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PHYLOGENETIC AFFINITIES OF SOUTH AMERICAN *ANEMONE* (RANUNCULACEAE), INCLUDING THE ENDEMIC SEGREGATE GENERA, *BARNEOUDIA* AND *OREITHALES*

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This study tests the phylogenetic affinities of 11 South American species of *Anemone* s.l., including the closely related endemic segregate genera *Barneoudia* and *Oreithales*. We analyzed combined sequence data (chloroplast *atpB-rbcL* spacer and nuclear ITS regions) for 51 species of *Anemone* s.l., using both likelihood and cladistic methods. The segregate genera, *Oreithales* and *Barneoudia*, nest within *Anemone* and are included in a clade (subgenus *Anemone*, sect. *Pulsatilloides*) consisting largely of South American taxa (*Anemone sellowii*, *Anemone helleborifolia*, and *Anemone rigida*) and other Southern Hemisphere species (e.g., *Anemone caffra*, *Knowltonia vesicatoria*, and *Anemone crassifolia*). As reported previously, *Anemone antucensis* (Chile and Argentina) is in a separate clade (subgenus and section *Anemonidium*) and is sister to *Anemone tenuicaulis* (New Zealand). *Anemone multifida*, *Anemone triternata*, and *Anemone decapetala* are embedded in a clade (subgenus and section *Anemone*) consisting largely of North American taxa. The tetraploid, *Anemone multifida*, was found in two separate, highly supported clades in the nuclear and chloroplast trees, suggesting a hybrid origin. Both sections *Pulsatilloides* and *Anemonidium* suggest that anemones originated in the Northern Hemisphere and subsequently spread to the Southern Hemisphere, a pattern found in common with other members of Ranunculaceae. Preliminary suggestions are offered for the reclassification of *Anemone*.

Keywords: *Anemone*, *Barneoudia*, *Oreithales*, South America, ITS, *atpB-rbcL* spacer.

Online enhancement: table.

Introduction

Previous phylogenies based on molecular data (Hoot et al. 1994; Ehrendorfer and Samuel 2001; Schuettpelz et al. 2002; Ehrendorfer et al. 2009) recovered several clades consisting of *Anemone* and related genera (e.g., *Knowltonia* of South Africa) with geographic distributions in the Southern Hemisphere. However, this work included few South American anemones and no representatives of the closely related endemic segregate genera *Barneoudia* and *Oreithales*. The work reported here substantially increases the sampling of South American anemones s.l. and provides further information for evaluating the present distribution of the genus.

The genus *Anemone* s.str. consists of approximately 150 species (Tamura 1995), mainly distributed in the Northern Hemisphere. However, approximately 30 *Anemone* species and several closely allied genera (e.g., *Knowltonia*, *Barneoudia*, *Oreithales*) are found discontinuously throughout mountainous and cooler regions of the Southern Hemisphere, including approximately 13 species from South America. There is considerable variation within *Anemone* s.l., but the genus is characterized by a rosette of basal leaves, an involucrate peduncle bearing a single flower or compound inflorescence, a perianth of petaloid sepals, an achene fruit, and x=7 or 8 (base chromosome number; Heimburger 1959; Tamura 1967; Hoot et al. 1994). Pollen ranges from tricolpate to pantocolpate, pantoporate to spiroaperturate (Huynh 1970*a*).

The South American anemones (*A. antucensis*, *A. helleborifolia*, *A. rigida*, *A. sellowii*, *A. multifida*, *A. decapetala*, and *A. triternata*) and the two segregate genera (*Barneoudia* and *Oreithales*) included in this study vary in distribution from the southeast portions of the continent, near the coast of Brazil, to various altitudes of the montane regions of the Andes (Lourteig 1951, 1956). Morphology and habitat vary considerably. *Anemone antucensis* occurs in montane forests of central Chile and Neuquén, Argentina. It has tripartite basal leaves with long petioles; one- to two-flowered inflorescences; flowers with five white, elliptical to suborbical sepals; and glabrous achenes with a relatively long hook (Lourteig 1951). *Anemone helleborifolia*, endemic to the moderate altitudes of the Peruvian Andes, is found in rocky habitats. It has ternate basal leaves that appear palmatifid; compound branching inflorescences; flowers with (4)–5, white-yellow sepals; and subovoid, glabrous achenes. *Anemone rigida* is endemic to the midelevation forests of the Chilean Andes, Mediterranean climate sector. It has 3–5-lobed basal leaves with mucronate teeth; 4-flowered cymes; flowers with 6–7 white, pink, or bluish sepals; and ovoid, slightly pubescent achenes. *A. sellowii* occurs in the coastal mountains of southeast Brazil. It has ternate basal leaves, 1–3 flowered inflorescences; flowers with 9–12 white (abaxially red) sepals; and subglobose, ribbed, glabrous achenes.

