

Cryptic intercontinental hybridization in *Daphnia* (Crustacea): the ghost of introductions past

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SUMMARY

The dearth of known cladoceran introductions is paradoxical, as their parthenogenetic lifestyle and desiccation-resistant resting eggs make them excellent candidates for invaders. Unfortunately, the biogeography and systematics of the group are poorly studied so natural species distributions and the importance of invasions are difficult to assess. In this study we conducted an allozyme analysis comparing populations of the common lacustrine cladoceran *Daphnia galeata* from North America and Europe. Four populations from the lower Laurentian Great Lakes were genetically intermediate between North American and European populations. This geographic pattern of genetic variation suggests that the introduction of European *D. galeata* has gone unnoticed, and that extensive hybridization with native *Daphnia* has resulted. North American and European populations were not only genetically distinct but mated non-randomly under sympatry, supporting a taxonomic split at the species level. Our study suggests that insights into evolutionary and ecological processes and conservation biology may be lost when genetic markers are ignored in the biogeographical study of morphologically conserved groups.

1. INTRODUCTION

Studies of the genetic consequences of biological introductions often provide insight into problems in evolution, ecology and conservation biology. Genetic investigations of plant invasions have revealed, for example, the importance of hybridization and introgression among native and alien taxa in the creation of genetic variation, polyploidy and new species (Abbott 1992). Among animals, aquatic taxa seem particularly vulnerable to the infusion of alien genes. For instance, the introductions of rainbow trout, *Oncorhynchus mykiss*, in North America have resulted in hybridization and partial replacement of the native cutthroat trout, *Oncorhynchus clarki* (Allendorf & Leary 1988). Similarly, hybridization involving introduced Pecos pupfish, *Cyprinodon pecoensis*, in Texas and New Mexico (Wilde & Echelle 1992) and introduced guppies, *Poecilia reticulata*, in Trinidad (Shaw *et al.* 1992) has rapidly eroded gene combinations of native populations.

Cladocerans (a term of convenience for the crustacean orders Anomopoda, Ctenopoda, Haplopoda and Onychopoda) are one aquatic group in which introduced taxa seem to have had relatively little biological impact. In the Great Lakes, for example, only two of the 139 non-indigenous Great Lakes species identified by Mills *et al.* (1993) were cladocerans. Yet the prevalence and biological significance of cladoceran introductions are probably underappreciated. First, cladoceran taxa are undoubtedly well exposed to the major mechanisms of invasion, such as ship-related introductions and accidental releases, as

they often possess desiccation-resistant resting eggs and dominate the plankton. In addition, their cyclic parthenogenetic lifestyle means that successful colonization can result from one propagule. Finally, discerning alien from native taxa on morphological grounds is difficult, as cladocerans are morphologically conserved (Fryer 1991), and taxonomic studies of the group are confused and more recent than intercontinental shipping. The result is that many narrowly distributed cladoceran taxa were falsely assigned cosmopolitan distributions (Frey 1987), allowing introductions to occur unnoticed. The following documented cladoceran invaders add credence to this notion because they are all morphologically conspicuous from the native taxa: *Bythotrephes cederstroemi* (Bur *et al.* 1986), *Daphnia ambigua* (Scourfield 1947), *Daphnia parvula* (Einsle 1978), *Daphnia lumholtzi* (Sorensen & Sterner 1992; Hebert & Havel 1993), and *Eubosmina coregoni* (Wells 1970).

This study focuses on the analysis of *Daphnia galeata*, a common lacustrine microcrustacean with a near-cosmopolitan distribution (Brooks 1957; Christie 1983; Flössner & Kraus 1986; Glagolev 1986; Tanaka 1992). Brooks (1957) divided the taxon into Eurasian (*D. galeata galeata*) and New World subspecies (*D. galeata mendotae*) by differences in head shape and number of anal teeth. The Eurasian subspecies also possesses a shorter and more pronounced antennular mound than *D. g. mendotae* (see Brooks 1957; Flössner & Kraus 1986). In addition, the *gracilis* Hellich form of *D. galeata* occurs in Eurasia but is absent from North America (Hrbáček 1987). The *gracilis* form differs from typical *D. galeata* in its possession of a broadly rounded,

