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# Genetic assessment of species boundaries in the North American *Daphnia longispina* complex (Crustacea: Daphniidae)

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Species boundaries in the North American *Daphnia longispina* group have proved difficult to establish on the basis of morphology alone. This confusion may be due to hydridization, phenotypic plasticity or the existence of sibling species. We therefore used genetic analysis to delineate species boundaries by examining 27 North American populations belonging to the *longispina* complex for variation at 15–26 allozyme loci. The populations consisted of *Daphnia thorata* from two western sites and two eastern sites, *Daphnia galeata mendotae* from its type location and seven sites across its range, and *Daphnia rosea* from eight temperate and seven arctic sites. Two populations from the Eurasian *longispina* complex were also included for reference. Populations assigned to *D. galeata mendotae* formed a genetically cohesive group, whereas a genetic dichotomy was found between temperate and arctic *D. rosea*, suggesting that this taxon includes two species. Genetic analysis also confirmed the distinctness of western *D. thorata* from other members of the *longispina* group. Unexpectedly, eastern populations resembling *D. thorata* were genetically more similar to temperate *D. rosea* than to any helmeted species (*D. galeata, Daphnia hyalina* or *D. thorata*). Our results suggest that the helmet character is a poor indicator of phylogenetic relationships, as the genetic ability to produce this feature has been lost or acquired several times in the evolution of the *longispina* group.

ADDITIONAL KEY WORDS:—Allozymes – genetic relationships – species boundaries.

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## INTRODUCTION

The systematics of the *Daphnia longispina* O. F. Müller, 1785, group is poorly known in North America as detailed studies of the group ceased after the



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extensive monograph of Brooks (1957). The greatest existing problems in the group concern the species boundaries of *Daphnia galeata mendotae* Birge, 1918, *Daphnia rosea* Sars, 1862, and *Daphnia thorata* Forbes, 1893. Typical populations of these species are rare and individuals showing combinations of Brooks' morphological characters often occur (e.g. Ueno, 1971; Brandlova, Brandl & Fernando, 1972; Anderson & Green, 1976; Edmondson & Litt, 1981; Taylor & Hebert, 1992). As a result, few populations can be keyed unambiguously to the species level.

Unfortunately the geographic distributions of these species are also more similar than Brooks (1957) proposed and the ranges overlap for much of the continent. *Daphnia galeata mendotae* is a putative geographic subspecies of the Eurasian *Daphnia galeata galeata* Sars, 1864, and is distributed from Peru to southern Alaska in the west and through to Newfoundland in the east (Brooks, 1957; Patalas, 1964; Glagolev, 1986). Similarly, *D. rosea* ranges from central Alaska to Newfoundland in North America, with its northern limit roughly coinciding with the treeline and its southern limit reaching into Oklahoma (Brooks, 1957; Carter *et al.*, 1980; Hebert unpublished). The sole record of *D. rosea* beyond this range in North America is that from Nettilling Lake on Baffin Island (Reed, 1963). The only endemic member of the North American *longispina* complex is the rare *D. thorata,* which was thought by Brooks (1957) to be restricted to deep lakes in temperate western North America. More recently *D. thorata* has been reported from eastern North America (Carter *et al.*, 1980; Hrbáček, 1987).

As with other cladoceran species problems (cf. Dodson, 1981), three explanations have been posited for the confusion in the North American *longispina.* First, the morphological variation could reflect the presence of a few highly polymorphic species. The strongest proponent of this view is Glagolev (1986) who, citing morphological overlap, synonymized both *D. galeata mendotae* and *D. thorata* with *D. galeata galeata.* Second, the variation could represent several relatively monomorphic but currently unrecognized species. Brandlova *et al.* (1972), for example, suggested that *D. galeata mendotae* was too variable to constitute a single species. Finally, hybridization and introgression of species might blur taxon boundaries (Brooks, 1957; Ueno, 1971).

Clearly the species boundaries problems of the *longispina* group are intractable to morphological analysis alone—genetic analysis of this variation is warranted. Allozyme studies of the *longispina* group in Europe have resolved some longstanding taxonomic problems. Wolf & Mort (1986) and Hebert, Schwartz & Hrbáček (1989) have shown that interspecific hybridization and limited introgression are significant sources of morphological variation in the European complex. In addition, Hobaek & Wolf (1991) have used allozymes to establish the presence of reproductive isolation among three ecologically differentiated groups of the Norwegian *longispina* complex. Allozyme investigations of a systematical nature in the North American *longispina* complex have thus far been limited to a survey of populations from temperate eastern North America. Here, Taylor & Hebert (1992) provided evidence that *Daphnia*, previously identified as *D. galeata mendotae,* actually represented two species and their interspecific hybrids. Taylor & Hebert (in press) then provided strong evidence of widespread nuclear gene flow (unaccompanied by mtDNA introgression) between *D. galeata mendotae* and *D. rosea.*

In this paper we use allozyme analysis to further address species boundary problems in the North American *longispina* group. The conclusions are based on the survey of populations of the two dominant taxa (*D. rosea* and *D. galeata mendotae*) in North America from sites across their distributions, as well as the analysis of both *D. thorata* and related European species. The analyses establish the presence of an undescribed member of the *longispina* group in the eastern Arctic. The results also establish a close genetic relationship between *D. thorata* and *D. rosea*, and suggest polyphyly of helmet formation in the *longispina* group.