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Anemone decapetala, *A. triternata*, and *A. multifida* all have disjunct distributions with North American populations or closely related species. They are found at lower altitudes in Chile, Argentina, Uruguay, and southeastern Brazil, occasionally reaching the alpine in the Andes. Based on morphology and molecular data, Hoot et al. (1994) placed *A. decapetala* and *A. triternata* in the *Coronaria* group, a group composed of tuberous anemones from southwestern North America and the Mediterranean region. *Anemone decapetala* and *A. triternata* differ from each other in presence or absence of deciduous sepals, number of veins in sepals, filament width, number of flowers/inflorescences, and similarity of basal and involucral leaves (Ziman et al. 2006). *Anemone multifida* of South America and North America are morphologically diverse; some workers recognize this diversity by splitting the species into multiple species or varieties (Hoot et al. 1994). As is characteristic of *A. decapetala* and *A. triternata* as well, *A. multifida* has tomentose achenes.

Barneoudia and *Oreithales* are treated as segregate genera of *Anemone* by Lourteig (1951, 1956). *Barneoudia* consists of four species, distributed exclusively in alpine zones of Chile and Argentina. The monotypic genus *Oreithales* is endemic to the high puna of Bolivia and Peru (Lourteig 1956). The two genera differ from each other and anemones in general by a few uncommon morphological characteristics (table 1), such as absence of basal leaves at flowering (*Barneoudia*) and absence of involucral leaves on the inflorescence (*Oreithales*).

Based on chloroplast DNA restriction site data and morphology, Hoot et al. (1994) derived a phylogeny of *Anemone* that included 36 species, three segregate genera (*Hepatica*, *Pulsatilla*, and *Knowltonia*), and several Southern Hemisphere species. This work resulted in a preliminary classification that recognized two subgenera (*Anemonidium* and *Anemone*), seven sections, and 12 informal subsectional groupings. The Southern Hemisphere species, *A. crassifolia* (Tasmania), *A. caffra* (South Africa), and *Knowltonia capensis* (=*A. knowltonia*; South Africa), formed a well-supported monophyletic group within subgenus *Anemone* (Hoot et al. 1994).

In more recent studies, workers (Ehrendorfer and Samuel 2001; Schuettpelz et al. 2002) tested the sectional affinities of two Southern Hemisphere anemones, *A. antucensis* (Andean Chile) and *A. tenuicaulis* (New Zealand), using plastid and nuclear DNA sequence data. It was found that *A. antucensis* and *A. tenuicaulis* belong to subgenus *Anemonidium* (x=7) rather than subgenus *Anemone* (x=8), indicating that southern anemones are polyphyletic. Ziman et al. (2006) published a revision of the Southern Hemisphere species of *Anemone* s.str. based on morphological data, recognizing 21 species and nine sections. Based on morphology and molecular data, Ehrendorfer et al. (2009) propose a taxonomic revision and molecular dating of *Anemone* section *Anemone*, including a few taxa from the Southern Hemisphere.

The principal objectives of this study were (1) to derive a phylogenetic tree with increased sampling of South American *Anemone* species (including *Barneoudia* and *Oreithales*), using chloroplast *atpB-rbcL* spacer region and nuclear internal transcribed spacer (ITS) data and employing multiple analytic methods (Bayesian inference, maximum likelihood, and maximum parsimony), and (2) to test and identify changes needed in the preliminary classification proposed in Hoot et al. (1994). Additionally, we consider biogeographic hypotheses related to the South American and other Southern Hemisphere anemones in light of our results.

Material and Methods

Taxon Sampling

The sampling of taxa within *Anemone* s.l. (=Anemoninae; Ehrendorfer and Samuel 2001) used in this study follows the

Table 1

Comparison of Key Morphological Characters for *Anemone*, *Barneoudia*, and *Oreithales* (Lourteig 1951, 1956; Duncan and Perez 1979; Huynh 1970*a*)

	Anemone s. str.	*Barneoudia*	*Oreithales*
Perennating structure	Upright stems, rhizomes, tubers	Ovoid, compact tuber	Upright short stem
Habit	Usually acaulescent rosette of leaves	Acaulescent rosette; leaves absent at flowering	Acaulescent rosette
Leaf dissection	Simple trilobed to ternate, sometimes pinnate	Simple trilobed to ternate	Simple, not trilobed
Number of involucral leaves on peduncle	Usually 3	One suborbicular, consisting of two to three subdivided segments	Absent
Involucral leaf morphology	Similar to basal leaves to bractlike	Bractlike	NA
Position of involucre	Varies from ~ halfway up peduncle to directly beneath perianth	Directly beneath perianth	NA
Sepal number	Usually 4 to 20	7–18	10–18
Pollen type	Tricolpate, polycolpate, polyporate, pantoporate, eupantocolpate, spiroaperturate	Polyporate (*Barneoudia major*)	Tricolpate
Style length	Short (<.5 mm) to long (>3 mm)	4–6 mm	3.5 mm
Style morphology	Straight to angled; hooked to straight	Straight; not hooked	Straight; hooked
Achene morphology	Glabrous to hairy to densely wooly; not winged to winged	Hairy; not winged	Hairy; not winged
Base chromosome number	x=7, 8	Unknown	x=8 (2n=48)

Note. NA = not applicable.

informal taxonomy presented by Hoot et al. (1994) of subgenera, sections, and species groups. For easy recognition of genera that should be subsumed within *Anemone* (Hoot et al. 1994; Ehrendorfer and Samuel 2001; Schuettpelz et al. 2002), we have retained the segregate names (i.e., *Hepatica*, *Knowltonia*, *Pulsatilla*) in our figures and discussion.

