Available online at www.sciencedirect.com
ScienceDirect
Molecular Phylogenetics and Evolution xxx (2008) xxx–xxx
MOLECULAR PHYLOGENETICS AND EVOLUTION
www.elsevier.com/locate/ympev

The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): Rapid radiations, long distance dispersals, and hybridizations

Carolina I. Calviño [a,b,*], Susana G. Martínez [c], Stephen R. Downie [a]

[a] *Instituto de Botánica Darwinion, Labardén 200, CC22, B1642HYD, San Isidro, Buenos Aires, Argentina*
[b] *Department of Plant Biology, University of Illinois at Urbana—Champaign, Urbana, IL 61801, USA*
[c] *Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina*

Received 5 August 2007; accepted 29 October 2007

Abstract

Eryngium is the largest and arguably the most taxonomically complex genus in the family Apiaceae. Infrageneric relationships within *Eryngium* were inferred using sequence data from the chloroplast DNA *trnQ–trnK* 5′-exon and nuclear ribosomal DNA ITS regions to test previous hypotheses of subgeneric relationships, explain distribution patterns, reconstruct ancestral morphological features, and elucidate the evolutionary processes that gave rise to this speciose genus. In total, 157 accessions representing 118 species of *Eryngium*, 15 species of *Sanicula* (including the genus *Hacquetia* that was recently reduced to synonymy) and the monotypic *Petagnaea* were analyzed using maximum parsimony and Bayesian methods. Both separate and simultaneous analyses of plastid and nuclear data sets were carried out because of the prevalence of polyploids and hybrids within the genus. *Eryngium* is confirmed as monophyletic and is divided into two redefined subgenera: *Eryngium* subgenus *Eryngium* and *E.* subgenus *Monocotyloidea*. The first subgenus includes all examined species from the Old World (Africa, Europe, and Asia), except *Eryngium tenue*, *E. viviparum*, *E. galioides*, and *E. corniculatum*. *Eryngium* subgenus *Monocotyloidea* includes all examined species from the New World (North, Central and South America, and Australia; herein called the "New World sensu stricto" clade) plus the aforementioned Old World species that fall at the base of this clade. Most sectional and subgeneric divisions previously erected on the basis of morphology are not monophyletic. Within the "New World sensu stricto" group, six clades are well supported in analyses of plastid and combined plastid and nuclear data sets; the relationships among these clades, however, are unresolved. These clades are designated as "Mexican", "Eastern USA", "South American", "North American monocotyledonous", "South American monocotyledonous", and "Pacific". Members of each clade share similar geographical distributions and/or morphological or ecological traits. Evidence from branch lengths and low sequence divergence estimates suggests a rapid radiation at the base of each of these lineages. Conflict between chloroplast and nuclear data sets is weak, but the disagreements found are suggestive that hybrid speciation in *Eryngium* might have been a cause, but also a consequence, of the different rapid radiations observed. Dispersal-vicariance analysis indicates that *Eryngium* and its two subgenera originated from western Mediterranean ancestors and that the present-day distribution of the genus is explained by several dispersal events, including one trans-Atlantic dispersal. In general, these dispersals coincide with the polytomies observed, suggesting that they played key roles in the diversification of the genus. The evolution of *Eryngium* combines a history of long distance dispersals, rapid radiations, and hybridization, culminating in the taxonomic complexity observed today in the genus.
© 2007 Published by Elsevier Inc.

Keywords: Apiaceae; Saniculoideae; *Eryngium*; cpDNA *trnQ–trnK* 5′-exon; nrDNA ITS; Phylogeny; Biogeography; Rapid radiation; Reticulate evolution; Long distance dispersals

* Corresponding author. Address: Instituto de Botánica Darwinion, Labardén 200, CC22, B1642HYD, San Isidro, Buenos Aires, Argentina. Fax: +54 11 4747 4748x101.
E-mail address: ccalvino@life.uiuc.edu (C.I. Calviño).

1055-7903/$ - see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.ympev.2007.10.021

Please cite this article in press as: Calviño, C.I. et al., The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): ..., Mol. Phylogenet. Evol. (2008), doi:10.1016/j.ympev.2007.10.021

UNCORRECTED PROOF

1. Introduction

> "It's hard to believe that I've spent as much of my time on this ungrateful genus as I have had, and still have such a weak grasp of it…".
>
> L. Constance, 1990.

When systematists think of complex or difficult genera, they might imagine a group widespread on every continent and with a huge number of species. These species would be morphologically variable and show integrating features, making it difficult to identify and delimit them or to interpret their interrelationships. To confound matters, the group may display different ploidy levels and natural hybrids, suggesting their recent and reticulate origin. *Eryngium* displays all of these characteristics and more.

The genus *Eryngium* comprises about 250 species. It is the largest genus in the family Apiaceae and accounts for approximately three-quarters of the species diversity within Saniculoideae, the subfamily to which it belongs. *Eryngium* is distributed in temperate regions of every continent. However, species richness is unequally spread between and within the eastern and western hemispheres. In each hemisphere, two centers of diversity are recognized: central-west Mexico and central-east South America (southern Brazil, northeast Argentina, and Uruguay); and western Mediterranean and southwest Asia (Turmel, 1948, 1949). About two-thirds of *Eryngium* species are distributed in North, Central and South America.

Eryngium is easily distinguished from other members of Apiaceae by its capitate inflorescences and single bract per flower. The genus, however, is extremely variable morphologically. Some plants are prostrate and only a few centimeters tall; others are erect and up to 3 m tall. Most species are herbaceous perennials, but many annual species also occur, and even a few are woody. Leaf morphology and venation are also variable. These plants may have long petiolated leaves or sessile ones, with entire to partite blades, entire, setose or spiny margins, and first order venation either pinnate, palmate or even parallel-veined. Floral bracts at the base of the capitule may be showy or indistinguishable from the other floral bracts. The distal floral bracts show a similar variation which, when modified, form the coma. Fruits display scales and/or vesicles arranged dorsally and/or laterally or may even be naked. These characters are displayed in a myriad of combinations, making it difficult to identify and delimit species or to interpret phylogenetic relationships. Other complications involve integrating characteristics and phenotypic plasticity. Cytologically, the genus is very diverse. The most common basic chromosome number is $x = 8$, but lower numbers also exist (i.e., $x = 5$–7) and variation in ploidy levels is widespread. Within the same species, different basic chromosome numbers or ploidy levels may occur (Bell and Constance, 1960, 1966; Constance et al., 1971, 1976).

Wolff's (1913) treatment of *Eryngium* is the most comprehensive and predominant. He grouped the species into 34 sections and numerous subsections (Table 1). He also recognized two major informal groups: "Species gerontogeae" and "Species americanae and australienses", the former representing 12 sections from the Old World and the latter, 22 sections from the Americas and Australia (designated here as the New World, for simplification). Subsequent taxonomic studies of *Eryngium* have been restricted to plants from specific geographic areas, but have each used Wolff's system of classification as their framework (Mathias and Constance, 1941; Breton, 1962; Irgang, 1974; Davis, 1972; Mathias et al., 1972; Pimenov and Tamamschian, 1987; Nieto Feliner, 2003; Martínez, 2005). A recent classification of *Eryngium* has been proposed by Wörz (2005) based on morphology, but it does not reflect phylogeny or solve problems of infrageneric relationships (Calviño and Downie, 2007).

Several authors have speculated on the evolutionary history of *Eryngium* (Decaisne, 1873; Wolff, 1913; Turmel, 1948; Cerceau-Larrival, 1973; Constance, 1977). In general, they all agree that the New World species have originated from Old World ancestors and that the origin of the genus was likely southwest Asian (Turmel, 1950, 1951; Cerceau-Larrival, 1971). Based on phylogenetic analyses of chloroplast DNA (cpDNA) *trnQ–trnK* 5′-exon sequences from accessions representing all genera of subfamily Saniculoideae and putatively allied taxa traditionally treated in subfamily Apioideae, Calviño and Downie (2007) confirmed the monophyly of *Eryngium* and revealed its sister group relationship to *Sanicula*. Both "Old World" and "New World" clades were identified within *Eryngium* and the phylogenetic positions of three species from the Iberian Peninsula (including Morocco) as successive sister lineages at the base of the "New World" clade suggested that *Eryngium* of the New World may have had their origin from western Mediterranean ancestors. No additional hypotheses on biogeography or taxonomy were formulated by Calviño and Downie (2007), given that their study focused on subfamily Saniculoideae as a whole and sampling of *Eryngium* was sparse.

The major objective of this study is to estimate phylogenetic relationships within *Eryngium* using molecular data. This phylogeny will be used to test previous hypotheses of subgeneric relationships proposed by Wolff (1913) and others. Ancillary objectives include elucidating the evolutionary processes that gave rise to this taxonomically complex genus, interpreting its biogeographic history, and reconstructing ancestral morphological characters within the group. To resolve phylogeny, we continue our examination of the cpDNA *trnQ–trnK* 5′-exon locus (hereafter, called *trnQ–trnK*), given its large number of parsimony informative characters and adequate levels of sequence divergence reported in a previous study of subfamily Saniculoideae (Calviño and Downie, 2007). We supplement these plastid data with additional data from the nuclear rDNA (nrDNA) internal transcribed spacer (ITS) region. Although strongly criticized (Álvarez and Wendel, 2003), this region is among the most popular markers used at

UNCORRECTED PROOF

Please cite this article in press as: Calviño, C.I. et al., The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): ..., Mol. Phylogenet. Evol. (2008), doi:10.1016/j.ympev.2007.10.021

Table 1
Infrageneric classification of *Eryngium* sensu Wolff (1913) showing the proportion of species sampled from each of his sections and subsections and listing the species sampled per section

Section subsection	Proportion sampled	Species sampled
"Species gerontogeae", Old World		
Alpina	2/2	*E. alpinum*, *E. giganteum*
Astrantiifolia	1/2	*E. palmatum*
Campestria	5/11	*E. amethystinum*, *E. bourgatii*, *E. campestre*, *E. glaciale*, *E. glomeratum*
Dilatata	1/2	
Eucampestria	3/6	
Palmatisecta	1/3	
Chamaeeryngium	1/1	*E. tenue*
Corniculata	1/1	*E. corniculatum*
Dryophylla	5/6	*E. aquifolium*, *E. bungei*, *E. duriaei*, (*E. caespitiferum*), *E. huteri*, *E. ilicifolium*
Carlinifolia	1/2	
Eudryophylla	4/4	
Gigantophylla	1/1	*E. pyramidale*
Halobia	2/2	*E. macrocalyx*, *E. maritimum*
Hygrobia	2/3	*E. galioides*, *E. viviparum*
Palmito	2/3	*E. serbicum*, *E. ternatum*
Plana	3/8	*E. caeruleum*, *E. creticum*, *E. planum*, *E. variifolium*
Thorifolia	1/1	*E. thoraefolium*
"Species americanae et australienses", New World		
Areata	4/8	*E. agavifolium*, *E. elegans*, *E. floribundum*, *E. weberbaueri*
Agavifolia	1/1	
Brevibracteata	3/5	
Aromatica	1/1	*E. aromaticum*
Carliniformia	2/13	*E. carlinae*, *E. lemmonii*
Comosa	2/11	
Diffusa	2/4	*E. diffusum*, *E. leavenworthii*
Eudiffusa	1/3	
Megalocephala	1/1	
Ebracteata	1/4	*E. ebracteatum*, (*E. incantatum*)
Foetida	6/9	(*E. buchtienii*), *E. coronatum*, *E. echinatum*, *E. foetidum*, *E. nudicaule*,
Eufoetida	4/7	*E. ombrophyllum*, *E. spiculosum*
Ombrophila	1/1	
Spiculosa	1/1	
Flaccida	2/3	*E. divaricatum*, *E. prostratum*
Fruticosa	1/2	*E. bupleuroides*, (*E. fernandezianum*, *E. inaccessum*)
Goyazensia	1/1	*E. goyazense*
Indiana	3/18	*E. articulatum*, *E. integrifolium*, *E. vaseyi*
Armata	2/13	
Virgata	1/1	
Madrensia	2/2	*E. madrense*, *E. mexicanum*, (*E. fluitans*)
Panniculata	31/46	*E. aloifolium*, *E. balansae*, (*E. brasiliense*), *E. canaliculatum*, *E. chamissonis*, *E. eburneum*, *E. eriophorum*, *E. eurycephalum*, *E. falcifolium*, *E. gramineum*, *E. hemsleyanum*, *E. horridum*, *E. junceum*, *E. juncifolium*, *E. koehneanum*, *E. lacustre*, *E. longifolium*, *E. luzulaefolium*, *E. megapotamicum*, *E. mesopotamicum*, (*E. mexiae*), *E. pandanifolium*, *E. paniculatum*, *E. pohlianum*, *E. pristis*, *E. purpusii*, (*E. rauhianum*), *E. regnellii*, *E. rojasii*, *E. scirpinum*, (*E. smithii*), *E. sellowii*, *E. sparganophyllum*, (*E. subinerme*, *E. venustum*), *E. yuccifolium*
Ensiformia	1/3	
Eupanniculata	30/43	
Petiolata	3/10	*E. bonplandii*, *E. gracile*, *E. ghiesbreghtii*
Eupetiolata	2/7	
Polycephala	1/1	
Pilularioides	1/1	*E. pilularioides*
Pseudojuncea	1/1	*E. pseudojunceum*
Pulchella	1/3	*E. coquimbanum*
Reptantia	2/3	*E. cervantesii*, *E. nasturtiifolium*
Rostrata	2/5	(*E. ovinum*), *E. rostratum*, *E. vesiculosum*
Eurostrata	1/1	
Stolonifera	1/1	
Sanguisorbiformia	3/6	*E. ciliatum*, *E. hemisphaericum*, *E. sanguisorba*
Marginata	1/3	
Sanguisorba	2/3	

(*continued on next page*)

UNCORRECTED PROOF

Table 1 (*continued*)

Section subsection	Proportion sampled	Species sampled
Serrata	1/2	*E. serratum*
Spinescentia	4/13	(*E. alternatum*), *E. crassisquamosum*, (*E. monocephalum*), *E. montanum*, *E. palmeri*, *E. Proteaflorum*
Euspinescentia	3/10	
Involucrata	1/3	
Stellata	3/8	*E. glossophyllum*, *E. humile*, *E. scaposum*
Eustellata	3/7	
Total	103/201	

Species numbers are after Wolff (1913). Species between brackets were not treated by Wolff (1913) or are incertae sedis; these species have not been included in the numbers of species per section or subsection.

low taxonomic levels, and within Apiaceae it yields the greatest number of informative characters compared to other examined loci (Downie et al., 2001). The main problem in using ITS data for inferring Apiaceae phylogeny arises when analyzing distant taxa, such as members spanning the entire family; at such deep levels of comparison, ITS sequences are just too divergent for phylogenetic analyses (Downie et al., 2001; Hardway et al., 2004; Calviño and Downie, 2007). On the contrary, at the infrageneric level, the accumulation of indels in the ITS region is usually insignificant, the alignment is unambiguous, and paralogs (if they occur) may readily be identified because of their sequence divergence (Spalik and Downie, 2007). Consideration of both nuclear and chloroplast markers is important because any significant discordance of relationships between data sets may serve to identify past hybridization or introgression events (Doyle, 1992; Rieseberg and Brunsfeld, 1992; Soltis and Kuzoff, 1995), phenomena which have likely played important roles in the evolution of *Eryngium*. We present the first explicit phylogenetic hypothesis for the genus and highlight the roles of long distance dispersal, hybridization, and rapid radiation in shaping the complex taxonomic relationships observed today in *Eryngium*.

