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The evolutionary history of *Boloria* (Lepidoptera: Nymphalidae): phylogeny, zoogeography and larval-foodplant relationships

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Research Article

The evolutionary history of *Boloria* (Lepidoptera: Nymphalidae): phylogeny, zoogeography and larval–foodplant relationships

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Boloria is restricted to cooler regions of the northern hemisphere, and represents a model for understanding recent diversification of phytophagous insects in the Holarctic region. We infer the phylogeny of the genus based on characters from adult morphology and three genes (COI, EF1- α and *wingless*). We revise the subgenus level classification, and divide the genus into three subgenera, corroborating earlier works. The largest subgenus, *Clossiana*, is divided into nine informal species groups. A relaxed Bayesian clock analysis combined with a dispersal–vicariance analysis shows that the genus originated and first diversified in the Central Palaearctic region in the Middle Eocene. This region has remained important throughout the evolution of the genus, but independent diversifications also occurred in the Nearctic region. At least nine independent dispersal events between the Palaearctic and Nearctic regions are needed to explain the current distribution of the genus, whereas vicariance appears to have played only a minor role. An analysis of the evolution of larval–foodplant associations shows that although monophagous Violaceae-feeding is the original feeding strategy within *Boloria*, numerous host-shifts or broadenings of diet have happened. This inherent plasticity in host choice has likely been an important factor in the diversification in alpine and arctic environments by *Boloria*.

Key words: adaptation, alpine environment, arctic environment, dispersal, divergence time, Holarctic

Introduction

The Holarctic nymphalid butterfly genus *Boloria* Moore (Fig. 1) has been the subject of systematic study and discussion for the better part of a century. The major controversies have related to the total number of recognized species versus subspecies (e.g. Warren, 1944; dos Passos & Grey, 1945; Higgins, 1975), and whether one, two or three genera should be recognized (e.g. Reuss, 1926; Warren *et al.*, 1946). Recently Simonsen (2005) presented a preliminary phylogeny of the genus based on genitalia of both sexes, and we refer to that publication for a more detailed discussion of the older literature. In contrast to other old world authors, Simonsen (2005) treated *Proclassiana* Reuss and *Clossiana* Reuss as subgenera of *Boloria* s.l. He included 28 species in the phylogenetic analysis, but acknowledged that his taxon sampling was far from comprehensive. He also found that

genitalia morphology was insufficient to resolve the internal phylogeny of the subgenera, and suggested that a study of additional characters was much needed. Tuzov & Bozano (2006) revised the taxonomy of the Palaearctic part of the genus and listed the species found outside the region. They recognized the three genera normally used by Old World authors (*Proclassiana*, *Boloria* and *Clossiana*) with 1, 17 and 28 species respectively. Pelham (2008), in his catalogue of the butterflies of the USA and Canada, followed earlier New World authors as well as Simonsen (2005) in treating all species of *Boloria* s.l. as members of a single genus, *Boloria*. However, he disagreed with Simonsen's decision to give subgenus status to *Proclassiana* and placed the single species *P. eunomia* (Esper) in the subgenus *Boloria*. Pelham (2008) recognized two North American species of *Boloria* s.str. (incl. *eunomia*), and 12 North American species of *Clossiana*, disagreeing with Tuzov & Bozano (2006) on the status of *C. selene myrina* (Cramer), a North American taxon given full species status by the latter authors, but not by Pelham.

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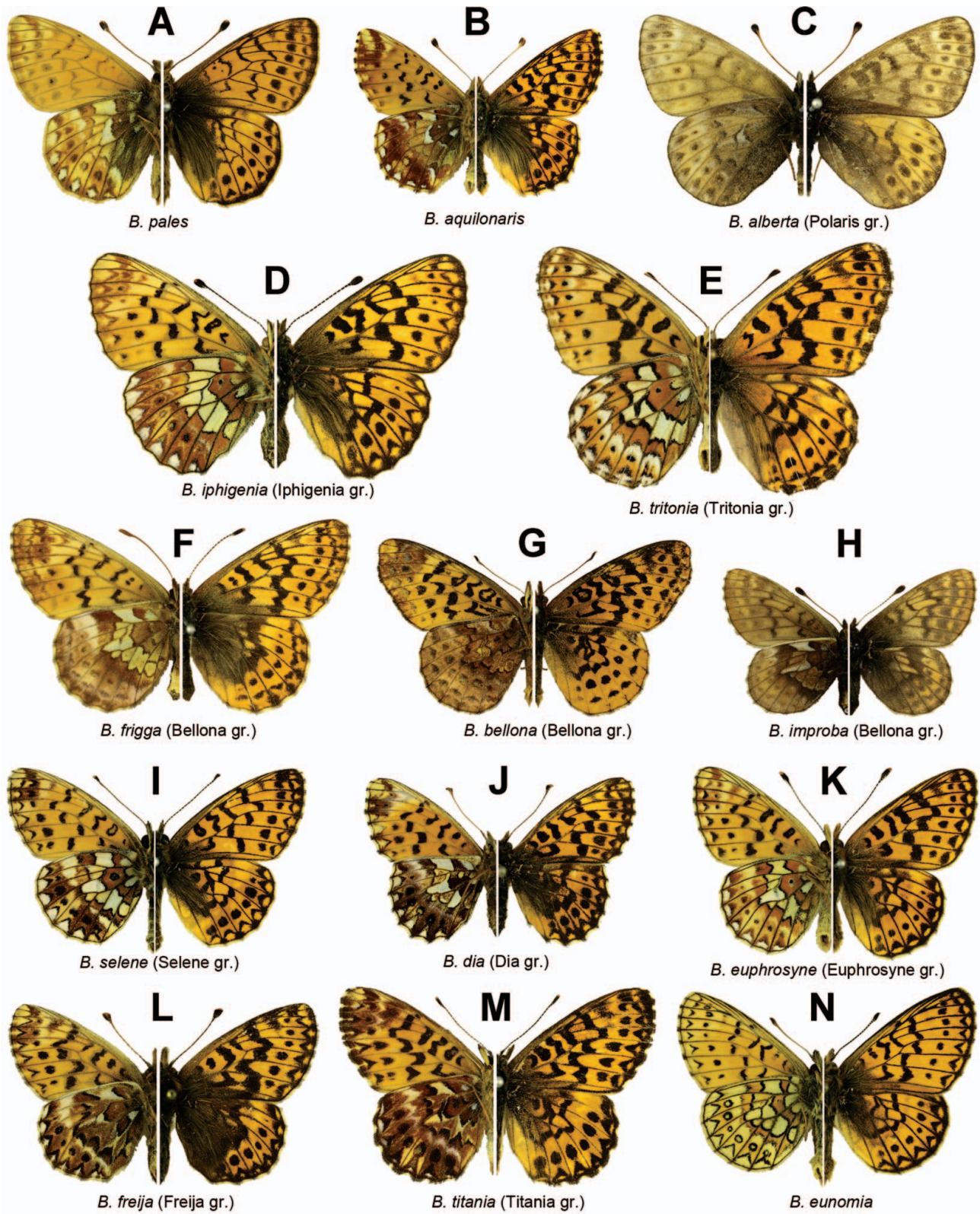


Fig. 1. Subspecies and species group diversity within *Boloria* sensu lato, left halves are underside, right halves are upper sides. **A–B.** *Boloria* sensu stricto. **C–M.** subgenus *Clossiana* N. subgenus *Proclassiana*.

Here we present a phylogenetic analysis of *Boloria* s.l. at the species level based on adult morphology in a re-examination of Simonsen's (2005) dataset and molecular characters from three genes. The phylogenetic results are subsequently used to address key questions about the historical zoogeography of the genus (incl. divergence times) and evolution of larval–foodplant associations. With their predominately montane, alpine or arctic distribution, *Boloria* species could be of conservation concern given current global climate change. Understanding the effect earlier climate changes have had on the genus could therefore aid in understanding of the effect that future climate changes could have on this and other groups of cold temperate/arctic groups of terrestrial invertebrates.

Materials and methods

Taxon sampling

A total of 81 specimens belonging to 37 potential species (out of 46 currently recognized species) were included in the analyses (Table 1). The taxon sampling is based partly on Simonsen (2005) and the most recent treatments of the genus in the Palaearctic and Nearctic regions (Tuzov & Bozano, 2006; Pelham, 2008). We strove to include as many species as possible, but some rarely collected species, especially from the Palaearctic region, were not available. Generally only species where both molecular and morphological data were available (for at least one gene and one sex respectively) were included in the analyses – the only exception is *B. (C.) astarte distincta* for which no morphological material was available. One species included by Simonsen (2005), *B. (C.) jerdoni* (Lang), was not available for molecular studies and left out of the analyses. Thirteen taxa not included in Simonsen (2005) were available for both molecular and morphological studies and were included here.

Re-examination of the photographs, dissection notes and drawings used by Simonsen (2005) revealed that the *B. (C.) kriemhild* (Strecker) specimens in that study were actually misidentified members of the North American *B. (C.) chariclea/titania* complex (confirmed by re-examinations of the pinned specimens by TJS). *B. (C.) kriemhild* from the E. H. Strickland Museum of Entomology were therefore dissected and used for scoring *B. (C.) kriemhild* for the present study.

The *B. (C.) titania/chariclea*, *B. (C.) improba/acrocneuma* and *B. (C.) freija/natazhati* species complexes in the subgenus *Clossiana* were considered to be of particular interest. These groups were therefore sampled more extensively to cover as much of their geographical and/or taxonomic range as possible. *Issoria lathonia* (L.) and *Yramea cytheris* (Drury) were used as outgroups since both genera are closely related to *Boloria* (Simonsen, 2006a; Simonsen *et al.*, 2006).

