Limnol. Oceanogr., 56(2), 2011, 440–450

doi:10.4319/lo.2011.56.2.0440

Radiation of European *Eubosmina* (Cladocera) from *Bosmina* (*E.*) *longispina*—concordance of multipopulation molecular data with paleolimnology

Markéta Faustová,[a,b,1,*] Veronika Sacherová,[b] Jan-Erik Svensson,[c,2] and Derek J. Taylor[a]

[a] Department of Biological Sciences, State University of New York at Buffalo, Buffalo, New York
[b] Department of Ecology, Charles University in Prague, Prague, Czech Republic
[c] University College of Borås, School of Engineering, Borås, Sweden

Abstract

We investigated the evolutionary radiation of a freshwater zooplankter that possesses a mixed breeding system and a detailed, dated, subfossil record. We estimated the phylogenetic relationships among the proposed species with complete nicotinamide adenine dinucleotide dehydrogenase subunit 2 mitochondrial gene sequence variation. We sequenced 374 *Eubosmina* specimens representing 10 out of 11 distinct morphospecies from 86 water bodies in three separate Holarctic regions. As expected for a Holocene radiation, there was a lack of monophyly for the proposed species and rare sharing of derived haplotypes among some species. Nevertheless, the proposed species exhibited little or no sharing of mitochondrial deoxyribonucleic acid haplotypes. Moreover, the phylogenetic and haplotype network results revealed a radiation pattern that is concordant with the subfossil record—with an older *Bosmina* (*E.*) *longispina* radiating into several new forms. Our results bolster the subfossil, morphometric, experimental, and within-population genetic evidence that *B.* (*E.*) *longispina* has radiated into several incipient species during the Holocene.

The best-known cases of recent radiations occur within strictly sexual organisms in newly created and isolated habitats. However, invertebrates with mixed breeding systems also appear to undergo rapid radiations (Peccoud et al. 2009; Faustová et al. 2010). Importantly, at least one of these groups *Eubosmina* (Crustacea: Bosminidae) has a detailed and dated subfossil record in the sediment of Holarctic lakes. These lakes appeared after the last glaciation (< 20,000 years ago) and were colonized from refugial waters. Coincident with the formation of glacial lakes and, in some cases, eutrophication (Hofmann 1978; Gasiorowski and Szeroczynska 2004), *Eubosmina* appears to have radiated into a taxonomically difficult species flock (Lieder 1983; De Melo and Hebert 1994; Taylor et al. 2002).

Although *Eubosmina* (Crustacea, Cladocera) is a common component of recent glacial lakes (< 20,000 years old), its taxonomy remains in a state of flux. Within-lake study has revealed genetic and morphometric differentiation among coexisting species of *Eubosmina*, but the among-lake relations of the proposed taxa in this radiation are unknown. Moreover, it is unknown if radiation from the Eurasian *B.* (*E.*) *longispina* morphospecies, apparent from the subfossil record, is supported by the genetic evidence. *Eubosmina* (< 1.5 mm) is a planktonic cladoceran that reproduces parthenogenetically for most of the year. When exposed to certain environmental conditions, most bosminids switch to producing sexual resting eggs stored in ephippia (protective capsules created from part of the carapace). Carapaces and head shields of *Eubosmina* are well preserved in sediments, often being the most common animal subfossils in late Pleistocene and early Holocene lakes (Frey 1960*a,b*). Subfossil remains normally retain the morphological features (carapace shape, antennules, mucro) that are commonly used to distinguish different morphospecies. There is growing evidence that the evolution of size and shape differences in the carapace, antennules, and mucro of bosminids is associated with invertebrate predation (Kerfoot 2006). The only *Eubosmina* morphospecies to be detected in the oldest sediments at the end of glaciation (late Pleistocene) in Europe and North America is *Bosmina* (*E.*) *longispina*—sedimentary records of other *Eubosmina* morphospecies are younger (Frey 1962; Hofmann 1978). The ancestral *B.* (*E.*) *longispina* hypothesis could be a sampling artifact, but the paleolimnological evidence continues to converge on this hypothesis.

Haney and Taylor (2003) attempted to test the ancestral *B.* (*E.*) *longispina* hypothesis using a phylogeographic approach, but their results indicated that *B.* (*E.*) *longispina sensu strictu* was likely absent from North America and that there was no resolution among European *Eubosmina* with the examined genes. They detected three phylogroups: two corresponded to the morphologically uniform *B.* (*E.*) *longispina* from eastern and western parts of North America, and a third group contained European *Eubosmina* with *B.* (*E.*) *longispina s.s.* and several members of the proposed Holocene species flock (Lieder 1996). This earlier genetic data and a later study (Kotov et al. 2009) lacked the phylogenetic resolution to test if the European *B.* (*E.*) *longispina* form is indeed the basal or ancestral species in Europe. Kerfoot (2006) has shown that there is a genetic component to the morphological features used for classification. Faustová et al. (2010) later provided morphometric and genetic evidence that coexisting morphospecies of

* Corresponding author: marfaustova@hotmail.com

Present addresses:
[1] Institute of Rheumatology, Prague, Czech Republic
[2] Medins Biology, Mölnlycke, Sweden

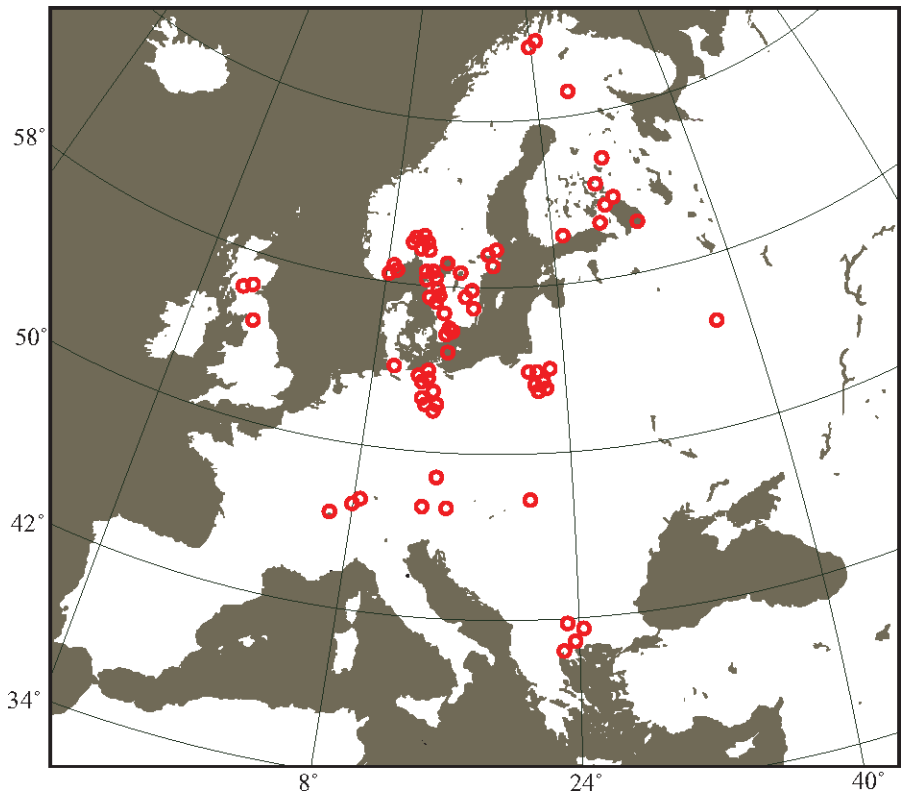

Fig. 1. Map showing localities of analyzed specimens of European *Eubosmina* (red circles). Additional sites from North America, lake names, and location coordinates are given in the Web Appendix.