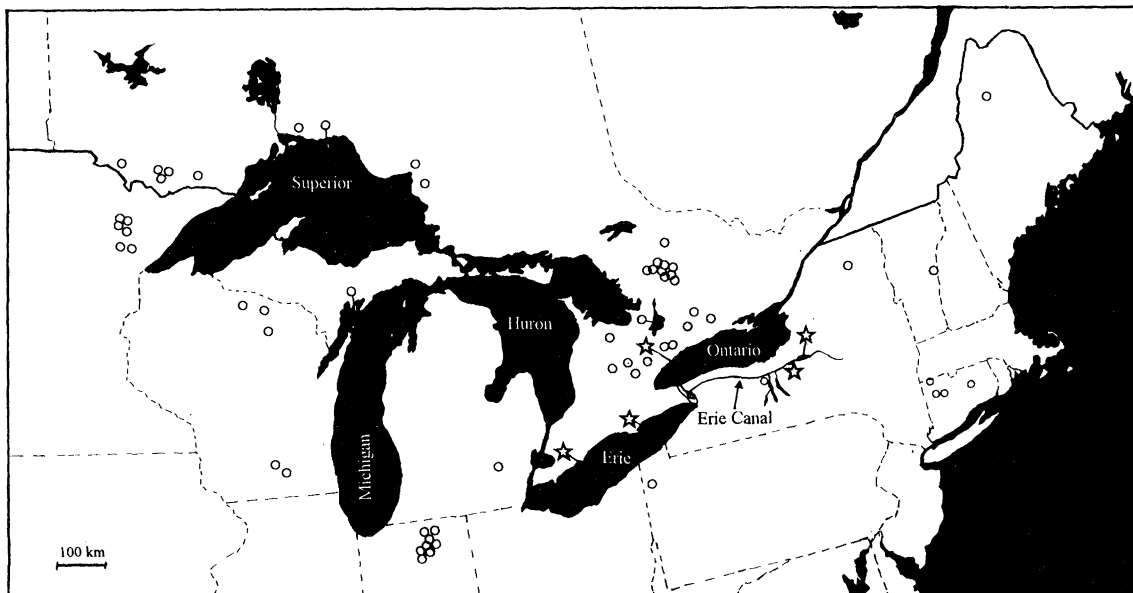


Figure 1. The Laurentian Great Lakes area showing sampling sites (lakes) for allozyme study of 64 populations of *Daphnia galeata*. Circles represent populations of pure *D. g. mendotae*; stars represent populations that show admixture between *D. g. mendotae* and Eurasian *D. g. galeata*.

unpointed helmet year round. Despite this modest regional variation, Negrea (1983) and Glagolev (1986) found morphological variation to be so great within each area that they unified all forms throughout the species range as *D. galeata*.

Our preliminary investigations showed that Lake Erie *D. g. mendotae* possessed different allelic arrays, heterozygosity and helmets from nearby populations (D. Taylor, unpublished observations). The purpose of this study was to determine if intercontinental introductions accounted for the anomalous Lake Erie *Daphnia*. The approach had three stages: (i) to assess the cosmopolitanism of *D. galeata*; (ii) to identify regional genetic markers within *D. galeata*; and (iii) to verify the occurrence of intercontinental introductions and assess their genetic implications

2. MATERIALS AND METHODS

A total of 64 populations of *D. g. mendotae* from North America and 10 populations of *D. g. galeata* from Europe were collected and analysed between 1990 and 1993. North American sampling focused on the Great Lakes watershed (figure 1), whereas the following widely distributed populations in Europe were collected: Derwentwater, Nanpanton Reservoir, Loch Oich, U.K.; Bodensee, Germany; Slapy, Hadí, Římov, Velký Pálenec, Žabinec, Czech Republic; and Turnianský Rybník, Slovakia. *Daphnia* species were identified according to Brooks (1957) and Flössner & Kraus (1986).

Cellulose acetate gel electrophoresis (Hebert & Beaton 1989) was used to examine allozyme diversity at the following nine loci: aldehyde oxidase (*Ao*, 1.2.3.1), aspartate aminotransferase (*sAat*, *mAat*; 2.6.1.1), dipeptidase (*Pep-A*, 3.4.13.11), fumarate dehydrogenase (*Fum*, 4.2.1.2), lactate dehydrogenase (*Ldh*, 1.1.1.27), phosphoglucosyltransferase (*Pgm*, 5.4.2.2), phosphoglucosylisomerase (*Gpi*, 5.3.1.9), and proline dipeptidase (*Pep-D*, 3.4.13.9). Substrates for *Pep-A* and *Pep-D* were L-leucylglycine and L-phenylalanyl proline, respectively. The minimum sample size for any population was 20 individuals per locus, with the exception of Římov