This work represents increased sampling over previous work on *Anemone*. Our sampling includes 52 species (including segregate genera; see table A1 in the online edition of the *International Journal of Plant Sciences*). In previous work, Hoot et al. (1994) sampled 42 species (one from South America [SA]), Ehrendorfer and Samuel (2001) sampled 19 species (one from SA), Schuettpelz et al. (2002) sampled 21 species (two from SA), and Ehrendorfer et al. (2009) sampled 19 species (one from SA). None of this work included *Barneoudia* or *Oreithales*. Of the 17 South American species of *Anemone* s.l. recognized by Lourteig (1951, 1956), our sampling includes 11 species: seven species of *Anemone*, three species of *Barneoudia*, and two accessions of *Oreithales* (table A1). We have multiple accessions of a number of *Anemone* species (e.g, *A. narcissiflora*, *A. obtusiloba*, *A. trullifolia*, *A. edwardsiana*, *A. berlandieri*, and *A. tuberosa*) that have identical or almost identical sequences; we included only one representative of each of these to keep the tree size manageable. Based on previous molecular work on the Ranunculaceae (Hoot 1995; Johansson 1995), *Ranunculus* (two species), *Trautvetteria* (one species), and *Clematis* (two species) were used as outgroups.

Our work follows the same subgeneric and sectional names as found in Hoot et al. (1994) except for section *Anemonospermos* DC. This name was incorrectly applied in Hoot et al. (1994) since this clade does not include the type specimen for the section, *A. dichotoma* (Ehrendorfer and Samuel 2001). Since *A. rivularis* is strongly supported as a member of this clade, in this article we replace the name *Anemonospermos* with section *Rivularidium* Janczewski.

DNA Sequencing and Analyses

DNA extraction, amplification, and sequencing was as described in Schuettpelz et al. (2002) with the following exception: automated DNA sequencing was performed on either an ABI model 373A-Stretch (Applied Biosystems, Foster City, CA) or a CEQ 2000XL capillary sequencer (Beckman Colter, Fullerton, CA), according to the respective manufacturers' protocols.

Alignment of DNA sequences was accomplished to a rough approximation using Sequencher 3.0 (Gene Codes, Ann Arbor, MI) with subsequent manual corrections. Alignment procedures were as described in Hoot and Douglas (1998), paying careful attention to repeated motifs (type Ib indels) and runs of the same nucleotide (type Ia indels). Using MacClade (Maddison and Maddison 2001), insertions/deletions (indels) were scored as binary characters, following the conservative simple gap coding method (Simmons and Ochoterena 2000). Regions of ambiguous alignment were removed from the data set without gap scoring.

Before combining the ITS and *atpB-rbcL* spacer data, several methods of assessing congruence were implemented: visual comparison of the various clades found in the minimal trees, their bootstrap support, and implementation of the incongruence length difference (ILD) test (Farris et al. 1995), which tests whether the predefined partitions in the data differ significantly from random partitions of the combined data set. The ILD analysis was conducted on the final pruned and edited data using PAUP*, version 4.0b2 (Swofford 2002), with the following settings: 100 replications, heuristic search with simple addition, TBR (tree bisection-reconnection) branch swapping, and saving up to 2000 trees per replicate.

Bayesian inference (BI) was conducted on the combined data using MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003). The data were partitioned into nucleotide and indel sets and analyzed with default prior distributions and applying the model GTR+G to the nucleotide data and the restriction site model (F81) to the indel data. Models for each of the nucleotide data sets (excluding taxa with large amounts of missing data) were determined by MrModeltest 2.2 using the Akaike Information Criterion (Nylander 2004). Two runs of four chains (three heated) were run for 1 million generations, sampling trees every 100 generations and allowing the analysis to reach stationarity. The first 100,000 generations (1,000 trees) were conservatively excluded as the burn-in phase as determined with plots of log likelihood scores versus generation time. After importing the trees into PAUP*, a 50% majority rule consensus tree with posterior probabilities (PP) was computed.

For the maximum likelihood (ML) analyses, GARLI (genetic algorithm for rapid likelihood inference), version 0.95 (Zwickl 2006), was used. GARLI performs heuristic searches under the general time reversible (GTR + I + G) model of nucleotide substitution. The analysis was conducted on the combined *atpB-rbcL* spacer and ITS regions excluding scored indels, using the default parameters with "save every improved topology" unchecked. The ML bootstrap values (MLBS) were calculated using the same parameters with 100 replicates; a majority rule consensus tree was derived using PAUP.

Maximum parsimony (MP) analyses of the *atpB-rbcL* spacer and ITS data (including scored indels) were conducted for each gene independently (results not shown) and combined using PAUP* (Swofford 2002), heuristic search option with 20 random addition sequences and simple addition, TBR branch swapping, and saving up to 4000 trees each replicate. To estimate the confidence to be placed in the topology, bootstrap values (MPBS; Felsenstein 1985) were calculated with 100 replicates, each with 10 random additions.

