Molecular Phylogenetics and Evolution 50 (2009) 423–436

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

The scale of divergence: A phylogenetic appraisal of intercontinental allopatric speciation in a passively dispersed freshwater zooplankton genus

Sarah J. Adamowicz [a,*], Adam Petrusek [b], John K. Colbourne [c], Paul D.N. Hebert [d], Jonathan D.S. Witt [a]

[a] Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, Ont., Canada N2L 3G1
[b] Department of Ecology, Faculty of Science, Charles University in Prague, Viničná 7, CZ-12844, Prague 2, Czech Republic
[c] The Center for Genomics and Bioinformatics, Indiana University, Bloomington, IN 47405, USA
[d] Biodiversity Institute of Ontario, University of Guelph, 579 Gordon Street, Guelph, Ont., Canada N1G 2W1

ARTICLE INFO

Article history:
Received 19 October 2007
Revised 24 November 2008
Accepted 26 November 2008
Available online 16 December 2008

Keywords:
Biogeography
Cladocera
Long-distance dispersal
Mitochondrial DNA
Molecular systematics
Passive dispersal
Zooplankton
COI
12S
16S

ABSTRACT

Molecular studies have enlightened our understanding of freshwater zooplankton biogeography, yet questions remain regarding the scale and commonality of geographic speciation. Here, we present a mtDNA-based phylogenetic hypothesis for 92 *Daphnia* species from all seven continents, with a focus on North and South America, Europe, and Australia, and use it to explore the frequency, scale, and geographical orientation of allopatric divergence events. Allopatric speciation can conservatively account for at least 42% of cladogenetic events among the species included in our study; most of these involve intercontinental splits. Closely related species pairs are concentrated in the circumarctic region and between northern and southern continents, aligned with bird migration routes, suggesting recent dispersal. By contrast, deeper phylogenetic patterns are consistent with vicariance scenarios linked to continental fragmentation. The possible reasons for the puzzling persistence of these ancient patterns in light of the eroding force of dispersal are considered. Our results demonstrate the high frequency and complex pattern of allopatric speciation in this ancient, passively dispersed genus.

1. Introduction

A historically close relationship exists between the study of biogeography and our understanding of speciation processes (Darwin, 1859). This association is especially clear within studies of the freshwater invertebrate fauna. The morphological similarity of species inhabiting different continents, combined with observations of strong dispersal mechanisms possessed by freshwater invertebrates, led to the early conclusion of cosmopolitan distributions (Darwin, 1859, 1882; Mayr, 1963). According to this view, global species diversity within many freshwater groups was low, and high dispersal rates limited opportunities for allopatric speciation. Yet detailed morphological (Frey, 1982, 1987) and extensive molecular (e.g. Colbourne et al., 1998; Černý and Hebert, 1999; Gómez et al., 2000; Schwenk et al., 2000; Petrusek et al., 2004; Fontaneto et al., 2008) evidence necessitated a reversal of this view. Despite broad geographic morphological similarities, most aquatic invertebrate species are confined to single continents. Moreover, species richness as well as intraspecific genetic diversity is often high within continents (De Melo and Hebert, 1994; Hann, 1995; Taylor et al., 1998; Cox and Hebert, 2001; Hebert et al., 2003; Penton et al., 2004) and even smaller regions (Hebert and Wilson, 1994, 2000; Kořínek and Hebert, 1996; Kořínek et al., 2003; Petrusek et al., 2007), and has led to a shift towards provincialism as the prevailing biogeographic hypothesis. Concordant with this view, detailed distributional information has revealed that allopatric divergence is likely to be an important mechanism of diversification for invertebrates inhabiting continental waters.

Despite these advances, the relationship between observed distributions and evolutionary processes remains a complex puzzle. The facts that new habitats are rapidly invaded (Louette and De Meester, 2005) and that some genotypes have vast geographic distributions (e.g. Weider et al., 1999a,b) suggest that dispersal potential is high, which is seemingly paradoxical given the high degree of genetic structuring among populations even at local scales (reviewed in De Meester et al., 2002). De Meester et al. (2002) presented a convincing synthesis that addressed this dispersal/gene flow problem. According to their "monopolization hypothesis", founder events, rapid population increase, local adaptation, resource monopolization, and the build-up of resting egg banks combine to make established populations highly resistant to invasion by new migrants. As such, patterns of genetic diversity

* Corresponding author. Present address: Biodiversity Institute of Ontario, University of Guelph, 579 Gordon Street, Guelph, Ont., Canada N1G 2W1. Fax: +1 519 824 5703.
E-mail address: sadamowi@uoguelph.ca (S.J. Adamowicz).

1055-7903/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.ympev.2008.11.026

are more related to priority effects than to contemporary dispersal opportunities. Genetic divergence among allopatric groups is often observed, but these groups most likely originated as a consequence of colonization priority rather than the presence of pervasive barriers to gene flow. Indeed, sharp demarcations can be observed in the distributions of refugial phylogroups, which appear to have dispersed rapidly until encountering habitats already occupied by other groups (Cox and Hebert, 2001; reviewed in De Meester et al., 2002). The importance of priority effects seems to be especially pronounced for cyclic parthenogens such as cladocerans and rotifers (Boileau et al., 1992; De Meester et al., 2002), which have the capacity for rapid local adaptation due to large population sizes, inter-clonal selection during parthenogenetic phases of reproduction, and sexual shuffling of genetic variation (De Meester et al., 2002).

Another important problem in the resolution of the dispersal/gene flow question is that of scale. Intuitively, it would seem that dispersal—followed by successful establishment—between continents would be even less likely than within them. As a result of differences in geology, climate, and habitat arrays, intercontinental migrants might be expected to have extra adaptive handicaps compared with incomers from the same continent, in addition to fewer candidate propagules that travel the distance. However, several species or closely related species pairs display close intercontinental genetic associations. Mitochondrial divergences of <5% are known between North and South America (Adamowicz et al., 2002, 2004; Hebert et al., 2003; Mergeay et al., 2008) and between North America and Europe (Schwenk et al., 2000; Ishida and Taylor, 2007) for several different species of *Daphnia*. Intercontinental associations are known within other cladoceran genera, such as *Bosmina* (Taylor et al., 2002), *Moina* (Petrusek et al., 2004), and *Holopedium* (Rowe et al., 2007), as well as in bryozoans (Freeland et al., 2000a) and rotifers (Fontaneto et al., 2008). The shallow divergences often observed are strongly suggestive of dispersal rather than ancient vicariance, if mitochondrial molecular clocks (e.g. Knowlton and Weigt, 1998) are correct even to within an order of magnitude. These successful intercontinental migrations suggest that additional factors might be fruitfully added to the monopolization hypothesis. For example, the degree of predator and pathogen adaptation to their local prey may be an important factor. Genetic evidence on presumed anthropogenic introductions indicates that intercontinental migrants can be more ecologically successful than would be expected based upon the numbers of newcomers alone (Havel et al., 2000; Mergeay et al., 2006). Understanding the dynamics of the dispersal/gene flow relationship across different spatial scales will require more complete knowledge of rates of intercontinental movement.

Daphnia is an especially useful target among freshwater zooplankton for a multi-continental study as a consequence of taxonomic insights gained from numerous genetic studies employing allozymes and mitochondrial DNA. These studies enable an interspecific phylogeographic analysis based upon genetically defined species boundaries, rather than on species lists derived only from morphological studies, which can overlook cryptic or recent speciation events (see Barraclough and Nee, 2001). Here, we quantify the frequency and phylogenetic depth of intercontinental shifts in *Daphnia*, compare patterns of divergence across different spatial scales, and assess the role of large-scale allopatric divergence in the global cladogenesis of this genus.

2. Methods

2.1. The sequence data

2.1.1. Species inclusion

All 92 *Daphnia* taxa included in this study are thought to be distinct species, even though some are not yet formally described. Undescribed species are referred to by the name of the species complex to which they belong, together with a number identifying the particular lineage and its region of origin (e.g. *D.* gr. *atkinsoni* sp2-ISR, referring to a previously unknown lineage in the *atkinsoni* complex from Israel). In some cases, genetic evidence has revealed that certain species are in fact divergent from a similar-looking morphospecies to which they have previously been assigned (e.g. North American lineages similar to *D. similis* or *D. pulex*). In these cases, where retaining information about the morphological similarity is useful, lineages differing from the nominate species are indicated by "cf." Species assigned invalid names or those which have not been formally described, but have received a name in previous literature (i.e. a *nomen nudum*), are designated by placing the specific epithet in quotation marks. Species names, localities, and abbreviations for regions, as well as taxonomic notes, are provided in Table 1.

Given this mixture of described and undescribed species, we briefly outline the species concept employed. Adamowicz and Purvis (2005) found that most authors of recent branchiopod genetic studies use a concordance species concept (Avise and Ball, 1990), or a proxy thereof (see below). Typically, a traditional morphospecies is employed as a starting point and then other types of evidence are considered to identify distinctive species with their own evolutionary trajectories. Here, a concordance species concept was also adopted, as it was expected that this approach would provide the best indication of species boundaries (i.e. where natural breaks lie in genetic, morphological, and ecological variation). We adopted a pragmatic set of quantitative criteria to delineate species following Adamowicz and Purvis (2005), who examined levels of genetic diversity typically observed within and among branchiopod species. Our preferred approach was to use multiple lines of concordant evidence when available, requiring that at least two of the following conditions be met: (1) a fixed allozyme allelic substitution at one or more loci; (2) overall divergence of allozyme allele frequencies; (3) mtDNA sequence divergence (>5% in sequences of the cytochrome *c* oxidase subunit I gene [COI], >4% in the 12S rRNA gene); (4) clear morphological difference; and (5) clear habitat difference or other ecological segregation. When multiple lines of evidence were unavailable, lineages showing substantial sequence divergence (>10% COI, >8% 12S) were treated as species, because in cases where there was more information, lineages differing by this magnitude were always classified as different species. However, *Daphnia "arenata"* Hebert (1995), did not conform to the above criteria, and was tentatively recognized as a distinct species because Pfrender et al. (2000) provided evidence of both a habitat shift and genetic differentiation from progenitor populations in allozyme, mtDNA, and microsatellite markers. This case may represent an example of incipient speciation. Two taxon names appearing in previous phylogenetic literature (*D. hyalina* and *D. thorata*) are not included here as they did not meet our species criteria; the former has been formally synonymized with *D. longispina* (for more details, see Petrusek et al., 2008).

The majority of the undescribed *Daphnia* species listed in Table 1, as well as many described species, have been characterized by allozymes (i.e. nuclear markers), which revealed that they possess fixed diagnostic differences (Hebert, 1977, 1995; Wolf and Mort, 1986; Hebert et al., 1989; Hobæk and Wolf, 1991; Taylor and Hebert, 1992, 1993a,b, 1994; Hebert and Finston, 1993, 1996, 1997; Dufresne and Hebert, 1994, 1997; Hebert and Wilson, 1994, 2000; Kořínek and Hebert, 1996; Taylor et al., 1996, 1998; Černý and Hebert, 1999; Kořínek et al., 2003; Michels et al., 2003; Adamowicz et al., 2004). In a few cases where allozyme data were lacking, the mtDNA divergence criterion was used instead. On the basis of mtDNA sequences, *D.* gr. *similis* sp2-EUR from Germany and *D.* cf. *"similis"* sp3-NA/SA from North America are very different from

Table 1
List of *Daphnia* species included, by subgenus, and their collection localities.