Voucher specimens and DNA extractions are deposited in the E. H. Strickland Museum of Entomology, Department of Biological Sciences, University of Alberta, and the Laboratory of Genetics, Department of Biology, University of Turku; some vouchers are in the collections of ADW, S. Van Campen (California) or D. Lawrie (Edmonton). Voucher images and label information are available online at the UASM website (<http://www.biology.ualberta.ca/facilities/strickland/Vouchers/>), and the Nymphalidae Systematics Group website (<http://nymphalidae.utu.fi/>).

Molecular methods

The mitochondrial gene COI, and the nuclear genes EF-1 α and *wingless* were sequenced for species not already available on GenBank. Genomic DNA was extracted from legs or thoracic tissue using the QIAGEN QIAamp DNA mini kit. PCRs were performed in 20 μ l volume reactions. Primers were generally from the Nymphalidae Systematics Group (<http://nymphalidae.utu.fi/>) or Simonsen *et al.* (in press), but a new internal reverse primer was designed for *wingless* (3'-TTG CAC CGT TCC ACT ACG-5') to successfully amplify problematic specimens. The general cycling and sequencing protocols followed Simonsen *et al.* (in press). Consensus sequences from the two sequencing directions were constructed using Sequencer 4.1. Consensus files were aligned using Clustal X (Thompson *et al.*, 1997) and checked for alignment quality. Sequence alignments were imported into MacClade and converted to NEXUS format.

Morphological methods and character scorings

The morphological character list and scorings are largely based on the matrix in Simonsen (2005). Specimens added in this study were dissected as described therein. The characters listed by Simonsen (2005) were re-examined for new taxa, and all old characters and potentially new characters were re-assessed by TJS.

Phylogenetic and divergence time analyses

Three different data combinations were analysed: morphology, combined molecular data, and total evidence (morphology and molecular data combined). All data sets were analysed in TNT (Goloboff *et al.*, 2008) using maximum parsimony (MP). Bremer Support values (BS) (Bremer, 1988; Bremer, 1994), and Partitioned Bremer Support values (PBS) (Baker & DeSalle, 1997; Baker *et al.*, 1998; Gatesy *et al.*, 1999) were calculated using the scripting feature in TNT (see Peña *et al.*, 2006; Simonsen *et al.*, 2006). The molecular dataset was subjected to a partitioned Bayesian likelihood analysis in the program BEAST v1.4.8

Table 1. Specimens included in this study, with extraction numbers, label data and GenBank accession numbers provided.

Species/ID#	Locality	COI	EF1-alpha	<i>wingless</i>
<i>Issoria lathonia</i> NW118-22	Pic de Nore, Aude, France	Genbank #	Genbank #	Genbank #
<i>Yramea cytheris</i> CH-10B-4	Chile	DQ922864	DQ922886	DQ922822
<i>Boloria (P) eunomia</i> FS-b-2407	Bragg Creek, AB, Canada	DQ922858	DQ922890	DQ922826
<i>Boloria (P) eunomia</i> FS-b-2456	Dempster Highway, YU, Canada	HQ161226	HQ161298	HQ161171
<i>Boloria (P) eunomia</i> FS-b-4203	Oxford Co., ME, USA	HQ161228	HQ161300	HQ161173
<i>Boloria (P) eunomia</i> NW151-13	Altai Mts., Siberia, Russia	HQ161248	HQ161320	HQ161191
<i>Boloria (P) eunomia</i> TS-11	AB, Canada	HQ161271	HQ161337	HQ161202
<i>Boloria (B) alaskensis</i> FS-b-2453	Pink Mountain, BC, Canada	DQ922869	DQ922801	DQ922837
<i>Boloria (B.) altiaca</i> FS-b-2465	Aktash Village, Siberia, Russia	HQ161227	HQ161299	HQ161172
<i>Boloria (B.) aquilonaris</i> TS-19	Sjælland, Denmark	HQ161230	HQ161302	HQ161175
<i>Boloria (B.) caucasica</i> FS-b-4215	Albga Mtn., Sochi dist., Russia	DQ922867	DQ922899	DQ922835
<i>Boloria (B.) generator</i> FS-b-4211	Tian Shan, Kirgizia	HQ266647	HQ266653	HQ266641
<i>Boloria (B.) graeca</i> FS-b-4220	Mt. Vamous, Pissoderi, Greece	HQ266648	HQ266654	HQ266642
<i>Boloria (B.) napaea</i> NW96-16	Kola Peninsula, Russia	HQ161252	HQ161324	HQ161193
<i>Boloria (B.) pales</i> NW151-21	Apennines, Italy	GQ864746	GQ864840	GQ864434
<i>Boloria (B.) pales</i> TS-20	Abruzzo, Italy	HQ161277	HQ161343	HQ161206
<i>Boloria (B.) palina</i> NW151-4	Tschingling Mts., Shanxi, China	DQ922866	DQ922898	DQ922834
<i>Boloria (B.) sifanica</i> NW144-12	N Sichuan, China	HQ161280	HQ161346	HQ161209
<i>Boloria (C.) alberta</i> FS-b-3757	Prospect Mtn., AB, Canada	HQ161266	HQ161334	-
<i>Boloria (C.) angarensis</i> NW151-7	Habarovska, Russia	HQ161243	HQ161315*	HQ161186
<i>Boloria (C.) astarte</i> FS-b-2461	Pink Mountain, BC, Canada	HQ161283	HQ161349	HQ161212
<i>Boloria (C.) astarte distincta</i> FS-b-4225	Dempster Highway, YU, Canada	HQ161229	HQ161301	HQ161174
<i>Boloria (C.) bellona</i> NY2	New York, USA	HQ161254	HQ161326	-
<i>Boloria (C.) chariclea</i> FS-b-2404	Itaska, AB, Canada	HQ161290	HQ161356	AF246530
<i>Boloria (C.) chariclea</i> FS-b-2474	Johnson Co., WY, USA	HQ161223	HQ161295	HQ161168
<i>Boloria (C.) chariclea</i> FS-b-3722	Stone Mountain PP, BC, Canada	HQ161231	HQ161303	HQ161176
<i>Boloria (C.) chariclea</i> FS-b-3723	Stone Mountain PP, BC, Canada	HQ266649	HQ266655	HQ266643
<i>Boloria (C.) chariclea</i> FS-b-3727	Stone Mountain PP, BC, Canada	HQ266650	HQ266656	HQ266644
<i>Boloria (C.) chariclea</i> FS-b-3727	Stone Mountain PP, BC, Canada	HQ266651	HQ266657	HQ266645
<i>Boloria (C.) chariclea</i> FS-b-3739	Sisimut, Greenland	HQ161234	HQ161306	HQ161178
<i>Boloria (C.) chariclea</i> FS-b-3740	Sisimut, Greenland	HQ161235	HQ161307	HQ161179
<i>Boloria (C.) chariclea</i> FS-b-3742	Amikfik, Greenland	HQ161236	HQ161308	HQ161180
<i>Boloria (C.) chariclea</i> FS-b-3754	Steese Highway, AK, USA	HQ161241	HQ161313	HQ161184
<i>Boloria (C.) chariclea</i> FS-b-3755	Dempster Highway, YU, Canada	HQ161242	HQ161314	HQ161185
<i>Boloria (C.) chariclea</i> FS-b-4201	Wathcom Co., WA, USA	HQ161247	HQ161319	HQ161190
<i>Boloria (C.) chariclea</i> FS-b-4208	Mt. Wash, NH, USA	HQ161250	HQ161322	HQ161192
<i>Boloria (C.) chariclea</i> FS-b-4222	Teton Co., WY, USA	HQ161253	HQ161325	HQ161194
<i>Boloria (C.) chariclea</i> FS-b-4232	Bragg Creek, AB, Canada	HQ161257	HQ161329	-
<i>Boloria (C.) chariclea</i> FS-b-4245	North Chatham, NB, Canada	HQ161259	HQ161331	HQ161196
<i>Boloria (C.) chariclea</i> FS-b-4247	North Chatham, NB, Canada	HQ161260	HQ161332	HQ161197
<i>Boloria (C.) chariclea</i> NW142-2	Chaffee Co., CO, USA	HQ161265	HQ161333	HQ161198
<i>Boloria (C.) chariclea</i> NW151-20	Schackenbergl, Greenland	HQ161276	HQ161342	HQ161205
<i>Boloria (C.) dia</i> JL5-6	Villanua, Aragon, Spain	HQ161262	-	-
<i>Boloria (C.) dia</i> NW156-24	Pradelles, Aude, France	HQ161287	HQ161353	HQ161216
<i>Boloria (C.) epithore</i> FS-b-3735	Waterton Lakes NP, AB, Canada	HQ161233	HQ161305	HQ161177
<i>Boloria (C.) epithore sierra</i> FS-b-4228	Tulare Co., CA, USA	HQ161255	HQ161327	-
<i>Boloria (C.) epithore sierra</i> FS-b-4230	Tulare Co., CA, USA	HQ161256	HQ161328	HQ161195
<i>Boloria (C.) erda</i> NW151-11	Anurskaya Province, Russia	HQ161269	HQ161336	HQ161200
<i>Boloria (C.) erubescens</i> NW151-5	Ganishou, Tadjikistan	HQ161281	HQ161347	HQ161210
<i>Boloria (C.) euphrosyne</i> JL5-11	Villanua, Aragon, Spain	HQ161261	-	-
<i>Boloria (C.) euphrosyne</i> NW156-23	Paradelles, Aude, France	HQ161286	HQ161352	HQ161215
<i>Boloria (C.) euphrosyne</i> NW163-2	Languedoc, France	HQ161289	HQ161355	HQ161218
<i>Boloria (C.) freija</i> FS-b-2405	Moose Mountain, AB, USA	HQ161224	HQ161296	HQ161169
<i>Boloria (C.) freija</i> FS-b-3733	Stone Mountain PP, BC, Canada	HQ161232	HQ161304	-
<i>Boloria (C.) freija</i> FS-b-3749	Stone Mountain PP, BC, Canada	HQ161239	HQ161311	HQ161182
<i>Boloria (C.) freija</i> FS-b-3750	Stone Mountain PP, BC, Canada	HQ161240	HQ161312	HQ161183
<i>Boloria (C.) freija</i> NW151-17	Chara district, Russia	HQ161273	HQ161339	-
<i>Boloria (C.) freija</i> NW151-18	Kurai range, Altai Mts, Russia	HQ161274	HQ161340	HQ161203
<i>Boloria (C.) frigga</i> FS-b-2406	Bragg Creek, AB, Canada	HQ161225	HQ161297	HQ161170

(Continued on next page)

Table 1. (Continued).

