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Mitochondrial sequence variation suggests extensive cryptic diversity within the Western Palearctic *Daphnia longispina* complex

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Abstract

We report on a discovery of six divergent lineages within the European *Daphnia longispina* complex from various localities in central to northeast Europe. The levels of divergence from well-known species of the complex suggest that they represent as yet unrecognized distinct taxa. These newly recognized lineages always coexisted in syntopy with widespread species of the complex. Particularly rich in cryptic diversity (with four of the six lineages present) was the delta of the River Pechora in northern European Russia, a region not covered by an ice sheet during most of the last glacial period. We suggest that ice-free regions of northeastern Europe may have been important refugia for planktonic species, and still are overlooked hot spots of diversity. Our findings confirm that the real diversity within widespread crustacean planktonic taxa is much higher than presently recognized. The potential presence of cryptic species should be considered in ecological studies.

Cryptic species, i.e., phenotypically similar but evolutionarily distinct (and often genetically highly divergent) species, are common throughout the animal kingdom (Pfenninger and Schwenk 2007), and cladocerans are not an exception (Forró et al. 2008). The boom of applications of genetic tools in ecology and taxonomy in recent decades revealed the existence of distinct lineages within numerous genera, particularly when seemingly widespread morphospecies were analyzed. Within the genus *Daphnia*, a widely used model in biological research, many previously overlooked lineages have been discovered in most biogeographical regions (Adamowicz et al. 2009). In several cases, in-depth morphological analyses allowed identification of diagnostic characters and subsequent species descriptions (Kotov et al. 2006; Juračka et al. 2010), but in most cases such cryptic lineages still await formal taxonomic recognition.

Assessing the variation of *Daphnia* species complexes had been often hampered by apparent or real overlap of phenotypic characters, which complicate identification using morphological traits, species delimitation, and taxonomical decisions (i.e., descriptions or synonymizations of taxa). *Daphnia* phenotypes are influenced by many factors, including among-population genetic variation (which might, due to local adaptations to varying environmental pressures, result in substantial differences in body shape or pigmentation even within a single genetic lineage; Petrusek et al. 2008*a*), or phenotypic plasticity such as predator-induced morphological traits (Laforsch and Tollrian 2004; Petrusek et al. 2009). Within the *Daphnia longispina* complex in particular, interspecific hybridization and introgression is an important additional factor that underlies substantial variation in body shapes and other phenotypic traits (Hobæk et al. 2004; Dlouhá et al. 2010). Thus, it is not surprising that numerous forms of the same lineages have been described as separate taxa (Petrusek et al. 2008*a*). However, some of the past descriptions of *Daphnia* species, later considered synonyms of the widespread ones, indeed referred to distinct biological species (Nilssen et al. 2007). The application of molecular methods allows successful reassessment of the status of various *Daphnia* morphotypes, and unraveling the true variation in morphologically similar populations.

In the framework of a recent study focusing on the taxonomic status of various members of the European *D. longispina* complex (Petrusek et al. 2008*a*), we discovered a highly divergent lineage of this complex in Lake Berse (southern Norway; Figs. 1, 2; Table 1) phenotypically similar to *Daphnia longispina*. Although this species has not been found as yet in any other locality, we assume that a more detailed sampling might reveal its presence in various lakes in the region of its origin. Other chance discoveries of distinct *Daphnia* lineages from single or few European localities (Petrusek et al. 2009; Juračka et al. 2010) suggest that more species await their discovery even in this seemingly well studied region, particularly if they are phenotypically cryptic, rare, or with restricted distributions.

In the present study, we document the coexistence of previously unrecognized lineages with common *Daphnia* species in several European lakes. In the framework of biogeographic, ecological, and phylogenetic projects on widespread taxa of the *D. longispina* complex, we repeatedly obtained sequences of studied mitochondrial genes that did not match to target species. Upon closer inspection, they turned out to represent several novel, highly divergent mitochondrial lineages of the *D. longispina* complex.

Methods

The analyzed samples originated from various lakes across Europe, spanning a latitudinal gradient from southern Italy to northern Scandinavia, and a longitudinal gradient from Portugal to Russia (Fig. 1). They were