## MATERIALS AND METHODS

### *Sampling*

Table 1 contains the sites, species and sampling dates for the specimens analysed. The samples included *D. galeata mendotae*, *D. rosea* and *D. thorata* as well as typical *Daphnia hyalina* Leydig, and *D. longispina* from Europe. Attempts were made to include geographically distant populations for each North American species. Two populations of *Daphnia longiremis* Sars, 1862, were also examined from the northern and southern limits of its range. Samples in arctic Canada were collected by oblique tows from a float-equipped Bell 206 helicopter taxiing slowly across the lake. Temperate populations were sampled either from shore

TABLE 1. Species, sites and sampling dates of *Daphnia* species used for electrophoresis.

| Species | Site | Sample Date |
|---|---|---|
| *D. galeata mendotae* | 1. MN Bello Lake | 7 July 1991 |
| | 2. CA Lake Berryessa | 2 April 1991 |
| | 3. IN Center Lake | 7 May 1990 |
| | 4. CO Lake Granby | 14 August 1991 |
| | 5. WI Lake Mendota | 5 July 1991 |
| | 6. VT Lake Morey | 9 July 1990 |
| | 7. ON Lake St. George | 25 June 1990 |
| | 8. AB Sundance Lake | 13 June 1991 |
| *D. hyalina* | 1. Austria Mondsee | November 1991 |
| *D. longispina* | 1. Slovakia Vysne Furkotske | October 1990 |
| *D. rosea* | 1. IN Bear Lake | 26 September 1989 |
| | 2. BC pond, Canal Flat | 15 June 1991 |
| | 3. ON Miller Lake | 14 September 1990 |
| | 4. ON pond, Guelph | 11 September 1990 |
| | 5. VT Lake Mitchell | 9 July 1990 |
| | 6. NY Round Pond | 20 April 1991 |
| | 7. ON Sunfish Lake | 22 December 1990 |
| | 8. BC pond, Wasa | 15 June 1991 |
| | 9. NWT pond, Baffin Island | 14 August 1990 |
| | 10. NWT pond, Ormonde Island | 11 August 1990 |
| | 11. NWT pond, Ormonde Island | 11 August 1990 |
| | 12. NWT pond, Melville Peninsula | 4 August 1990 |
| | 13. NWT pond, Melville Peninsula | 4 August 1990 |
| | 14. NWT Airiluq Lake | 20 August 1991 |
| | 15. NWT Lake, Melville Peninsula | 20 August 1990 |
| *D. thorata* | 1. MT Flathead Lake | 16 June 1991 |
| | 2. MI Lawrence Lake | 4 July 1991 |
| | 3. IN Pretty Lake | 4 October 1990 |
| | 4. WA Lake Washington | 6 October 1992 |
| *D. longiremis* | 1. IN Crooked Lake | 25 June 1990 |
| | 2. NWT Lake, Baffin Island | 14 August 1990 |

for ponds or from boats for lakes. North American taxa were identified according to Brooks (1957), with the following exceptions: Bear Lake specimens were designated as *D. rosea* after Taylor & Hebert (1992) and *D. galeata* × *D. rosea* hybrids were identified according to Taylor & Hebert (1992) and eliminated from the analysis. Arctic *D. rosea* in the present study often possessed cuticular pigmentation and coexisted with *Daphnia middendorffiana* Fischer, 1851. Brooks (1957) designated such specimens as *D. rosea* with introgression of *D. middendorffiana* genes. The Lawrence Lake MI specimens have been identified as *D. galeata mendotae* (Liebold, 1990), but this population exhibits neither the pointed-angulated helmets, which are typical of *D. galeata mendotae* in this region (Taylor & Hebert, 1992), nor the small rounded helmets and convex anterior heads which are typical of *D. rosea*. We have designated this population as eastern *D. thorata,* because these specimens often possessed the dorsal head concavity that is typical of *D. thorata* (Brooks, 1957) and because the Lawrence Lake specimens cannot be distinguished morphologically from the nearby Pretty Lake IN *D. thorata,* which were identified by Hrbáček (1987).

### *Allozyme electrophoresis*

In all, 26 putative loci were scored in 31 populations. When a locus was scored the minimum sample size was 20 individuals for each of the 29 populations, but the sample size ranged from 11 to 16 per locus for L. Washington *D. thorata,* and from 13 to 43 per locus for *D. hyalina*. For 31 populations the following 15 loci were scored: aldehyde oxidase (AO, 1.2.3.1), aspartate aminotransferase (sAAT, mAAT; 2.6.1.1), dipeptidase (PEP-A, PEP-A2; 3.4.13.11), fumarate hydratase (FUMH, 4.2.1.2), glucose-6-phosphate isomerase (GPI, 5.3.1.9), lactate dehydrogenase (LDH, 1.1.1.27), isocitrate dehydrogenase (IDH-1, 1.1.1.42), malate dehydrogenase (MDH-1, MDH-2; 1.1.1.37), malate dehydrogenase NADP+ (ME-2, 1.1.1.40), phosphoglucomutase (PGM-1, PGM-2; 5.4.2.2), and proline dipeptidase (PEP-D, 3.4.13.9). An additional 11 loci were scored for one or more populations of each *longispina* group taxon. These loci were: aconitase hydratase (ACOH-1, ACOH-2; 4.2.1.3), arginine phosphokinase (APK, 2.7.3.3), esterase (EST-1, EST-2; 3.1.1.-), glyceraldehyde-3-phosphate dehydrogenase (GAPDH, 1.2.1.12), isocitrate dehydrogenase (IDH-2), malate dehydrogenase NADP+ (ME-1), mannose-6-phosphate isomerase (MPI, 5.3.1.8), and phosphogluconate dehydrogenase (PGDH-1, PGDH-2; 1.1.1.44). All populations of *D. thorata* were scored for MPI.

Cellulose acetate electrophoresis was conducted according to Hebert & Beaton (1989), with the following exceptions: fast blue BB salt was used instead of fast red TR salt for EST staining; Tris-citrate III buffer (adjusted to pH 7.4) of Murphy *et al.* (1990) was used for EST and PGDH. Substrates used for PEP-D and PEP-A allozymes were phenylalanyl-proline and leucylglycine respectively. Alleles were given ascending letter designations, with the slowest anodal mobility designated 'a'.

### *Statistical treatment*

Hillis' (1984) modified genetic distance (D*) was used to summarise allozyme variation for UPGMA cluster analysis, as among-group differences in

polymorphism occur in the North American *longispina* (Taylor & Hebert, 1992). This distance measure is less distorted by among-group differences in polymorphism than other common measures (Hillis, 1984). The significance of among-group distances was tested by the NEIC program (Lessios, 1990), which uses the jackknife-based methods of Mueller & Ayala (1982). The data were also exposed to Distance-Wagner analyses, which do not possess the UPGMA assumption of equal divergence rates among taxa. For Distance-Wagner analysis, a metric distance measure was required, and again our selection, the chord distance of Cavalli-Sforza & Edwards, was based on its low sensitivity to among-group differences in polymorphism (see Swofford & Olsen, 1990). BIOSYS-1 was used for the Distance-Wagner analysis (Swofford & Selander, 1981).