2. Materials and methods

2.1. Accessions examined

In total, 157 accessions of Apiaceae were examined for cpDNA *trnQ–trnK* and/or nrDNA ITS sequence variation. In the phylogenetic analysis of *trnQ–trnK sequences*, 117 accessions were considered, which included 90 species of *Eryngium*, 15 species of *Sanicula* (including the genus *Hacquetia* that was recently reduced to synonymy; Calviño and Downie, 2007), and the monotypic *Petagnaea*. DNA sequences for 62 of these accessions were specifically obtained for this study (online Supplementary Appendix A); data for the remaining 55 accessions were obtained during a previous study (Calviño and Downie, 2007). In the ITS analysis, 136 accessions of *Eryngium* (representing 117 species), 15 accessions of *Sanicula* (representing 12 species including *Hacquetia*), and 2 accessions of *Petagnaea* were considered, all of which are new (online Supplementary Appendix A). One hundred and twelve accessions were common to both cpDNA and ITS data sets. The ingroup accessions represent all 34 sections of *Eryngium* recognized by Wolff (1913) (Table 1); for most of these sections, at least half of the species within each were sampled. When selecting taxa for inclusion in our analyses, their geographic diversity was also considered.

All phylogenetic trees were rooted with *Petagnaea gussonei*, as a previous study revealed a sister group relationship between this genus and *Eryngium* plus *Sanicula* (Calviño and Downie, 2007). As additional outgroups, we included representatives of *Sanicula*. The monophyly and sister group relationship of *Sanicula* and *Eryngium* were previously assessed based on cpDNA data (Calviño and Downie, 2007). *Sanicula* was included here to ascertain if nuclear ITS data support the same sister group relationship as that inferred by cpDNA.

2.2. Experimental strategy

Leaf material for DNA extraction was obtained from herbarium specimens, botanic gardens, or the field (online Supplementary Appendix A). For most accessions, total genomic DNA was obtained from about 20 mg of dried leaf tissue using a DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA). For several accessions extracted during previous studies, the modified hexadecyltrimethylammonium bromide (CTAB) protocol of Doyle and Doyle (1987) was used instead, as detailed in Downie and Katz-Downie (1996, 1999).

The *trnQ–trnK* region is bounded by chloroplast genes *trnQ* and *trnK* 5′-exon and includes the *rps16* gene (with intron) and its flanking intergenic spacer regions. In Saniculoideae the whole region is, on average, 3323 bp in size (Calviño and Downie, 2007). The strategies employed to obtain these cpDNA sequence data are presented elsewhere (Downie and Katz-Downie, 1996, 1999; Calviño et al., 2006; Calviño and Downie, 2007). The nrDNA ITS region encompasses two internal transcribed spacers (ITS1 and ITS2) and an intervening 5.8S gene. Upstream of ITS1 is the 18S rDNA gene; downstream of ITS2 is the 26S rDNA gene (Fig. 1). The entire ITS region was PCR-amplified using primers "18Sfor" and either "28Srev" or "C26A" (Fig. 1). For some accessions, the ITS1 and ITS2 regions

UNCORRECTED PROOF

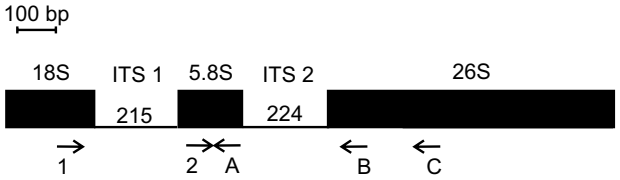

Fig. 1. Map of the 1568 bp locus of *Daucus carota* nrDNA (Yokota et al., 1989) showing the relative positions of genes 18S, 5.8S, and 26S and the two internal transcribed spacer regions (ITS1 and ITS2). The sizes of the two ITS regions are presented in base pairs (bp). Scale bar is 100 bp unit. The arrows represent the directions and approximate positions of the primers used in PCR amplification and/or DNA sequencing. Forward primers are designated 1–2; reverse primers are designated A–C. These primer sequences, written 5′–3′, are as follows: 1, GTC CAC TGA ACC TTA TCA TTT AG (18Sfor); 2, CGA TGA AGA ACG TAG CGA AAT G (ITS-3N); A, TTC TGC AAT TCA CAC CAA GTA T (5.8S-ITS1-R); B, GTT TCT TTT CCT CCG CT (C26A); C, TTG GAC GGA ATT TAC CGC CCG (28Srev).

were each amplified separately using internal primers "ITS-3N" and "5.8S-ITS1-R". Twenty-five microliters of PCRs were prepared according to Downie and Katz-Downie (1996), but with the addition of 5% v/v of dimethylsulfoxide (DMSO). For amplifications using primers "18Sfor" and "28Srev", the annealing temperature was decreased from 53 to 48 °C. The purification and sequencing strategies employed to obtain these ITS sequence data are the same as those described for the cpDNA region (Calviño et al., 2006; Calviño and Downie, 2007). Simultaneous consideration of both DNA strands across the entire cpDNA and ITS regions for most taxa permitted unambiguous base determination. All newly obtained cpDNA and ITS sequences have been submitted to GenBank.

2.3. Sequence comparisons and phylogenetic analyses

Sequence chromatograms were edited manually using Se-Al (Rambaut, 2002). DNA sequences were aligned initially using the default pairwise and multiple alignment parameters in the computer program ClustalX (gap opening cost = 15.00, gap extension cost = 6.66, DNA transition weight = 0.50; Jeanmougin et al., 1998) then rechecked and adjusted manually as necessary. Gaps were positioned to minimize nucleotide mismatches. A matrix of binary-coded indels was constructed for each locus (i.e., *trnQ–trnK* and ITS) to incorporate length-mutational information into the phylogenetic analysis. Gap coding was according to Downie and Katz-Downie (1999); for several regions, gap coding was problematic because of homopolymers or indirect duplications of adjacent elements in two or more taxa. These gaps were not scored and these ambiguous regions were excluded from subsequent analysis.

Some regions of the alignments were scored as missing. Data for portions of the *trnQ–rps16* or the *rps16–trnK* intergenic spacers could not be obtained for *Eryngium caeruleum*, *E. caespitiferum*, *E. fluitans*, *E. hemisphaericum*, *E. humile*, *E. pandanifolium* 2487, *E. pseudojunceum*, *E. ternatum*, and *Sanicula chinensis*. The *rps16–trnK* intergenic spacer in *E. pseudojunceum* and *E. rostratum* could not be PCR-amplified. Portions of the *rps16* 3′exon were missing data (between primers "3′exon-CR" and "3′exon-1"; Calviño and Downie, 2007), attributable to the positions of the primers anchored in this exon used to amplify the regions flanking it. However, this exon had little to no variation among all other accessions, hence the absence of these data did not affect the phylogenetic results. Overall, missing data represented 3.5% of the entire *trnQ–trnK* matrix. In the ITS matrix, only ITS1 and a part of 5.8S were scored as missing for *Eryngium duriaei* and *E. pyramidale*. Nineteen accessions of *Eryngium* displayed evidence of ITS sequence additivity at multiple nucleotide sites, as inferred by overlapping peaks on electropherograms from both forward and reverse sequencing runs. These polymorphic sites were scored using IUPAC nucleotide symbols for ambiguous bases.

The determination of boundary sequences for coding regions within the cpDNA *trnQ–trnK* locus was based on corresponding boundaries inferred previously for Saniculoideae which, in turn, were based on those of tobacco cpDNA (Shinozaki et al., 1986; Calviño and Downie, 2007). Boundaries of nuclear rDNA genes 18S, 5.8S, and 26S were determined by comparison of these DNA sequences to corresponding boundaries in *Daucus carota* rDNA (Yokota et al., 1989). Characterization of the *trnQ–trnK* and ITS regions was facilitated using BioEdit version 6.0.7 (Hall, 1999) and PAUP version 4.0b10 (Swofford, 2002). Uncorrected pairwise nucleotide distances of unambiguously aligned positions were determined using the distance matrix option of PAUP*.

The *trnQ–trnK* and ITS data matrices (with and without their corresponding scored indels) were each analyzed separately and combined using maximum parsimony (MP) as implemented by PAUP*. The heuristic search strategies employed by Calviño et al. (2006) were followed. Bootstrap values were calculated from 100,000 replicate analyses using "fast" stepwise-addition of taxa and only those values compatible with the majority-rule consensus tree were recorded. To examine the extent of conflict between the *trnQ–trnK* and ITS data sets for a comparable set of taxa, the incongruence length difference (ILD) test of Farris et al. (1995) was implemented using the partition homogeneity test of PAUP*. This test was carried out with 1000 replicate analyses, using the heuristic search option with simple addition of taxa and tree-bisection-reconnection (TBR) branch swapping. In addition, incongruence between the plastid and nuclear-derived trees was examined graphically using consensus networks as implemented by the program SplitsTree4 (Huson and Bryant, 2006). This method provides a visualization of the extent to which a collection of gene trees suggests contradictory taxon relationships. If a collection of gene trees has congruent topologies, consensus networks will be tree-like; where relationships are incongruent, these graphs will be net-like (McBreen and

UNCORRECTED PROOF

Lockhart, 2006). The starting point for this method is a collection of gene trees. In order to take into account phylogenetic uncertainty we used the bootstrap majority-rule consensus tree for each data set, so as to compare only those relationships that were most strongly supported. To explain the incongruent relationships displayed by a net-like consensus network in terms of reticulation events, a hybridization network was constructed. Alternative resolutions for the polytomies observed in MP strict consensus trees were explored using the program T.N.T. version 1.1 (Goloboff et al., 2003).

Bayesian inference of separate and combined data sets (all matrices excluding scored indels) was conducted using the program MrBayes version 3.1.1 (Huelsenbeck and Ronquist, 2001). This program was run in parallel on an IBM pSeries 690 system at the National Center for Supercomputing Applications at UIUC. Prior to analysis, Modeltest version 3.5 (Posada and Crandall, 1998) was used to select an evolutionary model of nucleotide substitution that best fits these data, as selected by the Akaike Information Criterion estimator (Posada and Buckley, 2004). The best-fit models selected were TVM + I + G and GTR + I + G for the *trnQ–trnK* and ITS matrices, respectively. Bayesian search strategies are the same as employed in Calviño and Downie (2007). For each data matrix and from different random starting trees, four independent analyses were run for 10 million generations and the trees saved to a file every 100 generations (i.e., a total of 400,000 trees was sampled). Variation in likelihood scores to determine apparent stationarity was examined graphically for each independent run using the program Tracer version 1.2.1 (A. Rambaut and A. Drummond, University of Oxford, unpublished data). The states of the chain that were sampled before stationarity (i.e., the "burn in" of the chain) were discarded and the posterior probability values for each bipartition of the phylogeny were determined from the remaining trees. To summarize and compare the samples from each analysis, the sump and sumt commands of MrBayes were used. MCMC convergence was also explored by examining the potential scale reduction factor (PSRF) convergence diagnostics for all parameters in the model (provided by the sump and sumt commands) and graphically using the cumulative, compare, and absolute difference options of the program AWTY online (Wilgenbusch et al., 2004).

2.4. Biogeographic analysis

To reconstruct the geographic distribution of the ancestor of *Eryngium* and of its major clades, a dispersal-vicariance analysis was carried out with the program DIVA version 1.1 (Ronquist, 1996), using the optimize command and default option settings. Because portions of the resultant phylogenies were unresolved, even in individual trees, we carried out three different analyses for individual subclades by entering the following simplified, fully resolved trees based on the results of the combined analyses: (1) "*Eryngium* origin"—(*Sanicula*, ((*Eryngium duriaei*, (*E. ilicifolium*, (*E. aquifolium*, (*E. glaciale*, other Old World *Eryngium*)))), (*E. tenue*, ((*E. viviparum*, *E. galioides*), (*E. corniculatum*, other New World *Eryngium*))))); (2) "Eastern USA"—(outgroup, (*E. prostratum*, (((*E. aromaticum*, *E. integrifolium*), (*E. diffusum*, *E. leavenworthii*)), ((*E. cervantesii*, *E. pilularioides*), *E. nasturtiifolium*)))); and (3) "South American"—(outgroup, (*E. glossophyllum*/*E. buchtienii*, (*E.incantatum*, other South American *Eryngium*))). The following areas were defined for each of the aforementioned analyses: (1) a—western Mediterranean (Iberian Peninsula, NW Africa), b—central-east Europe and Asia, c—Americas; (2) d—eastern USA, e—Mexico, f—rest of the world; and (3) f—rest of the world, g—southern South American Yungas in Argentina and Bolivia. For those nodes not subject to a dispersal-vicariance analysis (because resolution of relationships was poor), we speculate on the possible distribution of ancestors.

