Mar Biol (2008) 155:1–10
DOI 10.1007/s00227-008-0996-x

ORIGINAL PAPER

Comparative phylogeography of marine cladocerans

Alan Durbin · Paul D. N. Hebert · Melania E. A. Cristescu

Received: 25 July 2007 / Accepted: 2 May 2008 / Published online: 10 June 2008

Abstract We examined the population genetics of six species of marine cladocerans, using a ~600 bp fragment of the cytochrome oxidase subunit I gene sequence. Phylogenetic analysis revealed significant intraspecific, semi-allopatric phylogenetic breaks in four out of five species belonging to the Podonidae, supporting an ancient radiation and oceanic expansion for this group. By contrast, *Penilia avirostris* (Sididae) displayed no phylogeographic structure across a global sampling, suggesting a recent worldwide expansion. Our results also show a transoceanic distribution of identical or very similar haplotypes in several species of marine Cladocera, which may be interpreted as either natural transport or evidence of recent anthropogenic transport. If the latter is the case, marine cladocerans represent one of the first genetically documented cases of exotic or invasive marine zooplankton, likely an underreported group.

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under accession nos. EU675871–EU675924.

Communicated by T. Reusch.

Electronic supplementary material The online version of this article (doi:10.1007/s00227-008-0996-x) contains supplementary material, which is available to authorized users.

A. Durbin (✉)
Department of Marine Sciences,
University of North Carolina, 340 Chapman Hall,
CB# 3300, Chapel Hill, NC 27599, USA
e-mail: amdurbin@email.unc.edu

P. D. N. Hebert
Department of Integrative Biology,
University of Guelph, Guelph, ON, Canada N1G 2W1

M. E. A. Cristescu
Great Lakes Institute for Environmental Research,
University of Windsor, Windsor, ON, Canada N9B 3P4

Introduction

Although there are over 600 described species of cladocerans, only 8 are truly marine. The relative scarcity of marine cladocerans compared to the high diversity of their freshwater and brackish relatives has been remarked as curious (Rivier 1998) and has been attributed to their high dispersal ability which may inhibit vicariant speciation by enhancing genetic connectivity between distant populations (Egloff et al. 1997). On the other hand, the very broad distribution range of marine cladocerans suggests them as good candidates for phylogeographic and population genetic investigations. Cryptic speciation has been documented in many other "cosmopolitan" organisms, including species with high dispersal ability such as marine and freshwater zooplankton (Goetze 2003, 2005). To investigate questions of evolutionary history and population dynamics among marine zooplankton, we examined the phylogeography of six marine cladocerans of the families Podonidae (Order Onychopoda; *Evadne nordmanni*, *Pseudevadne tergestina*, *Podon leuckarti*, *Podon intermedius*, *Pleopis polyphemoides*) and Sididae (Order Ctenopoda; *Penilia avirostris*).

The two marine cladoceran families are convergent on marine adaptations, sharing a pelagic, neritic-to-oceanic habitat niche, and a similar reproductive biology that includes nursing embryos in a brood pouch (Egloff et al. 1997). The Podonidae further share a grasping mode of feeding, while *P. avirostris* is a filter feeder. With the exception of *P. intermedius*, all marine cladocerans included in this study have either a circumhemispheric distribution, or are reported worldwide (Table 1; Egloff et al.

Table 1 Collection locations and number of individuals sampled, and reported ranges

	GenBank	Collection locations (number of individuals sampled)	Reported ranges
Evadne nordmanni	EU675871–EU675873; EU675883–EU675893; EU675902–EU675905	NE Atlantic: Portugal (12), Netherlands (2), Copenhagen Harbor (2), Baltic Sea (12); NW Atlantic: Narragansett Bay (1); NE Pacific: Bering Strait (5), Vancouver (29); SW Pacific: Tasmania (3)	Subarctic to subtropical waters in the northern Pacific and Atlantic oceans[a]; also reported from the Argentine Sea[b]
Pseudevadne tergestina	EU675898–EU675900; EU675910–EU675911	NE Atlantic: Aegean Sea (1), Gulf of Naples (2); NW Pacific: Gulf of Thailand (1); SW Pacific: Sydney Harbor (4)	Worldwide neritic habitats of subtropical and tropical regions between 40–45°N and 40°S[a]
Podon leuckarti	EU675876; EU675878–EU675879; EU675906–EU675909;	NE Atlantic: Oslo Fjord (5), Copenhagen Harbor (2), Baltic Sea (3); NW Atlantic: Narragansett Bay (2); NE Pacific: Bering Strait (3), Vancouver (7), Oregon Coast (1)	Subarctic to subtropical waters of the northern Pacific and Atlantic oceans[a]
Podon intermedius	EU675874–EU675875; EU675877; EU675880–EU675882; EU675901	NE Atlantic: Aegean Sea (2), English Channel (24), Oslo Fjord (1), Copenhagen Harbor (2)	Atlantic Ocean and adjoining seas up to 40°N[a]
Pleopis polyphemoides	EU675894–EU675897; EU675912–EU675915	NE Atlantic: Black Sea (6), Portugal (3), Copenhagen Harbor (2), Baltic Sea (11); NW Atlantic: Bay of Fundy (2); NE Pacific: Vancouver (6)	Widely distributed in neritic habitats between 65 and 70°N and 40°S; also brackish estuaries, polluted harbors and nearly fresh riverine environments[a]
Penilia avirostris	EU675916–EU675924	NE Atlantic: Aegean Sea (2), Gulf of Naples (3); NW Pacific: Hong Kong Harbor (2); SW Pacific: Sydney Harbor (5); SE Indian: Perth Harbor (5)	Subtropical to tropical, neritic and oceanic habitats between 45°N and 40°S[a]

[a] Rivier, I. K. (1998)

[b] Egloff et al. (1997)

1997; Rivier 1998). High seasonal population densities of up to 100,000 individuals per square meter are achieved through a parthenogenetic reproduction phase, followed by the production of sexual resting eggs and population crash (Egloff et al. 1997). Since resting eggs must be laid in continental shelf areas to hatch, the marine cladoceran populations are anchored to near-shore habitats. However, *P. avirostris* has the ability to maintain planktonic populations without male or resting egg production, (Tang et al. 1995; Egloff et al. 1997).

