

Species diversity and endemism in the *Daphnia* of Argentina: a genetic investigation

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Although the temperate regions of South America are known to have a diverse daphniid fauna, there has been no genetic evaluation of the existing taxonomic system or of the affinities between the North and South American faunas. The present study analyses mitochondrial DNA sequences and allozyme variation to investigate species diversity in 176 *Daphnia* populations from Argentina. This work established the presence of at least 15 species in Argentina, six of which are either undescribed or are currently misidentified and two of which represent range extensions of North American taxa. Eleven of the Argentine species appear endemic to South America, while the remaining four also occur in North America. In the latter cases, the close genetic similarity between populations from North and South America indicates the recent exchange of propagules between the continents. While biological interactions and habitat availability have undoubtedly contributed to the observed species distributions, chance dispersal has apparently played a dominant role in structuring large-scale biogeographical patterns in this genus and probably in other passively-dispersed organisms. © 2004 The Linnean Society of London, *Zoological Journal of the Linnean Society*, 2004, 140, 171–205.

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INTRODUCTION

Our understanding of zooplankton biogeography has undergone a recent paradigm shift from ‘cosmopolitanism’ to ‘endemism’ (Frey, 1982a, 1987). Early investigators, such as Darwin (1859), were impressed by the morphological similarity of freshwater life from different continents, which they attributed to the long-distance dispersal capabilities of organisms adapted to discrete and relatively ephemeral habitats. As such, many species of invertebrates that were encountered in freshwater environments of the New World were assigned species names from Europe. However, more detailed comparisons subsequently revealed consistent morphological divergence in populations from different continents, leading to the recognition that few, if any, species are cosmopolitan (Frey, 1987). While a

few species have confirmed distributions spanning multiple continents (e.g. see Havel, Colbourne & Hebert, 2000; Schwenk, Posada & Hebert, 2000), most genetic analyses have further supported the generality of continental endemism and provincialism (e.g. Hebert & Wilson, 1994; Hann, 1995; Colbourne *et al.*, 1998; Černý & Hebert, 1999; Rowe, 2000; Schwenk *et al.*, 2000; Cox & Hebert, 2001), reinforcing the need for detailed regional studies.

The systematics and evolution of the cladoceran genus *Daphnia* have been studied particularly intensively. Species of this genus are dominant members of the microcrustacean communities of lakes and ponds throughout the world, except in the lowland tropics (Hebert, 1978; Fernando, Paggi & Rajapaksa, 1987). Furthermore, they are important model organisms for ecological, toxicological, and evolutionary studies. However, despite more than 200 years of attention, the taxonomy of this genus is still uncertain. Detailed morphological studies have shown that many variable traits (particularly helmet shape) are of limited taxo-

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nomic utility because of phenotypic plasticity and interspecific hybridization (Brooks, 1957a; Frey, 1982b; Flößner & Kraus, 1986; Dodson, 1988, 1989). To complicate matters further, morphological evolution in the genus has been characterized by stasis and convergence (Colbourne, Hebert & Taylor, 1997). While morphological analyses have often failed to resolve key aspects of the complex taxonomy of *Daphnia*, genetic markers have proven useful in delineating species boundaries, detecting hybridization, and clarifying phylogenetic relationships among taxa (e.g. Wolf & Mort, 1986; Taylor & Hebert, 1992, 1993a, 1994; Taylor *et al.*, 1996). The taxonomy of *Daphnia* has been investigated most comprehensively in North America. Allozyme studies were initially used to determine both species boundaries and the incidence of interspecific hybridization (e.g. Taylor & Hebert, 1993a; Hebert & Finston, 1996; see Hebert, 1995), and molecular phylogenetic analyses, employing mitochondrial DNA (mtDNA) sequences, later provided a systematic framework for the genus (Colbourne & Hebert, 1996; Taylor *et al.*, 1996). This work has indicated that North American members of the genus belong to three monophyletic subgenera: *Daphnia*, *Hyalodaphnia*, and *Ctenodaphnia* (Colbourne & Hebert, 1996). Each subgenus has also been divided into a number of species complexes, which were primarily defined by the detection or suspicion of hybridization potential among member species (Colbourne & Hebert, 1996). Genetic studies have established that hybridization is common between many closely allied *Daphnia* species and even occurs between more distant relatives, showing up to 14% divergence in the mitochondrial 12S rRNA gene (Colbourne & Hebert, 1996). Despite the high incidence of hybridization between some species, introgression is generally limited, and 'pure' parental genotypic arrays tend to remain largely intact (Schwenk & Spaak, 1997). However, extensive introgression has been documented and appears to have provoked speciation in at least one case (Taylor & Hebert, 1993b). Genetic evidence has also elucidated other factors of substantial evolutionary interest, such as breeding system shifts (Crease *et al.*, 1989; Černý & Hebert, 1993) and polyploidy (Dufresne & Hebert, 1997), which also contributed to taxonomic confusion in this genus. The key insights gained from such genetic analyses provide a valuable backdrop for investigating species diversity in *Daphnia* faunas that are virtually unexplored from a molecular point of view, such as that of Argentina.

Argentina possesses a large variety of landscapes and harbours a diverse daphniid fauna. Each of the three *Daphnia* subgenera is known to occur in this country (Paggi, 1998). All five species of the subgenus *Ctenodaphnia* known from the Neotropical region occur in Argentina (Paggi, 1998). These

are *D. dadayana* Daday emend. Paggi, *D. menucoensis* Paggi, *D. notacantha* Birabén, *D. ornithocephala* Birabén, and *D. spinulata* Birabén (Birabén, 1917, 1954; Paggi, 1996, 1999). All are large-bodied species that are usually restricted to fish-free, intermittent habitats in the arid, temperate regions of the country. While the taxonomy of *Ctenodaphnia* is considered well-resolved, species boundaries in the subgenera *Daphnia* and *Hyalodaphnia* are uncertain (Paggi, 1998). The only existing key (Olivier, 1962) includes many inaccurate records and questionable early descriptions. However, Paggi (1998) suggested that the subgenus *Hyalodaphnia* is represented by at least two taxa, *D. laevis* Birge and *D. gessneri* Herbst. Individuals of these small-bodied species inhabit lakes and reservoirs throughout subtropical and warm temperate South America (Arcifa, 1984; Infante, 1984; Matsuura-Tundisi, 1984). The taxonomy of the subgenus *Daphnia* is even more problematic, but at least five species have been reported from diverse pond and lake environments throughout the country, including *D. ambigua* Scourfield, *D. parvula* Forbyce, *D. peruviana* Harding, and *D. obtusa* Kurz (Scourfield, 1947; Marinone, 1979; Villagra de Gamundi, 1986; Zagarese, 1988, 1990; Paggi, 1998). Morphological analyses suggest that there may be several different species belonging to the 'obtusa' group (J. C. Paggi, pers. comm.). Finally, polyploid populations of hybrid origin, which are related to the mitochondrial lineages of *D. pulicaria* Forbes found in North America and arctic Europe (Colbourne *et al.*, 1998), have been reported from southern Argentina (Adamowicz *et al.*, 2002). While *D. pulex* L. emend. Leydig has been reported from Chile (Villalobos, 1994), it is not known from Argentina (Paggi, 1998).

To date, only two species of *Daphnia* from Argentina have been included in genetic studies. Hebert, Witt & Adamowicz (2003) found close genetic affinities between populations of *D. ambigua* from across North America and from southern South America, including Argentine and Chilean sites. Divergences between the South and North American phylogroups ranged from 3.2 to 5.7% in the mitochondrial COI gene (cytochrome C oxidase subunit I). Hebert *et al.* (2003) argued that these modest levels of divergence for daphniids indicate that the populations from the two continents may be considered conspecific, but are suggestive of a case of incipient allopatric speciation. Similarly, Adamowicz *et al.* (2002) detected very shallow divergences, an average of 2.9% at the quickly evolving mitochondrial ND5 gene (NADH dehydrogenase subunit V), between haplotypes of *D. pulicaria* from the northern hemisphere and southern South America. Together, these results suggest that intercontinental species distributions, and even 'cosmopolitanism', could be a reasonably common phenomenon in genera with highly

resistant dispersal stages, such as *Daphnia*. However, genetic work has not been done on the other South American species, many of which are thought on morphological grounds to be southern endemics.

In this study, mtDNA sequence data and allele frequencies at nuclear allozyme loci are used to clarify species boundaries, diagnose breeding systems, and screen for interspecific hybrids in populations of *Daphnia* from across Argentina. The validity of the current taxonomic system for Argentine *Daphnia* is evaluated by comparing sequence information from Argentine populations with corresponding data from all known North American species. Finally, general patterns of species diversity, dispersal, and endemism are discussed. We will address the phylogenetic position of the species that comprise the Argentine fauna in a future contribution. In addition, formal taxonomic descriptions will be prepared for all newly detected species (M. C. Marinone & J. C. Paggi, unpubl. data).

METHODS

COLLECTIONS

The majority of the collections were made during sampling campaigns in November–December, 1999, and January–February, 2001. *Daphnia* populations were sampled using a 280- μ m mesh net from 137 water bodies throughout Argentina, including ponds, road side ditches, playa lakes, alpine lakes, rivers, reservoirs, and saline lakes. Each site was assigned a unique number (see Appendix 1 for locality details). *Daphnia* were sorted alive and either flash-frozen in liquid nitrogen for allozyme surveys or preserved in 95% ethanol for DNA analysis. Initial species identifications for these populations were made in the field by M.C.M., P.D.N.H., and J. Paggi. As there is no modern key for Argentine *Daphnia*, the preliminary morphological identifications were based on original species descriptions and the personal experience of the investigators.

The above collections were augmented with 16 populations (sites 243–258, Appendix 1) from previous collections of M.C.M., preserved in either ethanol or trehalose, according to the protocol outlined in Taylor, Finston & Hebert (1994). Due to the age and method of preservation of these specimens, only DNA analysis was possible for these populations. Additionally, several ethanol-preserved specimens of *Daphnia peruviana* from a mountain lake in the province of Tucumán in northern Argentina (site 259) were provided by A. Villagra de Gamundi.

The genetic data from a few of these samples have been published elsewhere. Adamowicz *et al.* (2002) and Hebert *et al.* (2003) should be consulted for a detailed treatment of *D. pulicaria* and *D. ambigua*

populations, respectively. However, these populations are also included in the present study for completeness and for comparison of intra- and interspecific divergences among all taxa.

DNA SEQUENCE ANALYSIS

Total DNA was extracted from several individuals from each population by placing single animals in 50 μ L of proteinase-K-infused extraction buffer, according to the protocol of Schwenk *et al.* (1998). The extraction mixtures were incubated for 24 h at 50°C, after which the proteinase-K enzyme was denatured by a 10-min incubation at 95°C. Extractions were subsequently stored at –20°C. A 710-base pair (bp) fragment of the cytochrome *c* oxidase subunit I (COI) mitochondrial gene was amplified via the polymerase chain reaction (PCR) (Saiki, Gelfand & Stoffel, 1988) from a single individual from each population using universal primers LCOI490 and HCO2918 (Folmer *et al.*, 1994).

Each 50- μ L reaction consisted of 3–5 μ L of DNA template, 5 μ L of 10 \times PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl), 0.2 μ M of each primer, 1.5 mM MgCl₂, 0.2 mM of each dNTP, and 1 unit of *Taq* DNA polymerase. The PCR thermal regime was as follows: one cycle of 1 min at 94°C; 5 cycles of 1 min at 94°C, 1.5 min at 45°C, and 1.5 min at 72°C; 30 cycles of 1 min at 94°C, 1.5 min at 50°C, and 1.5 min at 72°C; and finishing with a final extension at 72°C for 5 min. PCR products were electrophoresed in 2% agarose gels, stained with ethidium bromide, and visualized with UV light. The desired fragment was excised, purified using the Qiaex II (Qiagen) kit, and subsequently subjected to dye terminator sequencing (25 cycles, 55°C annealing) using the Big Dye Terminator (version 3) sequencing kit (ABI Prism). Products were sequenced in one direction using primer LCOI490. Electrophoresis of sequencing-reaction products was performed on an ABI 377 automated sequencer (Applied Biosystems). Sequence electropherograms were inspected and aligned using the SeqApp 1.9 sequence editor (Gilbert, 1992), with the aid of the amino acid translation for the gene, resulting in a final alignment of 630 bp. All unique haplotypes encountered were deposited in GenBank under accession numbers AY323048–AY323126, while *D. ambigua* and *D. pulicaria* sequences are already published under numbers AF523691–2 and AF489523–5, respectively.

Phenetic analyses were performed on all unique COI haplotypes for each of the three *Daphnia* subgenera separately using the program MEGA 2.1 (Kumar *et al.*, 2001). Pairwise genetic distances were calculated using Kimura's (1980) two-parameter model (K2P) and pairwise deletion of missing sites. Pairwise

distance matrices were used to construct phenograms by the neighbour-joining (NJ) algorithm (Saitou & Nei, 1987), which does not assume equal evolutionary rates among lineages. North American taxa were included to root the trees, as indicated in the figure legends. Appropriate outgroups were selected based on the phylogenetic analyses of Colbourne & Hebert (1996). For example, since the subgenera *Daphnia* and *Hyalodaphnia* were found to be sister groups by Colbourne & Hebert (1996), a *Hyalodaphnia* species was used to root the *Daphnia* tree, and vice versa. Bootstrap values in all cases were based on 1000 pseudoreplicates. Distinct mitochondrial clades that corresponded to morphologically-identified groups of populations were tentatively designated as 'species' prior to allozyme analysis.