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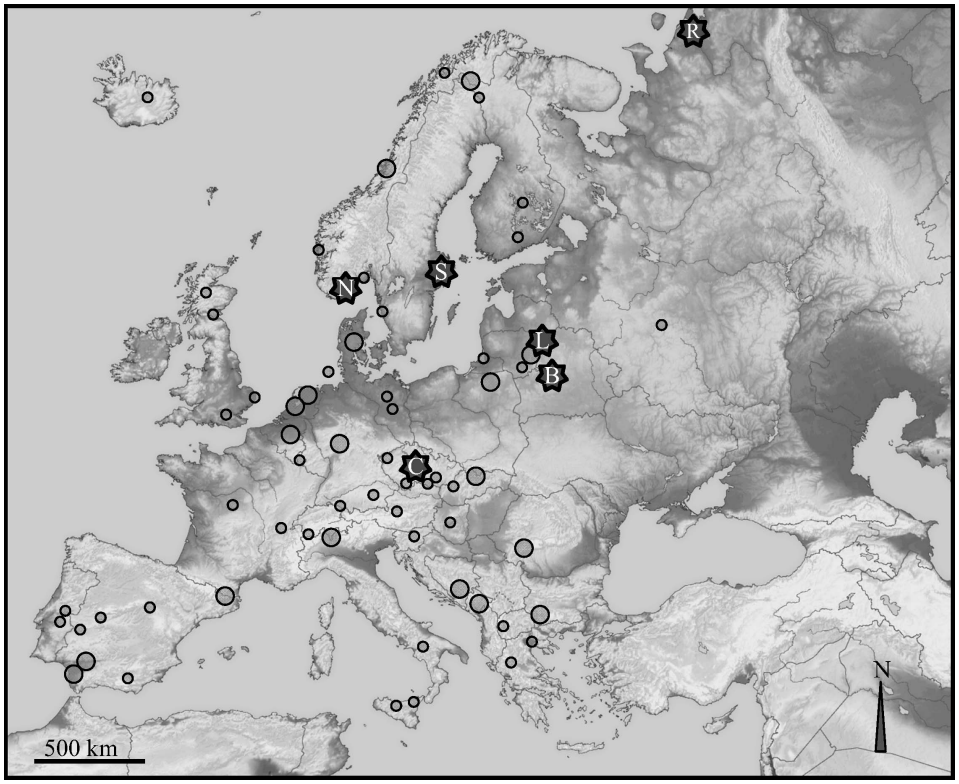

Fig. 1. European sites from which variation of the *Daphnia longispina* complex has been analyzed in the past by genetic tools including mtDNA sequencing (circles; large symbols indicate multiple sites), and geographic origin of the divergent lineages from the complex (stars). These are indicated by the first letter of the country name (B: Belarus, Kroman; C: Czech Republic, Želivka Reservoir; L: Lithuania, Druksiai; N: Norway, Berse; R: Russia, Pechora River delta; S: Sweden, Norrviken). Altogether, > 150 different sites have been studied in Europe; results were partly reported by Schwenk et al. (2004), Petrusek et al. (2007, 2008*a*), Thielsch et al. (2009), and Hamrová et al. (in press).

collected during several projects between the late 1990s and 2010, and many of them, particularly from Western, Central, and Southeastern Europe, were already processed in several studies (Petrusek et al. 2007, 2008*a*; Thielsch et al. 2009; Hamrová et al. in press). A particularly interesting area included in this study was the Pechora River delta in the northeast European part of Russia (indicated by R in Fig. 1). Apart from European sites, we also included in this study data from a reservoir in Siberia to demonstrate an extended range of one of the *Daphnia* lineages encountered in Europe.

Daphnids selected from ethanol-preserved zooplankton samples were treated according to standard protocols. Deoxyribonucleic acid (DNA) was isolated using proteinase K digestion (according to Schwenk et al. 1998), an ~ 560 base-pair (bp)–long fragment of the mitochondrial gene for 12S ribosomal ribonucleic acid (further abbreviated as 12S rRNA or 12S) was amplified by polymerase chain reaction (using the protocol from Petrusek et al. 2008*a*), and sequenced using the forward primer on a capillary sequencer. Randomly selected individuals were sequenced repeatedly and in both directions to verify data quality. In this study, we processed raw sequence data obtained between 2001 and 2011, specifically focusing on sequences not matching the reference data from known species of the *D. longispina* complex. The chromatograms were carefully checked for scoring errors, and only unambiguous part of the sequenced 12S fragment was used in subsequent analyses. If possible, multiple individuals per lineage were sequenced (Table 1) but if these originated from the same site (and carried identical haplotypes), only one was included in the tree reconstruction (Fig. 2). For a recently collected lineage originating from the Czech reservoir Želivka, we additionally obtained partial sequences of mitochondrial genes for the cytochrome *c* oxidase subunit 1 (COI) and for the reduced nicotine adenine dinucleotide (NADH) dehydrogenase subunit 2 (ND2), which we compared with other taxa of the complex. All newly obtained analyzed sequences have been submitted to GenBank under accession numbers JX069350 (for COI), JX069351 (for ND2), and JX069352-361 for 12S (*see* Table 1).

The 12S sequences were aligned with those representing all lineages of the complex for which sequences of the same gene were available, except of *Daphnia thorata* and *Daphnia mendotae*, often considered as distinct taxa but undistinguishable at mitochondrial DNA (mtDNA) level from *Daphnia dentifera* and *Daphnia galeata*, respectively. Apart