Similar to radiations of other predatory cladocerans, including the brackish Caspian podonids, the speciation of the marine podonids is likely related to the dynamic hydrogeologic history of the Ponto-Caspian region (Cristescu and Hebert 2002). In this scenario, cycles of habitat fragmentation and salinity fluctuations lead to the Miocene and Pliocene emergence of the podonid genera, which gained access to the world ocean soon after. A more recent expansion has been hypothesized for *P. avirostris* (Lochhead 1954; Egborge 1987) and also the Podonidae (Dumont 1998, 2000), wherein colonization of the world ocean took place in the late Pleistocene or Holocene. We used molecular data from worldwide distributed marine cladocerans to examine the evolutionary history of this group.

One confounding factor when interpreting population histories from mitochondrial phylogenies is the possibility of anthropogenic transport via transoceanic shipping (e.g., Roy and Sponer 2002). It has been postulated that small invertebrate taxa are underreported as invasive species (Ruiz et al. 2000), and this may be particularly the case for zooplankton species that are morphologically similar, thus making identification difficult. Marine cladocerans are good candidates for ballast-water transport because of their production of resistant resting eggs and asexual reproduction, which means only a single parthenogenetic female is required to populate a new habitat. Use of genetic methods suggested a 50,000-fold increase in the background rate of trans-oceanic dispersal in freshwater cladocerans due to ballast-water dispersal (Hebert and Cristescu 2002). Although it is difficult to genetically distinguish natural transport from human-mediated transport in an organism with unknown phylogeography, we examined this question using both the phylogeographic evidence from molecular data and available empirical data on ballast water transport.

Materials and methods

The study included six marine cladoceran species belonging to two families, Podonidae (Order Onychopoda; *Podon leuckarti, Podon intermedius, Pleopis polyphemoides, Evadne nordmanni,* and *Pseudevadne tergestina*) and Sididae (Order Ctenopoda; *Penilia avirostris*). Individuals

were collected from 19 sites around the globe, which were grouped together as the NE Atlantic (including adjoining seas), NW Atlantic, NE Pacific, SW Pacific, NW Pacific, and SW Indian (Table 1, supporting data online). Animals were identified using Rivier's 1998 key, and a mitochondrial protein coding gene, the cytochrome *c* oxidase subunit I (COI) was used to explore sequence diversity. DNA was extracted using a lysis buffer protocol following Schwenk et al. (1998). A 658 base pair (bp) fragment of CO1 was amplified using the primers LCO1490 (5′-GGT CAA ATC ATA AAG ATA TTG G-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) (Folmer et al. 1994). A total of 50 μL PCR reactions contained 4 μL of DNA, 5.2 μL of 10× buffer, 3.0 μL of 25 mM $Mg(OAC)_2$, 1.3 μL of 10 mM dNTP, 1.3 μL of 10 μM forward and reverse primers, and one unit of *Taq* polymerase. Conditions for PCR included: 5 min at 95°C, followed by five cycles of 1 min at 94°C, 1:15 min at 48°C, 1:15 min at 72°C, 30 cycles of 1 min at 94°C, 1:15 min at 50°C, and 1:15 min at 72°C, followed by an extension period at 72°C for 5 min. PCR product was purified using a SPRI protocol (Elkin et al. 2001), and DNA sequencing was carried out on ABI377 and ABI3730 automated sequencers, using the LCO1490 primer and the Big Dye 3 and Big Dye 3.1 sequencing kit, respectively (30 cycles and 55° annealing temperature).

Sequence alignment and editing was performed on CodonCode Aligner software (CodonCode Corporation) and was conducted separately for *P. avirostris* and the podonids. Phylogenetic analysis was conducted using both maximum parsimony (MP) and Bayesian analysis. To choose a model of sequence evolution for the Podonidae and for *Penilia*, MrModeltest 2.2 (Nylander 2004) and PAUP* (Swofford 1998) were used to calculate the maximum likelihood and parameter values of 24 candidate models of sequence evolution, given an inferred neighbor-joining tree and the data, followed by the selection of the best-fit model by hierarchical likelihood ratio tests (hLRTs) (Posada and Crandall 2001). The model chosen for the Podonids was a general-time-reversible model with γ-distributed rate heterogeneity ($\alpha = 1.913$) and a proportion of invariant sites (0.5953) (Tavaré 1986 1986; Waddell and Steel 1997). For *Penilia*, the hLRTs did not all select the same model, so the Akaike Information Criterion (Akaike 1974) was used to select a general time reversible model with a proportion of invariant sites, which was used to generate a phylogeny in MrBayes. The best-fit model and estimated parameter values for the Podonidae were then used to implement a LRT of the molecular clock hypothesis for this group in PAUP*. MrBayes 3.1 (Ronquist and Huelsenbeck 2003) was used to estimate model parameter values and Bayesian posterior probabilities of clades, under a general time reversible model with gamma-distributed rate variation and a proportion of invariant sites (GTR + I + G). Bayesian analyses were conducted for 4 million generations, with the first 25% of sampled trees discarded as burnin. PAUP* was used to infer a maximum parsimony tree using heuristic search with 100 random addition sequence replications, and clade support values were generated using bootstrap analysis at a 70% consensus level and 1,000 replications. The podonid tree was rooted using *Polyphemus pediculus* (Onychopoda: Polyphemidae) while *Sida crystalline* (Ctenopoda: Sididae) was used to root the *Penilia* tree.

Net between-clade pairwise distances were calculated for the Podonidae with MEGA 3.0 (Kumar et al. 2004), using the Tamura-Nei model (Tamura and Nei 1993) with a gamma rate distribution and $\alpha = 1.913$, which was the closest approximation to a GTR + I + G model available in MEGA. For three species (*Evadne nordmanni*, *Podon leuckarti* and *Pleopis polyphemoides*) which show not only significant sampling but also strong intraspecific phylogenetic structure, i.e. monophyletic groups that exceed the 3% divergence level accepted by many authors as defining phylogenetic species (Avise 2004), minimum spanning networks were calculated in Arlequin 3.0 with $\alpha = 0.05$ (Excoffier et al. 2005). Genetic diversity within species and within phylogroups was characterized by the standard diversity indices of haplotype diversity (Hd) and nucleotide diversity (Π) using Arlequin.