Eubosmina in European lakes are indeed genetically differentiated. However, the ancestral *B.* (*E.*) *longispina* hypothesis and the among-population relationships of European *Eubosmina* remain unaddressed.

In the present study, we test the ancestral *B.* (*E.*) *longispina* hypothesis for European *Eubosmina* (as predicted by paleolimnology) and assess the among-population differentiation of the European radiation. We follow the latest taxonomical system based on morphological features (Lieder 1996) and include 10 out of 11 main morphospecies from the system. We use the mitochondrial nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2) gene, as Faustová et al. (2010) have shown this region to be useful in differentiating species within lakes.

Methods

Samples collection—We analyzed 342 *Eubosmina* specimens from 72 water bodies in Europe and 32 specimens from 14 North American lakes (in the United States and Canada) collected from 1998 to 2006 (*see* Web Appendix, http://www.aslo.org/lo/toc/vol_56/issue_2/0440a.html). The North American specimens are included in our data, as existing studies suggest a sister group relation to the European radiation (Haney and Taylor 2003; Kotov et al. 2009). We tried to cover the geographic range in Europe (Fig. 1); most samples come from the circumbaltic area, where the morphological diversity is greatest. Samples were preserved in 96% ethanol or frozen at −80°C. We identified European specimens according to Lieder's (1996) identification system. We sampled all four species and 10 out of 11 subspecies. We included only adult specimens that could be assigned to 1 of 10 defined subspecies. All North American specimens (save the specimen from Lime Lake, New York) in this study are of the native form belonging to one "subspecies" defined by Lieder as *B.* (*E.*) *longispina longispina* (Haney and Taylor 2003).

Deoxyribonucleic acid extraction, polymerase chain reaction, sequencing, and cloning—We extracted total deoxyribonucleic acid (DNA) of single individuals using 25–30 μL of Quick-Extract (Epicentre) solution. We homogenized the specimens and then incubated samples at 65°C for 2 h and 98°C for 10 min. Samples were stored at −20°C. We performed polymerase chain reaction (PCR) in 50 μL reaction using 5 μL of template, 5 μL of 10× PCR buffer, 1.5 μL of each of 10-mmol L^{-1} deoxyribonucleotide triphosphate solution, 1.5 μL of each 10-μmol L^{-1} primer. The PCR conditions were 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min 20 s for 39 cycles, followed by 72°C for 7 min for ND2 gene sequences. Primers to amplify and sequence both strands of the ND2 gene were MetR1 (5′-CCGACCATAGAGTCAAATCTCCTCTCTG-3′) and COIMf (5′-ACGGATAAGCATTCTAAGTGCAGTACCC-3′) or ND2aRew (5′-GTGATTAGTAGAAAAGAGCCATCGTCGCAC3′) and MetR4 (5′-GCTTCAGCTTCGGCC-

ATCCTGTCAG-3′) or their combinations. The PCR products were sequenced in both directions by either Roswell Park Cancer Institute or Genaissance Pharmaceuticals. When multiple PCR bands appeared, we used a gel purification kit to isolate PCR products of the expected size. We used a DNA topoisomerase thymine, adenine (TOPO TA®) cloning kit (Invitrogen) to obtain a clear sequence of ND2 gene for one specimen from the United Kingdom (Loch Lubnaig), where ambiguities appeared after direct sequencing of PCR product.

DNA sequences alignments and analyses—We assembled and edited sequences using Sequencher 4.2. (Gene Code Corporation) and aligned sequences manually using Se-Al 2.0. We excluded 35 sequences of ND2 that had ambiguities or insertion(s) or that showed stop codons before the real stop codon from the next analyses because of the potential for pseudogenes. All sequences are deposited in GenBank under accession numbers HM194228–HM194601.

We performed maximum likelihood (ml) estimation of phylogenies using Phyml 3.0 with nearest neighbor interchange and subtree pruning and reconnection tree searches with five random starting trees. We used the best-fit nucleotide substitution model of Hasegawa-Kishino-Yano plus Gamma (Bayesian Information Criterion with jModeltest 0.1.1; Posada 2008). Support was measured with approximate likelihood ratios (aLRT; a measure of support similar to the bootstrap). We also performed Bayesian reconstruction using MrBayes 3.1.2. (Huelsenbeck and Ronquist 2001) with models partitioned by codon position. The analysis required 5 million generations for convergence (yielding 50,000 trees, of which the first 30,000 were considered burn-in). We applied a cutoff > 0.95 for posterior probability values and > 0.85 for aLRT values. We used the Templeton, Crandall, and Sing (TCS) 1.21 program (Clement et al. 2000) to estimate an ND2 haplotype network with connections that have a 95% probability of being the most parsimonious (in our case, 13 steps connecting parsimoniously two haplotypes).

To calculate the pairwise fixation index (F_{ST}; Reynolds et al. 1983; Slatkin 1995; Holsinger and Weir 2009), we used integrated software for population genetics data analysis (Arlequin 3.00; Excoffier et al. 2005). We tested the significance of F_{ST} values using a permutation procedure (1000 permutations). We used the same program to estimate the proportion of genetic variance explained by different morphological groupings using analysis of molecular variance (AMOVA; Weir and Cockerham 1984; Excoffier et al. 1992; Weir 1996) with 10,000 replications under Tamura-Nei and Kimura's model (the closest available fit to the selected models by Modeltest). We assigned specimens to four main morphospecies corresponding to Lieder's species list—*B.* (*E.*) *longispina*, including all North American samples, *B.* (*E.*) *coregoni*, *B.* (*E.*) *crassicornis*, and *B.* (*E.*) *longicornis*—and 10 morphospecies following Lieder's subspecies list—*B.* (*E.*) *longispina longispina*, *B.* (*E.*) *l. reflexa*, *B.* (*E.*) *coregoni coregoni*, *B.* (*E.*) *c. thersites*, *B.* (*E.*) *c. gibbera*, *B.* (*E.*) *crassicornis*, *B.* (*E.*) *longicornis longicornis*, *B.* (*E.*) *l. berolinensis*, *B.* (*E.*) *l. cederstroemi*, and *B.* (*E.*) *l. kessleri*, excluding all North American samples. In both analyses, populations corresponded to morphospecies from one lake. Whenever we analyzed two or more morphospecies from the same water body, we established them as different populations. We ran two different statistical analyses: one with all 374 analyzed sequences and one with the 342 European sequences. We also calculated the probability of identity (Melton et al. 1995) to quantify haplotype sharing between all pairs of morphospecies. The values of probability of identity can range from 0 to 1, where 1 corresponds to identical haplotypes.

