



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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ABSTRACT

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 south-western Chinese clades of *Aconitum* and *Delphinium* appear to be associated with transitions from short-lived to long-lived life histories.

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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.

<i>Delphinium</i>	<i>Aconitum</i>	<i>Consolida</i> + <i>Aconitella</i>	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 <i>Delphinium</i>	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 *Delphinium 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 *Oligophyllum* Dimitrova each have c. 150 species, of which we included 54 and 40 species, respectively, including their type species *Delphinium 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 gymnanthrum*, a facultative annual or biennial species from China (Liangqian and Kadota, 2001; Utelli et al., 2000; Wang et al., 2009a); subgenus *Lycotomum* (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 *trnL* intron and adjacent *trnL*–*trnF* intergenic spacer. The internal transcribed spacer region

Table 2

Species and genome regions sequenced, their sources, geographic origin, life cycle (A – annual, AB – facultative annual or biennial, P – perennial, PA – pseudo-annual), chromosome number (if known) and GenBank accession numbers. Information about chromosome numbers is from Simon et al. (1999), Yuan and Yang (2008), and references therein. – : missing data. Herbarium acronyms follow Index Herbariorum at <<http://sciweb.nybg.org/science2/IndexHerbariorum.asp>>.

Species	DNA source	Geographic origin	Life cycle	Haploid chromosome no.	ITS	<i>trnL</i> intron, <i>trnL</i> -F spacer
<i>Aconitella Spach</i>						
<i>A. aconiti</i> (L.) Soják	Buttler 20006 (M)	Turkey: Konya	A	?	JF331874	JF331679
<i>A. barbata</i> (Bunge) Soják	D. Podlech 11320 (M)	Afghanistan: Baghlan	A	8	JF331876	JF331681
<i>A. saccata</i> (Huth) Soják	P. Sintenis 1186 (M)	Kurdistan: Mardin	A	?	–	JF331683
<i>Aconitum L.</i>						
<i>A. albobolaceum</i> Kom.	Huang s.n. (PE)	China: Beijing	P	8	AY571363	–
<i>A. anthora</i> L.	Utelli & Horat (in Utelli et al., 2000), isolate 14417 (ZT)	France: Hautes-Pyrénées	PA	8	AF216547	–
<i>A. baicalense</i> Turcz. ex Rapaics	¹ 9027196 (TNS) ² Zedtwitz 1936 (M)	¹ Russia: Siberia ² Russia: Transbaikal	PA	8	¹ AB004941–5	² JF331723
<i>A. brunneum</i> Hand.-Mazz.	Yang 92025 (PE)	China: Sichuan	PA	?	AY571344	–
<i>A. cannabifolium</i> Franch. ex Finet & Gagnep.	1391652 (PE)	China: Anhui	PA	?	AY189801	–
<i>A. chienningense</i> W.T. Wang	Luo et al. (2002, unpublished)	–	PA	?	AY164646	AY164653
<i>A. chiisanense</i> Nakai	Kita 951000 Kita and Ito (2000)	Korea: Kyongsangnando	PA	8	AB004950-1	–
<i>A. chrysotrichum</i> W.T. Wang	Luo et al. (2002, unpublished)	–	P	?	AY164642	AY164649
<i>A. ciliare</i> DC.	¹ Kita 951120 (Kita and Ito, 2000) ² Herrmann 238 (M)	¹ Japan: Kumamoto ² China: Jilin	PA	8	¹ AB004952–4	² JF331724
<i>A. columbianum</i> Nutt.	N. Tsipoura 22 (WS)	USA: Idaho	PA	8, 9	AF258683	–
<i>A. crassiflorum</i> Hand.-Mazz.	¹ Y. Luo & Y.B. Luo 365 (PE) ² Luo et al. (2002, unpublished)	¹ China: Sichuan ² –	P	8	¹ AY150230	² AY150245
<i>A. decipiens</i> Vorosch & Anfalov	Wakab. et al. 9327102 (TNS)	Russia: Siberia	PA	8	AB004955-60	D86446
<i>A. delphinifolium</i> DC.	¹ Wells 1777 (WS) ² Coll. ign., 28.07.1996 (M)	¹ USA: Alaska: Kenai peninsula ² –	PA	8	¹ AF258681	² JF331725
<i>A. fengii</i> W.T. Wang	F.M. Zhang 00-003 (PE)	China: Yunnan	PA	8	AY189799	–
<i>A. ferox</i> Wall	¹ Minaki et al. 9100909 (TI) ² J. Pcelt 29 (M)	¹ Nepal: Mt. Shiwapuri ² Nepal: Khumbu	PA	10, 17	¹ AB004961–2	² JF331726
<i>A. finetianum</i> Hand.-Mazz.	Luo et al. (2002, unpublished)	–	P	?	AY164643	AY164650
<i>A. forrestii</i> Stapf	Luo et al. (2002, unpublished)	–	PA	8	AY164644	AY164651
<i>A. franchetii</i> Finet & Gagnep.	¹ Y. Luo & Y.B. Luo 390 (PE) ² Luo et al. (2002, unpublished)	¹ China: Sichuan ² –	PA	?	¹ AY150236	² AY150251
<i>A. gigas</i> H. Lév. & Vaniot	180011 (KANA)	Japan: Hokkaido	P	8	AB004963	D86453
<i>A. gymnantrum</i> Maxim.	¹ Luo et al. (2002, unpublished) ² B. Dickoré 9111 (MSB)	¹ – ² Tibet: Xizang	AB	8	¹ AY150238	² JF331727
<i>A. hemsleyanum</i> E. Pritz. ex Diels	¹ H.Z. Kong 99039 (PE) ² Luo et al. (2002, unpublished)	¹ China: Yunnan ² –	PA	8	¹ AY189793	² AY150252
<i>A. huiliense</i> Hand.-Mazz.	Y. Luo & Y.S. Chen 553 (PE)	China: Sichuan	PA	?	AY571362	–
<i>A. karakolicum</i> Rapaics	D.Y. Tan A002 (PE)	China: Xinjiang	PA	?	AY571358	–
<i>A. kitadakense</i> Nakai	191938 (KANA)	Japan: Yamanashi	PA	8	AB004977	D86463
<i>A. kusnezoffii</i> Rchb.	Q.E. Yang s.n. (PE)	China: Inner Mongolia	PA	8	AY571346	–
<i>A. legendrei</i> Hand.-Mazz.	Y. Luo & Y.S. Chen 448 (PE)	China: Sichuan	PA	?	AY571354	–
<i>A. liangshanicum</i> W.T. Wang	Y. Luo & Y.S. Chen 450 (PE)	China: Sichuan	PA	?	AY571364	–
<i>A. liljestrandii</i> Hand.-Mazz.	¹ Y. Luo & Y.B. Luo 344 (PE) ² Luo et al. (2002, unpublished)	¹ China: Sichuan ² –	PA	?	¹ AY164645	² AY164652
<i>A. lycocotum</i> L.	¹ M. Baltisberger & A. Widmer, isolate 13345 (Utelli et al., 2000) (ZT) ² Soininen et al. (2009) (no voucher)	¹ Romania: Zarnasti ² Norway	P	8	¹ AF216537	² GQ244533
<i>A. monanthum</i> Nakai	S. Ge 008 (PE)	China: Jilin	PA	?	AY189803	–
<i>A. nagarum</i> Stapf	¹ Y.B. Luo 468 (PE) ² Kadota 21473 (TNS)	¹ China: Yunnan ² –	PA	8	¹ AY571347	² D86450
<i>A. napellus</i> L.	Utelli & Soliva (in Utelli et al., 2000), isolate 14412 (ZT)	Italy: Lake Como	PA	82	AF216544	–
<i>A. nemorum</i> Popov	D.Y. Tan A001 (PE)	China: Xinjiang	PA	?	AY571361	–
<i>A. nipponicum</i> Nakai	180103 (KANA)	Japan: Nagano	PA	8	AB004989	D86462
<i>A. paskoi</i> Vorosch	Wakabayashi et al. 9327216 (TNS)	Russia: Siberia	PA	8	AB004997-5001	D86443
<i>A. pendulum</i> Busch	¹ Y. Luo & Y.B. Luo 411 (PE) ² D. Boufford et al. 40471 (MSB)	China: Sichuan	PA	8	¹ AY150235	² JF331728
<i>A. penterii</i> Hayek	D. Podlech & W. Lippert 26275 (M)	Serbia	PA	8	JF331905-18	JF331729
<i>A. piepunense</i> Hand.-Mazz.	F.M. Zhang 00-001 (PE)	China: Yunnan	PA	8	AY189800	–
<i>A. pilopetalum</i> W.T. & L.Q. Li	Y. Luo & Y.B. Luo 364 (PE)	China: Sichuan	PA	?	AY571343	–
<i>A. pulchellum</i> Hand.-Mazz.	¹ Y. Luo & Y.B. Luo 362 (PE) ² Luo et al. (2002, unpublished)	¹ China: Sichuan ² –	PA	8	¹ AY164647	² AY164654
<i>A. racemosum</i> Franch.	¹ Y. Luo & Y.B. Luo 360 (PE) ² Wang Wei 081 (PE)	¹ China: Sichuan ² China: Chongqing	PA	8	¹ AY150233	² FJ626533
<i>A. refractum</i> (Finet & Gagnep.) Hand.-Mazz.	Y. Luo & Y.B. Luo 406 (PE)	China: Sichuan	PA	?	AY571349	–
<i>A. sanyoense</i> Nakai	180095 (KANA)	Japan: Kyoto	PA	8	AB005002	D86263
<i>A. scaposum</i> Franch.	Luo et al. (2002, unpublished)	–	P	8	AY150231	AY150246