3. Results

3.1. Chloroplast DNA sequence comparisons and phylogenetic analyses

Sequence characteristics of the cpDNA *trnQ–trnK* region are presented in Table 2. Of the 117 sequences

Table 2
Sequence characteristics of the cpDNA *trnQ–trnK* and nuclear rDNA ITS regions for 117 and 153 accessions of Apiaceae, respectively

Sequence characteristic	*trnQ–trnK*	ITS[b]
Length variation (range/average in bp)		
Eryngium		
New World	3155–3298/3243	726–730/729
Old World	3213–3352/3310	718–730/726
Sanicula and *Petagnaea*	2770–3339/3298[c]	703–730/726
No. aligned positions	4104	743
No. positions eliminated	239	24
No. positions not variable	3087	484
No. positions autapomorphic	338	61
No. positions parsimony informative	440	174
No. unambiguous alignment gaps	161	19
No. unambiguous alignment gaps parsimony informative	83	10
Sequence divergence (range)		
All taxa included	0–5.14	0–15.72
Within *Eryngium*	0.03–4.90	0–9.37
Old World	0.19–3.14	0–6.03
New World	0.03–3.51	0–6.51
New World s. str.	0.03–1.41	0–4.68
Total no. parsimony informative characters[a]	523	184

[a] Number of parsimony informative nucleotide substitutions plus number of parsimony informative gaps.
[b] Includes 47, 164, and 68 bp of the 18S, 5.8S, and 26S regions, respectively.
[c] Average excluding *Petagnaea gussonei* accessions which are 2770 bp long.

UNCORRECTED PROOF

compared, this region varied in size from 2770 (*Petagnaea gussonei*) to 3352 bp (*Eryngium caespitiferum*). As a result of a few deletions in both intergenic spacers, the size of this region is, on average, smaller in New World *Eryngium* than it is in Old World *Eryngium* and *Sanicula*. Alignment of these sequences resulted in a matrix of 4104 positions. Of these, 239 were excluded from subsequent analysis because of alignment ambiguities. The remaining 3865 aligned positions yielded 440 parsimony informative characters. In addition, 161 unambiguous alignment gaps were inferred, of which 83 were parsimony informative. Of the latter, 15 occurred within the *rps16* intron, and 40 and 28 occurred within the *trnQ–rps16* and *rps16–trnK* intergenic spacer regions, respectively. Informative indels ranged in size from 1 to 588 bp. Most indels were 10 bp or shorter, but two exceptionally large deletions occurred in the *trnQ–rps16* region: a 62 bp deletion in all *Eryngium* of the New World plus Old World *E. corniculatum*, *E. galioides*, and *E. viviparum*; and a 588 bp deletion in *Petagnaea gussonei*. Pairwise sequence divergence estimates ranged from identity to 5.14% of nucleotides among all taxa. Among pairwise comparisons within "Old World" and "New World" *Eryngium*, maximum sequence divergence estimates were similar (3.14% and 3.51%, respectively), whereas for those taxa in the "New World sensu stricto" clade this value was much lower (1.41%).

MP analysis of 3865 unambiguously aligned *trnQ–trnK* nucleotide positions plus 83 binary-scored informative indels resulted in the preset maximum tree limit of 20,000 trees, each of 1309 steps (consistency indices, CIs = 0.7571 and 0.6638, with and without uninformative characters, respectively; retention index, RI = 0.9466). The relationships inferred in the strict consensus of these trees are largely identical to those resolved using Bayesian inference (Fig. 2). Repeating the MP analysis without the 83 scored gaps also resulted in the preset limit of 20,000 trees, each of 1197 steps (CIs = 0.7586 and 0.6535, with and without uninformative characters, respectively; RI = 0.9425). The topology of the strict consensus tree (not shown) was similar to that when gaps were included, but slightly less resolved especially at the tips.

The four independent Bayesian analyses showed MCMC convergence for all parameters in the best-fit model (PSRF reached one for all parameters). Moreover, the absolute difference graphic produced by AWTY online showed no significant variability among independent runs. Pairwise comparisons between tree files of each run showed no difference in the posterior probabilities of all splits for paired MCMC analyses. The first 50,000 trees of each run were discarded as "burn in" because after 5 million generations the likelihood values and the posterior probabilities of the splits were stable, indicating that the chains have reached stationarity. A majority-rule consensus tree that summarizes topology and branch length information was calculated based upon the remaining 200,000 trees and is presented in Fig. 2.

The phylogenies estimated using MP and Bayesian analyses of *trnQ–trnK* data are almost totally congruent with one another. The MP strict consensus tree is slightly less resolved than the Bayesian tree, with the differences between them denoted by dotted lines in Fig. 2. In the MP strict consensus tree *Eryngium foetidum* forms a well-supported clade with the two accessions of *E. coronatum*, whereas in the Bayesian tree the relationship between these species is unresolved. In all cpDNA-derived trees, the 97 accessions of *Eryngium* comprise a clade (99% bootstrap, 100% posterior probability) that is sister group to *Sanicula* (100% bootstrap and posterior probability). A major dichotomy is evident within *Eryngium* and this is designated as "Old World" *Eryngium* and "New World" *Eryngium*. Each of these clades is strongly supported by both high bootstrap (99–100%) and posterior probability (100%) values. The "New World" clade contains four western Mediterranean species (*Eryngium tenue*, *E. viviparum*, *E. galioides*, and *E. corniculatum*) as three successive basally branching lineages to a large group designated herein as the "New World sensu stricto (s. str.)" clade (99% bootstrap, 100% posterior probability). The "New World s. str." clade includes five subclades designated as "North American monocotyledonous", "South American monocotyledonous", "Mexican", "Pacific", and "Eastern USA" (Fig. 2). These five subclades are strongly supported in the Bayesian tree (each with 100% posterior probability values) but show poor to moderate bootstrap support (<50–75%) in the MP trees. The "North American monocotyledonous" clade comprises one branch of a polytomy that is made up of additional South American monocotyledonous *Eryngium* species. This assemblage forms a large polytomy at the base of the "New World s. str." clade in both Bayesian and MP trees with the four other aforementioned subclades plus *Eryngium coronatum* (two accessions), *E. foetidum*, and *E. glossophyllum*. Branch lengths at the base of both "Old World" and "New World" *Eryngium* clades are much longer than those of the distal branches within each of these major clades. With the exceptions of sections *Hygrobia* (*Eryngium galioides* and *E. viviparum*) and *Fruticosa* (*E. bupleuroides* and *E. inaccessum*), no other section recognized by Wolff (1913) is monophyletic in these trees (Table 1 and Fig. 2).

3.2. Nuclear rDNA ITS sequence comparisons and phylogenetic analyses

Nineteen accessions of *Eryngium* showed evidence of ITS sequence additivity at one to four nucleotide sites (*E. bourgatii*, *E. bungei*, *E. campestre*, *E. crassisquamosum*, *E. gracile*, *E. hemsleyanum*, *E. mesopotamicum* 2312, 1489, *E. montanum*, *E. paniculatum*, *E. pohlianum*, *E. rauhianum* 2467, 2543, *E. sellowii* 2470, 2471, *E. sparganophyllum*, *E. vaseyi*, *E. venustum* 2547, 2846). Trees resulting from MP analyses with and without these accessions are consistent, thus these sequences were retained in the analysis. Sequence characteristics of the ITS region are presented

UNCORRECTED PROOF

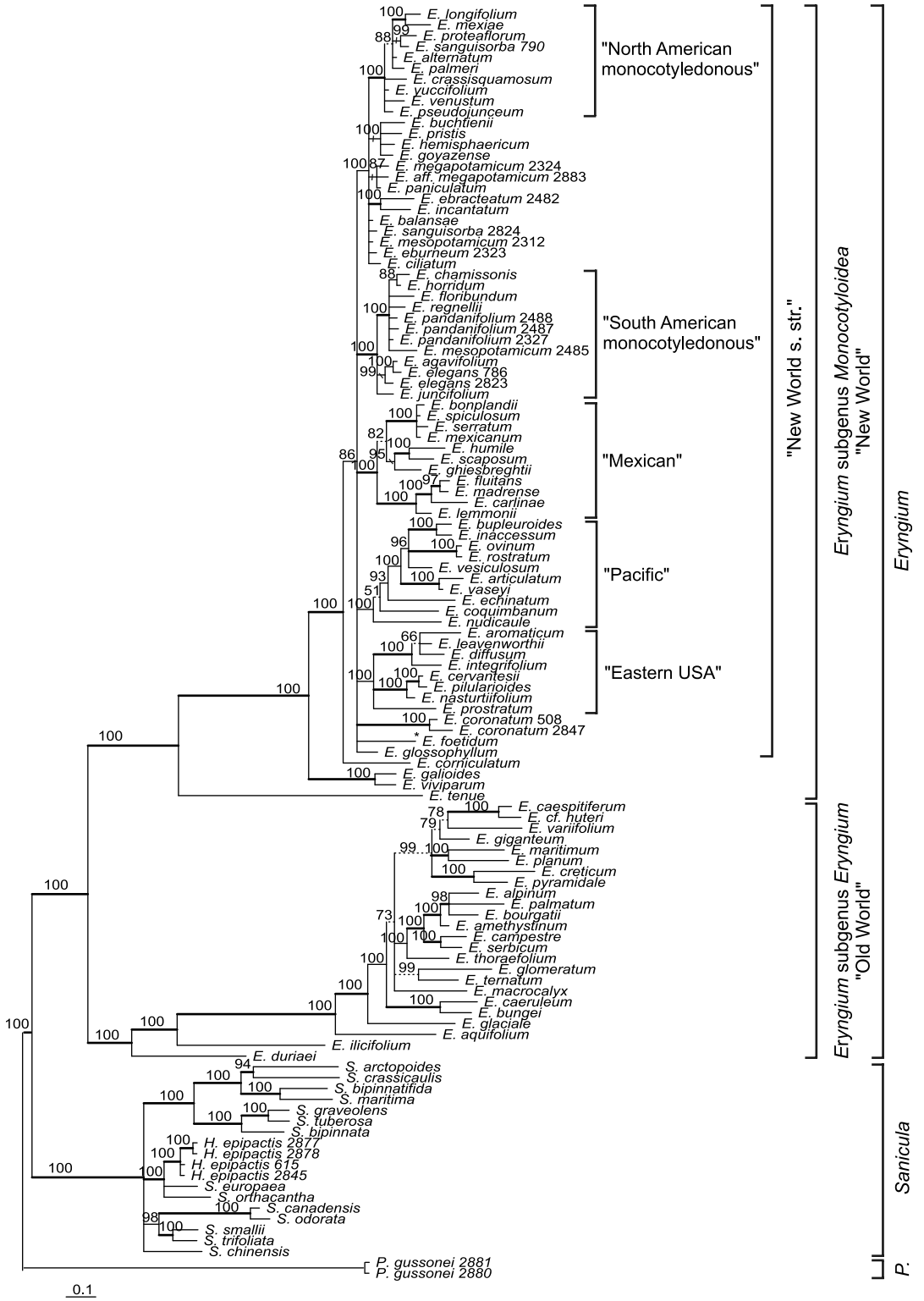

Fig. 2. Majority-rule consensus of 200,000 trees derived from Bayesian analysis of 117 cpDNA *trnQ–trnK* sequences. Numbers at nodes are posterior probability values. Dotted lines represent branches that collapse in the MP strict consensus of 20,000 minimal-length trees. Thick lines represent bootstrap support values >70%. Difference between the Bayesian and MP phylogenies are marked with asterisks and are discussed in the text.

in Table 2. Among the 153 sequences compared, the ITS region varied in size from 703 (*Sanicula arctopoides*) to 730 bp (*S. canadensis*, *Eryngium maritimum*, *E. humile*, *E. madrense*). There is little difference in average size of the ITS region between *Eryngium* and outgroups. Alignment of these sequences resulted in a matrix of 743 positions. Of these, 24 positions were excluded from subsequent analyses because of alignment ambiguities. The remaining 719

Please cite this article in press as: Calviño, C.I. et al., The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): ..., Mol. Phylogenet. Evol. (2008), doi:10.1016/j.ympev.2007.10.021

aligned positions yielded 174 parsimony informative characters. In addition, 19 unambiguous alignment gaps were inferred, of which 10 were parsimony informative. All informative indels were 1 bp in size, save two which were 2 and 9 bp in size. Pairwise sequence divergence estimates ranged from identity to 15.72% of nucleotides among all taxa. Within "Old World" and "New World" clades of *Eryngium*, maximum pairwise sequence divergence estimates were similar (6.03% and 6.51%, respectively); this value was lower in pairwise comparisons within *Eryngium* of the "New World s. str." clade (4.68%).

MP analysis of 719 unambiguously aligned ITS nucleotide positions plus 10 binary-scored informative indels resulted in the preset maximum tree limit of 20,000 trees, each of 606 steps (CIs = 0.5578 and 0.5055, with and without uninformative characters, respectively; RI = 0.8940). The relationships inferred in the strict consensus of these trees agree in part with those resolved using Bayesian inference (Fig. 3). Repeating the analysis without the 10 scored gaps also resulted in the preset limit of 20,000 trees, each of 591 steps (CIs = 0.5550 and 0.5009, with and without uninformative characters, respectively; RI = 0.8914). The topology of this strict consensus tree was identical to that of the previous analysis, with the exception of the collapse of a few branches in the "Old World" clade. For several nodes, bootstrap support values were lower when gaps were excluded from the analysis.

One of the four independent Bayesian runs did not converge to the same parameter values as the others and was discarded. The remaining three Bayesian analyses showed no significant variability among them, as determined by the absolute difference graphic produced by AWTY online, MCMC convergence for all parameters in the best-fit model (PSRF reached one for all parameters), and insignificant difference in the posterior probabilities of all splits for paired MCMC analyses. The first 50,000 trees of each run were discarded as "burn in" and a majority-rule consensus tree that summarizes topology and branch length information was calculated based upon the remaining 150,000 trees (Fig. 3).

The phylogenies estimated using MP and Bayesian analyses agree in part. Overall, however, the MP strict consensus tree is less resolved than that of the Bayesian tree, with the differences denoted by dotted lines in Fig. 3. In general, bootstrap support values are low, but some branches with low bootstrap values show posterior probability values >95%, such as the first splits within the "New World" and "Old World" *Eryngium* clades. Both reconstructions support the monophyly of *Eryngium* (59% bootstrap, 100% posterior probability), with this genus sister group to *Sanicula* (100% bootstrap and posterior probability). The "New World" clade contains the same four western Mediterranean species as successive basally branching lineages to the "New World s. str" clade, as inferred in the analyses of cpDNA data. Within the "New World s. str." clade, two subclades identified previously in the cpDNA trees are evident: "Mexican" (with the addition of *Eryngium gracile* and *E. montanum*); and "Eastern USA" (but with the removal of *E. prostratum*). All remaining New World *Eryngium* with the exceptions of *E. coquimbanum* and *E. prostratum* comprise a strongly supported clade designated herein as "South American". These three subclades along with *E. coquimbanum* and *E. prostratum* comprise a polytomy (Fig. 3), and each of the three subclades are supported with 70–100% posterior probability and <50–70% bootstrap support values. Both MP and Bayesian phylogenies show several polytomies, including a large, well-supported polytomy ("South American") that is sister group to *E. incantatum* (Fig. 3). Branch lengths within this clade are shorter relative to those of the first branching lineages within the "Old World" and "New World" *Eryngium* clades. In all MP trees, the two accessions of *Eryngium tenue* ally as a sister group to the "Old World" clade, whereas the Bayesian tree places them as a sister group to all other members of the "New World" clade. In both reconstructions, however, the placement of *E. tenue* is weakly supported (<50% bootstrap, 63% posterior probability). Other differences between MP and Bayesian trees are in the placements of some "Old World" taxa (i.e., *Eryngium pyramidale* and *E. palmatum* are placed as two successive basal lineages to the clade of *E. giganteum* to *E. campestre* in the MP trees). It is hard to evaluate the naturalness of the sections created by Wolff (1913) in such poorly resolved phylogenies, but sections *Diffusa* (*E. diffusum* and *E. leavenworthii*), *Fruticosa* (*E. bupleuroides*, *E. inaccessum*, and *E. fernandezianum*), and *Hygrobia* (*E. galioides* and *E. viviparum*) are each monophyletic based on the accessions sampled (Table 1 and Fig. 3).