To infer the population history (demographic parameters) of the European radiation, we used the frequency distribution of the number of pairwise differences (the mismatch distribution) among all European *Eubosmina* based on pairwise sequence differences calculated in ARLEQUIN. This method takes the advantage of mitochondrial DNA (mtDNA) characteristics (haploid maternal inheritance) and treats mtDNA haplotypes as the simple products of mutational divergence of evolutionary descendants without recombination. We used the bootstrap approach to derive confidence intervals around the estimated parameters and to test the observed data under the models of the pure demographic expansion (sudden expansion) and spatial expansion by comparing the sum of squared deviations between the observed (SSD_{OBS}) and simulated data. To estimate the time (t) of the expansion for both pure sudden (unsubdivided populations suddenly expand in population size) and spatial (subdivided populations expand the distribution range and increase the number of individuals) expansion models of the European clade, we used $Tau = 2ut$, where $u = m_T\mu$, m_T is number of nucleotides under the study, and μ is the mutation rate per time. The number of migrants (M) among neighboring demes per time (a parameter likely to be affected by priority effects in zooplankton) was calculated for a model of spatial expansion. We note that the 95% confidence intervals for Tau and M as well as for mismatch distribution were calculated using a parametric bootstrap approach with 1000 replications.

Results

The alignment was 1005 bases long with no gaps. We detected 116 unique haplotypes belonging to 342 European specimens, four unique haplotypes belonging to four eastern North American specimens, and 11 unique haplotypes belonging to 28 western North American specimens. There were three major geographic clades strongly supported by posterior probability values and aLRT values (Fig. 2). The mean genetic divergence within each group was very similar, ranging from 0.010 to 0.011 with Kimura's two-parameter distance. The western Palearctic (European) clade was comprised of two subclades. The first of these was comprised of numerous European haplotypes from many locations. Some branches with high posterior probability values (≥ 95) supported subgroups with several morphospecies. The second main subclade was basal and comprised of four haplotypes belonging to eight *B.* (*E.*) *longispina* specimens from two Austrian lakes and one *B.*

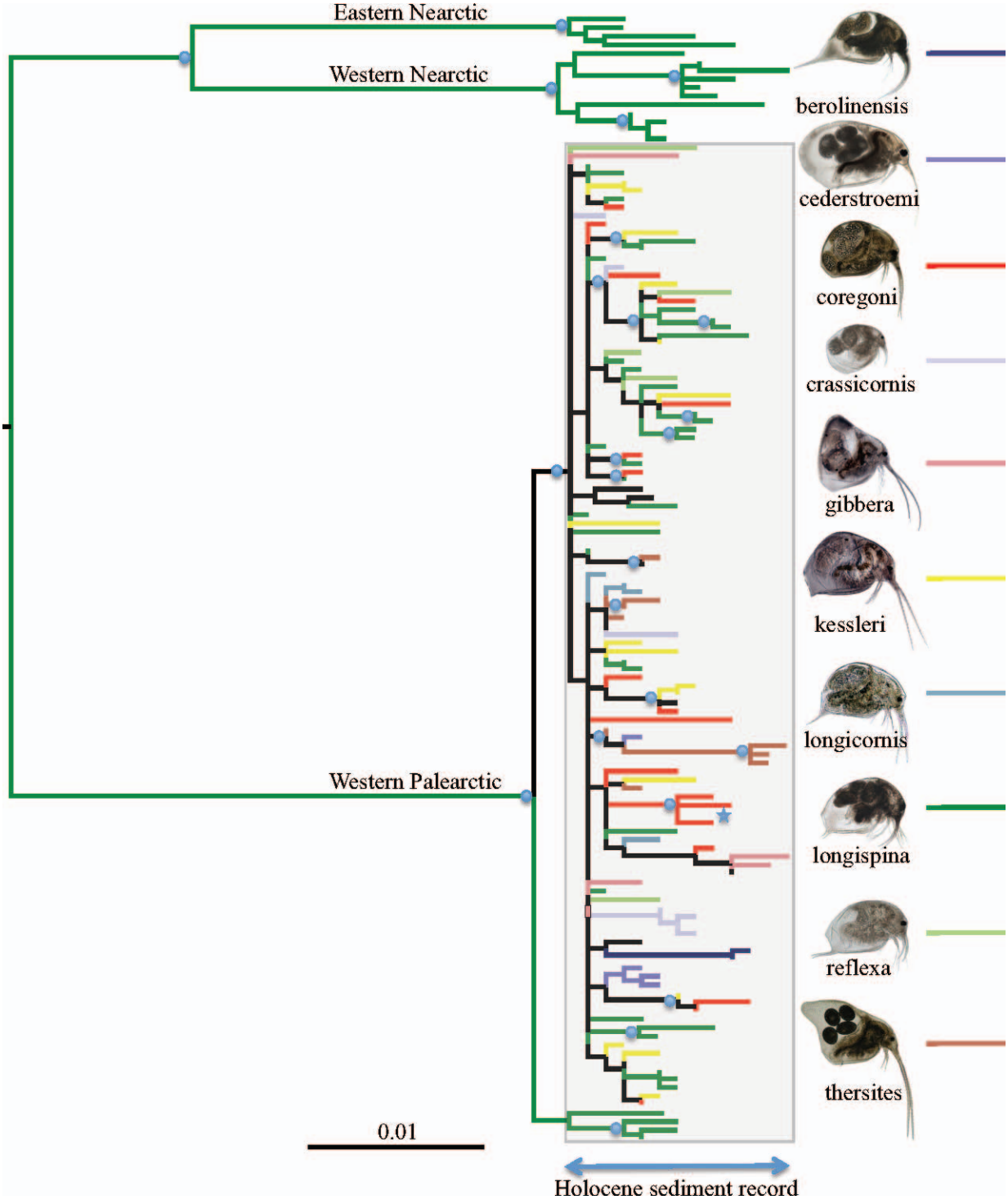

Fig. 2. Pictures of the 10 main morphospecies of European *Eubosmina* and the maximum likelihood phylogram of ND2 gene sequences belonging to 374 *Eubosmina* specimens from Europe and North America. Blue circles at a branch node indicate approximate likelihood ratio (aLRT) values > 0.85 and posterior probabilities > 0.95. Branch tips comprised of two or more different morphospecies are left black. The star symbol represents an ND2 sequence obtained from a whole mitochondrial genome amplicon from Lime Lake, New York. The star specimen is expected to be an invader from the western Palearctic. The gray shading indicates a clade with morphotypes that are unknown from Pleistocene subfossil records and postdate *longispina* in the Holocene subfossil record. Habitat, haplotype, and specimen details can be found in the Web Appendix.