Table 2 (continued)

Species	DNA source	Geographic origin	Life cycle	Haploid chromosome no.	ITS	<i>trnL</i> intron, <i>trnL</i> -F spacer
<i>A. senanense</i> Nakai	191943 (KANA)	Japan: Yamanashi	PA	8	AB005006	D86461
<i>A. septentrionale</i> Koelle	¹ Yarosh. & Maslin. ZZT5 (Utelli et al., 2000) (ZT)	¹ Russia: Ural	P	8	¹ AF216552	² JF331730
	² Dietrich 5808 (M)	² Norway				
<i>A. sessiliflorum</i> (Finet & Gagnep.) Hand.-Mazz.	Luo et al. (2002, unpublished)	–	PA	?	–	AY164655
<i>A. sinomontanum</i> Nakai	Luo et al. (2002, unpublished)	–	P	8	–	AY150247
<i>A. spiripetalum</i> Hand.-Mazz.	Y. Luo & Y.B. Luo 393 (PE)	China: Sichuan	PA	?	AY571345	–
<i>A. sungpanense</i> Hand.-Mazz.	H.Z. Kong k1001 (PE)	China: Shaanxi	PA	?	AY189795	–
<i>A. tanguticum</i> (Maxim.) Stapf	¹ Y. Luo & Y.B. Luo 309 (PE)	¹ China: Sichuan	PA	?	¹ AY150234	² AY150249
	² Luo et al. (2002, unpublished)	² –				
<i>A. tongolense</i> Ulbr.	Y. Luo & Y.B. Luo 375 (PE)	China: Sichuan	PA	?	AY571350	–
<i>A. tsaii</i> W.T. Wang	Zhang 00–014 (PE)	China: Yunnan	PA	8	AY189784	–
<i>A. villosum</i> Rchb.	9237144 (TNS)	Russia: Siberia	PA	8	AB005010-1	D86442
<i>A. volubile</i> Muhl.	S. Ge 004 (PE)	China: Jilin	PA	8	AY189802	–
<i>A. yamazakii</i> Tamura & Namba	180040 (KANA)	Japan: Hokkaido	PA	?	AB005012	D86260
<i>A. yuparensis</i> Takeda	180022 (KANA)	Japan: Hokkaido	PA	8	AB005019-20	D86262
<i>Consolida</i> S.F. Gray						
<i>C. ajacis</i> (L.) Schur	H. Merxmüller 8524 (M)	Germany: Bavaria	A	8	JF331880	JF331687
<i>C. axilliflora</i> (DC.) Schrödinger	Brachf. & Graben 42414 (MSB)	Turkey: Hatay	A	8	JF331885	JF331692
<i>C. divaricata</i> Hayek	G. Fayvush 1600 (M)	Armenia	A	8	–	JF331716
<i>C. flava</i> (DC.) Schrödinger ex Hand.-Mazz.	K.H. Rechinger 148 (M)	Iraq: Haswa desert	A	?	JF331887	JF331695
<i>C. glandulosa</i> (Boiss. & A. Huet) Bornm.	Nydegger 16741 (M)	Turkey	A	8	JF331888	JF331696
<i>C. incana</i> (E.D. Clarke) Munz	Coll. ign., 25.06.1979 (MSB)	Israel	A	?	–	JF331699
<i>C. mauritanica</i> (Coss.) Munz	D. Podlech 47566 (MSB)	Morocco: High Atlas	A	8	JF331894	JF331704
<i>C. olopetala</i> Hayek	Nydegger 43795 (MSB)	Turkey: Erzincan	A	8	JF331895	JF331706
<i>C. orientalis</i> (J.Gay) Schrödinger	Parishani 14382 (M)	Iran: Isfahan	A	8	JF331896	JF331707
<i>C. persica</i> (Boiss.) Grossh.	K.H. Rechinger 42445 (M)	Iran: Kurdistan	A	7	JF331897	JF331708
<i>C. regalis</i> Gray	H. Hertel 6560 (M)	Austria	A	8	JF331900	JF331714
<i>C. stocksiana</i> Nevski	Volk 1585 (M)	Afghanistan: Kabul	A	8	JF331903	JF331720
<i>C. tenuissima</i> Soó	Coll. ign., 1937 (M)	Greece: Attica	A	?	–	JF331721
<i>Delphinium</i> L.						
<i>D. afghanicum</i> Rech.f.	Grötzbach 1 (MSB)	Afghanistan: Takhar	P	?	–	JN573529
<i>D. albocoeruleum</i> Maxim.	Trippner 191 (M)	China: Kansu	P	8	–	JN573530
<i>D. anthriscifolium</i> Hance	D. Podlech 55468 (MSB)	China: Shanxi	A	8	JF331919-30	JF331731
<i>D. aquilegifolium</i> (Boiss.) Bornm.	Manutsheri 1066 (MSB)	Iran: Mazandaran	P	?	–	JN573531
<i>D. bakeri</i> Ewan	Snow 1230 (RPBG)	USA: California	P	?	AF258697	AF258652
<i>D. balansae</i> Boiss. & Reut.	Sammet & Illitz s.n., 1991 (MSB)	Morocco: Marrakech	P	8	JF331931-44	JF331732
<i>D. balcanicum</i> Pawl.	Rössler 6570 (M)	Serbia	A	8	JF331945-54	JF331733
<i>D. barbeyi</i> Huth	Siplivisky & Beck 4314 (WS)	USA: Colorado	P	8	AF258709	AF258639
<i>D. beesianum</i> W.W. Sm.	Farrer & Purdom 237 (M)	China: Kansu	P	8	–	JN573532
<i>D. bicolor</i> Nutt.	P. Soltis 2383 (WS)	USA: Idaho	P	8	AF258711	AF258653
<i>D. bicornutum</i> Hemsl.	Mendoza 1905 (ARIZ)	Mexico: Tejupan-Coixtlahuaca	P	8	AF258701	–
<i>D. biternatum</i> Huth	D. Podlech 15909 (M)	Afghanistan: Baghlan	P	?	–	JN573533
<i>D. bonvalotii</i> Franch.	Wang Wei 030 (PE)	China: Guizhou	P	?	–	FJ626542
<i>D. brachycentrum</i> Ledeb.	Voron. & Derviz-Sokolova s.n., 1967 (M)	Russia: Magadan	P	8	JN573515	JN573534
<i>D. bulleyanum</i> Forrest ex Diels	Cultivated in Munich Bot. Garden, 1975 (M)	China: Yunnan	P	8	–	JN573535
<i>D. caeruleum</i> Jacquem.	J. Pcelt s.n., 19.09.1962 (M)	Nepal: Khumbu	P	8	–	JN573536
<i>D. cardinale</i> Hook.	Mort 1374 (no voucher)	USA: Santa Barbara	P	8	AF258740	AF258648
<i>D. carduchorum</i> Chowdhuri & P.H. Davis	Vogel s.n., 16.08.1968 (M)	Turkey: Hakkari	P	?	–	JN573537
<i>D. cashmerianum</i> Royle	Anders 8118 (MSB)	Afghanistan: Badakhshan	P	8	–	JN573538
<i>D. cheilanthum</i> Fisch. ex DC.	Leuchtenb. s.n., 1851 (M)	Russia: Siberia	P	8	–	JN573539
<i>D. cossonianum</i> Batt.	D. Podlech 46655 (MSB)	Morocco: Meknes	A	8	JF331955-64	JF331734
<i>D. crassifolium</i> Schrad. ex Spreng.	Boyko & Starch. s.n., 23.07.1979 (M)	Russia: Amur	P	8	–	JN573540
<i>D. crispulum</i> Rupr.	K.H. Rechinger 57118 (M)	Azerbaijan: Mt. Sahand	P	?	–	JN573541
<i>D. cuneatum</i> Steven ex DC.	Litvinov 7219 (MSB)	Russia: Samara	P	?	–	JN573542
<i>D. cyphoplectrum</i> Boiss.	S. Zarre & Zarrei 1042 (MSB)	Iran: Bushehr	P	8	–	JN573543
<i>D. dasycaulon1</i> Fresen	Mbala 592 (M)	Zambia	P	?	–	JN573544
<i>D. dasycaulon2</i> Fresen	Kahurananga 2639 (M)	Tanzania: Nwese Npanda	P	?	–	JN573545
<i>D. decorum</i> Fisch. & C.A. Mey.	J. Koontz 63 (WS)	USA: California	P	8	AF258744	AF258631
<i>D. delavayi</i> Franch.	McBeath et al. CLD0895 (UCBG)	China: Yulong Shan	P	8	AF258705	AF258659
<i>D. denudatum</i> Wall.	D. Podlech 31929 (MSB)	Afghanistan: Ghazni	P	8, 10	–	JN573546
<i>D. depauperatum</i> Nutt.	Richter 59 (WS)	USA: Oregon	P	?	AF258689	AF258656
<i>D. dolichostachyum</i> Chowdhuri & P.H. Davis	Thoma s.n., 1971 (M)	Turkey: Munzur-Gebirge	P	?	JN573516	JN573547
<i>D. dubium</i> (Rouy & Fouc.) Pawl.	H. Merxmüller & Wiedmann 285/64 (M)	Italy: Cuneo	P	8	–	JN573548
<i>D. elatum</i> L.	Skvortsov et al. 10597 (M)	Russia: Ural	P	8	JN573517	JN573549