## RESULTS

Sixteen of the 26 loci examined were variable among populations of the *longispina* complex. Table 2 summarizes the among-taxon variation at these variable loci as well as at two additional loci that differed only in *D. longiremis*. Both *D. rosea* and *D. thorata* showed distinct geographic dichotomies. The Arctic *D. rosea* possessed at least nine fixed differences (*mAat, sAat, Est-1, Est-2, Gpi, Me-2, Pep-A2, Pep-D,* and *Pgm-1*) from temperate *D. rosea,* whereas the western *D. thorata* was separated from the eastern *D. thorata* by three fixed differences (*Ao, Mdh-1, Mpi*). In contrast, neither *D. galeata mendotae* nor *D. longiremis* showed allelic substitutions among populations that were geographically widespread. The arctic *D. rosea* and western *D. thorata* were also allozymically distinct from other taxa in the *longispina* complex. Arctic *D. rosea* possessed at least six fixed differences from any other taxon and western *D. thorata* showed at least two differences from other taxa. Surprisingly, there were no fixed allozyme differences between temperate *D. rosea* and eastern *D. thorata.* At least three fixed differences (*Est-1, Est-2, Pgm-1*) existed between temperate *D. rosea* and *D. galeata mendotae.* Allele frequency variation also increased the genetic divergence between *D. rosea* and *D. galeata mendotae* because each species had loci with uncommon alleles that were fixed in the opposite species (e.g. *Ao* in *D. rosea* and *Pep-A, Pep-D* and *mAat* in *D. galeata mendotae).*

### *Hierarchical analyses*

Among-population differences in allozyme variation are summarized in Figures 1–4. For 15 loci, UPGMA phenograms and Distance-Wagner trees yielded similar topologies for the North American *Daphnia longispina* complex. Four major clusters were apparent: *Daphnia galeata,* temperate *D. rosea,* arctic *D. rosea* and western *D. thorata.* In each case, the arctic *D. rosea* clustered outside the other populations of the *longispina* group. Statistical analysis using jackknifing revealed that the three major clusters (*D. galeata mendotae,* arctic *D. rosea* and temperate *D. rosea*) were significantly different (Table 3). For each comparison, the difference between the mean intergroup genetic distance and the mean intragroup distance (*U*) was significantly greater than zero. The western populations of *D. thorata* consistently clustered closest to, but outside, the temperate *D. rosea,* whereas the eastern *D. thorata* populations clustered within

TABLE 2. Mean population allele frequencies at 18 loci for species of the *Daphnia longispina* group. See text for the number of populations of each species for a locus. 'n.a.' indicates that no allozyme data is available for a locus.

| Locus | Allele | *D. galeata* | *D. hyalina* | *D. longi-spina* | *D. rosea* temp. | *D. thorata* western | *D. thorata* eastern | *D. rosea* arctic | *D. longiremis* |
|---|---|---|---|---|---|---|---|---|---|
| mAAT | *(n)* | 390 | 21 | 22 | 448 | 53 | 65 | 242 | 126 |
| | *a* | 0.011 | 0.000 | 0.000 | 1.000 | 1.000 | 1.000 | 0.000 | 0.000 |
| | *b* | 0.973 | 1.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| | *c* | 0.016 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *d* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| sAAT | *(n)* | 527 | 21 | 22 | 504 | 54 | 72 | 242 | 126 |
| | *a* | 0.595 | 1.000 | 0.000 | 1.000 | 1.000 | 1.000 | 0.000 | 0.000 |
| | *b* | 0.405 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 | 1.000 | 1.000 |
| AO | *(n)* | 561 | 21 | 22 | 502 | 66 | 72 | 259 | 126 |
| | *a* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| | *b* | 0.000 | 0.000 | 0.000 | 0.022 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *c* | 0.000 | 0.000 | 0.000 | 0.788 | 0.000 | 0.921 | 0.000 | 0.000 |
| | *d* | 0.000 | 1.000 | 1.000 | 0.081 | 1.000 | 0.000 | 0.000 | 0.000 |
| | *e* | 1.000 | 0.000 | 0.000 | 0.109 | 0.000 | 0.079 | 1.000 | 0.000 |
| EST-1 | *(n)* | 105 | 24 | 25 | 99 | 66 | 45 | 145 | n.a. |
| | *a* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | |
| | *b* | 0.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.000 | |
| | *c* | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| EST-2 | *(n)* | 105 | 24 | 25 | 99 | 66 | 45 | 145 | n.a. |
| | *a* | 0.000 | 0.000 | 0.000 | 0.091 | 0.040 | 0.000 | 0.000 | |
| | *b* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | |
| | *c* | 0.000 | 1.000 | 1.000 | 0.909 | 0.960 | 1.000 | 0.000 | |
| | *d* | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | |
| FUMH | *(n)* | 447 | 21 | 22 | 278 | 55 | 44 | 217 | 66 |
| | *a* | 0.022 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *b* | 0.978 | 1.000 | 1.000 | 0.991 | 1.000 | 1.000 | 1.000 | 1.000 |
| | *c* | 0.000 | 0.000 | 0.000 | 0.009 | 0.000 | 0.000 | 0.000 | 0.000 |
| GPI | *(n)* | 646 | 21 | 22 | 519 | 42 | 50 | 237 | 66 |
| | *a* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| | *b* | 0.000 | 0.000 | 0.159 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *c* | 0.000 | 0.000 | 0.000 | 0.002 | 0.012 | 0.023 | 0.000 | 0.000 |
| | *d* | 0.074 | 1.000 | 0.841 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| | *e* | 0.869 | 0.000 | 0.000 | 0.998 | 0.988 | 0.977 | 0.000 | 0.000 |
| | *f* | 0.057 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| LDH | *(n)* | 503 | 21 | 22 | 499 | 55 | 72 | 237 | 66 |
| | *a* | 0.000 | 0.000 | 0.000 | 0.028 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *b* | 1.000 | 1.000 | 1.000 | 0.972 | 1.000 | 1.000 | 1.000 | 0.000 |
| | *c* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| IDH-1 | *(n)* | 293 | 21 | 22 | 238 | 66 | 78 | 233 | 66 |
| | *a* | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.000 |
| | *b* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |

the temperate *D. rosea*. Geographic distance among populations was not a good predictor of subclustering. The Vermont population of *Daphnia galeata*, for example, clustered with populations from Alberta and California, while the Vermont *D. rosea* clustered with their conspecifics from British Columbia.