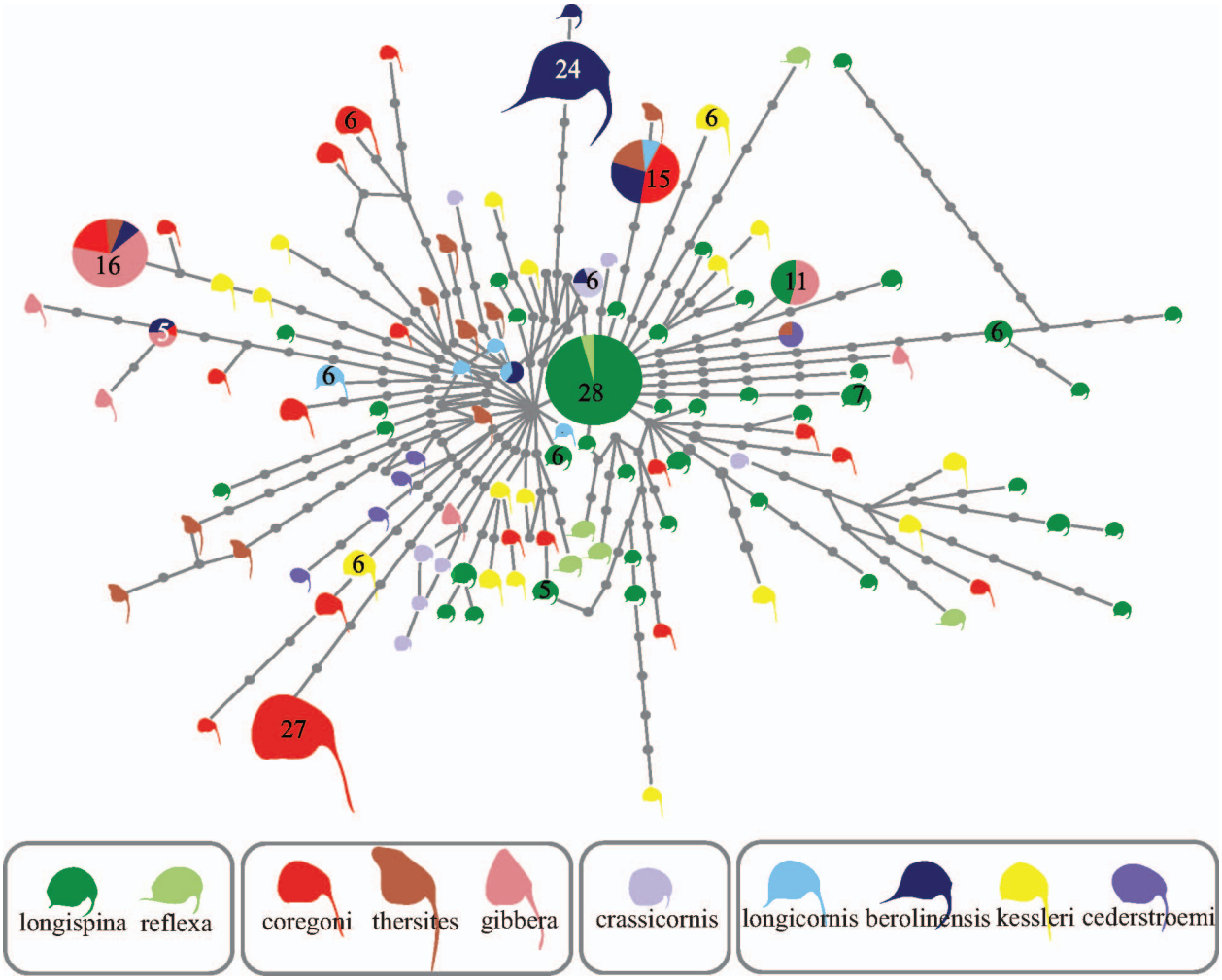

Fig. 3. The TCS most parsimonious network based on 342 European ND2 gene sequences of *Eubosmina*. Each of the 10 different shapes and colors corresponds to a different morphospecies (in accordance with Lieder's taxonomy for *Eubosmina*). When a haplotype is found in more than one morphospecies, a pie graph is used to depict frequencies. The size of the circle or the *Eubosmina* symbol corresponds to the number of individuals sharing the haplotype. Sample numbers are shown when five or more individuals share the haplotype. Each line between two haplotypes symbolizes one mutational step. Inferred haplotypes are marked as small circles. The four boxes correspond to the four species of Lieder's taxonomic system. The name of the first morphospecies in each box corresponds to the name of the species of Lieder's system.

(*E.*) *longispina* specimen from one British lake. Haplotype labels and details for each specimen on the phylogram can be found in the Web Appendix.

The maximum parsimony network of the 116 ND2 haplotypes (Fig. 3) belonging to the European specimens had a starlike topology. The central haplotype of the network corresponded to 27 specimens identified as *B.* (*E.*) *longispina* morphospecies from lakes within the large Scandinavian region (four Norwegian, three Swedish, and one Finnish lake) and one individual identified as the *B.* (*E.*) *reflexa* morphospecies from a Swedish lake.

There were five cases where a haplotype was found in two morphospecies, one case where a haplotype was found in three morphospecies, and two cases where a haplotype was found in four morphospecies. These shared haplotypes occurred mainly at the tips of the haplotype network (*see* Fig. 3). AMOVA revealed a weak association between morphospecies and ND2 haplotypes (Table 1). A statistically significant amount of genetic variation was explained by morphospecies for both the European species and the European subspecies data partitions. However, most of the genetic variation was partitioned among populations within morphospecies: 57.9% and 76.7% for European specimens and all specimens under the study, respectively. Pairwise F_{ST} values (Table 2) ranged from 0.06184 to 0.47792, and all contrasts were significant at the 0.05 level. One of the lowest F_{ST} values (0.07719) belonged to *B.* (*E.*) *longispina* and *B.* (*E.*) *reflexa*, whereas the highest differentiation (above 0.4) was found between *berolinensis* and several other morphospecies: *B.* (*E.*) *longispina*, *B.* (*E.*) *reflexa*, *B.* (*E.*) *thersites*, *B.* (*E.*) *gibbera*, *B.* (*E.*) *longicornis*, and *B.* (*E.*) *cederstroemi*. The probability of identity values equaled zero in cases of no sharing of haplotypes between two morphospecies and were smaller than 0.037 in all other cases, where haplotypes were shared between morphospecies (Table 2), with the

Table 1. Hierarchical AMOVA based on 342 mitochondrial ND2 sequences among four* taxa—*B.* (*E.*) *longispina*, *B.* (*E.*) *coregoni*, *B.* (*E.*) *crassicornis*, and *B.* (*E.*) *longicornis*—and 10 taxa of *Eubosmina*—*B.* (*E.*) *l. longispina* (excluding North American members), *B.* (*E.*) *l. reflexa*, *B.* (*E.*) *c. coregoni*, *B.* (*E.*) *c. thersites*, *B.* (*E.*) *c. gibbera*, *B.* (*E.*) *crassicornis*, *B.* (*E.*) *l. longicornis*, *B.* (*E.*) *l. berolinensis*, *B.* (*E.*) *l. kessleri*, and *B.* (*E.*) *l. cederstroemi*. F_{CT}, test by permuting whole populations among groups; F_{SC}, test by permuting genotypes among populations and among groups; F_{ST}, test by permuting genotypes among populations and among groups.

Hierarchical structure	Source of variation	Sum of squares	Variance component		Fixation index	*p*
			%	V		
1*	Among morphospecies	488.547	11.94%	Va=1.38097	F_{CT}=0.11937	<0.0049
2*	Among populations within morphospecies	3271.538	76.68%	Vb=8.87078	F_{SC}=0.87069	<0.0000
3*	Within populations	353.073	11.39%	Vc=1.31744	F_{ST}=0.88613	<0.0000
1	Among morphospecies	370.843	15.15%	Va=0.78011	F_{CT}=0.15146	<0.0000
2	Among populations within morphospecies	977.507	57.92%	Vb=2.98294	F_{SC}=0.68254	<0.0000
3	Within populations	346.851	26.94%	Vc=1.38740	F_{ST}=0.73063	<0.0000

highest value between *B.* (*E.*) *longispina* and *B.* (*E.*) *reflexa* morphospecies.