(continued on next page)

Table 2 (continued)

Species	DNA source	Geographic origin	Life cycle	Haploid chromosome no.	ITS	trnL intron, trnL-F spacer
<i>D. elbursense</i> Rech.f.	Manutshehri 983 (MSB)	Iran: Gilan	P	?	–	JN573550
<i>D. emarginatum</i> C. Presl	H. Ross 303 (M)	Italy: Blermo	P	8	–	JN573551
<i>D. exaltatum</i> Aiton	Bright 18948 (WS)	USA: Pennsylvania	P	?	–	AF258651
<i>D. favargeri</i> C. Blanché, Molero & Simon Pall.	Jury & Ait Lafkih 19781 (M)	Morocco: Middle Atlas	A	8	JF331965–76	JF331735
<i>D. fissum</i> Waldst. & Kit.	K.H. Rechinger 21506 (M)	Greece: Epirus	P	8	–	JN573552
<i>D. flexuosum</i> M. Bieb.	Gagnide et al. 887 (MO)	Georgia: Khevi	P	8	–	JN573553
<i>D. freynii</i> Huth	G. Fayvush 1631 (M)	Armenia: Vayotsdзор	P	?	–	JN573554
<i>D. glaciale</i> Hook.f. & Thomson	Miyamoto et al. 9592453 (MO)	Nepal: Sagarmatha	P	?	–	JN573555
<i>D. gracile</i> DC.	Lambinon 79/E/534 (MSB)	Spain: Teruel	A	8	JF331977–81	JF331736
<i>D. gracilentum</i> Greene	M.J. Warnock (WS 356763)	USA: California	P	8	AF258763	–
<i>D. grandiflorum</i> L.	Erskine et al. SICH205 (UCBG)	China: Songpan to Juizhaigou	P	8	AF258761	AF258630
<i>D. griseum</i> Gilli	Volk 1873 (M)	Afghanistan: Kabul	P	?	–	JN573556
<i>D. gyalanum</i> C. Marquand & Airy Shaw	Cultivated in Munich Bot. Garden, 1975 (M)	Tibet	P	8	–	JN573557
<i>D. gypsophilum</i> Ewan	J. Koontz 36 (WS)	USA: Washington	P	8	AF258721	AF258633
<i>D. halteratum</i> Sibth. & Sm.	Dunkel MTB3647.4 (M)	Italy: L'Aquila	A	8	JF331982–7	JF331737
<i>D. hansenii</i> Greene	M.J. Warnock (WS 355163)	USA: California	P	8	AF258760	–
<i>D. hesperium</i> A. Gray	Raiche 20005 (UCBG)	USA: California	P	8	AF258772	AF258634
<i>D. hirschfeldianum</i> Heldr. & Holzm. ex Boiss.	Coll. ign. 1604, 1901 (M)	Greece: Mykonos	A	?	JF331988–95	JF331738
<i>D. incisum</i> Wall.	J. Pcelt s.n., 09.09.1962 (M)	Nepal: Okhaldunga	P	?	–	JN573558
<i>D. kamaonense</i> Huth	Wuendish 303 (MSB)	Nepal: Langtang	P	8	–	JN573559
<i>D. kansuense</i> W.T. Wang	Q.E. Yang & Q. Yuan 386 (IBSC)	China: Fin'an	P	8	–	JN573560
<i>D. kohatense</i> (Brühl) Munz	Anders 3567 (MSB)	Afghanistan: Paktia	P	?	–	JN573561
<i>D. kurdicum</i> Boiss. & Hohen.	K.H. Rechinger 11017 (M)	Iraq: Erbil	P	?	–	JN573562
<i>D. leroyi1</i> Franch. ex Huth	Richards 25743 (M)	Tanzania: Arusha	P	?	–	JN573563
<i>D. leroyi2</i> Franch. ex Huth	De Wilde 6743 (M)	Ethiopia: Bale	P	?	–	JN573564
<i>D. luteum</i> A. Heller	J. Koontz 102 (WS)	USA: California	P	?	AF258777	–
<i>D. maackianum</i> Regel	Zedtwitz s.n., 17.06.1972 (M)	Russia	P	8	–	JN573565
<i>D. macrocentron1</i> Oliv.	W. Rauh 410 (M)	Kenya: Mt. Kenya	P	8	–	JN573566
<i>D. macrocentron2</i> Oliv.	Greenway & Kamrui 14526 (M)	Kenya: Nasampolai	P	8	–	JN573567
<i>D. macropetalum</i> DC.	D. Podlech 55412 (MSB)	Morocco	A	8	JF331996–2000	JF331739
<i>D. macrostachyum</i> Boiss. ex Huth	K.H. Rechinger 43020 (M)	Iran: Kurdistan	P	?	–	JN573568
<i>D. middendorffii</i> Trautv.	Korol. & Rjab. 6025 (M)	Russia: Magadan	P	8	–	JN573569
<i>D. minjanense</i> Rech.f.	Breckle 2998 (MSB)	Afghanistan: Kunar	P	?	–	JN573570
<i>D. montanum</i> DC.	Kalheber 85–2044 (M)	France: Pyrénées Orientales	P	8	JN573518	JN573571
<i>D. multiplex</i> (Ewan) C.L. Hitchc.	Hitchcock & Muhlick (WS 230765)	USA: Washington	P	?	AF258714	AF258654
<i>D. muscosum</i> Exell & Hillc.	A. Kress s.n., 1967 (M)	–	P	?	JN573519	JN573572
<i>D. nanum</i> DC.	Zubizaretta 42606 (M)	Spain: Marbella	A	8	JN573520	JN573573
<i>D. nevadense</i> Kunze	Bertel 11109 (M)	Spain: Granada	P	?	–	JN573574
<i>D. nudicaule</i> Torr. & A. Gray	J. Koontz 96–20 (WS), pop 4	USA: California	P	8	AF258728	AF258638
<i>D. nuttallianum</i> Pritz.	Strickler 91/2–6 (WS)	USA: Washington	P	8	AF258688	AF258646
<i>D. obcordatum</i> DC.	Nowotny 61.12.18 (M)	Spain: Torremolinos	A	8	JN573521	JN573575
<i>D. ochotense</i> Nevski	Soininen et al. (2009) (no voucher)	Norway	P	?	–	GQ244830
<i>D. oreophilum</i> Huth	Vassiljeva s.n. 19.08.1960 (M)	Uzbekistan	P	?	–	JN573576
<i>D. orthocentrum</i> Franch.	Luo et al. (2002 unpublished)	–	P	8	AY150242	AY150257
<i>D. oxycentrum</i> W.T. Wang	Luo et al. (2002, unpublished)	–	P	?	AY150240	AY150255
<i>D. oxysepalum</i> Pax & Borbás	Angerer s.n. 11.07.1994 (M)	Slovakia	P	8	JN573522	JN573577
<i>D. pachycentrum</i> Hemsl. ex Brühl	Luo et al. (2002, unpublished)	–	P	8	AY150243	AY150258
<i>D. parishii</i> A. Gray	Richter 53 (WS)	USA: California	P	8	AF258716	AF258635
<i>D. parryi</i> A. Gray	Edwards (RPB 88.240)	USA: California	P	8	AF258694	AF258636
<i>D. patens</i> Benth.	Richter 11 (WS)	USA: California	P	8	AF258734	AF258658
<i>D. pentagynum</i> Lam.	Lewalle 10967 (MSB)	Morocco: Rabat	P	8	JN573523	JN573578
<i>D. peregrinum</i> L.	Franzén et al. 870 (M)	Macedonia	A	8	JF332001–8	JF331740
<i>D. pictum</i> Willd.	Rumsey 15012 (M)	Balearic Islands: Majorca	AB	8	–	JF331741
<i>D. polycladon</i> Eastw.	R.C. Bacigalupi 6508 (WS)	USA: California	P	8	AF258743	AF258642
<i>D. potaninii</i> Huth	Q.E. Yang & Q. Yuan 805 (IBSC)	China: Zhuxi	P	8	–	JN573579
<i>D. pylzowii</i> Maxim.	Farrer & Purdom 253 (M)	China: Kansu	P	8	–	JN573580
<i>D. pyramidale</i> Royle	A. Kress s.n., 1966 (M)	–	P	?	JN573524	JN573581
<i>D. recurvatum</i> Greene	Brown (WS 355165) pop 36	USA: California	P	8	AF258775	AF258632
<i>D. requienii</i> DC.	Gavelle s.n., 12.06.1961 (M)	France: Porquerolles Island, Hyères	AB	8	–	JN573582
<i>D. saniculifolium</i> Boiss.	K.H. Rechinger 3838 (M)	Iran: Kerman	P	?	–	JN573583
<i>D. semibarbatum</i> Bien. ex Boiss.	Belianina et al. 9174 (MSB)	Russia: Kuhitang Mtns.	P	8	–	JN573584
<i>D. sparsiflorum</i> Maxim.	Trippner s.n., 1936 (M)	China: Kansu	P	?	JN573525	JN573585
<i>D. staphisagria</i> L.	Vitek 02–205 (M)	Greece: Crete	AB	8, 9	JF332022–3	JF331743
<i>D. suave</i> Huth	Peer s.n., 04.08.1993 (MSB)	Pakistan: Chitral	P	?	–	JN573586
<i>D. sutchuenense</i> Franch.	D. Boufford et al. 39837 (MSB)	China: Sichuan	P	?	JN573526	JN573587
<i>D. sylvaticum</i> Turcz.	Lager s.n., 18.07.1909 (G)	Algeria: Kabylia, Alger	P	8	–	JN573588
<i>D. szowitsianum</i> Boiss.	K.H. Rechinger 43500 (M)	Azerbaijan: Khalkal	P	?	–	JN573589
<i>D. tangkulaense</i> W.T. Wang	Q.E. Yang & Q. Yuan 409 (IBSC)	China: Madoi	P	8	–	JN573590

