequences (top left) and the *Eryngium* South American clade in detail (right). Maximum parsimony and maximum likelihood bootstrap support values are indicated above branches (left and right, respectively). Posterior probability values are indicated below branches. Nodes with ML bootstrap values <70% and PP values <0.95 are collapsed. Asterisks represent branches that collapse in the MP strict consensus of 20,000 minimal-length trees. Major clades of *Eryngium* are according to Calviño & al. (2008, 2010): A = South American clade, B = Mexican clade, C = Eastern U.S.A. clade.



between the MLE of the two species delimitation models analyzed. BF was calculated by subtracting the MLE values for two models and multiplying the difference by two: BF = 2 × (MLE splitter model − MLE lumper model). A positive BF value indicates support in favor of the splitter model, and a negative BF value favors the lumper model. We evaluated the strength of BF support for one competing model following Kass & Raftery (1995) where: 0–2 is not worth more than a bare mention, 2–6 is positive evidence, 6–10 is strong evidence, and >10 is very strong evidence.

RESULTS

Phylogenetic analyses. — The phylogenies estimated using MP, BI, and ML analyses of the *trnQ-trnK* and ITS data matrix are congruent with each other. The BI majority-rule consensus tree with MP bootstrap (MPBS), ML bootstrap (MLBS), and posterior probability (PP) support values is presented in Fig. 2, with branches that collapse in the MP trees denoted by asterisks. The accessions of *Eryngium regnellii* and the putative new species fall within the South American monocot-like clade recognized by Calviño & al. (2008; 67%

Fig. 3. Map showing the geographical distribution of *Eryngium* sp. nov. (orange diamonds) and *Eryngium regnellii* (purple squares). Diamonds and squares bordered in black correspond to accessions sampled for DNA.

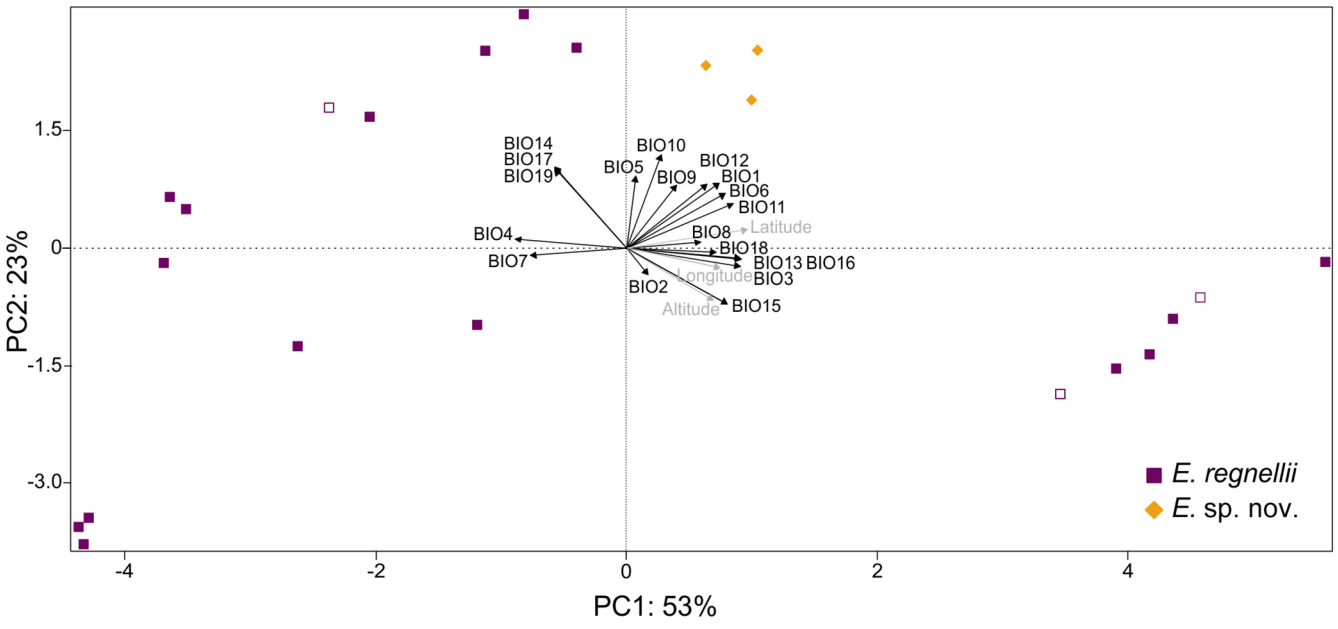


Fig. 4. Climatic principal component analysis (PCA): scatterplot of the first two axes (PC1, PC2) of 26 specimens of *Eryngium regnellii* and the putative new species studied based on 19 bioclimatic variables. Percentage of total variance associated to each PC is provided. Unfilled squares represent original material (types) of *E. regnellii*. Black arrows indicate the contribution of significant bioclimatic variables to the first two axes. Numbers correspond to the bioclimatic variables listed in Table 2. The gray arrows indicate the contribution of the illustrative geographic variables (latitude, longitude, altitude) to the first two axes.

MPBS, 93% MLBS, 1.0 PP; Fig. 2). Within this clade interspecific relationships are uncertain (MPBS and MLBS <70% and PP < 0.95). While the monophyly of *E. regnellii* is not resolved, the two accessions of the putative new species form a strongly supported monophyletic group (93% MPBS, 99% MLBS, 1.0 PP).

Geographic analyses. — *Eryngium regnellii* is distributed in Brazil (Goiás, Distrito Federal, Minas Gerais, Paraná, Santa Catarina, Rio Grande do Sul), Uruguay (Maldonado, Lavalleja, Montevideo, San José), and Argentina (Misiones, Buenos Aires) in relatively disjoint areas associated with the Tandilia and the Brazilian highlands that continue south as lower hills in Uruguay and in the Misiones Province of Argentina (Fig. 3). The putative new species is restricted to eastern Paraguay (Caaguazú, Canindeyú; Fig. 3). Both species grow in grasslands or savannas with *E. regnellii* occurring in the biogeographic provinces of Cerrado, Paranense and Pampeana (Cabrera & Willink, 1980) on rock outcrops, while the putative new species grows on sandy soils and is restricted to the Cerrado province.

Climatic analyses. — The PCA of climatic data (Fig. 4) for the two entities showed that the first two components account for 76% of the total variation of the data, with PC1 representing 53%, PC2, 23%, and PC3, 14%. Most of the bioclimatic variables contributed more to PC1 (BIO 1, 3, 4, 6–8, 11–19; Table 3), and five variables contributed the most to PC2 (BIO 5, 10, 14, 17, 19; Table 3). The three illustrative geographic variables (latitude, longitude, altitude) are correlated significantly with PC1 (Table 3). The specimens of *Eryngium regnellii* separate in PC1 into two groups: the specimens grouped to the left correspond to those that grow from Santa Catarina to the south, while those to the right correspond to those that grow from Minas Gerais to the north. The putative new species falls between these two groups in PC1, and does not overlap with *E. regnellii*. In PC2, the two entities overlap, but overall, the putative new species is restricted to the upper right quadrant (Fig. 4).

The univariate analyses of the bioclimatic variables showed that BIO5 and BIO10 are significantly different between *Eryngium regnellii* and the putative new species (although the two groups of *E. regnellii* do not differ in these variables; Table 2). The three groups differ in temperature seasonality (BIO4; Table 2). The putative new species grows in places with higher temperatures during summer and with intermediate temperature seasonality. All other climatic variables differ between the northern and southern specimens of *E. regnellii*, with the putative new species indistinguishable among these groups or with one or the other (Table 2).

Morphometric analyses. — The ANOVA and Kruskal-Wallis morphological analyses resulted in statistically significant differences among *Eryngium regnellii* and the putative new species for 7 of the 13 characters (3, 5, 9–11, 13, 14; Table 1). These characters include both, vegetative and reproductive variables. From the seven characters that statistically differ among the entities, the ranges of characters 10 and 14 do not overlap (Table 1, suppl. Fig. S1).

The PCA of morphology showed that the first two components account for 48% of the total variation of the data, with PC1 representing 29%, PC2, 19%, and PC3, 14%. The first two components show that on the first axis (PC1), from left to right, the putative new species separates completely from *Eryngium regnellii* (Fig. 5). There is substantial overlap on axis 2 (PC2) and the two entities are not separated in PC2 (Fig. 5). The characters that contributed most to PC1 are mainly related to the length and density of setae, and capitulum size (characters 6–10, 13, 14; Table 4), and the characters that contributed most to PC2 are related to the length of basal leaves (characters 1, 4; Table 4). Therefore, the specimens of the putative new species differ from those of *E. regnellii* mainly in that their basal leaves have shorter and denser setae, and that their capitula are smaller.

For the DA, only morphological variables 1, 2 and 4 showed a normal distribution. Therefore, to comply with the assumptions of DA, we applied log10 to each of the remaining variables. After this transformation, the following

Table 3. Correlations between each bioclimatic or illustrative geographic variable and the first two axes of the PCA of climatic data for *E. regnellii* and the putative new species.

Bioclimatic variables	PC1: 53%	PC2: 23%
BIO1. Annual mean temperature	**0.79**	0.57
BIO2. Mean diurnal range	0.19	−0.24
BIO3. Isothermality	**0.97**	−0.17
BIO4. Temperature seasonality	**−0.95**	0.08
BIO5. Max temperature of warmest month	0.08	**0.64**
BIO6. Min temperature of coldest month	**0.84**	0.48
BIO7. Temperature annual range	**−0.82**	−0.07
BIO8. Mean temperature of wettest quarter	**0.63**	0.05
BIO9. Mean temperature of driest quarter	0.43	0.56
BIO10. Mean temperature of warmest quarter	0.29	**0.83**
BIO11. Mean temperature of coldest quarter	**0.91**	0.39
BIO12. Annual precipitation	**0.69**	0.57
BIO13. Precipitation of wettest month	**0.97**	−0.09
BIO14. Precipitation of driest month	**−0.61**	**0.71**
BIO15. Precipitation seasonality	**0.86**	−0.50
BIO16. Precipitation of wettest quarter	**0.98**	−0.10
BIO17. Precipitation of driest quarter	**−0.61**	**0.72**
BIO18. Precipitation of warmest quarter	**0.76**	−0.04
BIO19. Precipitation of coldest quarter	**−0.61**	**0.69**
Ilustrative geographic variables		
Latitude	**0.97**	0.10
Longitude	**0.80**	−0.14
Altitude	**0.66**	−0.48

In bold: correlations ≥|0.6|, considered significant.