ALLOZYME ANALYSIS

Allozyme surveys were conducted on all populations for which frozen material was available (see Appendix 1). Variation was detected by subjecting whole-animal homogenate to cellulose acetate electrophoresis using a Tris-glycine buffer (pH 8.5) (Hebert & Beaton, 1993). Depending on the size of the individuals, populations were screened for variation at 4–7 of the following commonly polymorphic loci: aspartate amino transferase (AAT) (EC 3.2.1.1), fumarate hydratase (FUM) (EC 4.2.1.2), glucose-6-phosphate isomerase (GPI) (EC 5.3.1.9), glyceraldehyde-3-phosphate dehydrogenase (G3PDH) (EC 1.2.1.12), lactate dehydrogenase (LDH) (EC 1.1.1.27), malate dehydrogenase (ME) (EC 1.1.40), mannose-6-phosphate isomerase (MPI) (EC 5.3.1.8), and phosphoglucosmutase (PGM) (EC 5.4.2.2). All samples were electrophoresed for 15 min at 200 V, as pilot trials revealed that this run time maximized the separation of allelic variants while still maintaining resolution. Enzyme breakdown during longer trial runs produced smears that complicated scoring. From most sites, 22–44 individuals of each species were analysed, but in some cases fewer individuals were available. During each staining run, two individuals from a clonal stock of North American *Daphnia pulex* (from Lake Washington, Washington state, USA) were used to standardize scoring. All alleles encountered during allozyme screening were named according to their relative mobility compared with this standard.

It was known from morphological inspection that some currently-recognized morphospecies likely consisted of several species, somewhat complicating allozyme analysis. However, as the COI results were obtained first, mitochondrial clusters had already been tentatively designated as 'species' prior to allozyme analyses. Pilot allozyme runs revealed that samples that were in Hardy–Weinberg equilibrium, presumably constituting interbreeding (and therefore

single-species) populations, corresponded with the 'species' identified by mtDNA analysis. Furthermore, these allozyme trials revealed fixed allelic differences between each 'species' pair within the subgenera *Daphnia* and *Ctenodaphnia*. Therefore, for species in these subgenera, separation of individuals belonging to conspecific populations was straightforward, as hybrids were never detected at the diagnostic loci. Thus, allozyme data were analysed for each coexisting population separately for all *Daphnia* and *Ctenodaphnia* species. For the *Hyalodaphnia*, however, species boundaries were not clearly established, and so data from all individuals from each collection site were analysed together.

All analyses of allozyme data were performed using GDA (Genetic Data Analysis) software (Lewis & Zaykin, 2001). Genotypic frequencies within populations were compared with HW expectations using Fisher's exact test in order to screen for asexually-reproducing populations, mixed-species assemblages, and hybrids. Since multiple statistical tests were performed, a Bonferroni correction was applied by adjusting alpha for each species, such that: $\alpha = 0.05/(\text{No. of polymorphic loci in each population summed over all populations})$. Levels of genetic diversity and genetic substructure within populations were estimated using three basic statistics: per cent polymorphic loci, per cent individual heterozygosity, and F_{IS} (the inbreeding coefficient). The extent of genetic divergence among conspecific populations was estimated using Nei's (1978) genetic distances (D). F_{ST} statistics (i.e. fixation indices) were used to estimate the degree of genetic structure (i.e. gene pool fragmentation) among intraspecific populations. F_{ST} statistics were calculated for all sexually reproducing species for which three or more populations were surveyed. Interspecific divergences were also estimated using Nei's distances. Allozyme trees were constructed from pairwise distance matrices using the UPGMA method.

COMPARISON WITH NORTH AMERICAN SPECIES

COI sequences from Argentine populations were compared with sequences from most of the known North American species, the only missing species being *D. latispina* and *D. retrocurva*. Most sequences were provided by J. Colbourne or were sequenced from specimens from the archived collections of P.D.N.H. (see Appendix 2). While preliminary analyses included all North American species, only matches with possibly synonymous or closely-related species are reported in detail here. Analysis of the phylogenetic relationships among more distantly related species will be presented elsewhere (S. J. Adamowicz, J. K. Colbourne & P. D. N. Hebert, unpubl. data).

Preliminary genetic screening indicated a close relationship between *D. spinulata* from Argentina and the North American species *D. exilis*. Since extensive allozyme surveys had already been conducted on *D. exilis* (Hebert & Finston, 1993), and additional archived specimens were available for DNA sequencing, this species pair was selected for a more detailed investigation of patterns of intraspecific diversity and intercontinental relatedness. COI sequences from ten populations of North American *D. exilis* were obtained and analysed along with sequence data for *D. spinulata* (see Appendix 2 for collection localities). Sequence divergences were estimated using the K2P model and analysed by phenetic techniques, as described above. Additionally, allozyme variation was compared between *D. spinulata* populations from Argentina and *D. exilis* populations from North America. Three archived *D. exilis* populations from Mexico were electrophoresed in the present study. Live *D. exilis* populations from Oklahoma (provided by S. Schwartz) were used to standardize allozyme scoring between *D. spinulata* populations and similar data for *D. exilis* from Hebert & Finston (1993). Although these authors surveyed 11 allozyme loci, only seven loci were considered in the present study, so that *D. spinulata* and *D. exilis* were compared using the same markers. For the *D. exilis/D. spinulata* dataset, the NJ tree-building method was also used for the COI sequences, and the UPGMA technique was employed to analyse the allozyme data.

RESULTS

DIVERSITY IN THE SUBGENUS *DAPHNIA*

Initial morphological species assignments

Members of this subgenus were collected from 77 habitats (see Appendix 1; Fig. 1). Since some habitats contained more than one species, a total of 88 populations were available for analysis. Initial morphological identifications indicated the presence of at least five species. Three populations of *D. ambigua* were collected from lakes or reservoirs, while two individuals of *D. parvula* were collected from a river. A single population of *D. peruviana*, a darkly-melanized species, was collected from a mountain lake. The remaining 83 populations were assigned to either the *D. pulex* or *D. obtusa* complexes, which were separated, respectively, by their lack or possession of elongate setae along the internal margin of the carapace (Scourfield, 1942; Schwartz *et al.*, 1985). Populations belonging to the *D. pulex* complex were frequently encountered in southern Argentina, while members of the *D. obtusa* complex were collected throughout the country. Previous analyses have revealed that the members of the *pulex* complex are polyploid, asexual populations

closely related to North American populations of *D. pulicaria*, which will be referred to as *D. 'pulicaria'* because of the uncertain hybrid origin of these populations (see Adamowicz *et al.*, 2002). There was much morphological variability among populations assigned to the *obtusa* complex. Several populations from the Andean lakes and also from ponds in the southern parts of the country were melanized to varying degrees, but populations from other areas were not.

COI sequence variation

Phenetic analysis of all unique COI sequences revealed the presence of seven distinct clusters, which were tentatively assigned names based on morphological identifications (Fig. 2). Different numbers were used to designate cases in which divergent clades were initially identified as the same morphospecies. Members of the *obtusa* complex formed three distinct clusters (*D. obtusa* 1, 2, and 3), the third of which contained all of the melanized populations. Populations of *D. ambigua*, *D. parvula*, *D. peruviana*, and *D. 'pulicaria'* clearly formed separate clusters.

Bootstrap support for all seven clusters was high (100). The topology indicated that *D. obtusa* 1 and *D. obtusa* 2 were closely allied, an affinity supported by a moderately high bootstrap value (74). The relationships among the other species were not resolved by this analysis.

Maximum COI sequence divergences among individuals assigned to a single cluster (i.e. putative 'intraspecific' divergences) were generally small, ranging from a low of 0.3% in *D. 'pulicaria'* to a high of 4.3% in *D. obtusa* 1 (Table 1). By contrast, average pairwise distances between clusters were much larger. The lowest divergence (between *D. obtusa* 1 and 2) was 16.2%, while other pairwise divergences ranged from 20.3 to 28.3% (Table 2).

Allozyme variation

Allozyme analysis was not possible for *D. parvula* or *D. peruviana*, as only ethanol-preserved samples were available. However, allozyme analyses partitioned the other populations into the same five 'species' identified by mtDNA analysis. Allelic arrays and allele frequencies differed among species, and at least two fixed differences were detected between each pair of putative species (Table 3).

Nei's genetic distances were generally small among conspecific populations. Maximum intraspecific distances ranged from a low of 0.0 in *D. ambigua* to a high of 0.54 in *D. obtusa* 1 (Table 1). By contrast, genetic distances among the five species were large (Table 2). The allozyme results confirmed that *D. obtusa* 1 and 2 are most closely allied, with an

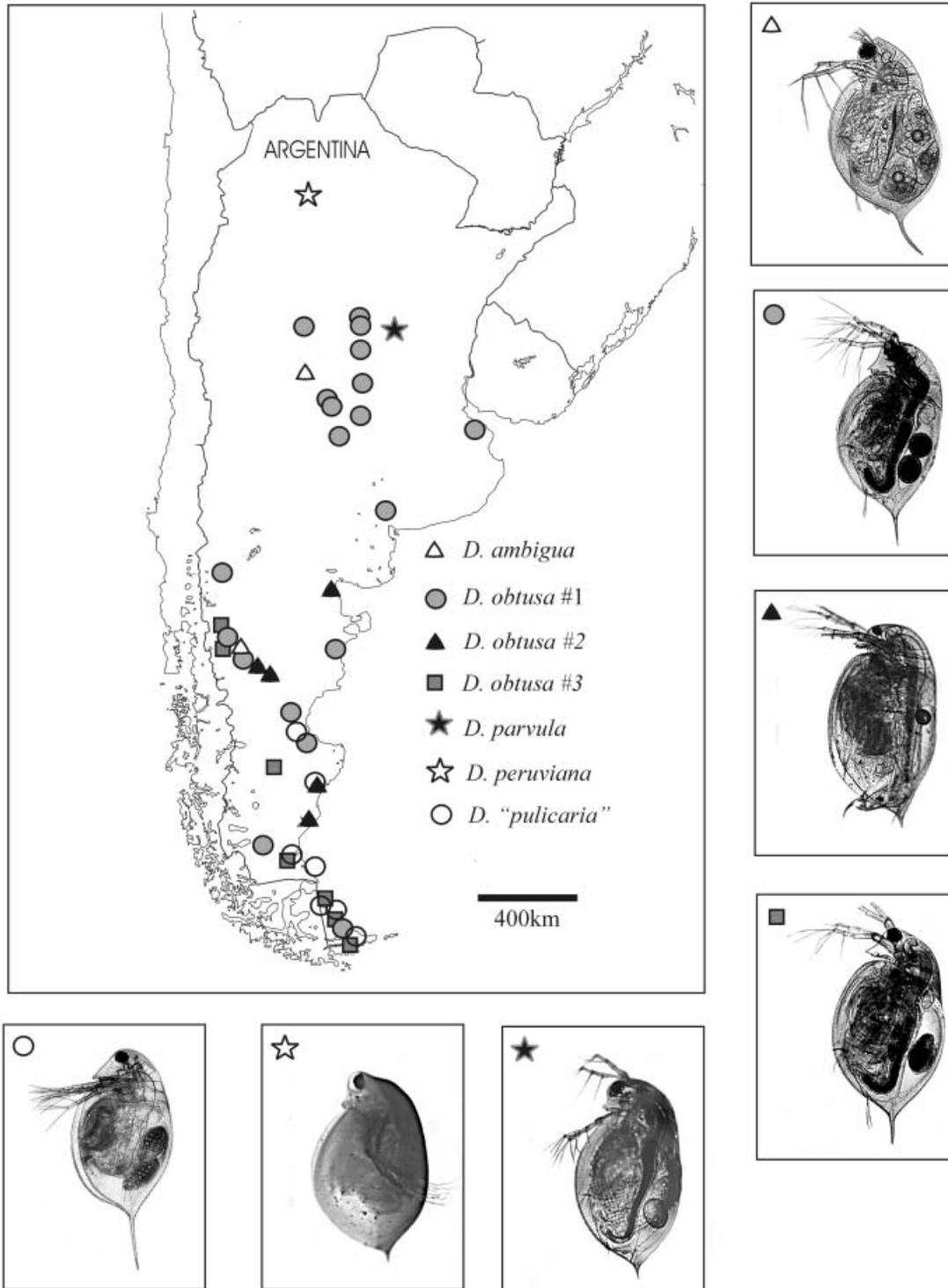


Figure 1. Collection sites for Argentine populations belonging to the subgenus *Daphnia*. Photographs are included for a single individual of each species. Species were identified based on genetic analyses (see text and Fig. 2). Animals are not shown to scale. In some cases, multiple collections of the same species within a small geographical region are not shown (see Appendix 1 for the complete list of collection localities).