Results

COI sequence data from the six species of the Podonidae were unambiguously aligned resulting in a 655-bp alignment. *Penilia avirostris* COI data were also unambiguously aligned as a 646 bp fragment. Size of the assembled and aligned contigs reflects the maximum extent of high-quality sequence data for each species. No insertions or deletions were detected. Of the 655 base pair total alignment for the Podonidae, there were 242 synonymous changes and 17 nonsynonymous changes, and 193 parsimony-informative changes. For *P. avirostris*, out of 646 sites, there were 16 variable sites, 4 of which were parsimony-informative, and 1 of which represented non-synonymous substitutions. The Bayesian analysis for the podonids produced tree topologies that were largely congruent with the MP analysis, with few exceptions (e.g., the MP analysis did not resolve hierarchical relations between species, and instead produced polytomies). Since these differences do not interfere with the interpretation of the phylogenetic structure, only the Bayesian trees were presented (Fig. 1). Four species with global or hemispheric distributions, namely *E. nordmanni*, *P. leuckarti*, *P. polyphemoides*, and *P. tergestina* showed significant intraspecific phylogenetic structure, approaching or exceeding the

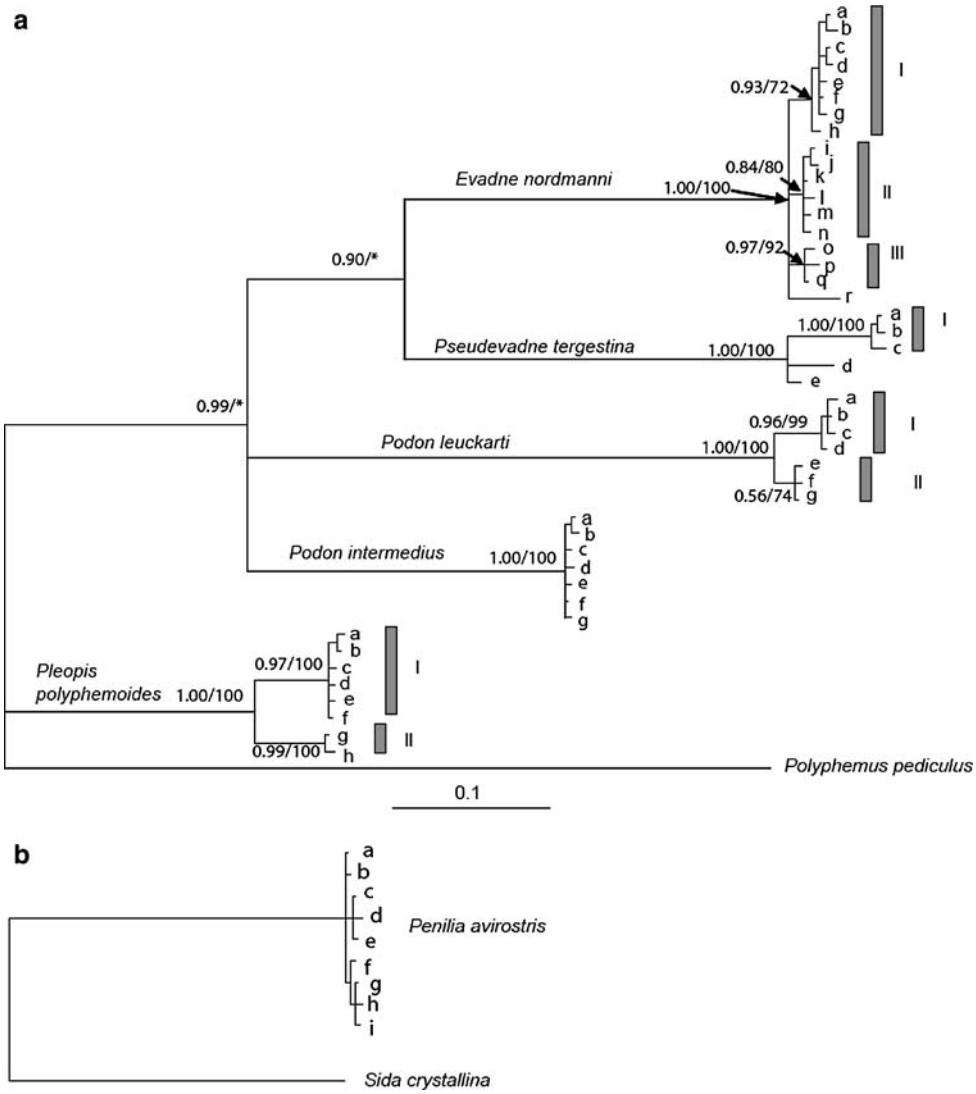

Fig. 1 **a** Bayesian trees of the marine Podonidae, and **b** *Penilia avirostris*. Scale is in units of expected substitutions per site. Support values are Bayesian posterior probabilities and MP bootstrap values, respectively, *stars* indicate no MP bootstrap support for the Bayesian topology. Major podonid intraspecific clades are indicated with *gray bars*, and haplotypes are labeled with *letters* (see S1 in ESM for collection information)

3% COI divergence level generally accepted as defining phylogenetic species (Avise 2004) (Fig. 1a). Although the molecular clock hypothesis was rejected using a hLRT model (-2 ln LR = 65.2; $df = 44$; $P < 0.05$), applying the CO1 molecular clock of 1.4% myr^{-1} calculated from *Synalpheus* shrimp (Knowlton and Weight 1998) suggested an approximate clade ages of 1–4 millions of years old (Tables 2, 3). While the intraspecific clades revealed for *E. nordmanni*, *P. leuckarti*, and *P. polyphemoides* were divergent, they contained limited internal genetic structure and low nucleotide diversity (Table 2). The single phylogroup recovered for *P. intermedius* contained nucleotide and haplotype diversity typical of the major intraspecific clades found in the other podonids.

There is a substantial degree of concordance between phylogroup and geographic region evident in the haplotype networks (Figs. 2 3, S1 in Electronic supplementary material). This concordance is most apparent in *E. nordmanni*, where a Pacific-Atlantic phylogeographic split is evident. All Atlantic *E. nordmanni* haplotypes fall into one phylogroup, clade II, with no representatives of this phylogroup recovered elsewhere. Most *E. nordmanni* haplotypes from the NE Pacific were also closely related, constituting all of Clade I. The split between clades in *P. leuckarti* and *P. polyphemoides* may also be associated with a Pacific/Atlantic phylogeographic split: clade I of *P. leuckarti* consisted solely of NE Pacific haplotypes, and clade I of *P. polyphemoides* consisted mostly of NE Atlantic haplotypes. Notably, only a single phylogroup was sampled from European waters for all three species, although this was by far the best sampled region, both geographically and numerically (Fig. 2).