Species	Collection location	References or collector	GenBank Accession Nos.		
			COI	12S	16S
Australodaphnia					
D. occidentalis Benzie, 1986	Northcliff, WA, AUS	C06	AY921424	AY921472	AY921457
Ctenodaphnia					
D. angulata Hebert, 1977	Lake Omeo, VIV, AUS	C06	AY921414	AY921460	AY921453
D. atkinsoni s.s. Baird, 1859	Pond in Kholon, ISR	A.P.	DQ166844	DQ116591	—
D. gr. atkinsoni sp2-ISR	Golan Heights, ISR	S.S.	FJ427481	FJ427403	FJ427455
D. gr. atkinsoni sp3-SA	Pond near Sarmiento, Chubut, ARG (site #209)	A04	AY323122	FJ427404	—
D. australis (Sergeev and Williams, 1985)*	Colac, VIC, AUS	H02; C06	AF217110	AF217122	AY921441
D. barbata Weltner, 1897	OI Bolossat ponds, KEN	M07	FJ427482	AM412584	—
D. carinata King, 1853	Maitland, NSW, AUS (COI, 16S); AS, AUS (12S)	H02; C06	AF217116	AY921461	AY921435
D. cephalata King, 1853	Sydney, NSW, AUS	H02; C06	AF308967	AF217135	AY921427
D. gr. chevreuxi sp1-ISR (not identical to *D. chevreuxi* s.s. Richard, 1896)	Pond close to El Rom, Golan Heights, ISR	A.P.	FJ427483	FJ427405	—
D. "citrina" AUS (formally undescribed species, name used in C06)	Coast, WA, AUS	C06	AY921419	AY921463	AY921432
D. dadayana Paggi, 1999	Pond near Esquel, Chubut, ARG (site #96)	A04	AY323084	FJ427406	FJ427456
D. ephemeralis (Schwartz and Hebert, 1985)*	Pond near Guelph, ON, CAN	Co06; C06	AY921422	AY921473	AY921439
D. exilis Herrick, 1895	Pond near Amarillo, TX, USA	H02; C06	AF308972	AY921465	AY921456
D. hispanica Glagolev and Alonso, 1990	Roadside ditch close to El Rocío, Andalusia, SP	A.P.	FJ427484	FJ427407	—
D. jollyi Petkovski, 1973	Pond near Mt. Hampton, WA, AUS	H02; C06	AF308969	AY921471	AY921449
D. longicephala Hebert, 1977	Wave, WA, AUS (16S); Ivanhoe, NSW, AUS (COI, 12S).	H02; C06	AF217114	AF217136	AY921426
D. lumholtzi Sars, 1885	Lyell Lake, NSW, AUS	C06	AY921417	AY921466	AY921451
D. lumholtzi NA	MI, USA (COI); Pomme de Terre Lake, MO, USA (12S and 16S)	H02; T96	AF308974	FJ427408	FJ427457
D. magna Straus, 1820	Crescent Lake, NE, USA	H02; C06	AF217106	AY921467	AY921452
D. magna EUR	COI haplotype H6 (found in EUR & ISR); Dora Pool, ISR (12S); Ring Sø, DEN (16S)	DD05 (COI); A.P. (12S); St06 (16S)	AY803045	DQ116603	DQ470575
D. "magniceps" sp2-AUS (this taxon corresponds to *D. magniceps* Sars, 1896 but needs a new name due to the priority of *D. magniceps* Herrick, 1884 from North America)	Hoskin, ACT, AUS	H02; C06	AF217117	AF217142	AY921433
D. mediterranea Alonso, 1985	Hypersaline pools close to Lucio de Membrillo lagoon, Doñana National Park, SP	A.P.	FJ427485	FJ427409	—
D. menucoensis Paggi, 1996	Lago Las Encadenadas, Buenos Aires province, ARG (site #124)	A04	AY323078	FJ427410	FJ427458
D. "muddensis" AUS (formally undescribed species, name used in C06)	Mt. Magnet, WA, AUS	C06	AY921415	AY921462	AY921447
D. "neocitrina" AUS (formally undescribed species, name used in C06)	Mt. Magnet, WA, AUS	C06	AY921420	AY921464	AY921431
D. "neosalinifera" AUS (formally undescribed species, name used in H02 and C06)	Colac, VIC, AUS	H02; C06	AY921416	AF217132	AY921429
D. nivalis Hebert, 1978	Lake Cootapatamba, NSW, AUS	H02; C06	AF217118	AF217143	AY921448
D. ornithocephala Birabén, 1954	Pond near Ulapes, La Rioja, ARG (site #229)	A04	AY323123	FJ427411	FJ427459
D. projecta Hebert, 1977	Nyngan, NSW, AUS	H02; C06	AF308966	AF217134	FJ427460
D. pusilla (Serventy, 1929)*	Rottnest Island, WA, AUS	H02; C06	AF217112	AF217124	AY921442
D. quadrangula (Sergeev, 1990)*	Colac, VIC, AUS	H02; C06	AF217108	AF217120	AY921444
D. queenslandensis (Sergeev, 1990)*	Lake Wyara, QLD, AUS	H02; C06	AF217109	AF217121	AY921440
D. "reflexa" (formally undescribed species, name used in H02 and C06)	Mugga, ACT, AUS	H02; C06	AF308968	AF217133	AY921428
D. salina Hebert and Finston, 1993	Shoe Lake, SK, CAN	H02; C06	AF308973	AY921469	AY921436
D. "salinifera" (formally undescribed species, name used in H02 and C06)	Lake Wyara, QLD, AUS	H02; C06	AF217113	AF217131	AY921430
D. similis s.s. Claus, 1876	Lake in Golan Heights, ISR	C06	AY921418	AY921470	AY921455
D. gr. *similis* sp2-EUR	Pools at Frotmanninger Heide, Munich, GER	A.P.	FJ427486	FJ427412	—
D. cf. *similis* sp3-NA/SA	Soap Lake, WA, USA	H02; C06	AF308971	AY921468	AY921446
D. spinulata Birabén, 1917	Pond in northern La Pampa, ARG (site #67)	A04	AY323108	FJ427413	FJ427461
D. studeri (Rühe, 1914)*	Lake Barkell, ANT	C06	AY921423	AY921474	AY921438
D. thomsoni Sars, 1895	Bombala, VIC, AUS	H02; C06	AF217119	AF217144	AY921450
D. tibetana (Sars, 1903)*	Lake in TIB	C06	AY921421	AY921475	AY921437
D. truncata s.s. (Hebert and Wilson, 2000)*	Coast, WA, AUS	H02; C06	AF308965	AF217125	AY921443
D. cf. *truncata* sp2-TAS*	Tasmania, AUS	P.H.	—	FJ427414	—
D. wardi (Hebert and Wilson, 2000)*	Lake Preston, WA, AUS	H02; C06	AF217111	AF217123	AY921445
Subgenus Daphnia: D. pulex group sensu lato					
D. ambigua Scourfield, 1947	Little Presa, MEX	H03 (COI, 12S); T98 (16S)	AF523687	AF523716	AF064188
D. ambigua SA	Lago Rosario, Chubut, ARG (site #255)	H03; A04	AF523692	FJ427423	FJ427464
D. "arenata" (name introduced in Hebert, 1995 but without formal description)	Pond near Florence, Oregon, USA	CH96	FJ427493	FJ427424	—
D. catawba Coker, 1926	Wren Lake, near Dorset, ON, CAN (COI, 12S); Prospect Lake, ON, CAN (16S)	P04 (COI); CH96 (12S); P.H. (16S)	AY380454	FJ427425	FJ427465

(*continued on next page*)

Table 1 (*continued*)

Species	Collection location	References or collector	GenBank Accession Nos.		
			COI	12S	16S
D. cheraphila Hebert and Finston, 1996	Presa la Calderon (2078N, 100.77W), MEX	P.H.	FJ427494	FJ427426	—
D. latispina Kořínek and Hebert, 1996	Pond near Lakeview, OR, USA	CH96	—	FJ427427	—
D. "*melanica*" (name introduced in Hebert, 1995 but without formal description)	Dune pond near Florence, OR, USA (COI); pond near Zoil, OR, USA (12S)	M.P. (COI); CH96 (12S)	FJ427495	FJ427428	—
D. cf. *middendorffiana* (not identical with *D. middendorffiana* Fischer, 1851)	Pond on Longstaff Bluff, Baffin Island, NU, CAN	CH96	FJ427496	FJ427429	—
D. minnehaha Herrick, 1884	Sault St. Marie, ON, CAN	P.H.	FJ427497	FJ427430	—
D. "*neoobtusa*"	Pond near Bend, OR, USA	P04 (COI); CH96 (12S)	AY380450	FJ427431	—
D. obtusa s.s. Kurz, 1874	Puddle near Blatná, CZ	A.P. & V.S.	FJ427498	FJ427432	FJ427466
D. gr. *obtusa* sp2-EUR	Ditch near Prášily, Bohemian Forest, CZ	A.P. & V.S.	FJ427499	FJ427433	FJ427467
D. gr. *obtusa* sp3-EUR	Wheeltrack in Flanders, BELG	Lab clone	FJ427500	FJ427434	—
D. gr. *obtusa* sp4-NA (=NA1 in P04)	Pond near Chandler, OK, USA (COI, 12S); temporary pond, IL, USA (16S)	CH96 (COI, 12S); C.P. (16S)	FJ427501	FJ427435	FJ427468
D. gr. *obtusa* sp5-NA (=NA2 in P04)	Coal Tipple, Portsmith, OH, USA	C.P.	FJ427502	FJ427436	FJ427469
D. gr. *obtusa* sp6-SA (=SA1 in A04)	Pond in La Pampa, ARG (site #37).	A04	AY323049	FJ427437	FJ427470
D. gr. *obtusa* sp7-SA (=SA2 in A04)	Laguna Quichaura, Chubut, ARG (site #98)	A04	AY323059	FJ427438	FJ427471
D. gr. *obtusa* sp8-SA (=SA3 in A04) (this species may correspond to *D. commutata*, but further work is required.)	Lago Rivadavia, Chubut, ARG (site #89)	A04	AY323065	FJ427439	FJ427472
D. oregonensis Kořínek and Hebert, 1996	Pond near Cow Creek, OR, USA	P.H.	FJ427503	FJ427440	—
D. parvula s.s. Fordyce, 1901	Little Presa, northeastern MEX (COI); Columbia Lake, Kitchener, ON, CAN (12S); Douglas, AZ, USA (16S)	P.H. (COI, 16S); CH96 (12S)	FJ427504	FJ427441	FJ427473
D. gr. *parvula* sp2-SA	Río Coronda, Santa Fe, ARG (site #9)	A04	AY323126	FJ427442	FJ427474
D. peruviana Harding, 1955	Andean Lake, Tucumán, ARG (site #259)	A04	AY323070	FJ427443	FJ427475
D. pileata Hebert and Finston, 1996	Mesa San Jose (21.17N, 101.08 W), MEX	P04 (COI); P.H.	AY380453	FJ427444	—
D. prolata Hebert and Finston, 1996	Amarillo, TX, USA	P04 (COI); P.H. (12S, 16S)	AY380452	FJ427445	FJ427476
D. pulex s.s. Leydig, 1860	Bourgoyen, BELG (COI); pond in Basel, SWI (12S)	M08 (COI); T.L.	EU152320	FJ427446	—
D. cf. *pulex* sp2-NA	Pond near Windsor, ON, CAN	Cr99	AF117817	AF117817	AF117817
D. pulicaria s.s. Forbes, 1893	Guelph Lake, ON, CAN	CH96	FJ427505	FJ427448	—
D. cf. *pulicaria* sp2-EUR	Fishpond, Bohemia, CZ	M08	EU152322	EU152312	—
D. cf. *pulicaria* sp3-SA (hybrid)	Lake Azul, BOL (haplotype BOLB2)	M08	EU152323	From author	—
D. cf. *pulicaria* sp4-SA (hybrid)	Lake Leche, BOL (haplotype BOLC2)	M08	EU152327	From author	—
D. retrocurva Forbes, 1882	Crooked Lake, IN, USA	CH96	—	FJ427449	—
D. tenebrosa Sars, 1898 (most likely junior synonym of *D. middendorffiana* Fischer, see M08)	Unmelanized individual from tundra pond near Churchill, MB, CAN	CH96	FJ427506	FJ427450	—
D. villosa Kořínek and Hebert, 1996	Pond in Okanagan Co., WA, USA	P.H.	FJ427507	FJ427451	FJ427477
Subgenus Daphnia: D. longispina group sensu lato (sometimes referred to as the subgenus "Hyalodaphnia")					
D. cristata Sars, 1862	Kroman, BEL (isolate CRI1)	S00	—	AF277281	—
D. cucullata Sars, 1862	Medlov Pond, CZ	S00, Pet08	EF375870	AF277270	—
D. curvirostris Eylmann, 1887	Pond near Tuktoyaktuk, NT, CAN	CH96	FJ427487	FJ427415	—
D. dentifera Forbes, 1893	Victoria Road, Guelph, ON, CAN	CH96	FJ427488	FJ427416	—
D. dubia Herrick, 1883	Wren Lake near Dorset, ON, CAN (COI); Huzzy Lake, Van Buren Co., MI, USA (site MI1)	C06 (COI); T98 (12S, 16S)	AY921411	AF064173	AF064181
D. galeata Sars, 1864	Lake Tjeukemeer, NL	Pet08	EF375867	EF375851	—
D. gessneri Herbst, 1967	Río Coronda, Santa Fe, ARG (site #9)	A04	AY323071	FJ427417	FJ427462
D. hyalina Leydig, 1860	This widely recognized taxon was not included, as Pet08 synonymized it with *D. longispina*, and it does not fulfil our criteria for species delimitation				
D. lacustris Sars, 1862	Lake Maridalsvann, NOR	N07, Pet08	DQ871251	DQ337943	—
D. laevis s.s. Birge, 1878	Reservoir near Mexico city, MEX (COI, 12S); pond near Truro, MA, USA (16S)	F.M.J. (COI, 12S); T98 (16S)	FJ427490	FJ427418	AF064179
D. gr. *laevis* sp2-SA	Río Coronda, Santa Fe, ARG (site #9) (E98)	A04	AY323072	FJ427419	FJ427463
D. longiremis Sars, 1862	Lake on Melville Peninsula, NT, CAN	T96; C06	AY921413	AY921459	AY921454
D. longispina O.F. Müller, 1785	Lake Constance, GER (isolate H29)	Pet08	EF375860	EF375829	—
D. gr. *longispina* sp2-EUR (taxon labelled *Daphnia* n.sp. A in Pet08)	Lake Berse, NOR	Pet08	EF375864	EF375848	—
D. magniceps s.s. Herrick, 1884	Pond in Rondeau Park, ON, CAN (COI, 12S) (specimen considered *D. laevis* in CH96, but *D. magniceps* was resurrected by T98); farm pond, Coconino Co., AZ, USA (16S)	CH96 (COI, 12S); T98 (16S)	FJ427491	FJ427420	AF064184
D. mendotae Birge, 1918	Guelph Lake, Guelph, ON, CAN	T96; C06	AY921412	FJ427421	AF064187
D. thorata Forbes, 1893	This taxon was not included, as it does not fulfil our criteria for species delimitation; Pet08 suggested it might be conspecific with *D. dentifera*				