Table 1. Measures of intraspecific and intrapopulation genetic diversity, divergence, and structure for 12 species of Argentine *Daphnia*. K2P distances are based on COI sequences, while all other statistics are based on allozyme variation. The same six allozyme loci were screened for all species (AAT, FUM, GPI, LDH, MPI, PGM) plus G3PDH for species in the subgenus *Daphnia* and ME for *Ctenodaphnia* species, with the exception of *D. ambigua*, for which four loci were used (AAT, GPI, LDH, PGM). Some values for *D. exilis* from North America are included for comparison with *D. spinulata* (data from the present study and Hebert & Finston, 1993). Missing statistics are due to small sample sizes for some species, poor resolution of species boundaries (subgenus *Hyalodaphnia*), or an asexual mode of reproduction (the polyploid species *D. 'pulicaria'*). Abbreviations: N = number of populations having individuals sequenced for COI; n = number of populations screened for allozyme variation; APP = average percentage of polymorphism; PH = percentage of heterozygosity; # = number of loci surveyed in each species; F_{ST} = fixation index; F_{IS} = inbreeding coefficient; H_O = average observed heterozygosity; H_E = average expected heterozygosity; n/a = not available

Species (N, n)	Max.% COI K2P distance	Max. Nei's distance	F_{ST}	APP (#)	PH H_O (H_E)	Average F_{IS}
SUBGENUS <i>DAPHNIA</i>						
<i>D. ambigua</i> (4, 2)	0.9	0.00	–	25.0 (4)	6.7 (5.9)	–0.14
<i>D. obtusa</i> 1 (41, 27)	4.3	0.54	0.50	29.6 (7)	11.4 (11.4)	0.001
<i>D. obtusa</i> 2 (8, 4)	1.3	0.04	0.29	25.0 (7)	6.7 (5.8)	–0.17
<i>D. obtusa</i> 3 (9, 8)	2.3	0.12	0.81	5.4 (7)	1.4 (1.9)	0.26
<i>D. 'pulicaria'</i> (8, 16)	0.3	0.01	–	57.1 (7)	57.1 (30.1)	–1.00
SUBGENUS <i>HYALODAPHNIA</i>						
<i>D. laevis/gessneri</i> (5, 3)	–	–	–	38.9 (6)	32.5 (17.8)	–0.85
SUBGENUS <i>CTENODAPHNIA</i>						
<i>D. dadayana</i> (21, 24)	3.6	0.87	0.52	29.4 (7)	10.0 (10.3)	0.03
<i>D. menucoensis</i> (12, 9)	1.4	0.33	0.33	21.5 (7)	8.3 (8.2)	–0.02
<i>D. ornithocephala</i> (4, 4)	0.3	0.18	0.49	12.5 (7)	6.2 (5.8)	–0.08
<i>D. similis</i> (1 individual)	–	–	–	0.0 (6)	–	–
<i>D. spinulata</i> (38, 37)	3.9	0.13	0.24	29.0 (7)	10.2 (9.3)	–0.10
<i>D. exilis</i> NA (11, 16)	1.9	0.36	0.43	n/a	n/a	n/a
<i>D. sp.</i> 1 (1, 1)	–	–	–	28.6 (7)	10.3 (8.7)	–0.19

Table 2. Estimates of genetic divergence between Argentine species within the subgenera *Daphnia* and *Ctenodaphnia*. Average pairwise K2P divergences in the COI gene are presented in the bottom half of the matrix. The top half of the matrix reports average Nei's genetic distances, based on allozymes (see Table 1 for loci included)

<i>DAPHNIA:</i>	<i>ambigua</i>	<i>obtusa</i> 1	<i>obtusa</i> 2	<i>obtusa</i> 3	<i>parvula</i>	<i>peruviana</i>	<i>'pulicaria'</i>
	–	1.71	3.20	1.27	n/a	n/a	3.25
<i>obtusa</i> 1	21.6	–	0.78	0.93	n/a	n/a	1.09
<i>obtusa</i> 2	21.2	16.2	–	1.07	n/a	n/a	0.85
<i>obtusa</i> 3	22.7	21.1	20.6	–	n/a	n/a	0.87
<i>parvula</i>	26.0	22.9	22.1	22.8	–	n/a	n/a
<i>peruviana</i>	26.2	24.1	28.3	27.3	27.3	–	n/a
<i>'pulicaria'</i>	25.3	23.8	20.3	22.2	25.3	27.5	–
<i>CTENODAPHNIA:</i>	<i>dadayana</i>	<i>menucoensis</i>	<i>ornithocephala</i>	<i>similis</i>	<i>spinulata</i>	sp. 1	
<i>dadayana</i>	–	2.33	*	1.38	1.60	2.51	
<i>menucoensis</i>	28.6	–	6.69	1.32	1.06	1.77	
<i>ornithocephala</i>	22.2	30.1	–	*	*	3.57	
<i>similis</i>	24.6	27.9	21.9	–	0.94	3.45	
<i>spinulata</i>	25.9	26.0	23.2	20.8	–	1.36	
sp. 1	23.6	26.1	20.9	22.2	25.5	–	

*Genetic distance could not be calculated as no alleles were shared.

Table 3. Mean allele frequencies at seven allozyme loci for five Argentine species in the subgenus *Daphnia*. Alleles are identified by their relative mobility compared with a laboratory clone of *D. pulicaria*. Abbreviations: *N* = number of populations; *n* = number of individuals

Alleles	<i>ambigua</i> (<i>N</i> = 2)	<i>obtusa</i> 1 (<i>N</i> = 27)	<i>obtusa</i> 2 (<i>N</i> = 4)	<i>obtusa</i> 3 (<i>N</i> = 8)	' <i>pulicaria</i> '* (<i>N</i> = 16)
AAT	(<i>n</i> = 64)	(<i>n</i> = 632)	(<i>n</i> = 132)	(<i>n</i> = 207)	(<i>n</i> = 328)
0.73	0.87	–	–	–	–
0.75	–	0.03	–	–	–
0.88	–	–	0.01	0.60	–
1.00	0.13	0.97	0.99	0.40	1.00
1.09	–	<0.01	–	–	–
FUM	(<i>n</i> = 0)	(<i>n</i> = 737)	(<i>n</i> = 153)	(<i>n</i> = 194)	(<i>n</i> = 328)
1.00	n/a	0.78	1.00	–	0.50
1.11	n/a	0.22	–	–	–
1.14	n/a	–	–	–	0.50
1.20	n/a	–	–	1.00	–
GPI	(<i>n</i> = 44)	(<i>n</i> = 622)	(<i>n</i> = 139)	(<i>n</i> = 193)	(<i>n</i> = 328)
88	–	<0.01	0.08	–	0.50
0.92	1.00	–	–	–	–
1.00	–	1.00	0.73	1.00	0.50
1.10	–	–	0.19	–	–
1.14	–	–	0.01	–	–
G3PDH	(<i>n</i> = 0)	(<i>n</i> = 585)	(<i>n</i> = 118)	(<i>n</i> = 149)	(<i>n</i> = 328)
0.87	n/a	1.00	–	–	–
1.00	n/a	–	1.00	1.00	1.00
LDH	(<i>n</i> = 37)	(<i>n</i> = 576)	(<i>n</i> = 126)	(<i>n</i> = 234)	(<i>n</i> = 328)
0.79	1.00	–	–	–	–
0.81	–	1.00	1.00	–	–
0.83	–	–	–	–	0.50
1.00	–	–	–	1.00	0.50
1.12	–	<0.01	–	–	–
MPI	(<i>n</i> = 0)	(<i>n</i> = 781)	(<i>n</i> = 138)	(<i>n</i> = 158)	(<i>n</i> = 360)
0.86	n/a	0.04	–	–	0.47
0.92	n/a	0.16	–	–	0.03
0.96	n/a	–	1.00	–	–
1.00	n/a	0.40	–	1.00	0.47
1.03	n/a	0.17	–	–	–
1.09	n/a	0.19	–	–	0.03
1.19	n/a	0.03	–	–	–
1.30	n/a	<0.01	–	–	–
PGM	(<i>n</i> = 44)	(<i>n</i> = 778)	(<i>n</i> = 159)	(<i>n</i> = 204)	(<i>n</i> = 328)
0.89	–	<0.01	–	–	–
1.00	–	0.03	0.02	–	1.00
1.06	–	0.45	0.96	0.04	–
1.12	1.00	0.51	0.02	0.96	–
1.22	–	<0.01	–	–	–

*These populations are tetraploid asexuals, with fixed heterozygosity at four loci (see Adamowicz *et al.*, 2002, for details).

Levels of genetic diversity within populations were compared for five species within the subgenus *Daphnia* using the same seven allozyme loci (AAT, FUM, GPI, G3PDH, LDH, MPI, and PGM), with the exception of *D. ambigua*, for which only four loci were available (Table 1). Observed levels of heterozygosity were similar to the values expected based on

allele frequencies in the sexual species. However, levels of polymorphism and heterozygosity for the obligately parthenogenetic *D. 'pulicaria'* were greatly elevated, due to fixed heterozygosity. Among the remaining species, *D. obtusa* 1 was the most genetically variable, while *D. obtusa* 3 was the least (Table 1).

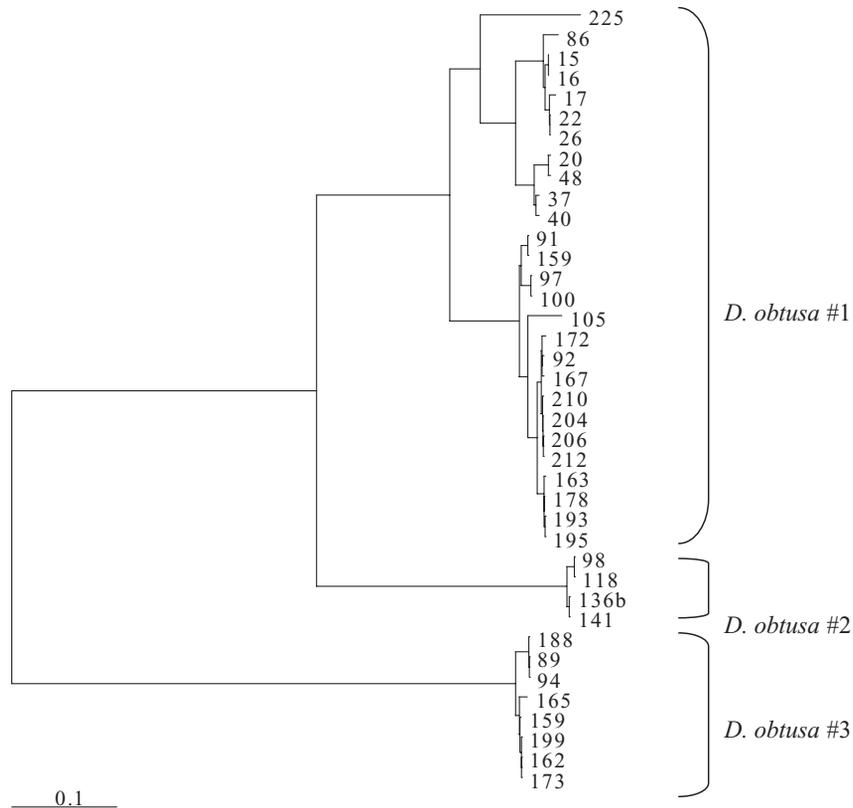


Figure 3. UPGMA tree based on allozyme variation at seven loci in three species of the *Daphnia obtusa* complex from Argentina. The scale bar represents Nei's genetic distance.

F_{ST} statistics for those species in which three or more populations were surveyed ranged from 0.29 in *D. obtusa* 2 to 0.81 in *D. obtusa* 3 (Table 1), the latter value indicating strong genetic differentiation among conspecific populations. The especially high value for *D. obtusa* 3 reflected the fixation or near fixation of alternate alleles at AAT in different populations. This one locus dominated the F_{ST} statistic because polymorphism was limited in this species (Table 1).

Evaluation of relatedness to North American Daphnia species

A comparison of COI sequences from Argentine and North American *Daphnia* revealed two cases in which taxa from the two continents were closely affiliated, and are probably conspecific. As mentioned above, in both *D. ambigua* and *D. pulicaria* divergences between North and South American populations were generally <5% (Adamowicz *et al.*, 2002; Hebert *et al.*, 2003).

A case of intermediate divergence between a South and North American species was detected. The two individuals of Argentine *D. parvula* were 12% divergent from a *D. parvula* individual from Mexico. Pair-

wise divergences between the remaining Argentine members of the subgenus *Daphnia* and all North American species were greater than 17%. Although two *Daphnia* species were missing from the COI dataset, recent analyses including the complete 12S rDNA dataset of Colbourne & Hebert (1996) and all Argentine species revealed no additional cases of close affinities between taxa from the two continents (S. J. Adamowicz, J. K. Colbourne & P. D. N. Hebert, unpubl. data).