However, phylogroups in these species contained closely related haplotypes collected from different ocean basins (clade II of *P. leuckarti* and clade I of *P. polyphemoides*) or distant regions of the same ocean basin (clade II of both species; Figs. 2, 3). The same phenomenon was observed among the Pacific haplotypes of *E. nordmanni*

Table 2 Number of individuals sampled (*n*), number of haplotypes (*H*), contig size, molecular diversity data, and divergence time estimates for intraspecific clades

	n	*H*	Contig size	Haplotype diversity (Hd)	Nucleotide diversity (π)	Substitution type		Range of intraspecific Tamura-Nei distances	Approximate age of divergences (1.4% myr^{-1})
						Synonomous	Nonsynonomous		
E. nordmanni	66	18	605	0.89 ± 0.022	0.013 ± 0.0067	38	3	0.016–0.036	1.1–2.6 myr
Clade I	29	8	605	0.86 ± 0.035	0.0032 ± 0.0021	9	0		
Clade II	30	6	605	0.60 ± 0.072	0.0013 ± 0.0011	4	2		
Clade III	5	3	605	0.70 ± 0.21	0.0033 ± 0.0026	5	0		
Haplotype r	2	1	605						
E. tergestina	8	5	642	0.86 ± 0.11	0.034 ± 0.019	41	3	0.028–0.058	2.0–4.1 myr
P. leuckarti	23	7	601	0.78 ± 0.063	0.0093 ± 0.0051	18	1	0.024	1.7 myr
Clade I	6	4	601	0.90 ± 0.16	0.0029 ± 0.0023	4	0		
Clade II	17	3	601	0.64 ± 0.074	0.0013 ± 0.0011	2	0		
P. intermedius	29	7	646	0.48 ± 0.11	0.0011 ± 0.00096	6	1		
P. polyphemoides	30	8	619	0.85 ± 0.034	0.020 ± 0.010	18	1	0.059	4.2 myr
Clade I	24	6	619	0.80 ± 0.051	0.0028 ± 0.0019	4	2		
Clade II	6	2	619	0.29 ± 0.20	0.00088 ± 0.00092	2	0		
P. avirostris	17	9	630	0.92 ± 0.037	0.0051 ± 0.0031	16	1		

Table 3 Net Tamura-Nei distances between species and between major intraspecific clades for Podonidae, below diagonal, and standard errors for distance calculations, above diagonal

	1	2	3	4	5	6	7	8	9	10	11	12
1. *Evadne nordmanni* clade I		0.005	0.005	0.008	0.027	0.026	0.026	0.031	0.029	0.025	0.026	0.025
2. *Evadne nordmanni* clade II	**0.016**		0.005	0.007	0.026	0.025	0.025	0.030	0.028	0.024	0.026	0.024
3. *Evadne nordmanni* clade III	**0.017**	**0.013**		0.007	0.025	0.026	0.026	0.030	0.028	0.025	0.026	0.025
4. *Evadne nordmanni* haplotype r	**0.036**	**0.030**	**0.029**		0.026	0.026	0.026	0.031	0.029	0.025	0.026	0.025
5. *Evadne tergestina* clade I	0.228	0.224	0.219	0.234		0.010	0.009	0.028	0.027	0.027	0.028	0.027
6. *Evadne tergestina* haplotype d	0.219	0.218	0.224	0.223	**0.058**		0.007	0.027	0.026	0.025	0.027	0.026
7. *Evadne tergestina* haplotype e	0.220	0.218	0.224	0.233	**0.044**	**0.028**		0.025	0.025	0.025	0.027	0.026
8. *Podon leuckarti* clade I	0.290	0.280	0.280	0.287	0.241	0.242	0.226		0.006	0.023	0.027	0.026
9. *Podon leuckarti* clade II	0.268	0.257	0.257	0.265	0.233	0.231	0.215	**0.024**		0.022	0.026	0.026
10. *Podon intermedius*	0.207	0.199	0.199	0.209	0.246	0.223	0.227	0.204	0.194		0.022	0.021
11. *Pleopis polyphemoides* clade I	0.229	0.228	0.234	0.235	0.250	0.241	0.240	0.233	0.239	0.209		0.011
12. *Pleopis polyphemoides* clade II	0.210	0.205	0.215	0.219	0.253	0.246	0.244	0.237	0.235	0.202	**0.059**	

Intraspecific distances are in bold

(clade III; Fig. 2). Further, within the relatively well-sampled NE Atlantic region, the most frequently occurring *E. nordmanni* haplotype, haplotype k, was recovered from disparate regions in the NE Atlantic, the Baltic Sea and Portugal, while haplotype i was recovered from both the NE and NW Atlantic (Fig. 3).

Although sampling in *P. tergestina* was more limited, three divergent haplogroups were recovered. A few haplotypes from the northwest and southwest Pacific grouped together, while the remaining two haplotypes, collected from the Mediterranean and southwest Pacific, respectively, grouped separately as highly divergent singletons (Fig. 1a). In contrast to the four podonid species with significant phylogenetic diversity, *P. intermedius*, reportedly a species restricted to the Atlantic (Rivier 1998), formed single phylogroups (Fig. 1a). Like *P. intermedius*, the globally distributed *P. avirostris* displayed little phylogenetic structure (Fig. 1b), and molecular diversity indices were again equivalent to those found for the intraspecific clades of the Podonidae (Table 2). Haplotype diversity for entire species as well as within major clades tended to be high, ranging from 0.60 for *E. nordmanni* clade II to 0.92 for *P. aviros-*

