

## Phylogeny and Reclassification of *Anemone* (Ranunculaceae), with an Emphasis on Austral Species

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**Abstract**—Previous phylogenies based on molecular data indicated that segregate genera from both the Northern and Southern Hemispheres (*Hepatica*, *Pulsatilla*, *Knowltonia*, *Oreithales*, and *Barneoudia*) are embedded within *Anemone* and should be subsumed within the genus. Based on a new phylogeny that substantially increases the sampling of the austral anemones (especially from Africa), we present a formal reclassification of *Anemone* s. l. We analyzed combined sequence data (chloroplast *atpB-rbcL* spacer and nuclear ITS regions) for 55 species of *Anemone* s. l., using Bayesian inference, maximum likelihood, and maximum parsimony. The segregate genera, *Oreithales* and *Barneoudia*, nest within *Anemone* and are included in a well supported clade (subgenus *Anemone*, section *Pulsatilloides*) consisting largely of Southern Hemisphere species. The Mexican *A. mexicana* is sister to all remaining members of section *Pulsatilloides*, which consists of two clades: a poorly supported South American and Tasmanian clade (*A. sellowii*, *A. helleborifolia*, *A. rigida*, *Barneoudia* and *Oreithales* species, and *A. crassifolia*) and a highly supported southern African clade including nine species of *Knowltonia* and eight species of *Anemone*. *Anemone antucensis* (Chile, Argentina) falls in a separate clade (subgenus and section *Anemonidium*) that is sister to *A. tenuicaulis* (New Zealand). *Anemone thomsonii* (eastern Africa) and *A. somaliensis* (Somalia) are in a clade (subgenus and section *Anemone*) composed largely of Northern Hemisphere species. *Anemone somaliensis* is further associated with other Mediterranean tuberous anemones in subsection and series *Anemone* (*A. coronaria*, *A. hortensis*, and *A. pavonina*). The topology of both sections *Pulsatilloides* and *Anemonidium* suggest that anemones originated in the Northern Hemisphere and subsequently spread to the Southern Hemisphere, a pattern that is shared with other members of Ranunculaceae. We present a formal reclassification of *Anemone* s. l., including the following new combinations and taxa of *Anemone* subgenus *Anemone*: subsections *Alchemillifoliae*, *Anemonanthea*, *Barneoudia*, *Crassifoliae*, *Helleborifoliae*, *Kilimansharicae*, *Mexicanae*, *Oreithales*, *Rigidiae*, and *Sellowii*; series *Carolinianae* of subsection *Anemone*; plus the new combination *Anemone balliana* (= *Barneoudia balliana*).

**Keywords**—*atpB-rbcL* spacer, *Barneoudia*, *Hepatica*, ITS, *Knowltonia*, *Oreithales*, *Pulsatilla*.

*Anemone* L. s. l. consists of approximately 200 species distributed throughout the world, but with the greatest diversity in the Northern Hemisphere. Molecular work indicates that several segregate genera are embedded within *Anemone* and therefore should be subsumed within the genus (Hoot et al. 1994; Ehrendorfer and Samuel 2001; Schuettpelez et al. 2002; Meyer et al. 2010). These include *Hepatica* (Northern Hemisphere), *Pulsatilla* (Northern Hemisphere), *Knowltonia* (South Africa), *Barneoudia* (Chile and Argentina), and *Oreithales* (Bolivia and Peru).

Species of *Anemone* are found in diverse habitats, including arctic and alpine regions, prairies, temperate woodlands, Mediterranean regions, and semideserts. Approximately 30 species of *Anemone* s. l. are found in more austral regions, including Africa, South America, New Zealand, and Tasmania. These more southerly species are usually found in montane to alpine regions, where the climate is cooler. Most species of African *Anemone* s. l. are found in grasslands or temperate forests and shrublands (fynbos), mainly in southern Africa (11 species). In addition, *A. thomsonii* occurs in eastern Africa, especially on the slopes of Mt. Kilimanjaro (alt. 2,500–4,500 m), *A. somaliensis* is endemic to the Al Hills region of Somalia (alt. 900–1,200 m), and the tuberous members of subsection *Anemone* (as defined by Ehrendorfer et al. 2009) have a Mediterranean distribution, including northern Africa. Other austral anemones are found in South America (including the segregate genera *Barneoudia* and *Oreithales*), Tasmania, and New Zealand (Schuettpelez et al. 2002; Meyer et al. 2010).

*Anemone* s. l. is characterized by rosettes of basal leaves with a variety of perennating structures, inflorescences with involucre leaves on the peduncle, a perianth composed of petaloid sepals of variable number, and achenes. Most species are diploid, with base chromosome numbers  $x = 7$  or  $x = 8$ . There is much variation in morphological characters and homoplasy is higher than with molecular characters (Hoot et al. 1994). Many current *Anemone* classifications are based mainly on morphology, especially fruit characters (e.g., Ulbrich 1905–06; Tamura 1995; Ziman et al. 2008).

Based on cpDNA restriction site data and morphology, Hoot et al. (1994) derived a phylogeny of *Anemone* s. l. that included 36 species, three segregate genera (*Hepatica*, *Pulsatilla*, and *Knowltonia*), and three African species. This work resulted in a preliminary classification that recognized the single genus *Anemone*, divided into two subgenera (*Anemonidium* and *Anemone*), seven sections, and 12 informal subsectional groupings. Base chromosome number was highly correlated with the two subgenera, with subgenus *Anemonidium* characterized by  $x = 7$  and subgenus *Anemone* by  $x = 8$ . Most Southern Hemisphere species formed a well-supported monophyletic group within subgenus *Anemone*, consisting of *A. crassifolia* (Tasmania), *A. caffra* (South Africa), and *Knowltonia capensis* (= *A. knowltonia*, South Africa).

In more recent studies (Ehrendorfer and Samuel 2001; Schuettpelez et al. 2002), the sectional affinities of two Southern Hemisphere anemones, *A. antucensis* (Andean Chile) and *A. tenuicaulis* (New Zealand), were tested using plastid and nuclear DNA sequence data. Ziman et al. (2006) published

a revision of the Southern Hemisphere species of *Anemone* (excluding *Knowltonia*, *Barneoudia*, and *Oreithales*) based on morphological data, recognizing 21 species and nine sections. Ehrendorfer et al. (2009) produced a taxonomic revision of the tuberous species of section *Anemone*, based on morphology and molecular data, including one African endemic. Meyer et al. (2010) recently addressed the phylogenetic affinities of many South American anemones using both chloroplast and nuclear sequence data. In Meyer et al., a weakly to moderately supported clade (section *Pulsatilloides*) was recovered, including seven South American species (including the segregate genera, *Oreithales* and *Barneoudia*) and three species from Africa and Tasmania. Based on molecular data (Hoot et al. 1994; Schuettpelz et al. 2002), *Knowltonia* has recently been formally subsumed within *Anemone* (Manning et al. 2009).

While several formal reclassifications of *Anemone* s. l. were recently published (Table 1; Tamura 1995; Ziman et al. 2008), these classifications were not phylogeny based, did not take into consideration the results of molecular work (e.g. Hoot et al. 1994; Schuettpelz et al. 2002; Meyer et al. 2010), and did not treat the segregate genera (*Hepatica*, *Pulsatilla*, *Knowltonia*, *Barneoudia*, and *Oreithales*) within *Anemone*. These papers do provide a good recently published history of classification within *Anemone* s. s. (Tamura 1995; Ziman et al. 2006, 2008), information that is not repeated here.

In this paper, we present a phylogeny-based classification for a broad sampling of anemones (including the segregate genera), with an emphasis on austral species. The principal objectives of this study are: 1) to further test the affinities of the austral *Anemone* species by increasing the sampling of African anemones (including *Knowltonia*), using molecular data (chloroplast *atpB-rbcL* spacer region and nuclear internal transcribed spacer), 2) to employ multiple analytic methods (Bayesian inference, maximum likelihood, and maximum parsimony) to test phylogenetic placements, and 3) to provide a reclassification of *Anemone* s. l. that is phylogeny driven.

## MATERIALS AND METHODS

**Taxon Sampling**—The sampling of taxa within *Anemone* s. l. used in this study follows the informal taxonomy presented by Hoot et al. (1994) of subgenera, sections, and species groups. For easy identification of segregate 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*, and *Pulsatilla*) in our Fig. 1.

This work represents increased sampling over any previous work. Our sampling includes 55 species, including all segregate genera, 21 species with austral distributions, 10 African endemics, and four Mediterranean species with distributions in northern Africa (Appendix 1). The largest sampling to date of endemic African anemones in terms of molecular studies included just three species (Hoot et al. 1994). Based on previous molecular work on the Ranunculaceae (Hoot 1995; Johansson 1995; Meyer et al. 2010), two species of *Clematis* were included as outgroups.

**DNA Sequencing and Analyses**—DNA extraction, amplification, and sequencing followed the protocols described in Schuettpelz et al. (2002) with the following exceptions: automated DNA sequencing was performed on either an ABI Model 373A-Stretch (Applied Biosystems, Foster City, California) or a CEQ 2000XL capillary sequencer (Beckman Coulter, Fullerton, California), according to the respective manufacturers' protocols.

Alignment of DNA sequences was accomplished to a rough approximation using Sequencher 3.0 (Gene Codes Corp., Ann Arbor, Michigan) 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). Insertions/deletions (indels) were scored as binary characters

using MacClade (Maddison and Maddison 2005), 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, two methods of assessing congruence were implemented: visual comparison of the various clades found in the minimal trees including 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 test was conducted on the final pruned and edited data sets 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 5,000 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), using the models for each individual data set as identified by MrModeltest 2.2 (Nylander 2004) and flat priors. Indels were treated using the binary (restriction site) model (MrBayes command "lset coding = variable") as recommended in the online manual (MrBayes.scs.fsu.edu/wiki/index.php/Manual\_3.2). 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 excluded as the burn-in phase, 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 (TreeBASE study number TB2:S10875).

A maximum likelihood (ML) search was conducted in GARLI vers. 0.95 (Zwickl 2006). The analysis was conducted on the combined *atpB-rbcL* spacer and ITS regions excluding scored indels, and using the default parameters with "save every improved topology" unchecked. The ML bootstrap values were not presented since indels cannot be included, leading to an underestimation of branch support.

Maximum parsimony (MP) analyses of the *atpB-rbcL* spacer and ITS data (including scored indels) were conducted for each marker independently (results not shown) and for the combined data set using PAUP\* version 4.0b2 (Swofford 2002), heuristic search option with 20 random addition sequences, TBR branch swapping, and saving up to 5,000 trees each replicate. To estimate the confidence to be placed in the topology, bootstrap values (BS; 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 gaps; whenever alignment was not ambiguous and gaps 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. We were unable to sequence the *atpB-rbcL* spacer for *K. cordata* and only half this region for *A. sellowii*; we sequenced only half the ITS region for *A. somalensis*.

The topologies resulting from the two individual data sets largely agreed with each other, especially where BS values were high (individual trees not presented). The partition homogeneity test (Farris et al. 1995) resulted in a *p* value of 0.94, indicating considerable congruence between the two data sets. Therefore, the data were combined. It is interesting to note that while many more MP trees were recovered from the *atpB-rbcL* spacer than the ITS data (> 10,000 as opposed to six), CI and RI values were markedly higher with the *atpB-rbcL* spacer data (Table 2).