Table 2 (continued)

Species	DNA source	Geographic origin	Life cycle	Haploid chromosome no.	ITS	<i>trnL</i> intron, <i>trnL</i> -F spacer no.
<i>D. tatsienense</i> Franch.	A. Kress s.n. (no collection date) (M)	China	P	8	–	JN573591
<i>D. thibeticum</i> Finet & Gagnep.	Rock 17338 (M)	China: Yunnan	P	8	–	JN573592
<i>D. trichophorum</i> Franch.	D. Boufford et al. 39746 (MSB)	China: Sichuan	P	8	–	JN573593
<i>D. tricorne</i> Michx.	Vincent 7734 (WS)	USA: Ohio	P	8	AF258770	–
<i>D. trollifolium</i> A.Gray	Edwards (RPBG 94.73)	USA: California	P	8	AF258686	AF258650
<i>D. tsarongense</i> Hand.-Mazz.	Handel-Mazzetti 7934 (M)	Tibet: Tsarong	P	?	–	JN573594
<i>D. tuberosum</i> Aucher ex Boiss.	K.H. Rechinger 47131 (M)	China: Sichuan	P	?	–	JN573595
<i>D. venulosum</i> Boiss.	Nydegger 15452 (MSB)	Turkey	A	?	JF332024-9	JF331744
<i>D. verdunense</i> Balb.	Krach & Koepff 3814 (MSB)	Morocco: Meknes	A	8	JN573527	JN573596
<i>D. virgatum</i> Poir.	S. Zarre 53 (MSB)	Turkey: Kayseri	A	8	JF332030-1	JF331745
<i>D. viride</i> S. Watson	Nesom 4969 (TEX/L)	Mexico: Chihuahua	P	?	AF258685	–
<i>D. viscosum</i> Hook.f. & Thomson	J. Pcelt s.n. 13.09.1962 (M)	Nepal: Okhaldunga	P	?	JN573528	JN573597
<i>D. wendelboi</i> Iranshahr	Anders 6143 (MSB)	Afghanistan: Baghlan	P	?	–	JN573598
Outgroups						
<i>Adonis annua</i> L.	Després A.an.JP2	In cultivation at Jardin des Plantes, Paris, France	A	?	AY148280	AY149010-1
<i>Helleborus niger</i> L.	McLewin K4, WM9519 (K)	Slovenia	P	8	AJ347898	AJ413290
<i>Nigella damascena</i> L.	¹ 041773 (MJG)	¹ In cultivation at Mainz Univ. Bot. Garden	A	6	¹ EU699446	² AY150260
	² Luo et al. (2002, unpublished)	² –				

(ITS-1-5.8S-ITS-2) of the nuclear ribosomal DNA was amplified with the primers of Balthazar et al. (2000). Direct amplification via polymerase chain reaction (PCR) yielded single bands and unambiguous base calls. PCR products were purified and sequenced, using the same primers. Sequencing relied on Big Dye Terminator kits (Applied Biosystems, Foster City, CA, USA) and an ABI 3100 Avant capillary sequencer (Applied Biosystems). Where multiple haplotypes of cloned ITS sequences had been reported in earlier studies, consensus sequences were computed using the program g2cef (Göker and Grimm, 2008). Sequence assembly of forward and reverse strands was carried out in MEGA v. 5 (Tamura et al., 2011), and all sequences were aligned with MAFFT v. 6 (Katoh et al., 2009), followed by manual adjustment. The alignment has been deposited in TreeBASE (www.treebase.org; accession number 12117).

2.3. Phylogenetic analyses

Tree searches relied on maximum likelihood (ML) as implemented in RAxML v. 7.2.6 (Stamatakis, 2006; Stamatakis et al., 2008). Analyses of the separate plastid and nuclear data partitions produced no topological discordance with ML bootstrap proportions >60%, and the data were therefore concatenated, yielding a matrix of 2088 characters and 191 taxa. Substitution model parameters were estimated separately for each partition, using the GTR + G model (with 4 rate categories) for the *trnL* region and the GTR model for the ITS region, these models being selected by FindModel (<http://hcv.lanl.gov/content/sequence/findmodel/findmodel.html>), which uses the ModelTest script (Posada and Crandall, 1998). Statistical support for nodes was assessed by bootstrapping the data under the same models (500 replicates).

2.4. Analysis of life history evolution

To analyse life history evolution, we used a reduced dataset of 73 species of Delphinieae that excluded species with identical or very short genetic branch lengths or that were widespread. Information about species' life history was compiled from literature and herbarium material, and we then defined four states: (i) annual, (ii) pseudo-annual (genetically a perennial; Section 1), (iii) perennial, and (iv) facultative annual or biennial. The last was the case for the three species of *Delphinium* subgenus *Staphisagria* and the single species of subgenus *Gymnaconitum*, which have vari-

ously been described as annual or biennial (Davis, 1965; Chater, 1993; Utelli et al., 2000; Bosch et al., 2001; Liangqian and Kadota, 2001; Orellana et al., 2009b; Wang et al., 2009a). In a separate run, we re-coded the 16 pseudo-annuals as perennials. To infer ancestral states, we used maximum likelihood as implemented in Mesquite v. 2.74 (Maddison and Maddison, 2009). Analyses were carried out on the preferred highest-likelihood tree, taking into account branch lengths, and using the Markov k-state one-parameter model, which is a generalization of the Jukes-Cantor model (Lewis, 2001) and assumes a single rate for all transitions between character states.

2.5. Molecular clock analyses

Divergence dating used on the same 73-species matrix and the program BEAST v. 1.6.2 (Drummond et al., 2006; Drummond and Rambaut, 2007), which employs a Bayesian Markov chain Monte Carlo (MCMC) approach to co-estimate topology, substitution rates, and node ages. All dating runs relied on the GTR + G model (with four rate categories), a Yule tree prior, and strict or relaxed clocks with rate variation across branches uncorrelated and log-normally distributed. The MCMC chains were run for 20 million generations (burn-in 20%), with parameters sampled every 5000th step. Results from individual runs were combined, and the program Tracer v. 1.5 (Rambaut and Drummond, 2007) was used to check that effective sample sizes for all relevant parameters were well above 200 and that stationarity probably had been reached. Final trees were edited in FigTree v. 1.3.1 (<http://tree.bio.ed.ac.uk/software/figtree/>). The crown group ages of *Consolida/Aconitella* and of *Delphinium* (including *Consolida/Aconitella* but excluding the three species of subgenus *Staphisagria*) have previously been estimated as 19.1 ± 2.7 Ma and 23.01 ± 3 Ma old (Jabbour and Renner, 2011a, using cross-validated calibration approaches), and these ages are here employed as secondary calibration points, with normal prior distributions and standard deviations of 3 Ma. These secondary calibrations were used because the phylogenetic placement of the few available Ranunculaceae fossils is too imprecise to use them as calibration points (cf. Jabbour and Renner, 2011a). A striking Ranunculaceae-like fossil from the Early Cretaceous Yixian Formation represents an extinct taxon along the stem lineage of this extant family (Sun et al., 2011) and can therefore not constrain the crown group of Ranunculaceae.