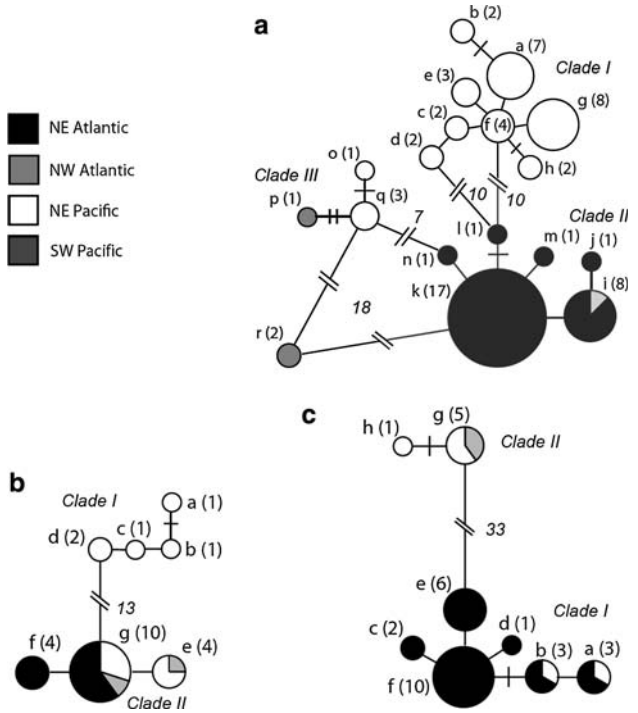

Fig. 2 Minimum spanning networks for **a** *Evadne nordmanni*, **b** *Podon leuckarti*, and **c** *Pleopis polyphemoides*. Each *circle* represents a haplotype, *size of circle* represents its sampling frequency with the *smallest circles* denoting a single individual, and *connecting lines* and *hache marks* each signify one mutational difference. Larger mutational distances are indicated by *gapped lines* and *numbers*. The *shading* and *partitioning* of each circle indicates the number of that haplotype collected from a specific ocean region

tris, with only *P. intermedius* having a low overall haplotype diversity of 0.48 (Table 2).

Discussion

The present study has revealed significant phylogeographic structure in four of six marine cladoceran species. According to rough calculations using the snapping shrimp CO1 clock (Knowlton and Weight 1998), this structure is the result of ~1–4 million years of isolated evolution. Based on CO1 divergences, the marine Podonidae appear to have occupied the world oceans since the late Pliocene to mid Pleistocene, contradicting the suggestion that colonization of the world oceans by this group took place in the latest Pleistocene or Holocene (e.g., Dumont 2000). On the other hand, the lack of phylogenetic structure and the low molecular diversity for *P. avirostris* (Fig. 1b; Table 2) appears to support the hypothesis of a recent invasion of the world ocean by this species (Lochhead 1954; Egborge 1987). Although a similar phylogenetic and molecular diversity pattern was found for *P. intermedius*, a recent oceanic expansion is unlikely, as *P. intermedius* is a sister species to *P. leuckarti*, which appears to be anciently oceanic (Cristescu and Hebert 2002; this study). The apparent lack of phylogeographic structure is most likely due to sampling restricted to the European coastal seas. It is possible that divergent phylogenetic groups for *P. intermedius* species exist in the Atlantic and elsewhere but were not recovered in this study.

Between-clade divergences of 1.3–3.6% for *E. nordmanni*, 2.8–5.8% for *P. tergestina*, 2.4% for *P. leuckarti*, and 5.9% for *P. polyphemoides* (Table 3) are similar to intraspecific divergence reported in other widely-distributed planktonic arthropods, including calanoid copepods (Bucklin et al. 2003), oceanic water striders (Andersen et al. 2000), and freshwater cladocerans (Hebert et al. 2003). These intraspecific divergences are significantly smaller than the genetic breaks found at the CO1 locus between celebrated examples of zooplankton cryptic species (e.g., the copepods of the genus Eucalanus and Acartia, and the chaetognath *Sagitta setosa*; Goetze 2003; Lee 2000; Caudill and Bucklin 2004; Peijnenburg et al. 2004; Taylor et al. 1998) which range between 15 and 30% divergent at COI. On the other hand, other morphological species of cladocerans, both marine, brackish (Cristescu and Hebert

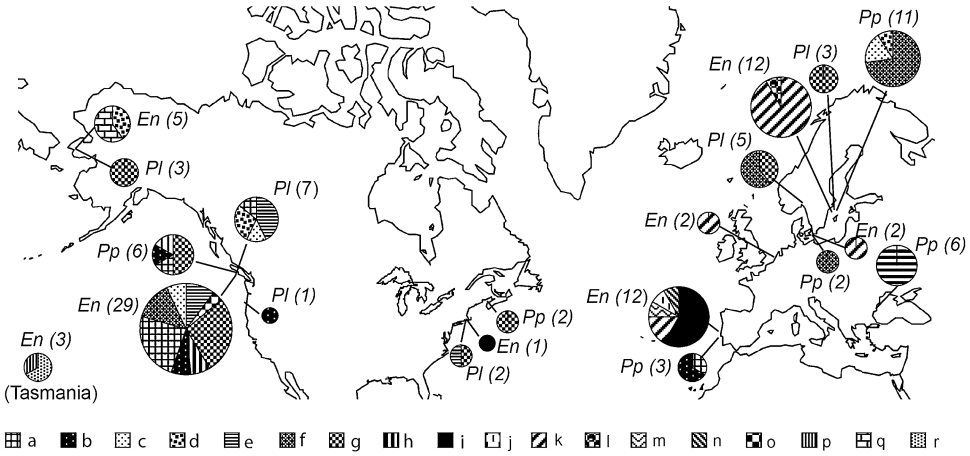

Fig. 3 Haplotype frequencies and locations for *E. nordmanni* (*EN,* haplotypes a-r), *P. leuckarti* (*PL*, haplotypes a-f), and *P. polyphemoides* (*PP,* haplotypes a-h). The same pattern refers to the same alphabetic haplotype name in each species, so all "a" haplotypes have the same pattern. Smallest circle corresponds to 1 individual, and the largest corresponds to 29 individuals

2002) and freshwater (Schwenk et al. 2000) show less than 6% divergence between sister species, or are even paraphyletic. Therefore, cryptic speciation cannot be confirmed or rejected for the marine cladocerans examined in this study.

The major phylogenetic splits evident in *E. nordmanni*, *P. leuckarti*, and *P. polyphemoides* appear to be associated with the North American continental barrier dividing Pacific and Atlantic populations. Support for this interpretation is best exhibited in the allopatric distribution of clades I and II of *E. nordmanni*, the best sampled species, with all individuals sampled from four European sites grouping together in clade I, and 30 of 33 individuals collected from two NE Pacific sites grouping in clade II of this species (Figs. 2, 3; Table SI in ESM). These divergences, dated tentatively as 1.2–4.2 millions of years old (Table 2), are younger than the opening of the Bering Strait ~4.8–7.4 mya (Marincovich and Gladenkov 1999), and coincide with a period during which the Arctic Ocean was often seasonally or perennially ice-free, which was followed by a period during which it was frequently ice-covered (Harris 2005). The ages of the intraspecific divergences are consistent with a scenario in which the Podonidae expanded to the Pacific via the Arctic Ocean, and possibly the Panamanian seaway, and subsequently became isolated due to the onset of more frequent severe glaciation ~0.8 mya (Harris 2005) and the rise of the Isthmus of Panama ~3 mya (Cronin and Dowsett 1996).