Results

Individual Data Analyses

Both the *atpB-rbcL* spacer region and ITS aligned sequences contained numerous indels; where alignment was not ambiguous and indels were informative, these were scored (table 2). Both data sets had regions of ambiguous alignment; these were especially prevalent in the *atpB-rbcL* spacer region (table 2). Sites were pruned due to large amounts of missing data at the beginning and end of the sequences, uninformative insertions with large amounts of missing data, or ambiguous alignments. For some of our *Anemone* species, we were able to produce only half of the *atpB-rbcL* spacer (*A. rupestris* and

Table 2

Tree Statistics for Analysis of *Anemone* s.l. for Separate and Combined *atpB-rbcL* Spacer and ITS Data

	atpB-rbcL spacer	ITS	Combined spacer/ITS
Alignment length	1325	684	...
No. bases pruned due to ambiguous alignment	69	33	...
Alignment length after pruning	789	598	1387
Missing data (%)	4.61	3.75	6.23
Variable characters (including gaps)	282	345	627
Informative characters (including gaps)	201	269	470
No. gaps scored	53	16	69
No. trees	>10,000	106	117
CI	.74	.47	.53
RI	.92	.78	.82

Note. The last three rows apply to maximum parsimony analyses only. CI = consistency index excluding uninformative characters; RI = retention index.

A. sellowi) and ITS regions (*A. nemorosa*, *A. rupestris*, and *A. triternata*) due to amplification or sequencing difficulties. The MPBS analyses of the combined data with removal of the above four species resulted in largely congruent trees and bootstrap values similar to those of the MPBS trees derived from the full data set (tree not presented).

The MP strict and bootstrap (BS) consensus trees for each individual data set were much less resolved than the trees resulting from the combined analyses (trees not presented). The topologies resulting from the two individual data sets largely agreed with each other, especially where bootstrap values were high. The one exception was in the placement of *A. multifida*. The *atpB-rbcL* spacer data placed this species in a clade with *A. sylvestris* and *A. virginiana* (BS = 100%); the ITS data placed it in a clade with *A. drummondii*, *A. parviflora*, and *A. lithophila* (BS = 78%). After verifying the accuracy of the *A. multifida* sequences, we decided to retain this species in our sampling but to include it as two separate entries in the combined analyses, one for each region sequenced (thus increasing the amount of missing data for these analyses; table 2). With the exclusion of *A. multifida*, the ILD test resulted in a *P* value of 0.77, supporting combination of the two data partitions.

Combined Data Analyses

MP analyses of the combined ITS and *atpB-rbcL* spacer data resulted in 117 most parsimonious trees with much greater resolution and branch support than resulted from analyses of the individual data sets (table 2). The BI, ML, and MP analyses produced largely congruent trees; ML and MP trees were less resolved than the BI tree.

In the BI tree resulting from the combined data (fig. 1), the monophyly of *Anemone* s.l. is highly supported (PP and MLBS = 100, MPBS = 90), as are the two subgenera *Anemonidium* and *Anemone* (PP $\geq$ 100, MLBS $\geq$ 88, MPBS $\geq$ 92). *Anemonidium* consists of four strongly supported (PP, MLBS, MPBS = 100) clades: sections *Hepatica*, *Keiskea*, *Anemonidium*, and *Homalocarpus* as defined in Hoot et al. (1994). The sister species, *A. antucensis* of SA and *A. tenuicaulis* of New Zealand (PP, MLBS, MPBS = 100), are within section *Anemonidium* along with the North American species, *A. canadensis* and *A. richardsonii* (fig. 1).

Within subgenus *Anemone* (fig. 1), three sections are weakly to well supported: *Pulsatilloides* (PP = 95, MLBS < 50, MPBS = 62), *Rivularidium* (PP, MLBS, MPBS = 100), and *Anemone* (PP, MLBS, MPBS = 100). *Pulsatilloides* includes the South American anemones (*A. sellowii*, *A. helleborifolia*, and *A. rigida*) as well as the segregate South American genera, *Oreithales* and *Barneoudia*.

Within section *Anemone*, *A. multifida* associates with two different clades, depending on the region sequenced. With nuclear ITS data, *A. multifida* is embedded in a clade with *A. drummondii*, *A. parviflora*, and *A. lithophila* (PP = 100, MLBS = 94, MPBS = 96). With chloroplast *atpB-rbcL* spacer data, *A. multifida* is associated with *A. sylvestris* and *A. virginiana* (PP, MLBS, MPBS = 100).

Also within section *Anemone*, *A. triternata* (=*A. decapetala* var. *foliosa* Eichler) from low elevations in Brazil, Bolivia, Uruguay, Argentina, and Chile, is sister to a clade of American tuberous species: *A. caroliniana*, *A. tuberosa*, *A. decapetala*, *A. edwardsiana*, and *A. berlandieri* (PP = 100, MLBS = 86, MPBS = 94).