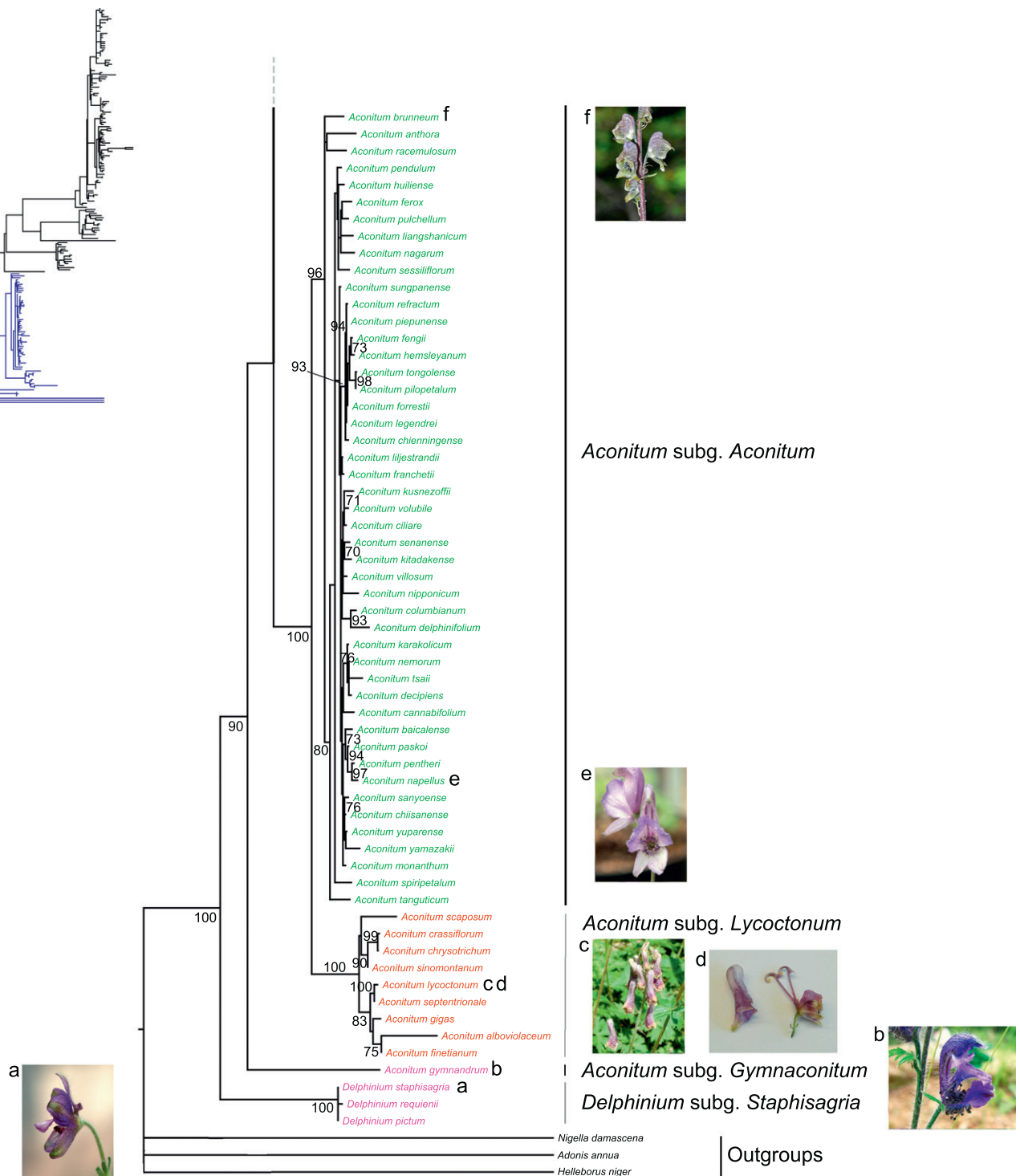


Fig. 2a. Partial maximum likelihood tree for Delphinieae obtained from 2088 aligned nucleotides of plastid and nuclear sequences. Likelihood bootstrap values $\geq 70\%$ are indicated. A tree overview is shown in the upper left-hand corner. Species names are colored according to life history: green: pseudo-annual, red: perennial, and pink: facultative annual or biennial. (a) *Delphinium staphisagria*, (b) *Aconitum gymmandrum*, (c) *A. lycoctonum*, (d) dissected flower of *A. lycoctonum* showing the hooded tepal and the two nectaries, e: *A. napellus*, (f) *A. brunneum*. Pictures: (a, c–e) F. Jabbour; (b and f) G. Lei.

2.6. Biogeographical analyses

To reconstruct the biogeographic history of Delphinieae, we used two methods, Bayesian ancestral area reconstruction (AAR) performed in BEAST and maximum likelihood inference performed in Mesquite. The Bayesian AAR relied on the 73-species Delphinieae dataset also used for life history reconstruction and dating. We used the continuous-time Markov chain (CTMC) model speci-

fied by Lemey et al. (2009) to infer discrete realizations of changes among four areas ($K = 4$): Asia, the broad Mediterranean region (including Europe and northern Africa), North America, and the East African Mts. The information about the current location of the species came from literature and herbarium material. The CTMC model is equivalent to the GTR model for nucleotide substitutions and allows for $K(K - 1)/2$ free parameters, i.e., diffusion rates. The model incorporates two main parameters, a relative

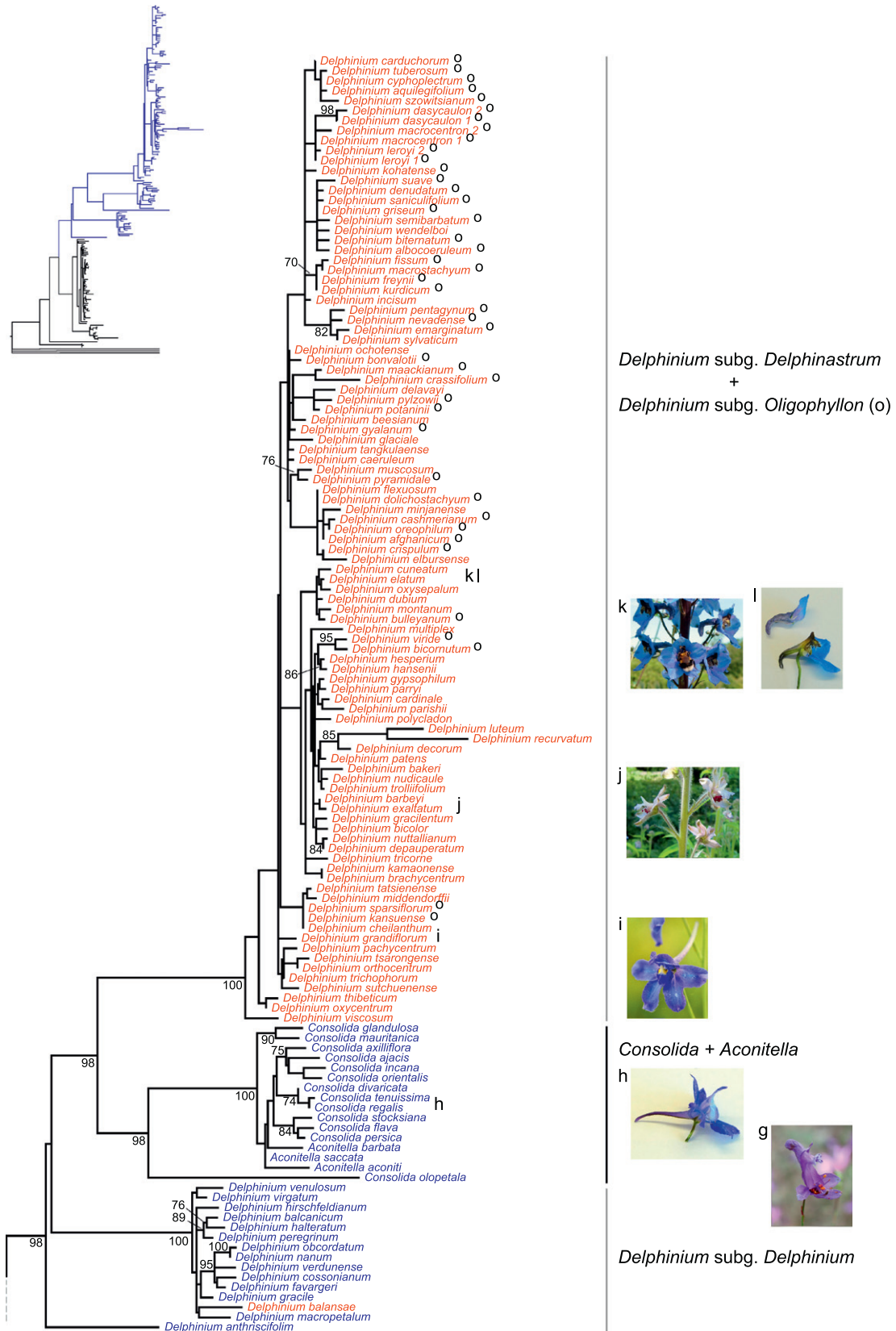


Fig. 2b. Continuation of the tree in (2a). A tree overview is shown in the upper left-hand corner. Species belonging to *D.* subg. *Oligophyllum* are marked with a black circle. Species names are colored according to their life history: red: perennial, blue: annual. (g) *Aconitella hohenackeri*, (h) *Consolida regalis*, (i) *Delphinium grandiflorum*, (j) *D. exaltatum*, (k) *D. elatum*, (l) dissected flower of *D. elatum* showing the spurred tepal and the two nectaries. Pictures: (g) A. Gröger; (h, j–l) F. Jabbour; (i) G. Lei.

rate parameter describing the frequency of spatial diffusion between two locations in relation to other location transitions and

a so-called geosite model parameter rescaling spatial transitioning in time units. We chose a gamma prior distribution (shape = 1.0)