When 26 loci were used for the analysis, UPGMA and Distance-Wagner methods produced slightly different tree topologies. UPGMA produced a tree with arctic *D. rosea* clustering outside the other *longispina* taxa both from North America and from Europe. Although the Distance-Wagner method also showed the arctic *D. rosea* to be the most genetically distinct taxon, the tree placed *D.*

TABLE 2.—*continued*

| Locus | Allele | *D. galeata* | *D. hyalina* | *D. longi-spina* | *D. rosea* temp. | *D. thorata* western | *D. thorata* eastern | *D. rosea* arctic | *D. longiremis* |
|---|---|---|---|---|---|---|---|---|---|
| ME-2 | *(n)* | 274 | 21 | 22 | 279 | 66 | 78 | 211 | 60 |
| | *a* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| | *b* | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.000 | 1.000 |
| MDH-1 | *(n)* | 280 | 21 | 22 | 268 | 66 | 75 | 215 | 118 |
| | *a* | 1.000 | 1.000 | 1.000 | 1.000 | 0.000 | 1.000 | 1.000 | 1.000 |
| | *b* | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 |
| MDH-2 | *(n)* | 300 | 21 | 22 | 280 | 55 | 78 | 215 | 120 |
| | *a* | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.000 |
| | *b* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| MPI | *(n)* | 110 | 24 | 25 | 111 | 66 | 78 | 198 | 60 |
| | *a* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| | *b* | 1.000 | 1.000 | 1.000 | 1.000 | 0.000 | 1.000 | 1.000 | 0.000 |
| | *c* | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 | 0.000 | 0.000 |
| PEP-A | *(n)* | 573 | 21 | 20 | 478 | 55 | 72 | 205 | 66 |
| | *a* | 0.000 | 0.000 | 0.375 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *b* | 0.132 | 1.000 | 0.675 | 1.000 | 1.000 | 0.989 | 1.000 | 0.000 |
| | *c* | 0.013 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *d* | 0.881 | 0.000 | 0.000 | 0.000 | 0.000 | 0.011 | 0.000 | 0.000 |
| | *e* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| PEP-A2 | *(n)* | 224 | 16 | 25 | 297 | 33 | 44 | 207 | 66 |
| | *a* | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.000 | 0.000 |
| | *b* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| | *c* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| PEP-D | *(n)* | 613 | 21 | 22 | 493 | 54 | 72 | 223 | 60 |
| | *a* | 0.735 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *b* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *c* | 0.265 | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 | 0.000 | 0.000 |
| | *d* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| | *e* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| PGM-1 | *(n)* | 224 | 13 | 25 | 316 | 55 | 55 | 242 | 100 |
| | *a* | 0.000 | 0.192 | 0.000 | 1.000 | 1.000 | 1.000 | 0.000 | 0.000 |
| | *b* | 1.000 | 0.808 | 1.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *c* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 | 0.000 |
| | *d* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| PGM-2 | *(n)* | 564 | 43 | 48 | 358 | 59 | 72 | 242 | 125 |
| | *a* | 0.006 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *b* | 0.006 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *c* | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 1.000 |
| | *d* | 0.434 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *e* | 0.000 | 0.000 | 0.000 | 0.069 | 0.000 | 0.000 | 0.000 | 0.000 |
| | *f* | 0.554 | 0.930 | 0.990 | 0.528 | 1.000 | 0.927 | 1.000 | 0.000 |
| | *g* | 0.000 | 0.070 | 0.010 | 0.373 | 0.000 | 0.073 | 0.000 | 0.000 |
| | *h* | 0.000 | 0.000 | 0.000 | 0.030 | 0.000 | 0.000 | 0.000 | 0.000 |

TABLE 3. Among- and between-group comparisons for *Daphnia galeata* and *Daphnia rosea* using Hillis' modified genetic distance for 15 loci. Seven populations of arctic *D. rosea* and eight populations each of temperate *D. rosea* and *D. galeata mendotae* were used. The *U* statistic of Mueller and Ayala (1982) is the difference between the intra- and inter-group distances.

| Groups Compared | Mean Intra-group D | Mean Inter-group D | *U* | Significance Level |
|---|---|---|---|---|
| *galeata* vs. arctic *rosea* | 0.036 | 0.560 | 0.524 | $P < 0.05$ |
| *galeata* vs. temperate *rosea* | 0.044 | 0.416 | 0.372 | $P < 0.01$ |
| *arctic rosea* vs. temperate *rosea* | 0.015 | 0.745 | 0.730 | $P < 0.05$ |