Discussion

Phylogenetic Relationships

While our current work represents increased and different sampling from previous work (Hoot et al. 1994; Ehrendorfer and Samuel 2001; Schuettpelz et al. 2002; Ehrendorfer et al. 2009), our phylogeny is largely congruent with past findings, recognizing subgenera and sections as defined in Hoot et al. (1994). Branch support values are also comparable, with all subgenera and sections well supported (PP $\geq$ 95, BS $\geq$ 70) except for section *Pulsatilloides*, which received weak bootstrap values in this work but significant PP values. The relationship of section *Rivularidium* also varies: in Hoot et al. (chloroplast restriction site data; 1994), *Rivularidium* is sister to *Pulsatilloides* (MPBS = 64); in this work and Schuettpelz et al. (2002), *Rivularidium* is sister to section *Anemone* with strong support (PP = 100, BS $\geq$ 91).

Our molecular results do not support the classification of the South American taxa based on morphology as proposed in Ziman et al. (2006). Superimposing their sectional designations for just the South American taxa (they did not treat

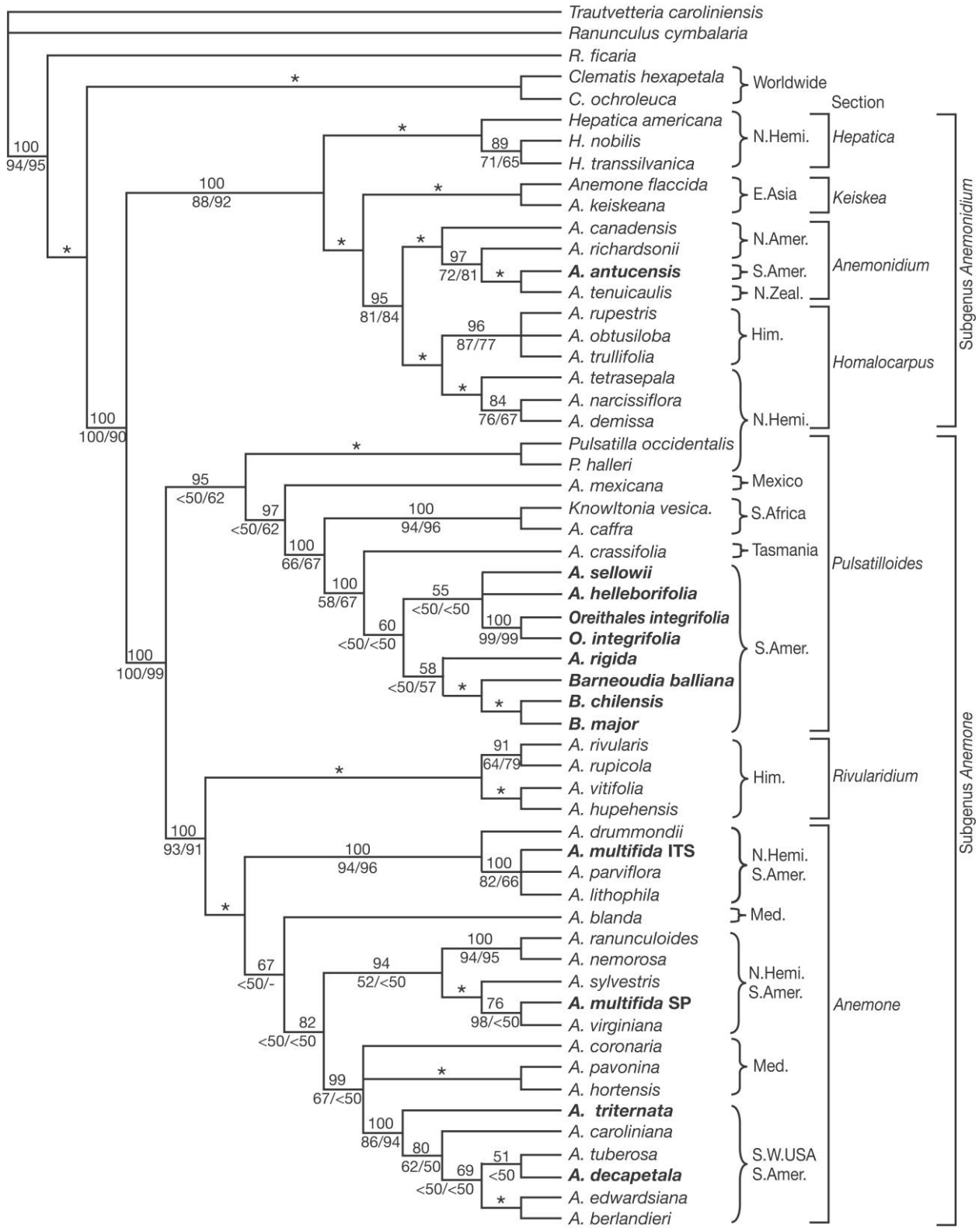

Fig. 1 Bayesian inference tree resulting from analyses of combined *atpB-rbcL* spacer region and ITS data. Posterior probabilities are above branches; maximum likelihood and maximum parsimony bootstrap values are below branches. Asterisk indicates that all support values = 100%. South American species are in bold. Geographic distributions, sectional, and subgeneric designations (as defined by Hoot et al. [1994]) are to the right of taxon names. N. Hemi. = Northern Hemisphere, N. Amer. = North America, S. Amer. = South America, N. Zeal. = New Zealand, S. Africa = South Africa, Him. = Himalayas, Med. = European Mediterranean region, S.W. USA = southwestern United States.

the taxonomic affinities of *Oreithales* and *Barneoudia*) on our ML tree (fig. 2), it is clear that their section *Rivularidium* is polyphyletic and that some of their sectional designations should be expanded (e.g., section *Alchimillifolia* should include *Knowltonia* species).

