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A phylogeny of Delphinieae (Ranunculaceae) shows that *Aconitum* is nested within *Delphinium* and that Late Miocene transitions to long life cycles in the Himalayas and Southwest China coincide with bursts in diversification

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A B S T R A C T

The tribe Delphinieae (Ranunculaceae) comprises two species-rich genera, *Aconitum* and *Delphinium*, the latter including *Consolida* and *Aconitella*. The 650–700 species are distributed in Eurasia and North America; three species occur on tropical African mountains. Maximum likelihood analyses of 2088 aligned nucleotides of plastid and nuclear sequences obtained from up to 185 species of Delphinieae from throughout the geographic range (plus relevant outgroups) show that three short-lived (facultative annual or biennial) Mediterranean species belonging to *Delphinium* subgenus *Staphisagria* are the sister clade to all other Delphinieae, implying that *Staphisagria* needs to be raised to genus status if *Delphinium* and *Aconitum* are to become mutually monophyletic. Molecular clock dating suggests an origin of the sampled Delphinieae in the Early Oligocene (c. 32.3 Ma) and expansion to North America of *Aconitum* and *Delphinium* around 3.3 and 2.9 Ma ago, respectively; the East African Mts. were reached by long-distance dispersal some 2.4 Ma ago, coincident with the major uplift of the East African Rift system. The ancestral growth form of the Delphinieae could not be reconstructed, but Late Miocene bursts in diversification rates in the Himalayan and southwestern Chinese clades of *Aconitum* and *Delphinium* appear to be associated with transitions from short-lived to long-lived life histories.

1. Introduction

The tribe Delphinieae comprises 650–700 species (Table 1), which amounts to some 25% of all Ranunculaceae (Tamura, 1993; also Watson and Dallwitz, 1992 onwards; Stevens, 2001 onwards; eFloras at http: www.eFloras.org). Its species share many anatomical and morphological characters, such as spurred or hooded zygomorphic flowers with hidden nectaries and characteristic seed ornamentation (Hoot, 1991; Johansson and Jansen, 1993, and references therein). Delphinieae are mostly holarctic, ranging from the Mediterranean basin to Korea and Japan, Siberia, and North America; a few species occur on mountains in tropical East and West Africa (Fig. 1). The tribe's center of diversity is in southwest China and the eastern Himalayas, where *Aconitum* has 166 species (Liangqian and Kadota, 2001) and *Delphinium* some 150 species (Wang and Warnock, 2001). Among Ranunculaceae, the tribe Delphinieae is relatively diverse in life histories, comprising strictly annuals, facultative annuals or biennials, perennials, and pseudo-annuals. In a pseudo-annual, the above ground parts die off after the first reproductive season, but the individual survives clonally via disconnected tubers (Krumbiegel, 2001); a pseudo-annual thus is quite different from an annual, which survives in the form of sexually produced seeds. Short-lived Delphinieae mostly occur in the Mediterranean and the xeric Irano-Turanian region, long-lived ones mostly in cold and wet high altitude areas in southeast Asia, but also in North America and on the tropical African mountains.

Many species of Delphinieae are of horticultural importance or medical use (e.g., Turabekova et al., 2010; Zhao et al., 2010), and a few are well-studied in terms of genetic diversity (Utelli et al., 1999; Zhang et al., 2005; Wang et al., 2009a; Orellana et al., 2009a,b) and floral morphology and pollination (Kosuge and Tamura, 1988; Bosch et al., 1997; Erbar et al., 1998; Jabbour et al., 2009). Phylogenetic relationships in the tribe, however, have never been properly analyzed. Here we address this deficiency, using plastid and nuclear DNA sequences from herbarium material covering the geographic range and the previously recognized generic and infra-generic taxa. We also use statistical area and state reconstruction, and molecular-clock dating, to infer the evolution of (i) life history, (ii) diversification rate changes, and (iii) the clade's geographic expansion through time.

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Table 1
Species number in the tribe Delphinieae as recorded from different sources.