Species/ID#	Locality	COI	EF1-alpha	wingless
<i>Boloria (C.) frigga</i> NW151-19	Chukotka, Siberia, Russia	HQ161275	HQ161341	HQ161204
<i>Boloria (C.) gong</i> NW151-14	Xiahe, Guangsu Prov., China	HQ161272	HQ161338	-
<i>Boloria (C.) improba</i> FS-b-2401	Cardinal Divide, AB, Canada	HQ161221	HQ161293	HQ161166
<i>Boloria (C.) improba</i> FS-b-3746	Pink Mountain, BC, Canada	HQ161238	HQ161310	-
<i>Boloria (C.) improba</i> FS-b-4217	Mackenzie Mountain, NWT	HQ161251	HQ161323	-
<i>Boloria (C.) improba</i> FS-b-4244	Cardinal Divide, AB, Canada	HQ161258	HQ161330	-
<i>Boloria (C.) improba acrocneuma</i> FS-b-3744	Uncam Peek, CO, USA	HQ161237	HQ161309	HQ161181
<i>Boloria (C.) improba harryi</i> FS-b-3764	NW Wind R. Mtns, WY, USA	HQ161244	HQ161316	HQ161187
<i>Boloria (C.) iphigenia</i> NW151-12	Tokoro, Japan	HQ161270	-	HQ161201
<i>Boloria (C.) krieghild</i> TJS-07-237	Caribon Co., ID, USA	HQ161292	HQ161358	HQ161220
<i>Boloria (C.) matveevi</i> NW151-10	Altai, Russia	HQ161268	HQ161335	HQ161199
<i>Boloria (C.) natazhati</i> SM0001	Stone Mountain PP, BC, Canada	HQ161291	HQ161357	HQ161219
<i>Boloria (C.) oscarus</i> NW151-24	Zanadovorovka, S Ussuri, Russia	HQ161279	HQ161345	HQ161208
<i>Boloria (C.) polaris</i> FS-b-3725	Stone Mountain PP, BC, Canada	HQ266652	HQ266658	HQ266646
<i>Boloria (C.) selene</i> FS-b-2402	Itaska, AB, Canada	HQ161222	HQ161294	HQ161167
<i>Boloria (C.) selene</i> FS-b-4204	Deer Lodge Co., MT, USA	HQ161249	HQ161321	-
<i>Boloria (C.) selene</i> NW76-13	Vallentuna, Sweden	AY090201	AY090167	AY090134
<i>Boloria (C.) selene</i> TS-6	Porte, France	DQ922868	DQ922900	DQ922836
<i>Boloria (C.) selenis</i> JM13-6	Habarovsk district, Russia	HQ161263	-	-
<i>Boloria (C.) thore</i> NW151-22	Polar Ural Mountains, Russia	HQ161278	HQ161344	HQ161207
<i>Boloria (C.) titania</i> FS-b-3797	La Punt, Switzerland	HQ161245	HQ161317	HQ161188
<i>Boloria (C.) titania</i> FS-b-3799	La Punt, Switzerland	HQ161246	HQ161318	HQ161189
<i>Boloria (C.) titania</i> NW151-9	Shuurmak, Tuva, Russia	HQ161285	HQ161351	HQ161214
<i>Boloria (C.) tritonina elatus</i> NW-151-8	Buryatia, S Siberia, Russia	HQ161284	HQ161350	HQ161213

*The Cho-Verdi5 fragment of the EF1-alpha sequence was from *Boloria (C.) alberta* FS-b-3758 (Yaha Tinda, AB, Canada) which was 100% identical to the two *Boloria (C.) alberta* FS-b-3757 fragments for the overlapping base pairs.

(Drummond & Rambaut, 2007), with times of divergence estimated simultaneously as part of the BEAST analysis. There are no known fossils of *Boloria*. However, a recent study on the family Nymphalidae estimates the age of the crown group of Argynnini as 24 ± 3 Mya based on fossil records and ages of host plant families (Wahlberg *et al.*, 2009). This age was used as the calibration point for the crown group Argynnini in the analysis of times of divergence. The data were partitioned by gene (three partitions). All three partitions were assigned the GTR+G model and the parameters were estimated separately for each partition. The relaxed molecular clock technique was used for the molecular dating, allowing branch lengths to vary according to an uncorrelated Lognormal distribution. The tree prior was set to the Birth-Death process, and the 'treeModel.RootHeight' prior (i.e. the age at the root of the tree) was set to 24 million years (with a standard deviation of 3 million years), in accordance with results from Wahlberg *et al.* (2009). All other priors were left to the defaults in BEAST. The analysis was run twice for 10 000 000 generations of MCMC analyses in BEAST and the chains were sampled every 1000 generations, yielding a total of 10 000 samples for each run. Whether the parameter estimates and tree topology were at equilibrium was determined by using the program Tracer (Drummond & Rambaut, 2007). The first 1 000 000 generations (or 1000 trees) were discarded as burn-in.

Biogeographical analysis

A dispersal-vicariance analysis was carried out in DIVA (Ronquist, 1997) based on the tree found in the Bayesian analysis of the combined molecular data set. Seven biogeographical zones were identified based on the literature (e.g. Sanmartin *et al.*, 2001; Kodandaramaiah & Wahlberg, 2009): Western Palaearctic; Central Palaearctic; Eastern Palaearctic; Western Nearctic; Eastern Nearctic; Greenland; South America. The last region is only relevant for the outgroup taxon *Yramea cytheris*. The regions are illustrated in Fig. 2. This is a relatively simple approach, and a more detailed subdivision of the zones followed by a phylogeographical study at the subspecies level would give a more detailed picture of the zoogeography of the genus. However, the taxon sampling of the present study does not allow such an approach, and the coarse division of the Holarctic used here is directly comparable to the division of the Palaearctic region by Kodandaramaiah & Wahlberg (2009). It is our hope that this study will inspire more detailed studies of *Boloria* phylogeography at the subspecies or population levels, since such studies will undoubtedly add important new information to our limited knowledge of biogeographical patterns in the region. Biogeographical regions as scored for each species are listed in Table 2. Information on distribution is from Scott (1986), Layberry *et al.* (1998), Bogdanov (2000a, 2000b, 2000c), and Tuzov

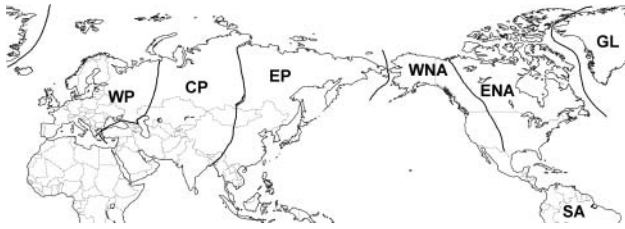


Fig. 2. Biogeographical ranges used in the DIVA analysis. CP: Central Palearctic; ENA: Eastern Nearctic; EP: Eastern Palearctic; GL: Greenland; SA: South America; WNA: Western Nearctic; WP: Western Palearctic.

& Bozano (2006). When ambiguous information on distribution exists the latest sources were followed (Layberry *et al.* (1998) for Nearctic, and Tuzov & Bozano (2006) for Palearctic).

Larval–foodplant relationships

Available foodplant information at family level for all species is listed in Table 2. The evolution of larval–foodplant relationship was analysed in Mesquite 2.71 (Maddison & Maddison, 2009) by scoring host plant families for each species in a nexus file and then analysing the distribution on the Bayesian tree using the Parsimony Reconstruction of Ancestral States option. Information on biology and foodplants is from Higgins & Riley (1970), Hovanitz (1970), Henriksen & Kreutzer (1982), Gall & Sperling (1980), Ferris (1984, 1986), Gall (1984), Scott (1986), Troubridge & Wood (1990), Bird *et al.* (1995), Layberry *et al.* (1998), Shepard *et al.* (1998), Bogdanov (2000a, 2000b, 2000c) and Gorbunov (2001).

Results

Molecular characters

Up to 3127 base pairs were sequenced for at least one specimen of each species: COI (max 1487 bp), EF-1 α (max 1240 bp), and *wingless* (max 400 bp). A complete list of sequences including GenBank accession numbers is given in Table 1.

Morphological characters

Two new characters were identified:

- New1: Phallus with small, lateral, sclerotized, triangular distal lobes: 0 = absent, 1 = present (Fig. 3a–b).
- New2: Ampulla of male valve with croquet-club shaped head: 0 = absent, 1 = present (Fig. 3c).

Nineteen of the 63 characters from Simonsen (2005) were omitted from the present analyses. Recent detailed studies of male genitalia in fritillary butterflies (Simonsen, 2006b,

Table 2. Available distribution and larval foodplant information for the species included in this study. Abbreviations.