Table 1 (*continued*)

Species	Collection location	References or collector	GenBank Accession Nos.		
			COI	12S	16S
D. "*umbra*" (name introduced in Hebert, 1995 but without formal description)	Pond near Richards Bay, NT, CAN	CH96	FJ427492	FJ427422	—
Outgroups					
Scapholeberis	Pond near Guelph, ON, CAN	C06	AY921410	AY921476	AY921458
Simocephalus sp1-NA	OR, USA	J.C.	FJ427478	FJ427400	FJ427452
Simocephalus sp2-NA	QC, CAN	J.C.	FJ427479	FJ427401	FJ427453
Simocephalus sp3-SA	Pond near Las Varillas, Córdoba, ARG	S.A.	FJ427480	FJ427402	FJ427454

Undescribed species that are genetically related to a morphologically similar species are referred to by their species group, an assigned species number, and region. Species which have been previously assigned to similar-looking morphospecies from which they are in fact distinct are designated by "cf.". Formally undescribed species for which a name has been used in previous literature (i.e. a *nomen nudum*), and those with invalid names, are designated by placing the specific epithet in quotation marks. Two taxa not meeting our criteria for species status (*D. hyalina* and *D. thorata*) were listed above along with taxonomic notes to facilitate comparison with previous work. Two divergent representatives of three widely distributed species which lack close relatives (*D. ambigua*, *D. lumholtzi*, and *D. magna*) are included to break up their long branches. Codes for countries or regions are: ARG, Argentina; AUS, Australia; BEL, Belarus; BELG, Belgium; BOL, Bolivia; CAN, Canada; CZ, Czech Republic; DEN, Denmark; EUR, Europe; GER, Germany; ISR, Israel; KEN, Kenya; MEX, Mexico; NA, North America; NL, Netherlands; NOR, Norway; PL, Poland; SA, South America; SK, Slovakia; SP, Spain; SWI, Switzerland; TAS, Tasmania; TIB, Tibet; USA, United States. Standard postal abbreviations are used for Canadian, Australian, and U.S. states and provinces. GenBank accession numbers are provided for all sequences. Species marked with an asterisk were formerly assigned to the genus *Daphniopsis*. Abbreviations for references are: A04—Adamowicz et al. (2004), CH96—Colbourne and Hebert (1996), C06—Colbourne et al. (2006), Cr99—Crease (1999), DD05—De Gelas and De Meester (2005); H02, H03—Hebert et al. (2002, 2003), M07, M08—Mergeay et al. (2007, 2008), N07—Nilssen et al. (2007), P04—Penton et al. (2004), Pet08—Petrusek et al. (2008), S00—Schwenk et al. (2000), St06—Stenderup et al. (2006), T96, T98—Taylor et al. (1996, 1998). Collectors of new specimens are: A.P., Adam Petrusek; C.P., Cheryl Prokopovich; D.T., Derek Taylor; F.M.J., Fernando Martínez Jerónimo; J.C., John Colbourne; K.S., Klaus Schwenk; M.P., Michael Pfrender; P.H., Paul Hebert; S.A., Sarah Adamowicz; S.S., Steven Schwartz; T.L., Tom Little; V.S., Veronika Sacherová. For more details on nomenclatural issues, see Ishida et al. (2006), Mergeay et al. (2008), and Petrusek et al. (2008).

D. similis s.s. from Israel (Petrusek, 2003; Colbourne et al., 2006). *Daphnia* gr. *atkinsoni* sp2-ISR from Israel and *D.* gr. *atkinsoni* sp3-SA from South America (this study) are distinct from *D. atkinsoni* s.s. Additionally, *Daphnia* gr. *parvula* sp2-SA from South America (Adamowicz et al., 2004), *D.* gr. *obtusa* sp. 5 from North America (Penton et al., 2004), and *D. truncata* sp2-TAS from Tasmania (this study) are divergent from their counterparts within the respective species complexes. *Daphnia* gr. *longispina* sp2-EUR from Norway (Petrusek et al., 2008) is distinguished because of its highly divergent mtDNA; however, restriction patterns in the nuclear internal transcribed spacer also suggest divergence from other members of the *D. longispina* complex (Skage et al., 2007).

Despite the inclusion of the largest number of *Daphnia* species in any phylogenetic study to date, we are aware of at least 15 species that are missing from our analysis (including species discussed or described in Hann, 1986; Flößner, 1987; Hrbáček, 1987; Valdivia Villar and Burger, 1989; Hudec, 1991, 1993; Kořínek, 1999; Kořínek and Villalobos, 2003; Dartnall et al., 2005; Ishida et al., 2006; Kotov et al., 2006). In addition, given the cryptic diversity revealed in the Americas and Australia, there are undoubtedly more cryptic lineages yet to be discovered, particularly in Africa and Asia.

The three subgenera identified by Colbourne and Hebert (1996) are well represented in our analysis, though we employ a different subgeneric concept. On the basis of the results and recommendations presented by Ishida et al. (2006), we pooled the subgenera "*Daphnia*" (=*D. pulex* group *sensu lato*, represented by 32 species in our analysis) and "*Hyalodaphnia*" (=*D. longispina* group *sensu lato*; 16 species in the analysis) into the single subgenus *Daphnia sensu* Johnson (1952). The subgenus *Ctenodaphnia* is represented by 43 species, and includes 10 species that were formerly considered members of the genus *Daphniopsis*; this pooling is supported by both morphological (Hrbáček, 1987) and genetic (Colbourne et al., 2006) evidence. The sole member of the recently proposed subgenus *Australodaphnia* (Colbourne et al., 2006), *D. occidentalis*, is also included in our dataset.

Sequences representing one species of *Scapholeberis* and three putative species of *Simocephalus* were included as outgroups. Also members of the family Daphniidae, these genera have been shown to be closely related to, but not part of, the genus *Daphnia* in morphological and molecular studies (Olesen, 1998, 2000; Taylor et al., 1999; Swain and Taylor, 2003; deWaard et al., 2006; Richter et al., 2007).

2.1.2. Marker selection and data compilation

Partial sequences for three mitochondrial genes were used: the protein-coding COI gene, as well as the ribosomal small (12S) and large (16S) subunits. These were selected on the basis of data availability and because they provide good phylogenetic resolution at different taxonomic levels. Previous studies have shown that COI evolves about 1.3 times faster than 12S (Schwenk et al., 2000), which in turn evolves about 1.5 times faster than 16S in *Daphnia* (Taylor et al., 1998). In addition, all three genes contribute phylogenetic signal under the maximum likelihood criterion (Colbourne et al., 2006). Sequences representing the three genes were gathered from published sources for as many *Daphnia* species as possible (see Table 1 for references and GenBank accession numbers).

In general, one representative of each gene was included per species for computational efficiency. Moreover, genetic divergences tend to be limited within species, relative to interspecific comparisons. According to data availability, we aimed to sub-divide long branches for those species lacking close relatives. We were able to include two divergent sub-lineages from different continents for the widespread species *D. ambigua*, *D. lumholtzi*, and *D. magna*.

2.1.3. New sequences obtained

Missing gene sequences were obtained whenever possible by sequencing DNA extracted from frozen or ethanol-preserved specimens (see Table 1), using a proteinase-K method (Schwenk et al., 1998). For the South American species, the two ribosomal genes were sequenced from previous extractions of known COI identity (from Adamowicz et al., 2004). A 710-base-pair (bp) fragment of COI was PCR-amplified using the primers LCO1490 and HCO2918 (Folmer et al., 1994), a ~600-bp fragment of 12S was amplified using primers designed specifically for *Daphnia* (Taylor et al., 1996), while a ~550-bp 16S fragment was obtained using the universal primers 16Sar and 16Sbr (Palumbi, 1996). PCR, gene purification, and sequencing-reaction protocols for COI followed Adamowicz et al. (2004). A "touch-down" PCR thermal regime (Palumbi, 1996) was employed for the ribosomal genes, consisting

of one cycle of 1 min at 94 °C; 10 cycles with declining annealing temperatures: 1 min at 94 °C, 1.5 min at 60/58/56/54/52 °C (2 cycles each), and 1.5 min at 72 °C; 25 cycles of 1 min at 94 °C, 1.5 min at 50 °C, and 1.5 min at 72 °C; and finally 5 min at 72 °C. Products were sequenced in one direction using primers LCO1490, 12S-A, or 16Sar.

2.1.4. Sequence alignments and composition

Sequences of COI were aligned by eye with the aid of the amino acid translation and combined with COI data from previous studies to obtain a final alignment of 646 bp. Preliminary alignments of the 12S and 16S sequences were constructed using ClustalW (Thompson et al., 1994) and then corrected in the DCSE editor (De Rijk and De Wachter, 1993), using the secondary structure models for rRNA molecules of *Daphnia* (Taylor et al., 1998; Crease, 1999). Regions that could not be aligned reliably were excluded from phylogenetic analysis. After excluding six short regions (varying in length from 0–2 bp among species to 9–14 bp), the final 12S data set consisted of 545 aligned positions. There were 501 aligned positions available for 16S, after the exclusion of two short regions (one 3–6 bp in length, the other 1–5 bp). The alignments are available from the authors upon request.

Nucleotide frequencies within each of the three genes were calculated in PAUP* 4.1.1 (Swofford, 2002). Chi-square tests were conducted for each gene to check for departures from homogeneous nucleotide composition. A partition homogeneity test was also conducted to assess congruence of phylogenetic signal among the three genes (based upon 10,000 replicates and nearest-neighbor interchange branch swapping), using those species for which all three genes were available. The partition homogeneity test was repeated omitting third codon positions in the COI gene, and again including just 12S and 16S.

2.2. Phylogenetic analyses

There was some variability in sequence length due to the inclusion of data from different studies, but 12S sequences were available for all 92 *Daphnia* species. There were COI sequences for 96% (88/92) species; however, the 16S data set was only 63% (58/92) complete, with missing taxa concentrated within the subgenus *Daphnia*. As a result, phylogenetic analyses were performed on several different data partitions: 12S alone for all species, 12S + 16S for species having data for both genes, COI + 12S + 16S for species having data for all genes, and COI + 12S + 16S including all species regardless of whether some data were missing. Wiens (2006) suggested that, in general, adding characters and taxa improves phylogenetic accuracy even if there are missing data, particularly using model-based methods and so long as there are many sampled characters overall.

Modeltest 3.7 (Posada and Crandall, 1998) and the Akaike Information Criterion were used to select the simplest adequate model of nucleotide substitution for each gene. Bayesian phylogenetic analyses were conducted in MrBayes version 3.1.2 (Huelsenbeck and Ronquist, 2001). Separate model parameters were estimated by MrBayes for each data partition. The Monte Carlo Markov chain (MCMC) analysis was run for 1,100,000 generations, with trees sampled every 10 generations. The first 10,000 trees were discarded as the "burn-in" phase, as pilot trials revealed this value to be well beyond the point of stabilization of the log likelihoods of sampled trees. The remaining 100,000 trees were used to construct a 50% majority-rule consensus tree, with other compatible nodes also included. Two independent runs of the MCMC analysis were performed to verify that separate runs converged on the same result.