where 16 was the minimum. Alleles were assigned ascending letter designations with the slowest mobility (relative to the anode) assigned to 'a'. Non-metric multidimensional scaling (MDS) of a Cavalli-Sforza & Edwards (1967) chord distance matrix was used to examine the genetic intermediacy of putative hybrid populations (Lessa 1990). Exact tests (Haldane 1954), pooling rare genotypes, were done for agreement of electrophoretic data to Hardy-Weinberg expectations (HWE). Hochberg's (1988) sequential Bonferroni procedure was used to control the family-wise error rate for multiple tests (see Lessios 1992).

3. RESULTS

With the exception of four North American populations, allelic arrays at five of the nine loci showed extensive differentiation between the two subspecies (table 1). Although *Pep-D* was variable within each subspecies, no alleles were shared between North American and European populations. Additional differentiation was detected at *Gpi*, *sAat*, *Pep-A*, and *Pgm*, where average gene frequencies between subspecies differed by 44% to 84%. On average, the chord genetic distance between the subspecies was $D = 0.522$ for nine loci.

The four exceptional North American populations were Erie, Onandaga, Grenadier and Oneida. The allelic arrays in these populations were intermediate between typical *D. g. mendotae* and *D. g. galeata*. That is, the presence of the diagnostic European alleles *Pep-D^{b,e,f}* and *Pgm^b*, as well as gene frequencies at *Pep-A*, *Gpi* and *sAat*, suggested that these populations were genetically closer to European populations than were neighbouring *D. g. mendotae* populations. This genetic intermediacy is apparent when genetic distances are exposed to multidimensional scaling (figure 2).

Dimension one ($R^2 = 0.965$), which we interpreted as genetic variation between subspecies, revealed that Lake Erie, Grenadier, Oneida and Onandaga were intermediate between European *D. g. galeata*

Table 1. Mean allele frequencies (AF) at nine allozyme loci for ten European populations of *Daphnia galeata galeata* and 60 North American populations of *Daphnia galeata mendotae*

(Allele frequencies are also presented for four populations representing intercontinental hybrid swarms. Asterisks represent significant heterozygote excesses at a locus from Hardy-Weinberg expectations.)

locus	alleles	<i>D. galeata galeata</i>		<i>D. galeata mendotae</i>		Lake Erie <i>D. galeata</i>		Grenadier Pond <i>D. galeata</i>		Onandaga Lake <i>D. galeata</i>		Oncida Lake <i>D. galeata</i>	
		AF	n	AF	n	AF	n	AF	n	AF	n	AF	n
<i>Aat-m</i>	a	—	(285)	0.025	(2244)	—	(85)	—	(41)	—	(42)	—	(83)
	b	1.000		0.975		1.000		1.000		1.000		1.000	
<i>Aat-s</i>	a	—	(307)	0.007	(3779)	—	(83)	—*	(63)	—	(87)	—	(83)
	b	—		0.666		0.416		0.349		0.557		0.247	
	c	1.000		0.327		0.584		0.651		0.443		0.753	
<i>Ao</i>	a	1.000	(262)	1.000	(3098)	1.000	(85)	1.000	(53)	1.000	(64)	1.000	(62)
<i>Fum</i>	a	—	(284)	0.040	(2868)	—	(22)	—	(22)	0.047	(86)	—	(52)
	b	0.999		0.957		1.000		1.000		0.953		1.000	
	c	—		0.003									
	d	0.001											
<i>Gpi</i>	a	0.036	(309)	—	(3873)	—*	(115)	—*	(61)	—	(86)	—*	(83)
	b	0.891		0.048		0.483		0.451		0.860		0.343	
	c	0.053		0.888		0.430		0.549		0.140		0.627	
	d	—		0.064		0.087		—		—		0.030	
	e	0.020											
<i>Ldh</i>	a	—	(279)	0.003	(2853)	—	(85)	—	(53)	—	(42)	—	(52)
	b	1.000		0.996		1.000		1.000		1.000		1.000	
	c	—		0.001									
<i>Pep-A</i>	a	0.100	(310)	—	(3476)	—*	(84)	—	(48)	—*	(86)	—	(81)
	b	0.011		—		—		—		—		—	
	c	0.849		0.152		0.494		0.688		0.500		0.142	
	d	—		0.039		—		—		—		—	
	e	0.040		0.809		0.506		0.312		0.500		0.858	
<i>Pep-D</i>	a	—	(276)	0.798	(3707)	0.302*	(91)	0.390*	(50)	0.151	(76)	0.831	(71)
	b	0.077		—		0.016		0.500		0.059		0.007	
	c	—		0.001		—		—		—		—	
	d	—		0.201		0.254		0.110		0.388		0.162	
	e	0.488		—		0.170		—		0.355		—	
	f	0.435		—		0.258		—		0.047		—	
<i>Pgm</i>	a	—	(287)	0.012	(3841)	—	(85)	—*	(60)	—	(72)	—*	(85)
	b	0.089		—		0.006		0.492		0.069		0.420	
	c	0.261		0.697		0.824		0.508		0.334		0.570	
	d	0.643		0.290		0.165		—		0.590		0.010	
	e	0.007		0.001		0.005		—		0.007		—	