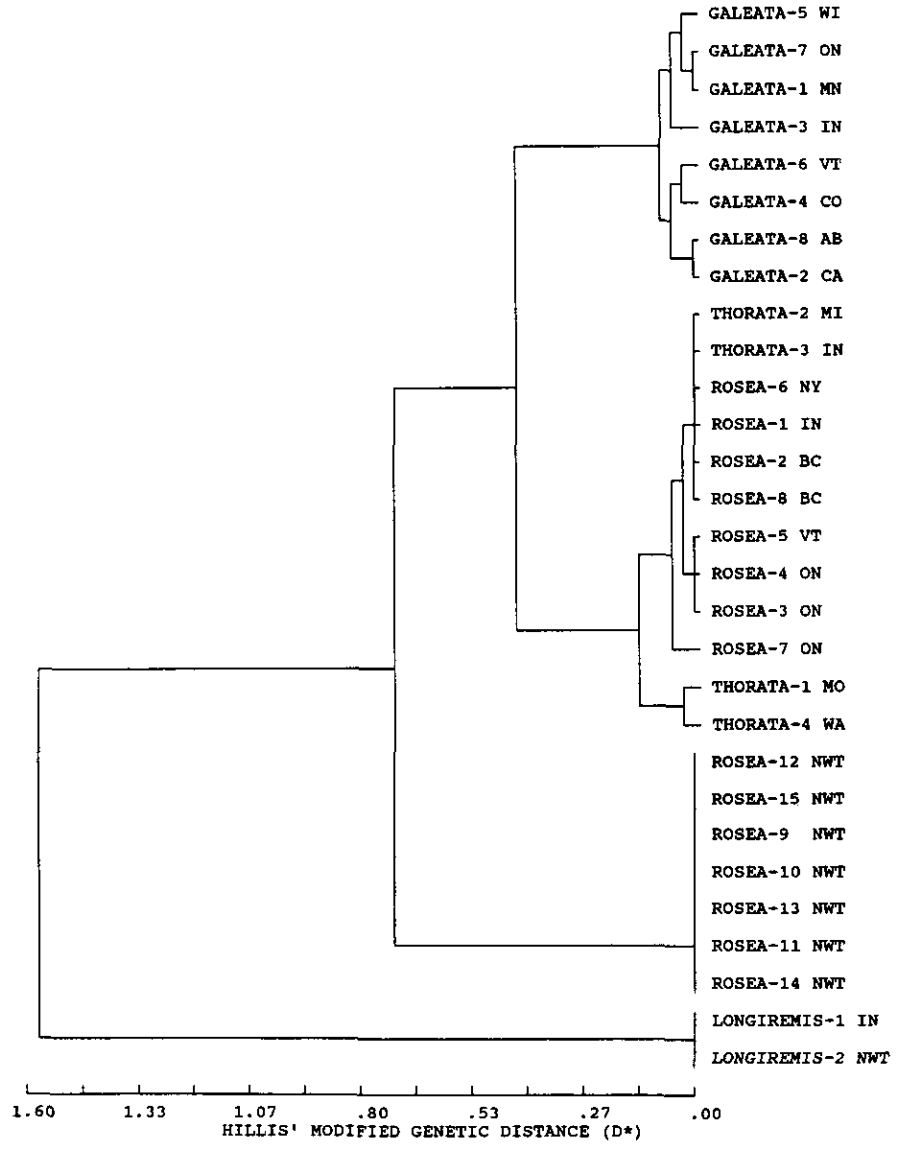


Figure 1. UPGMA phenogram of the North American *Daphnia longispina* group derived from Hillis' modified genetic distance for 15 loci. The species, population number (see Table 1 for key) and state/province abbreviations are given for each population.

*galeata mendotae* as the sister group to arctic *D. rosea*. The remaining *longispina* taxa from North America and Europe formed a second, tighter cluster.

## DISCUSSION

Our allozyme analyses indicate that populations of both *D. galeata mendotae* and *D. longiremis* show little genetic divergence over sites that are several thousand kilometers apart. In contrast, *D. rosea* is separated into two very genetically divergent groups—temperate and arctic. The allozyme data clearly

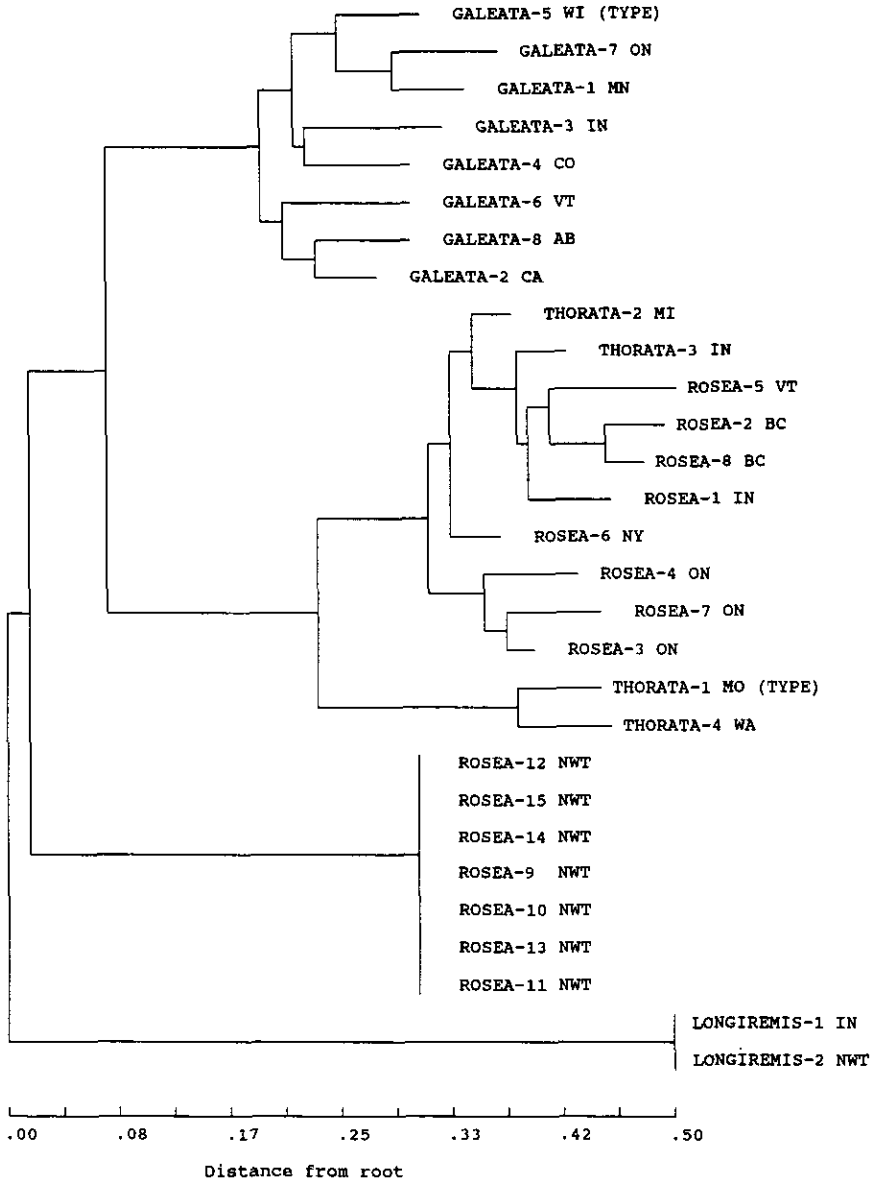


Figure 2. Distance-Wagner tree of 27 populations of the North American *Daphnia longispina* group derived from the chord distance for 15 loci. See Table 1 for species population key. *Daphnia longiremis* was included for outgroup rooting.

indicate the distinctness of arctic *rosea* from previously described species of the North American group. Among arctic populations and their putative conspecifics from the temperate zone, fixed or nearly fixed differences are present at more than 27% of the loci. In addition, the arctic *rosea* is more genetically divergent from each of the five examined species (two of which are from another continent) than is any one of these species from another.