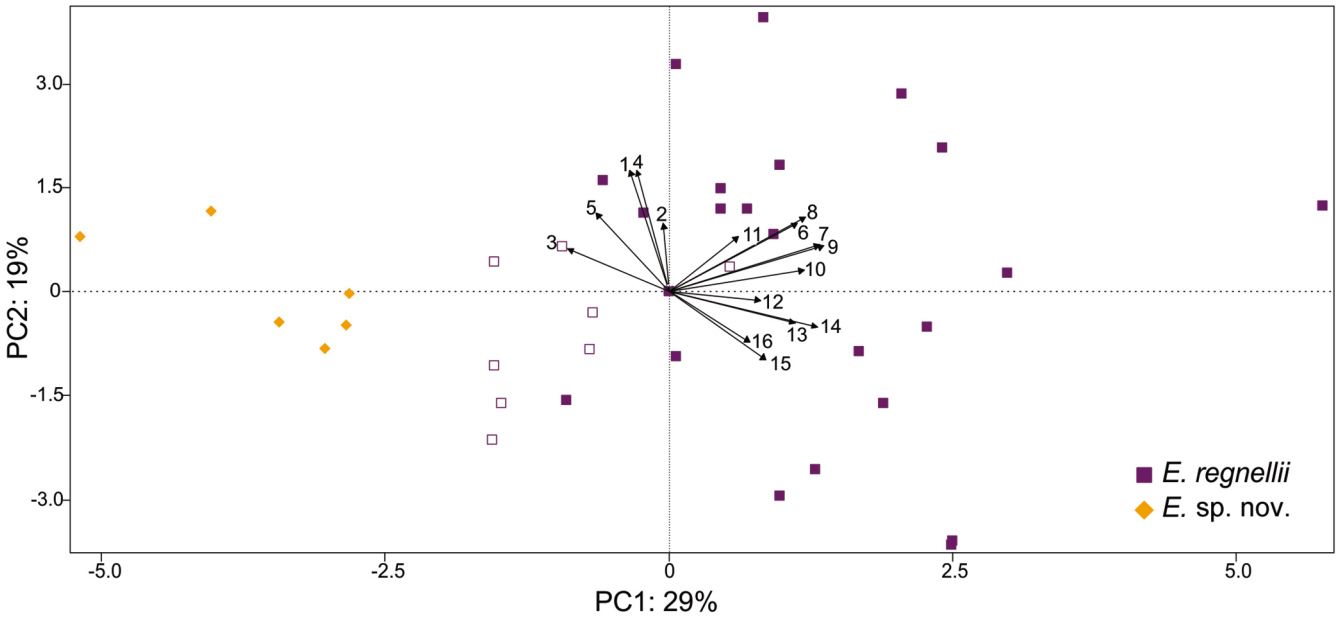


Fig. 5. Morphological principal component analysis (PCA): scatterplot of the first two axes (PC1, PC2) of 36 specimens of *Eryngium regnellii* and the putative new species based on 16 morphometric variables. Percentage of total variance associated with each PC is provided. Unfilled squares represent original material (types) of *E. regnellii*. Arrows indicate the contribution of significant morphological characters to the first two axes. Numbers correspond to the morphological characters listed in Table 1.

Table 4. Correlations between each morphometric variable and the first two axes of the PCA of morphological data for *Eryngium regnellii* and the putative new species.

Morphometric variables	PC1: 29%	PC2: 19%
1. Basal leaf (B.l.) length	−0.19	**0.81**
2. B.l. sheath length	−0.03	0.45
3. B.l. sheath width	−0.51	0.29
4. B.l. blade length	−0.16	**0.81**
5. B.l. blade width	−0.36	0.52
6. Setae length at the base of B.l. blade	**0.64**	0.45
7. Setae length at basal third of B.l. blade	**0.75**	0.31
8. Distance between setae at basal third of B.l. blade	**0.68**	0.49
9. Setae length at middle third of B.l. blade	**0.77**	0.30
10. Distance between setae at middle third of B.l. blade	**0.68**	0.14
11. Setae length at distal third of B.l. blade	0.35	0.37
12. Distance between setae at distal third of B.l. blade	0.46	−0.06
13. Capitulum length	**0.63**	−0.21
14. Capitulum width	**0.74**	−0.24
15. Peduncle length	0.48	−0.45
16. Involucre bract length	0.41	−0.33

In bold: correlations ≥|0.6|, considered significant.

characters showed normality: 3, 9, 10, 13–15. Pearson's correlation coefficients between all pairs of characters showed a significant ($P < 0.05$) and strong ($r \geq |0.9|$) association between characters 1 and 4, hence we eliminated character 4 from the analysis. The DA showed statistically significant differences between the two entities for four of the eight characters analyzed (characters 3, 9, 10, 14, Table 5; Wilks' $\lambda = 0.42$; $F = 10.63$; $\chi^2 = 27.63$, $P < 0.001$), with Mahalanobis distances (MD) between centroids of each entity (MD = 9.33; $P < 0.001$). The discriminant function explains 100% of the variance of the data, and clearly separates *Eryngium regnellii* from the putative new species without overlap (Fig. 6). The characters that contributed most to this function are related to the width of the basal leaf sheath, the length and the density of setae in the middle third of the basal leaf blade, and the

Table 5. Correlations of the morphometric variables that showed significant differences between *Eryngium regnellii* and the putative new species, with the function of the discriminant analysis (DA).

Morphometric variables	DA: 100%
3. Basal leaf (B.l.). sheath width	0.45
9. Setae length at middle third of B.l. blade	−0.66
10. Distance between setae at middle third of B.l. blade	−0.76
14. Capitulum width	−0.48

The percentage of the total variance of the data explained by the DA single function is indicated.

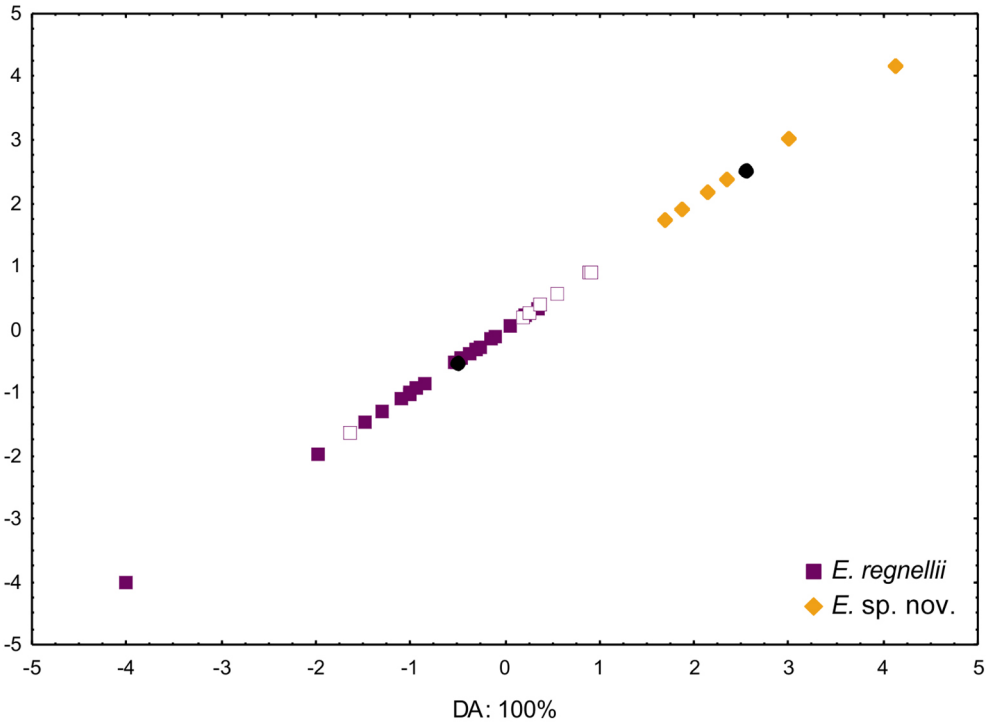


Fig. 6. Morphological discriminant analysis (DA): scatterplot of the only function of 36 specimens of *Eryngium regnellii* and the putative new species obtained from the four characters listed in Table 5 that showed significant differences between the species. Unfilled squares represent original material (types) of *E. regnellii*. Black circles correspond to the mean value of the discriminant function for each species.

capitulum width (3, 9, 10, 14, Table 5). In addition, results of the DA showed that out of a total of 30 individuals of *E. regnellii* and 6 individuals of the putative new species, all were classified correctly as such.

Coalescent-based species delimitation analyses. — Under many sets of priors, all BPP analyses supported the delimitation of two different species with high posterior probability (PP ≥ 0.95; Table 6). In addition, path sampling results from the BF delimitation approach supported the splitter species model (i.e., the recognition of two different species; BF > > 10; Table 7).

Table 6. Bayesian phylogenetics and phylogeography (BPP) analyses results from nine different prior schemes between *Eryngium regnellii* and the putative new species (*Eryngium* sp. nov.).

Priors		No. species best model	PP best model
θ	$\tau 0$		
IG(3, 0.001)	IG(3, 0.06)	2	**1.00** ± 0.00
IG(3, 0.001)	IG(3, 0.04)	2	**1.00** ± 0.00
IG(3, 0.001)	IG(3, 0.01)	2	**1.00** ± 0.00
IG(3, 0.01)	IG(3, 0.06)	2	0.89 ± 0.01
IG(3, 0.01)	IG(3, 0.04)	2	**0.99** ± 0.00
IG(3, 0.01)	IG(3, 0.01)	2	**0.99** ± 0.00
IG(3, 0.02)	IG(3, 0.06)	2	**1.00** ± 0.00
IG(3, 0.02)	IG(3, 0.04)	2	**0.98** ± 0.00
IG(3, 0.02)	IG(3, 0.01)	2	**0.99** ± 0.00

For each prior the number of species delimited in the best model and its posterior probability (PP) are indicated. Values of posterior probability reported here correspond to the mean ± standard deviation of three replicates, values ≥0.95 were considered strong support and are indicated in bold. Abbreviations: θ = effective population size, $\tau 0$ = root height, IG = inverse gamma.

■ DISCUSSION

The monocot-like *Eryngium* comprises groups of species that have diversified rapidly during the Late Pliocene and Quaternary (Calviño & al., 2008, 2010; Padin & Calviño, unpub.). As such, many of these species show low morphological and genetic differences, and therefore delimiting its species boundaries is often a difficult task that can be better addressed using an integrative approach with evidence from multiple data sources.

The phylogenetic results confirm our hypothesis based on morphology that the specimens of the putative new species from Paraguay are closely related to a group of robust species that comprise the South American monocot-like clade, which

Table 7. Bayes factor (BF) species delimitation results, and marginal likelihood estimates (MLE) based on path sampling method for different species delimitation hypotheses.