The larger number of species available here was used to expand upon Colbourne and Hebert's (1996) system of grouping species into species complexes. The species complex concept is a useful tool for discussing groups of species that are closely related and whose evolutionary trajectories may be linked via hybridization. Their proposed cut-off point corresponds to the maximum divergence observed between any hybridizing species pair within North America: 14% divergence (Kimura 2-parameter distances [K2P], Kimura, 1980) in the 12S gene. This guideline was used to group species from the other continents into putative complexes, but the 14% cut-off was relaxed for a few species complexes in order to accommodate all hybridizing species. We obtained the K2P distances in MEGA4 (Tamura et al., 2007) and employed pairwise deletion of gapped and missing sites. No 12S regions were excised prior to calculating these metrics, because sequences within species complexes could be readily aligned. This approach enabled a more direct comparison of our data to that presented by Colbourne and Hebert (1996).

2.3. Biogeographic analyses

2.3.1. Intercontinental allopatric speciation

The role of intercontinental allopatric speciation in the diversification of *Daphnia* was explored by using the maximum parsimony (MP) criterion to reconstruct potential geographical speciation events, using the program Mesquite version 2.0 (Maddison and Maddison, 2007). The biogeographic region(s) occupied by each species were scored as discrete characters. Seven regions were represented and treated as unordered characters: North America, South America, Palearctic (including most Eurasian specimens), Tibet, Australia, Africa, and Antarctica. Tibet was separated from the remainder of Eurasia because populations inhabiting this area may have been rafted northward on the Indian subcontinent during the breakup of Gondwanaland; therefore, potential links with the southern continents were investigated. Each species inhabiting more than one continent was assigned to the region shared with its sister species, resulting in a conservative estimate of the intercontinental speciation rate. The number of shifts in continent (MP "steps") was calculated using the assumption that ancestral species occupied a single continent. The number of shifts between each pair of regions was also calculated, averaged across equally parsimonious character-state reconstructions. Rooting was performed using the most distantly related species within the genus *Daphnia* itself, to avoid biasing the results based upon the use of primarily North American members of the outgroup genera.

Species distributed on more than one continent were considered secondly. Since the majority of (morphological) intercontinental cognates have been shown to be different species, only distributions that have been confirmed genetically are included (Colbourne et al., 1998; Taylor et al., 1996; Weider et al., 1999a,b; Černý and Hebert, 1999; Schwenk et al., 2000; Havel et al., 2000; Hebert et al., 2003; Adamowicz et al., 2002, 2004; De Gelas and De Meester, 2005; Colbourne et al., 2006; Marková et al., 2007; Mergeay et al., 2008; Petrusek et al., 2008). Distributions that likely represent recent anthropogenic invasions were not considered. The minimum number of intercontinental dispersal events was counted and the main dispersal corridors inferred.

2.3.2. Intracontinental allopatric speciation

Evidence for allopatric speciation within continents was compiled from the literature, using geographic distributions confirmed by genetic evidence (see references above). Additionally, intraspecific phylogeographic studies were reviewed to determine how often and at what spatial scale allopatric divergence is observed within species.

3. Results

3.1. Sequence composition

Thymine was over-represented in COI (23.2% A, 19.7% C, 21.8% G, 35.3% T), and there was significant heterogeneity among species in nucleotide composition (X^2 = 345.84, d.f. = 279, p = 0.0039), even when the outgroups were excluded (p = 0.0049). 12S was more A-T biased than COI (33.7% A, 13.6% C, 18.3% G, 34.5% T), yet was not found to deviate significantly from expected levels of heterogeneity among species (X^2 = 219.82, d.f. = 312, p = 1.000). 16S was also A-T biased (32.6% A, 13.2% C, 21.2% G, 33.0% T), with no significant heterogeneity in nucleotide composition (X^2 = 44.73, d.f. = 189, p = 1.00). The three-gene dataset exhibited significant heterogeneity in phylogenetic signal (partition homogeneity test; p = 0.001), which was not rectified by including only the 1st and 2nd codon positions in the COI sequences. By contrast, the 12S and 16S datasets did not exhibit significant heterogeneity (p = 0.166).

3.2. Chosen phylogenetic hypothesis

The Akaike Information Criterion indicated that the best models of nucleotide substitution were TIM + I + G for 12S, TVM + I + G for 16S, and GTR + I + G for COI and for all sequences assembled into a combined dataset. In the Bayesian analysis, nst was set to 6 and gamma and invariant sites parameters were also estimated, with separate models estimated for each partition. The two MCMC runs for each data partition produced the same majority-rule topology for each.

There was a large degree of similarity among the trees reconstructed from different partitions, but they were not identical. For several reasons, we present the all-species three-gene analysis as our preferred hypothesis of *Daphnia* phylogenetic relationships. The two primary issues are that of missing data and of phylogenetic congruence among partitions. Wiens (2006) found that including more characters and taxa (even when some data are missing) improves phylogenetic analysis, and our results seem to corroborate that assessment. For example, upon analyzing the complete 12S data set, *D. pileata* was recovered as the sister species to the remaining species within the *Daphnia pulex* group *sensu lato* (i.e., the subgenus *Daphnia* as in Colbourne and Hebert, 1996). However, in the three-gene tree, it grouped with the other members of the *D. obtusa* complex, to which it probably belongs based on morphology and interspecific hybridization with other members of that complex. Furthermore, most of the topological differences between the largest data set and the smaller (but more complete) datasets occurred among deeply divergent members of the *Ctenodaphnia*, for which data were nearly complete, and therefore the hypothesis based upon more data is preferred.

Since there was significant heterogeneity among the partitions, the validity of combining the three genes is an important question. However, a ridged adherence to doctrines suggesting that a partition should be deleted is not automatically warranted due to problems with the partition homogeneity test (see Barker and Lutzoni, 2002). Moreover, since all three genes are mitochondrial, and hence tightly linked, their relationships are almost certainly based upon the same underlying phylogeny. Colbourne et al. (2006) found that the three-gene dataset provided the greatest phylogenetic signal under the criterion of maximum likelihood (ML), but not maximum parsimony. They also found that COI is the most informative for the Australian *Daphnia carinata* complex, which has radiated recently, and therefore would also be expected to be most informative for the young *D. pulex* complex. The three-gene and 12S + 16S phylogenies were very similar, and we present only the former (Fig. 1).

3.3. Relationships and species complexes

The monophyly of the subgenera *Daphnia* and *Ctenodaphnia* is supported, as is the distinctiveness of the monotypic subgenus *Australodaphnia*, which was recently proposed by Colbourne et al. (2006) to accommodate *D. occidentalis* (Fig. 1). Our three-gene hypothesis groups *Australodaphnia* as the sister lineage to a clade including all other members of the genus *Daphnia*. All species formerly assigned to the genus *Daphniopsis* were unambiguously placed within the subgenus *Ctenodaphnia*, but they did not form a clade, and instead were placed within four distinctive complexes (the *D. pusilla*, *D. tibetana*, *D. atkinsoni*, and *D. ephemeralis* species complexes).

Few changes were necessary to the North American species complex classification of Colbourne and Hebert (1996) within the subgenus *Daphnia* (including the former *Hyalodaphnia*), as most species from South America and Europe belong to the groups known from the North American fauna (Fig. 1). This subgenus is absent from Australia. The sole addition to their system here is *D. peruviana* from South America, which was assigned to a unique species complex as a result of its divergence of >14% from all other species. This species is, however, consistently affiliated with the *D. villosa* complex. Within the *D. obtusa* complex, there was variability in branch lengths such that a number of pairwise sequence divergences of >14% were observed (up to a maximum of 16.9%). However, all species were retained within that complex because the average divergences between all major clusters in the group were <14%. Moreover, *D. pileata* in particular exhibits deep divergences, but it is known to hybridize with other members of this complex (Hebert and Finston, 1996). Similarly, divergences of up to 19% were present among species within the *D. longispina* complex, many of whom hybridize, although the majority of pairwise distances were <14%. All of the subgenus *Daphnia* complexes identified by Colbourne and Hebert (1996) based upon 12S were also recovered as being monophyletic here.

By contrast, while five *Ctenodaphnia* species complexes were recognized by Colbourne and Hebert (1996) in North America, at least fifteen complexes are required in order to accommodate the species from other regions (Fig. 1). Newly designated complexes are named according to the earliest described species within the group. Most of the complexes conformed to the rule of <14% nucleotide sequence divergence within complexes and >14% average divergence between complexes. Larger sequence divergences were permitted among those species that are putatively assigned to the Australian *D. pusilla* complex (formerly considered *Daphniopsis*) (maximum of 23.8%) and to the *D. carinata* complex (maximum of 16.8%) because of their patterns of hybridization (Hebert and Wilson, 1994, 2000). Moreover, elevated rates of molecular evolution are observed among their member species inhabiting saline habitats (Hebert et al., 2002; Colbourne et al., 2006).

Several complexes recognized in Colbourne and Hebert (1996) were assigned new names here because of new insights about relationships of their members. For example, it is now clear that so-called North and South American *D. similis* (*D.* cf. *similis* sp3-NA/SA in this paper) is not part of the *D. similis* complex, but instead belongs to a New World clade which we term the *D. exilis* complex for clarity. The *D. similis* complex includes *D. similis* s.s. collected from Israel (the country from which it was described), as well as a related species collected in Germany (Petrusek, 2003). We have also included *D. lumholtzi* in this complex due to its divergences of <14% from other member species. As a consequence of the inclusion of *D. atkinsoni* s.s. in our data, it is clear

that this name is appropriately applied to its clade, which also includes *D.* (formerly *Daphniopsis*) *studeri* from the Antarctic, as well as two undescribed species collected from Israel and Argentina.

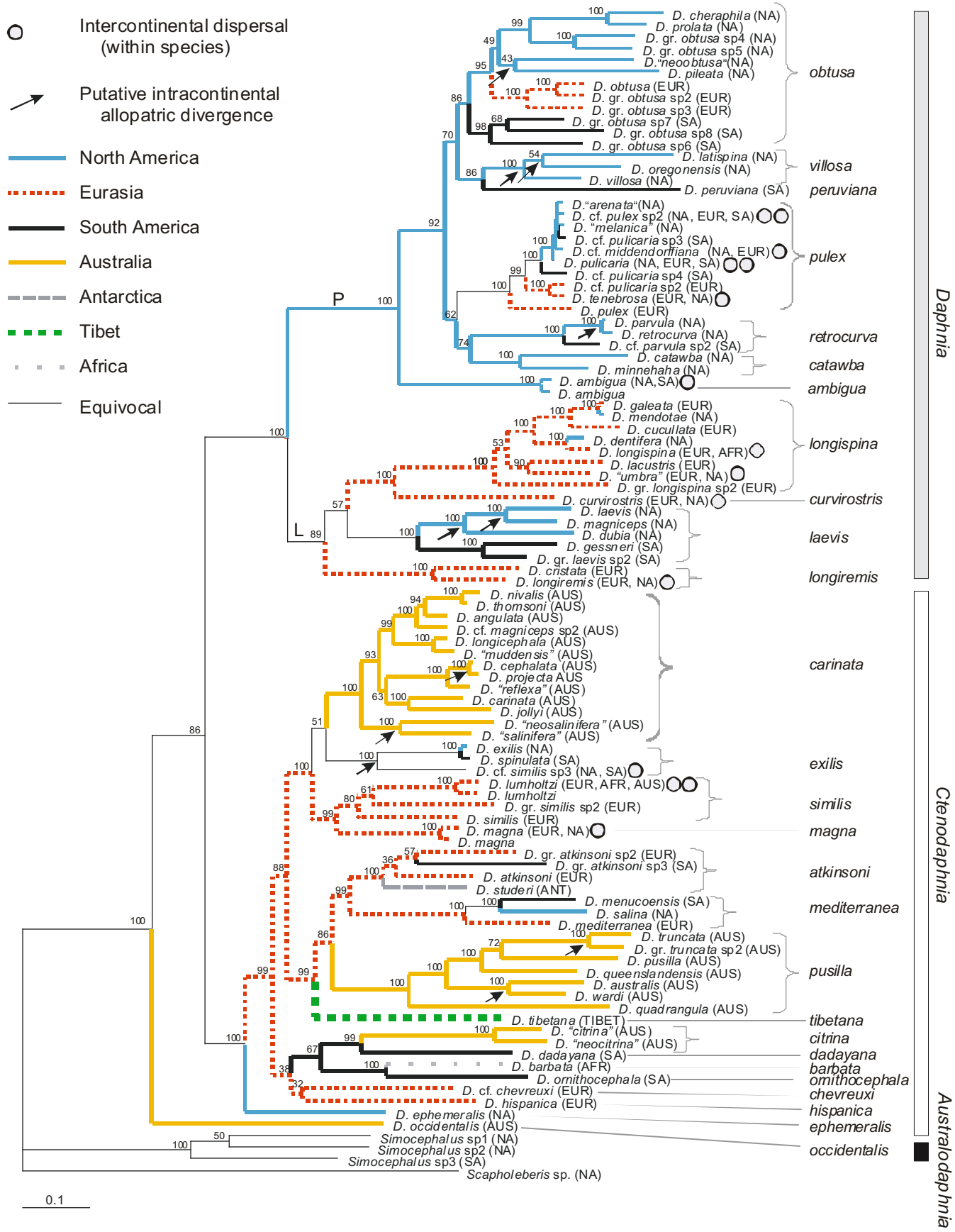

Table 2
Summary of intercontinental patterns of speciation and dispersal in *Daphnia*, based on MP analysis of continental occupancy (Fig. 1). Information about distributions of individual species was obtained from a large range of references (see Section 2 and References); only genetically confirmed distributions are included. *Abbreviations used:* NA, North America; SA, South America; AUS, Australia; EUR, Eurasia; AFR, subsaharan Africa; ANT, Antarctica; TIB, Tibet. Patterns are dependent upon species inclusion, and thus frequencies are likely to change as more data become available. In particular, data are lacking to a large degree for the African and Asian faunas.