It is unlikely that the divergent arctic *rosea* represent mere disjunct populations of a temperate North American species. First, our results show that

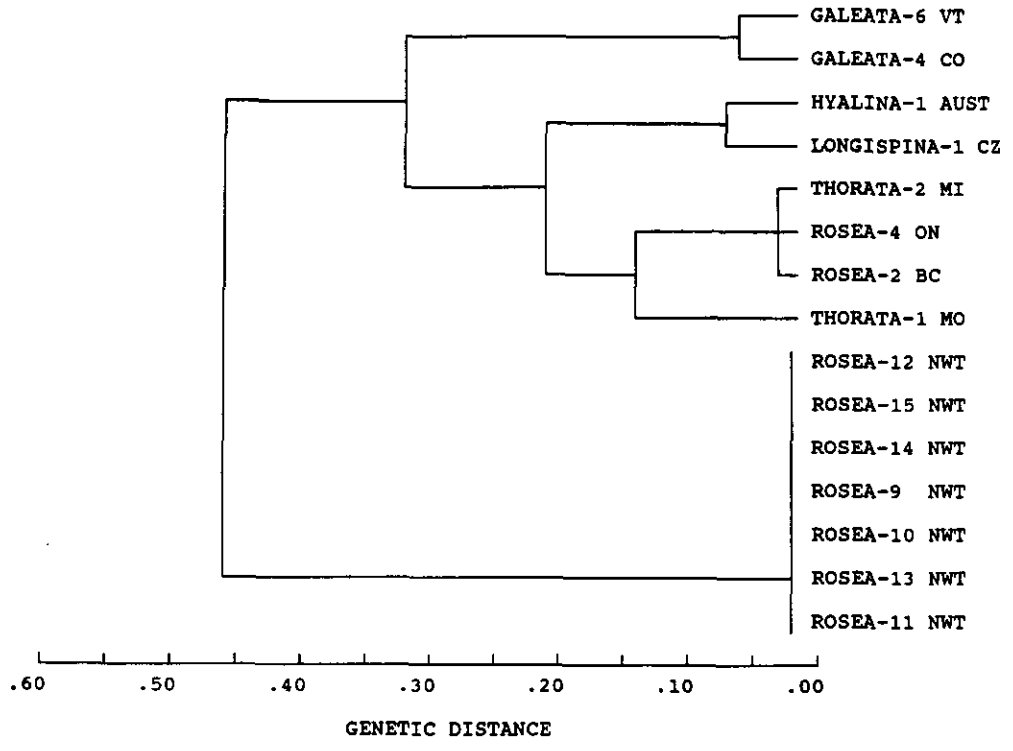


Figure 3. UPGMA phenogram of 15 populations of the *Daphnia longispina* group derived from Hillis' modified genetic distance for 26 allozyme loci. See Table 1 for species population key.

*D. galeata* and temperate *D. rosea* form genetically cohesive groups over greater geographic distances than the distances separating these temperate species from the arctic *rosea*. Second, as the arctic *rosea* habitat has been ice-free for less than 7000 years (Dredge, 1991), there has only been a brief period for divergence from any temperate refuge populations. The genetic homogeneity of temperate and arctic populations of *D. longiremis* found in this study may have resulted from such recent recolonization. The genetic distinctness, then, of the arctic *longspina* clearly suggests that such populations represent a taxon that is new to North America.

The present study shows that this undescribed species, which resembles *D. rosea*, is common on northern Baffin Island and the Melville Peninsula, suggesting that Reed's (1963) central Baffin Island record for *D. rosea* may be the same new species. The distribution of true *D. rosea* in North America seems to be limited to lakes and ponds below the treeline, as in Alaska (Haney & Buchanan, 1987), and in the Rocky Mountains (Patalas, 1964; Anderson, 1971), where its limit coincides with the treeline.

This absence of *rosea*-like animals in alpine and Western Arctic lakes and ponds suggests that the new taxon we found in the eastern High Arctic may have a restricted distribution in North America. If so, then colonization probably occurred either from refugia on Baffin Island or from Greenland, where *longispina*-like animals have also been found (Røen, 1977). East of Greenland, *longispina*-like animals have been found in Iceland, Svalbard and Scandinavia