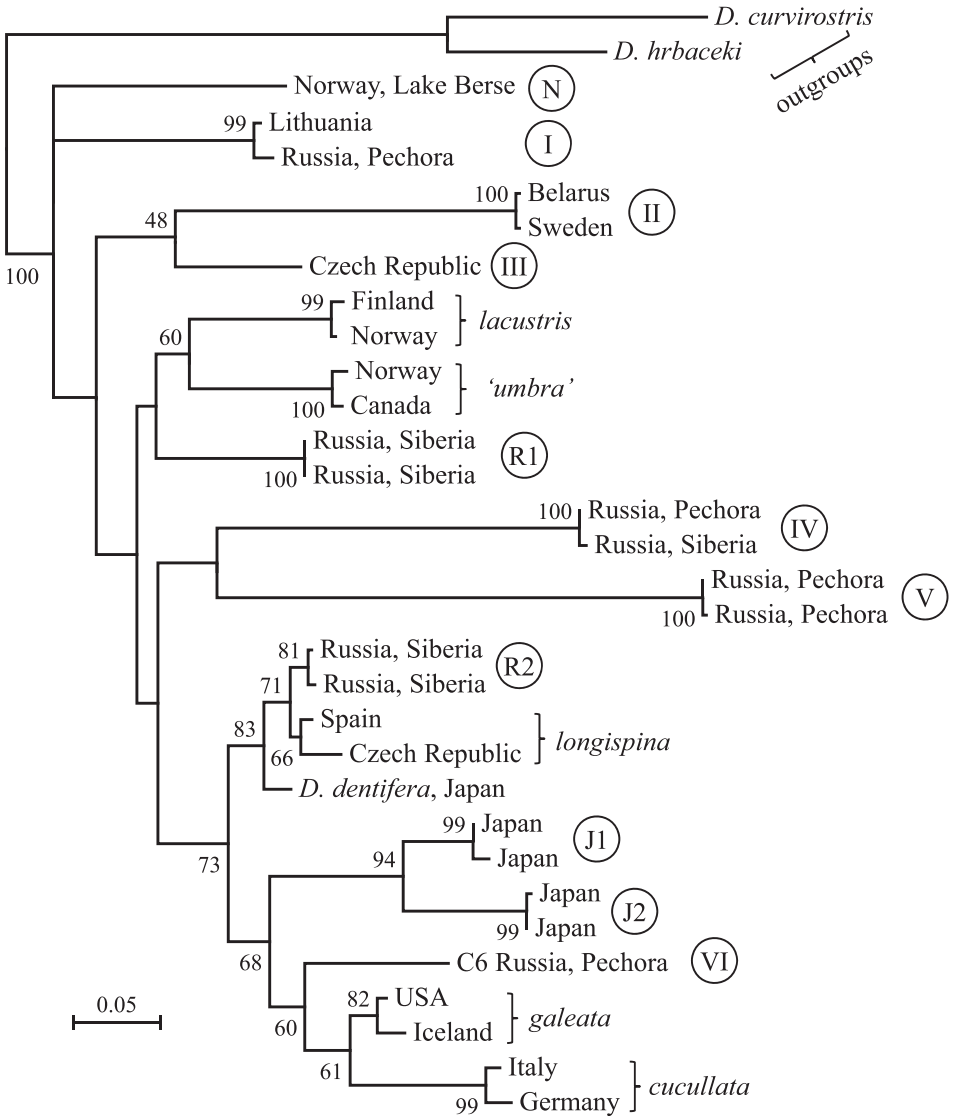

Fig. 2. Maximum likelihood tree representing the variation among and within lineages of the *Daphnia longispina* complex, with the newly reported ones indicated in Roman numerals in circles. Other as yet unnamed lineages are marked by capital letters according to their origin (N: southern Norway; J1, J2: Japan; R1, R2: Russia, Siberia). Two most divergent 12S haplotypes have been selected to represent taxa known from multiple sites (except *D. dentifera*). The scale indicates sequence divergence; its length was chosen to exceed the maximal known intraspecific variation of any European species of the complex (2.5% in *D. longispina* sensu stricto). The support values at individual nodes are based on 1000 bootstrap replications, values below 50% are not given. Note that due to limited performance of the analyzed gene fragment for resolving deeper evolutionary splits, the real phylogenetic relationships within the complex may differ, and the tree primarily serves for assessment of intra- and interspecific divergences.

from the relatively well recognized taxa (*D. longispina*, *D. galeata*, *Daphnia cucullata*, *D. dentifera*, *Daphnia lacustris*, and *Daphnia* "*umbra*"), we included also sequences of additional lineages reported in other studies and publicly available in GenBank: one lineage from Lake Berse in Norway (Petrusek et al. 2008*a*), two lineages relatively closely related to *D. galeata* and *D. cucullata* reported from Japan by Ishida et al. (2011), and two lineages from Siberia (Zuykova et al. in press). One of these Siberian lineages is substantially divergent from presently recognized taxa; the other one (of unclear taxonomic status) is very closely related to *D. dentifera* and *D. longispina* and very likely is identical to the clade showing corresponding patterns of variation of the ND2 gene in Ishida and Taylor (2007*a*). Two species of the *Daphnia curvirostris* complex, *Daphnia curvirostris* and *Daphnia hrbaceki*, representing the closest known sister clade to the *D. longispina* complex (Adamowicz et al. 2009; Juračka et al. 2010), were used as outgroup.

The alignment was created by the multiple sequence alignment software MUSCLE (Edgar 2004) with default settings, as incorporated in the Molecular Evolutionary

Table 1. List of sites in which the newly reported divergent clades of the *Daphnia longispina* complex have been found. Lineage reported from Lake Berse, Norway, by Petrusek et al. (2008*a*) is also included. Number of sequenced individuals (Inds seq.) of the respective lineage and the GenBank accession (acc.) number of the 12S sequence is given for each population.