**Combined Data Analyses**—The MP analyses of the combined ITS and *atpB-rbcL* spacer data resulted in six most

TABLE 1. Comparison of recent classifications of *Anemone* s. s. by Tamura (1995) and Ziman et al. (2008). In some cases, Tamura and Ziman et al. included more species in a taxon than are listed below; the number missing is indicated by + numeral. Infrageneric taxon names are given in the form used by Tamura and Ziman et al.

Tamura (1995)	Ziman et al. (2008)
<b>Subgen. Rivularidium</b>	<b>Subgen. Rivularidium</b>
<b>Sect. Rivularidium</b>	<b>Sect. Rivularidium</b>
<b>Ser. Rivulares</b> ( <i>A. angustiloba</i> , <i>A. hemsleya</i> , <i>A. mexicana</i> , <i>A. moorei</i> , <i>A. rivularis</i> , <i>A. sellowii</i> +3)	<b>Ser. Rivularidium</b> ( <i>A. filisecta</i> , <i>A. orthocarpa</i> , <i>A. rivularis</i> )
<b>Ser. Helleborifoliae</b> ( <i>A. antucensis</i> , <i>A. helleborifolia</i> , <i>A. jamesonii</i> , <i>A. peruviana</i> )	<b>Ser. Angustilobae</b> ( <i>A. angustiloba</i> , <i>A. sumatrana</i> )
	<b>Ser. Mexicanae</b> ( <i>A. helleborifolia</i> , <i>A. mexicana</i> )
	<b>Ser. Jamesonii</b> ( <i>A. antucensis</i> , <i>A. assisbrasiliensis</i> , <i>A. jamesonii</i> , <i>A. moorei</i> , <i>A. sellowii</i> , <i>A. tenuicaulis</i> +1)
	<b>Sect. Begoniifolia</b> ( <i>A. begoniifolia</i> , <i>A. hokouensis</i> , <i>A. chapaensis</i> , <i>A. howellii</i> )
<b>Sect. Crassifolia</b> ( <i>A. crassifolia</i> , <i>A. tenuicaulis</i> )	<b>Subgen. Crassifolia</b>
	<b>Sect. Crassifolia</b> ( <i>A. crassifolia</i> )
<b>Sect. Richardsonia</b> ( <i>A. richardsonii</i> )	<b>Subgen. Richardsonia</b>
<b>Subgen. Hepaticifolia</b> ( <i>A. hepaticifolia</i> )	<b>Sect. Richardsonia</b> ( <i>A. richardsonii</i> )
	<b>Subgen. Hepaticifolia</b>
	<b>Sect. Hepaticifolia</b> ( <i>A. hepaticifolia</i> )
<b>Subgen. Rigida</b> ( <i>A. rigida</i> )	<b>Subgen. Rigida</b>
	<b>Sect. Rigida</b> ( <i>A. rigida</i> )
<b>Subgen. Anemonidium</b> ( <i>A. dichotoma</i> , <i>A. canadensis</i> )	<b>Subgen. Anemonidium</b>
	<b>Sect. Anemonidium</b> ( <i>A. canadensis</i> , <i>A. dichotoma</i> )
<b>Subgen. Omalocarpus</b>	<b>Subgen. Omalocarpus</b>
<b>Sect. Begoniifolia</b> ( <i>A. begoniifolia</i> , <i>A. howellii</i> , <i>A. sumatrana</i> +2)	<b>Sect. Himalayicae</b>
<b>Sect. Himalayica</b> ( <i>A. obtusiloba</i> , <i>A. rupestris</i> , <i>A. trullifolia</i> +9)	<b>Ser. Rupestris</b> ( <i>A. rupestris</i> , <i>A. polycarpa</i> )
	<b>Ser. Obtusilobae</b> ( <i>A. obtusiloba</i> , <i>A. subpinnata</i> , <i>A. patula</i> , <i>A. rockii</i> , <i>A. geum</i> )
	<b>Ser. Trullifoliae</b> ( <i>A. coelestina</i> , <i>A. subindivisa</i> , <i>A. trullifolia</i> , <i>A. yulongshanica</i> )
<b>Sect. Omalocarpus</b> ( <i>A. demissa</i> , <i>A. narcissiflora</i> , <i>A. polyanthes</i> , <i>A. sikokiana</i> , <i>A. smithiana</i> , <i>A. speciosa</i> +9)	<b>Sect. Omalocarpus</b>
	<b>Ser. Involucratae</b> ( <i>A. demissa</i> , <i>A. narcissiflora</i> , <i>A. robusta</i> , <i>A. smithiana</i> +2)
	<b>Ser. Involucellatae</b> ( <i>A. elongata</i> , <i>A. polyanthes</i> , <i>A. tetrasepala</i> )
	<b>Ser. Fuscopurpurea</b> ( <i>A. fuscopurpurea</i> )
	<b>Sect. Imbricata</b> ( <i>A. imbricata</i> )
<b>Subgen. Anemonanthea</b>	<b>Subgen. Anemonanthea</b>
<b>Sect. Anemonanthea</b>	<b>Sect. Anemonanthea</b>
<b>Ser. Hylaelectryon</b> ( <i>A. altaica</i> , <i>A. davidii</i> , <i>A. debilis</i> , <i>A. deltoidea</i> , <i>A. griffithii</i> , <i>A. nemorosa</i> , <i>A. quinquefolia</i> , <i>A. ranunculoides</i> , <i>A. pseudoaltaica</i> , <i>A. stolonifera</i> +3)	<b>Ser. Anemonanthea</b> ( <i>A. amurensis</i> , <i>A. caerulea</i> , <i>A. debilis</i> , <i>A. nemorosa</i> , <i>A. ranunculoides</i> , <i>A. soyensis</i> , <i>A. trifolia</i> , <i>A. udensis</i> , <i>A. umbrosa</i> , <i>A. uralensis</i> )
<b>Ser. Reflexae</b> ( <i>A. reflexa</i> )	<b>Ser. Reflexae</b> ( <i>A. reflexa</i> )
	<b>Ser. Nikoenses</b> ( <i>A. nikoensis</i> )
	<b>Ser. Quinquefolia</b> ( <i>A. greyi</i> , <i>A. lancifolia</i> , <i>A. oregana</i> , <i>A. piperi</i> , <i>A. quinquefolia</i> ,)
	<b>Ser. Altaica</b> ( <i>A. altaica</i> , <i>A. pseudoaltaica</i> , <i>A. raddeana</i> )
<b>Sect. Tuberosa</b> ( <i>A. apennina</i> , <i>A. blanda</i> , <i>A. caucasica</i> )	<b>Sect. Rosulantes</b>
	<b>Ser. Rosulantes</b> ( <i>A. davidii</i> , <i>A. stolonifera</i> )
	<b>Ser. Exiguae</b> ( <i>A. exigua</i> , <i>A. griffithii</i> +1)
	<b>Sect. Tuberosa</b>
	<b>Ser. Tuberosae</b> ( <i>A. apennina</i> , <i>A. blanda</i> , <i>A. caucasica</i> )
	<b>Ser. Caucasicae</b> ( <i>A. caucasica</i> )
<b>Sect. Keiskea</b> ( <i>A. keiskeana</i> )	<b>Subgen. Keiskea</b>
	<b>Sect. Keiskea</b>
	<b>Ser. Keiskea</b> ( <i>A. keiskeana</i> )
	<b>Ser. Deltoidea</b> ( <i>A. deltoidea</i> )
<b>Sect. Stolonifera</b> ( <i>A. baicalensis</i> , <i>A. delavayi</i> , <i>A. falconeri</i> , <i>A. flaccida</i> +6)	<b>Subgen. Stolonifera</b>
	<b>Sect. Stolonifera</b>
	<b>Ser. Stolonifera = Baicalenses</b> ( <i>A. baicalensis</i> , <i>A. prattii</i> )
	<b>Ser. Flaccidae</b> ( <i>A. delavayi</i> , <i>A. flaccida</i> )
<b>Subgen. Anemoclema</b> ( <i>A. glaucifolia</i> )	<b>Subgen. Anemoclema</b>
	<b>Sect. Anemoclema</b> ( <i>A. glaucifolia</i> )
<b>Subgen. Pulsatilloides</b>	<b>Subgen. Pulsatilloides</b>
<b>Sect. Pulsatilloides</b> ( <i>A. capensis</i> )	<b>Sect. Pulsatilloides</b> ( <i>A. capensis</i> )
<b>Sect. Alchimillifolia</b> ( <i>A. caffra</i> , <i>A. fanninii</i> )	<b>Sect. Alchimillifolia</b> ( <i>A. caffra</i> , <i>A. fanninii</i> )
	<b>Subgen. Kilimandscharica</b>
<b>Sect. Kilimandscharica</b> ( <i>A. thomsonii</i> )	<b>Sect. Kilimandscharica</b> ( <i>A. thomsonii</i> )
<b>Subgen. Anemone</b>	<b>Subgen. Eriocapitella</b>
<b>Sect. Eriocapitella</b> ( <i>A. hupehensis</i> , <i>A. tomentosa</i> , <i>A. vitifolia</i> )	<b>Sect. Eriocapitella</b> ( <i>A. hupehensis</i> , <i>A. tomentosa</i> , <i>A. vitifolia</i> )
<b>Sect. Eriocephalus</b>	<b>Subgen. Eriocephalus</b>
<b>Subsect. Brevistylae</b>	<b>Sect. Eriocephalus</b>
<b>Ser. Rupicolae</b> ( <i>A. laceratoincisa</i> , <i>A. ochotensis</i> , <i>A. rupicola</i> , <i>A. sylvestris</i> )	<b>Ser. Rupicolae</b> ( <i>A. rupicola</i> , <i>A. laceratoincisa</i> , <i>A. tibetica</i> )

(Continued)



TABLE 1. Continued

Tamura (1995)	Ziman et al. (2008)
<b>Ser. Virginianae</b> ( <i>A. cylindrica</i> , <i>A. globosa</i> , <i>A. hudsoniana</i> , <i>A. multifida</i> , <i>A. riparia</i> , <i>A. virginiana</i> )	<b>Ser. Virginianae</b> ( <i>A. virginiana</i> , <i>A. cylindrica</i> )
<b>Subsect. Longistylae</b> ( <i>A. baldensis</i> , <i>A. drummondii</i> , <i>A. lithophila</i> , <i>A. pavoniana</i> , <i>A. tetonensis</i> )	<b>Ser. Sylvestres</b> ( <i>A. ochotensis</i> , <i>A. sylvestris</i> ) <b>Ser. Multifidae</b> ( <i>A. baldensis</i> , <i>A. drummondii</i> , <i>A. multiceps</i> , <i>A. multifida</i> , <i>A. pavoniana</i> )
<b>Subsect. Parviflorae</b> ( <i>A. parviflora</i> )	<b>Sect. Parviflora</b> ( <i>A. parviflora</i> )
<b>Sect. Anemone</b>	<b>Subgen. Anemone</b>
<b>Subsect. Anemone</b> ( <i>A. biflora</i> , <i>A. coronaria</i> , <i>A. gotschakovii</i> , <i>A. hortensis</i> , <i>A. palmata</i> +10)	<b>Sect. Anemone</b>
<b>Subsect. Carolinianae</b> ( <i>A. caroliniana</i> , <i>A. decapetala</i> , <i>A. sphenophylla</i> , <i>A. tuberosa</i> )	<b>Subsect. Anemone</b> ( <i>A. coronaria</i> , <i>A. hortensis</i> , <i>A. palmata</i> , <i>A. somaliensis</i> )
	<b>Subsect. Carolinianae</b> ( <i>A. berlandieri</i> , <i>A. caroliniana</i> , <i>A. edwardsiana</i> , <i>A. decapetala</i> , <i>A. okemnonii</i> , <i>A. triternata</i> , <i>A. tuberosa</i> )
	<b>Subsect. Biflorae</b> ( <i>A. biflora</i> , <i>A. bucharica</i> , <i>A. baissunensis</i> +2)
	<b>Subsect. Somaliense</b> ( <i>A. somaliensis</i> )

parsimonious trees with greater resolution and branch support than resulted from analyses of the individual data sets. The BI, ML, and MP analyses produced largely congruent trees; ML and MP trees were less resolved than the BI tree.