Delimitation model	No. species	MLE	BF
Split species	2	**−9097.37 ± 20.45**	–
(*E. regnellii* + *E.* sp. nov.)	1	−9150.65 ± 49.27	106.55

BF: Bayes factor = 2 × (MLE model 1 − MLE model 2), where model 1 corresponds to split species. The number of species recognized in each model is indicated. The model with the best ML score is highlighted in bold. Values correspond to the mean of three replicates with their standard deviation.

also includes *Eryngium regnellii*. Although interspecific relationships within this clade are not resolved with MP, ML and Bayesian methods, this is congruent with previous phylogenetic results and with the hypothesis of recent, rapid, and reticulate evolution within these groups (Calviño & al., 2008, 2010). For this reason, the use of Bayesian coalescent-based species delimitation analyses that contemplate incomplete lineage sorting was helpful to corroborate the presence of genetic structure compatible with the hypothesis of species divergence between *E. regnellii* and the entity from Paraguay.

Eryngium regnellii occupies a large geographic distribution in relatively disjoint areas associated with the Tandilia and the Brazilian highlands that continue south into Uruguay and in Misiones (Argentina). The putative new species, on the contrary, is endemic to a relatively small area of Cerrado in eastern Paraguay. Both entities grow in grasslands, and while the putative new species grows on sandy soils, *E. regnellii* is associated with rock outcrops along its large distribution. Rocky soils have very limited water storage capacity: they are often soaked during the rainy season and extremely dry during the dry season (Oliveira & Marquis, 2002). This difference in soil type (and in water availability) could have been a driver of ecological niche divergence and speciation of *E. regnellii* and the populations from Paraguay. This hypothesis is also supported by the high percentage of endemic flora and fauna found on rocky grasslands, as those of the *campos rupestres* or *campos de altitude* (Crisci-V. & al., 2001; Cardoso da Silva & Bates, 2002; Oliveira & Marquis, 2002; Vera & al., 2021).

The climatic niche of the specimens from Paraguay also differs from that of *Eryngium regnellii*, even considering the plasticity of the latter. Again, *E. regnellii* is found in disjoint climatic niches (and different biogeographic provinces) compatible with the latitudinal distribution of the species. The putative new species, restricted to the Cerrado biogeographic province, is found in grasslands with higher mean and maximum temperatures during summer (ca. 3°C higher) and intermediate temperature seasonality. During the Quaternary, grasslands were the major biomes in southern Brazil, covering large areas now occupied by different forest biomes (Behling, 1998, 2002; Simon & al., 2009). The climatic-vegetation changes during the Quaternary probably explain the large distribution of *E. regnellii* by expansion and colonization of equivalent ecological niches in soil type, and later, contraction and divergence in isolation.

South American monocot-like *Eryngium* species often differ in subtle morphological characteristics, among which are indumentum in leaf margins, shape of capitula and fruit ornamentation (Wolff, 1913; Calviño & Martínez, 2007; Martínez & Calviño, 2019; Esquivel Mattos & Calviño, 2022). The populations analyzed from Paraguay differ from *E. regnellii* in basal leaf consistency, but also showed differences in 7 of the 13 quantitative morphological variables explored, including both reproductive and vegetative characters. Basal leaves of *E. regnellii* are herbaceous and flaccid, with setae from the base to the apex of the blade margins. The capitula are globose or broadly ovoid. Basal leaves of the populations from the Cerrado of Paraguay are distinctive: subcoriaceous and erect, with setae occurring from the middle third of the blade to the apex (margins are glabrous at the basal third). Setae in the latter lineage are shorter and denser (suppl. Fig. S1). The capitula of the latter are ovoid and narrower than in *E. regnellii*. These combinations of morphological characteristics allow the identification of these distinct lineages based on morphology, as shown in our DA analyses, where all accessions were assigned to the correct lineage without error.

Divergence in morphology, environmental (or ecological) niche, and genetic structure between *Eryngium regnellii* and the lineage from Paraguay support the hypothesis of evolutionary independence of these two lineages. Therefore, based on an integrative approach, incorporating many types of data, we recognize an independent lineage of *E. regnellii* and describe it here as a new species from Paraguay: *Eryngium cerradense*.

■ TAXONOMIC TREATMENT

Eryngium cerradense Esquivel Mattos & C.I.Calviño, **sp. nov.** – Holotype: Paraguay. Canindeyú, Corpus Christi, Aguara Ñú, Reserva Natural del Bosque Mbaracayú, S24°11′01″, W55°16′04″, 16 Feb 2017, *P. Esquivel Mattos 85* (BCRU barcode BCRU000001V).

Description. – Perennial herb, erect, 1–2.5 m tall. Rhizome bearing fibrous roots. Main axis solitary, erect, very leafy, branching apically in a pleiochasium (5–8 rays) that successively branches in dichasia and sometimes then in monochasia; lateral cymes, distal, of equal or smaller order. Basal leaves rosulate, numerous, erect (ascending), 65–90 cm long, subcoriaceous, parallel-veined; sheath dilated, glabrous, 4–7 × 1.4 – 3 cm, margin entire; blade subulate, apex acuminate ending in a seta, 59–84 × 1.5–2 cm, margin entire and smooth in the lower third (no seta), densely setose from the middle third to the apex; setae in the middle third solitary or rarely with 1 accessory, 1.5–3 mm long, 1.5–3 mm apart, appressed; setae in the distal third solitary, 1.5–3 mm long, 3–5 mm apart, appressed to slightly ascending. Cauline leaves like the basal, numerous, ascending and gradually reduced upwards. Capitula pedunculated, peduncle 1–1.5 cm long; ovoid, 8–9 × 6–7 mm; involucral bracts inconspicuous, patent, 3 × 1–1.5 mm, narrowly ovate to narrowly triangular, margin entire, apex spinose; floral bracts subequal, 3–3.5 × 1–1.5 mm. Flowers sessile, sepals broadly ovate or broadly oblong, 1 × 0.8 mm, entire, apex obtuse, mucronate; petals oblong, 0.8–1 × 0.3–0.5 mm, entire, apex inflexed, fimbriate; stylopodium annular. Fruits obovoid to broadly obovoid, 2.5–3 × 1.5–2 mm, mericarps 2 mm long; surface scaly and papillate, lateral scales falcate, ascending, apex acute, calycine scales linear to ovate, in 1 or more rows, dorsal scales or papillae, smaller, totally or partially covering the surface. (Figs. 1A, 7)

Distribution and habitat. – This species is endemic to the departments of Caaguazú and Canindeyú, Paraguay. It grows on sandy soils of wet or dry grasslands of the Cerrado

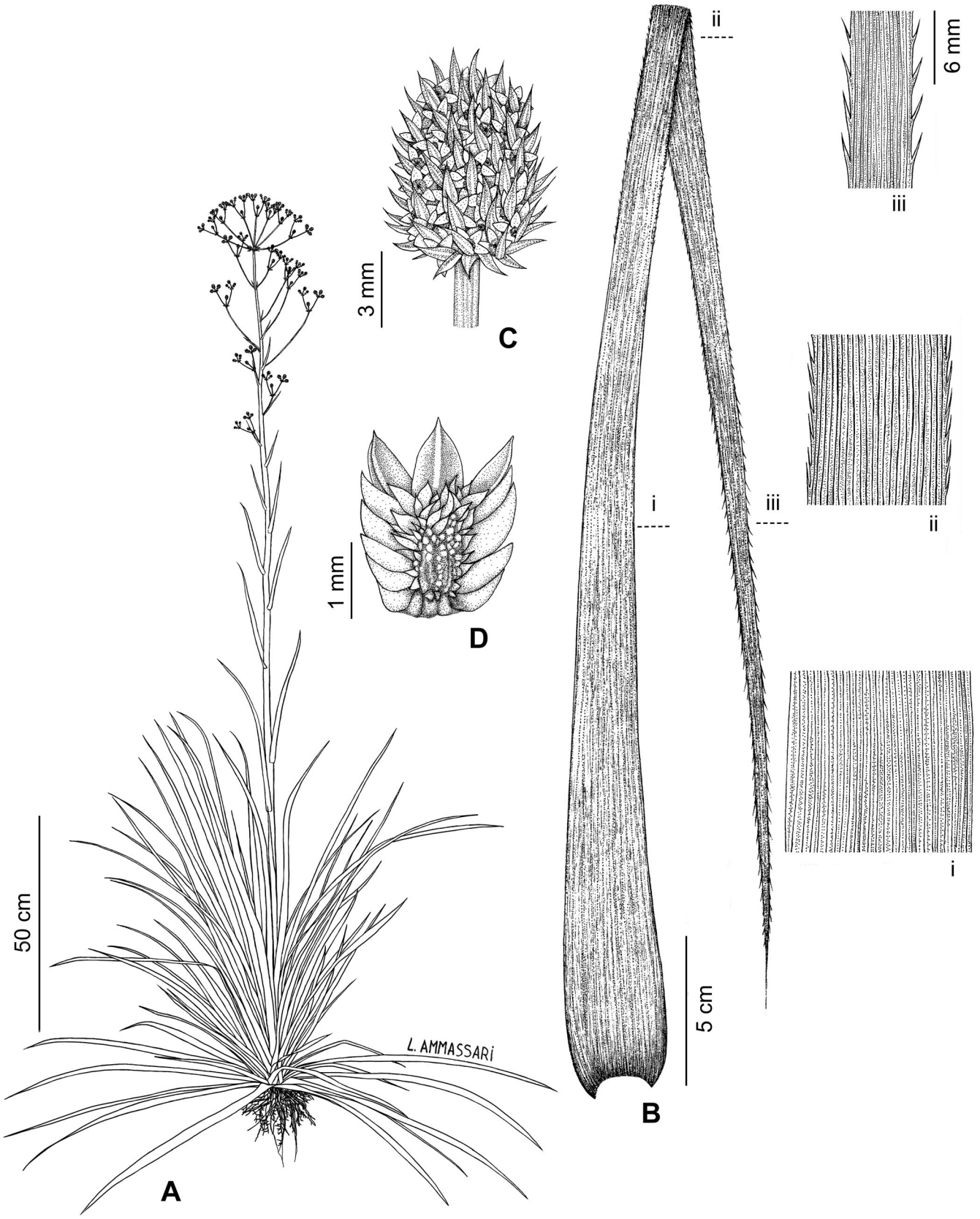


Fig. 7. *Eryngium cerradense*: **A**, Habit; **B**, Basal leaves (variation in leaf margin in the: **i**, basal third; **ii**, middle third; and **iii**, distal third of the blade); **C**, Capitulum; **D**, Fruit in dorsal view. — Illustrated by Luciana Ammassari from: A, photograph of specimen *P. Esquivel Mattos 169* in the field; B, *P. Esquivel Mattos 169* (BCRU); C, *A. Schinini & G. Caballero Marmori 30198* (CTES); D, *P. Esquivel Mattos 85* (BCRU).

phytogeographic province that occurs on gently rolling topographies in discontinuous areas of the Paranense phytogeographic province in the northeast of the Oriental Region of Paraguay (Cabrera & Willink, 1980; Rolón & al., 2017) (Fig. 3).