Clade	Country	Location	Latitude (N)	Longitude (E)	Altitude (m)	Approximate size	Habitat	Coexisting species	Inds seq.	GenBank acc. No.
I	Lithuania	Lake Druksiai	55.63	26.59	137	44.8 km^2	Deep stratified lake	*D. longispina*, *D. galeata*, *D. cucullata*	2	JX069355
I	Russia (Europe)	Pechora "4"	68.07	53.52	1	~2100×500–1500 m	Shallow lake in river delta	*D. galeata*	1	JX069354
II	Sweden	Lake Norrviken	59.46	17.93	11	~6200×400 m	Eutrophic, stratified lake	*D. galeata*, *D. cucullata*	1	JX069352
II	Belarus	Kroman	53.71	26.31	133	~1300×900 m	Lake	*D. cucullata*	1	JX069353
III	Czech Republic	Želivka Reservoir	49.72	15.10	379	29 km long, 14 km^2	Large, deep, stratified reservoir	*D. longispina*, *D. galeata*, *D. cucullata*	2	JX069358
IV	Russia (Europe)	Pechora—channel	68.06	53.59	1	—	Channel in river delta	*D. galeata*, lineage V	3	JX069361
IV	Russia (Siberia)	Irkutsk Reservoir	52.24	104.33	455	65 km long, 154 km^2	Reservoir on Angara River	*D. galeata*	1	JX069360
V	Russia (Europe)	Pechora—channel	*see* above					*D. galeata*, lineage IV	1	JX069357
V	Russia (Europe)	Pechora "Lake 7"	68.06	53.55	1	~520×310 m	Shallow lake in river delta	*D. galeata*	2	JX069356
VI	Russia (Europe)	Pechora Pond	68.07	53.58	1	~1200×480 m	Shallow lake in river delta	*D. galeata*	1	JX069359
B	Norway	Lake Berse	58.32	8.22	24	~1600×250 m	Lake	*D. cristata*	5	EF375848

Table 2. Mean pairwise genetic divergences (Kimura 2 parameter distance) between European clades of the *Daphnia longispina* complex (based on two most divergent sequences available of each clade), and maximal intraspecific divergences (on the diagonal, in italics), assessed from the analyzed fragment of the mitochondrial gene for the 12S rRNA. Intraspecific variation was not calculated (nc) when only a single haplotype was available for the respective clade. The lowest value of mean divergence from another clade is marked in bold for each lineage in its line; note that the values above and below diagonal are identical. Including non-European clades does not change the pattern substantially, but the lineage R2 (differing from *D. longispina* by no more than 3%, and possibly conspecific with it) becomes also the least divergent from *D. galeata* (Kimura 2 parameter distance 0.095), and lineages I (0.134), III (0.136), and IV (0.163).

	I	II	III	IV	V	VI	"Berse"	*longispina*	*galeata*	*cucullata*	*lacustris*	"*umbra*"
I	*0.013*	0.175	0.159	0.174	0.176	0.161	0.150	**0.148**	0.165	0.161	0.162	0.150
II	0.175	*0.004*	**0.139**	0.218	0.199	0.208	0.153	0.169	0.207	0.190	0.209	0.189
III	0.159	**0.139**	nc	0.186	0.183	0.152	0.140	0.143	0.167	0.166	0.156	0.143
IV	0.174	0.218	0.186	*0.004*	0.193	0.192	0.200	**0.167**	0.175	0.207	0.209	0.184
V	**0.176**	0.199	0.183	0.193	*0.002*	0.198	0.202	0.195	0.206	0.214	0.182	0.188
VI	0.161	0.208	0.152	0.192	0.198	nc	0.190	0.127	**0.108**	0.124	0.170	0.146
"Berse"	0.150	0.153	**0.140**	0.200	0.202	0.190	nc	0.164	0.188	0.179	0.194	0.152
longispina	0.148	0.169	0.143	0.167	0.195	0.127	0.164	*0.028*	**0.105**	0.121	0.156	0.142
galeata	0.165	0.207	0.167	0.175	0.206	0.108	0.188	0.105	*0.021*	**0.099**	0.164	0.161
cucullata	0.161	0.190	0.166	0.207	0.214	0.124	0.179	0.121	**0.099**	*0.021*	0.175	0.175
lacustris	0.162	0.209	0.156	0.209	0.182	0.170	0.194	0.156	0.164	0.175	*0.010*	**0.122**
"*umbra*"	0.150	0.189	0.143	0.184	0.188	0.146	0.152	0.142	0.161	0.175	**0.122**	*0.014*

Genetics Analysis software MEGA version 5.05 (Tamura et al. 2011). The resulting alignment was 533 bp long, out of which a 434 bp–long stretch was available for all taxa (including those for which sequences were retrieved from GenBank). We also compared the alignment with the 12S sequence of the American *Daphnia* "*pulex*" complex, for which a secondary structure has been proposed in Crease (1999). In particular, we carefully inspected the distribution of variable and conserved regions, and the nature of variation in proposed stem regions of the 12S secondary structure, to check whether these are congruent between well-characterized taxa, and the newly recorded lineages. Potential mismatches, particularly if an extensive variation in otherwise conserved regions or a substantial increase of non-compensatory mutations that disrupt the nucleotide pairing within the stem regions would be observed, could suggest that the divergent 12S sequences represent nuclear pseudogenes rather than distinct variants of a mitochondrial gene (*see* Web Appendix, www.aslo.org/lo/toc/vol_57/issue_6/1838a.pdf).