In the BI phylogeny resulting from the combined data (Fig. 1), the monophyly of *Anemone* s. l. is highly supported (PP, BS = 100), but some of the subgenera and sections recognized by Tamura (1995) and Ziman et al. (2008) are polyphyletic. Tamura's subgenus *Anemone*, *Rivularidium*, and *Anemonanthea* are polyphyletic (Fig. 1); Ziman et al.'s (2008) sections *Rivularidium*, *Erioccephalus*, and *Alchimillifolia* (= *Alchemillifoliae*) are polyphyletic. Comparisons of the classifications of Tamura and Ziman et al. (Table 1) with our BI tree indicate that polyphyly is evident at all taxonomic levels (information that could not be conveyed in Fig. 1 due to space limitations). In addition, some taxonomic groups of Tamura (1995) and Ziman et al. (2008) are paraphyletic (e.g. subgenus/section *Anemonidium*, sections *Pulsatilloides*, *Alchimillifolia*, and *Parviflora*; Fig. 1).

In contrast, subgenera *Anemone* and *Anemonidium* (PP = 100, BS ≥ 99) as recognized by previous workers (Hoot et al. 1994; Schuettelpelz et al. 2002; Meyer et al. 2010) are monophyletic when superimposed on our BI or ML trees (Fig. 2). Subgenus *Anemonidium* consists of four strongly supported clades (PP, BS = 100): sections *Hepatica*, *Keiskea*, *Anemonidium*, and *Omalocarpus* (= *Homalocarpus*) as originally defined in Hoot et al. (1994). The sister species, *A. antucensis* of South America and *A. tenuicaulis* of New Zealand (PP, BS = 100), are placed within section *Anemonidium* along with the North American species, *A. canadensis* and *A. richardsonii* (Fig. 2).

Within subgenus *Anemone* (Fig. 2), four sections are well supported: *Pulsatilloides* (PP = 100, BS = 71), *Pulsatilla* (PP, BS = 100), *Rivularidium* (PP, BS = 100), and *Anemone* (PP, BS = 100). Section *Pulsatilloides* includes the southern African anemones (*A. caffra*, *A. tenuifolia*, and *A. fanninii*), the southern African genus *Knowltonia*, the South American anemones (*A. sellowii*, *A. helleborifolia*, and *A. rigida*) and the segregate South American genera, *Oreithales* and *Barneoudia*. Within *Pulsatilloides*, the South African species are a well supported, monophyletic group (PP = 100, BS = 95), comprised of two well-supported sister clades, series *Knowltonia* and *Alchemillifolia* (Figs. 1, 2).

*Anemone thomsonii* from northeastern Africa is strongly supported (PP, BS = 100) as a member of subgenus *Anemone*, section *Anemone* (Fig. 1). However, this clade is poorly

resolved along its backbone, so its exact affinities within section *Anemone* are uncertain.

*Anemone somaliensis*, a tuberous species from Somalia, is well supported (PP = 100, BS = 82) as part of a clade consisting of tuberous species from the Mediterranean region (series *Anemone*, Fig. 2). All other species included in this clade (*A. coronaria*, *A. hortensis*, and *A. pavonina*) have broad distributions throughout the Mediterranean region, including northern Africa (Fig. 1).

#### DISCUSSION

##### *Phylogenetic Relationships and Past Classifications*—

Our molecular work strongly supports (PP, BS = 100) a monophyletic *Anemone* s. l., with *Hepatica* sister to all remaining species in subgenus *Anemonidium* (PP = 100; BS = 99). This result agrees with past work in the genus using diverse outgroups (Hoot et al. 1994; Schuettelpelz et al. 2002; Ehrendorfer et al. 2009; Meyer et al. 2010). In Lehnebach et al. (2007) and Wang et al. (2009), *Anemone* is not monophyletic and *Hepatica* is paraphyletic to clades consisting variously of *Anemone*, *Clematis*, *Knowltonia*, and *Pulsatilla* species. However, both of these works were not focused on *Anemone*, included small samplings of *Anemone* s. l. (four taxa each), and used conserved gene regions that were less likely to resolve relationships at the generic level.

The current work substantially improves the sampling of austral *Anemone* s. l. species (especially African anemones) compared with past molecular phylogenies (Hoot et al. 1994; Ehrendorfer and Samuel 2001; Schuettelpelz et al. 2002; Ehrendorfer et al. 2009; Meyer et al. 2010). Our molecular data do not support recent classifications (Tamura 1995; Ziman et al. 2008; Table 1) of the family based largely on morphology (Fig. 1). This incongruence is not dependent on taxonomic level. For example, a comparison of our results with the subgenera (rather than sections) proposed by Ziman et al. (2008) still reveals lack of monophyly in three subgenera (*Rivularis*, *Erioccephalus*, and *Anemonanthea*) plus a lack of taxonomic recognition of numerous clades that are well supported in our analyses. Significantly, the above classifications do not include the segregate genera *Hepatica*, *Knowltonia*, *Barneoudia*, *Oreithales*, and *Pulsatilla* within genus *Anemone* nor do they recognize many of the well supported clades (PP ≥ 95, BS ≥ 70) found with molecular data (e.g. Hoot et al. 1994; Meyer et al. 2010).

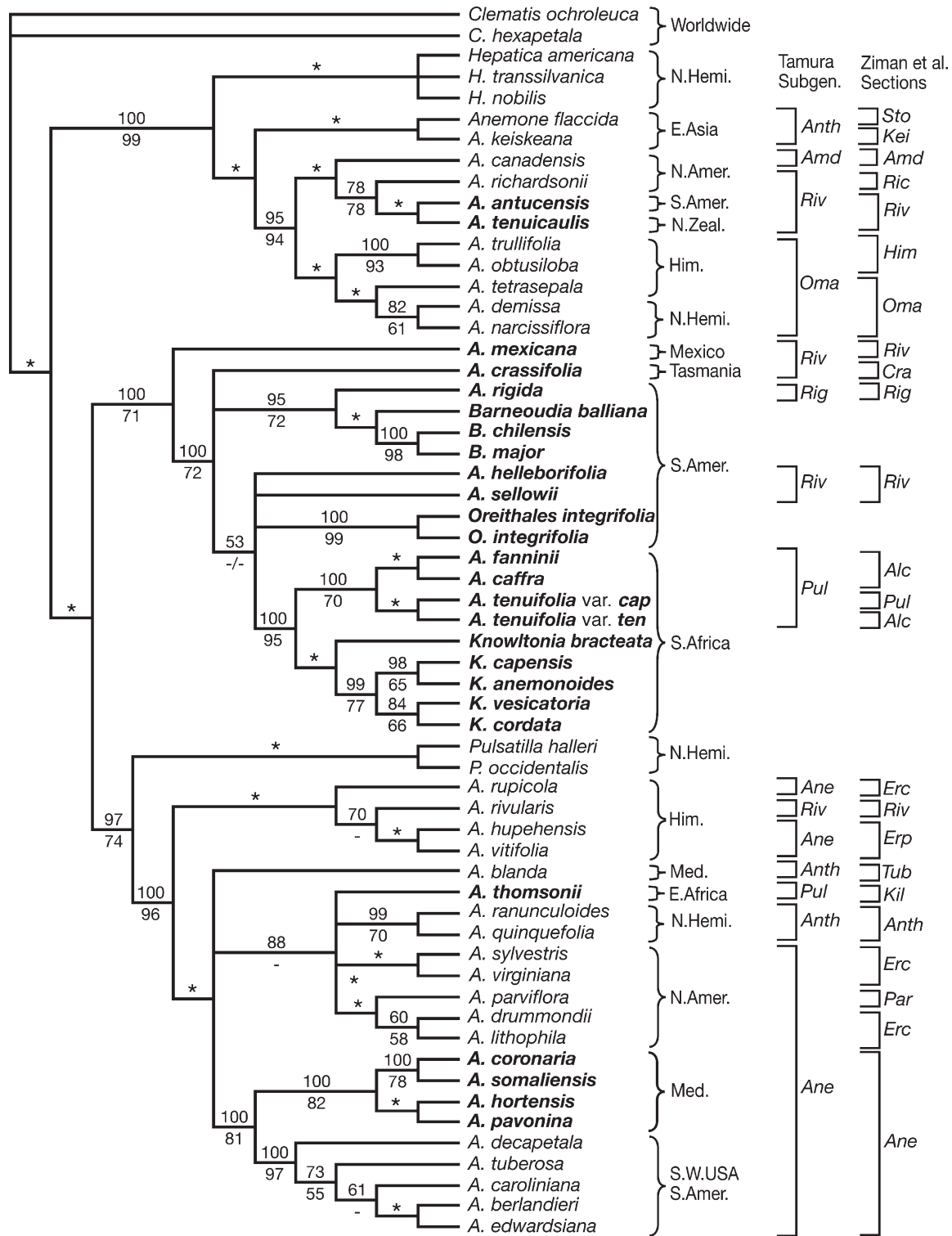


FIG. 1. Bayesian inference tree resulting from analyses of combined *atpB-rbcL* spacer region and ITS data. Posterior probabilities are above branches; maximum parsimony bootstrap values are below branches. An asterisk indicates that both support values = 100%. Austral species are in bold. Geographic distributions and subgeneric/sectional designations of Tamura (1995) and Ziman et al. (2008) are to the right of taxon names. Geographic distribution acronyms: 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. U. S. A. = southwestern U. S. A. Subgeneric and sectional acronyms: Alc = *Alchemillifoliae*, Ane = *Anemone*, Anth = *Anemonanthea*, Amd = *Anemonidium*, Cra = *Crassifoliae*, Erc = *Eriocapitella*, Erp = *Eriocapitella*, Him = *Himalayicae*, Kei = *Keiskea*, Kil = *Kilimandscharicae*, Oma = *Omalocarpus*, Par = *Parviflorae*, Pul = *Pulsatilloides*, Ric = *Richardsonia*, Riv = *Rivularidium*, Tub = *Tuberosae*. Variety acronyms: *cap* = *capensis*, *ten* = *tenuifolia*.

TABLE 2. Tree statistics for analyses of *Anemone* s. l. for separate and combined *atpB-rbcL* spacer and ITS data. The last three rows apply to MP analyses only.

	<i>atpB - rbcL</i> spacer	ITS	Combined spacer/ITS
Alignment length	1,260	651	—
Number of bases pruned due to ambiguous alignment	181	38	—
Number of bases pruned due to missing data	305	95	—
Alignment length after pruning	774	548	1,322
Missing data %	4.39	1.67	
Variable characters (including gaps)	269	320	589
Informative characters (including gaps)	182	250	432
Number of gaps scored	54	19	73
Number of trees	> 10,000	95	6
CI (excluding uninformative characters)	0.75	0.49	0.55
RI	0.93	0.81	0.84

Our phylogeny (Fig. 2) is largely congruent with past molecular work on *Anemone* (Hoot et al. 1994; Ehrendorfer and Samuel 2001; Schuettpelez et al. 2002; Meyer et al. 2010). The most important difference concerns the placement of *Pulsatilla*: in the present work, *Pulsatilla* species are sister to sections *Rivularidium* and *Anemone* (Figs. 1, 2; PP = 97; BS = 74) instead of being included within section *Pulsatilloides*, as was weakly to moderately supported in Hoot et al. (1994) and Meyer et al. (2010). Due to this differential placement of *Pulsatilla*, *Pulsatilla* is recognized here as a new section within subgenus *Anemone* with no further affinities specified (Fig. 2). The phylogenetic affinities of this new section need to be tested with increased sampling of taxa in both genus *Pulsatilla* and section *Anemone*.