E. nordmanni, which appears to be the sister species to the Ponto–Caspian-restricted, brackish-water *E. anonyx* (Cristescu and Hebert 2002), may have gained access to the world ocean during roughly the same period as *Pleopis* and *Podon*, or may have evolved during a later period of marine connection and high salinity in the Ponto-Caspian region 1–3 mya (Cristescu and Hebert 2002). The latter scenario, in which the worldwide net intraspecific divergence observed in CO1 in this study (1.65–3.6%) roughly equates to the interspecific mitochondrial divergence between *E. nordmanni* and *E. anonyx* (~2%; Cristescu and Hebert 2002), is consistent with a rapid period of colonization of the world ocean by *E. nordmanni* and subsequent isolation of phylogroups.

A problem with interpreting the phylogeographic structure in *P. polyphemoides, P. leuckarti*, and *E. nordmanni* as representing the simple allopatric evolution of populations is the appearance of identical haplotypes in both the Atlantic and Pacific (Figs. 2, 3). Significantly divergent phylogroups, which in the case of *E. nordmanni*, *P. polyphemoides* and *P. leuckarti* approach or exceed the 3% CO1 divergence level which is considered by some to define phylogenetic species, are the result of restricted gene flow, either by niche specialization or by geographic isolation (Avise 2004). If the phylogroups are indeed the result of geographic isolation, the appearance of identical haplotypes in different ocean basins requires additional explanation. This pattern may result either from recent anthropogenic dispersal superimposed over the evolutionary geographic isolation of phylogroups, or from intermittent or low-level natural dispersal between population centers of different phylogroups.

The hypothesis that the identical CO1 haplotypes in the Atlantic and Pacific are the result of natural dispersal is supported by the reported occurrence of *P. polyphemoides, P. leuckarti* and *E. nordmanni* in the seas of the Arctic Ocean (Rivier 1998), although this habitat, with typically a 1–5°C summertime maximum, is below the temperature optimum for the cold-tolerant cladoceran species, *P. leuckarti* (7–13°C) and *P. polyphemoides* (5–20°C) (Egloff et al. 1997). Poore et al. (1993) report a foraminifer species with temperature tolerances similar to the subpolar Podonidae in cores taken from the Arctic Ocean, which date to the interglacials of the last 400,000 years. Thus, cold-tolerant podonid species may disperse across the Arctic during interglacials such as the current one.

A phylogenetic structure in which a trans-Arctic species displays an Atlantic–Pacific clade split but one phylogroup occurs in both oceans has also been reported in the bivalve *Macoma balthica* (Luttikhuizen et al. 2003). This pattern is interpreted as the result of long-distance transport and colonization from the Pacific during a range expansion event, specifically when the Baltic became habitable for marine organisms following the last glacial maximum. Likewise, the appearance of haplotypes b and a of *P. polyphemoides* and haplotypes g and e of *P. leuckarti* in both the Atlantic and Pacific may represent a natural trans-Arctic interchange associated with the expansion of Atlantic temperate neritic habitat following the LGM. Genetic sampling from intermediate regions, such as the Nearctic region, may reveal whether natural dispersal between the Atlantic and Pacific is occurring, since the low ship traffic through these regions would be expected to minimize effects of anthropogenic dispersal.

The euryhaline nature of many marine cladocerans, some of which can tolerate near freshwater, as well as the wide temperature tolerances of the cool-temperate species (Rivier 1998), make them ideal candidates for transport in ballast water. Adult marine cladocerans have been shown to survive several days (*E. nordmanni*, *P. polyphemoides*) to a week (*P. avirostris*) in ballast water tanks (Gollasch et al. 2000), which at an average ship speed of 15 knots (~28 km h^{-1}), means theoretical transport ranges of thousands of kilometers in several days. Resting eggs may also be an important promoter of ballast water-mediated dispersal. Bailey et al. (2003) found viable resting eggs of *E. nordmanni* and *P. polyphemoides* in ballast tank sediments from transoceanic ships, even after mid-ocean purges of ballast water. Although it has been suggested that resting

eggs enhance invasion resistance and genetic insularity among freshwater cladoceran populations (De Meester et al. 2002), resting eggs may also enhance invasion success via ballast water transport (Panov et al. 2004). In this view, selection during ballast water transport favors strains that produce higher numbers of resting eggs, a trait which enables these strains to colonize a new environment despite intense competition from native cladocerans and novel predation regimes.

The invasive potential of cladocerans is well-documented (e.g., Hebert and Cristescu 2002), and prior studies provide several examples of marine cladocerans that recently colonized new habitats, possibly with the help of transoceanic shipping. *P. avirostris* has appeared suddenly in several areas, including of the Bay of Naples, Italy (Cattley and Harding 1949), Great Harbor, Woods Hole, USA (Lochhead 1954), and on George's Bank (Colton 1985). *P. intermedius* was newly found in the Chesapeake Bay in 1974 (Bryan and Grant 1974). *P. schmackeri* has been reported as a native of the warm waters of the North Pacific (Rivier 1998) but was also recorded in the port harbor of Santos, Brazil (da Rocha 1985). However, global occurrences of these species may go unreported because of their close similarity to congeners, which are difficult to distinguish morphologically. Knowledge of the native distributions of marine cladocerans is also incomplete, particularly in the southern hemisphere. For example, *E. nordmanni* is not reported as ranging to Tasmania in recent reviews of marine cladoceran ecology and biology (Egloff et al. 1997; Rivier 1998), although this was a collection site for this species in this study. This makes it difficult to determine which reports represent novel introductions.