Distributions: WP, West Palearctic; CP, Central Palearctic; EP, East Palearctic; WNA, West Nearctic; ENA, East Nearctic; GL, Greenland; SA, South America. Foodplants: Vi, Violaceae; Po, Polygonaceae; Ra, Ranunculaceae; Er, Ericaceae; Ro, Rocaceae; Sa, Salicaceae; Or, Orobanchaceae; Sx, Saxifragaceae; Be, Betulaceae; La, Laminaceae.

Species	Distribution	Foodplants
<i>Y. cytheris</i>	SA	Vi.
<i>I. lathonia</i>	WP, CP, EP	Vi.
<i>B. (P.) eunomia</i>	WP, CP, EP, WNA, ENA	Vi., Po., Ra., Er., Ro., Sa., Or.
<i>B. (B.) alaskensis</i>	CP, EP, WNA	Vi., Po., Er.
<i>B. (B.) altiaca</i>	CP, EP	?
<i>B. (B.) aquilonaris</i>	WP, CP	Er.
<i>B. (B.) caucasica</i>	CP	?
<i>B. (B.) generator</i>	CP	?
<i>B. (B.) graeca</i>	WP, CP	?
<i>B. (B.) napaea</i>	WP	Vi., Po.
<i>B. (B.) pales</i>	WP	Vi.
<i>B. (B.) palina</i>	CP, EP	?
<i>B. (B.) sifanica</i>	CP, EP	?
<i>B. (C.) alberta</i>	WNA	Ro.
<i>B. (C.) angarensis</i>	CP, EP	Er.
<i>B. (C.) astarte</i>	WNA	Sx.
<i>B. (C.) astarte distincta</i>	WNA	Sx.
<i>B. (C.) bellona</i>	WNA, ENA	Vi.
<i>B. (C.) chariclea</i>	WP, CP, EP, WNA, ENA, GL	Vi., Er., Ro., Sa., Po., Be.
<i>B. (C.) dia</i>	WP, CP, EP	Vi., Ro., La.
<i>B. (C.) epithore</i>	WNA	Vi.
<i>B. (C.) erda</i>	CP, EP	Er.
<i>B. (C.) erubescens</i>	CP	?
<i>B. (C.) euphrosyne</i>	WP, CP, EP	Vi., Er., Ro.,
<i>B. (C.) freija</i>	WP, CP, EP, WNA, ENA	Er., Ro.
<i>B. (C.) frigga</i>	WP, CP, EP, WNA, ENA	Ro., Sa., Be.
<i>B. (C.) gong</i>	CP, EP	?
<i>B. (C.) improba</i>	WP, CP, EP, WNA, ENA	Po., Sa.
<i>B. (C.) improba acrocnema</i>	WNA	Sa.
<i>B. (C.) improba harryi</i>	WNA	Sa.
<i>B. (C.) iphigenia</i>	EP	Vi., Er., Ro.
<i>B. (C.) kriemhild</i>	WNA	Vi.
<i>B. (C.) matveevi</i>	CP	Sx.
<i>B. (C.) natazhati</i>	WNA	?
<i>B. (C.) oscarus</i>	CP, EP	Vi.
<i>B. (C.) polaris</i>	WP, CP, EP, WNA, ENA, GL	Er., Ro.
<i>B. (C.) selene</i>	WP, CP, EP, WNA, ENA	Vi., Er.
<i>B. (C.) selenis</i>	WP, CP, EP	Vi.
<i>B. (C.) thore</i>	WP, CP, EP	Vi.
<i>B. (C.) titania</i>	WP, CP, EP	Vi., Po., Er., Ro., Ra.
<i>B. (C.) tritonia elatus</i>	WP, CP, EP	Sx.

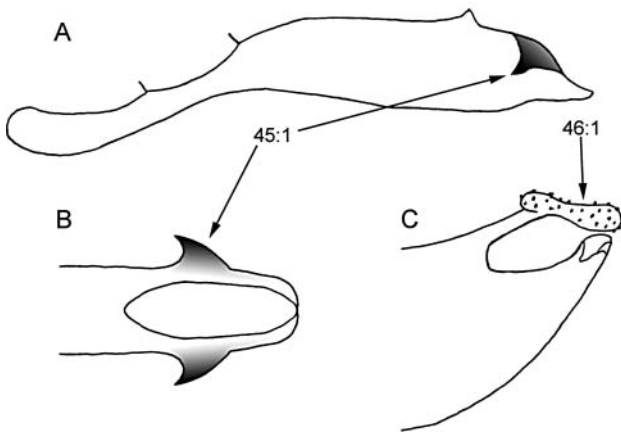


Fig. 3. Illustration of new morphological characters in *Boloria*. **A–B.** *C. freija* phallus showing lateral, sclerotized, triangular lobes, **A.** Entire phallus in lateral view **B.** Distal part of phallus in dorsal view **C.** Inner view of ampulla and distal half of the male valve in *C. tritonia* showing ampulla with crocheted-club shaped head.

2006c) have demonstrated that some characters were interpreted incorrectly, whereas other characters were found to be too variable, uncertain or poorly defined (characters omitted: 3, 6, 8, 14, 15, 17, 27, 29, 42, 43, 45, 47, 48, 50, 51, 57, 60). Three characters (characters 37, 38, 39) were merged into one (Phallus with large, sclerotized disto-ventral lobes: 0 = absent, 1 = present). Re-examination of TJS's photos and notes on *B. (C.) erda* revealed that this species should be scored 0 for this character (contrary to Simonsen, 2005). A full list of characters with character numbers from Simonsen (2005) is given in Appendix 1 (see supplementary material which is available on the Supplementary tab of the article's Informaworld page at <http://dx.doi.org/10.1080/14772000.2010.532833>), the morphological character matrix is given in Appendix 2 (see supplementary material which is available online), and a list of apomorphies for each clade is given in Appendix 3 (see supplementary material which is available online).

Phylogenetic relationships

BEAST analysis of the molecular dataset, shown in Fig. 4, and TNT analysis of the combined datasets (Appendix 4, see supplementary material which is available on the Supplementary tab of the article's Informaworld page at <http://dx.doi.org/10.1080/14772000.2010.532833>) yielded very similar results. BEAST analysis gave a considerably more resolved topology than TNT analysis, with the latter unable to resolve the following relationships: *B. (B.) generator* (Staudinger), *B. (B.) palina* (Fruhstorfer)+*B. (B.) sifanica* (Grum-Grshimailo)), and the remaining *Boloria* s.s. (clade 4 in Fig. 5); the basal relationship of the Selene and Bellona species groups, and the clade comprising the Dia, Euphrosyne, Freija and Titania groups (clade 20); and

the basal relationship of the Euphrosyne, Freija and Titania species groups (clade 28). Two other principal differences were that the TNT analysis placed *B. (C.) matveevi* Gorbunov and Korshunov as the sister of *B. (C.) astarte* (Doubleday) + *B. (C.) astarte distincta* (Gibson), and *B. (C.) chariclea* (Schneider) was paraphyletic with respect to *B. (C.) angarensis* (Erschoff). The topology of the BEAST analysis is used for the foodplant and zoogeography analyses, and the discussion.

Boloria s.l. (clade 1) is monophyletic and strongly supported in both analyses and receives high PBS from both morphological and molecular characters (strong support is defined as a posterior probability of 90% (Fig. 4), or a total BS of 5 (Appendix 4); high PBS is defined as a PBS of 3 from either the morphological or combined molecular dataset). The genus is supported by four unique autapomorphies (Appendix 3). The first split within the genus is between a clade comprising the monobasic subgenus *Proclossiana* + *Boloria* s.str., and the monophyletic subgenus *Clossiana*. The sister group relationship between *Proclossiana* and *Boloria* s.str. (clade 2) is strongly supported by both analyses and receives high PBS from both morphological and molecular characters.

Boloria s.str. (clade 3) is strongly supported in both analyses and receives high PBS from both morphological and molecular characters. Since we are missing seven species (40%) of *Boloria* s.str. we do not attempt to define species groups within the subgenus.

The subgenus *Clossiana* (clade 12) is monophyletic and well supported in both analyses. PBS from molecular characters is high, but low from morphological characters. One character (6: dent where uncus joins tegumen) has been identified as a unique autapomorphy for the subgenus. Only two recognized species (following Tuzov & Bozano, 2006; Pelham, 2008) of *Clossiana*, *B. (C.) jerdoni* and *B. (C.) perryi* (Butler) are not included in the present dataset. We are therefore confident that the present results can be used as a basis for dividing the subgenus further into species groups. Based on well-supported clades in Fig. 4, we identify species groups in Fig. 5 and in the Discussion (name based on the oldest name in the group). Three monobasic species groups (Iphigenia, Dia and Euphrosyne) are established for species that do not show unambiguous affinities with any other group; the remaining groups are monophyletic and supported in both the parsimony and Bayesian analyses. The Polaris species group (clade 15) comprised of *alberta* (Edwards), *polaris* (Boisduval) and *erda* (Christoph) is very well supported in both analyses and receives high PBS from both datasets. Despite this, no unique morphological apomorphy has been identified.

The Tritonia group (clade 17) comprised of *tritonia* (Böber), *matveevi* and the *astarte*-complex is well supported in both analyses. PBS from the molecular dataset is very high, but no unique morphological apomorphy has been identified.