The observed mismatch distribution of the European clade closely matched the expected distributions of both the sudden expansion model (SSD_{OBS} = 0.002211) and the spatial expansion model (SSD_{OBS} = 0.002772; not shown). The Tau parameters were 9.824 (6.946–13.478 in 95% confidence interval [CI]) and 8.514 (6.305–11.952 in 95% CI) in the sudden expansion model and the spatial expansion model, respectively. When assuming a divergence rate of 2.0% per million years for arthropod mitochondrial protein coding genes (DeSalle et al. 1987), the expansion time of the European clade was estimated to be 211,791 years BP (156,841–297,313 years BP in 95% CI) for the spatial expansion model and 244,378 years BP (172,787–335,274 years BP in 95% CI) for the sudden expansion model.

Discussion

Our genetic analysis of the *Eubosmina* complex is consistent with the predictions from the paleolimnological record—a rapid European radiation from *B.* (*E.*) *longispina* into several differentiated morphospecies. Phylogenetic reconstruction revealed three major clades of *Eubosmina*, one of which included haplotypes from all the European populations studied (Fig. 2). TCS analysis (Fig. 3) revealed that the basal haplotype was found only in the *B.* (*E.*) *longispina* morphospecies and more rarely in its closest relative *B.* (*E.*) *reflexa*. In the case of ancient polymorphism, one would expect starlike subnetworks with several related central haplotypes (Jakob and Blattner 2006). However, the ND2 haplotype network (Fig. 3) had an obvious starlike pattern, suggesting that one initial *B.* (*E.*) *longispina* haplotype was the relatively recent progenitor of all other haplotypes and that the *B.* (*E.*) *longispina* morphospecies gave rise to all other morphospecies. The starlike shape of the haplotype network also suggests that one main refugium existed for present-day European *Eubosmina*. The near identical within-group genetic divergences of the European clade and the other two proposed postglacial refugial expansions (eastern Nearctic and western Nearctic) support colonization from a main refugium for Europe. Weak haplotype sharing among morphospecies (a pattern that is also found within lakes (Faustová et al. 2010) could indicate that reproductive barriers among morphotypes are incomplete.

A starlike network pattern could result from a postglacial selective sweep of mitochondrial DNA, but selective homogenization cannot account for the significant morphospecies associations with mtDNA variation or the concordance of the mtDNA network with the paleolimnological record, that is, a radiation from an ancestral *B.* (*E.*) *longispina* morphospecies. Finally, selective sweeps of mtDNA are inconsistent with the similar patterns of genetic variation observed for mitochondrial and nuclear markers (allozymes, randomly amplified polymorphic DNAs [RAPD], and nuclear DNA sequences, though with less resolution and fewer samples than here; De Melo and Hebert 1994; Hellsten and Sundberg 2000; Haney and Taylor 2003). The converging evidence from nuclear, mitochondrial, paleolimnological, biogeographical, morphometric, and experimental sources is consistent with *B.* (*E.*) *longispina* as the ancestral morphospecies of a Holocene radiation of European *Eubosmina*.

The independent-multiple (polyphyletic) origin of several morphospecies is supported by the strong posterior probability and maximum parsimony values in the consensus tree (Fig. 2) and the appearance of the same morphospecies in several different branches of the TCS network (Fig. 3). Parallel formation may have contributed to the weak observed association between haplotypes and morphospecies in the AMOVA analysis (Table 1). Importantly, sharing of haplotypes among different morphospecies is low (eight from 116 haplotypes, about 7%), and probabilities of identity are low, indicating nonrandom mating and reproductive isolation between morphospecies as suggested by Hellsten and Sundberg (2000) from analyses of RAPD markers. Nonrandom mating and significant reproductive isolation among morphospecies has also been reported within single lake sites where morphospecies coexist (Faustová et al. 2010). In four of the lakes in this study (the nearby Swedish lakes of Björvattnet, Kärnsjön, Ragnerudssjön, and Vassbotten) and in six other lakes in the same drainage basin, we recorded the co-occurrence of the sexual stages of two or more *B.* (*E.*)

Table 2. F_{ST} values and probability of identity. The probability of identity between morphospecies (above the diagonal). Morphospecies pairwise F_{ST}s calculated in Arlequin with F_{ST} *p*-values, significance level 5% in parentheses (below the diagonal); if not stated, then 0.00000 ± 0.0000. Statistical significance of F_{ST} values was tested using a permutation procedure (1000 permutations). The number of haplotypes of each morphospecies is in bold, and the total numbers of individuals analyzed are in brackets. LS, *B.* (*E.*) *longispina*; REF, *B.* (*E.*) *reflexa*; COR, *B.* (*E.*) *coregoni*; THER, *B.* (*E.*) *thersites*; GIBB, *B.* (*E.*) *gibbera*; CRASS, *B.* (*E.*) *crassicornis*; LC, *B.* (*E.*) *longicornis*; BER, *B.* (*E.*) *berolinensis*; KESS, *B.* (*E.*) *kessleri*; CED, *B.* (*E.*) *cederstroemi*.

	LS	REF	COR	THER	GIBB	CRASS	LC	BER	KESS	CED
LS 40 (105)	—	0.03674	0	0	0.01143	0	0	0	0	0
REF 6 (7)	0.07719 (0.03604± 0.0148)	—	0	0	0	0	0	0	0	0
COR 19 (72)	0.19804	0.14833 (0.00901± 0.0091)	—	0.02315	0.02334	0	0.00810	0.01389	0	0
THER 12 (15)	0.14528	0.13407 (0.02703± 0.0194)	0.12491	—	0.024691	0	0.01667	0.02407	0	0.025
GIBB 8 (25)	0.18341	0.17797	0.17997	0.13559	—	0	0	0.01267	0	0
CRASS 8 (14)	0.20154	0.22530	0.18822	0.18421	0.21334	—	0	0.00992	0	0
LC 6 (12)	0.21155	0.26388	0.14244	0.16400 (0.00901± 0.0091)	0.19099	0.26533	—	0.01389	0	0
BER 7 (36)	0.44329	0.45390	0.38004	0.42197	0.42536	0.44401	0.47792	—	0	0
KESS 17 (48)	0.06184	0.07660	0.14902	0.10442	0.12756	0.14384	0.15042	0.38181	—	0
CED 5 (8)	0.15923	0.16700	0.15142	0.09202 (0.03604± 0.0201)	0.13852	0.24791	0.28799	0.47032	0.10143 (0.00901± 0.0091)	—

longispina, *B.* (*E.*) *coregoni*, *B.* (*E.*) *gibbera*, *B.* (*E.*) *kessleri*, and an intermediate *B.* (*E.*) *longicornis*–type morphospecies at varying densities. We have recorded the opposite sexes of at least two different morphospecies within the same sample from eight of 10 lakes during November. Thus, there are no large differences in the temporal or the spatial distribution that may prevent encounters between sexual stages of sympatric morphospecies. Other isolating mechanisms appear to be acting that enable genetic divergence under sympatry.