Phenology. – Flowering and fruiting October to January.

Etymology. – The specific epithet refers to the phytogeographic province where the species grows in Paraguay.

Paratypes. – Paraguay. Caaguazú, Yhú, Cnia. Pindó, Camino entre Itakyry y Curuguaty, 11 Oct 1995, *A. Schinini & G. Caballero Marmori 30198* (CTES barcode CTES 0551004); Canindeyú, Corpus Christi, Aguara Ñú, Reserva Natural del Bosque Mbaracayú, S24°11′01″, W55°16′04″, 15 Feb 2018, *P. Esquivel Mattos 169* (BCRU); Sierra de Maracayú [Cordillera de Mbaracayú], Nov 1898–99, *E. Hassler 5384* (G barcode G00448645!).

■ AUTHOR CONTRIBUTIONS

CIC and PEM conceived the ideas for this study. PEM found the new species during her field studies in Paraguay, collected, photographed, and analyzed the material, obtained all morphological data, performed geographical analyses, and wrote the manuscript. MF and PEM carried out the laboratory molecular work and the phylogenetic analyses. MF performed the climatic and morphometric analyses, and wrote the manuscript. CIC and MF performed the coalescent-based species delimitation analyses. CIC supervised all analyses, studied, and analyzed the material, designed, and wrote the manuscript. All authors analyzed and discussed the results, revised, improved, and approved the final manuscript. — MF, https://orcid.org/0000-0001-8531-1438; CIC, https://orcid.org/0000-0002-2672-4352

■ ACKNOWLEDGEMENTS

The authors thank Luciana Ammassari for line drawings of the new species; Diober B. Lucas and the following herbarium curators or assistants for sending photographs of specimens: Alan Paton (K), Johannes Lundberg (S), Kim Kersh (UC), Eduardo Marchesi (MVFA), Walter Medina and Gelina Pieszko (CTES), Meghann Toner (US), and the curators of the herbaria cited in the text for access to specimens. We also thank Danilo Salas, Research and Conservation Manager of Fundación Moisés Bertoni, for providing permission to access the Cerrado of Aguara Ñú, Reserva Natural del Bosque Mbaracayú; Humberto Sánchez, Juan José Resquín and Julio Torres for field assistance, and the staff of the FCQ herbarium for their help to process collected specimens. We also acknowledge CONICET, Argentina, for a Doctoral fellowship for Latin American countries to PEM directed by CIC, and Fátima Mereles as co-director of this fellowship. Specimens gathered for this study were collected legally with appropriate valid permits from the Ministerio del Ambiente y Desarrollo Sostenible (MADES) of Paraguay. This work was supported by the following institutions from Argentina to CIC.: ANPCyT-FONCyT [grant number PICT 2019 0770]; CONICET PIP [grant number 112-201301-00357]; Universidad Nacional del Comahue [grant number PIN B245].

■ LITERATURE CITED

Beentje, H. 2010. *The Kew plant glossary: An illustrated dictionary of plant terms*. Richmond: Royal Botanic Gardens, Kew.

Behling, H. 1998. Late Quaternary vegetational and climatic changes in Brazil. *Rev. Palaeobot. Palynol.* 99: 143–156. https://doi.org/10.1016/S0034-6667(97)00044-4

Behling, H. 2002. South and southeast Brazilian grasslands during Late Quaternary times: A synthesis. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* 177: 19–27. https://doi.org/10.1016/S0031-0182(01)00349-2

Bell, C.R. & Constance, L. 1960. Chromosome numbers in Umbelliferae II. *Amer. J. Bot.* 47: 24–32. https://doi.org/10.1002/j.1537-2197.1960.tb07089.x

Bell, C.R. & Constance, L. 1966. Chromosome numbers in Umbelliferae III. *Amer. J. Bot.* 53: 512–520. https://doi.org/10.1002/j.1537-2197.1966.tb07365.x

Bouckaert, R., Heled, J., Kühnert, D., Vaughan, T., Wu, C.-H., Xie, D., Suchard, M.A., Rambaut, A. & Drummond, A.J. 2014. BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computat. Biol.* 10(4): e1003537. https://doi.org/10.1371/journal.pcbi.1003537

Cabrera, A.L. & Willink, A. 1980. *Biogeografía de América Latina*. Washington, D.C.: Secretaria General de la Organización de los Estados Americanos.

Calviño, C.I. & Downie, S.R. 2007. Circumscription and phylogeny of Apiaceae subfamily Saniculoideae based on chloroplast DNA sequences. *Molec. Phylogen. Evol.* 44: 175–191. https://doi.org/10.1016/j.ympev.2007.01.002

Calviño, C.I. & Levin, G.A. 2019. A new species of *Eryngium* (Apiaceae, Saniculoideae) from the USA. *Syst. Bot.* 44: 446–450. https://doi.org/10.1600/036364419X15562052252261

Calviño, C.I. & Martínez, S.G. 2007. New records from Argentina and Uruguay, and notes on *Eryngium* sect. *Panniculata* (Apiaceae). *Darwiniana* 45(1): 68–76.

Calviño, C.I. & Martínez, S.G. 2019. Typifications of 22 names of Apiaceae subfamily Saniculoideae from southern South America. *Phytotaxa* 420: 65–71. https://doi.org/10.11646/phytotaxa.420.1.4

Calviño, C.I., Tilney, P.M., Van Wyk, B.E. & Downie, S.R. 2006. A molecular phylogenetic study of southern African Apiaceae. *Amer. J. Bot.* 93: 1828–1847. https://doi.org/10.3732/ajb.93.12.1828

Calviño, C.I., Martínez, S.G. & Downie, S.R. 2008. The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): Rapid radiations, long distance dispersals and hybridizations. *Molec. Phylogen. Evol.* 46: 1129–1150. https://doi.org/10.1016/j.ympev.2007.10.021

Calviño, C.I., Martínez, S.G. & Downie, S.R. 2010. Unraveling the taxonomic complexity of *Eryngium* L. (Apiaceae, Saniculoideae): Phylogenetic analysis of 11 non-coding cpDNA loci corroborates rapid radiations. *Pl. Diversity Evol.* 128: 137–149. https://doi.org/10.1127/1869-6155/2010/0128-0006

Cardoso da Silva, J.M. & Bates, J.M. 2002. Biogeographic patterns and conservation in the South American Cerrado: A tropical savanna hotspot. *BioScience* 52: 225–233. https://doi.org/10.1641/0006-3568(2002)052[0225:BPACIT]2.0.CO;2

Cardozo, A.L., Goldenberg, R., Fiaschi, P. & Labiak, P. 2021. *Eryngium* (Apiaceae, Saniculoideae) in the state of Paraná, southern Brazil. *Phytotaxa* 507: 1–50. https://doi.org/10.11646/phytotaxa.507.1.1

Carstens, B.C., Pelletier, T.A., Reid, N.M. & Satler, J.D. 2013. How to fail at species delimitation. *Molec. Ecol.* 22: 4369–4383. https://doi.org/10.1111/mec.12413

Constance, L., Chuang, T.I. & Bell, C.R. 1971. Chromosome numbers in Umbelliferae IV. *Amer. J. Bot.* 58: 577–587. https://doi.org/10.1002/j.1537-2197.1971.tb10007.x

Constance, L., Chuang, T.I. & Bell, C.R. 1976. Chromosome numbers in Umbelliferae V. *Amer. J. Bot.* 63: 608–625. https://doi.org/10.1002/j.1537-2197.1976.tb11849.x

Crisci-V., J., Freire-E., S., Sancho, G. & Katinas, L. 2001. Historical biogeography of the Asteraceae from Tandilia and Ventania

mountain ranges (Buenos Aires, Argentina). *Caldasia* 23: 21–41. http://www.jstor.org/stable/23641808

Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. 2012. jModelTest2: More models, new heuristics and parallel computing. *Nature, Meth.* 9: 772–772. https://doi.org/10.1038/nmeth.2109

Dayrat, B. 2005. Towards integrative taxonomy. *Biol. J. Linn. Soc.* 85: 407–417. https://doi.org/10.1111/j.1095-8312.2005.00503.x

De Queiroz, K. 2007. Species concepts and species delimitation. *Syst. Biol.* 56: 879–886. https://doi.org/10.1080/10635150701701083

Ellis, B., Daly, D.C., Hickey, L.J., Johnson, K.R., Mitchell, J.D., Wilf, P. & Wing, S.L. 2009. *Manual of leaf architecture*. New York: New York Botanical Garden Press.

El-Masri, M.M. & Fox-Wasylyshyn, S.M. 2006. Missing data: An introductory conceptual overview for the novice researcher. *Canad. J. Nursery Res.* 37: 156–171.