Delphinium	*Aconitum*	*Consolida* + *Aconitella*	Total for Delphinieae	Source
365	–	–	–	Malyutin (1987)
c. 300	c. 300	c. 40	c. 640	Flora of North America (eFloras online, accessed 04 August 2011)
c. 320	c. 300	43	c. 663	Tamura, 1993
365	300	Included in *Delphinium*	665	Angiosperm Phylogeny Website (accessed 04 August 2011)
>350	c. 350	50	>750	Verlaque and Aboucaya, 2001
451	331	52	834	The Plant List (source: Tropicos and WCSP, accessed 04 August 2011)

Fig. 1. Distribution area of the Delphinieae (wavy white lines).

2. Materials and methods

2.1. Taxon sampling

Table 2 lists the 188 accessions of Delphinieae, representing 185 species, included in this study (out of 650–700 accepted species in the tribe), with author names for all species, geographic origin of the sequenced vouchers, and GenBank accession numbers. Eighty-four sequences were newly generated for this study, 163 were available from our work on *Consolida* and *Aconitella* (Jabbour and Renner, 2011a), and 157 were downloaded from GenBank. The new sequences mostly come from herbarium material, with the oldest specimen 160 years old (*Delphinium cheilanthum*). Sampling comprises 57 species of *Aconitum* L., three of *Aconitella* Spach, 13 species of *Consolida* S.F. Gray, and 112 of *Delphinium* L. including relevant type species, such as *Aconitum napellus*, *Aconitella aconiti*, *Consolida regalis*, and *Delphinum peregrinum* as well as the type species of all subgeneric names. The subgeneric classification accepted for *Delphinium* is that of Malyutin (1987). Malyutin's subgenus *Staphisagria* (J. Hill) Peterm. includes three short-lived species (all sampled; *Results* and *Discussion*); subgenus *Delphinium* section *Anthriscifolium* W.T. Wang includes a single annual species (sampled), while section *Delphinium* includes 15 annual and one perennial species (most of them sampled here). Malyutin's perennial subgenera *Delphinastrum* (DC.) Peterm. and *Oligophyllon* Dimitrova each have c. 150 species, of which we included 54 and 40 species, respectively, including their type species *Delphinum elatum* and *D. fissum*. All North African and tropical African species (15 and three, respectively) of these subgenera are included, while the American species are selectively represented based on the results of Koontz et al. (2004). For the African species *D. dasycaulon* (in Cameroon and Nigeria as well as East Africa), *D. leroyi*, and *D. macrocentron* (distributed from Sudan and Ethiopia to Malawi), we included two samples per species to represent their geographic range. For *Aconitum*, we followed the classification of Tamura (1990), sampling each of his three subgenera: The monospecific subgenus *Gymnaconitum* (Stapf) Rapaics is represented by *Aconitum gymnandrum*, a facultative annual or biennial species from China (Liangqian and Kadota, 2001; Utelli et al., 2000; Wang et al., 2009a); subgenus *Lycoctonum* (DC.) Peterm. (c. 50 species) is represented by nine species, all perennials; and subgenus *Aconitum* (c. 250 species) is represented by 47 species that are mostly pseudo-annual (Liangqian and Kadota, 2001). The five North American species are represented by *A. delphinifolium* and *A. columbianum*.

In all, 37% of the c. 300 species of *Delphinium*, 19% of *Aconitum*, 30% of *Consolida*, and 30% of *Aconitella* are included in this study. As outgroups, we included *Adonis annua*, *Helleborus niger*, and *Nigella damascena* based on the Ranunculaceae phylogeny of Wang et al. (2009b).

2.2. DNA isolation, amplification, sequencing, and alignment

Total genomic DNA was isolated from herbarium specimens and silica-dried leaves using the NucleoSpin plant kit (Macherey–Nagel, Düren, Germany). DNA isolation and sequencing relied on commercial kits and the universal primers of Taberlet et al. (1991) for amplifying and sequencing the *trn*L intron and adjacent *trn*L–*trn*F intergenic spacer. The internal transcribed spacer region