The occurrence of the same haplotypes among reproductively isolated morphospecies can also be explained by shared ancestry in the case of central and other older haplotypes (Omland et al. 2006; Ritz et al. 2008). The possibility that gene flow occurs just for part of the genome and not for genes responsible for morphology is perhaps unlikely. Mutation in cis-regulatory sequences (Fondon and Garner 2004) or similar mechanisms can enable the rapid evolution of different morphospecies that can be reproductively isolated by a variety of mechanisms. A repeated evolution of adaptive traits has been shown in an experimental set by MacLean and Bell (2003), and parallelism of defensive characters, feeding morphology, or coloration appears quite often in the adaptive radiations of cichlids and other groups (Streelman and Danley 2003; Colosimo et al. 2005). Despite theoretical expectations of a weak capacity for radiation in cyclic parthenogens, *Eubosmina* and pea aphids appear to be among the fastest and the youngest radiations known (Peccoud et al. 2009; Faustová et al. 2010). The extrinsic factors and ecological selection models are known for many vertebrate radiations (Streelman and Danley 2003) but remain unclear in the *Eubosmina* radiation. Obviously, all young radiations exhibit similar genetic characteristics to those found in *Eubosmina* (ancestral sharing of haplotypes and incomplete lineage sorting). However, the *Eubosmina* radiation resembles those of sticklebacks (Taylor and McPhail 1999; Rundle et al. 2000; Bell 2001) and *Coregonus* fish (Douglas et al. 1999; Østbye et al. 2006; Mehner et al. 2010) in multiple origins, frequent co-occurrences, taxonomic difficulty, and habitat. As coexisting morphospecies are located in different clades, our genetic data suggest an allopatric origin of presently co-occurring morphospecies of *Eubosmina*. This pattern could be explained by the exceptional dispersal abilities of *Eubosmina* and the widespread anthropogenic disturbance of European lakes.

Eubosmina is presently missing from three (Iberian peninsula, Appenine peninsula in Italy, and the Balkans) of the five main proposed refugial regions (Hewitt 1999, 2004) for the last glaciation in Europe. While *Eubosmina* occurs in two proposed refugia (the area around Moscow and the Ponto-Caspian region), present-day populations of *B.* (*E.*) *longispina* morphospecies are found mainly in colder oligotrophic lakes in the northern part of Europe or high mountains, never southward near the main mountain ranges (the Pyrenees and Alps; Lieder 1996). The possibility that *B.* (*E.*) *longispina* has emerged from southern refugia and are absent in today's lakes appears to be unlikely because of the absence of the paleolimnological records from the region (this is, however, a negative result). Samples from Russia and the Ponto-Caspian region need to be examined to determine whether European *Eubosmina* followed similar colonization routes as some freshwater fish (Hewitt 2004).

Scandinavia is a candidate region for a refugium, as the oldest haplotype (including 28 specimens from nine lakes) is widespread and the genetic diversity is high here. However, Scandinavia was completely covered by a continental ice sheet during the last maximum glaciation. It is unlikely that *Eubosmina* managed to survive below the ice during this glaciation. It is more likely that Scandinavia, together with the Baltic Sea and its different stages (Yoldia Sea, Baltic Ice Lake, and Ancylus Lake), acted as a secondary late Pleistocene refugium. This hypothesis is also supported by the fact that some *Eubosmina* populations are known to withstand higher salinities (up to 5‰) and that one of these populations probably formed what has been known as *B.* (*E.*) *longispina maritima* from the Baltic Sea (present in the sedimentary record and recent samples; De Melo and Hebert 1994; Hofmann and Winn 2000). A possible aquatic refugium in Britain is suggested by a paleolimnological record of *B.* (*E.*) *longispina* from the most recent glaciation in the United Kingdom (Beales 1976; Gibbart and Aalto 1977). A similar refugium has been suggested for aquatic beetles (Mende et al. 2010) and for frogs (Teacher et al. 2009). A local British refugium would also explain the genetic distinctness of some specimens from Britain in our study as well in Haney and Taylor's (2003) study.

The close fit of the mismatch distributions for both models means that we cannot conclude whether an ancestral *B.* (*E.*) *longispina* underwent expansion in the refugium (primary or secondary) and then new haplotypes spread to newly created lakes (sudden expansion model) or whether some subpopulations with distinct haplotypes colonized different lakes from which they later colonized more distant habitats (spatial expansion model). More paleolimnological and genetic analyses are needed to assess the location(s) of Palearctic refugia.

Another remaining mystery of the *Eubosmina* radiation is how diversification occurred in such an apparently homogeneous habitat—the epilimnion of glacial lakes—and why the radiation occurred in Europe but is missing in the other Holarctic regions. The fact that the regional assemblages of invertebrate predators in North America and Europe differ and that contemporary predator communities may differ appreciably from historic communities (Kerfoot 2006; Kerfoot and McNaught 2010) has led Kerfoot (2006) to argue that the absence of *Epischura* or similar predators and exposure to *Leptodora* and *Mesocyclops* in Europe led to greater morphological variation of *Eubosmina* in Europe. Potential differences in large calanoid predation between North America and Europe may also be worth studying. Present-day predation by *Heterocope* (whose European distribution may also be related to the historic absence of *Epischura*) is important in many lakes of southern Scandinavia, and different species are associated with different *Eubosmina* morhospecies. *Heterocope appendiculata* co-occurs with *B.* (*E.*) *longispina*, *B.* (*E.*) *gibbera*, *B.* (*E.*) *longicornis*, and *B.* (*E.*) *kessleri*,

while *Heterocope borealis* may co-occur with *B.* (*E.*) *longispina*, *B.* (*E.*) *cederstroemi*, *B.* (*E.*) *longicornis*, and *B.* (*E.*) *kessleri* (Hellsten and Stenson 1995; J.-E. Svensson unpubl.).

Temporal changes from *B.* (*E.*) *longispina* to other morphospecies of *Eubosmina* have been associated with anthropogenic eutrophication (Szeroczynska 1991; Gasiorowski and Szeroczynska 2004; Alliksaar et al. 2005). Other planktonic cladocerans, such as the *Daphnia longispina* complex, have also shown taxonomic sensitivity to eutrophication (Brede et al. 2009). Most of the derived morphospecies of *Eubosmina* appeared to be associated with anthropogenic eutrophication and are more common in mesotrophic or eutrophic lakes compared to oligotrophic lakes (Hofmann 1978; Lieder 1996). The eutrophication process is much older in Europe compared to North America. Europe underwent significant deforestation and eutrophication over large areas as much as 15,000 years ago. Mesotrophic and eutrophic lakes are common, while oligotrophic lakes are restricted largely to high altitudes (mountains regions) and high latitudes (Norway, Sweden, and Finland). The lack of a radiation in other parts of the Holarctic could result from eutrophication there being only centuries old compared to the millennial-scale eutrophication in Europe. The present study estimated that European *Eubosmina* expansion occurred around 212,000 to 245,000 years ago. However, expansion time can be overestimated by more than 10 times because of the tendency of mtDNA clocks to overestimate recent divergence (Simon et al. 2005). There is very likely a further inflation of divergence times from our assumption of a 2% rate of change for a rapidly evolving gene such as ND2 in a very small cladoceran. We note that similar network shapes and overestimated divergence times (upper ranges are 113,000–189,000) are found for the apparent Holocene expansions of *Daphnia* (a larger cladoceran than *Bosmina*) using the homologous ND2 gene (Ishida and Taylor 2007*a,b*). Eutrophication is also associated with changes in predation regimes, so further studies will be required to establish the role of eutrophication and predation as the primary factor or factors leading to diversification in European *Eubosmina*.