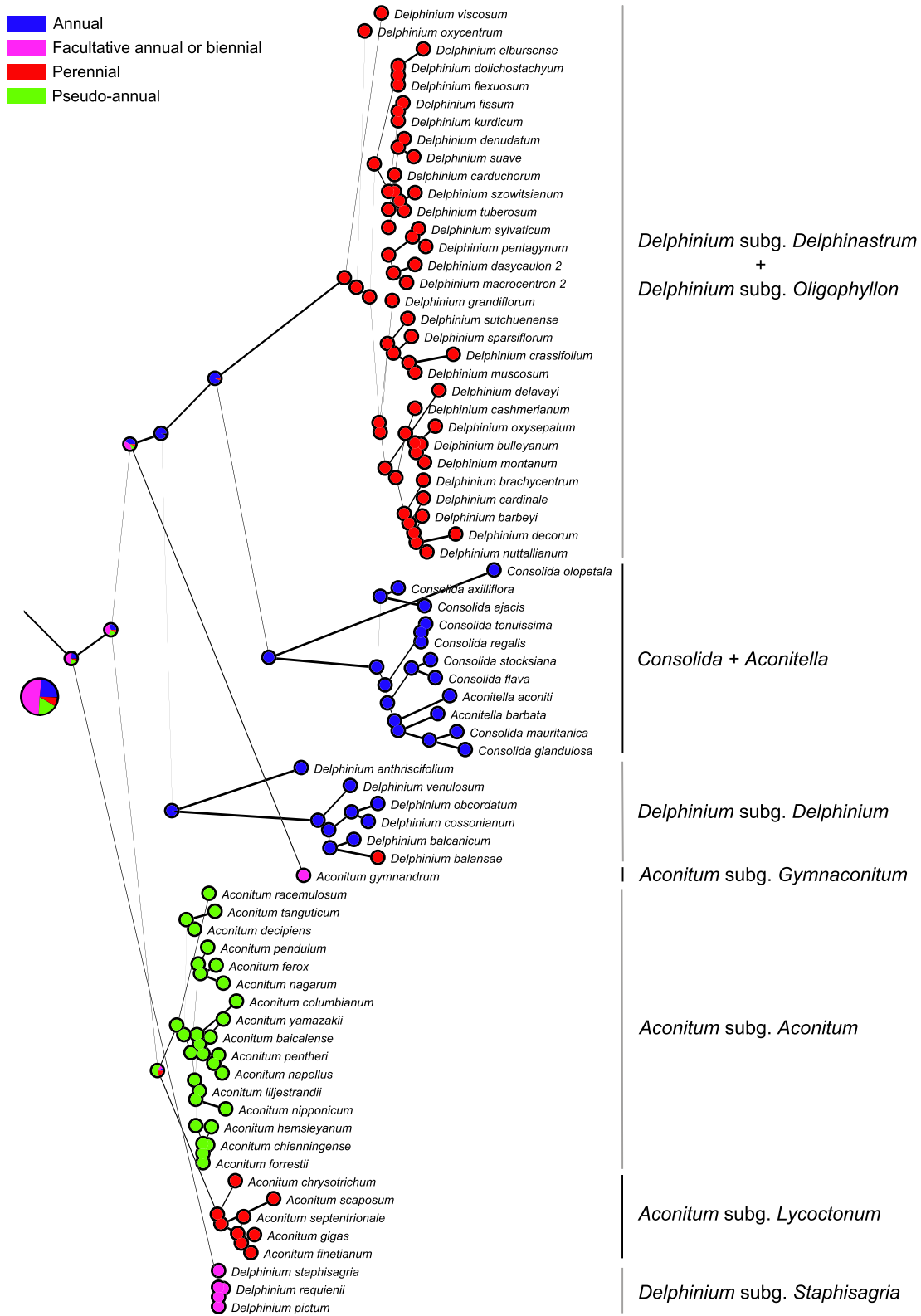


Fig. 3. The evolution of life histories in the Delphinieae inferred on a maximum likelihood tree for 73 species from 1990 aligned nucleotides of plastid and nuclear sequences.

for the relative rate parameter and an exponential prior (mean = 1) for the geosite parameter, following recommendations by Lemey et al. (2009). MCMC chain lengths, sampling frequencies and other settings were as detailed in Section 2.5 (Molecular clock analyses). Maximum likelihood AAR in Mesquite used the same input tree

and Markov k-state one-parameter model as used for life history reconstruction (Section 2.4). Transition parameters were estimated based on the tip trait states (i.e., regions assigned to the species). Geographic regions were coded as an unordered multi-state character, using the same four states as for the Bayesian AAR.

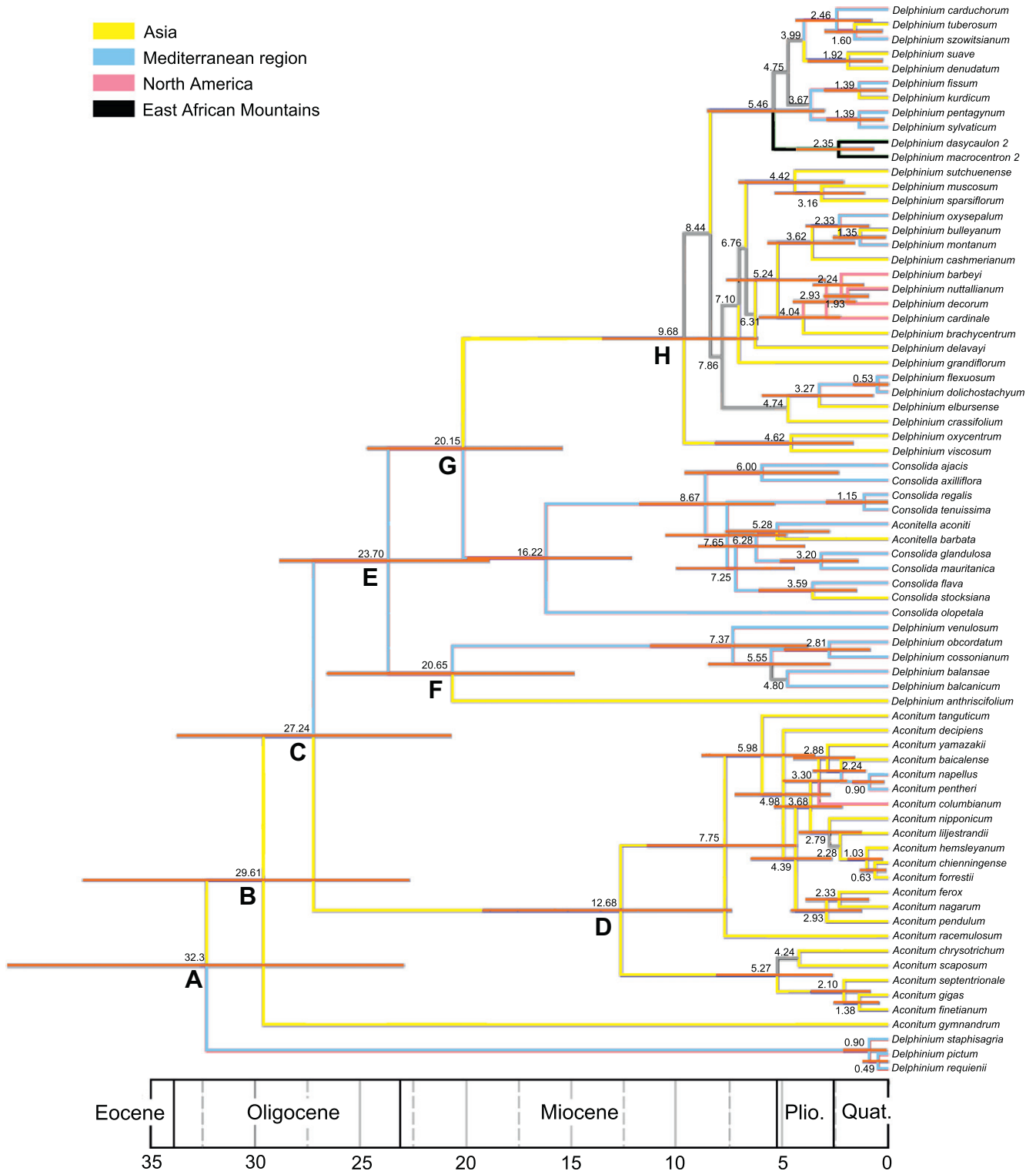


Fig. 4. Bayesian ancestral area reconstruction for the Delphinieae inferred on a Bayesian relaxed-clock chronogram from the same data as used in Fig. 3. Bars around node ages indicate the 95% highest posterior density interval for nodes with a posterior probability >0.95.

2.7. Net diversification rates

Net diversification rates (i.e., the result of speciation minus extinction) were calculated following Eq. (7) of Magallón and Sanderson (2001) for crown groups under the assumptions of either no extinction ($\epsilon = 0$) or high relative extinction ($\epsilon = 0.9$) to evaluate relative species diversification between clades. The equation

uses the full species number in a clade of interest and its age as inferred from a molecular clock model (Table 4). Although these rates may have little to do with real diversification rates, they may be comparable between closely related clades that diversified in the same region and over the same time period. Calculations were done using the Geiger R package (Harmon et al., 2008).