DIVERSITY IN THE SUBGENUS *HYALODAPHNIA*

Initial morphological species assignments

Members of the subgenus *Hyalodaphnia* were obtained from five sites, four of which were reservoirs or lakes and one, a river (Appendix 1; Fig. 4). Based on head morphology, these individuals were preliminarily identified as either the helmeted species *D. gessneri* (Herbst, 1967) or the unhelmeted species *D. laevis* (Birge, 1878). Three populations (30, 32, and 36) were identified as *D. laevis*, one population (226) was identified as *D. gessneri*, while the Coronda River (site 9) appeared to contain both

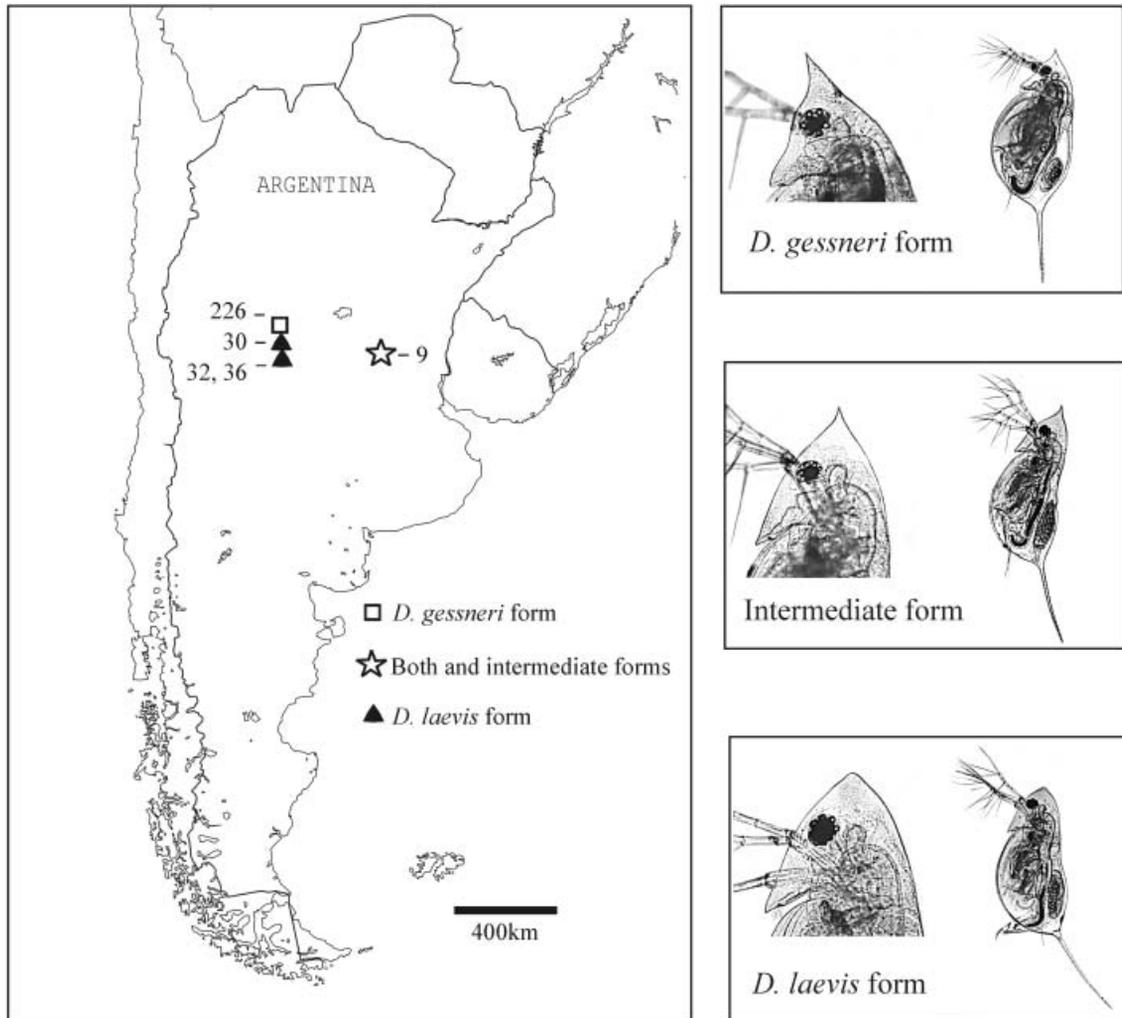


Figure 4. Collection sites for Argentine populations belonging to the subgenus *Hyalodaphnia*. Photographs are included to demonstrate the several head morphologies encountered. The morphological, not genetic, forms encountered at each site are indicated on the map.

taxa, as well as individuals with intermediate head morphologies (Fig. 4).

COI sequence analysis

Three COI haplotypes were found among the eight individuals examined from the five sites (Fig. 5). Two individuals of (morphological) *D. gessneri* from site 226 and three individuals of *D. laevis* from sites 30, 32, and 36 possessed the same haplotype. A similar sequence (only 0.6% divergent) was found in two individuals from the Coronda River (site 9), one with the head morphology of *D. laevis*, and the other with that of *D. gessneri*. However, a third individual from the same site, identified as *D. gessneri*, had a haplotype that showed an average of 13.6% divergence from the other group.

Allozyme variation

Three of the five *Hyalodaphnia* populations (30, 32, and 226) were screened for variation at six allozyme loci, three of which were polymorphic (Table 4). In all three populations, two loci were fixed, or nearly so, for heterozygotes. In all but one case (PGM at site 32), the polymorphic loci among these three populations were severely out of HW equilibrium ($P < 0.001$), always due to heterozygote excess (Tables 1 and 4).

Evaluation of relatedness to North American Hyalodaphnia species

Both groups of haplotypes found in Argentina, perhaps representing two species, were only distantly related to those present in the three North American species belonging to the *laevis* complex (*D. dubia*,

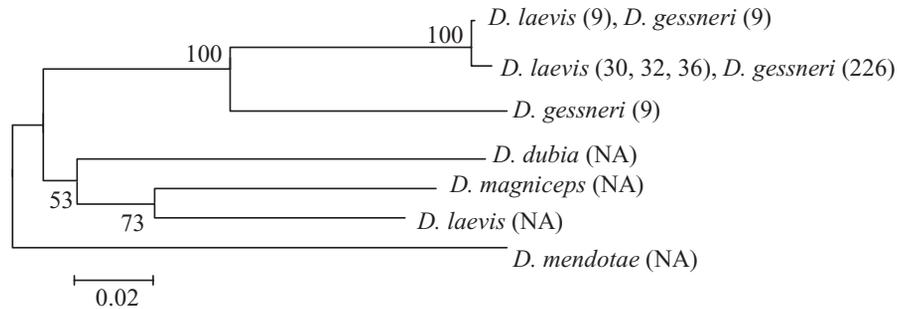


Figure 5. NJ tree based on COI sequence variation among Argentine populations identified as *D. laevis* and *D. gessneri*. The identifications, based on head morphology, are indicated in this tree, followed by the collection site numbers. Sequences from the North American members of the *D. laevis* complex (*D. dubia*, *D. laevis*, and *D. magniceps*) were included for comparison. *D. mendotae*, a *Hyalodaphnia* species belonging to a different species complex (Colbourne & Hebert, 1996), was used to root the tree. The scale bar represents K2P distance.

Table 4. Genotypic frequencies at six allozyme loci in three Argentine populations of *Hyalodaphnia*. Populations 30 and 32 were identified as *D. laevis*, while population 226 was identified as *D. gessneri*, based on head morphology. Complete genotype data are available from the authors

Genotype	30	32	226
AAT	(<i>n</i> = 19)	(<i>n</i> = 21)*	(<i>n</i> = 21)*
Homozygotes:			
0.79/0.79	1.00	–	–
Heterozygotes:			
0.79/0.90	–	1.00	1.00
FUM	(<i>n</i> = 22)	(<i>n</i> = 18)	(<i>n</i> = 20)
1.00	1.00	1.00	1.00
GPI	(<i>n</i> = 87)*	(<i>n</i> = 21)*	(<i>n</i> = 22)
Homozygotes:			
0.85/0.85	0.06	0.09	1.00
Heterozygotes:			
0.73/0.85	0.94	0.91	–
LDH	(<i>n</i> = 22)	(<i>n</i> = 22)	(<i>n</i> = 16)
0.87	1.00	1.00	1.00
MPI	(<i>n</i> = 21)	(<i>n</i> = 21)	(<i>n</i> = 8)
1.27	1.00	1.00	1.00
PGM	(<i>n</i> = 85)*	(<i>n</i> = 21)	(<i>n</i> = 42)*
Homozygotes:			
0.92/0.92	0.01	–	0.07
1.07/1.07	0.01	0.90	–
Heterozygotes:			
0.92/0.97	0.82	–	–
0.92/1.07	0.14	0.10	0.93
0.97/1.07	0.01	–	–

*Denotes statistically significant heterozygote excess ($P < 0.001$).

D. laevis, *D. magniceps*; see Taylor *et al.*, 1998). COI divergences between North and South American taxa ranged from 18 to 24% (see Fig. 5). North and South American *D. 'laevis'* are clearly not sister taxa, as each is more closely related to another *Hyalodaphnia* species from its home continent (Fig. 5).

DIVERSITY IN THE SUBGENUS *CTENODAPHNIA*

Initial morphological species assignments

A total of 83 *Ctenodaphnia* populations was sampled from 80 habitats (Appendix 1; Fig. 6). Preliminary morphological investigation suggested the presence of at least six species. Morphological species assignments were generally straightforward. *Daphnia spinulata* was collected in ponds throughout much of the country. Populations from four sites (73, 204, 206, and 229) were identified as *D. notacantha* because of the distinctive hump on their heads. However, smaller 'notacantha-like' humps were observed in several populations designated as *D. spinulata*. Twelve populations of *D. menucoensis* were collected from shallow saline lakes in the Patagonian steppes and in the province of Buenos Aires, while *D. dadayana* occurred at 24 sites in southern Argentina. Four populations of the distinctive *D. ornithocephala*, literally, 'bird head', were collected from ephemeral ponds in a restricted arid area of west-central Argentina (sites 299, 231, 233, and 234). Finally, a single population of a *Ctenodaphnia* species that was morphologically distinct from any described species was encountered at site 209.

COI sequence variation

Phenetic analysis of all unique COI sequences revealed six distinct clusters (Fig. 7). Four of these genetic groups corresponded to recognized morphospecies: *D. dadayana*, *D. menucoensis*, *D. ornithocephala*,

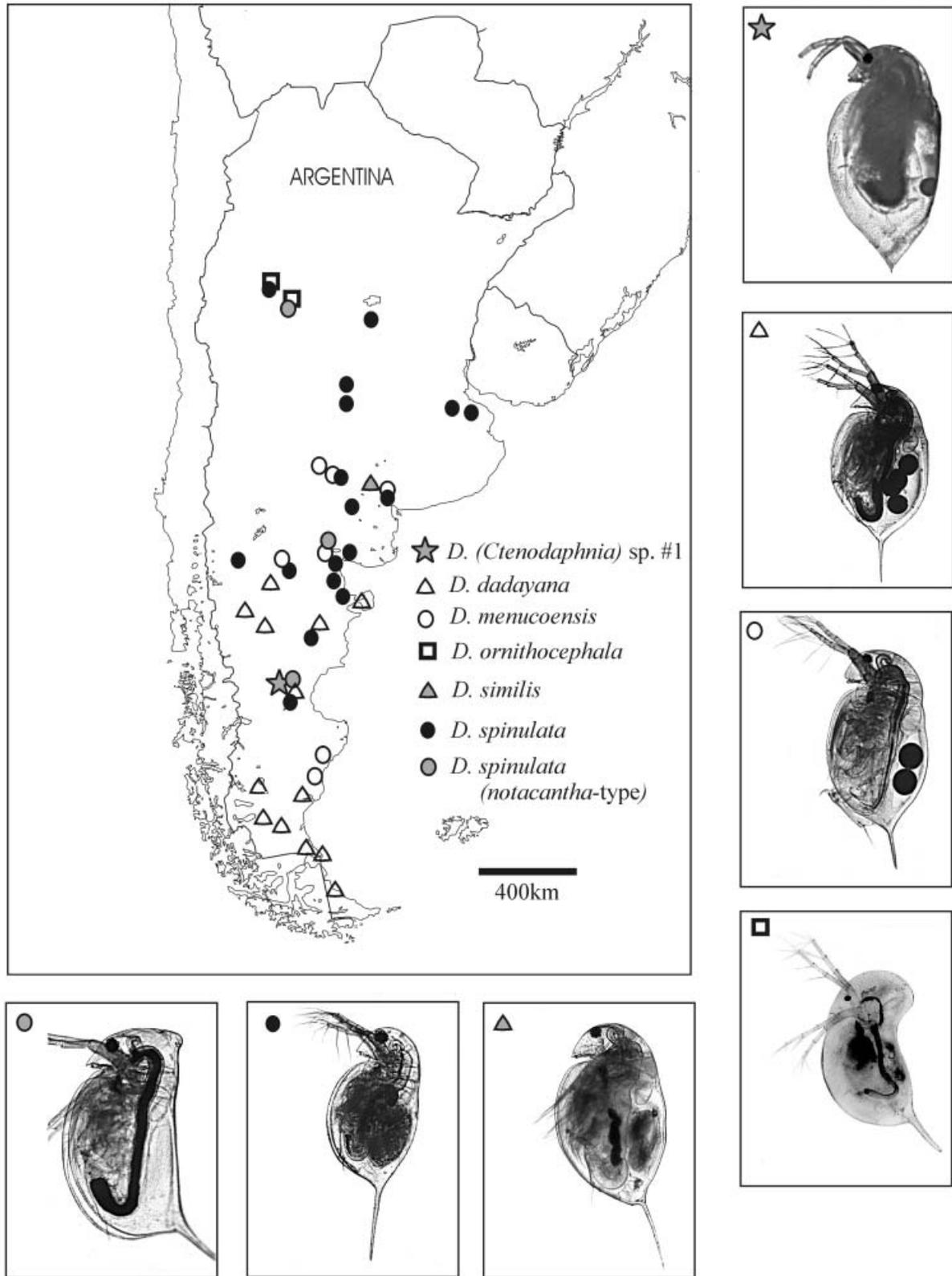


Figure 6. Collection sites for Argentine populations belonging to the subgenus *Ctenodaphnia*. Photographs are included for a single individual of each species. Species assignments are based on genetic analyses (see text and subsequent figures). Animals are not shown to scale, and not all sites are shown (see Appendix 1 for the complete collection list).