We have carried out a geographically broad molecular study of a dated radiation in cyclic parthenogens. Our genetic evidence is consistent with the sediment record in finding evidence for the radiation of *Eubosmina* morphospecies from *B.* (*E.*) *longispina*. The European *Eubosmina* appears to be a flock of very young morphospecies showing evidence of reproductive barriers both within and among lakes. As expected from an ongoing or Holocene animal radiation, the genetic differentiation is at the level of haplotype frequencies rather than reciprocal monophyly. Our results support the proposal that the *Eubosmina* species complex provides a rare system for detailed study of an animal radiation with both a mixed breeding system and a detailed subfossil record.

Acknowledgments

We thank Maria Belyaeva, Martin Černý, Helena Gogelová, Laura Hovind, Jaroslav Hrbáček, Igor Hudec, Seiji Ishida, Thomas Jankowski, Paavo Junttila, Vladimir Kořínek, Alexey Kotov, Astrid Löffler, Maria Leichtfried, Silvia Marková, Martin Meier, Jens Petter Nilssen, Adam Petrusek, Bernd Seidendorf, and Zuzana Sykorová for samples. We thank two anonymous reviewers for their valuable comments to the manuscript and constructive suggestions. Major research funding was provided by the National Science Foundation (Division of Environmental Biology grant 0331095 to D.J.T.). Additional research funding was provided by the Czech Ministry of Education (project MSM0021620828) to V.S. and M.F. (1867/41-202920) and the Swedish Environmental Protection Agency (dnr 3094664-06 Nv) to J.-E.S.

References

Alliksaar, T., A. Heinsalu, L. Saarse, J. Salujõe, and S. Veski. 2005. A 700-year decadal scale record of lake response to catchment land use from annually laminated lake sediments in southern Estonia. Verh. Int. Ver. Limnol. **29:** 457–460.

Brede, N., C. Sandrocka, D. Strailec, P. Spaak, T. Jankowskic, B. Streita, and K. Schwenk. 2009. The impact of human-made ecological changes on the genetic architecture of *Daphnia* species. Proc. Natl. Acad. Sci. USA **106:** 4758–4763, doi:10.1073/pnas.0807187106

Beales, P. W. 1976. Palaeolimnological studies of a Shropshire Mere. Ph.D. thesis. Cambridge Univ.

Bell, M. A. 2001. Lateral plate evolution in the threespine stickleback: Getting nowhere fast. Genetica **112–113:** 445–461, doi:10.1023/A:1013326024547

Clement, M., D. Posada, and K. Crandall. 2000. TCS: A computer program to estimate gene genealogies. Mol. Ecol. **9:** 1657–1659, doi:10.1046/j.1365-294x.2000.01020.x

Colosimo, P. F., and others. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. Science **307:** 1928–1933, doi:10.1126/science.1107239

De Melo, R., and P. D. N. Hebert. 1994. Allozymic variation and species diversity in North American Bosminidae. Can. J. Fish. Aquat. Sci. **51:** 873–880, doi:10.1139/f94-086

Desalle, R., T. Freedman, E. M. Prager, and A. C. Wilson. 1987. Tempo and mode of sequence evolution in mitochondrial-DNA of Hawaiian *Drosophila*. J. Mol. Ecol. **26:** 157–164, doi:10.1007/BF02111289

Douglas, M. R., P. C. Brunner, and L. Bernatchez. 1999. Do assemblages of *Coregonus* (Teleostei: Salmoniformes) in the Central Alpine region of Europe represent species flocks? Mol. Ecol. **8:** 589–603, doi:10.1046/j.1365-294x.1999.00581.x

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinf. Online **1:** 47–50.

———, P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics **131:** 479–491.

Faustová, M., V. Sacherová, H. D. Sheets, J.-E. Svensson, and D. J. Taylor. 2010. Coexisting cyclic parthenogens comprise a holocene species flock in *Eubosmina*. PLoS ONE **5:** e11623, doi:10.1371/journal.pone.0011623

Fondon, J. W., and H. R. Garner. 2004. Molecular origins of rapid and continuous morphological evolution. Proc. Natl. Acad. Sci. USA **101:** 18058–18063, doi:10.1073/pnas.0408118101

Frey, D. G. 1960*a*. The ecological significance of Cladocera remains in lake sediments. Ecology **41:** 790–795, doi:10.2307/1931802

———. 1960*b*. On the occurrence of cladoceran remains in lake sediments. Proc. Natl. Acad. Sci. USA **46:** 917–920, doi:10.1073/pnas.46.6.917

———. 1962. Cladocera from the Eemian interglacial of Denmark. J. Paleolimnol. **36:** 1133–1154.

Gasiorowski, M., and K. Szeroczynska. 2004. Abrupt changes in *Bosmina* (Cladocera, Crustacea) assemblages during the history of the Ostrowite Lake (northern Poland). Hydrobiologia **526:** 137–144, doi:10.1023/B:HYDR.0000041592.09564.e6

Gibbart, P. L., and M. M. Aalto. 1977. A Hoxnian interglacial site at Fishers Green, Stevenage, Hertfordshire. New Phytol. **78:** 505–523, doi:10.1111/j.1469-8137.1977.tb04857.x

Haney, R. A., and D. J. Taylor. 2003. Testing paleolimnological predictions with molecular data: The origins of Holarctic *Eubosmina*. J. Evol. Biol. **16:** 871–882, doi:10.1046/j.1420-9101.2003.00594.x

Hellsten, M. E., and J. A. Stenson. 1995. Cyclomorphosis in a population of *Bosmina coregoni*. Hydrobiologia **312:** 1–9, doi:10.1007/BF00018881

———, and P. Sundberg. 2000. Genetic variation in two sympatric European populations of *Bosmina* spp. (Cladocera) tested with RAPD markers. Hydrobiologia **421:** 157–164, doi:10.1023/A:1003975203884

Hewitt, G. M. 1999. Post-glacial re-colonization of European biota. Biol. J. Linn. Soc. **68:** 87–112, doi:10.1111/j.1095-8312.1999.tb01160.x

———. 2004. Genetic consequences of climatic oscillations in the Quaternary. Proc. R. Soc. Lond. B Biol. Sci. **359:** 183–195, doi:10.1098/rstb.2003.1388

Hofmann, W. 1978. *Bosmina* (*Eubosmina*) populations of the Grosserer Ploenersee and Schoehsee lakes during late-glacial and postglacial times. Pol. Arch. Hydrobiol. **25:** 167–176.