Esquivel Mattos, P. & Calviño, C.I. 2022. Collected but undetected for more than a century: A new species of *Eryngium* (Apiaceae, Saniculoideae) from Cordillera de Amambay, Paraguay. *Syst. Bot.* 47: 184–189. https://doi.org/10.1600/036364422X16442668423491

Fick, S.E. & Hijmans, R.J. 2017. WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *Int. J. Climatol.* 37: 4302–4315. https://doi.org/10.1002/joc.5086

Flouri, T., Jiao, X., Rannala, B., Yang, Z. & Yoder, A. 2018. Species tree inference with BPP using genomic sequences and the multispecies coalescent. *Molec. Biol. Evol.* 35: 2585–2593. https://doi.org/10.1093/molbev/msy147

Fujita, M.K., Leache, A.D., Burbrink, F.T., McGuire, J.A. & Moritz, C. 2012. Coalescent-based species delimitation in an integrative taxonomy. *Trends Ecol. Evol.* 27: 480–488. https://doi.org/10.1016/j.tree.2012.04.012

GIS Development Team 2021. QGIS Geographic Information System, version 3.28. http://qgis.osgeo.org

Kadereit, J.W. & Abbott, R.J. 2021. Plant speciation in the Quaternary. *Pl. Ecol. Diversity* 14: 105–142. https://doi.org/10.1080/17550874.2021.2012849

Kass, R.E. & Raftery, A.E. 1995. Bayes factors. *J. Amer. Statist. Assoc.* 90: 773–795. https://doi.org/10.1080/01621459.1995.10476572

Knowles, L.L. & Carstens, B.C. 2007. Delimiting species without monophyletic gene trees. *Syst. Biol.* 56: 887–895. https://doi.org/10.1080/10635150701701091

Knowles, L.L. & Chan, Y. 2008. Resolving species phylogenies of recent evolutionary radiations. *Ann. Missouri Bot. Gard.* 2: 224–231. https://doi.org/10.3417/2006102

Lartillot, N. & Philippe, H. 2006. Computing Bayes factors using thermodynamic integration. *Syst. Biol.* 55: 195–207. https://doi.org/10.1080/10635150500433722

Leaché, A.D. & Fujita, M.K. 2010. Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). *Proc. Roy. Soc. London, Ser. B, Biol. Sci*. 277: 3071–3077. https://doi.org/10.1098/rspb.2010.0662

Martínez, S.G. & Calviño, C.I. 2019. Apiaceae. Pp. 1–122 in: Zuloaga, F.O. & Belgrano, M.J. (eds.), *Flora vascular de la República Argentina*, vol. 20(2). Buenos Aires: Talleres Trama. https://doi.org/10.2307/j.ctvt7x6cb.4

Mathias, M.E., Constance, L. & Araujo, D. 1972. *Flora ilustrada Catarinense: Umbelíferas*. Santa Catarina: Herbario Barbosa Rodrigues.

Ogilvie, H.A., Bouckaert, R.R. & Drummond, A.J. 2017. StarBEAST2 brings faster species tree inference and accurate estimates of substitution rates. *Molec. Biol. Evol.* 34: 2101–2114. https://doi.org/10.1093/molbev/msx126

O'Leary, N., Calviño, C.I., Greizerstein, E., Martínez, S. & Poggio, L. 2004. Further cytogenetical studies on diploid and polyploid species of *Eryngium* L. (Saniculoideae, Apiaceae) from Argentina. *Hereditas (Lund)* 140: 129–133. https://doi.org/10.1111/j.1601-5223.2004.01795.x

Oliveira, P.S. & Marquis, R.J. 2002. *The cerrados of Brazil: Ecology and natural history of a Neotropical savanna*. New York: Columbia University Press. https://doi.org/10.7312/oliv12042

Padial, J.M., Miralles, A., De la Riva, I. & Vences, M. 2010. The integrative future of taxonomy. *Frontiers Zool.* 7: 16. https://doi.org/10.1186/1742-9994-7-16

Perthuy, G.Y., Martínez, S., Greizerstein, E.J. & Poggio, L. 2010. Genomic relationships among diploid and polyploid species of the genus *Eryngium* L. using genomic in situ hybridization. *Genome* 53: 824–831. https://doi.org/10.1139/G10-071

Rannala, B. & Yang, Z. 2013. Improved reversible jump algorithms for Bayesian species delimitation. *Genetics* 194(1): 245–253. https://doi.org/10.1534/genetics.112.149039

Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution* 14: 223–225. https://doi.org/10.2307/2409177

Rolón, C., Vera Jiménez, M. & Degen De Arrúa, R. (eds.) 2017. *Plantas nativas del Cerrado de Aguará Ñú, Reserva Natural del Bosque Mbaracayú, Paraguay.* Rojasiana, Serie Especial 3. San Lorenzo: Fundación Moisés Bertoni (FMB) y Rojasiana.

Shapiro, S.S. & Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52: 591–611. https://doi.org/10.1093/biomet/52.3-4.591

Shaw, J., Lickey, E.B., Schilling, E.E. & Small, R. 2007. Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: The tortoise and the hare III. *Amer. J. Bot.* 94: 275–288. https://doi.org/10.3732/ajb.94.3.275

Simon, M.F., Grether, R., de Queiroz, L.P., Skema, C., Pennington, R.T. & Hughes, C.E. 2009. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proc. Natl. Acad. Sci. U.S.A.* 106: 20359–20364. https://doi.org/10.1073/pnas.0903410106

SPAD 2012. *Base Aide à l'interprétation*, version 5.5. Paris: Cisia-Ceresta.

StatSoft 2014. STATISTICA (data analysis software system), version 7. http://www.statsoft.com, now https://www.tibco.com/products/data-science

Sukumaran, J. & Knowles, L.L. 2017. Multispecies coalescent delimits structure, not species. *Proc. Natl. Acad. Sci. U.S.A.* 114: 1607–1612. https://doi.org/10.1073/pnas.1607921114

Systematics Association Committee for Descriptive Terminology 1962. Terminology of simple symmetrical plane shapes (Chart 1). *Taxon* 11: 145–153, Addendum: 245–247. https://doi.org/10.2307/1216718, https://doi.org/10.2307/1217034

Turmel, J.M. 1949. Répartition géographique des *Eryngium*. II. Nouveau Monde. *Bull. Mus. Natl. Hist. Nat*., ser. 2, 21: 120–131.

Vera, D.G., Di Pietro, D.O., Tettamanti, G., Eirin, M., Trofino Falasco, C., Aranguren, M.F., Williams, J.D., Kacoliris, F.P. & Berkunsky, I. 2021. An annotated list of the reptiles of the highland grassland of Tandilia Mountains, Argentina. *Neotrop. Biol. Conservation* 16: 185–204. https://doi.org/10.3897/neotropical.16.e60629

Wiens, J.J. 2007. Species delimitation: New approaches for discovering diversity. *Syst. Biol.* 56: 875–878. https://doi.org/10.1080/10635150701748506

Wolff, H. 1913. Umbelliferae-Saniculoideae. Pp. 1–305 in: Engler, A. (ed.), *Das Pflanzenreich*, IV. 228. Leipzig: Wilhelm Engelmann.

Yang, Z. 2015. The BPP program for species tree estimation and species delimitation. *Curr. Zool.* 61: 854–865. https://doi.org/10.1093/czoolo/61.5.854

Yang, Z. & Rannala, B. 2010. Bayesian species delimitation using multilocus sequence data. *Proc. Natl. Acad. Sci. U.S.A.* 107: 9264–9269. https://doi.org/10.1073/pnas.0913022107

Yang, Z. & Rannala, B. 2014. Unguided species delimitation using DNA sequence data from multiple loci. *Molec. Biol. Evol.* 31: 3125–3135. https://doi.org/10.1093/molbev/msu279

Zhang, C., Zhang, D., Zhu, T. & Yang, Z. 2011. Evaluation of a Bayesian coalescent method of species delimitation. *Syst. Biol.* 60: 747–761. https://doi.org/10.1093/sysbio/syr071

Appendix 1. Specimens of *Eryngium cerradense* and *E. regnellii* examined for morphology and/or geography, with corresponding voucher information.

Species name and voucher information.

Eryngium cerradense Esquivel Mattos & C.I.Calviño, **sp. nov.: Paraguay.** Caaguazú, Compañía Pindó, camino entre Itakyry y Curuguaty, estribaciones de la Sra. de San Joaquín, alt. 300 m, 24°35′S, 55°20′W, 11 Oct 1995, *A. Schinini & G. Caballero Marmori 30198* (CTES nro. 211675 barcode 0551004); Canindeyú, [Cordillera de Mbaracayú] Sierra de Maracayú, Nov 1898–1899, *E. Hassler 5384* (G barcode G00448645); Dist. Corpus Christi, Reserva Natural del Bosque Mbaracayú, 24°11′01″S, 55°16′04″W, 16 Feb 2017, *P. Esquivel Mattos 85* (BCRU barcode BCRU000001V); 15 Feb 2018, *P. Esquivel Mattos 169* (BCRU). ***Eryngium regnellii*** Malme: **Argentina.** Buenos Aires, Pdo. Tandil, Sierra de Tandil, Sierra de las Ánimas, 21 Nov 1940, *A.L. Cabrera 6808* (LP045828); Pdo. Tandil, Sierra de Tandil, Cerro Albión, 22 Jan 1971, *J. Frangi 156* (UC barcode UC1382082); [Pdo. Tornquist], Sierra de la Ventana, cerca de Tornquist, 26 Dec 1988, *R.B. Selander 89-A-78* (SI069624); Misiones, Dpto. Cainguás, Campo Grande, alt. 408 m, 27°15′41.4″S, 54°57′31.3″W, 22 Oct 2011, *H.A. Keller, J.H. Pirelli & N.G. Paredes 10402* (CTES barcode CTES0051004); Dpto. Iguazú, Delicia, 8 Nov 1949, *E. Schwindt 2346* (US barcode 03076889); Dpto. Leandro N. Alem, R4, 8 Km S de Bonpland, entre Bonpland y Alem, alt. 221 m, 27°32′S, 55°25′W, 8 Dec 2004, *C. Calviño, S. Martínez, N. O'Leary & R. Olmstead 643* (SI069625). **Brazil.** Distrito Federal, [Brasilia], Córrego Jeriva, ca. 10 km E of Brasilia, 975 m, 15 Sep 1965, *H.S. Irwin, R.Souza & R. Reis dos Santos 8319* (NY barcode 00877753); Brasilia, 16 Nov 1958, *E. Pereira & G. Pabst 7418* (UC barcode UC1228838); Brasilia, 10 km W of Taguatinga, on road to Braslandia, alt. 1200 m, 25 Nov 1965, *H.S. Irwin, R. Souza & R. Reis dos Santos 10651* (NY barcode 00877751); Goiás, Apr 1840, *Gardner 3758* (K barcode K001130573); Mun. Cavalcante, *W.J. Burchell 7609* (K barcode K001130574); Mun. Santo Antônio do Descoberto, 24 Oct 1894, *A. Glaziou 21467* (P barcode P00130417); Rio de Gama, 31 Oct 1894, *A. Glaziou 21468* (P barcode P00130415); Minas Gerais, Oct 1848, *Regnell III-609* (US barcode 03076893); Mun. Caldas, 23 Nov 1861, *Regnell III-604* (S-R-9235); Mun. Caldas, 10 Nov 1876, *Regnell III-604* (US barcode 03076891); Mun. Caldas, 5 Dec 1873, *Mosen 890* (S14-43877); Mun. Caldas, 1 Oct 1873, *Mosen 541* (S06-14524); Mun. Uberaba, 15 Nov 1848, *Regnell III-604* (S14-43870, US barcode 03076892); Paraná, Mun. Piên, 13 Dec 1951, *G. Hatschbach 2635* (UC barcode UC956760); Rio Grande do Sul, Mun. Cachoeira do Sul, 1 May 1902, *G.O. Malme Ser. II No. 973* (S-R-9236, S06-14523); Mun. Santana do Livramento, Cerro Armour, 16 Nov 1975, *M.L. Porto & al. 1864* (CTES151072); Santa Catarina, Bom Jardim, S. Joaquim, alt. 1300 m, 15 Dec 1958, *Reitz & Klein 7964* (UC barcode UC1179203). **Uruguay.** Lavalleja, Polanco, 20 Nov 1963, *Del Puerto 3055* (MVFA); Cerro Arequita, 3 Oct 1937, *B. Rossengurtt B-2153* (MVFA); Maldonado, Sierra de las Animas, Dec 1937, *A. Lombardo 2080* (MVJB); Cerro Pan de Azúcar, 11 Feb 1940, *B. Rossengurtt B-3092* (MVFA); Montevideo, Jan 1941, *Gautier s.n.* (LP); San José, Sierra de Mahoma, 18 Oct 1941, *B. Rossengurtt B-3526* (MVFA).