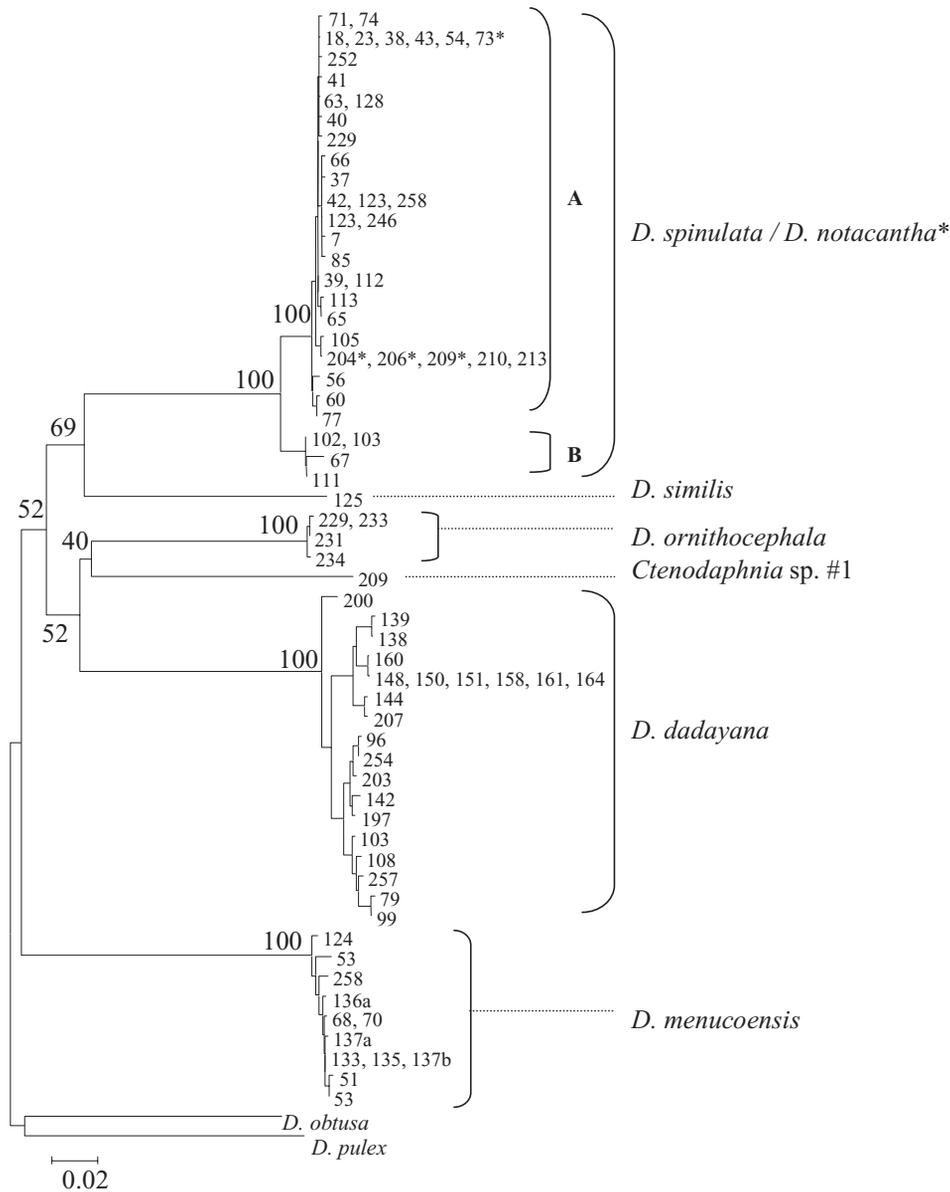


Figure 7. NJ tree based on COI sequence variation among all unique haplotypes of Argentine populations belonging to the subgenus *Ctenodaphnia*. Two members of the subgenus *Daphnia* (*D. obtusa* and *D. pulex*) were included to root the tree. Bootstrap values are presented for major clusters, and K2P distances are indicated by the scale bar. The collection site of each individual is indicated by its population code (see Appendix 1). Individuals morphologically identified as *D. notacantha* are indicated by an asterisk. This tree is not intended to represent a phylogenetic hypothesis for the subgenus.

and *D. spinulata*. On the other hand, populations of *D. notacantha* possessed haplotypes indistinguishable from those of *D. spinulata* (Fig. 7). A single individual from another population (125) was identified by its COI sequence (see below) as *D. similis*, as redescribed from North America by Hebert & Finston (1993). A morphologically unique population from site 209, temporarily designated *Ctenodaphnia* sp. 1, also constituted a distinct lineage.

Levels of COI sequence divergence within clusters were small, with maximum pairwise divergences ranging from 0.3% within *D. ornithocephala* to 3.9% in *D. spinulata* (Table 1). By contrast, divergences between clusters ranged from 20.8 to 30.1% (Table 2). The clusters were strongly supported, as evidenced by bootstrap values of 100. However, the relationships among clusters were poorly resolved (Fig. 7).

Allozyme variation

The allozyme results (Table 5) supported the recognition of all six 'species' identified by mtDNA analysis. Also paralleling the mtDNA results, *D. spinulata* and *D. notacantha* were not genetically distinct. Consequently, these taxa were pooled for all subsequent analyses and statistics. Allelic arrays of the putative species were distinct, and allelic substitutions were present at two or more loci between each species pair (Table 5).

Nei's genetic distances (*D*) between conspecific populations were smaller than distances between species. Maximum *D*-values between conspecific populations ranged from 0.13 in *D. spinulata* to 0.87 in *D. dadayana* (Table 1). By comparison, the average distances between pairs of species ranged from 0.94 (between *D. spinulata* and *D. similis*) to 6.7 (Table 2). In several species pairs, genetic distances could not be estimated as no alleles were shared.

In general, genotypic frequencies were close to HW equilibrium. Among the 24 populations of *D. dadayana*, only a single locus in one population (149) was out of HW equilibrium, due to a heterozygote deficit. Likewise, a single locus in one of the four populations of *D. ornithocephala* (229) differed significantly from HW equilibrium, due in this case to an excess of heterozygotes. There were no significant HW departures among the nine populations of *D. menucoensis*. HW deviations were similarly detected in only two of 27 *D. spinulata* populations. In one population (66), only a single locus was out of HW equilibrium, while another polymorphic locus in this same population was in HWE. The other population (from a road side puddle, 71) showed fixed heterozygosity at two loci but was monomorphic at other loci. The sole population of *Ctenodaphnia* sp. 1 had genotypic frequencies that were concordant with HW expectations. As only a single individual of *D. similis* was examined, genotype frequency analysis was not possible for this species.

Estimates of within-population variation were similar among most *Ctenodaphnia* species (Table 1).

Levels of polymorphism and heterozygosity were lowest in *D. ornithocephala*. Observed levels of heterozygosity were close to those expected based on allele frequencies. Evidence of strong local differentiation among conspecific populations was detected in all species, as F_{ST} statistics ranged from 0.24 to 0.52 (Table 1).

Evaluation of relatedness to North American Ctenodaphnia species

The comparison of COI sequences from Argentine *Ctenodaphnia* with those from all known North American species revealed two shared taxa. The individual of *D. similis* from Argentina was closely related to North American individuals, showing just 1.9% and 1.4% divergence from *D. similis* from Nevada and Washington, respectively. By comparison, the two North American sequences were 2.3% divergent from one another (Fig. 8).

The commonest *Ctenodaphnia* in Argentina, *D. spinulata*, was closely allied with the North American species *D. exilis* (Figs 8, 9). Two mitochondrial clusters were identified within *D. spinulata*, showing an average of 3.1% COI divergence from one another (Figs 7, 9). The commoner of these Argentine clusters, Group A, was only 1.6% divergent from haplotypes in North American populations of *D. exilis*, while Group B showed an average of 3.1% divergence from *D. exilis* (Fig. 9). By way of comparison, the maximum divergence among Argentine COI sequences was 3.9%, while maximum divergence among the North American *D. exilis* sequences was only 1.9% (Table 1). The NJ analysis grouped all North American haplotypes together, but with low bootstrap support (24) (Fig. 9).

In contrast to the mtDNA results, UPGMA analysis of allozyme variation revealed a clear genetic separation between *D. spinulata* and *D. exilis* populations (Fig. 10). The mean Nei's genetic distance between populations from these two taxa was 0.46. The maximum genetic distance among North American *D. exilis*

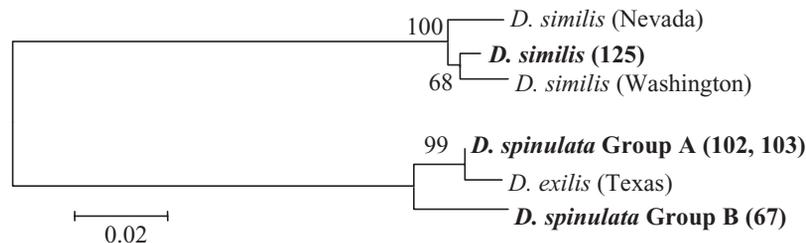


Figure 8. NJ tree based on COI sequences for two populations of North American and one population of South American *Daphnia similis*. Populations of Argentine *D. spinulata* and North American *D. exilis* are included for comparison. South American sequences are indicated in bold. The scale bar represents K2P distance.

Table 5. Mean allele frequencies at seven allozyme loci for six Argentine species of the subgenus *Ctenodaphnia*. Abbreviations: *N* = number of populations; *n* = number of individuals

Alleles	<i>spinulata</i> (<i>N</i> = 36)	<i>similis</i> (<i>N</i> = 1)	<i>menucoensis</i> (<i>N</i> = 9)	<i>dadayana</i> (<i>N</i> = 24)	<i>ornithocephala</i> (<i>N</i> = 4)	sp. 1 (<i>N</i> = 1)
AAT	(<i>n</i> = 1303)	(<i>n</i> = 1)	(<i>n</i> = 237)	(<i>n</i> = 649)	(<i>n</i> = 86)	(<i>n</i> = 39)
0.77	–	–	0.04	–	–	–
0.85	–	–	–	0.05	–	–
0.96	–	1.00	–	0.95	–	–
1.00	–	–	0.96	–	–	–
1.03	0.72	–	–	–	–	0.82
1.05	–	–	–	–	1.00	–
1.12	0.28	–	–	–	–	0.18
FUM	(<i>n</i> = 1153)	(<i>n</i> = 1)	(<i>n</i> = 187)	(<i>n</i> = 625)	(<i>n</i> = 86)	(<i>n</i> = 11)
0.70	–	–	0.95	–	–	–
0.76	–	–	–	<0.01	–	–
0.85	–	–	–	1.00	–	–
0.91	0.98	1.00	–	–	–	0.18
0.98	–	–	0.05	–	–	–
1.00	–	–	–	–	1.00	–
1.03	–	–	–	–	–	0.82
1.23	0.02	–	–	–	–	–
GPI	(<i>n</i> = 916)	(<i>n</i> = 1)	(<i>n</i> = 277)	(<i>n</i> = 871)	(<i>n</i> = 87)	(<i>n</i> = 33)
0.79	–	–	–	<0.01	–	–
0.92	–	–	1.00	0.49	–	1.00
0.95	–	–	–	–	1.00	–
1.00	–	1.00	–	0.39	–	–
1.11	1.00	–	–	0.12	–	–
LDH	(<i>n</i> = 384)	(<i>n</i> = 1)	(<i>n</i> = 51)	(<i>n</i> = 390)	(<i>n</i> = 22)	(<i>n</i> = 44)
0.72	<0.01	–	–	–	–	–
0.80	–	–	–	–	1.00	–
0.84	–	–	–	1.00	–	–
0.87	1.00	1.00	1.00	–	–	–
1.03	–	–	–	–	–	1.00
1.14	–	–	–	<0.01	–	–
ME	(<i>n</i> = 532)	(<i>n</i> = 0)	(<i>n</i> = 148)	(<i>n</i> = 157)	(<i>n</i> = 64)	(<i>n</i> = 11)
0.78	–	n/a	1.00	–	–	–
0.90	–	n/a	–	–	1.00	–
0.93	1.00	n/a	–	1.00	–	–
0.95	–	n/a	–	–	–	1.00
MPI	(<i>n</i> = 1196)	(<i>n</i> = 1)	(<i>n</i> = 313)	(<i>n</i> = 752)	(<i>n</i> = 103)	(<i>n</i> = 33)
0.92	–	–	0.01	–	–	–
0.97	–	–	0.01	0.02	–	–
1.06	–	–	0.01	–	0.78	–
1.11	–	–	0.40	–	–	–
1.12	–	–	–	0.73	–	–
1.16	–	–	–	–	0.22	–
1.18	–	–	–	0.25	–	–
1.23	0.20	1.00	0.49	–	–	–
1.30	0.80	–	0.08	–	–	1.00
PGM	(<i>n</i> = 962)	(<i>n</i> = 1)	(<i>n</i> = 315)	(<i>n</i> = 732)	(<i>n</i> = 82)	(<i>n</i> = 33)
0.92	–	1.00	–	–	–	–
0.98	–	–	–	–	0.82	–
1.04	–	–	<0.001	<0.01	0.18	1.00
1.11	1.00	–	1.00	0.09	–	–
1.18	<0.01	–	–	0.90	–	–