Most lineages of the complex known from multiple sites were represented by two 12S sequences that spanned the maximal known intraspecific variation of the respective taxon at that gene. The exception was *D. dentifera*, for which we kept a single sequence in the tree. This taxon has apparently its center of diversity in Japan, from which numerous subclades with the 12S sequences differing by up to 6.6% have been reported by Ishida et al. (2011). Even when analyzing these genetically distinct Japanese populations, *D. dentifera* forms a well-supported monophyletic clade at mtDNA level (Ishida and Taylor 2007*a*; Möst et al. in press); however, as its sister relationship with the closely related *D. longispina* could not be properly resolved unless substantial numbers of haplotypes of both taxa were included, we refrained from demonstrating this in the present analysis.

The variation within and among the lineages was shown in a tree constructed by Maximum Likelihood method in MEGA 5, applying the Tamura–Nei model of DNA evolution with gamma distributed rate heterogeneity (identified as the most suitable model by Bayesian Information Criterion). The node support was evaluated by 1000 bootstrap replications. Furthermore, we calculated mean pairwise sequence divergences between clades present in Europe, and the corresponding maximal intraspecific divergences, by Kimura 2 parameter model with pairwise deletion of indels.

Results

In addition to the six known European species of the *D. longispina* complex, we detected six more, mostly highly divergent clades (labeled with Roman numerals in Fig. 2 and Table 1), none of which is closely related to other cryptic lineages already detected in Siberia or Japan. These previously unknown European lineages originated from localities scattered in different regions of Central and North- to Northeastern Europe, including the Czech Republic, Lithuania, Sweden, Belarus, and Russia (Fig. 1; Table 1). Particularly rich in cryptic lineage diversity, with four new lineages detected (I, IV, V, and VI), was the Pechora Delta in northeast European Russia. Two lineages (III and VI) were found in single waterbodies (Želivka Reservoir in the Czech Republic and one of the shallow lakes in the Pechora Delta, respectively), others were detected in two different localities, either within the same region (lineage V in the Pechora Delta) or distantly apart (Sweden and Belarus, ~ 800 km; Lithuania and Pechora, 1900 km; and Pechora and Irkutsk Reservoir in Siberia, 3200 km). For all but one clade (the exception being lineage VI), sequences from more than one individual were obtained.

The levels of genetic differentiation (mean between-clade Kimura 2 parameter distance) of the newly recorded lineages (I–VI) from their closest sister taxa ranged from 10.8% to 17.6% (Table 2). The lowest divergence was found between lineage VI and *D. galeata*; the other lineages were substantially more divergent from presently recognized species and from each other (Table 1; Fig. 2). All

these values far exceed not only the maximal divergences of the same 12S fragment observed within relatively well studied taxa in Europe (ranging between 1.0% in *D. lacustris* to 2.8% in *D. longispina*; Table 1; Fig. 2) but also the outstanding variation observed among Japanese lineages of *D. dentifera* (up to 6.6%).

The 12S sequences of the newly obtained divergent clades shared conserved regions with those from other species of the complex as well as outgroups, and the distribution of compensatory and non-compensatory mutations in the stem regions of the proposed 12S secondary structure did not substantially differ between already known and newly characterized lineages of the complex (*see* Web Appendix for details). This suggests that the sequences originate from functional mitochondrial genes rather than from nuclear pseudogenes. Furthermore, the COI and ND2 sequences obtained from the lineage found in Želivka Reservoir also confirmed its distinct position within the *D. longispina* complex (differing from *D. galeata* as the nearest well-established taxon by no less than 12.4% at COI, and 16.6% at ND2). We thus conclude that the variation observed by us indeed reflects cryptic lineage diversity.

Discussion

The finding of six divergent clades in European samples of the *D. longispina* complex doubles the number of lineages known in this complex from the Western Palearctic region (Fig. 2). This increase is substantial also in the global context. Apart from species included in Fig. 2, there are two additional taxa often recognized in North America, *D. mendotae* and *D. thorata.* However, these fully overlap in mtDNA variation with *D. galeata* and *D. dentifera*, respectively (Schwenk et al. 2000; Ishida and Taylor 2007*a*), and the lack of divergence of *D. thorata* at nuclear markers might suggest that it is conspecific with *D. dentifera* (Petrusek et al. 2008*a*). Additional diversity within the *D. longispina* complex is likely to be found in understudied regions, including vast expanses of Asia in both Palearctic and Oriental regions, and sub-Saharan Africa. Presumed discoveries of rare new taxa based on the analysis of a single mitochondrial locus, however, must be interpreted particularly carefully to rule out the option that divergent sequences represent nuclear pseudogenes of mitochondrial genes rather than distinct evolutionary lineages.