Appendix 2. DNA accessions used in this study.

Species name, voucher information, DNA accession no., and GenBank reference no. for each data partition (*trnQ-rps16*, *rps16* intron, *rps16-trnK*^{UUU} 5′exon, *trnG*^{GCC}-*trnS*^{GCU}, *rpL32-trnL*^{UAG}, nuclear rDNA ITS). Accessions marked with an asterisk (*) were considered previously in Calviño & Downie (2007) and/or Calviño & al. (2008).

****Eryngium agavifolium*** Griseb. Argentina. Córdoba, Depto. Punilla, Pampa de Achala, 6 April 1977, *Pedersen 11703* (UC), DNA no. 2815, EU070416, EU070478, EU070540, –, –, EU070600. ****Eryngium alpinum*** L. Austria. Vienna, Heldenfriedhof, cult. Royal Botanic Garden Edinburgh (no. 19820697) from seeds obtained from Salzburg University, Austria, DNA no. 1189, DQ832445, DQ832445, DQ832445, –, –, EU070602. ****Eryngium alternatum*** J.M.Coult. & Rose. Mexico. Jalisco, Ciudad Guzman, Puerto de las Cruces, *H. Fuentes 654* (UC), cult. University of California, Berkeley, Constance pers. coll. no. C-2377, DNA no. 576, EU070417, EU070479, EU070541, –, –, EU070603. ****Eryngium amethystinum*** L. Cult. UIUC from seeds obtained from Hungarian Academy of Sciences Botanical Garden, Vácrátót, Hungary, *Downie 93* (ILL), DNA no. 93, DQ832446, DQ832446, DQ832446, –, –, EU070604. ****Eryngium aquifolium*** Cav. Spain. Cádiz, Sierra de Líjar, 11 July 1980, *Aparicio & al. s.n.* (MA 461716), DNA no. 3095, EU070418, EU070480, EU070542, –, –, EU070605. ****Eryngium aromaticum*** Baldwin. U.S.A. Florida, Dixie Co., 20 October 1977, *Kral 61212* (MO), DNA no. 2524, DQ832406, DQ832356, DQ832494, –, –, EU070606. ****Eryngium articulatum*** Hook. U.S.A. California, Tehama Co., 12 July 1995, *Oswald & Ahart 7062* (MO), DNA no. 2525, EU070419, EU070481, EU070543, –, –, EU070607. ****Eryngium balansae*** H.Wolff. Paraguay. Paraguarí, 25°37′S 57°07′W, 14 December 2003, *Múlgura & al. 3735* (SI), DNA no. 2966, EU070420, EU070482, EU070544, –, –, EU070608. ****Eryngium bonplandii*** F.Delaroche. Mexico. Michoacán, 5 October 1990, *García Ruiz 3256* (UC), DNA no. 2816, EU070421, EU070483, EU070545, –, –, EU070609. ****Eryngium bourgatii*** Gouan. Cult. UIUC from seeds obtained from National Botanic Gardens, Glasnevin, Ireland, *Downie 195* (ILL), DNA no. 195, DQ832447, DQ832447, DQ832447, –, –, EU070610. ****Eryngium buchtienii*** H.Wolff. Bolivia. Murillo, La Paz, Valle de Zonga, 11 December 1986, *Beck 13084* (UC), DNA no. 2818, EU070422, EU070484, EU070546, –, –, EU070612. ****Eryngium bungei*** Boiss. Turkmenistan. Karakamenskii Region, 20 June 1974, *Franukyevir s.n.* (MO), DNA no. 2527, DQ832448, DQ832448, DQ832448, –, –, EU070613. ****Eryngium bupleuroides*** Hook. & Arn. Chile. Isla Juan Fernández, cult. Conservatoire Botanique de la Ville de Mulhouse (no. 01-057a), 14 June 2002, *Hildenbrand s.n.* (ILL), DNA no. 2248, DQ832407, DQ832357, DQ832495, –, –, EU070614. ****Eryngium caeruleum*** Bieb. Russia. 8 August 1965, *Cnoiseancoba & al. s.n.* (US), DNA no. 2959, EU070423, EU070485, EU070547, –, –, EU070615. ****Eryngium caespitiferum*** Font Quer & Pau. Morocco. Montis Lexhab (Gomara), 21 July 1930, *Font Quer s.n.* (UC), DNA no. 2817, DQ832449, DQ832449, DQ832449, –, –, EU070616. ****Eryngium campestre*** L. Cult. UIUC from seeds obtained from Jardin botanique de Caen, France (no. 1453), *Downie 305* (ILL), DNA no. 305, EU070424, EU070486, EU070548, –, –, EU070617. ****Eryngium carlinae*** F.Delaroche. Mexico. Durango, 20 August 1979, *Wagner & Solomon 4294* (MO), DNA no. 2528, EU070425, EU070487, EU070549, –, –, EU070619. ***Eryngium cerradense*** Esquivel Mattos & C.I.Calviño, **sp. nov.** Paraguay. Canindeyú, Dist. Corpus Christi, Reserva Natural del Bosque Mbaracayú, 24°11′01″S, 55°16′04″W, 16 Feb 2017, *P. Esquivel Mattos 85* (BCRU barcode BCRU000001), DNA no. CC-602, ON326414, ON230063, ON326418, ON326426, ON326422, ON130147; Dist. Corpus Christi, Reserva Natural del Bosque Mbaracayú, 24°11′01″S, 55°16′04″W, 15 Feb 2018, *P. Esquivel Mattos 169* (BCRU), DNA no. CC-599, ON326413, ON230062, ON326417, ON326425, ON326421, ON130146. ****Eryngium cervantesii*** F.Delaroche. Mexico. *Constance pers. coll. no. C-2443*, DNA no. 791, DQ832450, DQ832450, DQ832450, –, –, EU070620. ****Eryngium chamissonis*** Urb. Argentina. Corrientes, Depto. Ituzaingó, 27°59′S 56°01′W, 22 January 2003, *Calviño & O'Leary 608* (SI), DNA no. 2491, EU070427, EU070489, EU070551, –, –, EU070623. ****Eryngium ciliatum*** Cham. & Schltdl. Argentina. Entre Ríos, Depto. Concordia, Parque Rivadavia, 31°22′S 57°59′W, 23 January 2003, *Calviño & O'Leary 613* (SI), DNA no. 2481, EU070428, EU070490, EU070552, –, –, EU070624. ****Eryngium coquimbanum*** Phil. ex Urb. Chile. Coquimbo, Elqui, Cuesta Porotitos, ca. 15 km N of La Serena, 2 December 1987, *Dillon & Teillier 5001* (UC), DNA no. 2820, DQ832408, DQ832358, DQ832496, –, –, EU070626. ****Eryngium corniculatum*** Lam. Cult. Hortus Botanicus Univ. Portugalensis, Campo Alegre, #134, *Constance C-542* (UC), DNA no. 2821, DQ832409, DQ832359, DQ832497, –, –, EU070627. ****Eryngium coronatum*** Hook. & Arn. Argentina. Entre Ríos, Depto. Concordia, Parque Rivadavia, 7 November 2000, *Calviño 104* (SI), DNA no. 2847, EU070429, EU070491, EU070553, –, –, EU070629; Paraguay. Parguarí, Arroyo Yuquyty, 1 km E of Nueva Italia, 14 December 1989, *Zardini & Velazquez 17068* (UC), cult. University of California, Berkeley, *Constance pers. coll. no. C-2389*, DNA no. 508, DQ832410, AF110586, DQ832498, –, –, EU070628. ****Eryngium crassisquamosum*** Hemsl. Mexico. Durango, 26 November 1970, *Soule 2190* (MO), DNA no. 2530, EU070430, EU070492, EU070554, –, –, EU070630. ****Eryngium creticum*** Lam. Jordan, 15 May 2002, *Lahham s.n.* (Yarmouk University Herbarium), DNA no. 2054, EU070431, EU070493, EU070555, –, –, EU070631. ****Eryngium diffusum*** Torr. U.S.A. Texas, Brazos Co., N Navasota, 24 July 1977, *Fryxell 2956* (UC), DNA no. 2822, EU070432, EU070494, EU070556, –, –, EU070632. ****Eryngium duriaei*** J.Gay ex Boiss. Spain. Oviedo, Puerto del Connio, Fuente Parada, 25 August 2001, *Serra & Bort 6121* (MA), DNA no. 3094, EU070433, EU070495, EU070557, –, –, EU070634. ****Eryngium ebracteatum*** Lam. Argentina. Corrientes, Depto. Itatí, RN 12, 28 km E Itatí, 22 January 2003, *Calviño & O'Leary*

Appendix 2. Continued.