There are a few cases reported in the literature of identical or nearly identical zooplankton haplotypes collected from distant locations (Goetze 2003; Bucklin et al. 2003; Taniguchi et al. 2004), such as nearly identical CO1 haplotypes in two copepod species found in New Zealand and the Aegean Sea (Bucklin et al. 2003). The distances and presumed dispersal barriers across which podonid phylogroups are distributed, such as Clade III of *E. nordmanni*, found over 13,000 km apart in Tasmania and Vancouver, BC, is remarkable for a neritic zooplankter. The same phenomenon has been reported in eukaryotic phytoplankton, including cases of identical haplotypes collected from opposite poles (Montresor et al. 2003; de Vargas et al. 1999; Darling et al. 2000). The ecological impact of invasive zooplankton is a growing area of research (Bollens et al. 2002), and questions such as the relative roles of anthropogenic transport versus natural long-distance gene flow in planktonic species have been little explored. Answering them will be important to our understanding of the evolution and current population genetics of marine plankton.

Conclusions

The current study examined the genetic structure in 6 species of marine cladocerans, of which four species, *E. nordmanni*, *P. tergestina*, *P. polyphemoides*, and *P. leuckarti* displayed significant phylogenetic structure, confirming an ancient oceanic expansion for the Podonidae (Cristescu and Hebert 2002). For three of these species, *E. nordmanni*, *P. polyphemoides*, and *P. leuckarti*, this phylogenetic structure appears to be associated with a phylogeographic split between Atlantic and Pacific populations. The presence of identical haplotypes in multiple oceanic regions could be explained either by natural dispersal or by anthropogenic introductions. The more parsimonious explanation may be recent anthropogenic dispersal superimposed upon evolutionary isolation of phylogroups. Further studies are necessary to distinguish these possibilities.

Acknowledgments Special thanks to D. Bonnet, J. Colbourne, B. Conner, E.G. Durbin, R. Dooh, M. Galbraith, E. Gorokhova, I. Grigorovich, D. Hardie, C. Hyatt, S. Jonasdottir, T. Klevjer, S. Leandro, M.G. Mazzochi, L. Omer, M. Pfrender, I. Siokou, J. Stzlecki, D.J. Taylor, G. Vlad, S. Vernooij, and C.K. Wong for providing samples. This work was supported by a HHMI Capstone Scholarship to AD, and Natural Sciences and Engineering Research Council grants to MEAC and PDNH.

References

Akaike H (1974) A new look at the statistical model identification. IEEE Trans Automat Contr 19:716–723. doi:10.1109/TAC.1974.1100705

Andersen NM, Cheng L, Damgaard J, Sperling FAH (2000) Mitochondrial DNA sequence variation and phylogeography of oceanic insects (Hemiptera: Gerridae: *Halobates* spp.). Mar Biol 136: 421–430. doi:10.1007/s002270050701

Avise JC (2004) Molecular markers, natural history and evolution, 2nd edn edn. Sinauer Associates, Sunderland

Bailey SA, Duggan IC, van Overdijk CDA, Jenkins P, MacIsaac HJ (2003) Viability of invertebrate diapason eggs collected from residual ballast sediment. Limnol Oceanogr 48:1701–1710

Bollens SM, Cordell JR, Avent S, Hooff R (2002) Zooplankton invasions: a brief review, plus two case studies from the Pacific Ocean. Hydrobiologia 480:87–110. doi:10.1023/A:1021233018533

Bryan BB, Grant GC (1974) The occurrence of Podon intermedius (Cladocera, Crustacea) in Chesapeake Bay, a new distributional record. Chesap Sci 15:120–121. doi:10.2307/1351274

Bucklin A, Frost BW, Bradford-Grieve J, Allen LD, Copley NJ (2003) Molecular systematic and phylogenetic assessment of 34 calanoid copepod species of the Calanidae and Clausocalanidae. Mar Biol 142:333–343

Cattley JG, Harding JP (1949) *Penilia*, a cladoceran normally found off tropical and sub-tropical coasts, recorded in North Sea plankton. Nature 164:238–239. doi:10.1038/164238a0

Caudill CC, Bucklin A (2004) Molecular phylogeography and evolutionary history of the estuarine copepod, *Acartia tonsa*, on the Northwest Atlantic coast. Hydrobiologia 511:91–102. doi:10.1023/B:HYDR.0000014032.05680.9d

Colton JB Jr (1985) Eastward extension of the distribution of the marine cladoceran Penilia avirostris in the Northwest Atlantic: a case of ecesis? J Northwest Atl Fish Sci 6:141–148

Cristescu MEA, Hebert PDN (2002) Phylogeny and adaptive radiation in the Onychopoda (Crustacea, Cladocera): evidence from multiple gene sequences. J Evol Biol 15:838–849. doi:10.1046/j.1420-9101.2002.00466.x

Cronin TM, Dowsett HJ (1996) Biotic and oceanographic response to the Pliocene closing of the Central American Isthmus. In: Jackson JBC, Budd AF, Coates AG (eds) Evolution and environment in tropical America. University of Chicago Press, Chicago, pp 76–104

Darling KF, Wade CM, Stewart IA, Kroon D, Dingle R, Leigh Brown AJ (2000) Molecular evidence for genetic mixing of Arctic and Antarctic subpolar populations of planktonic foraminifers. Nature 405:43–47. doi:10.1038/35011002

Da Rocha CEF (1985) The occurrence of *Pleopis schmackeri* (Poppe) in the southern Atlantic and other marine cladocerans on the Brazilian coast. Crustaceana 49:202–204

De Meester L, Gomez A, Okamura B, Schwenk K (2002) The monopolization hypothesis and the dispersal-gene flow paradox in aquatic organisms. Acta Oecologia 23:121–135. doi:10.1016/S1146-609X(02) 01145-1

De Vargas C, Norris R, Zaninetti L, Gibb SW, Pawlowski J (1999) Molecular evidence of cryptic speciation in planktonic foraminifers and their relation to oceanic provinces. Proc Natl Acad Sci USA 96:2864–2868. doi:10.1073/pnas.96.6.2864

Dumont HJ (1998) The Caspian cradle. In: Dumont HJF (ed) The predatory Cladocera (Onychopoda: Podonidae, Polyphemidae, Cercopagidae) and Leptodoridae of the world. Guides to the identification of the microinvertebrates of the continental waters of the world, Leiden, pp 9–15

Dumont HJ (2000) Endemism in the Ponto-Caspian fauna, with special emphasis on the Onychopoda (Crustacea). In: Rossiter A, Kawanabe H (eds) Advances in ecological research. ancient lakes: biodiversity ecology and evolution. Academic Press, London, pp 181–196

Egborge ABM (1987) Salinity and distribution of Cladocera in Warri River, Nigeria. Hydrobiologia 145:159–167. doi:10.1007/BF02530276