The present work confirms the previous hypothesis of Hoot et al. (1994) based on restriction site and morphological data that *Hepatica*, *Knowltonia*, and *Pulsatilla* should be subsumed within *Anemone*. As in Meyer et al. (2010), the segregate genera from South America, *Barneoudia* and *Oreithales*, are embedded within subgenus *Anemone* (section *Pulsatilloides*; Fig. 2) and should also be subsumed.

This work, with its increased sampling of southern African anemones (including *Knowltonia*), confirms their placement within subgenus *Anemone*, section *Pulsatilloides* (Fig. 2). Separation of *Knowltonia* from *Anemone* was based on the presence of more or less fleshy fruits, branched inflorescences, centrifugal stamen dehiscence, broad connectives and filaments, and presence of stigmatic papillae on the ventral sides of the styles (Rasmussen 1979; Manning et al. 2009). However, most of these characters are found in other anemones and only fleshy fruits are unique (Hoot et al. 1994; Manning et al. 2009). Previous classifications based on fruit types have proven unreliable in both *Anemone* and Ranunculaceae in general (Hoot et al. 1994; Hoot 1995).

*Anemone thomsonii* from eastern Africa has been variously associated with section *Pulsatilloides* of Hoot et al. (1994), subgenus *Pulsatilloides* section *Kilimandsharica* of Tamura (1995), and subgenus *Kilimandsharica* of Ziman et al. (2006; 2008). In our phylogeny *A. thomsonii* is placed with strong support in subgenus and section *Anemone* (Fig. 2). However, its association with various woodland and alpine anemones from the Northern Hemisphere (Fig. 2: subsect. *Parviflorae*, *Anemonanthea*, and *Virginanae*) is unresolved (Fig. 1); as a result it is provisionally classified in its own subsection *Kilimandsharica* (Fig. 2). The association of the Somalian tuberous species, *A. somalensis*, with other tuberous Mediterranean species within section *Anemone* (Hoot et al.

1994; Ziman et al. 2006, 2008; Ehrendorfer et al. 2009) is confirmed (Fig. 2).

**Morphological and Biogeographical Patterns**—All anemones from South America, Mexico, southern Africa, and Tasmania are found in section *Pulsatilloides*, with the exception of *A. antucensis* (South America) and *A. tenuicaulis* (New Zealand), which are placed in a clade composed of Northern Hemisphere species within subgenus *Anemonidium*. Hoot et al. (1994) incorrectly placed *A. tenuicaulis* with *A. crassifolia* (Tasmania) in section *Pulsatilloides*, based mainly on geographic distribution (Parkin and Sledge 1933). Ehrendorfer and Samuel (2001) and Schuettpelez et al. (2002) subsequently corrected this error. Significantly, the sole East African species, *A. thomsonii* is placed in section *Anemone*, indicating separate origins for the two sub-Saharan groups in the genus.

The fact that section *Pulsatilloides* is exclusively composed of species from Mexico, Tasmania, South America, and southern Africa is noteworthy (Fig. 1). There are several clear morphological trends apparent in all but the alpine species (sections *Barneoudia* and *Oreithales*). These include: leaves often coriaceous and serrated, compound (often umbellate) inflorescences, bractlike involucre leaves of varying numbers (two to five), presence of additional paired bracts on compound inflorescences, flowers with numerous linear sepals, and  $2n = 48$  (chromosome counts available only for *A. helleborifolia*, *A. integrifolia* (= *Oreithales integrifolia*), and several species within series *Knowltonia*; Hoot et al. 1994). In fact, the first speculations about the close relationships within section *Pulsatilloides* were based on morphology (Hoot et al. 1994). Although evident, these morphological trends are masked by the morphological diversity found within this group. This diversity is so marked that it has resulted in recognition of segregate genera (e.g. *Knowltonia*) and varied classifications based on morphology (e.g., Ulbrich 1905–06; Tamura 1995; Ziman et al. 2008).

Analyses of both subgenera *Anemonidium* and *Anemone* indicate a Northern Hemisphere origin for the austral section *Pulsatilloides*. The presence of Southern Hemisphere species in both subgenera and a possible previous Gondwanan distribution has been discussed in Meyer et al. (2010). The argument for a vicariant model is purely inferential, resting on similar distributions in other Ranunculacean genera (e.g. *Caltha*; Schuettpelez and Hoot 2004), the nature of fruits in sections *Pulsatilloides* (not conducive to long distance dispersal), and the relative proximity of Southern Hemisphere continents through the Late Cretaceous (up to 94 mya; Scotese 2001). Potential evidence against a vicariant Gondwanan

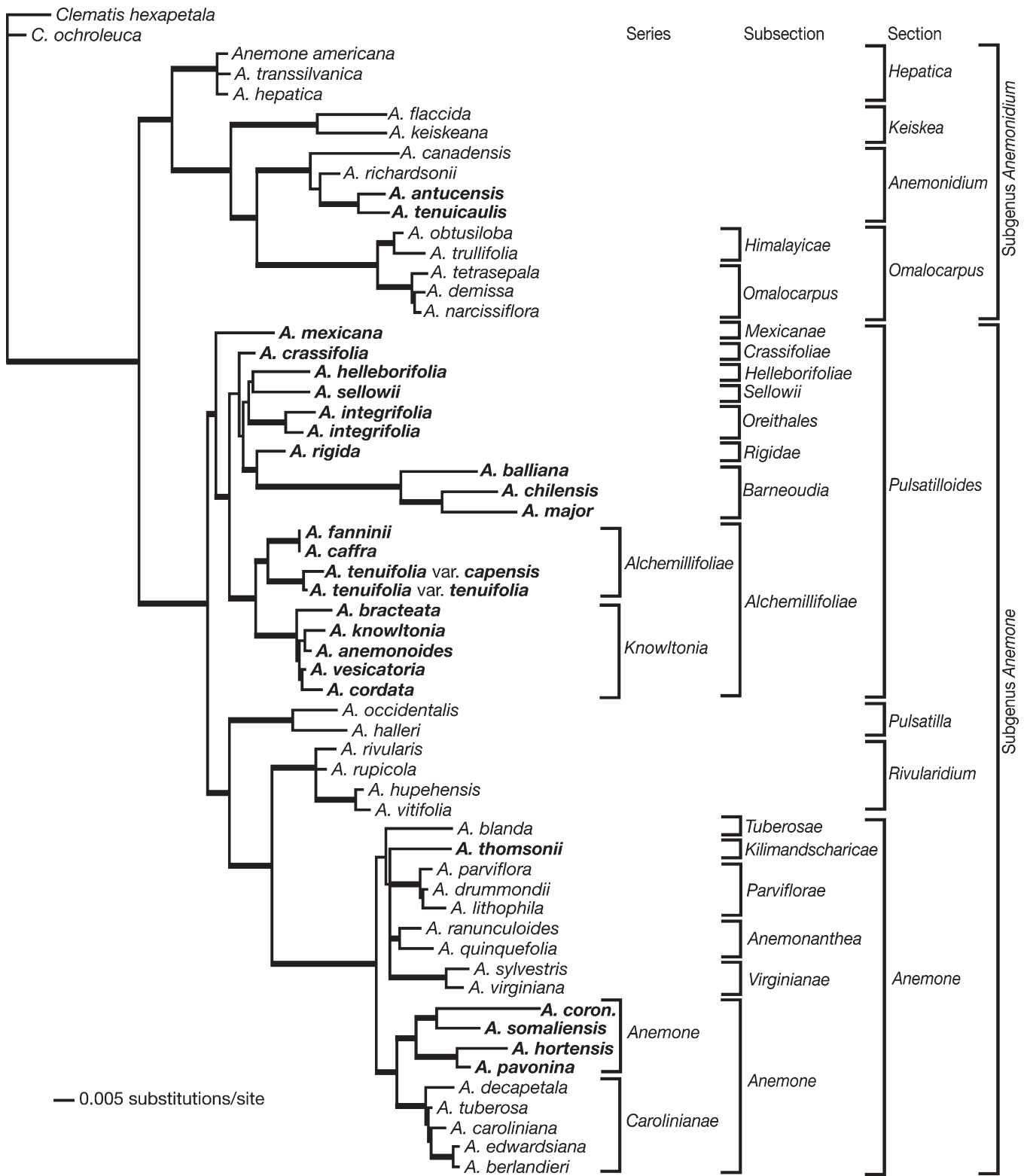


FIG. 2. Maximum likelihood phylogram resulting from analyses of combined *atpB-rbcL* spacer region and ITS data. Branches in bold had posterior probabilities  $\geq 95$  and bootstrap values  $\geq 70$  in Fig. 1. New classification of *Anemone* resulting from this work is to the right of species names.

model is indirect molecular dating (Paun et al. 2005; Anderson et al. 2005) that suggests more recent origins, but neither of these studies included any species of *Anemone*. Unfortunately, there is no reliable fossil record for the genus.

**Reclassification**—The first formal phylogeny-based classification and taxonomic revision of *Anemone* is presented

below. The classification (Table 3) draws much upon the present work, as well as previous molecular (e.g. Hoot et al. 1994; Schuettpelz et al. 2002; Meyer et al. 2010) and morphological data (e.g. Hoot et al. 1994; Tamura 1995; Ziman et al. 2006; Meyer et al. 2010). While the emphasis here is on the austral anemones, every attempt was made to provide a

TABLE 3. New taxonomic treatment of *Anemone* s. l. based on several molecular phylogenies (Hoot et al. 1994; Schuettepelz et al. 2002; Ehrendorfer et al. 2009; Meyer et al. 2010; this work). \*Indicates placement is based solely on morphology. †*Anemone multifida* appears twice in this table, in accordance with its putative hybrid origins (Meyer et al. 2010).