North–South	East–West	Circumarctic
Number of intraspecific dispersal events and direction		
4 NA-SA	2 NA-EUR[b]	7 arctic NA-EUR (numerous
- *D.* cf. *similis* sp3	- *D. magna*	dispersal events within some)
- *D. ambigua*	- *D. pulicaria*	- *D. longiremis*
- *D.* cf. *pulex* sp2		- *D. curvirostris*
- *D. pulicaria*	1 AFR-AUS	- *D. "umbra"*
	- *D. lumholtzi*[a]	- *D. tenebrosa*
2 EUR-AFR		- *D.* cf. *pulex* sp2
- *D. lumholtzi*[a]		- *D. pulicaria*
- *D. longispina*		- *D.* cf. *middendorffiana*
= 6	**= 3**	**= 7 +**
North–South hemisphere	East–West	Regions of former Gondwanaland
Number of putative allopatric speciation events (average number of intercontinental transitions among equally-parsimonious reconstructions)[c]		
7.13 NA-SA	8.80 NA-EUR	1.00 AUS-SA
1.24 AUS-NA	1.00 EUR-TIB	1.00 AFR-SA
2.10 AUS-EUR		
3.74 SA-EUR		
1.00 ANT-EUR		
=∼15.2	**=∼9.8**	**=∼2.0**

[a] Although Havel et al. (2000) suggested that the most plausible dispersal routes for *D. lumholtzi* are either AUS → AFR → EUR or AUS → EUR → AFR, our phylogenetic analysis suggests that this species is more related to Old World *Ctenodaphnia* of the *similis* complex than to any Australian *Daphnia*. The dispersal routes AFR → EUR → AUS or AUS ← EUR → AFR are therefore the more likely ones. Each possible route involves elements of both north–south and east–west dispersal, so one event in each category is scored for this species.

[b] In both *D. magna* and *D. pulicaria* s.s., part of the ranges of these species lie in the arctic, and therefore the dispersal could have involved the circumarctic route as in the next column (which is particularly likely for the latter species based upon its lineage distribution patterns).

[c] The values presented here sum to the total number of steps (27).

3.4. Intercontinental speciation

Among the 91 ingroup nodes within our *Daphnia* species phylogeny, 27 of these speciation events involved intercontinental geographic disjunctions (Table 2; Fig. 1). This is expected to be a conservative count (see Section 2), as many ancient events have certainly been obscured as a result of more recent dispersal. Thus, about 30% of speciation events may be attributed to allopatric divergence at an intercontinental scale.

Three primary patterns account for the continental shifts (Table 2). Among the equally-parsimonious reconstructions (which all involved 27 continental shifts), an average of 15 shifts were between continents lying in the northern vs. southern hemisphere, primarily North/South America disjunctions; about 9 nodes involved east/west shifts between North America and Eurasia; while 2 nodes were associated with reconstructed shifts among continents formerly comprising Gondwanaland. The true number of Gondwanaland-linked divergence events is suspected to be higher due to uncertainties in the biogeographic shifts deep in the phylogeny and poor representation of African species. Six nodes (four North/South America and two Europe/North America) involved shallow 12S K2P divergences of <5%.

3.5. Recent intercontinental dispersal

There have been at least 15 recent intercontinental dispersal events among the 12 *Daphnia* species that possess multicontinental distributions supported by genetic evidence (Table 2; Fig. 1). In some cases these may represent incipient speciation, but all indicate dispersal within the last few million years (e.g. see Knowlton and Weigt, 1998), due to genetic divergences of <∼5% in COI or ND5 and <∼3% in 12S (e.g. Taylor et al., 1996; Colbourne et al., 1998; Schwenk et al., 2000; Hebert et al., 2003; Adamowicz et al., 2002, 2004; De Gelas and De Meester, 2005; Marková et al., 2007; Mergeay et al., 2008). Six of these species demonstrated that dispersal occurred along a north–south axis (particularly North/South America), while 3 species provide evidence of east–west intercontinental dispersal in nonpolar regions. Finally, 7 species showed intercontinental dispersal in the circumarctic regions, with phylogeographic evidence indicating that long-range dispersal happened repeatedly within some.

3.6. Intracontinental allopatric speciation

On the basis of the available distributional evidence, at least 11 allopatrically distributed pairs of sister *Daphnia* species or clades are apparent within single continents (Fig. 1), representing possible examples of intracontinental allopatric speciation. This number is almost certainly a substantial underestimate since current species distributions are most likely to reflect recent occurrences, and because the distribution ranges of recently discovered cryptic species are often not sufficiently known. The inclusion of intracontinental patterns increases our conservative estimate of the proportion of *Daphnia* speciation events that have been allopatric to at least 42%. The evidence for relatively common intracontinental allopatric divergence is further supported by the observation that at least eight species show phylogeographic structure within single continents (Table 3).

4. Discussion

This study has used a multi-continental phylogenetic analysis to assess the role of allopatric speciation in the diversification of the freshwater zooplankton genus *Daphnia*. Allopatric speciation at large spatial scales has played a dominant role in cladogensis in this taxon, with intercontinental (at least 30%) and regional (at least 12%) divergence accounting for a large fraction of speciation events. The true fraction of total allopatric speciation is likely to

Fig. 1. Phylogenetic hypothesis for 92 species of the genus *Daphnia*, based upon Bayesian analysis of COI, 12S, and 16S mtDNA sequences. Proposed species complexes are marked to the right of the parentheses, with the three subgenera indicated at the far right. "P" and "L" designate the *D. pulex* and *D. longispina* groups *sensu lato*. Posterior probabilities indicate the percentage of 100,000 sampled trees containing the node (see text for other analysis details). The scale bar shows maximum likelihood distances. The history of continental occupancy is reconstructed using the MP criterion, with *D. occidentalis* used to root tree. Arrows designate putative cases of allopatric speciation within continents. Circles following species names indicate cases of recent intercontinental dispersal within species. The continent(s) or country occupied by each species is given after each taxon name, with only those distributions confirmed by genetic analyses included. The first continent listed for species having multi-continental distributions was the one chosen for the MP reconstruction, based on comparison with its closest relatives (see text), resulting in a conservative estimate of rates of allopatric speciation. *Abbreviations used:* AFR, Africa; ANT, Antarctica; AUS, Australia; EUR, Eurasia; NA, North America; SA, South America. This reconstruction represents one plausible biogeographic history, but the specific shifts are highly dependent upon taxon inclusion and are particularly uncertain for deeper nodes.

Table 3
Table of divergences among allopatric phylogroups within *Daphnia* species, obtained from detailed phylogeographic studies within single continents. Abbreviations for the types of genetic distances are: K2P—Kimura's (1980) two-parameter distance, based on nucleotide sequences; ML—maximum likelihood, also based on sequences; Nei—Nei's (1978) genetic distances, based on allozyme loci.

Species	Geographic regions and scale	Measure of genetic distance	Genetic divergence	Reference
Daphnia ambigua	Four phylogroups across North America	K2P-COI	2.6–5.0%	Hebert et al. (2003)
D. cephalata	Two groups in southeastern Australia	Nei (10 loci)	0.53	Hebert and Wilson (1994)
D. magniceps s.s. NA	Two phylogroups within North America (central and Pacific USA)	ML-12S	~2%	Taylor et al. (1998)
D. minnehaha	Two groups (Great Lakes and Atlantic)	Nei (9 loci)	0.38	Hebert and Finston (1997)
D. "neoobtusa"	Two phylogroups in western USA	K2P-COI	5.2%	Penton et al. (2004)
D. gr. *obtusa* sp4-NA	Four mtDNA phylogroups in USA	K2P-COI	1.2–2.0%	Penton et al. (2004)
	Three allozyme groups in USA	Nei (7 loci)	0.20–0.27	Hebert and Finston (1996)
D. gr. *obtusa* sp7-SA	Two groups showing a north–south split within Argentina	Nei (7 loci)	0.18	Adamowicz et al. (2004)
D. pulicaria s.s. NA	Three phylogroups across Holarctic	K2P-ND5	2.4–3.5%	Colbourne et al. (1998)

be larger because genetic divergence can be observed at even smaller spatial scales in *Daphnia*, and range shifts are likely to obscure some ancient events. We consider the potential roles of dispersal vs. vicariance in generating intercontinental patterns of clade distribution, as well as the geographical orientation of main dispersal routes, and conclude with a brief comparison of biogeographic patterns across freshwater taxa with varying biological traits.

4.1. Intercontinental allopatric speciation: dispersal and vicariance

Our phylogenetic hypothesis permits a detailed examination of allopatric speciation patterns, including the exploration of previous taxonomy-based hypotheses of vicariance (e.g. Hrbáček, 1987). Vicariance is indeed suggested by some of the deepest nodes within the *Daphnia* phylogeny. The dominance of the subgenus *Ctenodaphnia* in the southern hemisphere and *Daphnia* in the north, as well as the confinement of *Australodaphnia* to that continent, suggests ancient splits that most likely correspond to the break-up of Pangaea into Gondwanaland and Laurasia. A continued role for vicariance is implied by the distribution of clades occupying the continents formerly comprising Gondwanaland. The biogeographic patterns are complex, with several ancient clades detected among samples from each of South America, Australia, and the Mediterranean region, indicating that *Daphnia* had already diversified to a certain extent by the time of the break-up of Gondwanaland. Mesozoic fossil ephippia from daphniids and other anomopods (Fryer, 1991; Smirnov, 1992) are consistent with this estimate of an ancient age for this genus, but the exact time when it originated remains unknown. It would be desirable to estimate node ages from the phylogenies for a comparison to specific geological events using a molecular clock or relaxed clock approach (e.g. Drummond et al., 2006), but the lack of calibration points for *Daphnia* limits opportunities for developing a local clock. The dates estimated for our tree using Lynch and Jarrell's (1993) global clocks are consistent with vicariance. However, interpretation of these values is difficult due to large confidence intervals and rate heterogeneity across taxa.

Intercontinental dispersal has also been important for allopatric speciation, and this mechanism is fairly certain for recent divergences. At least six intercontinental speciation events are associated with young divergences of <5% in the 12S gene fragment, and six more with distances of <12%. Moreover, the twelve species known to inhabit more than one continent are generally composed of divergent phylogroups, a pattern suggestive of incipient allopatric speciation following dispersal. Although small levels of sequence change can accumulate rapidly (e.g. Denver et al., 2000; Ho et al., 2005; but see Emerson, 2007), there is a degree of consensus among studies that mitochondrial DNA tends to diverge at rates on the order of 2% per million years during the first few million years of isolation (e.g. 2%, Brown et al., 1979; 2.3%, Brower, 1994; 1.4%, Knowlton and Weigt, 1998). Therefore, the within-species distances (<3% 12S) and the shallower of the between-species intercontinental splits can only be explained by dispersal. The mechanisms that operated at deeper nodes in the tree are less certain, and it is possible that both vicariance and dispersal played a role.