3.3. Comparison of cpDNA and nuclear rDNA ITS phylogenies and a total evidence analysis

A visual comparison of plastid and nuclear-derived trees indicates that there is some discordance of relationships between them; most of these differences, however, are weakly supported. A consensus network constructed from bootstrap majority-rule consensus trees of cpDNA and ITS data suggests contradictory taxon relationships. A hybridization network, which explains the differences between source trees as reticulation events, is presented in Fig. 4 (for simplification, only the accessions of *Eryngium* are shown). Ten hybridization events are proposed: six in the "New World s. str." clade and four in the "Old World" clade. The placement of taxa involved in these contradictory positions is supported weakly in the source trees, for a consensus network of clades supported with >70% bootstrap values in each source tree shows no contradiction between the chloroplast and nuclear-derived phylogenies, except for the placement of *E. aquifolium*. Both *trnQ–trnK* and ITS phylogenies are consistent in the reconstruction of those well-supported clades. There is also close correspondence in relative branch lengths between the trees (Figs. 2 and 3), as well as a general agreement in the placement of the many polytomies observed in both source phyloge-

UNCORRECTED PROOF

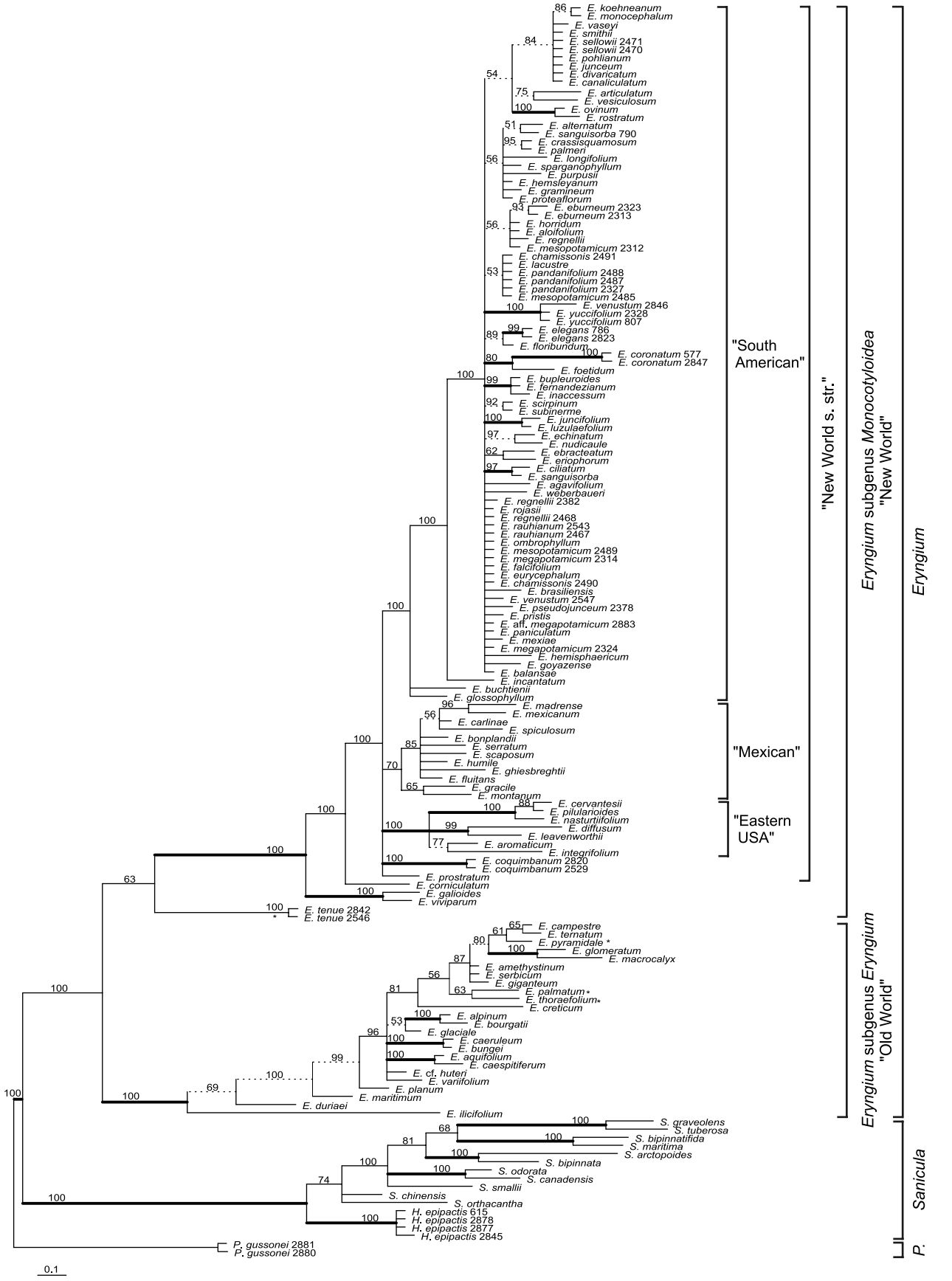

Fig. 3. Majority-rule consensus of 150,000 trees derived from Bayesian analysis of 153 nrDNA ITS sequences. Numbers at nodes are posterior probability values. Dotted lines represent branches that collapse in the MP strict consensus of 20,000 minimal-length trees. Thick lines represent bootstrap support values >70%. Differences between the Bayesian and MP phylogenies are marked with asterisks and are discussed in the text.

Please cite this article in press as: Calviño, C.I. et al., The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): ..., Mol. Phylogenet. Evol. (2008), doi:10.1016/j.ympev.2007.10.021

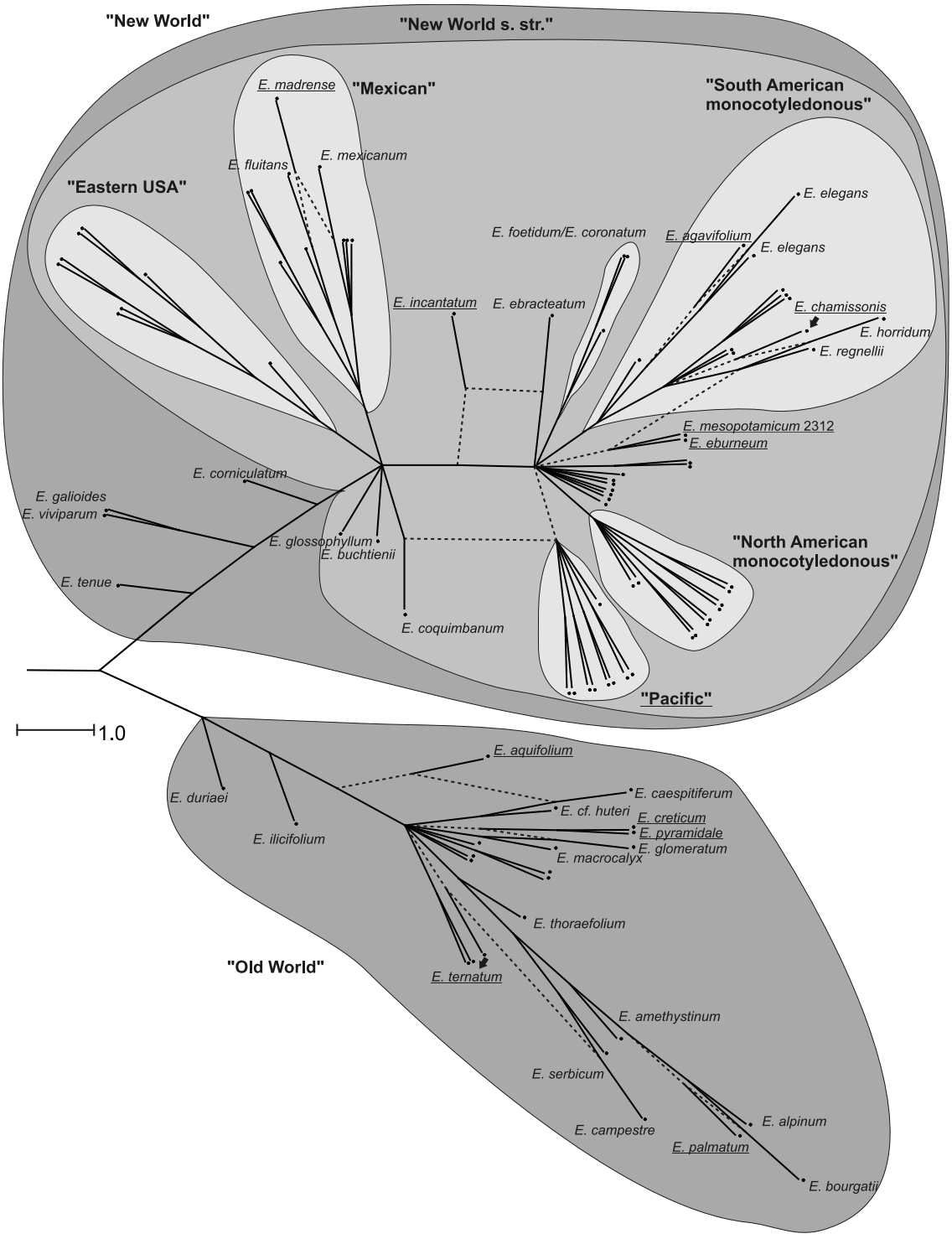

Fig. 4. Hybridization network from bootstrap majority-rule consensus trees from *trnQ–trnK* and ITS data. Dotted lines represent hybridization events. *Sanicula* and *Petagnaea* accessions are not shown for simplification. Taxa involved in hybridization events are underlined.

nies. The following major clades and subclades are apparent in both *trnQ–trnK* and ITS phylogenies: "Old World", "New World", "New World s. str.," "Mexican", and "Eastern USA". In the ITS trees, the "South American" clade is poorly resolved, but some of its accessions form well-supported subclades in the *trnQ–trnK* trees (e.g., "North American monocotyledonous", "South American monocotyledonous", and "Pacific"). Also, basal lineages within the "Old World" *Eryngium* clade are weakly supported in the ITS MP trees, whereas these same relationships are strongly supported in the *trnQ–trnK* phylogenies. Given the strengths and weaknesses of each data set, it was desirable to combine chloroplast and nuclear data for a "total evidence" analysis. The results

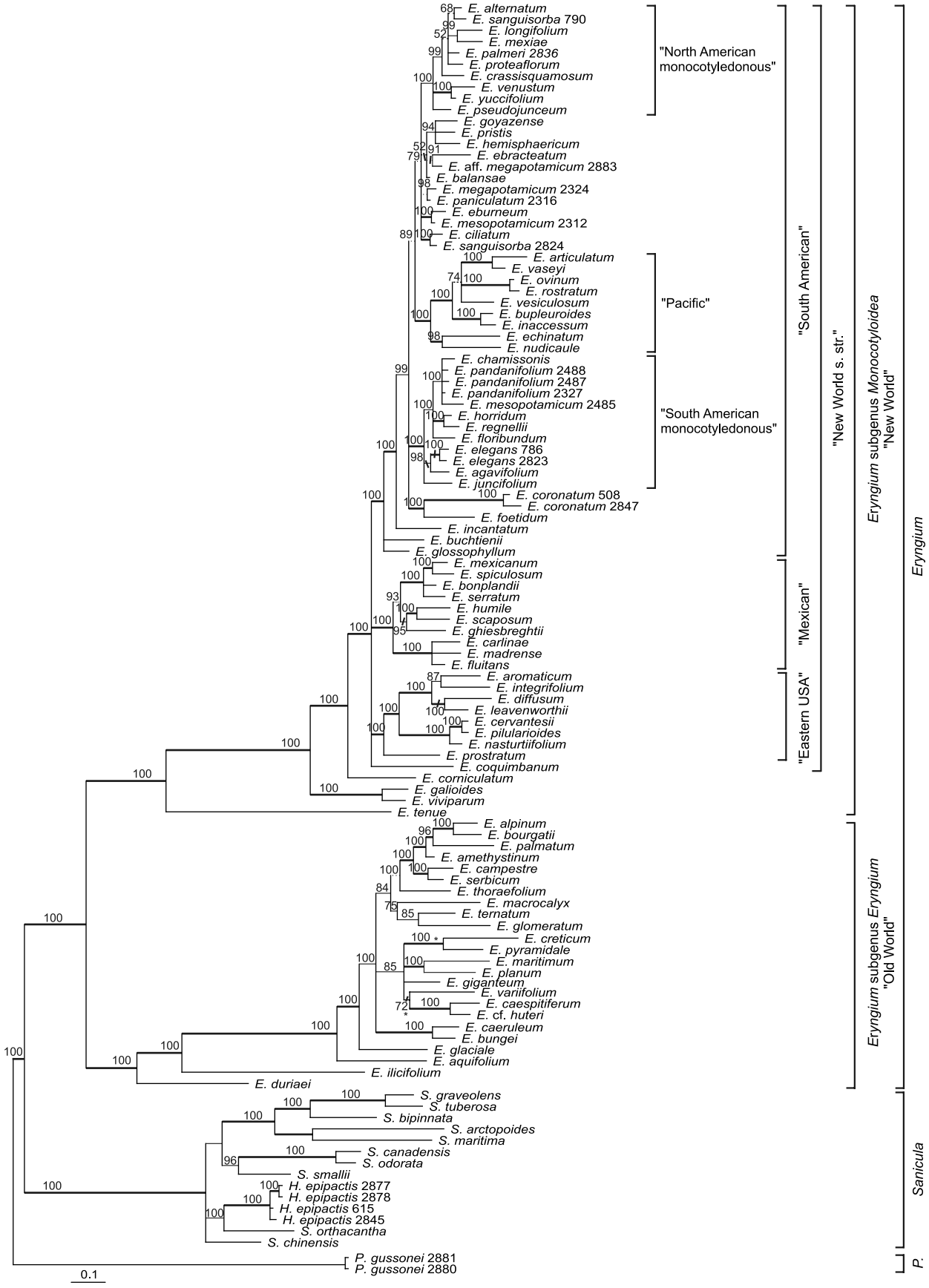

Fig. 5. Majority-rule consensus of 200,000 trees derived from Bayesian analysis of 112 accessions common to both *trnQ–trnK* and ITS data sets. Numbers at nodes are posterior probability values. Dotted lines represent branches that collapse in the MP strict consensus of 20,000 minimal-length trees. Thick lines represent bootstrap support values >70%. Differences between the Bayesian and MP phylogenies are marked with asterisks and are discussed in the text.