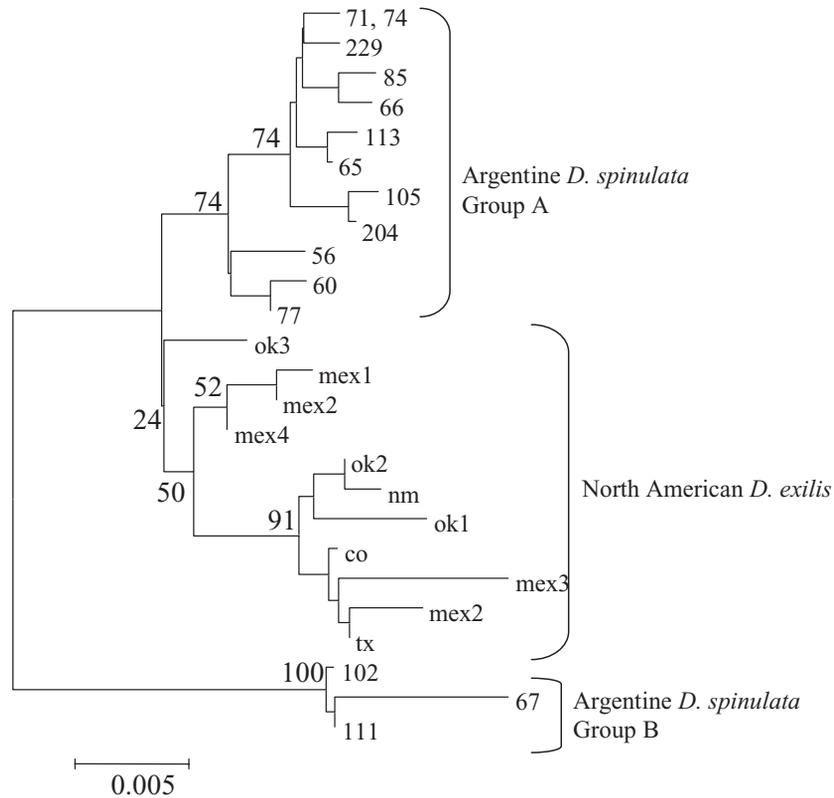


Figure 9. NJ tree based on COI sequence variation among a sample of *Daphnia spinulata* populations from Argentina and *D. exilis* populations from North America. The scale bar represents K2P distance. The codes for Argentine populations are provided in Appendix 1, while *D. exilis* codes are found in Appendix 2.

populations was 0.45, compared with a maximum of 0.15 for *D. spinulata*, based on the same seven loci (Table 1).

The remaining *Ctenodaphnia* species (*D. dadayana*, *D. menucoensis*, *D. ornithocephala*, and *Ctenodaphnia* sp. 1) appeared to have no close North American allies, as they were 18–28% divergent from all North American *Ctenodaphnia* species. However, an affiliation between *D. menucoensis* from Argentina and *D. salina* from North America, which showed 23% divergence in the COI gene, was detected by phylogenetic analysis using the slower-evolving 12S gene (S. J. Adamowicz, J. K. Colbourne & P. D. N. Hebert, unpubl. data).

DISCUSSION

SPECIES DIVERSITY OF ARGENTINE DAPHNIA: INTERPRETATION OF GENETIC EVIDENCE

This study provides the first genetic evaluation of species boundaries in the *Daphnia* of Argentina and presents a comprehensive survey of habitats throughout the country. This analysis has revealed the presence of

15 species among the 176 populations assayed. While additional rare species probably await discovery, the current results constitute a solid foundation for a taxonomic revision of the genus in Argentina and provide the necessary framework for biogeographical and evolutionary studies.

Interpretation of the genetic results was generally straightforward. Ten of the 11 species in the subgenera *Ctenodaphnia* and *Daphnia* that were characterized by allozyme surveys were diploid and reproduced by cyclic parthenogenesis, the sole exception involving polyploid populations in the *D. pulex* complex (Adamowicz *et al.*, 2002). Moreover, intra- and interspecific divergences among COI sequences were consistent with divergence values previously reported in *Daphnia* (e.g. Schwenk *et al.*, 2000). Although allozyme divergences were generally larger than values previously reported for *Daphnia*, this reflects the deliberate analysis of a small number of polymorphic loci. Furthermore, divergences among conspecific populations were always smaller than interspecific divergences, supporting the present taxonomic conclusions.

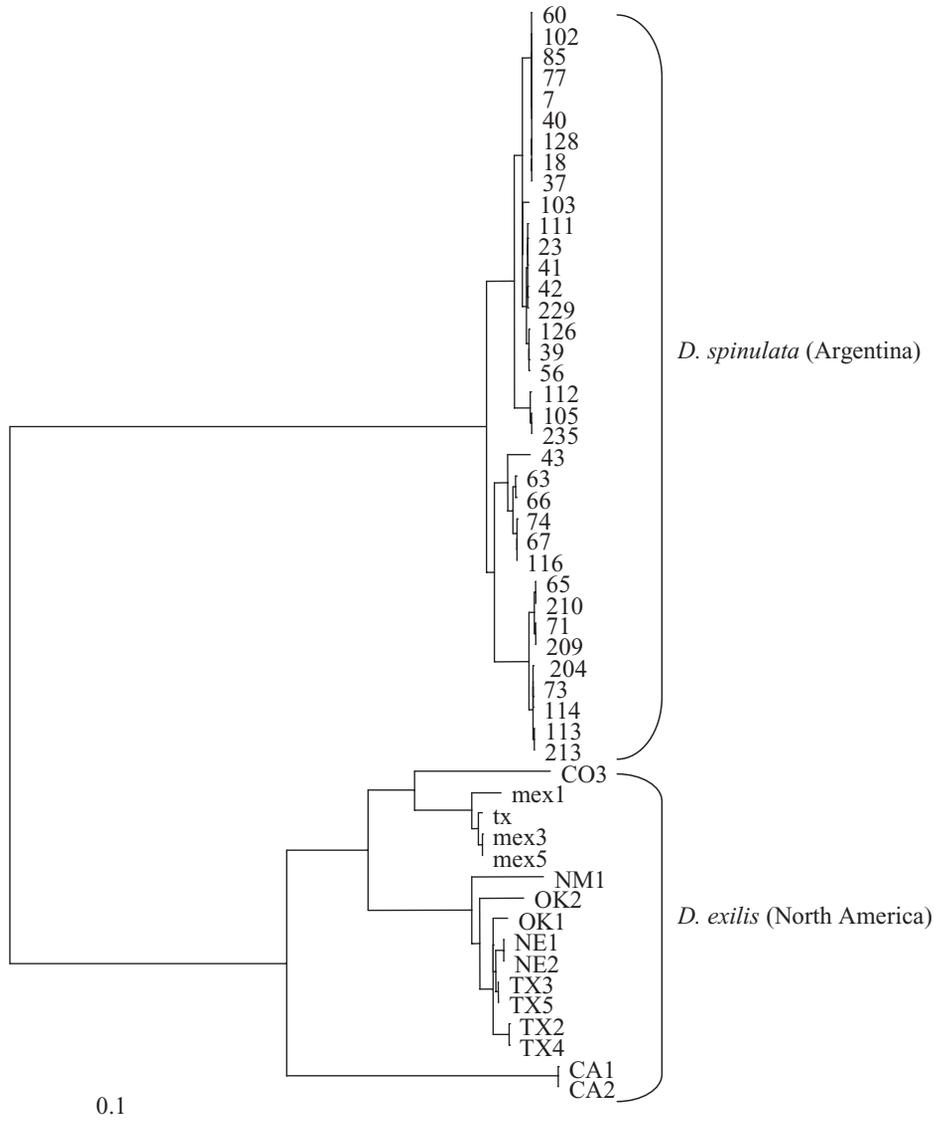


Figure 10. UPGMA tree based on allozyme variation at seven loci in *Daphnia spinulata* populations from Argentina and *D. exilis* populations from North America. Data for most of the *D. exilis* populations are from Hebert & Finston (1993), but trimmed to the same seven loci surveyed in the Argentine populations. Codes for their populations are in capital letters and indicate the state where each was collected. Codes in small letters represent new *D. exilis* data and are found in Appendix 2, while the *D. spinulata* codes are in Appendix 1. The scale bar represents Nei's genetic distance.

Subgenus Daphnia

The present genetic analyses suggest that the interpretation of species boundaries among the Argentine members of subgenus *Daphnia* is simple. This fact is remarkable considering the confused taxonomic history of this group in South America. The older literature is rife with dubious varieties of *D. obtusa*, *D. pulex*, and other poorly-described species that are mistrusted by modern investigators. By contrast, the genetic evidence provides clear support for the recognition of seven species.

The genetic analyses show that specimens historically identified as *D. obtusa* actually comprise three species. The congruence between the mitochondrial and allozyme results suggests that each taxon possesses monophyletic mitochondrial lineages and an isolated nuclear gene pool. Although the three species formerly assigned to *D. obtusa* are allied, their large interspecific divergences suggest a long period of evolutionary independence. Furthermore, no evidence of interspecific hybridization was detected among these species, as heterozygotes were never detected at loci

exhibiting fixed differences between these species. Reproductive isolation was even apparent in the case of sympatry of *D. obtusa* 1 and 3 (site 159). Thus, the genetic evidence strongly supports the conclusion that these three groups warrant recognition as separate species. The melanized species (*D. obtusa* 3) corresponds to a taxon that has been previously called '*D. middendorffiana*' (Paggi, 1973). However, *D. middendorffiana* s.s. is a member of the *D. pulex* complex, while its Argentine namesake possesses elongate setae along its internal carapace margin, indicating its membership in the *obtusa* complex (Paggi, 1998). *D. obtusa* 3 is morphologically distinctive, but differences between *D. obtusa* 1 and 2 are more subtle, although individuals of *D. obtusa* 2 ordinarily possess smaller heads than the other taxon (Fig. 1).

The three Argentine species in the *D. obtusa* complex are not closely related to any North American members of this group, as they show more than 17% divergence at COI from all northern species (*D. cheraphila*, *D. obtusa*, *D. neoobtusa*, *D. pileata*, *D. prolata*). While phylogenetic relationships among North and South American species require investigation, it is clear that populations from the two continents are not conspecific and that new names should be assigned to the three Argentine species. The proper identification of *D. obtusa* 1 is probably *D. brasiliensis* (Lubbock, 1855). It is also possible that species names assigned by Daday (1902) or varietal names listed in Olivier (1962) correspond to the other two taxa and should perhaps be resurrected.

Although members of the *obtusa* complex are dominant, a member of the *D. pulex* complex is also common in southern Argentina. The mitochondrial results suggest that these populations are closely allied to North American *D. pulicaria* (Adamowicz *et al.*, 2002). However, as allozyme evidence indicated that these are tetraploid asexuals of hybrid origin, the classification of these populations remains problematic. These lineages are probably *D. pulex* × *D. pulicaria* hybrids, but the paternal species is not known with certainty (see Adamowicz *et al.*, 2002).

Daphnia ambigua from Argentina is closely related to North American *D. ambigua* (Hebert *et al.*, 2003). Thus, this species is now known to be widely distributed in southern South America, as well as in North America. However, the clustering of mitochondrial haplotypes suggested that populations of *D. ambigua* on the two continents may represent a case of incipient divergence (Hebert *et al.*, 2003).