Subgeneric classification	Type
<b>1. <i>Anemone</i> subgen. <i>Anemonidium</i> (Spach) Juz. 1937:208</b>	<i>A. dichotoma</i> L.
<b>A. A. sect. <i>Hepatica</i> Spreng. 1825:660</b>	<i>A. hepatica</i> L.
<i>A. acutiloba</i> (DC.) Laws.	
<i>A. americana</i> (DC.) H. Hara	
<i>A. hepatica</i> L. = <i>Hepatica nobilis</i> Miller	
<i>A. transilvanica</i> (Fuss) Heuff	
All other <i>Hepatica</i> species*	
<b>B. A. sect. <i>Keiskea</i> Tamura 1967:26</b>	<i>A. keiskeana</i> Ito
<i>A. flaccida</i> F. Schmidt	
<i>A. keiskeana</i> Ito	
<b>C. A. sect. <i>Anemonidium</i> Spach 1839:248</b>	<i>A. dichotoma</i> L.
<i>A. antucensis</i> Poeppig	
<i>A. canadensis</i> L.	
<i>A. dichotoma</i> L.	
<i>A. richardsonii</i> Hook. f.	
<i>A. tenuicaulis</i> (Cheeseman) Parkin and Sledge	
<b>D. A. sect. <i>Omalocarpus</i> DC. 1817:212</b>	<i>A. narcissiflora</i> L.
<b>1) A. subsect. <i>Omalocarpus</i> (DC.) Tamura 1967:27</b>	<i>A. narcissiflora</i> L.
<i>A. demissa</i> Hook. f. & Thomson	
<i>A. narcissiflora</i> L.	
<i>A. tetrasepala</i> Royle	
<b>2) A. subsect. <i>Himalayicae</i> (Ulbr.) Tamura</b>	<i>A. obtusiloba</i> D. Don
<i>A. coelestina</i> Franch.*	
<i>A. obtusiloba</i> D. Don	
<i>A. rupestris</i> Hook. f. & Thomson*	
<i>A. trullifolia</i> Hook. f. & Thomson	
<b>2. A. subgen. <i>Anemone</i></b>	<i>A. coronaria</i> L.
<b>A. A. sect. <i>Pulsatilloides</i> DC. 1817:195</b>	<i>A. capensis</i> L.
<b>1) A. subsect. <i>Mexicanae</i> (Starod.) Hoot, new subsection</b>	<i>A. mexicana</i> Kunth
<i>A. mexicana</i> Kunth	
<b>2) A. subsect. <i>Crassifoliae</i> (Ulbr.) Hoot, new subsection</b>	<i>A. crassifolia</i> Hook.
<i>A. crassifolia</i> Hook.	
<b>3) A. subsect. <i>Helleborifoliae</i> (Starod.) Hoot, new subsection</b>	<i>A. helleborifolia</i> DC.
<i>A. helleborifolia</i> DC.	
<i>A. peruviana</i> Britton*	
<b>4) A. subsect. <i>Sellowii</i> Hoot, new subsection</b>	<i>A. sellowii</i> Pritz.
<i>A. assisbrasiliiana</i> Kuhl. & Porto*	
<i>A. sellowii</i> Pritz.	
<b>5) A. subsect. <i>Oreithales</i> (Schldtl.) Hoot, new subsection</b>	<i>A. integrifolia</i> Humb. & Bonpl. ex DC.
<i>A. integrifolia</i> Humb. & Bonpl. ex DC.	
<b>6) A. subsect. <i>Rigidae</i> (Ulbr.) Hoot, new subsection</b>	<i>A. rigida</i> Gay
<i>A. hepaticifolia</i> Hook. f.*	
<i>A. moorei</i> Espinosa*	
<i>A. rigida</i> Gay	
<b>7) A. subsect. <i>Barneoudia</i> (Gay) Hoot, new subsection</b>	<i>Barneoudia chilensis</i> Gay
<i>A. balliana</i> (Britton) Hoot	
<i>A. chilensis</i> (Gay) Reiche	
<i>A. major</i> (Phil.) Meigen	
<b>8) A. subsect. <i>Alchemillifoliae</i> (Ulbr.) Hoot, new subsection</b>	<i>A. caffra</i> Harv.
<b>a. A. ser. <i>Alchemillifoliae</i> Ulbr. 1905:201</b>	<i>A. caffra</i> Harv.
<i>A. caffra</i> Harv.	
<i>A. fanninii</i> Harv.	
<i>A. tenuifolia</i> (L. f.) DC. = <i>A. capensis</i>	
<b>b. A. ser. <i>Knowltonia</i> (Salisb.) J. C. Manning &amp; Goldblatt</b>	<i>Knowltonia rigida</i> Salisb.
<i>A. anemonoides</i> (H. Rasm.) J. C. Manning & Goldblatt	
<i>A. bracteata</i> (Harv. ex J. Zahlbr.) J. C. Manning & Goldblatt	
<i>A. cordata</i> (H. Rasm.) J. C. Manning & Goldblatt	
<i>A. knowltonia</i> Burt Davy	
<i>A. vesicatoria</i> (L. f.) Prantl	
All remaining <i>Knowltonia</i> species (Manning et al. 2009)*	
<b>B. A. sect. <i>Pulsatilla</i> (Mill.) DC. 1817:193</b>	<i>Pulsatilla vulgaris</i> Mill.
<i>A. patens</i> L.	
<i>A. halleri</i> All.	
<i>A. occidentalis</i> S. Watson	
<i>A. pulsatilla</i> L. (= <i>P. vulgaris</i> Mill.)	
+ all remaining species formerly in genus <i>Pulsatilla</i> *	
<b>C. A. sect. <i>Rivularidium</i> Jancz. 1892:251</b>	<i>A. rivularis</i> Buch.-Ham. ex DC.
<i>A. hupehensis</i> Lemoine	
<i>A. rivularis</i> Buch.-Ham. ex DC.	

(Continued)



TABLE 3. Continued

Subgeneric classification	Type
<i>A. rupicola</i> Camb.	
<i>A. tomentosa</i> (Maxim.) Pei	
<i>A. vitifolia</i> Buch.-Ham. ex DC.	
<b>D. A. sect. <i>Anemone</i></b>	
<b>1) A. subsect. <i>Tuberosae</i></b> Ulbr. 1905:194	<i>A. palmata</i> L.
<i>A. apennina</i> L.	<i>A. apennina</i> L.
<i>A. blanda</i> Schott & Kotschy	
<b>2) A. subsect. <i>Kilimandscharicae</i></b> (Ulbr.) Hoot, new subsection	<i>A. thomsonii</i> Oliv.
<i>A. thomsonii</i> Oliv.	
<b>3) A. subsect. <i>Parviflorae</i></b> (Ulbr.) Juz. 1937:267	<i>A. parviflora</i> Michx.
<i>A. drummondii</i> S. Watson	
<i>A. lithophila</i> Rydb.	
( <i>A. multifida</i> DC.)†	
<i>A. parviflora</i> Michx.	
<b>4) A. subsect. <i>Anemonanthea</i></b> (DC.) Hoot, new subsection	<i>A. nemorosa</i> L.
<i>A. lyallii</i> Britton*	
<i>A. nemorosa</i> L.	
<i>A. piperi</i> Britton*	
<i>A. quinquefolia</i> L.	
<i>A. ranunculoides</i> L.	
<i>A. trifolia</i> L.*	
<b>5) A. subsect. <i>Virginianae</i></b> (Ulbr.) Starod. 1991:120	<i>A. virginiana</i> L.
<i>A. cylindrica</i> A. Gray	
( <i>A. multifida</i> DC.)†	
<i>A. riparia</i> Fernald	
<i>A. sylvestris</i> L.	
<i>A. virginiana</i> L.	
<b>6) A. subsect. <i>Anemone</i></b>	<i>A. coronaria</i> L.
<b>a. A. ser. <i>Anemone</i></b>	<i>A. coronaria</i> L.
<i>A. coronaria</i> L.	
<i>A. hortensis</i> L.	
<i>A. palmata</i> L.	
<i>A. pavonina</i> Lam.	
<i>A. somaliensis</i> Hepper	
<b>b. A. ser. <i>Carolinianae</i></b> (Starod) Hoot, new series	<i>A. caroliniana</i> Walter
<i>A. berlandieri</i> Pritz.	
<i>A. caroliniana</i> Walt.	
<i>A. decapetala</i> Ard.	
<i>A. edwardsiana</i> Tharp	
<i>A. triternata</i> Vahl	
<i>A. tuberosa</i> Rydb.	

robust framework that will be useful with the addition of more taxa. The classification is missing many species from the Northern Hemisphere, an area of active research in the Hoot lab. We include only the more significant synonyms, for a more complete synonymy see Tamura (1995) and Ziman et al. (2006).

#### TAXONOMIC TREATMENT

ANEMONE subgen. ANEMONIDIUM (Spach) Juz. Flora USSR: 208. 1937. *Anemone* sect. *Anemonidium* Spach, Hist. Nat. Vég. Phanér. 7: 248. 1839.—TYPE: *Anemone dichotoma* L. Sp. Pl. 1: 540. 1753.

*A.* sect. *Anomospermos* DC. Syst. Nat. [Candolle] 1: 208. 1817. No type designated.

Achene heads globose, usually wider than long, with few (< 50) achenes/head; achenes nearly glabrous or with short, straight hairs with thick walls (Hoot et al. 1994). Base chromosome number  $x = 7$  (Baumberger 1970).

Subgenus *Anemonidium* comprises a strongly supported monophyletic group (Figs. 1, 2) that is characterized by  $x = 7$ . The subgenus is greatly expanded compared to previous authors, who included just *A. dichotoma* and *A. canadensis* (e.g. Tamura 1995, Ziman et al. 2008). Subgenus *Anemonidium*

includes four sections: *Hepatica* (= genus *Hepatica*), *Keiskea*, *Anemonidium* (containing the sister species *A. antucensis* of South America and *A. tenuicaulis* of New Zealand), and *Omalocarpus* (Table 3; Hoot et al. 1994; Schuettpelez et al. 2002; Meyer et al. 2010). Distribution of this taxon is mainly in the Northern Hemisphere.

ANEMONE sect. HEPATICA (Mill.) Spreng. Syst. Veg. (ed. 16) [Sprengel]: 660. 1825. *Hepatica* Mill. Gard. Dict. Abr., ed 4. 1754. *A.* subgen. *Hepatica* (Mill.) Peterm. Analytischer Pflanzenschlüssel für botanische Excursionen in der Umgegend von Leipzig, p. 5. 1846.—TYPE: *Hepatica nobilis* Mill. Gard. Dict., ed. 8. 1768 = *Anemone hepatica* L.

Basal leaves overwintering, simple and entire, 3-lobate (sometimes 5–7 lobate), coriaceous; involucre leaves 3, close to flower, sessile, entire; flowers single; sepals 6–10; achenes hispid, style short with capitate stigma. Pollen usually tricolpate (Huynh 1970a); chromosome number  $2n = 14$  (28, 42) (Baumberger 1970).

Although first recognized at the generic level by Dillenius (1719), Linnaeus (1753) included *Hepatica* in *Anemone* as *Anemone hepatica* L. Later, Ulbrich (1905–06) credited Dillenius as author when subsuming *Hepatica* within *Anemone* (*Anemone* subgen. and sect. *Hepatica* Dill.). However, the Dillenius publication is pre-Linnaean and Phillip Miller (1768) is correctly

attributed as the author of the genus. Authorship for *Anemone* sect. *Hepatica* is thus (Mill.) Sprengel (1825) and not Sprengel as rendered by Prantl (1887).

**ANEMONE** subgen. **ANEMONE**—CONSERVED TYPE: *A. coronaria* L. Jarvis, Taxon 41:557.

Achene heads various in shape, usually numerous (> 50) achenes/head; achenes often covered with long hairs, straight or twisted; achene trichome walls thin or thick (Hoot et al. 1994). Base chromosome number  $x = 8$ .

Subgenus *Anemone* is expanded here compared to past workers (Table 1; Tamura 1995; Ziman et al. 2008). Taxa in this strongly supported group are characterized by  $x = 8$ . Included are the diverse section *Pulsatilloides* with distributions mainly in the Southern Hemisphere, sections *Pulsatilla* (formerly genus *Pulsatilla*) and *Rivularidium* of the Northern Hemisphere, and the highly diverse and less resolved section *Anemone*, with a largely northern distribution.