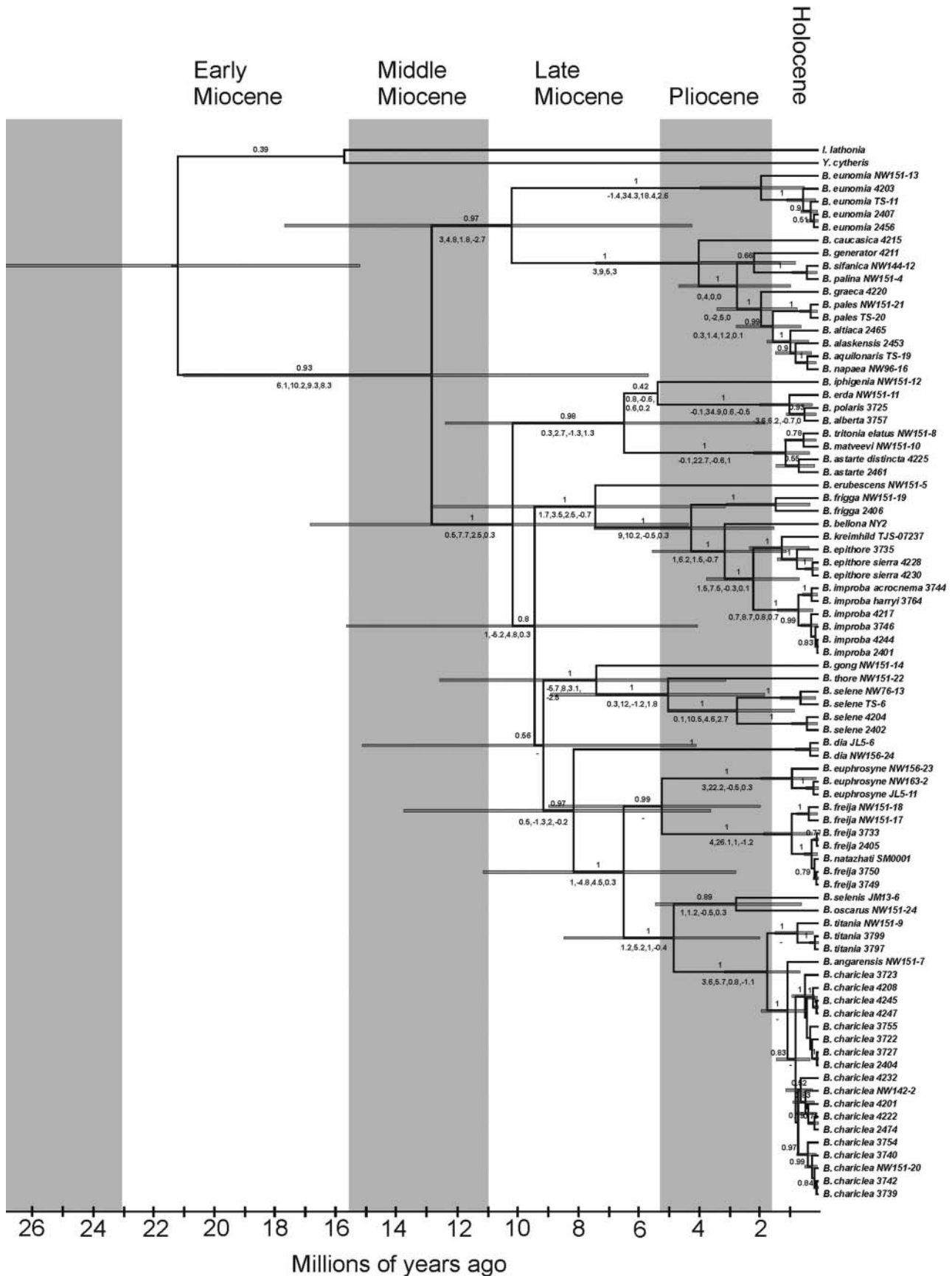


Fig. 4. Results from the BEAST analysis of the combined molecular dataset with estimated divergence times and confidence intervals. Numbers above the nodes are Posterior Probabilities from the BEAST analysis, numbers below the nodes are Partitioned Bremer Support values (morphology, COI, EF1- α and *Wingless* respectively) from the TNT analysis of the combined dataset.

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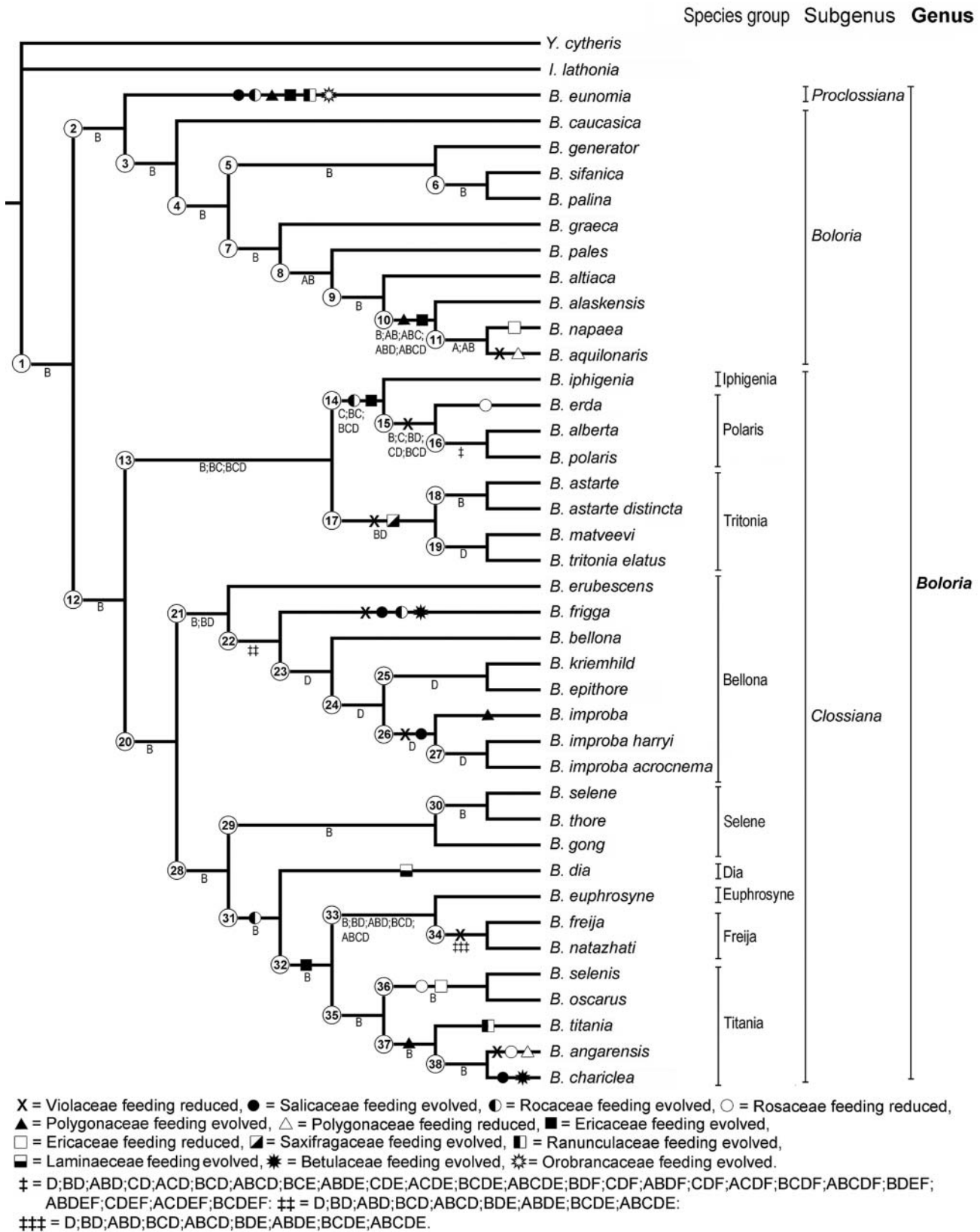


Fig. 5. Evolution of larval hostplant associations, zoogeography, and proposed subgenus and species group level classification within *Boloria* s.l. A = WP, B = CP, C = EP, D = WNA, E = ENA, F = GL.

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The Bellona group (clade 21) comprised of *erubescens* (Staudinger) (= *hegemone* Staudinger), *frigga* (Thunberg), *bellona* (F.), *kriemhild*, *epithore* (Edwards) and the *improba*-complex is well supported in both analyses with moderate to high PBS from both datasets. Character 28 (phallus with two membranous, disto-lateral lobes) is identified as a unique autapomorphy.

The Selene group (clade 29) comprised of *gong* (Oberthür), *selenis* (Denis and Schiffermüller) and *thore* (Hübner) is moderately well supported in both analyses with high PBS coming from the molecular dataset, but the clade is strongly contradicted by morphology. No unique morphological apomorphy has been identified.

The Freija group (clade 34) comprised of *freija* (Thunberg) and *natazhati* (Gibson) is strongly supported in both analyses with high PBS from both datasets. Morphological character 45 (phallus distally with lateral, sclerotized triangular lobes) is identified as a unique autapomorphy.

The Titania group (clade 35) comprised of *oscarus* (Eversmann), *selenis* (Eversmann), *titania* (Esper), *angarensis* and *chariclea* is well supported in both analyses with low PBS from morphology and good PBS from the molecular dataset. Morphological characters 23 (coecum [on phallus] very long and pointed downwards) and 27 (dorsal ridge [on phallus] sigmoid) are identified as unique autapomorphies, the latter is reduced in *oscarus*.

Biogeographical patterns

The most parsimonious optimization in DIVA was reached with a maximum number of ancestral areas of 5 or more. These optimizations required 62 independent dispersal events, but optimizations with max areas set to 6 or 7 (= unconstrained) were slightly more ambiguous than the optimization with max areas set to 5 (the former had ambiguous ancestral distribution for *Boloria* s.l. and *Boloria* s.s. + *Proclossiana*, both of which included the entire Palaearctic region in various combinations). The latter, and simpler, optimization is used in the discussion and plotted on Fig. 5.