The comparison with the divergence levels among and within currently recognized species of the *D. longispina* complex found in the region (Fig. 2; Table 2), as well as with interspecific divergences found in other *Daphnia* species complexes (Adamowicz et al. 2009), suggests that the newly recognized lineages likely represent distinct biological species. The morphological characteristics of sequenced individuals could not be retrospectively evaluated. However, given the extensive phenotypic variation within the *D. longispina* complex that often results in unreliable species identification, particularly if a general phenotype is assessed (Dlouhá et al. 2010), it is not particularly surprising that misidentification (as *D. galeata* or *D. longispina*) occurred. To evaluate to what extent these are truly cryptic lineages, detailed morphological examination, mostly based on new collections, is necessary.

Interestingly, divergent *Daphnia* lineages were found not only in understudied regions, from which hardly any genetic work on *Daphnia* has been reported (but *see* Schwenk et al. 2004), but also in a Czech reservoir (Želivka), in which numerous limnological surveys have been conducted. In particular, the taxonomic structure of the local population of the *D. longispina* complex was recently repeatedly analyzed using allozyme markers (Petrusek et al. 2008*b*), but no patterns suggesting a presence of a cryptic lineage were observed. It is possible that the new lineage has recently invaded this waterbody, or that its abundance only increased to the detectable levels between 2004–2005 (the time period of allozyme analyses) and summer 2010 (when the new lineage was collected). Although the taxon composition of the *D. longispina* complex in studied Czech reservoirs did not significantly differ between consecutive summers (Petrusek et al. 2008*b*), among-year changes in proportions of different *Daphnia* taxa have been repeatedly reported from various lakes, including a complete replacement of a dominant taxon from one year to another (Yin et al. 2010).

Temporal changes in the frequency of lineage III could indeed explain its recent discovery in Želivka. However, there are also alternative explanations of the apparent lack of divergence at allozyme loci despite high divergence at the mtDNA level. In particular, nuclear introgression from common species of the *D. longispina* complex, similar to that from *D. dentifera* to *D. galeata* in North America or Japan (Ishida and Taylor 2007*b*; Ishida et al. 2011), could result in high similarity of nuclear genomes between mtDNA lineages. In that case, the frequency of the divergent lineage could remain high within the *Daphnia* community but it would still escape detection.

Interspecific hybridization has been documented between relatively highly divergent lineages within the complex (*D. galeata* and *D. lacustris*; Hobæk et al. 2004; Nilssen et al. 2007); thus, it might also be possible between similarly divergent *Daphnia* from the Želivka Reservoir and coexisting *D. longispina* or *D. galeata* (*see* Fig. 2; Table 2). An analysis of more variable nuclear markers than allozymes, coupled with identification of the maternal lineage by mtDNA, may be thus conducted to test for the taxonomic and evolutionary status of this particular lineage. Even more likely than in the Želivka Reservoir is hybridization between the lineage VI and closely related *D. galeata*, with which it coexisted within the same waterbody in the Pechora Delta (Table 1). However, given its geographic origin, this lineage might be less accessible for future studies.

The very high lineage richness of the *Daphnia longispina* complex from the Pechora Delta suggests that this boreal region is an important center of *Daphnia* diversity. The number of newly detected lineages is particularly striking given the low number of analyzed samples, which come from a very small area ($< 3 \times 2$ km). Most of the Pechora Basin remained ice-free during the last glacial period, and a periglacial lake was adjacent to the ice sheet during the

period of maximal ice expansion (Astakhov 2011). It is thus possible that this region was a part of a refugium for aquatic taxa during that period, or that these taxa colonized the region from other areas further away from the Weichselian ice shield soon after the local conditions became suitable at the end of the last glacial. While the importance of extra-Mediterranean glacial refugia for plants and terrestrial animals is now widely recognized (Varga 2010), little is known about potential refugial areas of European planktonic species. Petrusek et al. (2007) speculated that the predominantly boreal species *D. lacustris* may have persisted during the glacials in Eastern Europe or Siberia. Although we did not record this particular species in the Pechora Delta, the finding of high diversity of *Daphnia* in this formerly unglaciated region of northeast Europe supports the existence of such a refugium for aquatic taxa. We presume that more detailed sampling in waterbodies of northern European Russia would likely reveal the presence of *D. lacustris*, as well as more localities with the presently reported lineages.

An interesting aspect of the spatial distribution of these *Daphnia* lineages is their apparent absence from any Western or Southern European regions. This does not seem to be due to limited sampling effort, as there were numerous samples analyzed from Spain, Italy, Germany, United Kingdom, the Netherlands, and other Western European countries, as well as from the mountain ranges of the Balkan Peninsula (Fig. 1). Rather, the lack of such rare lineages in Western Europe might reflect the fact that the Western Palearctic biogeographic region, which has been severely affected by Pleistocene climate oscillations, is at or beyond the edge of the distribution of taxa that survived in eastern rather than southern glacial refugia and encountered other ecologically similar and competitively superior species when dispersing westward. We thus suppose that the current distribution of these daphnids in Europe suggests their dispersal from more easterly located regions, where they might be still found at present.