South American species are found in both subgenera *Anemonidium* and *Anemone* and in sections *Anemonidium*, *Pulsatilloides*, and *Anemone*. Only *A. antucensis* of southern Chile, Brazil, and Argentina occurs in subgenus *Anemonidium*, as sister to *A. tenuicaulis* (PP = 100, BS = 100). This result is consistent with previous work in the genus (e.g., Schuettpelz et al. 2002).

Most South American anemones included in this study and the segregate genera *Barneoudia* and *Oreithales* occur in section *Pulsatilloides* (fig. 1) with moderate to weak support (PP = 95, MLBS < 50, MPBS = 62). Within section *Pulsatilloides*, all the South American taxa are found in one clade (fig. 1). While support for this clade is weak, it is clear that *Barneoudia* and *Oreithales* are embedded within *Anemone*, and this should be reflected nomenclaturally; all species except *Barneoudia balliana* already have combinations within genus *Anemone* (table A1). Similar results were previously found for genera *Hepatica*, *Pulsatilla*, and *Knowltonia* (Hoot et al. 1994).

Hoot et al. (1994) had originally proposed four informal groups within *Pulsatilloides*: *Crassifolia* (*A. crassifolia*, *A. tenuicaulis*), *Caffra* (all African anemones + *Barneoudia* and *Oreithales*), *Knowltonia* (all *Knowltonia* species, *A. hepaticifolia*, *A. helleborifolia*, *A. moorei*, and other species), and *Pulsatilla* (all *Pulsatilla* species). Most of these groups are not supported by our current work. The *Crassifolia* group is polyphyletic; *A. tenuicaulis* (New Zealand) is closely aligned with *A. antucensis* (SA) within sect. *Anemonidium* (subgenus *Anemone*). The South African *Knowltonia* and *Caffra* groups need to be combined into one group based on our current work (fig. 1). All remaining taxa except for the *Pulsatilla* species and *A. mexicana* should be lumped into one taxon consisting mainly of SA species (*A. crassifolia*, *A. sellowi*, *A. helleborifolia*, and *A. rigida* and all *Barneoudia* and *Oreithales* species). Preliminary sequence data (not presented) also indicate that *A. moorei* Espinosa and *A. hepaticifolia* Hook.f. (both South American) should be included in this last clade as well. A formal taxonomy must await the addition of more molecular data, increased sampling, and higher branch support.

The remaining genera with distributions within South America—*A. multifida*, *A. triternata*, and *A. decapetala*—occupy derived positions within the phylogeny within section *Anemone* (figs. 1, 2). The differing positions found for the *atpB-rbcL* spacer and ITS sequences of *A. multifida* (figs. 1, 2) are most likely due to an ancestral hybridization event with subsequent polyploidization. Also supporting this hypothesis is the tetraploid status of *A. multifida* (2n=32; Boriah and Heimburger 1964), the presence of two sets of chromosomes distinguishable by size, and crossing experiments (Heimburger 1961). Previous work with nuclear ribosomal restriction site data (Hoot et al. 1994) had not detected hybrid origins for *A. multifida*, but the amount of variation within these data was limited. Our nuclear ITS data did not pick up strong indications of a hybrid origin either—there were two sites exhibiting double-banding that could be explained as evidence of hybridity, but there was no evidence of sequence misalignment due to the inheritance of differing gaps from the two parental species. However, the evidence for hybridization in the ribosomal data may have largely disappeared due to concerted evolution (Hamby and Zimmer 1992). Our results (fig. 1) indicate that *A. multifida* may be a cross between a member of the montane/alpine clade consisting of *A. drummondii*, *A. parviflora*, and *A. lithophila* and a member of the more temperate clade consisting of *A. sylvestris*, *A. virginiania*, *A. riparia*, and *A. cylindrica*, all North American species. If true, this helps to explain the diverse habitats (from sand dunes to montane and alpine environments) and geographical range (discontinuous range in North America and South America) occupied by *A. multifida*.

The tuberous anemones of the Americas, *A. berlandieri* of the southeastern United States and *A. decapetala*/*A. triternata* of South America, are regarded as very closely related or conspecific. Lourteig (1951) treated *A. triternata* as equivalent to *A. decapetala* var. *foliolosa*, while Ziman et al. (2006) elevated it to species rank. Our molecular data weakly support the species rank of *A. triternata* and its separation from *A. decapetala* (fig. 1).