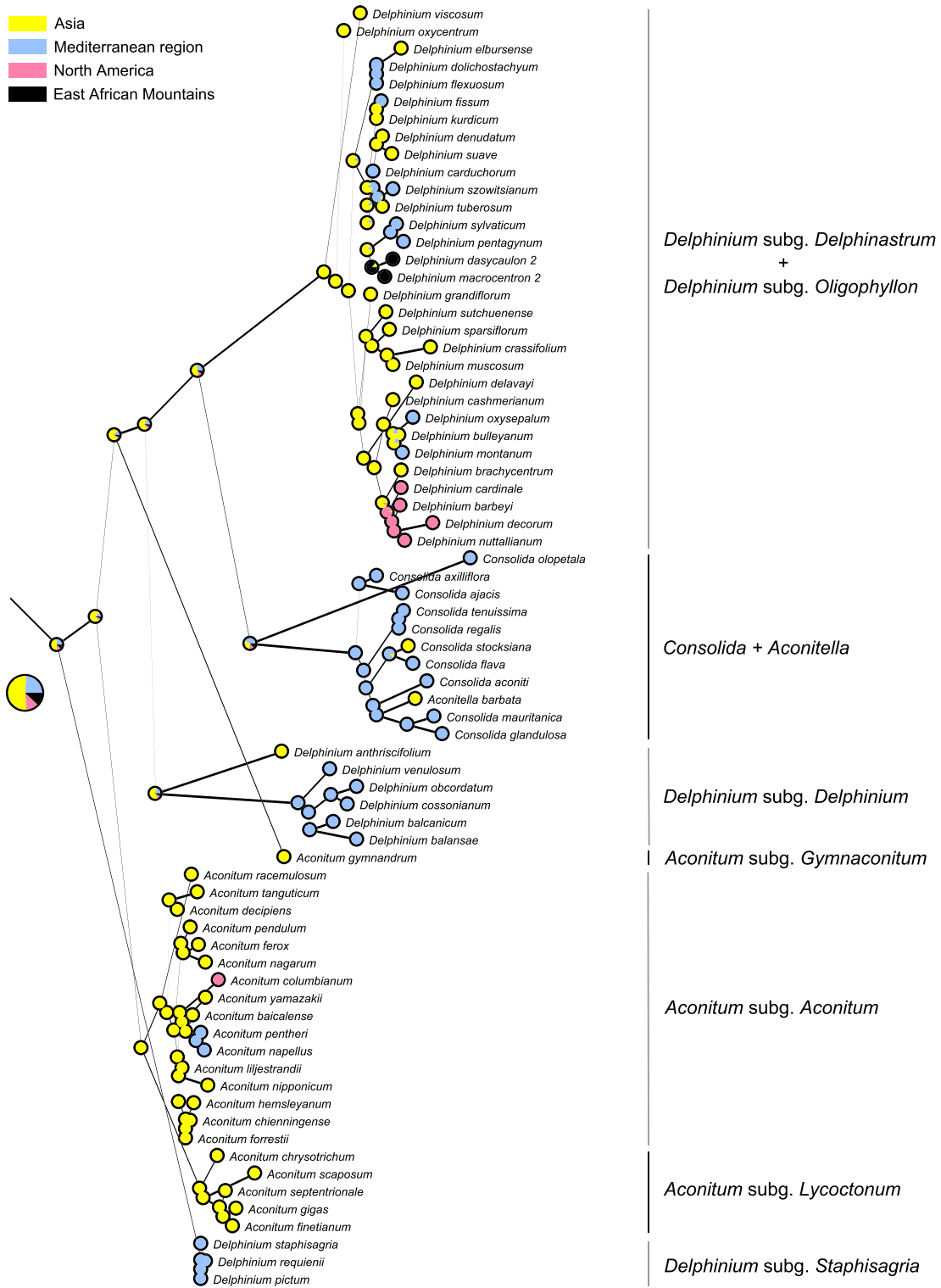


Fig. 5. The historical biogeography of the Delphinieae inferred on the preferred maximum likelihood 73-taxon tree under maximum likelihood optimization.

3. Results

3.1. Phylogenetic analyses

The plastid dataset consisted of 1424 aligned nucleotides, obtained for 159 accessions representing 156 species of Delphinieae.

The nuclear dataset consisted of 664 aligned nucleotides and 122 species of Delphinieae. The combined matrix of 2088 aligned nucleotides thus contained 25% empty cells. The maximum likelihood tree from the combined data (Figs. 2a and 2b) shows that the three species of *Delphinium* subgenus *Staphisagria* form the sister clade to all other Delphinieae, that *Aconitum* as currently

circumscribed is very likely monophyletic, and that the subgenera *Lycoctonum* and *Aconitum* are sister clades. *Consolida* and *Aconitella* are nested within *Delphinium* as found previously (Jabbour and Renner, 2011a), and the species that Malyutin (1987) assigned to the subgenera *Delphinastrum* and *Oligophyllum* (marked by the letter o in Fig. 2b) are intermixed in a single clade. Malyutin's *Delphinium* subgenus *Delphinium* (17 species, 15 of them here included) is monophyletic, and the single species of section *Anthriscifolium* is sister to the species placed in section *Delphinium*. The three species represented by multiple accessions (*D. dasycaulon*, *D. leroyi*, *D. macrocentron*, Fig. 2b) are monophyletic. Additional nuclear sequences of *A. gymnantrum* (GenBank accession numbers FJ418136 to FJ418148) also all clustered together (tree not shown).

3.2. Life history evolution

The ancestral state for the Delphinieae remained ambiguous, with short life histories (facultative annual or biennial growth) having a likelihood of 51%, strictly annual growth 24%, pseudo-annual growth 17%, and perennial growth 8%. When the pseudo-annual species were re-coded as perennials (reconstruction not shown), the inferred ancestral states became facultative annual/biennial with a likelihood of 46%, strictly annual 22%, and perennial (including pseudo-annual) 32%. Perennial and pseudo-annual life histories apparently evolved from short life histories in *Aconitum* subgenera *Lycoctonum* and *Aconitum*. The early-diverging subg. *Delphinium* comprises annuals, with the exception of *Delphinium balansae*, a perennial species nested among short-lived species. The *Consolida/Aconitella* clade is annual, and its sister clade consists of perennials (Fig. 3, top section of tree).

3.3. Biogeography and divergence times

Ancestral area reconstruction (AAR) under Bayesian (Fig. 4) or maximum likelihood (Fig. 5) optimization yielded similar results. The ancestral area of the tribe Delphinieae remained ambiguous, with the probabilities being: Asia 49% with Bayesian AAR (52% with ML AAR), Mediterranean 48% (24%), North America 2% (12%), and East African Mts. 1% (12%; see Table 3). *Aconitum* is inferred as having originated in Asia (Figs. 4 and 5) and to have col-

onized Europe and North America from there (Figs. 4 and 5). The early-diverging subg. *Delphinium* is distributed in the Mediterranean region (including the Irano-Turanian region). The only exception is *Delphinium anthriscifolium* which is found in China. The *Delphinastrum/Oligophyllum* clade is mostly Asian, but also colonized North America and the East African Mts. as well as expanding towards the Mediterranean basin (Fig. 4 and 5). Whether the ancestor of the tropical African *Delphinium* clade originated from North Africa or Asia is still unclear.

Divergence time estimates for Delphinieae are shown in Fig. 4. The split between the *Staphisagria* clade and the remaining members of the tribe, i.e. crown Delphinieae, is estimated as 32.3 Ma old (95% CI 23.0–41.8). Expansion to Asia in *D. anthriscifolium* and the ancestor of the *Delphinastrum/Oligophyllum* clade apparently occurred sometime after 20.7 (14.9–26.6) and 20.2 (14.4–24.7) Ma. The pseudo-annual subgenus *Aconitum* (c. 250 species) began diversifying around 7.8 (4.4–11.5) Ma ago and the perennial *Delphinastrum/Oligophyllum* clade (c. 300 species) around 9.7 (6.2–13.6) Ma ago. The dispersals to North America are dated to 2.9 (1.6–4.5) Ma (*Delphinium*) and 3.3 (2.0–5.0) Ma (*Aconitum*) and that to East Africa to 2.4 (0.7–4.4) Ma (*Delphinium*).

3.4. Net diversification rates

The diversification rates for selected nodes that were comparable in age and distributional range are shown in Table 4. The *Delphinastrum/Oligophyllum* clade and the *Aconitum* subgenera *Aconitum* and *Lycoctonum* experienced high net diversification, with respective rates of 0.35–0.52, 0.41–0.62, and 0.33–0.61 species per million years (Section 2, assuming either homogeneously high extinction or no extinction).

4. Discussion

The present phylogeny of the Delphinieae includes about 25% of the estimated species in the tribe (Table 1), with sampling designed to represent the geographic range and major morphological groups. Previous studies had suggested that *Aconitum* and *Delphinium* were sister groups, but the imbalanced sampling of these genera precluded testing their mutual monophyly (Kita and Ito, 2000; Utelli et al., 2000; Koontz et al., 2004; Wang et al.,

Table 3

Probabilities associated with each of the four areas, as estimated by the Bayesian ancestral area reconstruction applied to the Delphinieae dataset.

Nodes	Asia	Mediterranean area	North America	East African Mountains
A	0.4935	0.4784	0.0166	0.0115
B	0.5155	0.4691	0.0093	0.0061
C	0.5276	0.4643	0.0060	0.0021
D	0.8954	0.1019	0.0018	0.0009
E	0.4706	0.5264	0.0021	0.0009
F	0.4341	0.5614	0.0033	0.0012
G	0.4447	0.5532	0.0015	0.0006
H	0.9171	0.0829	–	–

Table 4

Net diversification rates calculated for crown group (CG) ages obtained by divergence dating using a relaxed molecular clock approach.