———, and K. Winn. 2000. The Littorina transgression in the Western Baltic Sea as indicated by subfossil Chironomidae (Diptera) and Cladocera (Crustacea). Int. Rev. Ges. Hydrobiol. **85:** 267–291, doi:10.1002/(SICI)1522-2632(200004)85:2/3<267::AID-IROH267>3.0.CO;2-Q

Holsinger, K. E., and B. S. Weir. 2009. Genetics in geographically structured populations: Defining, estimating and interpreting FST. Nat. Rev. Genet. **10:** 639–650, doi:10.1038/nrg2611

Huelsenbeck, J. P., and F. Ronquist. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics **17:** 754–755, doi:10.1093/bioinformatics/17.8.754

Ishida, S., and D. J. Taylor. 2007*a*. Genetic divergence associated with mature habitats in a passively dispersing arthropod. BMC Evol. Biol. **7:** Article 52, doi:10.1186/1471-2148-7-52

———, and ———. 2007*b*. Quaternary diversification in a Holarctic sexual zooplankter, *Daphnia galeata.* Mol. Ecol. **16:** 569–582, doi:10.1111/j.1365-294X.2006.03160.x

Jakob, S. S., and F. R. Blattner. 2006. A chloroplast genealogy of *Hordeum* (Poaceae): Long-term persisting alleles, incomplete lineage sorting, regional extinction, and the consequences for phylogenetic inference. Mol. Biol. Evol. **23:** 1602–1612, doi:10.1093/molbev/msl018

Kerfoot, W. C. 2006. The Baltic *Eubosmina* radiation: Sensitivity to invertebrate predators (induction) and observations on genetic differences. Arch. Hydrobiol. **167:** 147–168, doi:10.1127/0003-9136/2006/0167-0147

———, and A. S. McNaught. 2010. "Two-step" dialogue between the cladoceran *Bosmina* and invertebrate predators: Induction and natural selection. Limnol. Oceanogr. **55:** 403–419.

Kotov, A. A., S. Ishida, and D. J. Taylor. 2009. Revision of the Genus *Bosmina* Baird, 1884 (Cladocera, Bosminidae), based on evidence from male morphological characters and molecular phylogenies. Biol. J. Linn. Soc. **156:** 1–51.

Lieder, U. 1983. Revision of the Genus *Bosmina* Baird, 1845 (Crustacea, Cladocera). Int. Rev. Ges. Hydrobiol. **68:** 121–139, doi:10.1002/iroh.19830680108

———. 1996. Crustacea. Cladocera, Bosminidae, p. 1–80. *In* J. Schwoerbel and P. Zwick [eds.], Süßwasserfauna von Mitteleuropa [Freshwater fauna of Central Europe]. G. Fisher Verlag.

Maclean, R. C., and G. Bell. 2003. Divergent evolution during an experimental adaptive radiation. Proc. R. Soc. Lond. B Biol. Sci. **270:** 1645–1650, doi:10.1098/rspb.2003.2408

Mehner, T., K. Pohlmann, C. Elkin, M. T. Monaghan, B. Nitz, and J. Freyhof. 2010. Genetic population structure of sympatric and allopatric populations of Baltic ciscoes (Coregonus albula complex, Teleostei, Coregonidae). BMC Evol. Biol. **10:** 85, doi:10.1186/1471-2148-10-85

Melton, T., R. Peterson, A. J. Redd, N. Saha, A. S. Sofro, J. Martinson, and M. Stoneking. 1995. Polynesian genetic affinities with Southeast Asian populations as identified. Am. J. Hum. Genet. **57:** 403–414.

Mende, M., O. Biström, E. Meichssner, and G. Kölsch. 2010. The aquatic leaf beetle *Macroplea mutica* (Coleoptera: Chrysomelidae) in Europe: Population structure, postglacial colonization and the signature passive dispersal. Eur. J. Entomol. **107:** 101–113.

Omland, K. E., J. M. Baker, and J. L. Peters. 2006. Genetic signatures of intermediate divergence: Population history of Old and New World Holarctic ravens (*Corvus corax*). Mol. Ecol. **15:** 795–808, doi:10.1111/j.1365-294X.2005.02827.x

Østbye, K., P. A. Amundsen, L. Bernatchez, A. Klemetsen, R. Knudsen, R. Kristoffersen, T. F. Næsje, and K. Hindar. 2006. Parallel evolution of ecomorphological traits in the European whitefish *Coregonus lavaretus* (L.) species complex during postglacial times. Mol. Ecol. **15:** 3983–4001, doi:10.1111/j.1365-294X.2006.03062.x

Peccoud, J., J. C. Simon, H. J. McLaughlin, and N. A. Moran. 2009. Post-Pleistocene radiation of the pea aphid complex revealed by rapidly evolving endosymbionts. Proc. Natl. Acad. Sci. USA **106:** 16315–16320, doi:10.1073/pnas.0905129106

Posada, D. 2008. jModelTest: Phylogenetic model averaging. Mol. Biol. Evol. **25:** 1253–1256, doi:10.1093/molbev/msn083

Reynolds, J., B. S. Weir, and C. C. Cockerham. 1983. Estimation of the coancestry coefficient: Basis for a short-term genetic distance. Genetics **105:** 767–779.

Ritz, M. S., and others. 2008. Phylogeography of the southern skua complex—rapid colonization of the Southern Hemisphere during a glacial period and reticulate evolution. Mol. Phylogenet. Evol. **49:** 292–303, doi:10.1016/j.ympev.2008.07.014

Rundle, H. D., L. Nagel, J. W. Boughman, and D. Schluter. 2000. Natural selection and parallel speciation in sympatric sticklebacks. Science **287:** 306–308, doi:10.1126/science.287.5451.306

Simon, Y. W. H., M. J. Phillips, A. Cooper, and A. J. Drummond. 2005. Time dependency of molecular rate estimates and systematic overestimation of recent divergent times. Mol. Biol. Evol. **22:** 1561–1568, doi:10.1093/molbev/msi145

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics **139:** 457–462.

Streelman, J. T., and P. D. Danley. 2003. The stages of vertebrate evolutionary radiation. Trends Ecol. Evol. **18:** 126–131, doi:10.1016/S0169-5347(02)00036-8

Szeroczynska, K. 1991. Impact of prehistoric settlements on the Cladocera in the sediments of Lakes Suszek, Błcedowo, and Skrzetuszewskie. Hydrobiologia **225:** 105–114, doi:10.1007/BF00028389

Taylor, D. J., C. R. Ishikane, and R. A. Haney. 2002. The systematics of Holarctic bosminids and a revision that reconciles molecular and morphological evolution. Limnol. Oceanogr. **47:** 1486–1495, doi:10.4319/lo.2002.47.5.1486

Taylor, E. B., and J. D. McPhail. 1999. Evolutionary history of an adaptive radiation in species pairs of threespine sticklebacks (Gasterosteus): Insights from mitochondrial DNA. Biol. J. Linn. Soc. **66:** 271–291, doi:10.1111/j.1095-8312.1999.tb01891.x

Teacher, A. G. F., T. W. J. Garner, and R. A. Nichols. 2009. European phylogeography of the common frog (*Rana temporaria*): Routes of postglacial colonization into the British Isles, and evidence for an Irish glacial refugium. Heredity **102:** 490–496, doi:10.1038/hdy.2008.133

Weir, B. S. 1996. Genetic data analysis II: Methods for discrete population genetic data. Sinauer Associates.

———, and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population. Evolution **38:** 1358–1370, doi:10.2307/2408641

Associate Editor: Luc De Meester

Received: 11 May 2010
Accepted: 28 October 2010
Amended: 29 November 2010