Morphology

The inclusion of the alpine genera, *Oreithales* and *Barneoudia*, within *Anemone* and their placement within section Pulsatilloides suggest that the alpine species have evolved from lowland species (fig. 1). Similar results were recently shown for the segregate dioecious alpine genus *Hamadryas*, which is firmly embedded in a *Ranunculus* grade (Hoot et al. 2008). Like *Hamadryas*, *Oreithales*, and *Barneoudia* both have adaptations to montane and alpine habitats that make them appear morphologically unique, such as a low-growing acaulescent rosette habit. While both *Oreithales* and *Barneoudia* do have a few unique characteristics that set them apart from *Anemone* s.str., they also share numerous characters with other anemones (table 1).

Superimposing the pollen types described by Huynh (1970*a*, 1970*b*) and Meacham (1981) on the phylogeny, it appears that various pollen types have arisen numerous times within the genus (fig. 2). It had been hoped that pollen type might be a reliable character for assigning anemones to various clades within the genus, but this does not appear to be the case. Looking at just the South American taxa of sect. *Pulsatilloides*, three pollen types are found: tricolpate, eupantocolpate, and polyporate (fig. 2). The group composition and pollen characteristics suggested by Hoot et al. (1994) for this section need revision.

Biogeography

South American anemones are found in three sections on our tree: *Anemonidium*, *Pulsatilloides*, and *Anemone* (fig. 1). In each clade containing South American species, the direction of dispersal is most likely from the Northern Hemisphere to the Southern Hemisphere, suggesting a Northern Hemisphere origin for the family (fig. 1).

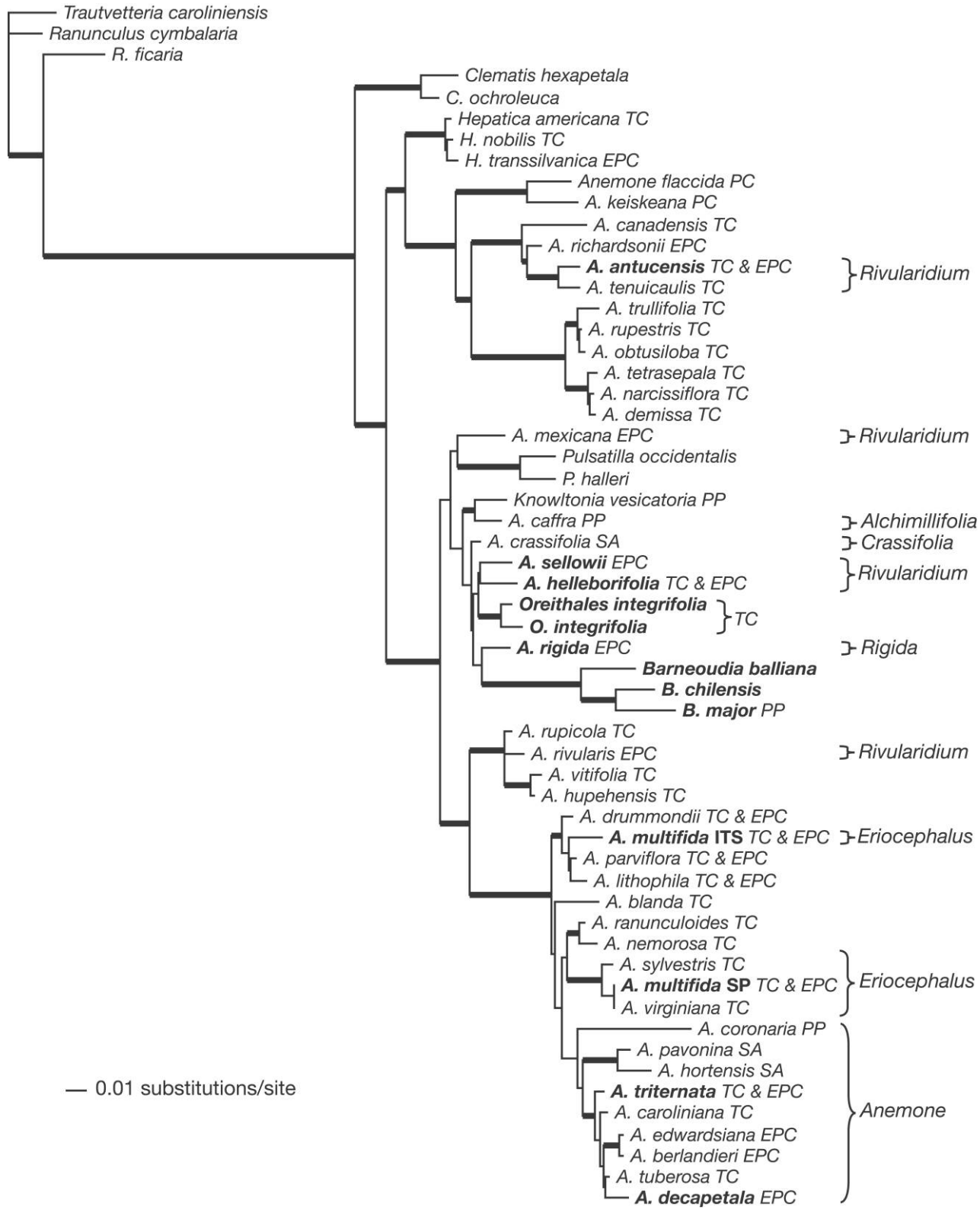

Fig. 2 Maximum likelihood phylogram resulting from analyses of combined *atpB-rbcL* spacer region and ITS data. Branches in bold had posterior probabilities ≥95 and bootstrap values ≥70 in figure 1. Acronyms after species names designate pollen types (as defined by Huynh [1970*a*, 1970*b*]: TC = tricolpate, EPC = eupantocolpate, PC = polycolpate, PP = pantoporate, SA = spiroaperturate. Sectional designations for South American species (in bold) are as in Ziman et al. (2006).