Please cite this article in press as: Calviño, C.I. et al., The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): ..., Mol. Phylogenet. Evol. (2008), doi:10.1016/j.ympev.2007.10.021

of a partition homogeneity test for 112 accessions common to both *trnQ–trnK* and ITS data sets revealed that these loci do yield significantly different phylogenetic estimates (ILD probability value = 0.001667). Nevertheless, these data were combined for simultaneous analysis for it has been argued that when the characters of two data matrices evolve at different rates (and thus display different amounts of noise), the ILD test could suggest significant heterogeneity despite the two matrices having similar underlying topologies (Dolphin et al., 2000). It seems likely that the marked differences in divergence rates of the genes we analyzed have influenced the ILD test results.

Alignment of the *trnQ–trnK* and ITS regions from 112 common accessions resulted in a matrix of 4847 positions. Of these, 263 were excluded from subsequent analyses because of alignment ambiguities. The remaining 4584 aligned positions yielded 597 parsimony informative and 390 autapomorphic characters. In addition, 93 informative indels were scored. MP analysis of the combined *trnQ–trnK* and ITS partitions plus 93 indels resulted in the preset maximum tree limit of 20,000 trees, each of 1911 steps (CIs = 0.6771 and 0.5862, with and without uninformative characters, respectively; RI = 0.9201). The relationships inferred in the strict consensus of these trees are largely identical to those resolved using Bayesian inference (Fig. 5). The four independent Bayesian analyses of combined data (without indels) showed MCMC convergence and the splits reached stationarity after 5 million generations. Given these results, the first 50,000 trees of each run were discarded as "burn in" and a majority-rule consensus tree that summarizes topology and branch length information was calculated based upon the remaining 200,000 trees (Fig. 5).

The phylogenies estimated using MP and Bayesian analyses are consistent except for the following: in the MP trees, the clade of *Eryngium creticum* and *E. pyramidale* is sister group to *E. glomeratum* (<50% bootstrap); and the clade of *E. caespitiferum* and *E.* cf. *huteri* is sister group to *E. glaciale*. Both MP and Bayesian analyses of combined data indicate that *Eryngium* forms a clade (97% bootstrap, 100% posterior probability) that is sister group to *Sanicula* (100% bootstrap and posterior probability). *Eryngium* is divided into both "Old World" and "New World" clades (100% bootstrap and posterior probability). Within the "Old World" clade, the first splits are highly supported, with *Eryngium duriaei*, *E. ilicifolium*, and *E. aquifolium* as three successive basally branching lineages (97–100% bootstrap, 100% posterior probability). Within the "New World" clade, *Eryngium tenue*, *E. viviparum* plus *E. galioides*, and *E. corniculatum* occupy the first three diverging lineages (98–100% bootstrap, 100% posterior probability). Sister group to *E. corniculatum* is the "New World s. str." clade (82% bootstrap, 100% posterior probability). The latter comprises a polytomy made up of the "Eastern USA", "Mexican", and "South American" subclades, and*E. coquimbanum*. The first two of these subclades are well supported (78–84% bootstrap, 100% posterior probability). The "South American" subclade is supported with 53% bootstrap and 100% posterior probability.

3.4. Polytomies

The phylogenies resulting from MP and Bayesian analyses of combined plastid and nuclear DNA data are not fully resolved (Fig. 5). Three of these polytomies are remarkable because they appear irrespective of the method or data source used. These polytomies include a trichotomy in the "Old World" *Eryngium* clade, a quadritomy at the base of the "New World s. str." clade, and a 12-tomy within the "South American" clade. The latter is observed in the Bayesian tree by collapsing branches with posterior probabilities <94%. Inspection of the 20,000 MP trees using the program T.N.T. shows six and 212 different resolutions for the unresolved nodes of the "New World s. str." and "South American" clades, respectively. For the trichotomy in the "Old World" clade, two different resolutions are displayed. Branch lengths for these alternative resolutions are null or one-step long.

3.5. Biogeographic analyses

The results of the three dispersal-vicariance analyses are shown in Fig. 6. All reconstructions were unambiguous except for the origin of the ancestor of the "Eastern USA" clade. In this reconstruction, two alternative biogeographic scenarios were obtained. One scenario is that the ancestor of this clade had a widespread Mexican-eastern USA distribution, as a result of a dispersal event from an ancestor from eastern USA. The other scenario suggests that the ancestor of the "Eastern USA" clade was distributed in eastern USA and dispersal to Mexico occurred in the next ancestor. Both alternative reconstructions indicate that the Mexican species belonging to this clade came from ancestors from eastern USA. According to the three DIVA reconstructions, at least four dispersal events were required to explain the present geographic distribution of species in *Eryngium*. The ancestor of *Eryngium* originated in the western Mediterranean, and from there, two independent dispersal events occurred, one in the "New World" clade to the Americas, and the other in the "Old World" clade to central-east Europe and Asia. The ancestor of the "South American" clade was unambiguously reconstructed in the southern South American Yungas, and from there at least one dispersal event occurred to eastern South America, Chile or North America.

4. Discussion

4.1. Polytomies: hybrid speciation and/or rapid radiations?

Polytomies in a phylogeny may result from either character conflict or short branches, the latter zero in length or nearly so, relative to other branches of the phylogeny. Each

UNCORRECTED PROOF

Please cite this article in press as: Calviño, C.I. et al., The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): ..., Mol. Phylogenet. Evol. (2008), doi:10.1016/j.ympev.2007.10.021

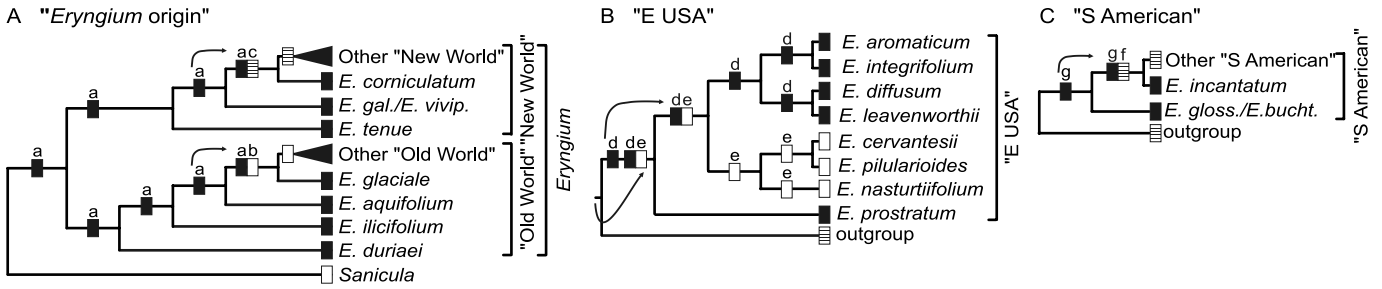

Fig. 6. Optimal reconstructions of the ancestral distributions of three subclades of *Eryngium* using dispersal-vicariance analysis: (A) "*Eryngium* origin"; (B) "Eastern USA"; (C) "South American". Historical biogeography of each subclade was analyzed in terms of the following areas: (A) western Mediterranean–Iberian Peninsula, NW Africa- ("a" and solid bars), central-east Europe and Asia ("b" and open bars), Americas ("c" and striped bars); (B) eastern USA ("d" and solid bars), Mexico ("e" and open bars), rest of the world (striped bars); (C) rest of the world ("f" and striped bars), southern South American Yungas in Argentina and Bolivia ("g" and solid bars). At each node, the optimal distribution prior to vicariance is given; alternative and equally optimal distributions are separated with a space. Arrows represent dispersal events.

of these cases may reflect artifacts of the methods or data used, or evolutionary processes that are not congruent with a bifurcating pattern of species diversification. Character conflict results in a lack of resolution when different characters within a particular gene, or characters from different lines of evidence, provide support for incompatible trees because of similar amounts of conflicting phylogenetic and non-phylogenetic (noise) signal. In these cases, polytomies can be resolved by using strategies that reduce noise without altering the genuine phylogenetic signal, such as increasing taxon sampling, replacing fast with more slowly evolving gene sequences, and applying appropriate methods and substitution models in phylogenetic reconstruction (Baurain et al., 2007, and references cited therein). Another reason for character conflict may reflect reticulate events in the evolutionary history of the taxa involved, such as hybridization, horizontal gene transfer, or recombination, which stems from the fact that different character sets can have different underlying evolutionary histories. In these cases, the inference of a reticulate evolutionary history depends on the reliability of the individual gene trees (Seelanan et al., 1997; McBreen and Lockhart, 2006), and only strongly supported incongruent relationships from different source gene trees should be taken into consideration. Polytomies may also result from short branch lengths. Branches may be short because of insufficient data (i.e., by using molecular or morphological data that are not variable enough at the appropriate taxonomic level considered, or not having enough data to solve the problem), or because of a hard multifurcation (i.e., simultaneous or rapid splitting of several lineages). These two scenarios are sometimes difficult to tease apart. Rapid radiation will tend to defy resolution using most types of data. In contrast, if the polytomy is not caused by truly short times between divergences, relationships should ultimately be resolvable using data sources with appropriate levels of variation for the target age of divergence (Whitfield and Lockhart, 2007). Deciphering patterns of rapid radiations, as well as those of reticulate evolution, often require an array of data sources and analytical techniques. In this study, we ascertain if the polytomies observed in the phylogenies are the result of artifacts of the data or methods used, or if they represent the results of rapid radiation and/or reticulation during the evolutionary history of *Eryngium*.

All phylogenetic analyses of *Eryngium* divide the genus into strongly supported sister groups. Within each of these clades, the first divisions are also well supported, except in the "Old World" clade based on ITS data only. However, after the first 4–5 splits in each of these major clades, many accessions of *Eryngium* fall into three major polytomies comprised of several moderately to strongly supported clades. One of these polytomies is a trichotomy in the "Old World" clade, another is a quadritomy at the base of the "New World s. str." clade, and the third is a 12-tomy within the "South American" clade. The occurrence of these polytomies is puzzling, especially because they include morphologically diverse and widespread groups of species. The chloroplast and nuclear regions examined in this study represent a matrix of 4584 characters, including 597 parsimony informative nucleotide positions and 93 informative indels. Among those loci considered to date in molecular phylogenetic studies of Apiaceae, the ITS region is the most variable (Downie et al., 2001). It has been used successfully to resolve interspecific relationships, even in the speciose genus *Bupleurum* (Neves and Watson, 2004) and the morphologically uniform genus *Cicuta* (Lee and Downie, 2006). The *trnQ–trnK* region has also been useful to resolve infrageneric relationships in subfamilies Apioideae and Saniculoideae (Lee and Downie, 2006; Calviño and Downie, 2007). In *Eryngium*, however, relationships at the base of the "New World s. str." clade and those within the "South American" and "Old World" clades remain unresolved, irrespective of the marker used or phylogenetic method considered. We examined 118 of the approximately 250 species recognized in *Eryngium*, a substantial increase in sampling over any previous study (Downie and Katz-Downie, 1999; Valiejo-Roman et al., 2002; Calviño and Downie, 2007). Because these species represent most of the morphological diversity within the genus and were collected from throughout its geographic range, we discard incomplete or inadequate taxon sampling

UNCORRECTED PRC

as the cause for these polytomies. An inspection of all MP trees shows that these polytomies are not the result of character conflict, but rather of insufficient data because the individual trees are also unresolved. Similarly, in the Bayesian tree, mean branch lengths for these unresolved relationships are very short. One hypothesis to explain the lack of phylogenetic signal in these portions of the phylogenies is that insufficient data have been acquired or the regions examined are inappropriate for the level of taxonomic divergence considered. In the analysis of combined chloroplast and ITS sequence data, there is strong support for several phylogenetically related taxa, both at the base and tips of the trees. Additional data may or may not increase resolution or branch support in the unresolved portions of the tree, but there is no strong reason to suppose that *trnQ–trnK* and ITS are inappropriate markers to resolve infrageneric relationships in *Eryngium*.

An alternative explanation for the lack of phylogenetic signal in portions of the trees is a rapid diversification of the ancestors involved in the polytomies. Short periods of time between speciation events would readily explain the polytomies observed. Polytomies at the base of the "Old World" and "New World s. str." clades coincide with the colonization of new territories from western Mediterranean ancestors. These new territories include east Mediterranean/southwest Asia/northern Europe in the "Old World" clade and the Americas in the "New World s. str." clade. The radiation within the "South American" clade is likely correlated with the colonization of new lands, as well. The subclades occurring within this clade are concentrated in central-east South America ("South American monocotyledonous"), Mexico and central-east USA ("North American monocotyledonous"), and the Pacific coasts of Chile, Australia, and California ("Pacific"). The ancestors involved in the three major polytomies inferred herein were able to diversify and spread over very long distances in short periods of time. Long distance dispersal involves a founder effect which is often the driver for rapid morphological change (Milne and Abbott, 2002). Many species of *Eryngium* are considered important weeds and first colonizers of disturbed areas, and the results of DIVA suggested that dispersal was frequent in the evolutionary history of *Eryngium* (see below, "Biogeographical origin of the genus *Eryngium* and its major clades").