**ANEMONE** sect. **PULSATILLOIDES** DC. Syst. Nat. [Candolle] 1: 195. 1817. *A.* subgen. *Pulsatilloides* (DC.) Juz. Flora URSS VII: 256. 1937.—LECTOTYPE designated by Tamura (1995): *Anemone capensis* Lam., Encyl. (Lamarck) 1 (1): 164. 1783 = *A. tenuifolia* (L. f.) DC.

Leaves often coriaceous, serrated; inflorescence often compound (solitary flowers in alpine species), umbellate to cymose; involucre leaves bractlike, most commonly 3 (2–5 possible), paired additional bracts often present on compound inflorescences; sepals sometimes numerous (> 10), often linear; achene heads usually wider than long; achene trichome walls usually thin (Hoot et al. 1994).

Section *Pulsatilloides*, with our current sampling, consists of species with austral distributions. Based on morphology, Hoot et al. (1994) included *A. glaucifolia* from the Himalayan regions in this section but this needs further testing. They also included the Northern Hemisphere genus *Pulsatilla* in this section based on restriction site data but this is not supported by our current work (Figs. 1, 2).

Our section *Pulsatilloides* is greatly expanded over subgenus *Pulsatilloides* as defined by Tamura (1995) and Ziman et al. (2008), which included only the African *Anemone* species (Table 1). Our molecular results strongly support the inclusion of the segregate genera *Barneoudia*, *Oreithales*, and *Knowltonia* within section *Pulsatilloides*, as previously suggested by Hoot et al. (1994), and Meyer et al. (2010). Also included are species from Mexico, Tasmania, southern Africa, and both eastern and western South America (Figs. 1, 2).

**Anemone** subsect. **Mexicanae** (Starod.) Hoot, stat. nov. *Anemonidium* sect. *Mexicanae* Starod. Bot. Zhurn. (Moscow & Leningrad) 74(9): 1345. 1989. *A.* ser. *Mexicanae* (Starod.) Ziman, Balakh & Kadota. J. Jap. Bot. 81(4): 196. 2006.—TYPE: *Anemone mexicana* Kunth, Nov. Gen. Sp. [H.B.K.] v. 33.

Basal leaves petiolate, trifoliate, with each leaflet slightly petiolate, not coriaceous or fleshy, leaflets lobed, margins irregularly serrated; inflorescence usually compound with two to three involucre leaves similar to basal leaves, 1–5 flowered; flowers with usually five white sepals; achenes glabrous to slightly pubescent, style short and reflexed. Pollen eupantocolpate (Huynh 1970a); chromosome number  $n = 16$  (Baumberger 1970).

Tamura (1995) and Ziman et al. (2008) place *A. mexicana* in subgenus/section *Rivularidium*; both taxa are polyphyletic

on our tree (Fig. 1). *Anemone mexicana* is well supported as a member of section *Pulsatilloides* and as sister to all remaining species in that section (Figs. 1, 2).

**Anemone** subsect. **Crassifoliae** (Ulbr.) Hoot, stat. nov. *Anemone* ser. *Crassifoliae* [as ‘*Crassifolia*’] Ulbr. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 37: 188. 1905–06. *A.* sect. *Crassifoliae* (Ulbr.) Tamura. Acta Phytotax. Geobot. 42: 178. 1991.—TYPE: *Anemone crassifolia* Hook. Icones Plantarum 3: 257. 1840.

Basal leaves petiolate, slightly coriaceous or fleshy, 3–5 lobed with additional incisions; coarsely toothed; inflorescence sometimes compound, one to several flowered; involucre of 2–3 sessile, irregularly lobed or entire leaves; flowers with 6–8 white sepals; stamens with filaform filaments and narrow connectives; achenes essentially glabrous, style erect with a hooked to coiled apex. Pollen spiroaperturate (Huynh 1970b); chromosome number unknown.

This subsection, consisting solely of *A. crassifolia* (Tasmania), differs from Tamura’s (1991, 1995) section *Crassifoliae* in its exclusion of *A. tenuicaulis*, which we place in subgen. and sect. *Anemonidium* (Fig. 2).

**Anemone** subsect. **Helleborifoliae** (Starod.) Hoot, stat. nov. *Anemonidium* subsect. *Helleborifoliae* [as ‘*Helleborifolia*’] Starod. Bot. Zhurn. 74: 1345. 1989. *A.* ser. *Helleborifoliae* Tamura, Acta Phytotax. Geobot. 42: 178. 1991.—TYPE: *Anemone helleborifolia* DC. Syst. Nat. [Candolle] 1: 211. 1817.

*A.* ser. *Rivulares* [as ‘*Rivularis*’] Ulbr. Bot. Jahrb. 37: 188. 1905. No type designated.

*Anemonidium* sect. *Meridium* Starod. Bot. Zhurn. 74: 1345. 1989.—TYPE: *Anemone helleborifolia*.

Basal leaves with long petioles (15–25 cm), triternate but superficially 5-parted; inflorescences 3–4 times compound, many flowered; usually 5 yellowish white sepals; filaments basally expanded; glabrous achenes slightly compressed with lateral ribs and basally curved style. Pollen 3–6 colpate (Huynh 1970a); chromosome number  $n = 24$  (*A. helleborifolia*, Baumberger 1970).

This subsection is less inclusive than *Anemone* series *Helleborifolia* of Tamura (1991), consisting of only *A. helleborifolia* and *A. peruviana*. Ziman et al. (2008) include *A. helleborifolia* with *A. mexicana* in Ser. *Mexicanae* (Table 1).

**Anemone** subsect. **Sellowii** Hoot, subsect. nov.—TYPE: *Anemone sellowii* Pritz. Linnaea 15: 667. 1842.

Folia radicalia ternate; involucre 3 bracteae; inflorescentia floribus 1–3; sepala 8–16 alba, abaxiales rubae to violaceae; achenia glabra, compressa, lateralis costatae.

Radical leaves ternate; involucre leaves 3 and bract-like; inflorescence of 2–3 large (~7.5 cm diameter) flowers; sepals 8–16, adaxially white, abaxial surface pink to violet; carpels laterally flattened, glabrous with ribbing along lateral edges. Geographic distribution: Brazil, in montane forests (1,500–1,800 m). Pollen eupantocolpate (Huynh 1970a; chromosome numbers unknown).

*Anemone sellowii* from Brazil was previously included in the highly polyphyletic section *Rivularidium* (Fig. 1) by many workers (e.g., Ulbrich 1905–06; Tamura 1995; Ziman et al. 2008). Our data indicate that it is in a largely unresolved clade within section *Pulsatilloides* (Fig. 2, Table 3). Based on morphology, *A. assisbrasiliensis* is also included in this section

(Table 3), sharing with *A. sellowii* ternate leaves, 3-bracted involucre, inflorescences of 1 or 2 white flowers with 9–15 sepals, glabrous achenes with lateral ribs, and eupantocolpate pollen (Huynh 1970a).

**Anemone** subsect. **Oreithales** (Schltdl.) Hoot, comb. et stat. nov. *Oreithales* Schltdl. Linnaea 27: 559. 1856.—TYPE: *Anemone integrifolia* Kunth ex DC. Syst. Nat. [Candolle] 1: 217. 1817.

*Capethia* Britton. Ann. New York Acad. Sci. 6: 235. 1891, nom. illegit. superfl.—TYPE: *Anemone integrifolia* Kunth ex DC., Syst. Nat. [Candolle] 1: 217. 1817.

Radical leaves coriaceous, tomentose, and simple; involucre leaves absent; flowers 1–2, sessile; sepals 10–18, linear, white to violet; achenes hairy with filiform reflexed styles (Lourteig 1956). Pollen tricolpate (Meacham 1981); chromosome number  $n = 24$  (Duncan and Perez 1979).

The taxonomic history of *A. integrifolia*, the sole representative of subsection *Oreithales*, is complex. De Candolle (1817) lists this species as synonymous with *Hepatica integrifolia* Kunth (originally collected by Humboldt). The Humboldt voucher verifies that the species in question is indeed *A. integrifolia* (Röpert 2000–), not *Hepatica*. Sprengel (1825) incorrectly lists *A. integrifolia* within *Hepaticae* (Dill.) Cand., characterizing the plants as having a three leaved involucre (absent in this species) and found in Mexico (rather than Ecuador, Peru, and Bolivia). Pritzel (1841) lists *A. integrifolia* Spreng. but rather than follow Sprengel's description, he largely follows Kunth's original description. Von Schlechtendal (1854) erected the new genus *Oreithales*, citing Pritzel (1841) and with a new Latin diagnosis that is correct for this species.

**Anemone** subsect. **Rigidae** (Ulbr.) Hoot, stat. nov. *Anemone* ser. *Rigidae* [as '*Rigida*'] Ulbr. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 37: 199. 1905. *Anemone* sect. *Rigidae* (Ulbr.) Tamura, Sci. Rep. Osaka Univ. 16: 28. 1967. *A.* subgen. *Rigidae* (Ulbr.) Tamura, Acta Phytotax. Geobot. 42: 178. 1991. *Anemonidium* subsect. *Rigidae* (Ulbr.) Strarod. *Anemone*: Systematics and Evolution. Leningrad: Nauka 119. 1991.—TYPE: *Anemone rigida* Gay, Fl. Chil. i. 25. 1845.

Morphology and incomplete DNA data suggest that *A. rigida* is in a clade including two sister species, also from Chile, *A. hepaticifolia* and *A. moorei* (tree not presented). These three species share: radical leaves petiolate, either ternate (*A. moorei*) or 3-lobed with dentate/serrate margins and mucronate teeth, coriaceous; involucre of 3 sessile leaves similar to the radical leaves; paired, sessile bracteoles often present; compound inflorescence 1–5 flowered; flowers white, pink, to bluish, large (diameter 2.0–4.0 cm.), usually 5–7 sepals; stamens usually filiform (sometimes slightly expanded at base), anthers pink (*A. hepaticifolia*) or yellow (*A. rigida*, *A. moorei*), connectives thin with no extension (*A. moorei*) to wide with extended subglobose (*A. hepaticifolia*) or mucronate appendages (*A. rigida*); achenes not noticeably flattened, no lateral ridging, sparsely pubescent, styles straight, more or less apically hooked. *Anemone rigida* has basal hairs that are slightly spinose. Pollen eupantocolpate (*A. rigida*), pantoporate (*A. hepaticifolia*), or unknown (*A. moorei*) (Huynh 1970a); chromosome numbers unknown.

*Anemone rigida* and *A. hepaticifolia* have each been placed in monotypic taxa at various ranks by Tamura (1967, 1991), and

Ziman et al. (2006, 2008). *Anemone moorei* has been placed in series *Rivulares* (Tamura 1995) or in section *Rivularidium* series *Jamesonii*, along with *A. assisbrasiliensis*, *A. jamesonii*, *A. peruviana*, and *A. sellowii* (Table 1; Ziman et al. 2008). Our molecular phylogeny places *A. rigida* as sister to *Barneoudia*. However, morphological and molecular support are not yet sufficient (Fig. 1) to justify its inclusion within subsection *Barneoudia*.

**Anemone** subsect. **Barneoudia** (Gay) Hoot, comb. et stat. nov. *Barneoudia* Gay, Flora chilena i. 29. t. 1 (1845).—TYPE: *Barneoudia chilensis* Gay, Fl. Chil. i. 29. t. 1. 1845 = *Anemone chilensis* (Gay) Reiche.

Plants tuberous; leaves absent during anthesis; involucre one-leaved, involucre leaves diversely parted or lobed, fleshy, placed directly below the perianth; flowers solitary with 7–18 sepals; numerous pubescent achenes. Pollen polyporate (*Barneoudia major*; Meacham 1981), chromosome number unknown.