600 (SI), DNA no. 2482, DQ832411, DQ832360, DQ832499, –, –, EU070635. ****Eryngium eburneum*** Decne. Argentina. Entre Ríos, Depto. Concordia, Concordia, Parque Rivadavia, February 2002, *O'Leary 11* (SI), DNA no. 2323, DQ832451, DQ832451, –, –, EU070637. ****Eryngium echinatum*** Urb. Argentina. Entre Ríos, Depto. Concordia, Concordia, Parque Rivadavia, 31°22′S 57°59′W, 23 January 2003, *Calviño & O'Leary 611* (SI), DNA no. 2483, EU070434, EU070496, EU070558, –, –, EU070638. ****Eryngium elegans*** Cham. & Schltdl. Argentina. Corrientes, Depto. Itatí, 28 km E Itatí, 27°36′S 56° 40′W, 22 January 2003, *Calviño 601* (SI), DNA no. 2823, EU070435, EU070497, EU070559, –, –, EU070640; Jujuy, near San Salvador de Jujuy, 14 February 1983, *Ornduff 8996* (UC), cult. University of California, Berkeley (no. 90.0536), *Constance pers. coll. no. C-2245*, DNA no. 786, DQ832452, DQ832452, DQ832452, –, –, EU070639. ****Eryngium floribundum*** Cham. & Schltdl. Argentina. Buenos Aires, Pdo. Tigre, Isla Vieja del Buenos Aires, 27 January 2003, *Calviño & al. 621* (SI), DNA no. 2484, EU070436, EU070498, EU070560, –, –, EU070645. ****Eryngium fluitans*** M.E.Jones. Mexico. Durango, El Salto, 8 August 1959, *Bell & Duke 16612* (LL), DNA no. 2953, EU070437, EU070499, EU070561, –, –, EU070646. ****Eryngium foetidum*** L. U.S.A. Illinois, Champaign Co., Illinois Natural History Survey, in greenhouse, 4 April 1999, *Hill 31238* (ILLS), DNA no. 2569, EU070438, EU070500, EU070562, –, –, EU070647. ****Eryngium galioides*** Lam. Spain, Guadalajara, Puebla de Beleña, Beleña Great Lake, 4 September 1996, *Goldman & al. 1066* (LL), DNA no. 2954, EU070439, EU070501, EU070563, –, –, EU070648. ****Eryngium ghiesbreghtii*** Decne. Mexico. Jalisco, 10 miles S of Autlan, 19 August 1949, *R.L. & C.R. Wilbur 2426* (WIS), DNA no. 2825, DQ832412, DQ832361, DQ832500, –, –, EU070649. ****Eryngium giganteum*** M.Bieb. Armenia. Mt. Tehenis, above Tsakhadzor, N Erevan, 5 August 1984, *Mc Neal 472* (UC), DNA no. 2826, EU070440, EU070502, EU070564, –, –, EU070650. ****Eryngium glaciale*** Boiss. Spain. Mount Valeta, Sierra Nevada, 5 October 1991, *Cannon s.n*., *Constance pers. coll. no. C-2222* (UC), DNA no. 2827, EU070441, EU070503, EU070565, –, –, EU070651. ****Eryngium glomeratum*** Lam. Jordan, 28 June 2002, *Lahham s.n*. (Yarmouk University Herbarium), DNA no. 2252, EU070442, EU070504, EU070566, –, –, EU070652. ****Eryngium glossophyllum*** H.Wolff. Argentina. Salta, Rd. to Pueblo Lizote, 22°30′S 65°W, 7 March 1993, *Funk & Katinas 11155* (US), DNA no. 2965, EU070443, EU070505, EU070567, –, –, EU070653. ****Eryngium goyazense*** Urb. Brazil. Goiás, Serra dos Cristais, ca. 5 km S of Cristalina, 5 March 1966, *Irwin & al. 13576* (UC), DNA no. 2829, DQ832413, DQ832362, DQ832501, –, –, EU070654. ****Eryngium hemisphaericum*** Urb. Brazil. Minas Gerais, Serra do Cabral, W Cantoni, 9 March 1970, *Irwin & al. 27260* (MO), DNA no. 2534, EU070444, EU070506, EU070568, –, –, EU070657. ****Eryngium horridum*** Malme. Argentina. Entre Ríos, Depto. Concordia, Concordia, Parque Rivadavia, 17 February 2002, *O'Leary 5* (SI), DNA no. 2325, EU070445, EU070507, EU070569, –, –, EU070659. ****Eryngium humile*** Cav. Costa Rica. San José, RN 2, km 87–88, 29 February 1984, *Khan & al. 1402* (MO), DNA no. 2537, EU070446, EU070508, EU070570, –, –, EU070660. ****Eryngium*** cf. ***huteri*** Porta & Rigo ex Porta. Spain. Granada, Huéscar, Sierra de la Sagra, 27 July 1996, *Martínez Ortega & al. SALA94907* (MA), DNA no. 3093, EU070426, EU070488, EU070550, –, –, EU070621. ****Eryngium ilicifolium*** Lam. Spain. Alicante, Sierra de Orihuela, 21 June 2001, *Serra & al. 5778* (MA), DNA no. 3092, EU070447, EU070509, EU070571, –, –, EU070661. ****Eryngium inaccessum*** Skottsb. Chile. Isla Juan Fernández, cult. Conservatoire Botanique de la Ville de Mulhouse (no. 99048), 14 June 2002, *Hildenbrand s.n*. (ILL), DNA no. 2249, EU070448, EU070510, EU070572, –, –, EU070662. ****Eryngium incantatum*** Lucena, Novara & Cuezzo. Argentina. Salta, Depto. Chicoana, Valle Encantado, 15 March 2002, *Calviño 400* (SI), DNA no. 2363, EU070449, EU070511, EU070573, –, –, EU070663. ****Eryngium integrifolium*** Walter. U.S.A. South Carolina, Charleston Co., WNW McClellanville, 2 September 1989, *MacDougal 4620* (MO), DNA no. 2538, EU070450, EU070512, EU070574, –, –, EU070664. ****Eryngium juncifolium*** (Urb.) Mathias & Constance. Argentina. Misiones, Depto. San Ignacio, Parque Prov. Teyú Cuaré, 19 December 2001, *Mello-Silva & al. 1931* (SI), DNA no. 2364, EU070451, EU070513, EU070575, –, –, EU070666. ****Eryngium leavenworthii*** Torr. & Gray. U.S.A. Texas, Grayson, Snead Environmental Research Area, 1.6 km N of Hwy 82 and 1417, 33°41′14″N, 96°47′12″E, 11 September 1986, *Perches & al. 8* (WIS), DNA no. 2832, DQ832453, DQ832453, DQ832453, –, –, EU070669. ****Eryngium longifolium*** Cav. Mexico. Michoacán, Jarahuaro, *Rzedowski 46148* (UC), cult. University California, Berkeley, *Constance pers. coll. no. C-2373*, DNA no. 575, EU070452, EU070514, EU070576, –, –, EU070670. ****Eryngium macrocalyx*** Schrenk. Kyrgyzstan. Chuy Region, S of Bishkek, Thon-Aryk (Boz-Peldek Mountain), 42°47′06″N, 74°34′37″E, 2 July 2000, *Phillippe & al. 31793* (ILLS), DNA no. 2570, DQ832415, DQ832364, DQ832503, –, –, EU070672. ****Eryngium madrense*** S.Watson. Mexico. Chihuahua, Guerrero, between Cuauhtemoc and La Junta, 19 August 1978, *Bye 8688* (LL), DNA no. 2955, EU070453, EU070515, EU070577, –, –, EU070673. ****Eryngium maritimum*** L. Portugal. Algarve, Amacao de Pera, Praia Grande, 7 June 2001, *Medina & al. MP1656* (LL), DNA no. 2957, EU070454, EU070516, EU070578, –, –, EU070674. ****Eryngium megapotamicum*** Malme. Argentina. Entre Ríos, Depto. Concordia, Concordia, Parque Rivadavia, February 2002, *O'Leary 4* (SI), DNA no. 2324, EU070455, EU070517, EU070579, –, –, EU070676; aff. Uruguay. Maldonado, Piriápolis, Cerro Maldonado, 13 January 2005, *Martínez 300* (SI), DNA no. 2883, EU070456, EU070518, EU070580, –, –, EU070677. ****Eryngium mesopotamicum*** Pedersen. Argentina. Buenos Aires, Pdo. Tigre, Isla Vieja del Buenos Aires, 27 January 2003, *Calviño & al. 619* (SI), DNA no. 2485, EU070458, EU070520, EU070582, –, –, EU070679; Depto. Campana, Otamendi, 15 December 2001, *O'Leary 19* (ILL, SI), DNA no. 2312, EU070457, EU070519, EU070581, –, –, EU070678. ****Eryngium mexiae*** Constance. *Constance pers. coll. no. C-2418*, DNA no. 809, EU070459, EU070521, EU070583, –, –, EU070681. ****Eryngium mexicanum*** S.Watson. Mexico. *Constance pers. coll. no. C-2428*, DNA no. 794, DQ832454, DQ832454, DQ832454, –, –, EU070682. ****Eryngium nasturtiifolium*** Juss. ex F.Delaroche. Mexico. Sonora, N Bahia San Carlos, 4 June 1962, *Hutchison 2449* (WIS), DNA no. 2834, EU070460, EU070522, EU070584, –, –, EU070685. ****Eryngium nudicaule*** Lam. Argentina. Entre Ríos, Depto. Concordia, Concordia, Parque Rivadavia, 31°22′S, 57°59′W, 23 January 2003, *Calviño & O'Leary 610* (SI), DNA no. 2486, EU070461, EU070523, EU070585, –, –, EU070686. ****Eryngium ovinum*** A.Cunn. Australia. Canberra, 35°21′S, 149°10′E, 5 December 1980, *Canning 5025* (US), DNA no. 2963, EU070473, EU070535, –, –, –, EU070711; Victoria, Winchelsea, S Lake Murdeduke, 5 February 1963, *Aston 895* (US), DNA no. 2962, EU070462, EU070524, EU070586, –, –, EU070688. ****Eryngium palmatum*** Pancic & Vis. Cult. UC Berkeley Botanical Garden from seeds obtained from Hortus Botanicus, Acad. Sci. Bulgariae, Sofia, Bulgaria, *Constance C-99* (UC), DNA no. 2835, DQ832416, DQ832365, DQ832504, –, –, EU070689. ****Eryngium palmeri*** Hemsl. Mexico. Jalisco, Jocotepec, Co La Lima, 26 March 1987, *Machuca Nuñez 5652* (UC), DNA no. 2836, DQ832455, DQ832455, DQ832455, –, –, EU070690. ****Eryngium pandanifolium*** Cham. & Schltdl. Argentina. Entre Ríos, Depto. Concordia, Calabacillas to Pto. Yeruá, March 2000, *Martínez & al. 6* (SI), DNA no. 2327, EU070463, EU070525, EU070587, –, –, EU070691; Concordia, Parque Rivadavia, 31°22′S, 57°59′W, 23 January 2003, *Calviño & O'Leary 616* (SI), DNA no. 2487, EU070464, EU070526, EU070588, –, –, EU070692; *Calviño & O'Leary 617* (SI), DNA no. 2488, EU070465, EU070527, EU070589, –, –, EU070693. ****Eryngium paniculatum*** F.Delaroche. Argentina. Neuquén, Depto. Confluencia, 11 August 2002, *O'Leary 1* (SI), DNA no. 2316, EU070466, EU070528, EU070590, –, –, EU070694. ****Eryngium pilularioides*** Hemsl. & Rose. Mexico. Huichapan, Hidalgo, 1.7 km SE R45, road to Nopala, 27.5 km E R57, 4 July 1983, *Cowan 3997* (UC), DNA no. 2837, DQ832417, DQ832366, DQ832505, –, –, EU070695. ****Eryngium planum*** L. Cult. UIUC from seeds obtained from National Botanic Gardens, Glasnevin, Ireland, *Downie 191* (ILL), DNA no. 191, DQ832456, DQ832456, DQ832456, –, –, EU070696. ****Eryngium pristis*** Cham. & Schltdl. Argentina. Misiones, Depto. San Ignacio, 27°16′S, 55°33′W, 18 March 2002, *Múlgura & al. 3449* (SI), DNA no. 2367, EU070467, EU070529, EU070591, –, –, EU070698. ****Eryngium prostratum*** Nutt. ex DC. U.S.A. Illinois, Saline Co., 19 September 2002, *Calviño & al. 501* (ILL, SI), DNA no. 2329, EU070468, EU070530, EU070592, –, –, EU070699. ****Eryngium proteaflorum*** F.Delaroche. Mexico. Vera Cruz, Cofre de Perote, 19°30′N, 97°11′W, 27 June 1982, *Diggs & al. 2630* (MO), DNA no. 2541, EU070469, EU070531, EU070593, –, –, EU070700. ****Eryngium pseudojunceum*** Clos. Argentina. Neuquén, Parque Nacional Lanín, 11 February 1968, *Eskuche & Klein 1443-1* (CORD), DNA no. 2378, EU070470, EU070532, –, –, –, EU070701. ****Eryngium pyramidale*** Boiss. & Hausskn. ex Boiss. Turkey. 40 km SE Varto, 16 July 1982, *Sorger & Buchner 82-74-47* (W), DNA no. 3096, EU070471, EU070533, EU070594, –, –, EU070703. ***Eryngium regnellii*** Malme. Argentina. Buenos Aires, Pdo. Balcarce, Sierra La Larga, entrando por Estancia San Juan, 4 Mar 2014, *Calviño 827* (SI), DNA no. CC-605, ON326416, ON230065, ON326420, ON326427, ON326423, ON130149; Misiones, Dpto. Leandro N. Alem, R4, 8 Km S de Bonpland, entre Bonpland y Alem, alt. 221 m, 27°32′S, 55°25′W, 8 Dec 2004, *Calviño, S. Martínez, N. O'Leary & R. Olmstead 643* (SI069625), DNA no. 2882, EU070472*, EU070534*, EU070595*, ON326428, ON326424, EU070709*. Uruguay. Maldonado, Piriápolis, Cerro Maldonado, 13 Jan 2005 (det. 2 Feb 2005), *S.G. Martínez 301* (SI), DNA no. CC-604, ON326415, ON230064, ON326419, –, –, ON130148. ****Eryngium sanguisorba*** Cham. & Schltdl. Argentina. Corrientes, Depto. Santo tomé, Garruchos, 7 December 1997, *Múlgura & al. 1582* (SI), DNA no. 2824, EU070474, EU070536, EU070596, –, –, EU070713; *Constance pers. coll. no. C-2444*, DNA no. 790, DQ832457,