In addition to the interspecific patterns, our review of intraspecific phylogeographic studies also revealed a propensity for intercontinental dispersal in a north–south orientation (e.g. between North and South America, with emerging evidence echoing this pattern in Europe and Africa) and for high dispersal rates in the circumarctic region. Recent east–west dispersal in temperate regions appears to be more limited, but a few cases of temperate sister species inhabiting North America and Europe are known.

4.2. Ancient biogeographic patterns: how are they maintained?

The persistence of the ancient, subgeneric north–south split presents an apparent paradox. Although this pattern appears to have arisen during the breakup of Pangaea, it remains strong despite the evidence for high vagility in the genus *Daphnia*. Moreover, the majority of recent dispersal events (with the exception of the circumarctic region) involved north–south migration, following major bird migration routes, which should have acted to erode the continental affinities established by the fragmentation of Gondwanaland and Laurasia. There are three principal explanations that might account for the intriguing persistence of these ancient biogeographic patterns.

First, the actual rates of propagule migration may have been higher in recent times compared with the distant past. Although anomopods have produced ephippia (protective, dispersive structures) at least since the Cretaceous (Fryer, 1991), opportunities for long-distance dispersal may have changed. The alignment of the continents has shifted gradually since the origin of *Daphnia*, and biotic dispersal agents have moved and evolved as well. Although aquatic and wading birds have a long evolutionary history (Hedges et al., 1996; Cooper and Fortey, 1998; Cracraft, 2001), changing migration pathways and intensity may be associated with shifts in zooplankton dispersal rates. For example, postglacial population and range expansions have been associated with increases in avian migration distance (Milá et al., 2006; Ruegg et al., 2006).

Second, ancient biogeographic patterns may have persisted due to changing probabilities of establishment vs. extinction of intercontinental migrants over time. In general, the odds are stacked against a newcomer (but see Ebert et al., 2002), as evidenced by strong genetic structuring among cladoceran populations (reviewed in De Meester et al., 2002). The lone or small number of

propagules would usually be entering habitats occupied by well-adapted locals already utilizing available resources (De Meester et al., 2002). Furthermore, the main freshwater habitat types and their frequencies vary on different continents. However, the question is whether the (undoubtedly low) probability of success on a new continent has shifted over time, such that a greater number of colonizations are observed in more recent times. There are geological events to suggest that such shifts are likely. Only a few truly colossal glacial episodes are known from the Phanerozoic, and the glaciations of the Pleistocene were thus quite striking events in the geological history of Earth. There were several brief intervals enabling "free for all" events as new habitats were created, in which incoming migrants could gain a foothold with greater success than had previously been possible.

Finally, neutral types of explanation may also explain the observation of increasing intercontinental migration through time. It is possible that actual intercontinental dispersal rates have occurred at a fairly consistent rate, but that over time, one member of the resulting intercontinental pair may go extinct, making it seem that migration rates are actually higher in the present. In the absence of an explicit mathematical framework exploring the likelihoods of these different scenarios, we favor a combination of the first two factors; it is expected that background extinction would similarly affect east–west pairs of lineages, such as those arising with the fragmentation of Gondwanaland. Thus, it would seem that the different ancient versus recent patterns are best explained by an actual shift in process, such as the Pleistocene scenario outlined above.

4.3. The special biogeography of the Arctic

In addition to the high dispersal rates observed along the north–south axis, elevated rates of exchange were also observed in the arctic. In fact, 8 of the 9 arctic species are shared between North America and Eurasia, whereas the majority of temperate taxa are endemic to a single continent. Additionally, within single species, there is evidence of several intercontinental exchanges (Weider et al., 1999a,b). The high rates of migration in this biogeographic region, compared with other intercontinental comparisons, are likely due to environmental similarity and close physical proximity (Colbourne et al., 1998). However, enhanced dispersal opportunities probably also play a role, for example via migratory bird species having large arctic distributions. Weider et al. (1999a) also suggest that some of the intraspecific phylogeographic patterns are compatible with dispersal via ice floes. Moreover, the fact that many habitats were recently vacant due to glacial retreat may contribute to especially high rates of establishment in this region.

Considering such high rates of dispersal and the existence of vast regions characterized by similar habitats, the high diversity of arctic species and lineages is remarkable. Nevertheless, it appears that intercontinental allopatric divergence has been involved in the major split between the so-called *tenebrosa* group within the *D. pulex* complex, which is dominant in Eurasia, and the *pulicaria* group, which dominates North and South America and is common in the European arctic (Colbourne et al., 1998; Weider et al., 1999a,b; Adamowicz et al., 2002; Mergeay et al., 2008). Furthermore, allopatric divergence is apparent among young arctic lineages within *Daphnia* species, many of which may be associated with distinct refugia or recent dispersal events (Dufresne and Hebert, 1997; Colbourne et al., 1998; Černý and Hebert, 1999; Weider et al., 1999a,b; Weider and Hobæk, 2003).

Glacial advances and retreats have also been implicated in provoking more exotic evolutionary processes than allopatric divergence. After fostering population isolation and divergence in refugia, subsequent admixture may promote hybridization and polyploidization of lineages (Stebbins, 1984). While selection appears important in maintaining polyploids in the arctic (Dufresne and Hebert, 1998) and could similarly operate in high-altitude environments (Mergeay et al., 2008), the dynamic geological history of the area is instrumental in generating the patterns in both ploidy levels and phylogeography observed in arctic daphniids (Beaton and Hebert, 1988; Ward et al., 1994; Dufresne and Hebert, 1997; Weider et al., 1999a,b).

4.4. Intracontinental allopatric speciation

Allopatric speciation is observed both within single continents and at an intercontinental scale. This pattern has been studied in detail in a few cases: particularly among members of the *laevis* complex in North America (Taylor et al., 1998), among arctic lineages in the *pulex* complex (Colbourne et al., 1998; Weider et al., 1999a,b; Weider and Hobæk, 2003), and among members of the *carinata* complex in Australia (Hebert and Wilson, 1994). Interspecific phylogeographic evidence to date indicates at least 11 cases consistent with geographic speciation within single continents, generally—but not always—between large regions. However, this figure is probably an underestimate, as most of these cases occur at shallow nodes. Older geographic speciation events have likely been masked by subsequent range shifts, which cautions against drawing conclusions about the frequencies of speciation modes based on current geographic distributions (see Losos and Glor, 2003). However, this minimum estimate is an informative indicator of the commonness of this speciation mode.

Intraspecific phylogeographies also demonstrate allopatric divergence within continents for at least eight species. Although it is expected that phylogroups will sometimes go extinct or be homogenized by migration, some of these examples may represent instances of incipient speciation. Interestingly, geographical patterning among species and lineages in the *laevis* complex mirrors phylogeographical results within the single species *Daphnia ambigua* (Taylor et al., 1998; Hebert et al., 2003), and is somewhat similar to the very shallow mtDNA structure within the species "*D. obtusa* NA 1" (Penton et al., 2004). In these taxa, divergences within temperate North America largely follow the major drainages of the continent, which also coincide with the main bird migratory pathways. These phylogeographic similarities at different depths of divergence suggest that diversifying processes observed within single species have also been involved in cases of speciation in the past. However, phylogroup distributions are not exactly congruent, suggesting that it is not particular boundaries such as mountain ranges that have produced these patterns. Interestingly, bird movements are associated with patterns of genetic structuring in several different zooplankton taxa having dispersive resting stages (Figuerola et al., 2005), highlighting the importance of biotic factors in determining allopatric divergence tendencies in freshwater invertebrates.

4.5. Comparison with biogeographic patterns in other groups

Our results are not unique to the genus *Daphnia*. For example, similar large-scale phylogeographic patterning has been observed in other members of the cladoceran order Anomopoda, at the subgeneric level within the genus *Bosmina* (Lieder, 1983; Taylor et al., 2002), and at the subfamilial level in the family Chydoridae (see Sacherová and Hebert, 2003). All of these taxa produce diapausing resting eggs housed in sturdy structures (ephippia). Other members of the class Branchiopoda, which have simpler resting eggs thought to be less resistant to digestion by dispersal vectors, may display different biogeographic patterns and would be an interesting target for future comparative work. For example, in the fairy shrimp (order Anostraca) continental endemism is observed at higher, even familial, taxonomic levels (Brtek and Mura, 2000). These contrasting patterns in depth of endemism may be due to differing ages of families in the two groups. However, the similarity

of branch lengths among families in the two orders (see ML phylogenies in deWaard et al., 2006) suggests that the difference in the geographic scale of diversification is probably the result of differing dispersal ability or some other biological feature.

Other small, passively-dispersed invertebrates (such as freshwater copepods, bryozoans, and rotifers) also show geographical structure or phylogroup allopatry within and among continents (Boileau and Hebert, 1988, 1991; Freeland et al., 2000b; Gómez et al., 2000; Fontaneto et al., 2008). By contrast, large actively-dispersing animals like fishes, crayfishes, and turtles can show divergence among watersheds at a provincial scale, particularly in geologically stable (i.e. unglaciated) regions of North America (Bernatchez and Wilson, 1998; Walker and Avise, 1998; Avise, 2000). On the opposite side of the size spectrum, eukaryotic microbes often have cosmopolitan distributions (Finlay and Clarke, 1999; Finlay, 2002; Finlay and Fenchel, 2004). Although lineage diversity is often higher than morphospecies diversity in microbes, molecular studies are revealing cases of cosmopolitanism of genetic lineages (Finlay et al., 2006). Thus, biological attributes, such as body size and dispersal ability, are important in determining the geographic scale of diversification, despite the shared freshwater habitat of these organisms.

4.6. Concluding remarks

As long recognized, a proper understanding of the distributions of species and clades has enlightened our study of the processes of diversification. Our results have established that intercontinental allopatric divergence is a dominant mode of cladogenesis in *Daphnia*, and both long-distance dispersal and vicariance have likely been involved. It is interesting that the apparent signature of the ancient movements of continents is still seen in the biogeography of this vagile genus. Intercontinental dispersal, especially in more recent times, is common—but generally not common enough to hold species together. Allopatric divergence is also observed within single continents, both within and between species.

We have considered just one mechanism of diversification within *Daphnia*, albeit a major one. However, habitat shifts, polyploidy, and reticulate evolution have also been implicated in daphniid speciation (Taylor et al., 1996; Colbourne et al., 1997; Dufresne and Hebert, 1997; Schwenk et al., 2000; Forró et al., 2008). The general long-term morphological stasis observed in this genus, and in many other freshwater organisms, is fascinating considering the prolific cladogenesis that occurs through diverse mechanisms. A joint consideration of the roles of allopatric divergence and habitat shifts in cladogenesis and morphological diversification, using species-level phylogenies of *Daphnia* and other taxa, is expected to be a fruitful avenue for future work.

Acknowledgments

Our sincere thanks to María Cristina Marinone, Agustín Bachmann, Silvina Menu Marque, Juan Paggi, Santiago Echaniz, and Alicia Vignatti for assistance with the field work in South America, and Tereza Petrusková and Martina Petrů for help in the Mediterranean. Cheryl Prokopovich, Veronika Sacherová, Klaus Schwenk, and Fernando Martínez Jerónimo provided prepared DNA, specimens, or sequences. We are also grateful to Angela Holliss for DNA sequencing, and to Teri Crease, Denis Lynn, Jinzhong Fu, and Andy Purvis for discussion and helpful comments on various drafts of the paper. Funding was provided by a Natural Sciences and Engineering Research Council of Canada (NSERC) postgraduate scholarship and post-doctoral fellowship, an Overseas Research Scholarship (UK), and a Beit Research Fellowship (Imperial College London) to S.J.A., by grants from NSERC and the Canada Research Chairs program to P.D.N.H., and by Czech Ministry of Environment (project MSM0021620828), EuroCORES/EuroDIVERSITY project BIOPOOL (supported through the Czech Science Foundation grant DIV/06/E007), and ECODOCA (Access to Research Infrastructure action of the Improving Human Potential Programme in Doñana Biological Station) to A.P.