This subsection includes the three species (as defined by Lourteig 1951) formerly included in *Barneoudia*. Most workers recognize genus *Barneoudia* (e.g. Britton 1892; Ulbrich 1905–06; Lourteig 1951; Tamura 1991; Ziman et al. 2006). However, Meigen (1893) and Kurtz (1886) subsumed some *Barneoudia* species within *Anemone*, a treatment that was followed by Reiche (1894). While these species do exhibit some unusual features for *Anemone* (e.g. one involucre leaf directly beneath the flower, no basal leaves at anthesis), many of the features fall within the range of characters found within *Anemone* (Meyer et al. 2010). Furthermore, molecular data strongly support the placement of *Barneoudia* species within *Anemone* (Figs. 1, 2).

**Anemone balliana** (Britton) Hoot, comb. nov. *Barneoudia balliana* Britton, Ann. New York Acad. Sci. 6: 236. 1981.—TYPE: Argentina, Sierra de Famatina, Cueva de Pérez, leg. Hieronymus et Niederlein (385) 26–28-I-1879 (K, hol.).

**Anemone** subsect. **Alchemillifoliae** (Ulbr.) Hoot, stat. nov. *Anemone* ser. *Alchemillifoliae* Ulbr. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 37: 201. 1905–06.—LECTOTYPE: designated by Tamura (1995): *Anemone caffra* Harv. Gen. S. Afr. Pl. 9. 1838.

*A.* ser. *Pinnatifoliae* Ulbr. Bot. Jahrb. 37: 200. 1905. No type designated.

Basal leaves usually compound, toothed; inflorescences usually compound and multiflowered; flowers with numerous linear sepals.

*Anemone* section/series *Alchemillefoliae* (e.g., Ulbrich 1905–06) or *Alchimillefolia* (Tamura 1995; Ziman et al. 2008) included two southern African species, *A. caffra* and *A. fanninii*, in past classifications (Table 1). Our *Anemone* subsect. *Alchemillifoliae* expands this taxon to include all southern African anemones, including those previously within genus *Knowltonia*.

**ANEMONE** ser. **ALCHEMILLIFOLIAE** Ulbr. Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 37: 179. 1905.—TYPE: as for subsect. *Alchemillifoliae*.

Plants with ascending caudices (lignified in *A. tenuifolia*); basal leaves palmately to pinnately compound, toothed; usually two bract-like involucre leaves; inflorescence usually



once-compound; flowers with numerous linear sepals (> 14); numerous achenes/head (> 140), achenes usually four times longer than wide, tomentose, styles straight; achene walls thin and not berry-like; pollen pantoporate (*A. capensis*; Huynh 1970a); chromosome numbers unknown.

*Anemone* subsection *Alchemillifoliae* is recognized by various workers (e.g. Ulbrich 1905–06; Tamura 1995; Ziman et al. 2006, 2008) as including *A. caffra* and *A. fanninii* but not *A. tenuifolia* (= *A. capensis*; see below). Our DNA data indicate that *A. tenuifolia* should also be included (Fig. 1) and that the two varieties of *A. tenuifolia* are more different from one another (differing at six sites) than *A. fanninii* and *A. caffra*, which have essentially identical sequences (Fig. 2). *Anemone tenuifolia* is a variable species and more molecular and morphological work is needed. The source of the name “alchemillifolia” appears to be *A. alchemillifolia* E. Mey. ex Pritz, (Linnaea 15: 758. 1842), synonymous with *Pulsatilla* (*Anemone*) *caffra* Eckl. & Zeyh. (Enum. Pl. Afric. Austral. [Ecklon & Zeyher] 1: 1. 1934–35).

ANEMONE TENUIFOLIA (L. f.) DC. Prodr. 1: 18 (1824). *Atragene tenuifolia* L.f., Suppl.: 270 (1781). *Anemone capensis* var. *tenuifolia* (L. f.) Harv. Fl. Cap. 1: 3 (1859).—TYPE: South Africa, without precise locality or date, *Thunberg s. n.* (UPS-THUNB 12999, holo.).

*Atragene capensis* L., Sp. Pl.: 543 (1753). *Anemone capensis* (L.) Harv. Fl. Cap. 1: 3 (1859), nom. illegit. non Lam. (1783).—TYPE: South Africa, without locality, date or collector (LINN 711.3, holo.).

*Anemone capensis* Lam. Encycl. 1: 164 (1783).—TYPE: South Africa, without locality or collector, specimen in Herb. Jussieu (P, holo.).

The authorship and priority of *Anemone tenuifolia/capensis* is complicated, thus warranting a special discussion here. Based on Killick (1977), Ziman et al. (2006) incorrectly attributed the name *A. capensis* (L.) Lam. to Lamarck. Lamarck makes no mention of Linnaeus’ name *Atragene capensis* and is thus considered to be describing the species independently in the genus *Anemone*, with a separate type. The existence of *A. capensis* Lam. precludes the transfer of Linnaeus’ name to *Anemone*, and the next available name is *Atragene tenuifolia* L. f., which was transferred to *Anemone* by DC.

ANEMONE ser. KNOWLTONIA (Salisb.) J. C. Manning & Goldblatt, Bothalia 39: 218. 2009. *Knowltonia* Salisb., Prodromus stirpium in horto ad Chapel Allerton vigintium: 372 (1796). *Anemone* sect. *Knowltonia* (Salisb.) Prantl, Die natürlichen Pflanzenfamilien 3(2): 62. 1891.—TYPE: *Knowltonia rigida* Salisb. Prodr. Stirp. Chap. Allerton 372. 1796, nom. illeg. = *Anemone knowltonia* Burt Davy, Ann. Transvaal Mus. iii. 121. 1912.

This series comprises all species formerly included in genus *Knowltonia*. See Manning et al. (2009) for diagnosis and descriptions. Pollen pantoporate (*K. capensis*; Meacham 1981); chromosome number  $2n = 48$  (Rasmussen 1979).

ANEMONE sect. PULSATILLA (Mill.) DC. Syst. Nat. [Candolle] 1: 193. 1817. *Pulsatilla* Mill. Gard. Dict. Abr., ed. 4. 1754.—LECTOTYPE: here designated: *Pulsatilla vulgaris* Mill. Gard. Dict. Ed. 8 [unpagged]. 1768 = *Anemone pulsatilla* L.

*Anemone* sect. *Preonanthos* DC. Syst. Nat. [Candolle] 1: 193. 1817, syn. nov.—TYPE: *Anemone alpina* L. Sp. pl. 1: 539. 1753.

Basal leaves often pinnately compound; inflorescence simple; involuclal leaves 3; sepals 6–8; achenes with straight, elongated hairy styles (> 5 mm); achene trichomes thick-walled (> 1.5  $\mu\text{m}$ ). Pollen tricolpate, eupantocolpate, pantoporate (Huynh 1970c), chromosome numbers  $2n = 16, 32$  (Baumberger 1970).

De Candolle’s (1817) section *Preonanthos*, previously placed in genus *Pulsatilla* but later treated as a distinct section, is formally included here under *Anemone* section *Pulsatilla*.

*Anemone* sect. *Anemone*—Involuclal leaves usually three; achene heads usually longer than wide; achenes usually 2.0–3.5 mm in length, achene walls thin (< 1.5  $\mu\text{m}$ ). A variable group found mainly in North America.

*Anemone* subsect. *Kilimandsharicae* (Ulbr.) Hoot, stat. nov. *Anemone* series *Kilimandsharicae* Ulbr., Botanische Jahrbücher für Systematik, Pflanzengeschichte und Pflanzengeographie 37: 201. 1905. *A. sect. Kilimandsharicae* [as ‘*Kilimandscharica*’] (Ulbr.) Tamura, Acta Phytotax. Geobot. 42: 180. 1991.—TYPE: *Anemone thomsonii* Oliv. J. Linn. Soc., Bot. 21: 397. 1885.

Plants rhizomatous, basal leaves 2–3 ternate, the ultimate lobes more or less deeply incised, more or less hairy on lower surface. Involuclre of usually 3 bract-like leaves; flowering stems 0.7–7.0 dm. tall; flowers 2.5–6.5 cm. in diameter; sepals 10–20, linear, white or white tinged pink on inner surface, outer surface often pink, red, or purple; connectives slightly projecting; achenes with dense silky pubescence, style less than 1 mm. long, short, thick, and spatulate. Pollen eupantocolpate (Huynh 1970a); chromosome number unknown.

This subsection is identical to series *Kilimandsharicae* of Ulbrich (1905/06), section *Kilimandsharica* of Tamura (1995), and subgenus *Kilimandsharica* of Ziman et al. (2008; never formally described). All taxonomic levels are monotypic, consisting of *A. thomsonii* of eastern Africa (especially Mt. Kilimanjaro region). While some workers (Ulbrich 1905–06; Tamura 1995) placed this taxon in subgen. *Pulsatilloides* s. l., or within its own subgenus (Table 1; Ziman et al. 2008), our results indicate that it is firmly embedded within subgenus and section *Anemone* (Fig. 2).

*Anemone* subsect. *Anemonanthea* (DC.) Hoot, stat. nov. *Anemone* sect. *Anemonanthea* DC. Syst. Nat. [Candolle] 1: 196. 1817. *Anemonanthea* Gray, Nat. Arr. Brit. Pl. ii. 724. 1821. *Anemone* subgen. *Anemonanthea* (DC.) Juz. Flora URSS VII: 241. 1937.—LECTOTYPE: designated by Tamura (1995): *Anemone nemorosa* L. Sp. pl. 1: 541. 1753.

Rhizomatous; involuclal leaves petiolate, similar to basal leaves; inflorescence usually simple; sepals usually 5–6, ovoid; achenes few (< 40) with short trichomes, styles usually upright. Pollen tricolpate (Huynh 1970a); chromosome number  $n = 8$  (polyploidy in some species).

Subgenus or section *Anemonanthea*, as defined by many workers (e.g. De Candolle 1817; Tamura 1995; Ziman et al. 2008), includes numerous species and taxonomic groups (Table 1). In our *Anemone* subsection *Anemonanthea*, the tuberous species *A. blanda* and *A. apennina*, have been removed and placed in subsection *Tuberosa* (Table 3; Fig. 2). Preliminary molecular data suggest that *A. piperi*, *A. lyalii*, and *A. trifolia* also belong in this subsection (tree not presented). Further work is needed to determine the phylogenetic affinities of the many species often included in this taxon.



*Anemone* series *Anemone*—Tuberous; basal leaves 3-parted to 3-lobed; involucre of three leaves, either similar or dissimilar to basal leaves; 6–18 sepals; achenes lanate. Pollen tricolpate, pantocolpate, pantoporate, or spiroperurate (Huynh 1970a; Ziman et al. 2006); chromosome number  $2n = 16$  (occasionally  $2n = 32$  or  $48$ ; Baumberger 1970).

Series *Anemone* is similar in composition and description to *Anemone* subsect. *Anemone* recently described in Ehrendorfer et al. (2009). It differs in including *A. somaliensis* within the series rather than erecting a separate taxon for this species. *Anemone somaliensis* differs from other tuberous European and African species by several characteristics outlined in Ehrendorfer et al. (2009). Our phylogeny does not support a separate status for *A. somaliensis* at the present time (it is well supported as sister to *A. coronaria*; Fig. 1).