Appendix 2. Continued.

DQ832457, DQ832457, –, –, EU070712. ***Eryngium scaposum*** Turcz. Mexico. Oaxaca, near summit of Sierra San Felipe, 9 July 1969, *Niehus 889* (UC), DNA no. 2839, DQ832458, DQ832458, DQ832458, –, –, EU070714. ***Eryngium serbicum*** Pancic. Cult. University California, Berkeley from seeds obtained from Gardini de Villa Taranto, Pallanza, Italy, *Constance C-436* (UC), DNA no. 2840, DQ832459, DQ832459, DQ832459, –, –, EU070718. ***Eryngium serratum*** Cav. Mexico. Guanajuato, 5 km NE of Guanajuato, 11 November 1989, *R. & J. Galvan 3462* (MO), DNA no. 2544, DQ832418, DQ832367, DQ832506, –, –, EU070719. ***Eryngium spiculosum*** Hemsl. Mexico. Michoacán, Jiquilpan, *Ruiz 3402* (UC), cult. University California, Berkeley (no. 94.0960), *Constance pers. coll. no. C-2415*, DNA no. 559, DQ832460, DQ832460, DQ832460, –, –, EU070722. ***Eryngium tenue*** Lam. Morocco, 1930, *Font Quer s.n*. (UC), DNA no. 2842, DQ832419, DQ832368, DQ832507, –, –, EU070725. ***Eryngium ternatum*** Poir. Greece. Crete, 15 June 1942, *Rechinger 13795* (US), DNA no. 2964, EU070475, EU070537, EU070597, –, –, EU070726. ***Eryngium thoraefolium*** Boiss. Turkey. Prov. Vil. Mughla (Caria), Sandras Dag above Agla, 25 July 1947, *Davis 13591* (UC), DNA no. 2843, DQ832420, DQ832369, DQ832508, –, –, EU070727. ***Eryngium variifolium*** Coss. *Constance pers. coll*., DNA no. 784, EU070476, EU070538, EU070598, –, –, EU070728. ***Eryngium vaseyi*** J.M.Coult. & Rose. U.S.A. California, San Diego Co., Camp Pendleton, *Bliss s.n*. (UC), *Constance pers. coll. no. C-2394*, DNA no. 562, DQ832461, DQ832461, DQ832461, –, –, EU070729. ***Eryngium venustum*** Bartlett ex Constance. Mexico. Tamaulipas, NE Victoria, cult. University California, Berkeley (no. 91.0149), *Calviño & Keller 627* (ILL, SI), DNA no. 2846, EU070477, EU070539, EU070599, –, –, EU070731. ***Eryngium vesiculosum*** Labill. Australia. Sydney, New South Wales, 1 km NE of Collit's Inn near Hartley Vale at start of Locker's Road walking trail, 14 May 1984, *Coveny & Bishop 11849* (MO), DNA no. 2548, DQ832421, DQ832370, DQ832509, –, –, EU070732. ***Eryngium viviparum*** J.Gay. France, Morbiham, Belz, cult. Conservatoire Botanique de la Ville de Mulhouse (no. 20-109), 1 July 2002, *Hildenbrand s.n*. (ILL), DNA no. 2251, DQ832422, DQ832371, DQ832510, –, –, EU070733. ***Eryngium yuccifolium*** Michx. U.S.A. Illinois, Brown Co., *Tyson s.n*. (UC), cult. University California, Berkeley (no. 86.0104), DNA no. 807, DQ832462, DQ832462, DQ832462, –, –, EU070735. ***Hacquetia epipactis*** DC. Croatia. Gerovo, 8 km above town, 8 August 1984, *M.F. & S.G. Gardner 2590* (E), DNA no. 2877, DQ832423, DQ832372, DQ832511, –, –, EU070739; Cult. Royal Botanic Garden Edinburgh (no. 19694625), DNA no. 615, DQ832463, DQ832463, DQ832463, –, –, EU070737; Hungary. Comit. Turoc, in silvis ad balneas Stubnyafurdo, May 1916, *Margittai s.n*. (UC), DNA no. 2845, DQ832464, DQ832464, DQ832464, –, –, EU070738; Slovenia. S of Borovnica, Gorges du Pekel, 6 April 1976, *Greuter 13733* (E), DNA no. 2878, DQ832424, DQ832373, DQ832512, –, –, EU070740. ***Petagnaea gussonei*** (Spreng.) Rauschert. Italy. Nebrodi Mountains, cult. Botanical Garden of Palermo, *Donila 2005* (PAL), DNA no. 2880, DQ832466, DQ832466, DQ832466, –, –, EU070741; Cult. Royal Botanic Garden Edinburgh (no. 19695641), DNA no. 2881, DQ832465, DQ832465, DQ832465, –, –, EU070742. ***Sanicula arctopoides*** Hook. & Arn. U.S.A. California, Marin Co., pastured coastal slopes near Tomales, 18 February 1940, *Gould 868* (MO), DNA no. 2561, DQ832433, DQ832375, DQ832521, –, –, EU070743. ***Sanicula bipinnata*** Hook. & Arn. U.S.A. California, Tehama Co., Tehama, slopes beside US Hwy 99, 4 mi N of Red Bluff, 21 March 1953, *Bell 1394* (ILL), DNA no. 2575, DQ832434, DQ832376, DQ832522, –, –, EU070744. ***Sanicula canadensis*** L. U.S.A. Illinois, Champaign Co., Urbana, *Downie 737* (ILL), DNA no. 737, DQ832467, DQ832467, DQ832467, –, –, EU070746. ***Sanicula chinensis*** Bunge. Korea. Kyunggido, Mt. Chungrung, July 1996, *Lee s.n*. (ILLS), DNA no. 2699, DQ832436, DQ832378, DQ832524, –, –, EU070747. ***Sanicula graveolens*** DC. U.S.A. California, Plumas Co., in Red Clover Valley, Overland Inc. Ranchos, ca. 0.75 mi N of Genesee-Backwourth Rd, 10 June 1981, *Taylor & Foster 3886* (MO), DNA no. 2564, DQ832438, DQ832380, DQ832526, –, –, EU070748. ***Sanicula maritima*** S.Watson. U.S.A. California, Monterey Co., Cape San Martin (31a), *Vargas 15-96* (JEPS), DNA no. 2615, DQ832469, DQ832469, DQ832469, –, –, EU070749. ***Sanicula odorata*** (Raf.) K.M.Pryer & L.R.Phillippe. U.S.A. Illinois, Lasalle Co., Starved Rock State Park, South Ottawa; E of Salt Wells Stream; along N side of IL Rt. 71, S bank of Illinois River, 20 June 2000, *Hill 32457A* (ILLS), DNA no. 2572, DQ832439, DQ832381, DQ832527, –, –, EU070750. ***Sanicula orthacantha*** S.Moore. China. Jiangxi Province, Wuyuan Co., Zhangkou Mountain, 10 May 1998, *Lai & Shan 4763* (MO), DNA no. 2565, DQ832440, DQ832382, DQ832528, –, –, EU070751. ***Sanicula smallii*** E.P.Bicknell. U.S.A. Tennessee, Blount Co., Great Smoky Mountains National Park, Lower Panther Creek Watershed, 19 September 2001, *Feist & al. 1286* (ILLS), DNA no. 2573, DQ832441, DQ832383, DQ832529, –, –, EU070752. ***Sanicula tuberosa*** Torr. U.S.A. California, Marin Co., Mt. Tamalpais (27d), *Vargas 3-96* (JEPS), DNA no. 2616, DQ832471, DQ832471, DQ832471, –, –, EU070753.