One potential consequence of recent rapid radiations is that there may be too little time for effective intrinsic prezygotic and postzygotic isolating mechanisms to have evolved, leaving these species subject to introgressive hybridization (i.e., hybridization in which there is an actual exchange of genes between species and not merely the production of inviable or infertile offspring; Wiens et al., 2006). However, it has also been argued that hybridization may be an important factor driving these rapid radiations (Seehausen, 2004). A fundamental difference between these hypotheses is that the former predicts introgressive hybridization among the tips of radiation, whereas the latter predicts introgression at the base of the radiation. It seems likely that in the evolutionary history of *Eryngium,* these two hypotheses are not mutually exclusive. The plastid- and nuclear-derived phylogenies inferred herein are generally consistent with one other, with the apparent conflict only moderately to weakly supported. The hybridization network revealed three reticulate events that involve taxa at the base of the radiation (Fig. 4). These involve *Eryngium coquimbanum* and the "Pacific" clade, *E. incantatum* and *E. ebracteatum*, and *E. aquifolium* and the clade of *E. caespitiferum*/*E. huteri*. All other hybridization events involve taxa at the tips of the radiations. Many species of *Eryngium* are polyploids and natural hybrids are common (Constance, 1977; Pimenov et al., 2003). In fact, more than half of the sampled species belonging to the "South American" clade are polyploids. It is evident that hybrid speciation has played a key role in the diversification of these plants, as has been suggested previously for Mexican and South American *Eryngium* based on chromosome counts and karyology (Constance, 1977; Calviño et al., 2002; O'Leary et al., 2004). The phylogenies presented herein suggest that hybridization events occurred both at the base of the radiation and its tips, suggesting that hybrid speciation might have been the cause, but also a consequence, of the rapid diversification of the group. Ancient hybridization in the "South American" clade might have led to accelerated diversification rates, which in turn led to extensive hybridization among the rapidly generated species. The same is postulated for the "Old World" clade; polyploidy, however, occurs less often in this clade but several natural hybrids have been reported (Wolff, 1913; Molinas and Perdigó, 1981). The cpDNA and ITS data obtained to date do not conflict strongly, thus these hypotheses need to be corroborated with further studies. Cloning of the low copy nuclear gene GBSSI (*waxy*) is resulting in paralogs that might help estimate the parentage of these polyploid species (C.I. Calviño and S.R. Downie, unpublished data).

4.2. Biogeographical origin of the genus Eryngium and its major clades

Traditionally, it was hypothesized that *Eryngium* had an Asiatic origin, with subsequent migrations northwestward to colonize Europe (Turmel, 1950, 1951; Breton, 1962; Cerceau-Larrival, 1971). However, based on the results of DIVA, the ancestor of *Eryngium* was inferred to have occurred in the western Mediterranean, whereupon the two major clades of the genus split, perhaps as a result of adaptation to different habitats. The basal lineages in the "New World" clade show semi-aquatic preferences, while those equivalent lineages in the "Old World" clade grow in arid or semiarid, rocky areas. At least four dispersal events are required to explain the present-day distribution of *Eryngium*. In the "Old World" clade, the first dispersal event from the western Mediterranean is no earlier than in the ancestor of the clade of *Eryngium glaciale* to *E. alpinum* (Fig. 5), but because of the lack of resolution in sub-

UNCORRECTED PROOF

Please cite this article in press as: Calviño, C.I. et al., The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): ..., Mol. Phylogenet. Evol. (2008), doi:10.1016/j.ympev.2007.10.021

sequent splits, further estimates cannot be reconstructed using DIVA. Most species in the *E. bungei* to *E. alpinum* clade are distributed in Middle-Eastern Asia, but some are also from eastern Europe and these are scattered throughout the clade, suggesting several extra dispersal events or waves of migrations. The *Eryngium* of the New World (Americas and Australia) have been previously suggested to have originated from western Mediterranean ancestors based on morphological similarities (Turmel, 1949; Wolff, 1913; Cerceau-Larrival, 1971) and phylogenetic study (Calviño and Downie, 2007). Our results corroborate this hypothesis. However, a problem with this scenario is that it requires a long distance dispersal event across the Atlantic Ocean. At one time, a hypothesis of long distance dispersal was unpopular because it is highly random in nature, almost impossible to falsify, and unlike vicariance cannot normally be linked to specific abiotic events (McGlone, 2005). Recently, however, a shift in opinion has come about (Milne, 2006). Trans-Atlantic dispersals are now commonly reported for Arctic plant species (Hagen et al., 2001; Abbott and Brochmann, 2003) and for tropical South American and African taxa (reviewed in Renner, 2004), and at least one case of dispersal from the Mediterranean to the New World has been reported for *Senecio* (Asteraceae; Coleman et al., 2003). While a hypothesis of trans-Atlantic dispersal between the Mediterranean and New World seems unusual, it is the one that best explains the available evidence for *Eryngium*. The morphology of *Eryngium* facilitates dispersal through water. Its fruits are covered with vesicles and can float, and in *E. maritimum* it has been reported that 55% of seeds kept in sea-water for 40 days remained viable (Ridley, 1930). The western Mediterranean species of the "New World" clade are adapted to semi-aquatic habitats. Seeds germinate and plants grow in wet areas that dry out in summer (Breton, 1962; Nieto Feliner, 2003). The fruits are small and light and can also be wind dispersed, and its spiny sepals can attach to feathers of birds. These upward-pointing spines can also anchor the fruits in the sand, as they are blown in the wind (Ridley, 1930). The lack of resolution at the base of the "New World s. str." clade precludes a definite statement on where in America the ancestor arrived from the western Mediterranean. Based on the reconstruction of the ancestors of the three subclades found at the base of the "New World s. str." clade, such possibilities include the southern South American Yungas, Mexico, or eastern USA. The first is probably the most complex scenario based on distance of dispersal, morphological similarities, and habitat preferences of ancestral species. Such long dispersal from the western Mediterranean to the South American Yungas could only be explained by birds as the agent of dispersal. According to Ridley (1930) and Renner (2004), there are no transverse bird migration routes from Tropical Africa to South America, nor are there routes from Europe to North America. On the other hand, dispersal from the western Mediterranean to Mexico or the eastern USA may have been by water. A list of 110 trans-Atlantic disjunct angiosperm genera considered by Renner (2004) reveals several cases of trans-oceanic dispersals by sea- or wind-currents. The north equatorial current (NEC) is a broad westward-flowing current that is fortified by the Atlantic trade wind belt. This current originates from the northwestern coast of Africa, where it is fed mainly by the cooler waters flowing from the northeast Atlantic. Once in the Americas, the water flows northwest to feed the Guiana and the Caribbean Currents (Bourles et al., 1999). *Eryngium viviparum*, *E. galioides*, and *E. corniculatum* grow in semi-aquatic conditions and similar ecological preferences are observed for plants from the "Eastern USA" and "Mexican" clades. According to Cerceau-Larrival (1973), *E. nasturtiifolium*, *E. gracile*, and *E. carlinae* (species representing both of these clades) show similarities in pollen morphology and type of cotyledons with species from the western Mediterranean. Dispersal from the western Mediterranean to Mexico or the eastern USA remains unclear; nevertheless, the lack of resolution among subclades at the base of the "New World s. str." clade suggests that subsequent dispersal events in the New World were rapid. The results of DIVA for the "Eastern USA" clade indicate that, even if the previous ancestor of the clade had a widespread northern American (i.e., Mexico and eastern USA) distribution, the Mexican species included in this clade originated from a dispersal event from the eastern USA. The "Mexican" clade includes species primarily from Mexico and Central America, but also *E. humile* that has a broader distribution south to Ecuador, Venezuela, and Peru. Relationships within this clade are not fully resolved, thus an analysis of dispersal-vicariance must await further taxon sampling. Based on these preliminary data, however, it seems that a migration southwards through the Andes might explain the present-day distribution of *E. humile*. The arrival of this species in South America is independent from the distribution of the ancestor of the "South American" clade. DIVA reconstructs the origin of the "South American" clade in the South American Yungas in Bolivia and Argentina. This comes as a surprise, given that it has been generally assumed based on species richness that the center of origin of *Eryngium* in South America was in central-east South America (southern Brazil, northeast Argentina, and Uruguay; Cerceau-Larrival, 1971; Constance, 1977). Because of the lack of resolution within the "South American" clade, we could not analyze its historical biogeography; nevertheless, the phylogenetic relationships observed suggest at least five extra dispersal events within the group (to central-east South America, Chile, Australia, the western coasts of California, and Mexico/eastern USA). The origin(s) of these dispersals is (are) ambiguous, but phylogenetic reconstructions together with morphological similarities suggest the following biogeographic scenarios: Mexican and eastern USA monocotyledonous *Eryngium* originated from central-east South American ancestors; the Australian species likely originated from a long trans-Pacific dispersal from Chile; and the hypothesis

UNCORRECTED PROOF

Please cite this article in press as: Calviño, C.I. et al., The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): ..., Mol. Phylogenet. Evol. (2008), doi:10.1016/j.ympev.2007.10.021

of a North American origin (Mexican or eastern USA) for those species from California can be refuted. Whether the Californian species originated from a dispersal from Australia or Chile cannot be ruled out, however. Numerous cases of dispersals have been inferred between the mediterranean floras of Chile and California for different plant families (Raven and Axelrod, 1974; Carlquist, 1981), so a dispersal from Chile may be possible. What seems apparent from our study is that the present-day distribution of the genus is the result of several dispersals, some of which imply long distances across both the Atlantic and Pacific Oceans.

4.3. Systematics of Eryngium

The results presented here continue to support our earlier finding that *Eryngium* is monophyletic and sister group to the genus *Sanicula* (Calviño and Downie, 2007). The hypothesis that *Eryngium* is paraphyletic, as proposed by Valiejo-Roman et al. (2002) on the basis of deep-level ITS sequence comparisons, is rejected (Calviño and Downie, 2007). Morphological synapomorphies for *Eryngium* include non-palmate leaves, capitate elemental inflorescences, showy involucral bracts, and monoclinous flowers, each of which is subtended by a single bract (Calviño et al., submitted for publication).

Wolff's (1913) revision of *Eryngium* is comprehensive and commonly used as the framework for systematic studies of the genus. Many of the approximately 50 new species described after this revision have been referred to his system. Wolff recognized two major informal groups, "Species gerontogeae" and "Species americanae and australienses", that included 12 sections from the Old World and 22 sections from the New World, respectively. He provided keys to the sections, subsections, series and species, as well as Latin descriptions for all of these. Hypotheses of phylogenetic relationships were also provided. Although, in general, Wolff's system has proved useful, the naturalness of the groupings have not been corroborated based on independent data, until recently. The informal groups "Species gerontogeae" and "Species americanae and australienses" are very similar to the "Old World" and "New World" clades estimated based on chloroplast *trnQ–trnK* sequence data (Calviño and Downie, 2007). Upon expanded sampling, the same groupings appear here based on analyses of nuclear ITS and combined *trnQ–trnK* and ITS sequence data. The only difference between Wolff's treatment and the major clades recognized herein is the placement of *Eryngium tenue*, *E. viviparum*, *E. galioides*, and *E. corniculatum*. Wolff included these western Mediterranean species in his "Species gerontogeae", whereas in the molecular phylogenies these species fall as successively basal branching taxa within the "New World" clade. Wolff did indicate, however, that these three species are closely related to those of North America. This major split within *Eryngium* has been recognized for well over a century (Decaisne, 1873; Wolff, 1913; Turmel, 1948; Cerceau-Larrival, 1971; Constance, 1977). Molecular data corroborate their monophyly and sister group relationship, therefore we recognize these two lineages as subgenera of *Eryngium*. The "Old World" clade is automatically established as *Eryngium* subgenus *Eryngium*. It includes *Eryngium maritimum*, the type species of the genus, and all species from Africa, Europe, and Asia, except *Eryngium tenue*, *E. viviparum*, *E. galioides*, and *E. corniculatum*. The "New World" clade is considered here as *Eryngium* subgenus *Monocotyloidea* Wörz emend. C.I. Calviño and S.R. Downie. It includes all species from the Americas and Australia, plus *Eryngium tenue*, *E. viviparum*, *E. galioides*, and *E. corniculatum*. In a subgeneric classification of *Eryngium*, Wörz (2005) divided the *Eryngium* species now attributable to the "New World" clade into four subgenera: *Eryngium* subgenus *Monocotyloidea* Wörz, *E.* subgenus *Fruticosa* (H. Wolff) Wörz, *E.* subgenus *Semiaquatica* Wörz and *E.* subgenus *Foetida*. These subgenera do not reflect phylogenetic relationships based on the available evidence (Calviño and Downie, 2007; and this study). We consider *E. Fruticosa*, *E. Semiaquatica*, and *E. Foetida* synonyms of *E. Monocotyloidea*. Subgenus *Fruticosa* was first effectively published in a preliminary classification (Wörz, 2004), however, the name was not validly published given that the author explicitly stated that the nomenclature proposed was provisional (Art. 34.1b, International Code of Botanical Nomenclature (ICBN) Vienna, McNeill et al., 2006). Wörz (2005) defined subgenus *Monocotyloidea* to include the species of *Eryngium* with exclusively monocotyledoneous habit. Our circumscription of subgenus *Monocotyloidea* is radically different and broader, so to differentiate it from his concept, we indicate the nature of our change by adding the words "emendavit (or emend.)", as specified in recommendation 47 A.1. of the ICBN, Vienna (McNeill et al., 2006).

Most of the sections recognized by Wolff (1913) are not monophyletic. Eight sections are monotypic, and probably only two of these (*Chamaeeryngium* and *Corniculata*) deserve to be maintained as such because they occupy isolated lineages in the phylogenetic trees. Based on taxonomic sampling, sections *Diffusa*, *Fruticosa*, and *Hygrobia* are each monophyletic. A formal, and modern infrasubgeneric classification for *Eryngium* is still pending further phylogenetic and taxonomic studies and, no doubt, most of the sections recognized by Wolff (1913) or Wörz (2004) will need to be redefined. In this study, we recognize several subclades that are strongly supported based on chloroplast and nuclear data, as well as share several ecological, biogeographical and/or morphological traits. These subclades are treated as informal and unranked groups.