Two of the sections, *Anemonidium* and *Pulsatilloides*, include other Southern Hemisphere species, suggesting a Gondwanan distribution (Hoot et al. 1994; Schuettpelz et al. 2002). A similar pattern of Gondwanan distribution with North American origin is also found in *Caltha* (Ranunculaceae; Schuettpelz and Hoot 2004) and within a basal *Ranunculus* clade that includes the South American genus *Hamadryas* (Hoot et al. 2008). A vicariance explanation for the distribution of Southern Hemisphere anemones is supported by fruit morphology, which is not conducive to long-distance dispersal, especially in the South American species found in sections *Anemonidium* and *Pulsatilloides*.

If the vicariance model is true, the most likely time that *Anemone* and other Ranunculaceae taxa could move via short-distance dispersal from North America into the Southern Hemisphere is during the Late Cretaceous, when austral land masses (South America, Antarctica, Australia, and New Zealand) were still relatively contiguous. During this time, a Cretaceous Island Arc (Iturralde-Vinent and MacPhee 1999) is hypothesized to have moved from the Pacific into the Caribbean basin (Ross and Scotese 1988; Pindell and Barrett 1990; Pindell and Kennan 2001), possibly forming a temporary land bridge during dry periods (70–80 Myr).

However, recent molecular dating studies do not support a vicariance explanation. Molecular dating of the closely related *Ranunculus* group (Paun et al. 2005) and Ranunculales (Anderson et al. 2005) suggest a more recent origin for *Ranunculus* during the Eocene (42.0 Myr). Due to lack of *Ranunculus* fossils, the Paun et al. (2005) analysis was calibrated with an age interval of 51–55 Myr for the split of *Ranunculus* and *Xanthorhiza*, as determined in a previous angiosperm molecular dating study by Wikström et al. (2001) using penalized likelihood (PL; Sanderson 2002). As Paun et al. (2005) suggest, PL does not perform well in the absence of multiple calibration points, and therefore, their inferred dates may be inaccurate.

Subsequent dating of the basal eudicots using multiple calibration points determined by the fossil records (Anderson et al. 2005) found considerably older dates for Ranunculaceae: stem group ages of 90 Myr using PL and 104 Myr based on nonparametric rate smoothing (Sanderson 2003); crown group ages were 73 and 87 Myr, respectively. While these crown group ages are within the time frame (~80–95 Myr) hypothesized above for a vicariance explanation, the crown group age of the genera most closely related to *Anemone* (*Clematis*, *Ranunculus*, and *Myosaurus*) is estimated at 47 Myr, based on PL (C. L. Anderson, personal communication). So the role of long-distance dispersal between continents, as is now hypothesized to be the case in many austral plant groups (e.g., Sanmartin and Ronquist 2004), must be considered for Southern Hemisphere anemones. In particular, the split between *A. antucensis* (South America) and *A. tenuicaulis* may be the result of dispersal, given that New Zealand was severely peneplained and largely submerged during the Oligocene (Cooper and Millener 1993; Trewick and Morgan-Richards 2005)

Unfortunately, we are unable to test the vicariance hypothesis for *Anemone* with our own molecular dating because there are no reliable fossils within *Anemone* to use as calibration points. The nearest reliable fossils for dating, such as *Teixeiraea* and *Prototinomiscium*, have affinities with Ranunculales or families within Ranunculales (von Balthazar et al. 2005). To use these fossils in conjunction with *Anemone* for molecular dating, we would need to analyze all of Ranunculales using more conserved sequence data than the ITS and the *atpB-rbcL* spacer region employed for this study. This work is in progress and will be the topic of a future paper.

The two cases of disjunction between North America and South America, *A. multifida* and the *A. decapetala/berlandieri* complex, are most likely more recent in timing. This is based on the similarity of both morphological and molecular data within each of these groups. In previous work (Hoot et al. 1994), *A. multifida* from Argentina had identical restriction sites with four North American representatives of this species. In this work, Jukes-Cantor distances for the *A. decapetala/berlandieri* complex vary between 0.01–0.02 (compared to the *Pulsatilloides* clade, where interspecific distances range between 0.03–1.14). To explain such disjunctions, Raven and Axelrod (1974) proposed a pathway along mountain ranges between North America and South America during the cooler Pleistocene. In the case of these *Anemone* disjunctions, movement was probably from North America to South America (fig. 1). It is interesting to note that both of these species have the tomentose achenes characteristic of some members of sections *Anemone* and *Rivularidium*, facilitating longer-distance dispersal (although these achenes are not easily airborne and probably do not often travel great distances).

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