Divergence times

Estimated divergence times with confidence intervals are given in Fig. 4. *Boloria* s.l. probably arose in the early Miocene, with the first split between the *Proclossiana* + *Boloria* s.s. lineage and *Clossiana* occurring in the middle Miocene. The split between *Proclossiana* and *Boloria* s.s., and much of the deep divergence within *Clossiana* are inferred to have happened during the late Miocene. Though some species are inferred to have originated in the Pliocene, much of the current species-level diversity apparently has originated during the past 2 My from the late Pliocene throughout the Holocene period.

Larval–foodplant associations

The results of the Mesquite analysis of the evolution of larval–foodplant associations are shown in Fig. 5. Violaceae feeding is found to be the ancestral feeding strategy within the genus, but this habit has been lost independently on seven occasions. Diet broadening or shifts to other plant families have happened on more than a dozen occasions.

Discussion

Phylogenetic relationships

Overall phylogenetic pattern. The genus *Boloria* is monophyletic and well supported by both molecular and morphological characters. The first split within the genus is between a clade comprising the subgenera *Proclossiana* and *Boloria* s.s., and a clade comprising the subgenus *Clossiana*. Both clades are well supported by both molecular and morphological characters. *Boloria* s.s. is monophyletic and well supported, but due to the relatively poor taxon sampling within the subgenus, the internal phylogeny remains uncertain. The relationships between the subgenera recovered here are similar to that found by Simonsen (2005, 2006a) and Simonsen *et al.* (2006), and the morphological characters supporting the relationships are, not surprisingly, the same as identified by Simonsen (2005).

The subgenus *Clossiana* is divided into three major lineages with nine well-defined species groups. One lineage (clade 13 in Fig. 5) comprising the species groups Iphigenia, Tritonia and Polaris, is the sister group to the remaining two lineages. One of these (clade 21) is comprised only by the well defined and strongly supported and predominantly North American Bellona species group. The last lineage (clade 28) is considerably more heterogeneous and comprised of the Selene, Dia, Euphrosyne, Freija and Titania species groups. In the following we highlight three species groups of particular interest: the *improba/acrocneuma*, *freija/natazhati* and *chariclea/titania* complexes.

The *improba/acrocneuma* complex. Following the descriptions of *B. (C.) acrocneuma* Gall & Sperling and *B. (C.) improba harryi* Ferris there has been considerable interest in their biology and conservation (Gall, 1984; Ferris, 1986), as well as the relationships between these North American lineages (Scott, 1986; Britten & Brussard, 1992), especially their taxonomic status. Gall & Sperling's (1980) decision to name *acrocneuma* from Colorado as a full species rather than a subspecies of *improba* (Butler) was based partly on a detailed phenetic comparison between *acrocneuma* and *improba*, and partly on *acrocneuma*'s isolated geographical position. Although the geographical isolation of *acrocneuma* was approximately halved with the discovery of *improba harryi* in Wyoming, Ferris (1984) treated *acrocneuma* as a full species and *harryi* as a subspecies of *improba* partly based on the fact that *acrocneuma* has a somewhat different

UV reflection pattern on the underside of the wings. Scott (1986) treated both *acrocnema* and *harryi* as subspecies of *improba*.

Based on allozymes, Britten & Brussard (1992) concluded that *acrocnema* and *harryi* were glacial relicts, each other's closest relatives, and nested within other North American *B. (C.) improba*. Despite this they did not revise the taxonomic status of *acrocnema*. In the latest catalogue of North American butterflies, Pelham (2008) lists both *acrocnema* and *harryi* as subspecies of *improba*.

Our results clearly confirm that *acrocnema* and *harryi* are each other's closest relatives, and closely related to other North American *B. (C.) improba*. However, since we were unable to obtain material from Palaearctic *improba* populations and have limited molecular sampling of *acrocnema* and *harryi*, we cannot be conclusive about the origin of *acrocnema* and *harryi* with respect to the remaining North American *improba* populations. The question of which taxonomic rank should be assigned to *acrocnema* and *harryi* is more difficult. Given that the specimens examined here for morphology (3 ♂♂, 1 ♀ of each taxon) are very similar, the molecular differences between the two specimens sequenced are negligible (COI: 0.3%; EF1- α : 0.08%; WG: 0.00%), they diverged only about 0.2 Mya (Fig. 4), and they are each other's closest relatives, it is clear that they should have the same rank.

The *improba/acrocnema*-complex forms a closely related and homogeneous group. The molecular differences within the group are minimal: COI: 0.8%; EF1- α : 0.3%; WG: 0.00%. Furthermore, there are no clear biological or ecological differences between *acrocnema*, *harryi* and remaining Rocky Mountain *improba* (Ferris, 1986), or indications that they inhabit the same overall geographic area without interbreeding as is the case for the *freija/natazhati* complex (see below). We therefore maintain that both *acrocnema* and *harryi* at the present should be considered subspecies of *B. (C.) improba*.

The *freija/natazhati* complex. Some controversy has surrounded the status of *B. (C.) natazhati* relative to *B. (C.) freija*. For a time considered a subspecies of *freija* (e.g. dos Passos 1964), *natazhati* was recognized as a full species by Scott (1986) based mainly on sympatric occurrence with *freija* at Coppermine, NWT, without evidence of hybridization (Scott, 1986: 323–324). Troubridge & Wood (1990) and Shepard *et al.* (1998) revised the status of *natazhati* based on detailed morphological studies and a considerable amount of new biological observations (especially when both species occur in the same area). Based on overall morphological differences and notable differences in biology and habitat choice, both studies concluded that *freija* and *natazhati* are distinct species.

Whereas *natazhati* is restricted to subarctic and arctic western Canada and neighbouring Alaska (e.g. Shepard *et al.*, 1998), *freija* is Holarctic in its distribution with five subspecies recognized in the Palaearctic region

(Tuzov & Bozano, 2006) and two additional in the Nearctic (Pelham, 2008). Although our results confirm (Fig. 4) that *freija* and *natazhati* form a well-supported monophyletic group, they also highlight a phylogenetic problem with recognizing both species as currently identified since the two Palaearctic specimens form a well-supported group which is sister to a group comprising the North American *freija* and the single specimen of *natazhati* included here. The molecular differences are very small; less than 0.5% within either the Palaearctic clade or the Nearctic clade (including *natazhati*), and less than 1% for the entire *Freija* species group for COI. The differences for the nuclear genes are negligible.

The divergence time between the Palaearctic and Nearctic lineages is 0.8 Mya, and only 0.2 Mya between Nearctic *freija* and *natazhati* (Fig. 4). The slight morphological differences in the male genitalia scored here have only been confirmed in few specimens of both species and cannot at the present be considered reliable diagnostic differences. Nevertheless, the clear differences in biology and habitat choice described by Troubridge & Wood (1990) and Shepard *et al.* (1998) (difference in habitat choice was confirmed at Summit Lake, BC by TJS and J. J. Dombroskie in 2007 when collecting material for this study) convince us that *natazhati* should be considered a full species, and *freija* is yet another example of a potentially paraphyletic species (Funk & Omland, 2003). A detailed study of the *Freija* species group with much broader taxon sampling (for both morphological and molecular characters) across its range should be conducted to determine whether some subspecies should be given full species-level status.

The *chariclea/titania* complex. The Nearctic component of the *B. (C.) chariclea/titania* complex has been the subject of considerable debate over the years (e.g. dos Passos & Grey, 1945; Scott, 1986; Layberry *et al.*, 1998; Shepard, 1998; Pelham, 2008 – see Shepard, 1998 for an overview) with a number of names ascribed to the non-arctic populations. Most general disagreement has centred on whether these populations are conspecific with the arctic (and circumpolar) *B. (C.) chariclea* (e.g. Layberry *et al.*, 1998; Shepard, 1998; Tuzov & Bozano, 2006; Pelham, 2008), or conspecific with the Palaearctic cold-temperate and montane *B. (C.) titania* (e.g. Scott, 1986; Bogdanov, 2000b).

All 18 Nearctic members of the *chariclea/titania* complex in our analyses (representing specimens from Alaska to Greenland, and New Hampshire to Colorado and Washington State) form a single clade, clearly distinct from the Palaearctic *B. (C.) titania*. The *chariclea* clade is also more closely related to the temperate/high altitude Eastern Palaearctic *B. (C.) angarensis* than to Eurasian *B. (C.) titania*. Since we were unable to include specimens from *B. (C.) chariclea chariclea* from the subarctic and polar regions of Europe, we are unable to say with 100% certainty that the Nearctic populations belong to the same phylogenetic species as the *B. (C.) chariclea* type subspecies. But

based on the available evidence, this seems most likely. The estimated divergence times between *titania* and *angarensis/chariclea*, and *angarensis* and *chariclea* are 1.6 Mya and 1.1 Mya respectively (Fig. 4).

Species with Holarctic distributions

In addition to the species complexes discussed above, three species with a wide Holarctic distribution are included with representatives from both Nearctic and Palaearctic regions in our study: *B. (C.) selene* with two Nearctic and two Palaearctic (western) specimens, *B. (C.) frigga* with one Nearctic and one Palaearctic (eastern) specimen, and *B. (P.) eunomia* with five Nearctic and one Palaearctic (eastern) specimens. All three species have a number of named subspecies in both regions (e.g. Scott, 1986; Bogdanov, 2000b; Tuzov & Bozano, 2006; Pelham, 2008).