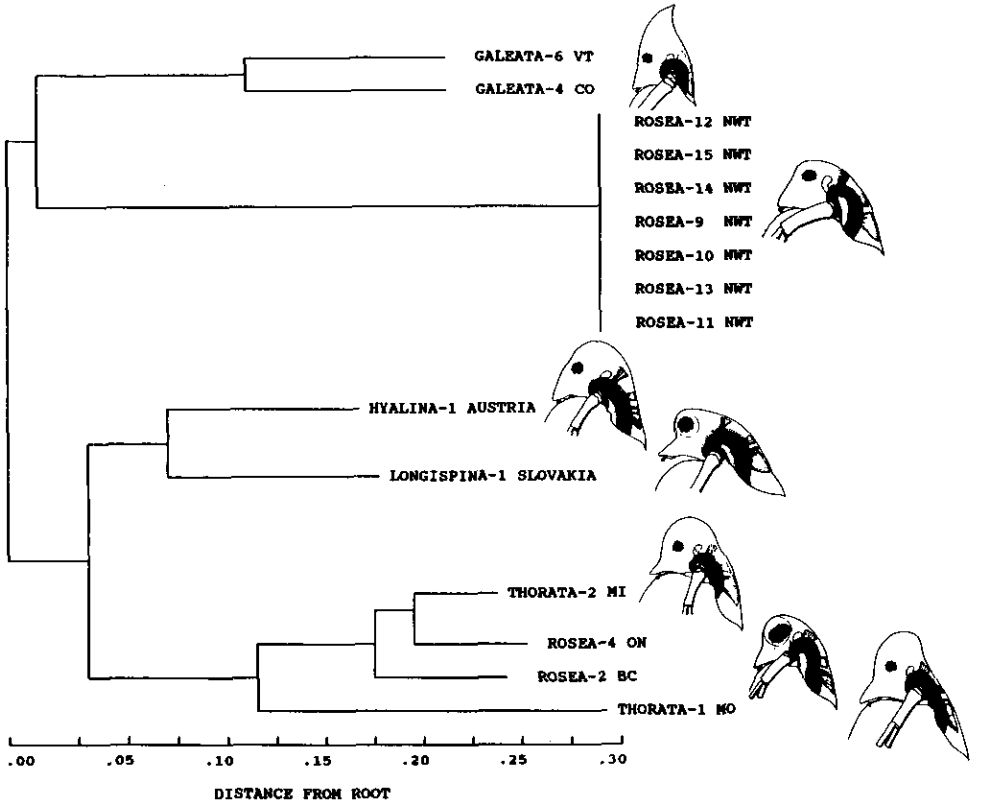


Figure 4. Distance-Wagner tree of 15 populations of the *Daphnia longispina* group derived from the chord distance for 26 loci. See Table 1 for species population key. This tree was midpoint rooted. Head profiles depict maximum forms of mature females.

(Røen, 1977; Hobaek & Wolf, 1991). Direct genetic comparisons are necessary to confirm the affinities of the Canadian arctic *longispina* with the neighbouring *longispina* stocks to the east.

Our results challenge the hypothesis of introgression as an explanation for cuticular pigmentation in the North American *longispina* group. Brooks (1957) described such pigmentation as a diagnostic character of *D. middendorffiana*. As a consequence, he and subsequent authors (e.g. Ueno, 1971) attributed dorsal pigmentation in *D. rosea* and *D. pulex* to introgression of *D. middendorffiana* genes. Hebert & McWalter (1983) provided good evidence that cuticular pigmentation is polyphyletic in the North American *D. pulex* group. In our study, the monomorphic genetic structure of the pigmented *longispina* populations similarly argues against introgression with *D. middendorffiana*. More importantly, at least six fixed or nearly fixed allozyme differences occur between these two species at sites in the eastern Canadian arctic (D. Taylor, unpublished).

The midpoint rooted Distance-Wagner analysis using 26 loci produced the only exceptional topology as *D. galeata mendotae* grouped with the new arctic species. This grouping may be due to a lower sensitivity of the Distance-Wagner method to introgressed *D. rosea* genes in *D. galeata mendotae* (Taylor & Hebert, in press). In any event, our data suggest that the historical dichotomy of the

*longispina* group (see Hrbáček, 1987) into a helmeted (*galeata* or *hyalina*) and an unhelmeted (*longispina*) complex is unfounded. Different clusters contain both helmeted and unhelmeted species, suggesting a polyphyletic origin for helmets (Fig. 4). Given this polyphyly of helmets and the phenotypic plasticity in species capable of expressing this character, we propose that all the species of the *longispina* group be united as the *longispina* complex.

We present two examples where the artificial division of the *longispina* group has been counterproductive. The *longispina* group *Daphnia* in Lawrence Lake has until now been classified as *D. galeata mendotae* because it is capable of producing a helmet (Leibold, 1990). However, our results show that this population is much more closely related to *D. rosea* than to *D. galeata*. Inclusion of helmeted populations of this species in comparative studies with *D. galeata mendotae* may increase the variation in measured variables (e.g. Leibold & Tessier, 1991). Second, Hobaek & Wolf (1991) examined the genetic structure of the *longispina* complex in Norway. In this area, *D. galeata* is restricted to lakes less than 800 m above sea level, whereas pigmented *D. longispina* is restricted to water bodies more than 900 m above sea level. Although recognizing morphological overlap between *D. galeata* and *D. longispina* in this area, the authors chose not to include *D. galeata* in their genetic analysis—presumably because this species is not a member of the *longispina* complex. This is unfortunate because our results suggest that *D. galeata* may be the closest relative to the pigmented arctic-alpine *longispina*.

One of the clusters containing both helmeted and unhelmeted species in our analysis is the *rosea–thorata* cluster. This finding contradicts previous proposals that placed *D. thorata* in synonymy with, or in closest relation to, other helmeted species such as *D. galeata mendotae* and *D. hyalina* (Forbes, 1893; Brooks, 1957; Edwards, 1980; Glagolev, 1986; Hrbáček, 1987). Our results indicate that *D. thorata* from the type locality and Lake Washington are more closely related to *D. rosea* than to either *D. galeata mendotae* or *D. hyalina*. The original evidence linking *D. thorata* with other helmeted species is weak. The evidence, for example, of Forbes (1893) and Brooks (1957) relies mainly on the shaky assumption of helmet monophyly in *longispina*. Edwards (1980) and Glagolev (1986) later provided further evidence for the association based on overlapping juvenile helmet and mandible morphology. Yet both of these studies relied on specimens from Lake Washington, and it is probable that these individuals overlapped in morphology because they were hybrids between *D. galeata* and *D. thorata*. Allozyme and mtDNA analyses have shown that *D. thorata* × *D. galeata* hybrids are present in this lake (D. Taylor, unpublished).

The three or more fixed genetic differences between western *D. thorata* and other sympatric *longispina* taxa and the genetic homogeneity of *D. thorata* from its type location and Lake Washington (which are 675 km apart) support Brooks' (1957) elevation of *D. thorata* to a species. Nevertheless, our results also show that eastern populations resembling *D. thorata* are in fact closely related to *D. rosea*. Brooks (1957) suggested that western *D. thorata* was older than the Pleistocene because its distribution lies outside the area covered by the last glaciation. Carter *et al.* (1980) then proposed that the rarity of *D. thorata* in glacial lakes of eastern North America (where it was detected in less than 1% of 698 lakes) reflected recent colonization of the area from the western refuge. Yet the type *D. thorata* possesses derived characters at *Mdh-1*, *Mpi* and *Ao*, while those populations in

the younger eastern lakes possess the ancestral *longispina* group characters at these loci. Such a pattern suggests that helmeted lake-dwelling forms have arisen from *D. rosea* more than once. The genetic similarity to *D. rosea*, rarity of occurrence, disjunct distribution and differing habitat preference of putative eastern *D. thorata* suggest that *D. thorata sensu stricto* is restricted to western North America.