Within *Eryngium* subgenus *Eryngium*, *E. duriaei, E. ilicifolium*, and *E. aquifolium* form three successive lineages basal to all other examined members of the subgenus. These three species are distributed in dry, rocky areas in northwestern Africa and the Iberian Peninsula. The next splits within this clade are conflicting. The trichotomy that is sister group to *E. glaciale* in the Bayesian

UNCORRECTED PROOF

trees (*E. glaciale* was part of this polytomy in the MP trees) was interpreted as a rapid radiation that coincided with the colonization of eastern portions of southwest Asia and central Europe. Major subclades within *Eryngium* subgenus *Eryngium* are not identified because the data do not support strongly or unequivocally any particular hypothesis. Of the three subclades forming the trichotomy in the Bayesian trees, only the clade of *Eryngium caeruleum* and *E. bungei* is strongly supported. The other two subclades show posterior probability values of 84–85% and bootstrap support of <50%. Moreover, there are no obvious morphological characteristics or ecological preferences uniting the members of each group. Within subgenus *Eryngium*, the chloroplast- and nuclear-derived phylogenies show contradictory relationships. Though these differences are weakly supported, the incongruities are in agreement with the several cases of natural hybrids reported for Old World *Eryngium* species (Wolff, 1913; Molinas and Perdigó, 1981), as are the overlapping peaks observed in the ITS sequence electropherograms. The evolutionary history of this subgenus is very complex. What is clear from our studies is that the foliar characters used by Wolff (1913), and accepted by Wörz (2004, 2005), to delimit sections (or subgenera) find no support from our analyses. Indeed, Wolff (1913) accepted the poor cohesion of species within many of his sections and proposed affinities of species from one section to those of several others.

Within *Eryngium* subgenus *Monocotyloidea*, sections *Chamaeeryngium* (*E. tenue*), *Hygrobia* (represented by *E. galioides* and *E. viviparum*), and *Corniculata* (*E. corniculatum*) form three successive lineages basal to the "New World s. str." clade. The "New World s. str." clade includes all *Eryngium* species from the Americas and Australia and is divided in three major subclades: "Eastern USA", "Mexican", and "South American". These three subclades and *E. coquimbanum* form a quadritomy, and we propose that this lack of resolution reflects a rapid diversification of their ancestors. Dispersal to different geographic areas may have been rapid and colonization of these new areas favored the radiation observed. The position of the Chilean species *E. coquimbanum* is puzzling and requires further investigation. The nuclear and chloroplast gene trees place this species in contradictory positions, suggesting an ancient hybridization event. This difference, however, is supported weakly (especially in the ITS trees).

The "Eastern USA" clade comprises mostly low, prostrate to erect herbs possessing small capitules arranged in monochasia and bearing a coma (a differentiation of the distal floral bracts in a capitule). These plants often grow in moist soils or meadows. Within this clade, two sister subclades are evident: one with species from Mexico (*Eryngium cervantesii*, *E. pilularioides*, and *E. nasturtifolium*); the other with species from eastern and central USA. *Eryngium prostratum*, sister group to these two subclades, is distributed in eastern and central USA.

The "Mexican" clade includes herbaceous plants with a conspicuous and highly reflective involucre (involucral bracts are green on the abaxial face and silver or white on the adaxial face). Their capitules are often blue and have a coma. These showy heads superficially resemble an asteraceous capitule and suggest specialization for insect pollination (Constance, 1977). While affinities among the members of this clade have been proposed previously (Wolff, 1913; Constance and Bye, 1976), the authors were reluctant to put too much classificatory weight "on such purely vegetative structures as the involucre" (Constance, 1977). Silver involucral bracts are a synapomorphy of this clade, although the state reverts in *Eryngium bonplandii* and *E. serratum*. Another synapomorphy for this group is the reduction in basic chromosome number. The number $x = 8$ is plesiomorphic in *Eryngium*, but all members of the "Mexican" clade where counts have been made have a basic chromosome number of $x = 7$, or both, within the same species (Bell and Constance, 1960). These plants are common in moist habitats (damp slopes, marsh lakes, and forests) but also occur in open grassy slopes. Their distribution ranges from east Texas southwards to Mexico, Central America, and northern South America (Peru, Ecuador, Venezuela), via the Andes. Within this clade, *Eryngium madrense* is placed sister group to either *E. mexicanum* or *E. fluitans* depending upon the phylogeny, suggesting a possible hybrid origin of the species.

The "South American" clade is the largest of the New World clades and is extremely diverse morphologically and ecologically. We designate the name of this clade as South America in reference to the biogeographical origin of its ancestor, even though the group includes species from Australia and North, Central, and South America. *Eryngium glossophyllum*, *E. buchtienii*, and *E. incantatum* form three successive lineages at the base of the clade. These three species are poorly known and rarely collected, and their distributions are restricted to high altitude valleys (2500–4000 m) of the Yungas in Argentina and Bolivia. *Eryngium buchtienii* is a flaccid, tall perennial known from cloud forests in Bolivia (2800–3200 m), *E. glossophyllum* is a low acaulescent or subcaulescent perennial distributed in cloud prairies of Tarija (Bolivia) and of Salta and Jujuy (Argentina) (2500–4000 m), and *E. incantatum* is a low perennial endemic to the "Valle Encantado", a cloud prairie in Salta (3000–3200 m). Their phylogenetic positions are of relevance for the reconstruction of the biogeographical history of the genus. Sister group to *E. incantatum* is a large clade which includes both weakly and well-supported groups of uncertain relationship, the latter recognized as subclades "North American monocotyledonous", "Pacific", "South American monocotyledonous", and "*E. foetidum–E. coronatum*". This large polytomy is hypothesized as a second major radiation event within *Eryngium* subgenus *Monocotyloidea*, but the incongruence observed at several positions within the "South American" clade could also be due to reticulation. Most of the polyploid

UNCORRECTED PROOF

Please cite this article in press as: Calviño, C.I. et al., The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): ..., Mol. Phylogenet. Evol. (2008), doi:10.1016/j.ympev.2007.10.021

species reported for *Eryngium* fall within this "South American" clade.

Eryngium coronatum and *E. foetidum* are most similar morphologically to *E. glossophyllum*, *E. incantatum*, and especially to *E. buchtienii*. They share petiolated reticulate-veined leaves and fruits covered with subequal vesicles, which are all probably plesiomorphic character states. *Eryngium foetidum* and *E. coronatum* have wide distributions. The first ranges from tropical forests in Central America to Rio de Janeiro (Brazil) and Bolivia. *Eryngium coronatum* has a more southern distribution in Paraguay, Uruguay, and north and central Argentina. Both species have a coma and their foliage tastes like coriander; indeed, *E. foetidum* is commercially used for culinary purposes. The phylogenetic position of this species confirms previous ideas that the presence of this herb in the paleotropics is exotic (it was likely introduced because of its popularity as a spice; Constance, 1977) and not relictual of a Malaysian-tropical African bridge between the Old and New Worlds (Cerceau-Larrival, 1971).

The "Pacific" clade contains two sister groups. One group includes several subclades comprising all species sampled from the Pacific coasts of Australia, California, and Chile (including the Juan Fernandez Islands). The other group includes two species (*Eryngium nudicaule, E. echinatum*) that do not reach the Pacific coasts. Instead, they grow in central-east South America (Argentina, Brazil, Paraguay, Uruguay), with *E. nudicaule* also extending north-westward into Bolivia and Peru. Despite their different distributions, these two widespread species share several morphological similarities with their sister group (save the Juan Fernandez Islands species), such as their types of basal leaves and involucral bracts and the presence of a coma. The Californian species find closest affinities with the Australian and Chilean members of the group, a relationship suggested by Wolff (1913) based on leaf morphology. *Eryngium vesiculosum*, *E. ovinum* and *E. rostratum* grow in disturbed or damp areas in western Australia (*E. rostratum* also occurs in Chile) and *E. articulatum* and *E. vaseyi* occupy the unique "vernal pool" habitat on the Pacific coast of California (Constance, 1977). These species (particularly, *E. vesiculosum* and the Californian species) share rigorous ecological conditions of temporary inundated and well drained and dry soils. Their leaves are seasonally dimorphic. During summer, the leaves are petiolate, laminoid and have spiny margins, whereas during winter, they are fistulate, linear and septate. Webb (1984) showed that heterophylly in *E. vesiculosum* is cued by day-length and that total immersion in water affects leaf form to a lesser extent. *Eryngium rostratum* and *E. ovinum* also show variation in leaf morphology, including fistulose septate forms; however, correlations of leaf form with ecological conditions have not been reported for these species. Septate, fistulose leaves are common in many aquatic plants, and in *Eryngium* the trait appears in several independent lineages (i.e., *E. corniculatum*, *E. pseudojunceum*, and the "Pacific" clade). Within the "Pacific clade", the bizarre species from the Juan Fernandez Islands (*Eryngium inaccessum, E. fernandezianum* and *E. bupleuroides*; sect.*Fruticosa*) form a strongly supported monophyletic group. Their peculiar characters (woody habit, parallel-veined leaves, and big capitules) precluded previous authors from hypothesizing possible relationships with taxa from the mainland. In the phylogenies inferred herein, these species show a close relationship to the Australian, Californian and Chilean species. These relationships, however, are not well resolved and further sampling of Chilean species is necessary. The sessile, parallel-veined leaves of the Juan Fernandez Island species have evolved independently from the ones observed in monocotyledonous *Eryngium*.

The monocotyledonous *Eryngium* species are characterized by the possession of sessile, generally linear, parallel-veined leaves and a well-developed cauline axis (erect and with several internodes). This group of exclusively North, Central and South American species has always evoked special attention and has long been considered a natural group (Decaisne, 1873; Wolff, 1913; Constance, 1977). The phylogenetic reconstructions presented here are not conclusive about the monophyly of this unique group of species. At least two different clades include these monocotyledonous members of *Eryngium*, and these clades comprise part of a large polytomy within the "South American" clade. The "North American monocotyledonous" clade comprises mostly Mexican polyploids with conspicuous involucral bracts. All the North American monocotyledonous species sampled in this study are placed within this clade. The latter is strongly supported in the plastid-derived trees and in the Bayesian trees inferred by combined data, but is only weakly supported in the other analyses. The "South American monocotyledonous" clade includes species primarily from northern Argentina, southern Brazil and Uruguay, many of which are also polyploids with inconspicuous involucral bracts. The clade is strongly supported, but it does not include all the South American monocotyledoneous species sampled. These other South American monocotyledonous *Eryngium* species ally in small groups in the polytomy that is sister group to the "North American monocotyledonous" clade. This relationship, however, is only seen in the Bayesian combined trees and it is supported very weakly. The relationships among the monocotyledonous species of *Eryngium* remain obscure. Sequence divergence estimates between these species are very low, and the lack of parsimony informative characters, together with the multiple peaks observed in some electropherograms, suggests that speciation in this group was recent and rapid. Moreover, the phylogeny reconstructed for this group is complicated due to reticulation. The cloning of low copy nuclear genes may hold promise in finding a way to elucidate the possible role of hybridization in the evolution of these plants (C.I. Calviño and S.R. Downie, unpublished data).

In summary, an estimate of phylogenetic relationships within the genus *Eryngium* is presented using data from

UNCORRECTED PROOF

Please cite this article in press as: Calviño, C.I. et al., The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): ..., Mol. Phylogenet. Evol. (2008), doi:10.1016/j.ympev.2007.10.021

the cpDNA *trnQ–trnK* 5′-exon and nrDNA ITS regions. Approximately half of the 250 species of *Eryngium* were sampled. In total, 4584 unambiguously aligned nucleotide positions were considered, and these yielded 597 parsimony informative characters and 93 informative indels. *Eryngium* was confirmed as monophyletic, with *Sanicula* its sister genus. Two subgenera are recognized and redefined within *Eryngium*: subgenus *Eryngium* and subgenus *Monocotyloidea*. The first subgenus includes all species from the Old World (Africa, Europe, and Asia), except *Eryngium tenue*, *E. viviparum*, *E. galioides*, and *E. corniculatum*. The second subgenus is redefined to include all species from the New World (Americas and Australia), plus the four aforementioned Old World species. Based on the results of these phylogenetic analyses, most sectional and subgeneric divisions recognized by Wolff (1913) and Wörz (2005), respectively, are not monophyletic. Within each subgenus, the first splits are resolved and several subclades are erected. Members of each of these subclades share similar geographical distributions and/or morphological or ecological traits; however, the relationships among them are not resolved. We interpreted the three major polytomies observed as radiation events that coincided with the colonization of new territories. The results of dispersal-vicariance analyses indicated that the genus *Eryngium* and its two subgenera originated from western Mediterranean ancestors. The widespread distribution of the genus is the result of several dispersal events, including trans-oceanic dispersals, and other long distance dispersals that probably occurred in short periods of time relative to one another. This resulted in a lack of accumulated molecular changes between common ancestors of the different subclades and also a rapid morphological divergence between them driven by the establishment of new populations in new and disjunct territories. The radiations observed in the phylogenies are suggestive of hybridization events, which in *Eryngium* may have been the cause, but also a consequence, of the rapid diversification of the group. Deciphering the evolutionary history of *Eryngium* remains a difficult task given that it combines several complex evolutionary processes, such as rapid radiations, reticulate evolution, and long distance dispersals.

5. Uncited reference

Q1 Bell and Constance (1957).

Acknowledgments

The authors thank the curators of herbaria BA, CORD, CTES, E, ILL, ILLS, JACA, JEPS, MA, MO, PAL, SI, TEX-LL, UC, US, W, WIS, for access to specimens, F.O. Zuloaga, and anonymous reviewers for comments on the manuscript. This work was supported by grants to S.R. Downie from the National Science Foundation (DEB 0089452) and the National Center for Supercomputing Applications (DEB 030005N), utilizing the IBM pSeries 690 system at the University of Illinois at Urbana-Champaign (UIUC). Travel funds to C.I. Calviño were provided by UIUC's School of Integrative Biology Enhancement Fund and the Department of Plant Biology John R. Laughnan Award. A collection trip to Paraguay (C.I. Calviño and S.G. Martínez) was supported by the Myndel Botanica Foundation. This paper represents part of a Ph.D. dissertation (C.I.C.), for which funding from CONICET is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2007.10.021.