Although complete sampling of all subspecies falls outside the scope of this study, our results allow us to recognize several interesting patterns. First, all three species appear monophyletic in our analyses. Second, the genetic differences between Nearctic and Palaearctic specimens are considerably larger than between specimens from the same region. For COI the values are 0.4% (within a region) vs. 3.3% (between regions) for *B. (C.) selene*, and 0.6% vs. 2.6% for *B. (P.) eunomia* (internal variation between Nearctic specimens only). The COI difference between the single Nearctic and single Palaearctic *B. (C.) frigga* specimens is 2.1%. The difference in nuclear genes between the *B. (C.) frigga* specimens is minimal (<0.3% for both genes), but considerably larger between Nearctic and Palaearctic specimens for *Wingless* in *B. (P.) eunomia* (1.25%) and for both genes in *B. (C.) selene* (c. 1.5% for both genes). The divergence times between Nearctic and Palaearctic specimens are more than 1 Mya for all three species: *B. (C.) selene*, 2.5 Mya; *B. (P.) eunomia*, 1.7 Mya; *B. (C.) frigga*, 1.2 Mya.

The status of Nearctic *B. (C.) frigga* (as currently defined) and *B. (P.) eunomia* has never seriously been questioned, but the Nearctic *B. (C.) selene* subspecies *myrina* Cramer has previously been considered a full species (e.g. Holland 1896). However, since dos Passos & Grey (1945) concluded that Nearctic and Palaearctic *B. (C.) selene* populations are conspecific, most authors have considered *myrina* and other Nearctic populations conspecific with *selene* (e.g. Scott, 1986; Layberry *et al.*, 1998; Pelham, 2008; but see Tuzov & Bozano, 2006). Our taxon sampling is too small and too geographically restricted to test whether the observed differences in *B. (C.) selene* and *eunomia*, respectively, are disjunctive or continuous across their substantial ranges. Furthermore, before any taxonomic changes are proposed (i.e. elevating Nearctic subspecies to full species-level status) detailed examinations of the genitalic morphology of all subspecies should be undertaken. Preliminary comparisons between *B. (C.) selene selene* and *B. (C.) selene my-*

rina by TJS in preparation for the 2005 publication showed no discernible differences.

Comparisons to earlier studies

Though Warren (1944) did not publish an explicit systematic hypothesis, his species and subspecies arrangement of *Boloria* s.s. (= the *pales* group *sensu* Warren) can be interpreted as a basic phylogenetic hypothesis for the subgenus. Many of Warren's subspecies are included as full species in our analyses and most were given full species-level status by Tuzov & Bozano (2006). It therefore seems appropriate to consider Warren's three species comparable to species groups in the latter works. Of the species included here, *B. (B.) graeca* (Staudinger) is the sole member of the species as defined by Warren (1944), whereas *B. napaea* (Hoffmannsegg) is represented by *B. (B.) napaea*, *B. (B.) altaica* (Grum-Grshimailo) and *B. (B.) alaskensis* (Holland), and *B. pales* (Denis & Schiffermüller) is represented by *B. (B.) pales*, *B. (B.) caucasica* (Lederer), *B. (B.) palina*, *B. (B.) sifanica*, *B. (B.) generator* and *B. (B.) aquilonaris* (Stichel). Neither of the last two groups is supported by our phylogenetic results.

Unlike Warren (1944), dos Passos & Grey (1945) divided *Boloria* s.l. into two major groups; the *selenis* group including the species in the Euphrosyne, Freija and Titania species groups in the present study, excluding *B. (C.) natazhati*; and the *thore* group (including *B. (P.) eunomia* [= *aphiraphe*] and *B. (B.) pales*). The former group is defined by the large, heavily sclerotized dorso-distal, vertical lobes on the male phallus; the latter by much smaller and membranous phallic lobes. We agree with the definition of the first group, but *B. (C.) natazhati* (not known to dos Passos & Grey) must be included to retain monophyly. The large, sclerotized lobes are presumed reduced in *B. (C.) freija* and *B. (C.) natazhati*. It is worth noting here that neither of these species have any trace of lobes left (membranous or otherwise) unless the small, sclerotized, lateral lobes that are identified as a synapomorphy for the two species are derivatives of the much larger (but less pointed) lobes found in the rest of this clade. The second group is paraphyletic in the present analysis, and the small, membranous phallic lobes are a symplesiomorphy (and a misinterpretation in the cases of *B. (P.) eunomia* and *B. (B.) pales*). Dos Passos & Grey (1945) also considered *B. (C.) improba* to be close to (but not conspecific with) *B. (C.) frigga*. *B. (C.) bellona* (= *toddi*) was also considered to be close to *B. (C.) frigga*, whereas *B. (C.) kriemhild* and *B. (C.) epithore* were considered subspecies of *B. (C.) frigga*. *B. (C.) kriemhild* and *B. (C.) epithore* have since been raised to full species status (e.g. dos Passos 1964). Our results confirm that all these taxa deserve full species-level status, but also corroborate with dos Passos & Grey's (1945) hypothesis that these species are closely related. Grey's (1989) synonymization

of *Proclassiana* with *Clossiana* cannot be justified based on our results.

Our results have an overall pattern that is similar to the morphology-based phylogeny in Simonsen (2005). *Boloria* s.l. is recognized as monophyletic with strong support and the same three characters are identified as unique autapomorphies for the genus. The first split in the genus between the subgenera *Proclassiana* and *Boloria* s.s. in one clade and the monophyletic *Clossiana* in another is recovered, as is the sister group relationship between *Proclassiana* and *Boloria* s.s. A monophyletic *Clossiana* is recovered and two of the three autapomorphies from Simonsen (2005) are confirmed as unique autapomorphies for the subgenus. The internal phylogeny of *Clossiana* differs considerably, however, from Simonsen (2005), although the latter was acknowledged to be highly uncertain. The main reason for the differences between the two results is undoubtedly the high level of homoplasy in the morphology dataset (also discussed by Simonsen, 2005) illustrated by the generally very low contributed PBS values, and the fact that 14 of the clades in Fig. 5 do not have morphological apomorphies (unique or homoplastic) supporting them. Although new morphological examinations of all species focusing on a broader set of characters undoubtedly would reveal identification characters for most of these clades, this study clearly underpins the value of including molecular characters in species-level studies of recently diverged groups.

Tuzov & Bozano (2006) identified four groups within *Boloria* s.s.: *pales* group (*pales*, *pyrenemiscens* Warren, *alaskensis*, *eupales* Fruhstorfer), *napaea* group (*napaea*, *altaica*, *frigidalis* Warren, *purpurea* Churkin, *palina*, *generator*), *graeca* group (*graeca*, *caucasica*, *sipora* (Moore)), and *aquilonaris* group (*aquilonaris*, *banghaasi* (Seitz), *roddi* Kosterin, *sifanica*). It is not possible with the current taxon sampling to confirm these groups. Seven of the *Boloria* s.s. species recognized by Tuzov & Bozano (2006) were omitted from our dataset, but the remaining ten species represent the four species groups suggested by these authors with at least two species from each group. A phylogenetic analysis of *Boloria* s.s. with a complete taxon sampling based on both molecular and morphological characters is thus still much needed and should be the focus of a future study.

The two *Boloria* s.str. taxa *B. (B.) napaea* and *B. (B.) alaskensis* have often been considered conspecific (e.g. Bird *et al.*, 1995; Layberry *et al.*, 1998). However, our results endorse Tuzov & Bozano (2006) and Pelham (2008) that *B. (B.) alaskensis* is the only Nearctic representative of *Boloria* s.str. (as defined here).

Biogeography and divergence time

Biogeographical patterns. Despite the fact that the results of the dispersal-vicariance analyses are ambiguous some interesting trends can be observed. Regardless of constraints on maximum number of ancestral areas, the Central

Palaeartic region appears to have been the most important region during the evolution of *Boloria* s.l. In the preferred '5 areas' analysis both *Boloria* s.l., *Proclassiana* + *Boloria* s.s., and *Boloria* s.s. are hypothesized to have originated in Central Palaeartic. Most clades within *Boloria* s.s. are also hypothesized to have originated in this area. This result seems probable despite the omission of several *Boloria* s.s. species from the dataset; with the exception of *B. (B.) pyrenemiscens* from Western Palaeartic, all omitted species are either strictly central Palaeartic (*B. (B.) sipora*), or central Palaeartic and Eastern Palaeartic (*B. (B.) banghaasi*, *B. (B.) eupales*, *B. (B.) purpurea*, *B. (B.) roddi*) in their distribution.

Interpreting the historical biogeography of *Clossiana* is difficult as more clades have ambiguous ancestral area combinations. However, the Central Palaeartic region seems to have played a major role in the evolution of this subgenus as well. The ancestors of the Titania group, the Selene group, and all higher clades (with the exception of clade 13), including *Clossiana* itself, are all inferred to have had a distribution restricted to the Central Palaeartic region.

Within *Clossiana*, both the Iphigenia + Polaris + Tritonia clade, and the Bellona group have a strong Nearctic influence. The first split within the Tritonia group between *B. (C.) tritonia* + *B. (C.) matveevi*, and the *B. (C.) astarte*-complex is inferred to have happened *c.* 1.5 Mya which roughly coincides with the formation of the third Beringian Land Bridge (Sanmartin *et al.*, 2001). It is thus likely that the ancestor of the *B. (C.) astarte*-complex spread to North America across the third bridge shortly after its formation. It is equally likely that the ancestor of either the *B. (C.) polaris* + *B. (C.) alberta*, or the ancestor of *B. (C.) alberta* spread to North America via the third bridge (both scenarios fit the estimated divergence times).