One of the lineages (V), which was found in both the Pechora Delta and Irkutsk Reservoir in Central Siberia (located on the outflow of Lake Baikal), has certainly a very wide distribution. However, it does not seem that any of these lineages dominates Siberian waterbodies. The presence of common Palearctic species, *D. longispina*, *D. galeata*, and *D. cucullata*, in Siberia has been confirmed by genetic tools (Zuykova et al. 2010; Zuykova et al. in press) and another highly divergent mitochondrial lineage of the *D. longispina* complex (indicated as R1 in Fig. 2) was found in this region by Zuykova et al. (in press). We suppose that at least in the waterbodies examined in detail by these authors, a widespread presence of other distinct *Daphnia* lineages would not be missed. Thus, we assume that many of the lineages recorded by us may have either relatively restricted or scattered distributions, as indicated by the fact that none of them has been recorded as yet in more than two localities. This apparent paradox of substantial genetic divergence (which suggests sufficient evolutionary age for successful long-range dispersal) but small presently known distributional areas does not seem to be exceptional. Various other members of the *D. longispina* complex (e.g., the Japanese lineages reported by Ishida et al. 2011) as well as other *Daphnia* species often show similar restricted distributions, and it remains to be seen whether these are real biogeographic patterns or simply reflect a limited sampling effort.

Altogether, our records of several divergent *Daphnia* lineages in Eastern and Northern European waterbodies suggests that the lineage diversity of the *D. longispina* complex in the Western Palearctic is much higher than assumed until recently. Such high diversity within a genus that served as model in many ecological and evolutionary studies further supports the indications that a substantial proportion of cladoceran taxa still awaits discovery (Forró et al. 2008), and the same is likely true for other freshwater planktonic crustaceans (Boxshall and Defaye 2008; Hamrová et al. in press). We may thus expect that species-rich groups that have mostly escaped the interest of molecular taxonomists and molecular ecologists (e.g., cladoceran genera *Ceriodaphnia*, *Diaphanosoma*, *Moina*, most members of the cladoceran families Chydoridae and Macrothricidae, as well as a wide range of freshwater copepods) hide enormous diversity that remains to be discovered. Furthermore, when interpreting results of ecological studies, the potential that the presence of cryptic species may influence the observed patterns should be kept in mind.

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References

Adamowicz, S. J., A. Petrusek, J. K. Colbourne, P. D. N. Hebert, and J. D. S. Witt. 2009. The scale of divergence: A phylogenetic appraisal of intercontinental allopatric speciation in a passively-dispersed freshwater zooplankton genus. Mol. Phylogenet. Evol. **50:** 423–436, doi:10.1016/j.ympev.2008.11.026

Astakhov, V. 2011. Ice margins of Northern Russia revisited, p. 323–336. *In* J. Ehlers, P. L. Gibbard, and P. D. Hughes [eds.], Quaternary glaciations—extent and chronology. Part IV: A closer look. Developments in quaternary sciences, v. 15. Elsevier.

Boxshall, G. A., and D. Defaye. 2008. Global diversity of copepods (Crustacea: Copepoda) in freshwater. Hydrobiologia **595:** 195–207, doi:10.1007/s10750-007-9014-4

Crease, T. J. 1999. The complete sequence of the mitochondrial genome of *Daphnia pulex* (Cladocera: Crustacea). Gene **233:** 89–99, doi:10.1016/S0378-1119(99)00151-1

Dlouhá, Š., A. Thielsch, R. H. S. Kraus, J. Seda, K. Schwenk, and A. Petrusek. 2010. Identifying hybridizing taxa within the *Daphnia longispina* species complex: A comparison of genetic methods and phenotypic approaches. Hydrobiologia **643:** 107–122, doi:10.1007/s10750-010-0128-8

Edgar, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. **32:** 1792–1797, doi:10.1093/nar/gkh340

Forró, L., N. M. Korovchinsky, A. A. Kotov, and A. Petrusek. 2008. Global diversity of cladocerans (Cladocera, Crustacea) in freshwater. Hydrobiologia **595:** 177–184, doi:10.1007/s10750-007-9013-5

Hamrová, E., M. Krajicek, T. Karanovic, M. Černý, and A. Petrusek. In press. Congruent patterns of lineage diversity in two species complexes of planktonic crustaceans, *Daphnia longispina* (Cladocera) and *Eucyclops serrulatus* (Copepoda), in East European mountain lakes lakes. Zool. J. Linn. Soc.

Hobæk, A., M. Skage, and K. Schwenk. 2004. *Daphnia galeata* × *D. longispina* hybrids in western Norway. Hydrobiologia **526:** 55–62, doi:10.1023/B:HYDR.0000041614.68315.ec

Ishida, S., A. Takahashi, N. Matsushima, J. Yokoyama, W. Makino, J. Urabe, and M. Kawata. 2011. The long-term consequences of hybridization between the two *Daphnia* species, *D. galeata* and *D. dentifera*, in mature habitats. BMC Evol. Biol. **11:** 209, doi:10.1186/1471-2148-11-209

———, and D. J. Taylor. 2007*a*. Mature habitats associated with genetic divergence despite strong dispersal ability in an arthropod. BMC Evol. Biol. **7:** 52, doi:10.1186/1471-2148-7-52

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

Juračka, P. J., V. Kořínek, and A. Petrusek. 2010. A new Central European species of the *Daphnia curvirostris* complex, *Daphnia hrbaceki* sp. nov. (Cladocera, Anomopoda, Daphniidae). Zootaxa **2718:** 1–22.