References

Adamowicz, S.J., Purvis, A., 2005. How many branchiopod crustacean species are there? Quantifying the components of underestimation. Global Ecol. Biogeogr. 14, 455–468.
Adamowicz, S.J., Gregory, T.R., Marinone, M.C., Hebert, P.D.N., 2002. New insights into the distribution of polyploid *Daphnia*: the Holarctic revisited and Argentina explored. Mol. Ecol. 11, 1209–1217.
Adamowicz, S.J., Hebert, P.D.N., Marinone, M.C., 2004. Species diversity and endemism in the *Daphnia* of Argentina: a genetic investigation. Zool. J. Linn. Soc. 140, 171–205.
Avise, J.C., 2000. Phylogeography: The History and Formation of Species. Harvard University Press, Cambridge, MA, USA.
Avise, J.C., Ball, R.M., 1990. Principles of genealogical concordance in species concepts and biological taxonomy. In: Futuyma, D.J., Antonovics, J. (Eds.), Oxford Surveys in Evolutionary Biology. Oxford University Press, Oxford, pp. 45–67.
Barraclough, T.G., Nee, S., 2001. Phylogenetics and speciation. Trends Ecol. Evol. 16, 391–399.
Barker, F.K., Lutzoni, F.M., 2002. The utility of the incongruence length difference test. Syst. Biol. 51, 625–637.
Beaton, M.J., Hebert, P.D.N., 1988. Geographical parthenogenesis and polyploidy in *Daphnia pulex*. Am. Nat. 132, 837–845.
Bernatchez, L., Wilson, C.C., 1998. Comparative phylogeography of nearctic and palearctic fishes. Mol. Ecol. 7, 431–452.
Boileau, M.G., Hebert, P.D.N., 1988. Genetic differentiation of freshwater pond copepods at arctic sites. Hydrobiologia 167–168, 393–400.
Boileau, M.G., Hebert, P.D.N., 1991. Genetic consequences of passive dispersal in pond-dwelling copepods. Evolution 45, 721–733.
Boileau, M.G., Hebert, P.D.N., Schwartz, S.S., 1992. Nonequilibrium gene-frequency divergence–persistent founder effects in natural populations. J. Evol. Biol. 5, 25–39.
Brower, A.V.Z., 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc. Natl. Acad. Sci. USA 91, 6491–6495.
Brown, W.M., George, M., Wilson, A.C., 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76, 1967–1971.
Brtek, J., Mura, G., 2000. Revised key to families and genera of the Anostraca with notes on their geographical distribution. Crustaceana 73, 1037–1088.
Černý, M., Hebert, P.D.N., 1999. Intercontinental allozyme differentiation among four holarctic *Daphnia* species. Limnol. Oceanogr. 44, 1381–1387.
Colbourne, J.K., Hebert, P.D.N., 1996. The systematics of North American *Daphnia* (Crustacea: Anomopoda): a molecular phylogenetic approach. Philos. Trans. R. Soc. Lond. B 351, 349–360.
Colbourne, J.K., Hebert, P.D.N., Taylor, D.J., 1997. Evolutionary origins of phenotypic diversity in *Daphnia*. In: Givnish, T.J., Sytsma, K.J. (Eds.), Molecular Evolution and Adaptive Radiation. Cambridge University Press, pp. 163–188.
Colbourne, J.K., Crease, T.J., Weider, L.J., Hebert, P.D.N., Dufresne, F., Hobæk, A., 1998. Phylogenetics and evolution of a circumarctic species complex (Cladocera: *Daphnia pulex*). Biol. J. Linn. Soc. 65, 347–365.
Colbourne, J.K., Wilson, C.C., Hebert, P.D.N., 2006. The systematics of Australian *Daphnia* and *Daphniopsis* (Crustacea: Cladocera): a shared phylogenetic history transformed by habitat-specific rates of evolution. Biol. J. Linn. Soc. 89, 469–488.
Cooper, A., Fortey, R.A., 1998. Evolutionary explosions and the phylogenetic fuse. Trends Ecol. Evol. 13, 151–156.
Cox, A., Hebert, P.D.N., 2001. Colonization, extinction, and phylogeographic patterning in a freshwater crustacean. Mol. Ecol. 10, 371–386.
Cracraft, J., 2001. Avian evolution, Gondwana biogeography and the Cretaceous-Tertiary mass extinction event. Proc. R. Soc. Lond. B 268, 459–469.
Crease, T.J., 1999. The complete sequence of the mitochondrial genome of *Daphnia pulex* (Cladocera: Crustacea). Gene 233, 89–99.
Dartnall, H.J.G., Hollwedel, W., de Paggi, J.C., 2005. The freshwater fauna of Macquarie Island, including a redescription of the endemic water-flea *Daphnia gelida* (Brady) (Anomopoda: Crustacea). Polar Biol. 28, 922–939.
Darwin, C., 1859. On the Origin of Species by Means of Natural Selection. John Murray, London.
Darwin, C., 1882. On the dispersal of freshwater bivalves. Nature 15, 529–530.
De Gelas, K., De Meester, L., 2005. Phylogeography of *Daphnia magna* in Europe. Mol. Ecol. 14, 753–764.
De Meester, L., Gómez, A., Okamura, B., Schwenk, K., 2002. The monopolization hypothesis and the dispersal-gene flow paradox in aquatic organisms. Acta Oecol. 23, 121–135.
De Melo, R., Hebert, P.D.N., 1994. Allozymic variation and species diversity in North American Bosminidae. Can. J. Fish. Aquat. Sci. 51, 873–880.
Denver, D.R., Morris, K., Lynch, M., Vassilieva, L.L., Thomas, W.K., 2000. High direct estimate of the mutation rate in the mitochondrial genome of *Caenorhabditis elegans*. Science 289, 2342–2344.

De Rijk, P., De Wachter, R., 1993. DCSE, an interactive tool for sequence alignment and secondary structure research. CABIOS 9, 735–740.
deWaard, J.R., Sacherová, V., Cristescu, M.E.A., Remigio, E.A., Crease, T.J., Hebert, P.D.N., 2006. Probing the relationships of the branchiopod crustaceans. Mol. Phylogenet. Evol. 39, 491–502.
Drummond, A.J., Ho, S.Y.W., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4 (5), e88 (pp. 699–710).
Dufresne, F., Hebert, P.D.N., 1994. Hybridization and origins of polyploidy. Proc. R. Soc. Lond. B 258, 141–146.
Dufresne, F., Hebert, P.D.N., 1997. Pleistocene glaciations and polyphyletic origins of polyploidy in an arctic cladoceran. Proc. R. Soc. Lond. B 264, 201–206.
Dufresne, F., Hebert, P.D.N., 1998. Temperature-related differences in life-history characteristics between diploid and polyploid clones of the *Daphnia pulex* complex. Écoscience 5, 433–437.
Ebert, D., Haag, C., Kirkpatrick, M., Riek, M., Hottinger, J.W., Pajunen, V.I., 2002. A selective advantage to immigrant genes in a *Daphnia* metapopulation. Science 295, 485–488.
Emerson, B.C., 2007. Alarm bells for the molecular clock? No support for Ho et al'.s model of time-dependent molecular rate estimates. Syst. Biol. 56, 337–345.
Figuerola, J., Green, A.J., Michot, T.C., 2005. Invertebrate eggs can fly: evidence of waterfowl-mediated gene flow in aquatic invertebrates. Am. Nat. 165, 274–280.
Finlay, B.J., 2002. Global dispersal of free-living microbial eukaryote species. Science 296, 1061–1063.
Finlay, B.J., Clarke, K.J., 1999. Ubiquitous dispersal of microbial species. Nature 400, 828.
Finlay, B.J., Fenchel, T., 2004. Cosmopolitan metapopulations of free-living microbial eukaryotes. Protist 155, 237–244.
Finlay, B.J., Esteban, G.F., Brown, S., Fenchel, T., Hoef-Emden, K., 2006. Multiple cosmopolitan ecotypes within a microbial eukaryotic morphospecies. Protist 157, 377–390.
Flößner, D., 1987. A pedomorphic form type of *Daphnia triquetra* Sars, 1903 (Cladocera, Daphniidae) from Mongolia. Hydrobiologia 145, 47–49.
Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome *C* oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.
Fontaneto, D., Barraclough, T.G., Chen, K., Ricci, C., Herniou, E.A., 2008. Molecular evidence for broad-scale distributions in bdelloid rotifers: everything is not everywhere but most things are very widespread. Mol. Ecol. 17, 3136–3146.
Forró, L., Korovchinsky, N.M., Kotov, A.A., Petrusek, A., 2008. Global diversity of cladocerans (Cladocera; Crustacea) in freshwater. Hydrobiologia 595, 177–184.
Freeland, J.R., Romualdi, C., Okamura, B., 2000a. Gene flow and genetic diversity: a comparison of freshwater bryozoan populations in Europe and North America. Heredity 85, 498–508.
Freeland, J.R., Noble, L.R., Okamura, B., 2000b. Genetic consequences of the metapopulation biology of a facultatively sexual freshwater invertebrate. J. Evol. Biol. 13, 383–395.
Frey, D.G., 1982. Questions concerning cosmopolitanism in Cladocera. Arch. Hydrobiol. 93, 484–502.
Frey, D.G., 1987. The taxonomy and biogeography of the Cladocera. Hydrobiologia 145, 5–17.
Fryer, G., 1991. A daphniid ephippium (Branchiopoda: Anomopoda) of Cretaceous age. Zool. J. Linn. Soc. 102, 163–167.
Gómez, A., Carvalho, G.R., Lunt, D.H., 2000. Phylogeography and regional endemism of a passively dispersing zooplankter: mitochondrial DNA variation in rotifer resting egg banks. Proc. R. Soc. Lond. B 267, 2189–2197.
Hann, B.J., 1986. Revision of the genus *Daphniopsis* Sars, 1903 (Cladocera: Daphniidae) and a description of *Daphniopsis chilensis*, new species, from South America. J. Crust. Biol. 6, 246–263.
Hann, B.J., 1995. Genetic variation in *Simocephalus* (Anomopoda: Daphniidae) in North America: patterns and consequences. Hydrobiologia 307, 9–14.
Havel, J.E., Colbourne, J.K., Hebert, P.D.N., 2000. Reconstructing the history of intercontinental dispersal in *Daphnia lumholtzi* by use of genetic markers. Limnol. Oceanogr. 45, 1414–1419.
Hebert, P.D.N., 1977. A revision of the taxonomy of the genus *Daphnia* (Crustacea: Daphniidae) in south-eastern Australia. Aust. J. Zool. 25, 371–398.
Hebert, P.D.N., 1995. The *Daphnia* of North America—An Illustrated Fauna. CD-ROM Distributed by the Author. University of Guelph, Guelph, Ont., Canada.
Hebert, P.D.N., Finston, T.L., 1993. A taxonomic reevaluation of North American *Daphnia* (Crustacea: Cladocera). I. The *D. similis* complex. Can. J. Zool. 71, 908–925.
Hebert, P.D.N., Finston, T.L., 1996. A taxonomic reevaluation of North American *Daphnia* (Crustacea: Cladocera). II. New species in the *Daphnia pulex* group from the south-central United States and Mexico. Can. J. Zool. 74, 632–653.
Hebert, P.D.N., Finston, T.L., 1997. A taxonomic reevaluation of North American *Daphnia* (Crustacea: Cladocera). III. The *D. catawba* complex. Can. J. Zool. 75, 1254–1261.
Hebert, P.D.N., Wilson, C.C., 1994. Provincialism in plankton: endemism and allopatric speciation in Australian *Daphnia*. Evolution 48, 1333–1349.
Hebert, P.D.N., Wilson, C.C., 2000. Diversity of the genus *Daphniopsis* in the saline waters of Australia. Can. J. Zool. 78, 794–808.
Hebert, P.D.N., Schwartz, S.S., Hrbáček, J., 1989. Patterns of genotypic diversity in Czechoslovakian *Daphnia*. Heredity 62, 207–216.
Hebert, P.D.N., Remigio, E.A., Colbourne, J.K., Taylor, D.J., Wilson, C.C., 2002. Accelerated molecular evolution in halophilic crustaceans. Evolution 56, 909–926.
Hebert, P.D.N., Witt, J.D.S., Adamowicz, S.J., 2003. Phylogeographical patterning in *Daphnia ambigua*: regional divergence and intercontinental cohesion. Limnol. Oceanogr. 48, 261–268.
Hedges, S.B., Parker, P.H., Sibley, C.G., Kumar, S., 1996. Continental breakup and the ordinal diversification of birds and mammals. Nature 381, 226–229.
Ho, S.Y.W., Phillips, M.J., Cooper, A., Drummond, A.J., 2005. Time dependency of molecular rate estimates and systematic overestimation of recent divergence times. Mol. Biol. Evol. 22, 1561–1568.
Hobæk, A., Wolf, H.G., 1991. Ecological genetics of Norwegian *Daphnia*. II. Distribution of *Daphnia longispina* genotypes in relation to short-wave radiation and water colour. Hydrobiologia 225, 229–243.
Hrbáček, J. 1987. Systematics and biogeography of *Daphnia* species in the northern temperate region. In: Peters, R.H., de Bernardi, R., (Eds.) ''*Daphnia*". Mem. Ist. Ital. Idrobiol. 45, 37–76.
Hudec, I., 1991. A comparison of populations from the *Daphnia similis* group (Cladocera, Daphniidae). Hydrobiologia 225, 9–22.
Hudec, I., 1993. Redescription of *Daphnia deserti* (Gauthier, 1937) (Crustacea, Daphniiformes, Daphniidae). Hydrobiologia 264, 153–158.
Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Ishida, S., Taylor, D.J., 2007. Quaternary diversification in a sexual Holarctic zooplankter, *Daphnia galeata*. Mol. Ecol. 16, 569–582.
Ishida, S., Kotov, A.A., Taylor, D.J., 2006. A new divergent lineage of *Daphnia* (Cladocera: Anomopoda) and its morphological and genetical differentiation from *Daphnia curvirostris* Eylmann, 1887. Zool. J. Linn. Soc. 146, 385–405.
Johnson, D.S., 1952. The British species of the genus *Daphnia* (Crustacea: Cladocera). Proc. Zool. Soc. London 122, 435–462.
Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.
Knowlton, N., Weigt, L.A., 1998. New dates and new rates for divergence across the Isthmus of Panama. Proc. R. Soc. Lond. B 265, 2257–2263.
Kořínek, V., 1999. A guide to limnetic species of Cladocera of African inland waters (Crustacea, Branchiopoda). Societias Internationalis Limnologiae Occasional Publication 1, 55.
Kořínek, V., Hebert, P.D.N., 1996. A new species complex of *Daphnia* (Crustacea, Cladocera) from the Pacific Northwest of the United States. Can. J. Zool. 74, 1379–1393.
Kořínek, V., Villalobos, L., 2003. Two South American endemic species of *Daphnia* from Andean lakes. Hydrobiologia 490, 107–123.
Kořínek, V., Černý, M., Petrusek, A., 2003. *Daphnia obtusa* Kurz, 1874: Komplex kryptických druhů—nový problém populační ekologie. [*Daphnia obtusa* Kurz, 1874: a complex of cryptic species—new challenge for population ecology.]. Acta Facultatis Ecologiae (Zvolen) 10 (Suppl. 1), 113–114 (in Czech).
Kotov, A.A., Ishida, S., Taylor, D.J., 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.
Lieder, U., 1983. Revision of the genus *Bosmina* Baird, 1845 (Crustacea, Cladocera). Int. Rev. Ges. Hydrobiol. 68, 121–139.
Losos, J.B., Glor, R.E., 2003. Phylogenetic comparative methods and the geography of speciation. Trends Ecol. Evol. 18, 220–227.
Louette, G., De Meester, L., 2005. High dispersal capacity of cladoceran zooplankton in newly founded communities. Ecology 86, 353–359.
Lynch, M., Jarrell, P.E., 1993. A method for calibrating molecular clocks and its application to animal mitochondrial DNA. Genetics 135, 1197–1208.
Maddison, W.P, Maddison, D.R., 2007. Mesquite: a modular system for evolutionary analysis, version 2.0. Available from: <http://mesquiteproject.org/>.
Marková, S., Dufresne, F., Rees, D.J., Černý, M., Kotlík, P., 2007. Cryptic intercontinental colonization in water fleas *Daphnia pulicaria* inferred from phylogenetic analysis of mitochondrial DNA variation. Mol. Phylogenet. Evol. 44, 42–52.
Mayr, E., 1963. Animal Species and Evolution. Harvard University Press, Cambridge, Massachusetts, USA.
Mergeay, J., Verschuren, D., De Meester, L., 2006. Invasion of an asexual American water flea clone throughout Africa and rapid displacement of a native sibling species. Proc. R. Soc. B 273, 2839–2844.
Mergeay, J., Vanoverbeke, J., Verschuren, D., De Meester, L., 2007. Extinction, recolonization, and dispersal through time in a planktonic crustacean. Ecology 88, 3032–3043.
Mergeay, J., Aguilera, X., Declerck, S., Petrusek, A., Huyse, T., De Meester, L., 2008. The genetic legacy of polyploid Bolivian *Daphnia*: the tropical Andes as a source for the North and South America *D. pulicaria* complex. Mol. Ecol. 17, 1789–1800.
Michels, E., Audenaert, E., Ortells, R., De Meester, L., 2003. Population genetic structure of three pond-inhabiting *Daphnia* species on a regional scale (Flanders, Belgium). Freshw. Biol. 48, 1825–1839.
Milá, B., Smith, T.B., Wayne, R.K., 2006. Postglacial population expansion drives the evolution of long-distance migration in a songbird. Evolution 60, 2403–2409.
Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89, 583–590.
Nilssen, J.P., Hobæk, A., Petrusek, A., Skage, M., 2007. Restoring *Daphnia lacustris* G.O. Sars, 1862 (Crustacea, Anomopoda): a cryptic species in the *Daphnia longispina* group. Hydrobiologia 594, 5–17.
Olesen, J., 1998. A phylogenetic analysis of the Conchostraca and Cladocera (Crustacea, Branchiopoda, Diplostraca). Zool. J. Linn. Soc. 122, 491–536.
Olesen, J., 2000. An updated phylogeny of the Conchostraca—Cladocera clade (Branchiopoda, Diplostraca). Crustaceana 73, 869–886.