The split between *B. (C.) frigga* and the Nearctic part of the Bellona group is inferred to have happened *c.* 3.7 Mya, which roughly fits the break-up of the second Beringian Land Bridge (3.5 Mya; Sanmartin *et al.*, 2001). The latter clade, which includes the Holarctic *B. (C.) improba*, is inferred as being completely Nearctic in its origin. The first split within this clade is between the predominantly Eastern Nearctic *B. (C.) bellona* and the remaining Western Nearctic species. It is not clear whether this split was due to a vicariance event, long-distance dispersal, or an allopatric speciation event. However, climatic vicariance has recently been shown to have had considerable effect on speciation in the nymphalid genus *Charaxes* (Aduse-Poku *et al.*, 2009; Müller *et al.*, 2010). It is quite likely that the phenomenon has had an important effect on the speciation in *Boloria* as well, not only in this particular case, but on speciation in Asian taxa.

Several dispersal events between Central/Eastern Palaeartic and Nearctic, and from Central/Eastern Palaeartic into Western Palaeartic are also needed to explain the current distributional pattern of *Boloria* s.l.

Probable dispersals from Central/Eastern Palaearctic to Nearctic include *B. (P.) eunomia*, *B. (B.) alaskensis*, *B. (C.) frigga*, and *B. (C.) chariclea*. Probable dispersals the other way from Nearctic to Central/Eastern Palaearctic include *B. (C.) improba* and *B. (C.) polaris/erda*. The third Beringian land bridge, which existed from *c.* 1.5 Mya until approximately 10 kya, was dominated by barren tundra vegetation (Sanmartin *et al.*, 2001). Thus it likely acted as a corridor for tundra species (e.g. *B. (B.) alaskensis*, *B. (C.) improba*, *B. (C.) polaris*), and could also have aided chance dispersals for species with a more Boreal distribution (e.g. *B. (C.) selene*). But long-distance oceanic dispersals (either trans-Pacific or trans-Atlantic) for those species cannot be ruled out entirely.

When divergence times are included, only the split between *B. (C.) frigga* and the Nearctic part of the Bellona group fits a classical biogeographical vicariance event; other distribution patterns found in *Boloria* are best explained as results of dispersals. Our results thus support recent results (e.g. Yoder & Nowak, 2006; de Jong & van Achterberg, 2007) which have demonstrated that dispersal is generally more important than vicariance for explaining current distributional patterns in flying insects.

The general importance of the Central Palaearctic region in the evolutionary history of *Boloria* s.l. is in agreement with the few other zoogeographical studies of insects in the Holarctic biome. Recently, Kondandaramaiah & Wahlberg (2009) reached a similar conclusion for the more temperate (and less Nearctic) but otherwise comparable nymphalid genus *Coenonympha* Hübner. In an insect-based overview, Varga (1995: fig. 1, p. 76) hypothesized that the south-central and east-central Palaearctic are hotspots in Palaearctic biogeography, and that major faunal migration routes have either been from the south central Palaearctic, south of the Aral, Caspian, and Black Seas through the Balkan Peninsula into Western Palaearctic (Mediterranean-xeromontane fauna elements), or from the east central Palaearctic, east to eastern Siberia and Japan, and north and north west (north of the Aral, Caspian and Black Seas) into northern Central Palaearctic and Western Palaearctic (Boreo-montane elements).

One interesting side observation is that the clades with the most ambiguous ancestral area combinations (the clades comprising *B. (C.) polaris* + *B. (C.) alberta*, *B. (C.) frigga* + the Nearctic Bellona group, the Freija group, and the Euphrosyne + Freija groups respectively) are ones that have immediate descendants with very wide distributions (*B. (C.) polaris*, *B. (C.) frigga* and *B. (C.) freija* respectively), thus confirming the reservations made by the program author (Ronquist, 1997).

The inferred divergence times for *Boloria* correspond roughly to those found for *Coenonympha* by Kondandaramaiah & Wahlberg (2009), indicating the importance of this period for species-level diversification in Holarctic butterflies.

Evolutionary aspects

Members of *Boloria* feed on at least 22 genera of plants in 10 different families (Fig. 5, Table 2). Though foodplant records were not available for some species, several interesting conclusions can be made based on the results. Eighteen of these foodplant genera are utilized by one or two species (13 plant genera are only utilized by single species), and five families are only utilized by one or two species. The original larval feeding strategy in *Boloria* was on Violaceae, the basal and most utilized foodplant for the entire tribe Argynnini (Simonsen, 2006a). A number of host shifts and/or diet broadenings have happened during the evolution of the genus. Importantly, Violaceae-feeding has been lost in the *Polaris*, *Tritonia* and *Freija* species groups, as well as in the species *B. (B.) aquilonaris*, *B. (C.) frigga*, *B. (C.) angarensis* and *B. (C.) improba* s.l. It is remarkable that with the exception of *B. (B.) aquilonaris*, these are all species or clades which are either fully or partly adapted to arctic and/or high altitude environments. In several other clades, Violaceae-feeding seems to play only a minor role based on the literature. Partial or full foodplant shifts to Rosaceae, Salicaceae, Ericaceae, Polygonaceae and Betulaceae appear to have occurred in several groups. *Boloria (P.) eunomia* exhibits the most extreme diet broadening, feeding on ten different foodplant genera in seven different families, a fact that probably is an important explanation of this species' very broad habitat range from lowland temperate marshes through boreal muskeg (Henriksen & Kreutzer, 1982; Bogdanov, 2000a), to subarctic willow patches well above tree line (TJS pers. obs.), and arctic lowlands (Layberry *et al.*, 1998).

Reconstructing ancestral abilities to utilize several foodplant families is difficult, especially since host shifts to very different plant genera and families seem to have happened independently several times across *Boloria*. However, the genus appears to have an inherited plasticity when it comes to foodplant choice. Twelve of the 28 species for which foodplant data were available are reported to feed on at least two different families, and nine species are reported to feed on three different families or more. Furthermore, it is important to bear in mind that although foodplant records exist for most *Boloria* species, some are based on few observations or successful larva rearing. It is therefore likely that the ability to utilize additional plant genera and families has been overlooked, especially in species with broad habitat preferences in little-collected areas such as Siberia and the Central Palaearctic.

Boloria in general seems to have considerably broader foodplant preferences than its closest relatives, the other fritillary subtribes Argynnina and Yrameina (Simonsen *et al.*, 2006), which feed either on few species in Rosaceae and Fabaceae (three species in the genus *Brenthis* Hübner, Argynnina) or exclusively on Violaceae (all other species for which foodplants are known) (e.g. Simonsen, 2006a). This broadening of diets may indeed be a major explanation for *Boloria*'s success in alpine and arctic

environments compared to the remainder of the tribe. Among other fritillaries, only a few, scattered species appear in alpine habitats (e.g. Hovanitz, 1970; Scott, 1986; Tuzov, 2000; Lamas & Grados, 2004), but almost all *Boloria* s.l. occur at least in part in arctic and subarctic environments and/or in high altitude (>2500 metres) habitats (e.g. Scott, 1986; Layberry *et al.*, 1998; Bogdanov, 2000a, 2000b, 2000c; Gorbunov, 2001). Plasticity and broadening of foodplant choices has recently been shown to be an important driving force in generating diversity in phytophagous insects (e.g. Janz *et al.*, 2006; Nylin & Wahlberg, 2008). Here it appears that a broadening of the Violaceae-feeding habit found basally in the clade comprising Yrameina, Bolorina and Argynnina (Simonsen, 2006a), in particular to include Salicaceae, Rosaceae, Ericaceae, Saxifragaceae and Polygonaceae, has enabled *Boloria* species to colonize alpine and arctic habitats in the northern hemisphere where Violaceae do not occur. In some taxa with restricted ranges and/or habitat choice a secondary specialization to a single non-Violaceae foodplant has occurred (e.g. *B. (C.) natazhati* and the *B. (C.) improba* subspecies *acrocne* and *harryi*). Such local diet specializations following earlier diet broadenings are to be expected as a part of 'dynamic oscillations of host range' (Janz *et al.*, 2006).

Classification

We agree with the classification proposed by Simonsen (2005) and recommend that all species treated herein should be placed in the genus *Boloria*, and that three subgenera *Boloria*, *Proclassiana* and *Clossiana* are recognized.

Subtribe Bolorina Warren, dos Passos, and Grey, 1946
Genus *Boloria* Moore, 1900
Subgenus *Boloria* Moore, 1900

The subgenus *Boloria* is comprised by the species in clade 3 in Fig. 5, and the following species not included in the study: *pyrenesmiscens* Warren; *eupales* (Fruhstorfer); *frigidalis* Warren; *purpurea* Churkin; *sipora* (Moore); *banghaasi* (Seitz); *roddi* Kosterin. These seven species are assigned to the subgenus based on Tuzov & Bozano (2006).

Subgenus *Proclassiana* Reuss, 1921

The subgenus *Proclassiana* comprises the single species *P. eunomia*. Although *Proclassiana* is most closely related to subgenus *Boloria*, we do not agree with Pelham (2008) that they should be synonymized. *Proclassiana eunomia* is morphologically distinct from both *Boloria* and *Clossiana*, and does not share any of *Boloria*'s striking apomorphies in the male or female genitalia (e.g. Simonsen, 2005, 2006a). There is therefore sufficient justification to uphold the subgenus *Proclassiana* for the species *eunomia*.

Subgenus *Clossiana* Reuss, 1920

The subgenus *Clossiana* is comprised by the species in clade 12 in Fig. 5, and the following species not included in this study: *jerdoni* (Lang) and *perryi* (Butler). Nine informal species groups are identified as illustrated in Fig. 5 and discussed above. *B. (C.) jerdoni* is assigned to the Bellona species group based on a re-examination of the dissection notes and photos used by Simonsen (2005) which confirmed that the species is very close to *B. (C.) erubescens*. *Boloria (C.) perryi* is tentatively assigned to the Selene species group based on the illustrations of male genitalia in Tuzov & Bozano (2006) which are similar to those of *B. (C.) selene*.

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