Egloff DA, Fofanoff PW, Onbe T (1997) Reproductive biology of marine cladocerans. Adv Mar Biol 31:79–167

Elkin CJ, Richardson PM, Fourcade HM, Hammon NM, Pollard MJ, Predki PF et al (2001) High-throughput plasmid purification for capillary sequencing. Genome Res 11:1269–1274. doi:10.1101/gr.167801

Excoffier LG, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform 1:47–50

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

Goetze E (2003) Cryptic speciation on the high seas: global phylogenetics of the copepod family Eucalanidae. Proc R Soc Lond B 270:2321–2331. doi:10.1098/rspb.2003.2505

Goetze E (2005) Global population genetic structure and biogeography of the oceanic copepods Eucalanus *hyalinus* and *E. spinifer*. Evolution 59:2378–2398

Gollasch S, Rosenthal H, Botnen H, Hamer J, Laing I, Leppakoski E et al (2000) Fluctuations of zooplankton taxa in ballast water during short-term and long-term ocean-going voyages. Int Rev Hydrobiol 85:597–608. doi:10.1002/1522-2632(200011) 85:5/6<597::AID-IROH597>3.0.CO;2-4

Harris SA (2005) Thermal history of Arctic Ocean environs adjacent to North America during the last 3.5 million Ma and a possible mechanism for the cause of the cold events. Progr Phys Oceanogr 29: 218–237. doi:10.1191/0309133305pp444ra

Hebert PDN, Cristescu MEA (2002) Genetic perspectives on invasions: the case of the Cladocera. Can J Fish Aquat Sci 59:1229–1234. doi:10.1139/f02-091

Hebert PDN, Witt JDS, Adamowicz S.J. (2003) Phylogeographical patterning in *Daphnia ambigua*: regional divergence and intercontinental cohesion. Limnol Oceanogr 48:261–268

Knowlton N, Weight LA (1998) New dates and new rates for divergence across the Isthmus of Panama. Proc R Soc Lond B 265:2257–2263. doi:10.1098/rspb.1998.0568

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163. doi:10.1093/bib/5.2.150

Lee CE (2000) Global phylogeography of a cryptic copepod species complex and reproductive isolation between genetically proximate "populations". Evolution 54:2014–2027. doi:10.1111/j.0014-3820.2000.tb01245.x

Lochhead JH (1954) On the distribution of a marine cladocera, *Penilia avirostris* Dana (Crustacea, Branchiopoda), with a note on its reported bioluminescence. Biol Bull Woods Hole 107: 92–105. doi:10.2307/1538633

Luttikhuizen PC, Drent J, Baker AJ (2003) Disjunct distribution of highly diverged mitochondrial lineage clade and population subdivision in a marine bivalve with pelagic larval dispersal. Mol Ecol 12:2215–2229. doi:10.1046/j.1365-294X.2003.01872.x

Nylander JAA (2004) MrModeltest v2. Evolutionary Biology Centre, Uppsala University

Marincovich L, Gladenkov AY (1999) Evidence for an early opening of the Bering Strait. Nature 397:149–151. doi:10.1038/16446

Montresor M, Lovejoy C, Orsini L, Procaccini G, Roy S (2003) Bipolar distribution of the cyst-forming dinoflagellate *Polarella glacialis*. Polar Biol 26:186–194

Panov VE, Krylov PI, Riccardi N (2004) Role of diapause in dispersal and invasion success by aquatic invertebrates. J Limnol 63:56–59

Peijnenburg KTCA, Breeuwer JAJ, Pierrot-Bults AC, Menken SBJ (2004) Phylogeography of the planktonic chaetognath *Sagitta setosa* reveals isolation in European seas. Evolution 58:1472–1487

Poore RZ, Phillips RL, Rieck HJ (1993) Paleoclimate record for Northwind Ridge, Western Arctic Ocean. Paleoceanography 8:149–159. doi:10.1029/93PA00146

Posada D, Crandall KA (2001) Selecting the best-fit model of nucleotide substitution. Syst Biol 50:580–601. doi:10.1080/106351501750435121

Rivier IK (1998) The predatory Cladocera (Onychopoda: Podonidae, Polyphemidae, Cercopagidae) and Leptodorida of the world. In: Dumont HJF (ed) Guides to the identification of the microinvertebrates of the continental waters of the world. Backhuys Publishers, Leiden

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574. doi:10.1093/bioinformatics/btg180

Roy MS, Sponer R (2002) Evidence of a human-mediated invasion of the tropical western Atlantic by the 'world's most common brittlestar'. Proc R Soc Lond B 269:1017–1023. doi:10.1098/rspb.2002.1977

Ruiz GM, Fofonoff PW, Carlton JT, Wonham MJ, Hines AH (2000) Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. Annu Rev Ecol Syst 31:481–531. doi:10.1146/annurev.ecolsys.31.1.481

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. doi:10.1023/A:1009939901198

Schwenk K, Posada D, Hebert PDN (2000) Molecular systematics of European Hyalodaphnia: the role of contemporary hybridization

in ancient species. Proc R Soc Lond B 267:1833–1842. doi:10.1098/rspb.2000.1218

Swofford DL (1998) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512–526

Taniguchi M, Kanehisa T, Sawabe T, Christen R, Ikeda T (2004) Molecular phylogeny of *Neocalanus* copepods in the subarctic Pacific Ocean with notes on non-geographical genetic variations for *Neocalanus cristatus*. J Plankton Res 26:1249–1255. doi:10.1093/plankt/fbh115

Tang KW, Chen QC, Wong CK (1995) Distribution and biology of marine cladocerans in the coastal waters of southern China. Dev Hydrobiol 107:99–107. doi:10.1007/BF00032001

Tavaré S (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. In: Miura RM (ed) Some mathematical questions in biology—DNA sequence analysis. Am Math Soc, Providence, RI, pp. 57–86

Taylor DJ, Finston TL, Hebert PDN (1998) Biogeography of a widespread freshwater crustacean: pseudocongruence and cryptic endemism in the North American *Daphnia laevis* complex. Evolution 52:1648–1670. doi:10.2307/2411338

Waddell PJ, Steel MA (1997) General time-reversible distances with unequal rates across sites: mixing Γ and inverse Gaussian distributions with invariant sites. Mol Phylogenet Evol 8:398–414. doi:10.1006/mpev.1997.0452