Daphnia parvula was represented in the present collections by just two individuals from the Coronda River, which may have been flooded out of reservoirs located upstream in the Paraná River system. Among all species from North America, these individuals

were most closely related to *D. parvula* from Mexico, from which they showed 12% COI divergence. Unfortunately, COI data are unavailable for *D. retrocurva*, the only other known North American member of the *retrocurva* complex (Colbourne & Hebert, 1996). However, other evidence suggests that Argentine and North American *D. parvula* are not sister taxa, and should perhaps be considered different species. At the slower-evolving mitochondrial gene (12S rRNA), the Argentine individuals display 5% divergence from both *D. parvula* and *D. retrocurva* (data from Colbourne & Hebert, 1996; S. J. Adamowicz, unpubl. data). On the other hand, several *D. parvula* isolates from a wide geographical range in North America show <1% 12S divergence from one another and only 1–1.5% divergence from *D. retrocurva*. Thus, South American *D. parvula* probably represents a new species belonging to the *retrocurva* complex, but further sampling of this taxon is necessary.

Finally, *D. peruviana*, a highly-melanized mountain species described from Peru (Harding, 1955), was confirmed to represent a divergent lineage. As this species does not have any close North or South American relatives, it is apparently a southern endemic.

Subgenus *Hyalodaphnia*

Although the taxonomy of the *Hyalodaphnia* was not clearly resolved in the present analysis, some important insights were obtained. All *Hyalodaphnia* populations were morphologically identified as either *D. gessneri* or *D. laevis*, species differing only in head shape. Previous studies on members of this subgenus have shown that head shape is an unreliable taxonomic feature, as it is highly plastic and seasonally variable (Dodson, 1988). The present study confirmed incongruence between morphological identifications and mitochondrial lineages. Prior work has revealed that interspecific hybridization often underlies phenotypic variability in *Hyalodaphnia* (Wolf & Mort, 1986; Taylor & Hebert, 1993a), and this also appears likely in Argentina. Both populations of '*D. laevis*' and one of '*D. gessneri*' showed extremely high levels of heterozygosity, suggesting that these assemblages were comprised largely of hybrids. A more detailed investigation, combining morphological, mtDNA, and allozyme analyses on the same individuals, would be necessary to verify the occurrence of hybridization and to clarify the taxonomy. It would also be desirable to analyse additional populations. Former studies have shown that the detection of 'pure' parental populations, as evidenced by their conformity to HW expectations, is useful for elucidating species boundaries and the incidence of introgression in hybridizing species complexes (Taylor & Hebert, 1993a,b, 1994). Although the current study examined only a few pop-

ulations, the detection of two mitochondrial lineages showing 13% COI divergence, along with the elevated levels of allozyme heterozygosity, does suggest that the *laevis* complex in Argentina includes two divergent 'species' that frequently hybridize.

Although Argentine '*D. laevis*' may be a valid species, this name is not appropriate, since the South American populations are highly divergent (>17% at COI) from those of North America. Paggi (1977) first described Argentine *D. laevis* from a population from the province of Tucumán, in the northern part of the country. He noted several subtle differences between the Argentine animals and their North American counterparts (Brooks, 1957b), including a longer tail spine, more extensive setation along the valve margin, and a slightly different head shape. Since these characters are known to be variable and subject to change during cyclomorphosis, he concluded that the Argentine populations should be assigned to *D. laevis*. However, genetic evidence indicates that Argentine and North American lineages of *D. 'laevis'* are highly divergent, suggesting the need for description of a new species from Argentina.

The name *D. gessneri* is appropriate for the second lineage in the Argentine *laevis* complex, since this species was described from South America (Herbst, 1967). However, it is clear that further genetic work and a re-examination of its diagnostic morphological features are necessary. Although based on a small sample size, it is interesting that several individuals with the head morphology of *D. gessneri* possessed '*laevis*'-type mitochondrial haplotypes, but not the reverse. Taken alone, this result could indicate that the helmeted forms represent either pure *D. gessneri* or *D. gessneri* × *D. laevis* hybrids, while unhelmeted forms represent pure '*D. laevis*', an interpretation consistent with results on hybridizing *Hyalodaphnia* in the northern hemisphere (Taylor & Hebert, 1993a). However, the allozyme data suggest a more complex situation, since both *D. 'gessneri'* and *D. 'laevis'* populations displayed elevated levels of heterozygosity, suggestive of a hybrid origin. Cyclomorphosis may confound the interpretation of these genetic results, as it is possible that helmets are seasonally present in some of the populations that were found to be 'unhelmeted' during the present study. A complete understanding of the taxonomy of hybridizing *Hyalodaphnia* species complexes is possible, but generally requires an intensive approach, involving repeated sampling and genetic and morphological analyses.

Subgenus *Ctenodaphnia*

Of the three subgenera, the *Ctenodaphnia* have been the most amenable to traditional taxonomy. As opposed to the situation in the *Daphnia* and

Hyalodaphnia, the different Argentine *Ctenodaphnia* species are generally separated by large and concordant morphological, mitochondrial, and allozymic divergences. Genetic evidence supports the species status of *D. dadayana*, *D. menucoensis*, *D. ornithocephala*, and *D. spinulata*, which constitute four of the five *Ctenodaphnia* species previously recognized by morphologists (Paggi, 1998). However, neither mtDNA nor allozyme evidence support the recognition of a fifth morphospecies, *D. notacantha*.

Daphnia notacantha is morphologically similar to *D. spinulata*, barring a conspicuous hump on the dorsal surface of its head. However, the present results establish that individuals of *D. notacantha* and *D. spinulata* possess identical, or nearly so, mitochondrial haplotypes, and are allozymically indistinguishable. A possible explanation for this genetic similarity is that *D. spinulata* and *D. notacantha* speciated only very recently, and that allozyme and COI divergence has not yet occurred. However, some adults of *D. spinulata* possessed a hint of a *notacantha*-like hump, indicating a gradation in head morphology. Moreover, since juvenile *D. spinulata* often resemble the *notacantha* form (M. C. Marinone, pers. observ.), this distinct head shape in adults may represent a neotenic maintenance of the trait, which could be either environmentally induced or genetically determined. The fact that other *Daphnia* species possess inducible head structures, such as helmets and neck-teeth, suggests the possibility that the '*notacantha*' head protuberance is a predator-induced morphological form of *D. spinulata*. If so, its presence may coincide with that of a particular predator. Interestingly, in their recent description of *D. inca*, Kořínek & Villalobos (2003) note that this Andean species coexists with juveniles and some adult females resembling *D. notacantha*. They point out that the *notacantha* form may represent a predator-induced morphology in several South American *Ctenodaphnia* species, but for now recognize *D. inca* and *D. notacantha* as separate species. Genetic information would be helpful in resolving the species boundaries among *D. spinulata*, *D. inca*, and the enigmatic *D. notacantha*.

Genetic analysis suggests that the taxonomic status of *D. spinulata* itself requires examination because of its close affiliation with the North American species, *D. exilis*. As these taxa show just 1.5–3% COI sequence divergence, they are clearly closely allied. Moreover, the fact that all COI sequences for *D. exilis* are nested within the greater diversity present in *D. spinulata* suggests that one or a few dispersal events from South America could have established the North American populations (Fig. 9). However, allozyme variation suggests a different history. North and South American populations appear to be differenti-

ated at nuclear markers, and, furthermore, there is greater nuclear diversity among North American populations (Table 1; Fig. 10). The discrepancy between levels of mitochondrial and allozyme diversity might be explained by invoking a hypothetical scenario involving several dispersal events of (Group A) *D. spinulata* from South to North America. This would account for the similarity between Group A and North American mitochondrial haplotypes, while admixture of diverging gene pools would explain the elevated allozyme diversity in North America. While the actual evolutionary history of these populations remains unknown, it is clear that *D. spinulata* and *D. exilis* are closely related. However, given their divergence at nuclear loci, along with their allopatric distributions, it is likely that populations on the two continents are now on independent evolutionary pathways. Moreover, there are diagnostic morphological traits that distinguish the males of these species (M. C. Marinone, pers. observ.). Thus, it seems appropriate to maintain recognition of the Argentine and North American populations as distinct species.

The present study is the first to document the presence of *D. similis* in Argentina. This species is clearly rare in most of Argentina as it was only detected at a single locality. The sole Argentine individual sequenced shows less than 2% COI divergence from two North American isolates, suggesting that populations from the two continents are conspecific. Moreover, the other Argentine individual possessed allozyme alleles that were consistent with those reported among North American populations of *D. similis* (Hebert & Finston, 1993). A genetic comparison of populations of *D. similis* from the Americas with those from the Old World is necessary to validate the use of this taxon name in the New World.

A single population of a new *Ctenodaphnia* species was detected ('*Ctenodaphnia* sp. 1') and is supported by both allozyme and mitochondrial results. This taxon appears to constitute an old lineage that may be endemic to South America. Preliminary morphological study indicated that individuals of this species possess morphological features (e.g. very large antennular mounds, weak tail spines, unusual shape of the post-abdomen of males) that distinguish them from other *Ctenodaphnia* from Argentina.

SUMMARY OF SPECIES DIVERSITY

Fifteen species of *Daphnia* were identified by genetic criteria in Argentina. The genetic results revealed that some species are consistent with previously described morphospecies. However, other species are incorrectly named or were previously unrecognized. Given that at least four undescribed or inadequately described species were revealed during this survey, as

well as two species that had not been documented in Argentina, a few additional species may await discovery. Studies in the high alpine and northern regions of the country may be particularly fruitful.

CONTINENTAL ENDEMISM AND INTERCONTINENTAL DISPERSAL IN *DAPHNIA*

Among the 15 species identified in this study, four (*D. ambigua*, *D. 'pulicaria'*, *D. similis*, *D. spinulata*) are either conspecific or very closely allied with North American species. A population of a fifth shared species, *D. pulex*, has been detected in Chile by a molecular survey of populations (S. J. Adamowicz, unpubl. data) and, recently, in the Argentine province of Neuquén, based on morphology (M. C. Marinone, pers. observ.). On the other hand, 11 species were found to be South American endemics. Additionally, three species known from morphological study are considered to be Andean endemics: *Daphnia inca* Kořínek & Villalobos (2003), *Daphniopsis chilensis* Hann (1986), and *D. marcahuasensis* Valdivia Villar & Burger (1989). Since members of the genus *Daphniopsis* are likely properly assigned to *Ctenodaphnia* (Hrbáček, 1987; Colbourne & Hebert, 1996), it would be desirable for future genetic studies on South American daphniids to include these taxa. Although further South American surveys are necessary to reveal additional narrowly-distributed species, it is likely that the majority of *Daphnia* species on this continent is now known either genetically or morphologically, as most major geographical regions harbouring suitable habitats have been studied. Thus, current evidence suggests that 14 of 19 species now known from South America (or 74%) are endemic to this continent.

North America is home to nearly twice as many *Daphnia* species as South America, as 34 taxa have been recorded (Hebert, 1995). Part of this difference is attributable to the higher intensity of sampling in North America. However, the difference probably also reflects a real difference in species richness. The lower diversity of South America can be explained by the fact that much of this continent lies in the tropics, a setting where *Daphnia* diversity is low (Fernando, 1980; Fernando *et al.*, 1987; Fernando & Paggi, 1998). Interestingly, a similar proportion of North American species (66%) is endemic to this continent, while its other species are shared with South America or Eurasia. Australia, a much smaller continent, harbours approximately the same number of species as South America. About 21 Australian species are currently known (P. D. N. Hebert, unpubl. data), all belonging to the subgenus *Ctenodaphnia*. Five of these species have also been reported from Asia or Africa (Hebert, 1978), although only one of

these shared species, *D. lumholtzi*, has been genetically confirmed (Havel *et al.*, 2000). The remaining shared records are dubious, as the former morphospecies *D. carinata* s.l. has been shown to comprise a species complex of several species within Australia alone (Hebert, 1977; Hebert & Wilson, 1994). Thus, Australia's fauna contains a higher proportion (at least 76%, probably closer to 90–95%) of endemics than North or South America, reflecting the greater isolation of this continent.

Patterns of species richness and endemism cannot yet be compared between South America and Africa or the Eurasian landmass, as the daphniid faunas of the latter area have not been adequately characterized using genetic tools. Patterns of morphological similarity indicate that this would be an interesting endeavour. The nature of daphniid species distributions could be better understood by verifying the identity of those species nominally shared between South America and Africa: *D. gessneri*, *D. laevis*, *D. ornithocephala*, *D. pulex*, *D. pulicaria*, and *D. similis* (Kořínek, 1999; 2002). Moreover, opposing biogeographical hypotheses could be tested using genetic data from species from different continents. It has been argued that the distributions of *Daphnia* clades reflect ancient vicariance events in Earth's history, especially the break-up of Gondwanaland (Benzie, 1987). Recent molecular studies have confirmed the pre-Gondwanan age of this genus (Colbourne & Hebert, 1996; Schwenk *et al.*, 2000), indicating that a phylogenetic study of the faunas of South America, Australia, and Africa might expose a Gondwanan signature. On the other hand, the present study revealed that intercontinental dispersal events do occur, suggesting that modern movements of taxa have also influenced clade distributions. Thus, an interesting avenue for future work would be to compare the roles of ancient geological events and recent bird-mediated dispersal in structuring contemporary biogeographical patterns in daphniids.