Clades	Species number	Clade CG age	Net diversification rates (Ma ⁻¹)			
			$\epsilon = 0$	95% HPD	$\epsilon = 0.9$	95% HPD
Subg. <i>Delphinastrum</i> + <i>Oligophyllum</i>	300	9.68 [6.19–13.56]	0.52	0.37–0.81	0.35	0.25–0.55
<i>Consolida</i> + <i>Aconitella</i>	52	16.22 [12.16–19.96]	0.20	0.16–0.28	0.11	0.09–0.14
Subg. <i>Delphinium</i>	17	20.65 [14.88–26.60]	0.10	0.08–0.14	0.04	0.03–0.06
Subg. <i>Aconitum</i>	250	7.75 [4.35–11.45]	0.62	0.42–1.11	0.41	0.28–0.74
Subg. <i>Lycoctonum</i>	50	5.27 [2.64–8.15]	0.61	0.40–1.22	0.33	0.21–0.65

2009a,b). Our results now reveal that for *Aconitum* and *Delphinium* to become mutually monophyletic, *Staphisagria* needs to be recognized as a separate genus (Fig. 6; Jabbour and Renner, 2011b). Its three species indeed possess traits that do not fit *Delphinium*, such as production of C19 aconitine-type alkaloids (De La Fuente and Reina, 1990), stomata and pollen similar to *Aconitum* not *Delphinium* (Blanché, 1991), and spontaneous selfing (Bosch et al., 2001). They also have eight chromosome pairs of staggered size (see Fig. 3 in Verlaque and Aboucaya, 2001), while most other species of *Delphinium* have two long and six short chromosome pairs (Gregory, 1941; Kurita, 1955; Blanché and Simon, 1987; Yang, 1996, 2001), except for a few dysploid species in *Consolida/Aconitella* (Blanché et al., 1997). After resurrection of the genus *Staphisagria* J. Hill (Jabbour and Renner, 2011b), the Delphinieae now consist of three natural genera: *Staphisagria* (three species), *Aconitum* (c. 300 spp.), and *Delphinium* (c. 350 spp., including *Consolida* and *Aconitella*, which will require about a dozen name changes because names in *Delphinium* are available for most species of *Consolida* and *Aconitella*).

Based on our dating results, the Delphinieae evolved in the Early Oligocene, but their ancestral area cannot yet be inferred because their sister-group is unresolved. Our reduced sampling of 73 species could also have led to an overly simplistic view of the biogeographic history of the tribe (Figs. 4 and 5). The inferred time for the split between *Aconitum gymnandrum* and the remaining species of *Aconitum* (c. 30 Ma, Fig. 4) matches roughly with the age inferred for this node by Wang et al. (2009a) when they used a low

average ITS substitution rate to obtain absolute ages (38.6 ± 3.3 Ma). Our inferred time for the split between the two *Aconitum* subgenera (c. 13 Ma) does not match as well with their estimate (20.7 ± 2.52 Ma), but species sampling in the two studies is quite different. These latter two clades, namely subgenus *Aconitum* (c. 250 species) and subgenus *Lycoctonum* (c. 50 species), both centered in the Qinghai-Tibetan Plateau, Sichuan, and Yunnan, apparently experienced increased diversification simultaneous with the acquisition of perennial or pseudo-annual growth (both involving the multi-annual persistence of individual plants) at around 5.3 and 7.8 Ma ago (Fig. 4; Table 4). By that time, the Tibetan Plateau had more or less its present elevation (Molnar and Stock, 2009), and the East Asian monsoon system already brought wet summers (Molnar et al., 2010), favorable for perennials.

The same correlations as in *Aconitum* are observed in *Delphinium*: The evolution of perennial growth and expansion in Asia at c. 9.7 Ma (Fig. 4) appear associated with a burst of diversification (Table 4). Rapid diversification in mountain ranges and the evolution of perennial growth are also correlated in the Neotropical genus *Lupinus* (Drummond, 2008; Drummond et al., in press). Cold high-altitude habitats may generally favor perennial growth, as exemplified also by the case of *D. balansae* in subg. *Delphinium*, which grows at 2400–2800 m, while related annual species grow below 1500 m (Munz, 1967; and information from herbarium notes). Longer vegetative growth (over several years) may be required to invest in extensive root systems and tubers in the pseudo-annual *Aconitum* species (Krumbiegel, 2001). In contrast,

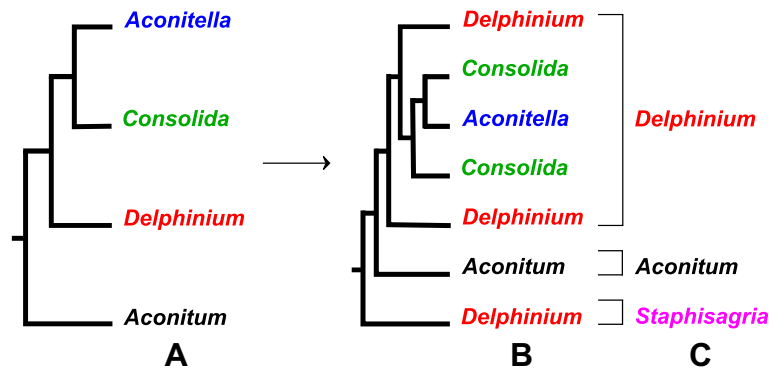


Fig. 6. Summary of the phylogenetic relationships in the Delphinieae (a) as suggested by previous studies, (b) as revealed by our molecular data, and (c) as proposed to achieve mutually monophyletic genera.

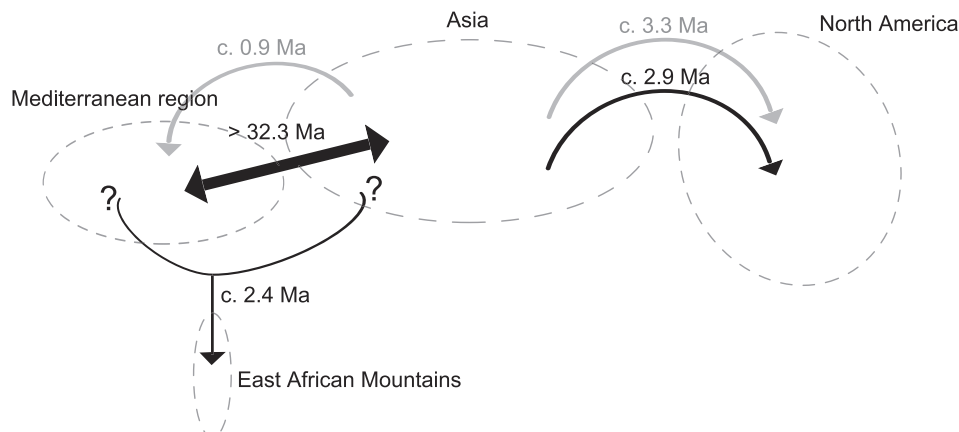


Fig. 7. Key divergence times and dispersal events in the Delphinieae involving their major regions of diversity, the Mediterranean region, Asia, North America and the East African Mts. Gray: *Aconitum*. Black: *Delphinium*.

an annual life cycle may often be favored under strongly seasonal rainfall (Bosch et al., 1997, 2001), fitting with Raunkiaer's (1918) finding that annuals are especially common in the Mediterranean.

Delphinium and *Aconitum* crossed into North America during the Pliocene (Figs. 4 and 7). Of the five American *Aconitum* species, only two are included here (the sister species *A. columbianum* and *A. delphinifolium*), both belonging to subgenus *Aconitum* (Fig. 2a). Future work will need to add the three other American aconites (viz. *A. maximum* and *A. uncinatum* from subg. *Aconitum* and *A. reclinatum* from subg. *Lycocotnum*). At around 3 Ma, when *Aconitum* and *Delphinium* colonized North America, the Bering Strait was already submerged (Brigham-Grette, 2001), and the mean annual temperature at the coldest location in Beringia was c. -8°C (Table S8 in Vila et al., 2011). Today, several American species of *Aconitum* and *Delphinium* occur between 1000 and 3600 m alt. in the Californian mountains (Warnock, 1996), where they sustain 6 months with temperatures below zero (<http://www.usclimatedata.com/>).

The East African mountains were colonized by *Delphinium* between 0.7 and 4.4 Ma ago (Figs. 4 and 7) during the major uplift of the East African Rift system (dated between 2 and 5 Ma, Sepulchre et al., 2006). All three East African *Delphinium*, *D. dasycaulon*, *D. leroyi*, and *D. macrocentron*, are perennials and grow at altitudes of 1000 to 3400 m, mostly in moist grasslands and open woodlands (Hutchinson et al., 1954; Munz, 1967; Agnew, 1974). The mountains in Nigeria and Cameroon were only colonized by *D. dasycaulon* (Hutchinson et al., 1954; Munz, 1967), but our sampling does not include a West African specimen, so we cannot infer the source of the West African colonization and the level of genetic divergence.

The phylogenetic and biogeographic framework for the Delphinieae provided here implies evolutionary transitions from annual to perennial growth that would be interesting to address with comparative ecophysiological approaches, using common garden experiments with annual, perennial, or pseudo-annual species of known relationships. Another desideratum is a better-resolved phylogeny of the Ranunculaceae that would identify the sister group of Delphinieae, resulting in more confident ancestral area and life history reconstructions.

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