populations and 60 North American *D. g. mendotae* populations. A second dimension was not used because its addition improved the R^2 by only 0.013. The anomalous North American populations are all associated directly with either Lake Erie or Lake Ontario (figure 1).

Such a geographic pattern of genetic diversity is consistent with the introduction of *D. g. galeata* into North America and its subsequent hybridization with *D. g. mendotae*. As only one diagnostic locus is available and recombinant genotypes were present, categorization of hybrid individuals as F_1 , backcross, or advanced generation is uncertain. Nevertheless, mating between the North American and European individuals is markedly non-random, as genotype frequencies at 10 of 21 polymorphic loci in these four populations deviated significantly from HWE (table 1) after controlling for the family-wise error rate. All these deviations were due to heterozygote excesses involving alleles that are diagnostic or differ greatly in frequency between North American and European *D. galeata*. In

contrast, there is little evidence that 'pure' populations from either continent are mating non-randomly. Among the 28 variable loci in European populations, only one showed genotype frequencies that were significantly different from HWE: *Pep-D* in Nanpanton Reservoir showed a heterozygote deficiency. Similarly, only 7% (21 out of 287) of the total comparisons of variable loci in North American populations showed Hardy-Weinberg deviations. Of these deviations, 15 were due to heterozygote excesses involving alleles that Taylor & Hebert (1993) suggested were introgressed from *D. rosea* to *D. g. mendotae*.

If a hybrid is defined as an individual of mixed subspecies ancestry (i.e. clones that possess combinations of diagnostic alleles) then intercontinental hybrids are clearly very successful, as they constituted 90–100% of the swarms in the four 'mixed' lakes. The balance of the individuals in such lakes was either *D. g. mendotae* or advanced generation hybrids, but pure *D. g. galeata* was not detected in any of the North American samples. There was a clinal pattern of

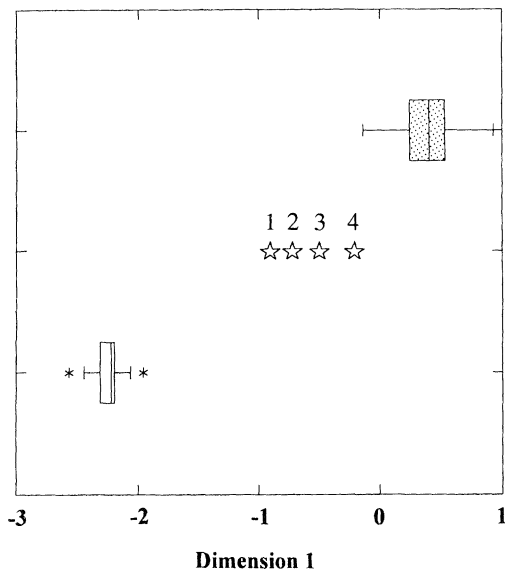


Figure 2. Box plots of a non-metric one-dimensional scaling of genetic distances (chord) derived from nine protein loci in *Daphnia galeata*. Dimension one is interpreted as a hybrid index between Eurasian and North American subspecies. A total of 60 North American populations (stippled box plot) and 10 European populations (open box plot) are represented. Asterisks represent outside values. North American populations showing admixture between *D. g. mendotae* and *D. g. galeata* are represented by stars instead of box plots, and are numbered as follows: 1, Lake Erie (two nearshore sites from the Ontario side); 2, Grenadier Pond, Ontario; 3, Onandaga Lake, New York; and 4, Oneida Lake, New York.

genetic distances as admixed populations were genetically more similar to *D. g. mendotae* (see figure 2) when they were geographically more distant from Lake Erie. It should also be noted that hybrid clonal diversity is high with different clonal arrays present in each lake.