The present study has provided the first broad overview of genetic relationships among North American members of the *D. longispina* group. The results suggest that taxonomic confusion has arisen in part from the presence of undescribed taxa, although the sole new species detected appears to have a restricted geographic distribution. The important role of interspecific hybrids in complicating taxonomic decisions was minimized in the current study by excluding $F_1$ hybrids from the analyses. Nevertheless, the detection of rare alleles in both *D. rosea* and *D. galeata mendotae*, which appear to owe their origin to introgression, makes it likely that genes controlling morphological traits will show similar leakage across species boundaries. Finally, and perhaps more importantly, our study indicates that the loss or acquisition of helmets has occurred on a number of occasions and that genetic variability in this trait is apparent even in a single taxon.

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## REFERENCES

**Anderson RS. 1971.** Crustacean plankton of 146 alpine and subalpine lakes and ponds in western Canada. *J. Fish. Red. Bd. Canada*, **28:** 311–321.

**Anderson RS, Green RB. 1976.** *Limnological and planktonic studies in the Waterton Lakes, Alberta.* Occasional Paper No. 27. Ottawa: Canadian Wildlife Service.

**Brandlova J, Brandl Z, Fernando CH. 1972.** The Cladocera of Ontario with remarks on some species and distribution. *Canadian Journal of Zoology* **50:** 1373–1403.

**Brooks JL. 1957.** The systematics of North American *Daphnia*. *Memoirs of the Connecticut Academy of Arts and Sciences* **13:** 1–180.

**Carter JCH, Dadswell MJ, Roff JC, Sprules WG. 1980.** Distribution and zoogeography of planktonic crustaceans and dipterans in glaciated eastern North America. *Canadian Journal of Zoology* **58:** 1355–1387.

**Dodson SI. 1981.** Morphological variation of *Daphnia pulex* Leydig (Crustacea: Cladocera) and related species from North America. *Hydrobiologia* **83:** 101–114.

**Dredge LA. 1991.** Raised marine features, radiocarbon dates, and sea level changes, eastern Melville Peninsula, Arctic Canada. *Arctic* **44:** 63–73.

**Edmondson WT, Litt AH. 1982.** *Daphnia* in Lake Washington. *Limnology and Oceanography* **27:** 272–293.

**Edwards C. 1980.** The anatomy of *Daphnia* mandibles. *Transactions of the American Microscopal Society* **99:** 2–24.

**Forbes SA. 1893.** A preliminary report on the aquatic invertebrate fauna of the Yellowstone National Park, Wyoming, and the Flathead region of Montana. *Bulletin of the United States Fisheries Commission* **11:** 207–256.

**Glagolev SM. 1986.** Species composition of *Daphnia* in Lake Glubokoe with notes on the taxonomy and geographical distribution of some species. *Hydrobiologia* **141:** 55–82.

**Haney JF, Buchanan C. 1987.** Distribution and biogeography of *Daphnia* in the Arctic. *Memorie Dell'Istituto Italiano di Idrobiologia* **45:** 77–105.

**Hebert PDN, Beaton MJ. 1989.** *Methodologies for allozyme analysis using cellulose acetate electrophoresis.* Beaumont, Texas: Helena Laboratories.

**Hebert PDN, McWalter DB. 1983.** Cuticular pigmentation in arctic *Daphnia*: adaptive diversification of asexual lineages? *American Naturalist* **122:** 286–291.

**Hebert PDN, Schwartz SS, Hrbáček J. 1989.** Patterns of genotypic diversity in Czechslovakian *Daphnia*. *Heredity* **62:** 207–216.

**Hillis DM. 1984.** Misuse and modification of Nei's genetic distance. *Systematic Zoology* **33:** 238–240.

**Hobaek A, Wolf HG. 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.

**Hrbéček J. 1987.** Systematics and biogeography of *Daphnia* species in the northern temperate region. *Memoire Dell'Instituto Italiano di Idrobiologia* **45:** 37–76.

**Leibold MA. 1990.** Resources and predators can affect the vertical distributions of zooplankton. *Limnology and Oceanography* **35:** 938–944.

**Leibold MA, Tessier AJ. 1991.** Contrasting patterns of body size for *Daphnia* species that segregate by habitat. *Oecologia* **86:** 342–348.

**Lessios HA. 1990.** A program for calculating Nei's genetic distances and their jackknifed confidence intervals. *Journal of Heredity* **81:** 490.

**Mueller LD, Ayala FJ. 1982.** Estimation and interpretation of genetic distance in empirical studies. *Genetical Researches Cambridge* **40:** 127–137.

**Murphy RW, Sites JW, Buth DG, Haufler CH. 1990.** Proteins I: Isozyme electrophoresis. In Hillis DM & Moritz C (eds.) *Molecular Systematics*. Sunderland Massachusetts: Sinauer, 45–126.

**Patalas K. 1964.** The crustacean plankton communities in 52 lakes of different altitudinal zones of northern Colorado. *Verh. Internat. Verein. Limnol.* **15:** 719–726.

**Reed EB.** 1963. Records of freshwater Crustacea from arctic and subarctic Canada. *National Museum of Canada Bulletin* **199:** 29–62.

**Røen U. 1977.** Revision of freshwater entomostraca fauna in the Thule area, Angmassalik area, and Southwest Greenland. *Folia Limnologica Scandinavia* **17:** 107–110.

**Swofford DL, Olsen GJ.** 1990. Phylogeny reconstruction. In Hillis DM & Mortiz C (eds). *Molecular Systematics:* Sunderland Massachusetts: Sinauer, 411–501.

**Swofford DL, Selander RK. 1981.** *Biosys-1*. A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* **72:** 281–283.

**Taylor DJ, Hebert PDN. 1992.** *Daphnia galeata mendotae* as a cryptic species complex with interspecific hybrids. *Limnology and Oceanography* **37:** 658–665.

**Taylor DJ, Hebert PDN. In press.** Habitat dependent hybrid parentage and differential introgression between neighboringly sympatric *Daphnia* species. *Proc. Natl. Acad. Sci. USA*.

**Ueno M. 1971.** Hybrid populations of *Daphnia* from some lakes on Vancouver Island, British Columbia. *Researches on Crustacea* **4 and 5:** 50–60.

**Wolf HG, Mort MA. 1986.** Inter-specific hybridization underlies phenotypic variability in *Daphnia* populations. *Oecologia* **68:** 507–511.