Within series *Anemone*, we also recognize *A. pavonina*. Based on morphology, this species is included within a polymorphic *A. hortensis* in Ehrendorfer et al. (2009). Our DNA data indicate that *A. pavonina* is strongly supported as sister to *A. hortensis* but the branch lengths for these two species indicate a number of unique substitutions (Fig. 2), suggesting species status may be correct for *A. pavonina*.

**Anemone** ser. **Carolinianae** (Starod.) Hoot, comb. et stat. nov. *Anemone* subsect. *Carolinianae* Starod. Bot. Zhurn. 74:1345. 1989.—TYPE: *Anemone caroliniana* Walter, Fl. Carol. [Walter] 157. 1788.

*A. subser. Tuberosa* Ulbr. Bot. Jahrb. 37: 206. 1905. No type designated.

Tuberous; basal leaves 1–2 ternate, often heteromorphic; involucre leaves three, usually different from basal leaves; sepals often linear; fruiting heads elongate; achenes lanate. Pollen 3-colpate or pantocolpate (Huynh 1970a); chromosome number  $2n = 16$  (24) (Baumberger 1970).

*Anemone* ser. *Carolinianae* is virtually identical in composition to *A. subsect. Carolinianae* as described in Ehrendorfer et al. (2009). The inclusion of the South American species, *A. triternata*, was confirmed by recent molecular work (Meyer et al. 2010).

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APPENDIX 1. *Anemone* species and outgroup taxa sampled for this work, including voucher information, geographic location, and GenBank numbers (*atpB-rbcL* spacer, ITS respectively). RBGE = Royal Botanical Garden, Edinburgh.

*Anemone antucensis*, L. & F. Ehrendorfer, 24.01.98 (WU); Chile, Concepcion, Nahuelbuta; AF311735, AY056049. *Anemone berlandieri*, S. Hoot 89-3 (MICH); U. S. A., Texas, Travis Co., Babcock Rd.; FJ639841, FJ639876. *Anemone blanda*, S. Hoot s. n. (UWM); Cultivated, Matthaei Bot. Gard., Ann Arbor, Michigan; AY055422, AY055402. *Anemone caffra*, Cultivated, RBGE 770617; Natal, Kamberg Mt. (C. Puff); AY055420, AY055399. *Anemone canadensis*, S. Hoot 86-7 (MICH); Canada, Ontario, Bruce Co., McGregor Prov. Park; AY055408, AY055387. *Anemone caroliniana*, L. Raymond s. n. (MICH); U. S. A., Louisiana, Bossier Parish Black Bayou Rec. Area; AY055423, AY055403. *Anemone coronaria*, S. Hoot 005 (UWM); Cultivated, U. S. A., Wisconsin, S. Hoot garden; FJ639842, FJ639877. *Anemone crassifolia*, S. Hoot 88-55 (MICH); Cultivated, Tasmania, Kingston, Woodbank Nurseries; AY055419, AY055398. *Anemone decapetala*, M. & K. Weigend 2000/50 (NY) (UWM); Peru, Puno, Macusani Rd from Rosario to Macusani; FJ639843, FJ639878. *Anemone demissa*, Cultivated, RBGE 19940561 (UWM); China, Yunnan (Kunming, Gothenburg Botanical Expedition 55); FJ639844, FJ639879. *Anemone drummondii*, R. Meyers 88-7 (MICH); U. S. A., Alaska, Nome Quad, Kougarok Rd.; AY055424, AY055404. *Anemone edwardsiana*, S. Hoot s. n. (MICH); U. S. A., Texas, Kendall Co., Edge Falls; FJ639845, FJ639880. *Anemone fanninii*, J. C. Manning s. n. (NBG); South Africa, KwaZulu-Natal Prov., Sani Pass; JF810695, JF810685. *Anemone flaccida*, S. Hoot 89-52 (MICH); Cultivated, Japan, T. Rokujo garden; AY055412, AY055411. *Anemone helleborifolia*, Weigend 2000/133 (UWM); Peru, Puno, Macusani, Road from Ollachea to Macusani; FJ639846, FJ639881. *Anemone hortensis*, Cultivated, RBGE 820949 (MICH); Italy, Nerano (W. Tait); FJ639847, FJ639882.

*Anemone hupehensis*, S. Hoot 91-1 (MICH); Cultivated, seed from Berlin Bot. Garden, China, Szechuan Prov.; AY055418, AY055397. *Anemone keiskeana*, S. Hoot 89-51 (MICH); Cultivated, Japan, T. Rokujo garden; AY055411, AY055390. *Anemone lithophila*, S. Hoot 88-16 (MICH); U. S. A., Montana, Banff NP, Sulphur Mt. below Lake Agnes Teahouse; FJ639848, FJ639883. *Anemone mexicana*, H. D. Barriga 6782 (MO); Mexico, Michoacan, Zinapécuaro, Las Adjuntas, carretera Querénd aro Mavavatio; FJ639849, FJ639884. *Anemone narcissiflora*, R. Meyers 88-8 (MICH); U. S. A., Alaska, Nome Quad, Anvil Mt.; AY055414, AY055393. *Anemone obtusiloba*, Cultivated, RBGE 19851867; India, Himachal Pradesh, Rhotang Pass; FJ639850, FJ639886. *Anemone parviflora*, S. Hoot 88-14 (MICH); Canada, Alberta, Banff NP, Sulphur Mt.; FJ639851, FJ639887. *Anemone pavonina*, Cultivated, RBGE 19821939; Greece, Samos, Mt. Kerkes; FJ639852, FJ639888. *Anemone quinquefolia*, S. Hoot 881 (MICH); U. S. A., Michigan, Washtenaw Co., Ann Arbor, Bird Hills Park; JF810696, JF810686. *Anemone ranunculoides*, S. Hoot s. n. (MICH); Cultivated, U. S. A., Michigan, Ann Arbor, H. Elkins Garden; FJ639853, FJ639889. *Anemone richardsonii*, C. L. Parker 9801 (ALA); U. S. A., Alaska, Shagway Quad; AY055409, AY055388. *Anemone rivularis*, S. Hoot 88-53 (MICH); Cultivated from seed, Germany, Jena, Bot. Garten der Friedrich-Schiller; AY055417, AY055396. *Anemone rupicola*, Cultivated, RBGE 19841895; Bhutan, Upper Mo Chu Dist., (Sinclair & Long); FJ639856, FJ639892. *Anemone sellowii*, L. B. Smith & R. Klein 7913 (NY); Brazil, Santa Catarina Mun., Bom Retiro; FJ639857, FJ639893. *Anemone somaliensis*, P. R. O. Bally & R. Melville 15763 (MO); Somalia, Galgallo, 64 km. SSW of Besaso, Al Mado Range; JF810697, JF810687. *Anemone sylvestris*, S. Hoot 88-54 (MICH); Cultivated from seed, Germany, Univ. of Bonn Bot. Garden; FJ639858, FJ639894. *Anemone tenuicaulis*, P. Garnock-Jones 2147 (CHR); New Zealand, Southland, Garvie Mt., N.E. Shore Lake Scott; AY055410, AY055389. *Anemone tenuifolia* 'var. *capensis*' (= *A. capensis*), P. Goldblatt 4357 (MO); South Africa, Western Cape Prov., Saw Edge Peak; JF810698, JF810688. *Anemone tenuifolia* 'var. *tenuifolia*' (typical *A. tenuifolia*), J. C. Manning s. n. (NBG); South Africa, Western Cape Prov., Table Mountain; JF810699, JF810689. *Anemone tetrasepala*, S. Hoot 01-7 (UWM); France, Grenoble, Rhône-Alpes, Station Alpine du Lautere; FJ639859, FJ639895. *Anemone thomsonii*, E. Knox 2513 (EA); Kenya, Aberdare Mts., Wanderi Track; JF810700, JF810690. *Anemone trullifolia*, Cultivated, RBGE 19812614; China, Dali Co., Cangshan, Huadianba (Sino-British Expedition); AY055416, AY055395. *Anemone tuberosa*, S. Hoot 95-5 (UWM); U. S. A., Texas, Val Verde Co.; FJ639861, FJ639897. *Anemone virginiana*, A. Reznicek 7706 (MICH); Canada, Simcoe Co., N. of Orillia; FJ639862, FJ639898. *Anemone vitifolia*, S. Hoot 90-51 (MICH), Cultivated, Berlin Bot. Garden, India, Himalaya, Shila Margi (Polunin); FJ639863, FJ639899. *Barneoudia balliana* = *A. balliana*, Arroyo 201443 (CONC); Chile, RM, Santuario de la Naturaleza Yerba Loca, Cordillera de los Andes; FJ639864, FJ639900. *Barneoudia chilensis* = *A. chilensis*, Arroyo & Humañá 25207 (CONC); Chile, Cordillera de los Andes, Cerro El Colorado; FJ639865, FJ639901. *Barneoudia major* = *A. major*, Arroyo & Humañá 25113 (CONC); Chile, Cordillera de los Andes, Sector alto de Valle Nevado; FJ639866, FJ639902. *Hepatica americana* = *A. americana*, S. Hoot 88-3 (MICH); U. S. A., Michigan, Oakland Co., Highland Recreation Area; AY055407, AY055386. *Hepatica nobilis* = *A. hepatica*, S. Hoot 00-19 (UWM); France, Grenoble, Rhône-Alpes, Station Alpine du Lautere; FJ639868, FJ639904. *Hepatica transsilvanica* = *A. transsilvanica*, M. Chase 10334 (K); Cultivated, England, Kew, Royal Botanical Gardens; FJ639869, FJ639905. *Knowltonia anemonoides* = *A. anemonoides*, J. C. Manning 3077 (NBG); South Africa, Western Cape Prov., Grootbos Nature Reserve; JF810701, JF810691. *Knowltonia bracteata* = *A. bracteata*, B. L. Burt 7727 (E); Cultivated, Scotland, Edinburgh, Royal Botanical Gardens, EBG19760898, Collected S. Africa, Natal Province; JF810702, JF810692. *Knowltonia capensis* = *A. knowltonia*, J. C. Manning 3229 (NBG); South Africa, Western Cape Prov., Signal Hill; JF810703, JF810693. *Knowltonia cordata* = *A. cordata*, R. D. A. Bayliss 7185 (MO); South Africa, Prov. Eastern Cape Prov., Niekersberg Forest Reserve; No seq., JF810694. *Knowltonia vesicatoria* = *A. vesicatoria*, Cultivated, Univ. of Copenhagen Bot. Garden, South Africa, Western Cape Prov., Cape Peninsula; AY055421, AY055401. *Oreithales integrifolia* = *A. integrifolia*, A. Hofreiter C36 (UWM); Peru, Dept. Ayacucho, above Quinua on road to Tambo; FJ639870, FJ639906. *Oreithales integrifolia*, A. Hofreiter C19 (UWM); Peru, Dept. Cusco, above Paucartambo on road to Pilcopata; FJ639871, FJ639907. *Pulsatilla halleri* = *A. halleri*, S. Hoot s. n. (MICH); Cultivated, U. S. A., Michigan, Ann Arbor, Hoot garden; FJ639872, FJ639908. *Pulsatilla occidentalis* = *A. occidentalis*, S. Hoot 88-17 (MICH); Canada, Alberta, Jasper National Park, Cavell Mt.; AY055426, AY055400.

**Outgroups:** *Clematis hexapetala*, S. Hoot 91-50 (MICH); Cultivated, U. S. A., North Carolina, Marion, WeDu Nurseries; AY055406, AY055385. *Clematis ochroleuca*, S. Hoot 90-50 (MICH); Cultivated, U. S. A., North Carolina, Marion, WeDu Nurseries; FJ639867, FJ639903.