Palumbi, S., 1996. Nucleic acids II: the polymerase chain reaction. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), Molecular Systematics. Sinauer Associates Inc., Sunderland, MA, USA, pp. 205–247.

Penton, E.H., Hebert, P.D.N., Crease, T.J., 2004. Mitochondrial DNA variation in North American populations of *Daphnia obtusa*: continentalism or cryptic endemism? Mol. Ecol. 13, 97–107.

Petrusek, A., 2003. The population of *Daphnia similis* species complex in Germany after 110 years—a new case of species introduction? Senck. biol. 82, 11–14.

Petrusek, A., Černý, M., Audenaert, E., 2004. Large intercontinental differentiation of *Moina micrura* (Crustacea: Anomopoda): one less cosmopolitan cladoceran? Hydrobiologia 526, 73–81.

Petrusek, A., Černý, M., Mergeay, J., Schwenk, K., 2007. *Daphnia* in the Tatra Mountain lakes: multiple colonisation and hidden species diversity revealed by molecular markers. Fundam. Appl. Limnol. 169, 279–291.

Petrusek, A., Hobæk, A., Nilssen, J.P., Skage, M., Černý, M., Brede, N., Schwenk, K., 2008. A taxonomic reappraisal of the European *Daphnia longispina* complex (Crustacea, Cladocera, Anomopoda). Zool. Scr. 37, 507–519.

Pfrender, M.E., Spitze, K., Lehman, N., 2000. Multi-locus genetic evidence for rapid ecologically based speciation in *Daphnia*. Mol. Ecol. 9, 1717–1735.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Richter, S., Olesen, J., Wheeler, W.C., 2007. Phylogeny of Branchiopoda (Crustacea) based upon a combined analysis of morphological data and six molecular loci. Cladistics 23, 301–336.

Rowe, C.L., Adamowicz, S.J., Hebert, P.D.N., 2007. Three new cryptic species of the freshwater zooplankton genus *Holopedium* (Crustacea: Branchiopoda: Ctenodpoda), revealed by genetic methods. Zootaxa 1656, 1–49.

Ruegg, K.C., Hijmans, R.J., Moritz, C., 2006. Climate change and the origin of migratory pathways in the Swainson's thrush, *Catharus ustulatus*. J. Biogeogr. 33, 1172–1182.

Sacherová, V., Hebert, P.D.N., 2003. The evolutionary history of the Chydoridae (Crustacea: Cladocera). Biol. J. Linn. Soc. 79, 629–643.

Schwenk, K., Sand, A., Boersma, M., Brehm, M., Mader, E., Offerhaus, D., Spaak, P., 1998. Genetic markers, genealogies and biogeographic patterns in the cladocera. Aquat. Ecol. 32, 37–51.

Schwenk, K., Posada, D., Hebert, P.D.N., 2000. Molecular systematics of European *Hyalodaphnia*: the role of contemporary hybridization in ancient species. Proc. R. Soc. Lond. B 267, 1833–1842.

Skage, M., Hobæk, A., Ruthová, Š., Keller, B., Petrusek, A., Seda, J., Spaak, P., 2007. Intraspecific rDNA-ITS restriction site variation and an improved protocol to distinguish species and hybrids in the *Daphnia longispina* complex. Hydrobiologia 594, 19–32.

Smirnov, N.N., 1992. Mesozoic Anomopoda (Crustacea) from Mongolia. Zool. J. Linn. Soc. 104, 97–116.

Stebbins, G.L., 1984. Polyploidy and distribution of arctic-alpine flora: new evidence and a new approach. Botanica Helvetica 94, 1–13.

Stenderup, J.T., Olesen, J., Glenner, H., 2006. Molecular phylogeny of the Branchiopoda (Crustacea)—multiple approaches suggest a "diplostracan" ancestry of the Notostraca. Mol. Phylogenet. Evol. 41, 182–194.

Swain, T.D., Taylor, D.J., 2003. Structural rRNA characters support monophyly of raptorial limbs and paraphyly of limb specialization in water fleas. Proc. R. Soc. Lond. B 270, 887–896.

Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony, Version 4.1.1. Sinauer Associates, Sunderland, MA, USA.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) Software Version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Taylor, D.J., Hebert, P.D.N., 1992. *Daphnia galeata mendotae* as a cryptic species complex with interspecific hybrids. Limnol. Oceanogr. 37, 658–665.

Taylor, D.J., Hebert, P.D.N., 1993a. Habitat-dependent hybrid parentage and differential introgression between neighboringly sympatric *Daphnia* species. Proc. Natl. Acad. Sci. USA 90, 7079–7083.

Taylor, D.J., Hebert, P.D.N., 1993b. A reappraisal of phenotypic variation in *Daphnia galeata mendotae*: the role of interspecific hybridization. Can. J. Fish. Aquat. Sci. 50, 2137–2146.

Taylor, D.J., Hebert, P.D.N., 1994. Genetic assessment of species boundaries in the North American *Daphnia longispina* complex (Crustacea: Daphniidae). Zool. J. Linn. Soc. 110, 27–40.

Taylor, D.J., Hebert, P.D.N., Colbourne, J.K., 1996. Phylogenetics and evolution of the *Daphnia longispina* group (Crustacea) based on 12S rDNA sequence and allozyme variation. Mol. Phylogenet. Evol. 5, 495–510.

Taylor, D.J., Finston, T.L., Hebert, P.D.N., 1998. Biogeography of a widespread freshwater crustacean: pseudocongruence and cryptic endemism in the North American *Daphnia laevis* complex. Evolution 52, 1648–1670.

Taylor, D.J., Crease, T.J., Brown, W.M., 1999. Phylogenetic evidence for a single long-lived clade of crustacean cyclic parthenogens and its implications for the evolution of sex. Proc. R. Soc. Lond. B 266, 791–797.

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

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. ClustalW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Valdivia Villar, R.S., Burger, L., 1989. Descripción de *Daphniopsis marcahuasensis* sp. nov. (Cladocera: Daphniidae) del Perú, con la inclusión de una clave de identificación de las especies del género. Amazoniana 10, 439–452.

Walker, D., Avise, J.C., 1998. Principles of phylogeography as illustrated by freshwater and terrestrial turtles in the southeastern United States. Annu. Rev. Ecol. Syst. 29, 23–58.

Ward, R.D., Bickerton, M.A., Finston, T.L., Hebert, P.D.N., 1994. Geographical cline in breeding systems and ploidy levels in European populations of *Daphnia pulex*. Heredity 73, 532–543.

Weider, L.J., Hobæk, A., 2003. Glacial refugia, haplotype distributions, and clonal richness of the *Daphnia pulex* complex in arctic Canada. Mol. Ecol. 12, 463–473.

Weider, L.J., Hobæk, A., Colbourne, J.K., Crease, T.J., Dufresne, F., Hebert, P.D.N., 1999a. Holarctic phylogeography of an asexual species complex I. Mitochondrial DNA variation in arctic *Daphnia*. Evolution 53, 777–792.

Weider, L.J., Hobæk, A., Hebert, P.D.N., Crease, T.J., 1999b. Holarctic phylogeography of an asexual species complex-II. Allozymic variation and clonal structure in Arctic *Daphnia*. Mol. Ecol. 8, 1–13.

Wiens, J.J., 2006. Missing data and the design of phylogenetic analyses. J. Biomed. Inform. 39, 34–42.

Wolf, H.G., Mort, M.A., 1986. Inter-specific hybridization underlies phenotypic variability in *Daphnia* populations. Oecologia 68, 507–511.