References

Abbott, R.J., Brochmann, C., 2003. History and evolution of the arctic flora: in the footsteps of Eric Hulten. Mol. Ecol. 12, 299–313.
Álvarez, I., Wendel, J.F., 2003. Ribosomal ITS sequences and plant phylogenetic inference. Mol. Phylogenet. Evol. 29, 417–434.
Baurain, D., Brinkmann, H., Philippe, H., 2007. Lack of resolution in the animal phylogeny: closely spaced cladogeneses or undetected systematic errors? Mol. Biol. Evol. 24, 6–9.
Bell, C.R., Constance, L., 1957. Chromosome numbers in Umbelliferae. Am. J. Bot. 44, 565–572.
Bell, C.R., Constance, L., 1960. Chromosome numbers in Umbelliferae II. Am. J. Bot. 47, 24–32.
Bell, C.R., Constance, L., 1966. Chromosome numbers in Umbelliferae III. Am. J. Bot. 53, 512–520.
Bourles, B., Molinari, R.L., Johns, E., Wilson, W.D., Leaman, K.D., 1999. Upper layer currents in the western tropical North Atlantic (1989–1991). J. Geophys. Res. 104, 1361–1375.
Breton, A., 1962. Revision des *Eryngium* d'Afrique du Nord. Mém. Soc. Sci. Nat. Phys. Maroc. Bot. Nouv. Sér. 2, 1–86.
Calviño, C.I., O'Leary, N., Greizerstein, E., Martínez, S., Poggio, L., 2002. Chromosome studies on eight species of *Eryngium* L. (Saniculoideae, Apiaceae) from Argentina. Caryologia 55, 315–321.
Calviño, C.I., Tilney, P.M., van Wyk, B.E., Downie, S.R., 2006. A molecular phylogenetic study of southern African Apiaceae. Am. J. Bot. 93, 1828–1847.
Calviño, C.I., Downie, S.R., 2007. Circumscription and phylogeny of Apiaceae subfamily Saniculoideae based on chloroplast DNA sequences. Mol. Phylogenet. Evol. 44, 175–191.
Calviño, C.I., Martínez, S.M., Downie, S.R., submitted for publication. The morphology and biogeography of Apiaceae subfamily Saniculoideae as inferred by phylogenetic analysis of molecular data.
Carlquist, S., 1981. Chance dispersal. Am. Sci. 69, 509–516.
Cerceau-Larrival, M.T., 1971. Plantules et pollens dans le genre *Eryngium* L. Relations avec biogéographie. C.R. Somm. Séances Soc. Biogéogr. 424, 103–112.
Cerceau-Larrival, M.T., 1973. Corrélations de caractères pour une étude biologique du genre *Eryngium* L. In: Anonymous Comptes rendus du 96 Congrès National des Sociétés Savantes, Section des Sciences, Toulouse, 1971, Bibliothèque Nationale, Paris, vol. V, pp. 451–477.
Coleman, M., Liston, A., Kadereit, J.W., Abbott, R.J., 2003. Repeat intercontinental dispersal and Pleistocene speciation in disjunct Mediterranean and desert *Senecio* (Asteraceae). Am. J. Bot. 90, 1446–1454.
Constance, L., Chaung, T.I., Bell, C.R., 1971. Chromosome numbers in Umbelliferae IV. Am. J. Bot. 58, 577–587.
Constance, L., Bye, R.A.J., 1976. New Chihuahuan Umbelliferae. Bot. Mus. Leafl. Harv. Univ. 24, 225–240.

UNCORRECTED PROOF

Constance, L., Chuang, T.I., Bell, C.R., 1976. Chromosome numbers in Umbelliferae V. Am. J. Bot. 63, 608–625.

Constance, L., 1977. Some problems in New World *Eryngium*. In: Cauwet Marc, A.M., Carbonnier, J. (Eds.), Les Ombelliferes. Contributions pluridisciplinaires à la systématique. Missouri Botanical Garden, Michigan, pp. 7–19.

Davis, P.H., 1972. *Eryngium* L. In: Davis, P.H. (Ed.), Flora of Turkey and the East Aegean Islands, vol. 4. Edinburgh University Press, Great Britain, pp. 292–304.

Decaisne, J., 1873. Remarques sur les especes du genre *Eryngium*, a fuilles parallelinerves. Bull. Soc. Bot. Fr. 20, 19–27.

Dolphin, K., Belshaw, R., Orme, C.D.L., Quicke, D.L.J., 2000. Noise and incongruence: interpreting results of the incongruence length difference test. Mol. Phylogenet. Evol. 17, 401–406.

Downie, S.R., Katz-Downie, D.S., 1996. A molecular phylogeny of Apiaceae subfamily Apioideae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. Am. J. Bot. 83, 234–251.

Downie, S.R., Katz-Downie, D.S., 1999. Phylogenetic analysis of chloroplast *rps16* intron sequences reveals relationships within the woody southern African Apiaceae subfamily Apioideae. Can. J. Bot. 77, 1120–1135.

Downie, S.R., Plunkett, G.M., Watson, M.F., Spalik, K., Katz-Downie, D.S., Valiejo-Roman, C.M., Terentieva, E.I., Troitsky, A.V., Lee, B.Y., Lahham, J., El Oqlah, A., 2001. Tribes and clades within Apiaceae subfamily Apioideae: the contribution of molecular data. Edinb. J. Bot. 58, 301–330.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Doyle, J.J., 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. Syst. Bot. 17, 144–163.

Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1995. Testing significance of incongruence. Cladistics 10, 315–319.

Goloboff, P., Farris, J.S., Nixon, K., 2003. T.N.T.: Tree Analysis Using New Technology. Version 1.0. Program and documentation, available from the authors, and at www.zmuc.dk/public/phylogeny.

Hagen, A.R., Giese, H., Brochmann, C., 2001. Trans-Atlantic dispersal and phylogeography of *Cerastium arcticum* (Caryophyllaceae) inferred from RAPD and SCAR markers. Am. J. Bot. 1, 103–112.

Hall, T., 1999. BioEdit. Biological Sequence Alignment Editor for Win95/98/NT/2K/XP. Version 6.0.7.

Hardway, T.M., Spalik, K., Watson, M.F., Katz-Downie, D.S., Downie, S.R., 2004. Circumscription of Apiaceae tribe Oenantheae. S. Afr. J. Bot. 70, 393–406.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Huson, D.H., Bryant, D., 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23, 254–267.

Irgang, B.E., 1974. Umbelliferae II. Gênero *Eryngium*. In: Schultz, A.R. (Ed.), Flora Ilustrada do Rio Grande do Sul. Bol. Inst. Ciencias Nat. Univ. Rio Grande do Sul, vol. 32, pp. 1–86.

Jeanmougin, F., Thompson, J.D., Gouy, M., Higgins, D.G., Gibson, T.J., 1998. Multiple alignment with Clustal X. Trends Biochem. Sci. 23, 403–405.

Lee, C.S., Downie, S.R., 2006. Phylogenetic relationships within *Cicuta* (Apiaceae tribe Oenantheae) inferred from nuclear rDNA ITS and cpDNA sequence data. Can. J. Bot. 84, 453–468.

Martínez, S., 2005. Apiaceae, parte 2. Subfamilia III. Saniculoideae. In: Anton A.M., Zuloaga, F.O. (Eds.), Flora Fanerogámica Argentina. Gráfica Gutierrez, Córdoba, vol. 94.

Mathias, M.E., Constance, L., 1941. A synopsis of the North American species of *Eryngium*. Am. Midl. Nat. 25, 361–387.

Mathias, M.E., Constance, L., Araujo, D., 1972. Umbelíferas. In: Reitz P.R. (Ed.), Flora Ilustrada Catarinense. Herbario Barbosa Rodrigues, vol. UMBE, Santa Catarina, pp. 1–205.

McBreen, K., Lockhart, P.J., 2006. Reconstructing reticulate evolutionary histories of plants. Trends Plant Sci. 11, 398–404.

McGlone, M.S., 2005. Goodbye Gondwana. J. Biogeogr. 32, 739–740.

McNeill, J., Barrie, F.R., Burdet, H.M., Demoulin, V., Hawskword, D.L., Marhold, K., Nicolson, D.H., Prado, J., Silva, P.C., Skog, J.E., Wiersema, J.H., 2006. International Code of Botanical Nomenclature (Vienna Code). Regnum Vegetabile 146, 1–568.

Milne, R.I., Abbott, R.J., 2002. The origin and evolution of Tertiary relict floras. Adv. Bot. Res. 38, 281–314.

Milne, R.I., 2006. Northern hemisphere plant disjunctions: a window on Tertiary land bridges and climate change? Ann. Bot. 98, 465–472.

Molinas, M.L., Perdigó, M.T., 1981. Aportació a l'estudi d'algunes espècies del gènere *Eryngium* de la Flora Pirenenca. An. Secc. Cienc. Col. Univ. Gerona 5, 179–187.

Neves, S.S., Watson, M.F., 2004. Phylogenetic relationships in *Bupleurum* (Apiaceae) based on nuclear ribosomal DNA ITS sequence data. Ann. Bot. 93, 379–398.

Nieto Feliner, G., 2003. *Eryngium* L. In: Nieto Feliner, G., Jury, S.L., Herrero, A. (Eds.), Araliaceae-Umbelliferae. Real Jardín Botánico, vol. X, CSIC, Madrid, pp. 36–60.

O'Leary, N., Calviño, C.I., Greizerstein, E., Martinez, S., Poggio, L., 2004. Further cytogenetical studies on diploid and polyploid species of *Eryngium* L. (Saniculoideae, Apiaceae) from Argentina. Hereditas (Lund) 140, 129–133.

Pimenov, M.G., Tamamschian, S., (1987). *Eryngium* L. In: Rechinger, K.H. (Ed.), Flora Iranica, Akademische Druck und Verlagsanstalt, Graz, vol. 162, pp. 45–60.

Pimenov, M.G., Vasil'eva, M.G., Leonov, M.V., Daushkevich, J.V., 2003. Karyotaxonomical Analysis in the Umbelliferae. Science Publishers, Enfield, New Hampshire.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Posada, D., Buckley, T.R., 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst. Biol. 53, 793–808.

Rambaut, A., 2002. Se-Al: Sequence Alignment Editor. Version 2.0a11. Available from: <http://evolve.zoo.ox.ac.uk/> (Accessed 31 May 2005).

Raven, P.H., Axelrod, D.I., 1974. Angiosperm biogeography and past continental movements. Ann. Mo. Bot. Gard. 61, 539–673.

Renner, S., 2004. Plant dispersal across the tropical Atlantic by wind and sea currents. Int. J. Plant Sci. 165, S23–S33.

Ridley, H.N., 1930. The Dispersal of Plants Throughout the World. Reeve L. & Co., Ltd., Kent.

Rieseberg, L.H., Brunsfeld, S.J., 1992. Molecular evidence and plant introgression. In: Soltis, P.S., Soltis, D.E., Doyle, J.J. (Eds.), Molecular Systematics of Plants. Chapman and Hall, New York, pp. 151–176.

Ronquist, F., 1996. DIVA, version 1.1 for MacOS and Win32. Computer program and documentation distributed by the author. Available from: <http://www.ebc.uu.se/systzoo/research/diva/diva.html/> (Accessed 21 June 2005).

Seehausen, O., 2004. Hybridization and adaptive radiation. Trends Ecol. Evol. 19, 198–207.

Seelanan, T., Schnabel, A., Wendel, J.F., 1997. Congruence and consensus in Cotton tribe (Malvaceae). Syst. Bot. 22, 259–290.

Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chunwongse, J., Obokata, J., Yamaguchi-Shinozaki, K., Ohto, C., Torazawa, K., Meng, B.Y., Sugita, M., Deno, H., Kamogashira, T., Yamada, K., Kusuda, J., Takaiwa, F., Kato, A., Tohdoh, N., Shimada, H., Sugiura, M., 1986. The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J. 5, 2043–2049.

Soltis, D.E., Kuzoff, R.K., 1995. Discordance between nuclear and chloroplast phylogenies in *Heuchera* group (Saxifragaceae). Evolution 49, 727–742.

Spalik, K., Downie, S.R., 2007. The evolutionary history of*Sium* sensu lato (Apiaceae): dispersal, vicariance, and domestication inferred from ITS rDNA phylogeny. Am. J. Bot. 93, 5–747.

Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (and other methods), Version 4.0 b10. Sinauer Associates, Sunderland, Massachusetts, USA.

UNCORRECTED PROOF

Please cite this article in press as: Calviño, C.I. et al., The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): ..., Mol. Phylogenet. Evol. (2008), doi:10.1016/j.ympev.2007.10.021

Turmel, J.M., 1948. Répartition géographique des *Eryngium*. I. Ancien Monde. Bull. Mus. Nat. Hist. Nat. 2a Sér. 20, 395–401.

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

Turmel, J.M., 1950. Évolution des Saniculoidées II. Évolution du genre *Alepidea* (Ombellifères). Bull. Mus. Nat. Hist. Nat. 2a Sér. 1, 120–126.

Turmel, J.M., 1951. Liaison Euro-Afrique et biogéographie des *Eryngium*. I. Ancien Monde. C.R. Somm. Séances Soc. Biogéogr. 241, 57–60.

Valiejo-Roman, C.M., Terentieva, E.I., Samigullin, T.H., Pimenov, M.G., 2002. Relationships among genera in Saniculoideae and selected Apioideae (Umbelliferae) inferred from nrITS sequences. Taxon 51, 91–101.

Webb, C.J., 1984. Heterophylly in *Eryngium vesiculosum* (Umbelliferae). N.Z. J. Bot. 22, 29–33.

Wiens, J.J., Engstrom, T.N., Chippindale, P.T., 2006. Rapid diversification, incomplete isolation, and the "speciation clock" in North American salamanders (genus *Plethodon*): testing the hybrid swarm hypothesis of rapid radiation. Evolution 60, 2585–2603.

Wilgenbusch, J.C., Warren, D.L., Swofford, D.L., 2004. AWTY: a system for graphical exploration of MCMC convergence in Bayesian phylogenetic inference. Available from: <http://ceb.csit.fsu.edu/awty/> (Accessed 17 June 2005).

Whitfield, J.B., Lockhart, P.J., 2007. Deciphering ancient rapid radiations. Trends Ecol. Ecol. 22, 258–265.

Wolff, H., 1913. Umbelliferae-Saniculoideae. In: Engler, A. (Ed.), Das Pflanzenreich, vol. IV 228. Wilhelm Engelmann, Leipzig and Berlin, pp. 1–305.

Wörz, A., 2004. On the distribution and relationships of the South-West Asian species of *Eryngium* L. (Apiaceae–Saniculoideae). Turk. J. Bot. 28, 85–92.

Wörz, A., 2005. A new subgeneric classification of the genus *Eryngium* L. (Apiaceae, Saniculoideae). Bot. Jahrb. Syst. 126, 253–259.

Yokota, Y., Kawata, T., Iida, Y., Kato, A., Tanifuji, S., 1989. Nucleotide sequences of 5.8S rRNA gene and internal transcribed spacer regions in carrot and broad bean ribosomal DNA. J. Mol. Evol. 29, 294–301.

UNCORRECTED PROOF

Please cite this article in press as: Calviño, C.I. et al., The evolutionary history of *Eryngium* (Apiaceae, Saniculoideae): ..., Mol. Phylogenet. Evol. (2008), doi:10.1016/j.ympev.2007.10.021