4. DISCUSSION

The geographic pattern of allozyme variation in North American populations of *D. galeata* supports the hypothesis that Eurasian *D. galeata* has been introduced into the Great Lakes watershed unnoticed. Alleles that are common in Eurasian *D. galeata* populations are restricted in North American *D. galeata* to four populations (Erie, Grenadier, Onandaga and Oneida), all of which are connected to the Great Lakes intercontinental shipping routes. Alternative hypotheses of convergence and symplesiomorphy do not predict such a geographic pattern. In addition, convergence occurring simultaneously at the five marker loci is improbable. The genetic intermediacy of these populations between European *D. g. galeata* and North American *D. g. mendotae* suggests that, along with the introductions, extensive hybridization between the subspecies has occurred. The *Pep-D* locus is the most useful locus for diagnosing such hybrids as fixed differences occur between subspecies. The great number of different hybrid genotypes suggests that numerous hybridization events have occurred, and the homozygosity at *Ao* shows that none of the other

members of the *D. longispina* complex or their hybrids (Wolf & Mort 1986; Taylor & Hebert 1992) are prevalent in eastern North American lakes.

Our study supports Brooks' (1957) taxonomic separation of North American *D. galeata* from Eurasian forms. The fixed genetic difference at *Pep-D* and the multiple gene frequency divergences between populations from the two continents are inconsistent with one panmictic cosmopolitan species. Taylor & Hebert (1993) have shown that much of the differentiation between these taxa is the result of reticulate evolution involving *D. g. mendotae* and *D. rosea*. The detection of successful interbreeding does not by itself establish subspecific status because interspecific hybrids have been detected between *D. galeata* and five other species (Wolf & Mort 1986; Hebert *et al.* 1989; Taylor & Hebert 1992; D. J. Taylor, unpublished results). When 'true' allopatric conspecifics interbreed, Hardy-Weinberg expectations and gametic phase equilibrium are rapidly attained at diagnostic loci (see, for example, Avise & Smith 1974; Forbes & Allendorf 1991; Shaw *et al.* 1992). Thus the discovery that *D. g. galeata* and *D. g. mendotae* do not interbreed freely in sympatry suggests that intrinsic reproductive barriers exist and that each taxon should be accorded species status. Recombinant genotypes involving diagnostic alleles were present in mixed populations, showing that the F_1 generation is fertile. Yet heterozygote excesses prevailed at diagnostic loci, suggesting that a reproductive barrier to advanced generation hybridization exists. Such a breakdown of advanced generation hybrids is common in *Daphnia* hybrid systems (Hebert 1985; Wolf 1987; Taylor & Hebert 1993).

Heterozygote excesses at diagnostic loci and hybrid success may also be due to the increased hybrid fitness relative to the parent subspecies. Previous investigations of *Daphnia* have linked hybrid success both to differential use of the environment and to hybrid vigour (Weider & Stich 1992; Taylor & Hebert 1993). The rarity and relative ecological equivalence of the parent taxa in this study suggest that hybrid vigour may be important. Indeed Hebert *et al.* (1982) provided experimental evidence that laboratory-produced clones of intercontinental *Daphnia magna* hybrids were more fit than parent clones under conditions of thermal and osmotic stress. These authors also predicted that such hybrids would be successful if introduced into disturbed lakes because clonal reproduction would be rapid and perpetual. This scenario may have been realized with *Daphnia* hybrids in lakes of the present study.