Kotov, A. A., S. Ishida, and D. J. Taylor. 2006. A new species in the *Daphnia curvirostris* (Crustacea: Cladocera) complex from the eastern Palearctic with molecular phylogenetic evidence for the independent origin of neckteeth. J. Plankton Res. **28:** 1067–1079, doi:10.1093/plankt/fbl041

Laforsch, C., and R. Tollrian. 2004. Inducible defenses in multipredator environments: Cyclomorphosis in *Daphnia cucullata*. Ecology **85:** 2302–2311, doi:10.1890/03-0286

Möst, M., A. Petrusek, R. Sommaruga, P. J. Juračka, M. Slusarczyk, M. Manca, and P. Spaak. In press. At the edge and on the top: Molecular identification and ecology of *Daphnia dentifera* and *D. longispina* in high-altitude Asian lakes. Hydrobiologia.

Nilssen, J. P., A. Hobæk, A. Petrusek, and M. Skage. 2007. Restoring *Daphnia lacustris* G. O. Sars, 1862 (Crustacea, Anomopoda)—a cryptic species in the *Daphnia longispina* group. Hydrobiologia **594:** 5–17, doi:10.1007/s10750-007-9076-3

Petrusek, A., M. Černý, J. Mergeay, and K. Schwenk. 2007. *Daphnia* in the Tatra Mountain lakes: Multiple colonisation and hidden diversity revealed by molecular markers. Fund. Appl. Limnol. **169:** 279–291, doi:10.1127/1863-9135/2007/0169-0279

———, A. Hobæk, J. P. Nilssen, M. Skage, M. Černý, N. Brede, and K. Schwenk. 2008*a*. A taxonomic reappraisal of the European *Daphnia longispina* complex (Crustacea, Cladocera, Anomopoda). Zool. Scr. **37:** 507–519, doi:10.1111/j.1463-6409.2008.00336.x

———, J. Seda, J. Macháček, Š. Ruthová, and P. Šmilauer. 2008*b*. *Daphnia* hybridisation along ecological gradients in pelagic environments: The potential for the presence of hybrid zones in plankton. Phil. Trans. R. Soc. B **363:** 2931–2941, doi:10.1098/rstb.2008.0026

———, R. Tollrian, K. Schwenk, A. Haas, and C. Laforsch. 2009. A "crown of thorns" is an inducible defense that protects *Daphnia* against an ancient predator. Proc. Natl. Acad. Sci. USA **106:** 2248–2252, doi:10.1073/pnas.0808075106

Pfenninger, M., and K. Schwenk. 2007. Cryptic animal species are homogeneously distributed among taxa and biogeographical regions. BMC Evol. Biol. **7:** 121, doi:10.1186/1471-2148-7-121

Schwenk, K., P. Junttila, M. Rautio, F. Bastiansen, A. Knapp, O. Dove, R. Billiones, and B. Streit. 2004. Ecological, morphological, and genetic differentiation of *Daphnia* (*Hyalodaphnia*) from the Finnish and Russian subarctic. Limnol. Oceanogr. **49:** 532–539, doi:10.4319/lo.2004.49.2.0532

———, D. Posada, and P. D. N. Hebert. 2000. Molecular systematics of European *Hyalodaphnia*: The role of contemporary hybridization in ancient species. Proc. R. Soc. B **267:** 1833–1842, doi:10.1098/rspb.2000.1218

———, A. Sand, M. Boersma, M. Brehm, E. Mader, D. Offerhaus, and P. Spaak. 1998. Genetic markers, genealogies and biogeographic patterns in the Cladocera. Aquat. Ecol. **32:** 37–51, doi:10.1023/A:1009939901198

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. **28:** 2731–2739, doi:10.1093/molbev/msr121

Thielsch, A., N. Brede, A. Petrusek, L. De Meester, and K. Schwenk. 2009. Contribution of cyclic parthenogenesis and colonization history to population structure in *Daphnia*. Mol. Ecol. **18:** 1616–1628, doi:10.1111/j.1365-294X.2009.04130.x

Varga, Z. 2010. Extra-Mediterranean refugia, post-glacial vegetation history and area dynamics in eastern Central Europe, p. 57–87. *In* J. C. Habel and T. Assmann [eds.], Relict species: Phylogeography and conservation biology. Springer.

Yin, M., J. Wolinska, and S. Gießler. 2010. Clonal diversity, clonal persistence and rapid taxon replacement in natural populations of species and hybrids of the *Daphnia longispina* complex. Mol. Ecol. **19:** 4168–4178, doi:10.1111/j.1365-294X.2010.04807.x

Zuykova, E. I., N. A. Bochkarev, and A. V. Katokhin. In press. Molecular genetic identification and phylogeny of *Daphnia* species (Crustacea: Cladocera) from the water bodies of Chany Lake basin. Russ. J. Genet.

———, ———, A. S. Semenova, and A. V. Katokhin. 2010. Morphological differentiation, mitochondrial and nuclear DNA variability between geographically distant populations of *Daphnia galeata* and *Daphnia cucullata* (Anomopoda, Daphniidae). J. Sib. Fed. Univ. Biol. **3:** 434–453.

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