Nevertheless, previous studies have shown that there are often seasonal shifts in abundance of parent taxa and hybrids (see, for example, Wolf 1987). Although we were unable to assess the seasonal variance in hybrid domination, reinterpretation of evidence from Phipps (1987) suggests that such a phenomenon occurs in western Lake Erie. Phipps (1987) analysed *Gpi* phenotypes and size at maturity in *D. g. mendotae* from June to August 1986. As in this study, three alleles (95, 100, 105) were detected, with the two common genotypes being $Gpi^{95/100}$ and $Gpi^{100/100}$. Phipps (1987) found that $Gpi^{100/100}$ individuals (which had a smaller

size at maturity than other genotypes) prevailed during the spring, whereas *Gpi*^{95/100} genotypes dominated after June, and interpreted this pattern as a response to fluctuating selection at the *Gpi* locus. Yet, as *Gpi* is a partly diagnostic locus between *D. g. galeata* and *D. g. mendotae*, heterozygotes in Lake Erie often represent clones of hybrid origin. Therefore, if selection occurs, it is more likely to be acting on the entire hybrid genome with shifts in genotype frequencies at the *Gpi* locus being a consequence of hitchhiking. It is worth noting that the only other reported case of selection at *Gpi* in *D. g. mendotae* (Mort & Jacobs 1981) has also been reinterpreted as an artefact of hybridization (Taylor & Hebert 1993). Interpretation of the factors responsible for the increasing abundance of hybrids during the summer months is complicated by the sustained dilution of hybrids in western Lake Erie by inflow of *D. g. mendotae* from the Detroit River. In effect, a reduction of putative hybrids in winter and early spring may simply be a consequence of the greater impact of dilution when local reproduction is slowed by low water temperatures.

Although the introduction of *D. g. galeata* passed unnoticed, some historical information is useful for inferring its details. Bigelow (1922), for example, reported that forma *galeata* (i.e. *D. g. mendotae* with pointed helmets) prevailed at our collection sites of Port Dover and Rondeau on Lake Erie early in this century. Examination of intermittent formalin-preserved samples from 1958 to 1992 showed that summer forms with a low-rounded helmet (typical of the European *gracilis* form) do not dominate until the early 1980s (D. J. Taylor, unpublished results). Therefore, it is likely that the introduction of Eurasian *D. galeata* occurred in Lake Erie during the late 1970s or early 1980s. Sprules *et al.* (1990) concluded that *Bythotrephes* also invaded the Great Lakes at this time, and they noted that this period represents the all-time high of ships in ballast entering the Great Lakes. The timing of the introduction of Eurasian *D. galeata*, together with its limited distribution near shipping lanes, suggests that ballast water was the probable mode of introduction into North America. It should be noted that the introgressed specimens from Onandaga and Oneida Lakes possessed helmet shapes that are typical of *D. g. mendotae*. This clinal pattern of morphology and genetic distances from Lake Erie suggests that Eurasian genes probably spread secondarily to Onandaga and Oneida Lakes through the Erie Canal, a route taken by several other introduced invertebrate taxa (Harmon & Forney 1970; May & Marsden 1992). Grenadier Pond was probably colonized by hybrids as a result of transfer of Lake Erie or Lake Ontario water.

A longstanding problem in ecology is predicting the outcome of invasions and determining the qualities of successful invaders. At present, strong conclusions are hampered by the lack of information on unsuccessful invasions (see, for example, Simberloff 1989). Most cladocerans possess the attributes of good invaders such as asexual reproductive stages and desiccation-resistant propagules, but only morphologically distinct species seem to be successful invaders. This pattern

may be due to vacant niches as cladoceran invaders such as *Bythotrephes*, *Eubosmina coregoni*, *D. ambigua*, and *D. lumholtzi* have no ecological equivalents among native taxa and have spread rapidly from their invasion sites (Brooks 1957; Yan *et al.* 1992; Havel & Hebert 1993; D. Taylor, unpublished observations). Alternatively, taxonomic confusion may have created an underestimate of invasions by alien taxa that are morphologically and ecologically similar to native taxa. Our study suggests that both ecological factors and the resolution of taxonomic confusion are important for understanding cladoceran invasions. 'Pure' Eurasian *D. g. galeata* has been unsuccessful in invading North American lakes that are often dominated by its ecological equivalent *D. g. mendotae*. Nevertheless, *D. g. galeata* genes have become well established in four lakes through the success of hybrid clones.

Our study highlights the need to determine genetically the geographic distributions of species to assess the impact of artificial introductions. As the number of intercontinental introductions has been exceptionally high over the last century, information about ecology, evolution and genetic diversity may be lost. It is well documented that hybrid swarms resulting from introduced taxa often replace or deplete populations of native congeners (see, for example, Abbott 1992; Wilde & Echelle 1992). Planktonic organisms are particularly susceptible to this sort of scientific loss, as they are morphologically conserved and poorly studied with respect to biogeography and systematics.

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