CONCLUDING REMARKS

This genetic investigation has significantly advanced our knowledge of the taxonomy and biogeography of the genus *Daphnia*. While formal taxonomic descriptions are needed for several newly-recognized species, and further study will likely reveal additional species, a solid foundation now exists for future studies on South American daphniids. Moreover, with the delineation of species boundaries, investigations of interesting evolutionary phenomena and patterns, such as phylogenetic relationships, phylogeography, processes of speciation, and the evolutionary consequences of long-distance dispersal, are possible.

The present taxonomic evaluation has already revealed some interesting biogeographical patterns.

While most Argentine daphniids are endemic to South America, migration events between North and South America are surprisingly common given the great distances involved. Such intercontinental migration is apparently of substantial evolutionary significance. Phylogeographical analyses of the shared North and South American taxa suggest that many populations from the two continents are in the early stages of divergence, indicating that intercontinental dispersal rates are not high enough to homogenize gene pools. This evidence illustrates that allopatric speciation at a global scale is an important mechanism of diversification in this genus, a finding contrary to the predictions of earlier investigators (e.g. Mayr, 1963), who thought that the dispersal powers of many zooplankton taxa would preclude a significant role for allopatric divergence in their evolution.

The taxonomic framework established in this study builds on the previous extensive work done on the genus *Daphnia*. There is now comprehensive knowledge of the taxonomy and distributions of species from several major regions of the world, including North America, Europe, Australia, and now, southern South America. This basic information provides the opportunity for far-reaching explorations of the diverse factors that have influenced the evolutionary diversification of this prominent freshwater organism.

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APPENDIX 1
COLLECTION SITES FOR ARGENTINE *DAPHNIA* POPULATIONS

Most species designations are determined by genetic analyses. However, members of the subgenus *Hyalodaphnia* (*D. laevis* and *D. gessneri*) are listed by morphological identification, as many of these populations appear to consist of hybrids, which require further attention (see text). Also, populations morphologically identified as *D. notacantha* are indicated as such, although these populations are genetically indistinguishable from *D. spinulata*. The type of data collected from each population is indicated in parentheses after each species name as follows: A = allozymes only; D = DNA only (COI sequence); B = both. Abbreviations for Argentine provinces: BA = Buenos Aires; CH = Chubut; CO = Córdoba; LP = La Pampa; LR = La Rioja; ME = Mendoza; NE = Neuquén; RN = Río Negro; SC = Santa Cruz; SF = Santa Fe, SJ = San Juan; SL = San Luis; TF = Tierra del Fuego; TU = Tucumán. Translations of informative Spanish words that are found in many of the locality names: cabo = cape; chorrillo = stream; Ea. (estancia) = ranch; embalse = reservoir; lago = lake; laguna = small, generally shallow, lake; puerto = port; punto = point; río = river; valle = valley. Not all numbered sites are listed, since only those sites from the larger Argentina survey containing *Daphnia* are included here. Sample 10 was provided by J. Paggi, 247 and 248 by H. E. Zagarese, and 259 by A. Villagra de Gamundi. GenBank accession numbers are provided for all unique haplotypes, except for nine sequences which contained a large number of undetermined nucleotide positions.

Site	<i>Daphnia</i> species present (data type)	Sampling locality (directions)	Habitat type	Conductivity (in µS)/Turbidity	Lat. (S)	Long. (W)	COI GenBank No
1	<i>D. obtusa</i> 1 (D)	San Francisco Reservoir, BA	reservoir	1480/high	31°40'	58°52'	AY323050
7	<i>D. spinulata</i> (B)	Laguna Navarro, BA	deflation lake	2330/slight	35°02'	59°17'	AY323099
9	<i>D. parvula</i> (D)	El Río Coronda, SF (pier, town of Coronda)	river	320/slight	31°58'04"	60°54'49"	AY323126
10	<i>D. gessneri</i> (B)	lab culture established from small lake near Colastiné River, SF	small lake	—/—	—	—	AY323071
13	<i>D. obtusa</i> 1 (D)	Las Varillas, CO (haplotype)	roadside pond	1130/—	31°51'36"	62°43'07"	AY323072
15	<i>D. obtusa</i> 1 (B)	Villa María, CO (W side of Rd. 157, 11 km N of town)	roadside pond	600/—	31°55'41"	62°47'38"	AY323048
16	<i>D. obtusa</i> 1 (B)	La Francia, CO (W side of Rd. 3, 3 km S of town)	roadside pond	390/black	31°24'19"	62°38'45"	same as 1
17	<i>D. obtusa</i> 1 (B)	Las Varillas, CO (W side of Rd. 158, S of town)	roadside pond	370/—	31°53'45"	62°44'57"	same as 1
18	<i>D. obtusa</i> 1 (D)	Villa María, CO (N side of Rd. 2, ~20 km W of town)	roadside ditch	480/—	32°21'48"	62°56'53"	same as 1
20	<i>D. spinulata</i> (B)	Bell Ville, CO (W side of Rd. 3, ~30 km N of town)	pond	160/—	32°25'31"	62°41'04"	AY323100
22	<i>D. obtusa</i> 1 (B)	Bell Ville, CO (E side of Rd. 3, ~24 km N of town)	roadside pond	330/—	32°24'37"	62°41'07"	same as 1
23	<i>D. spinulata</i> (B)	Bell Ville, CO (by stream near village of Cintra)	roadside pond	390/—	32°20'12"	62°52'17"	same as 18
26	<i>D. obtusa</i> 1 (B)	Villa María, CO (S side of Rd. 2, ~15 km W of town)	large pond	—/—	32°21'25"	62°55'10"	same as 1

27	<i>D. obtusa</i> 1 (D)	Bell Ville, CO (W side of Rd. 3, ~20 or 30 km N of town)	roadside pond	440/-	32°27'31"	62°49'35"	same as 1
30	<i>D. laevis</i> (B)	Embalse Río Tercero, CO	reservoir	150/clear	32°10'50"	64°25'10"	AY323073
32	<i>D. laevis</i> (B)	José de la Quintana, CO (5 km S of village, near San Agustín)	small reservoir	105/fairly clear	31°50'15"	64°26'04"	same as 30
36	<i>D. laevis</i> (D)	Almafuerte, CO (Rd. 36, just W of town)	small reservoir	-/-	32°11'	64°17'	same as 30
37	<i>D. obtusa</i> 1 (B)	Highway 35, LP (W side, ~165 km N of Santa Rosa)	large roadside pond	800/-	35°05'47"	64°15'53"	AY323049
38	<i>D. spinulata</i> (B)	Highway 35, CO (E side, southern CO province)	large pond	3400/-	34°57'25"	64°18'07"	AY323101
39	<i>D. spinulata</i> (B)	Highway 35 CO (W side, southern CO province)	roadside ditch	1280/-	34°36'34"	64°22'34"	AY323114
40	<i>D. obtusa</i> 1 (B)	Highway 35, southern CO	roadside ditch	580/-	34°20'23"	64°22'53"	same as 1
41	<i>D. spinulata</i> (B)	Highway 35, southern CO	roadside ditch	580/-	34°11'47"	64°22'58"	AY323102
42	<i>D. obtusa</i> 1 (D)	35, CO (E side, southern CO)	roadside ditch pond	2750/muddy	34°06'36"	64°22'24"	AY323103
43	<i>D. spinulata</i> (B)	Highway 35, southern CO	marshy pond	880/-	34°05'13"	64°22'23"	AY323104
46	<i>D. obtusa</i> 1 (D)	Highway 35 (W side, southern CO)	roadside ditch	270/muddy	33°47'52"	64°25'50"	same as 18
48	<i>D. obtusa</i> 1 (B)	Highway 35, CO (58.7 km S of intersection of 35 and 8)	roadside ditch pond	95/-	33°43'32"	64°25'38"	same as 1
51	<i>D. menucoensis</i> (B)	Laguna El Carancho, LP	shallow saline lake	24 000/-	37°26'46"	65°04'24"	AY323074
53	<i>D. menucoensis</i> (B)	Highway 35, LP (W side, just N of intersection of 35 and I54)	saline lake	14 000/-	37°06'44"	64°17'09"	AY323075
54	<i>D. spinulata</i> (D)	Highway 35, LP	marsh	1720/-	37°16'03"	64°17'14"	AY323076
56	<i>D. spinulata</i> (B)	Highway 154, LP (W side, ~46 km S of intersection of 35 and 154)	swamp	720/-	38°08'48"	64°04'12"	same as 18
60	<i>D. spinulata</i> (B)	Highway 251, LP (E side, N of General Conesa)	culvert pond	2270/-	40°26'27"	65°01'11"	AY323105
63	<i>D. spinulata</i> (B)	Road 23, LP (S side, 4.9 km W of turn-off from Highway 3)	mud puddle	1480/-	40°49'26"	65°23'46"	AY323117
65	<i>D. spinulata</i> (B)	Rd. 23, LP (N side, 34.6 km W of turn-off from Highway 3)	mud puddle	2230/-	40°51'40"	65°44'19"	AY323106
66	<i>D. spinulata</i> (B)	Rd. 23, LP (S side, 40.5 km W of turn-off from Highway 3)	mud puddle	650/-	40°51'40"	48°26'52"	AY323107
67	<i>D. spinulata</i> (B)	Rd. 23, LP (S side, ~65 km W of Highway 3)	sandy pond	650/-	40°46'09"	66°02'21"	AY323108

APPENDIX 1 Continued

Site	<i>Daphnia</i> species present (data type)	Sampling locality (directions)	Habitat type	Conductivity (in μS)/Turbidity	Lat. (S)	Long. (W)	COI GenBank No
68	<i>D. menucoensis</i> (B)	Valcheta, RN (W side of Rd. 4, 27.9 km N of Valcheta)	clay puddle	43 000/turbid	40°26'28"	66°03'22"	AY323077
70	<i>D. menucoensis</i> (B)	Los Menucos, RN (N side of Rd. 23, ~11 km W of town)	shallow salt lake	22 500/clear	40°47'24"	68°00'40"	same as 68
71	<i>D. spinulata</i> (B)	Valcheta, RN (S side of Rd. 23, 6.9 km W of town)	clay pond	460/turbid	40°38'34"	66°14'48"	AY323109
73	<i>D. notacantha</i> (B)	Valcheta, RN (S side of Rd. 23, 11.6 km W of town)	clay puddle	700/very turbid	40°37'49"	66°17'46"	same as 18
74	<i>D. spinulata</i> (B)	Valcheta, RN (S side of Rd. 23, 37.7 km W of town)	mud puddle	8000/murky	40°32'47"	66°31'11"	same as 71
77	<i>D. spinulata</i> (B)	Los Menucos, RN (S side of Rd. 23, 7.2 km E of rail tracks)	pond	1890/-	40°47'23"	68°01'01"	AY323110
79	<i>D. dadayana</i> (B)	Maquinchao, RN (Rd. 23, 38.4 km W of town)	muddy pond	600/-	41°18'12"	69°08'40"	AY323083
85	<i>D. spinulata</i> (B)	Junín de los Andes, NE (W side of Highway 40, 44 km W of city)	marsh	1800/moderately turbid	40°18'28"	70°38'46"	AY323111
86	<i>D. obtusa</i> 1 (B)	Junín de los Andes, NE (E side of Rt. 234, S of city, N of San Martín)	pond	250/nearly clear	40°04'32"	71°06'44"	AY323051
89	<i>D. obtusa</i> 3 (B)	Lago Rivadavia, CH	Andean lake	55/very clear	42°36'04"	71°38'36"	same as 94
91	<i>D. obtusa</i> 1 (B)	Cholila, CH (W side Rd. 71, just N of town sign)	mud puddle	620/nearly clear	42°29'33"	71°27'52"	AY212052
92	<i>D. obtusa</i> 1 (B)	Cholila, CH (E side of Rd. 71, a few km S of town)	pond	260/nearly clear	42°30'29"	71°29'27"	AY323053
93	<i>D. obtusa</i> 3 (D)	Lago Futalaufquen (north arm), CH (sample taken at Playa del Francés)	Andean lake	50/very clear	42°47'25"	71°43'40"	same as 94
94	<i>D. obtusa</i> 3 (B)	Lago Futalaufquen, CH (sample taken near Hotel Futalaufquen)	Andean lake	-/very clear	42°51'41"	71°37'27"	AY323065
96	<i>D. dadayana</i> (B)	Esquel, CH (N side of Rt. 40, 11.7 km E of town)	marshy pond	2580/-	42°57'46"	71°11'38"	AY323084
97	<i>D. obtusa</i> 1 (B)	Esquel, CH (E side of Rt. 40, 68.8 km E of town)	roadside pond	1300/fairly clear	43°17'48"	70°53'21"	AY323054
98	<i>D. obtusa</i> 2 (B)	Laguna Quichaura, CH (~1 km in from rd., S side of Rt. 62, 15.3 km E of Rt. 40)	shallow, weakly saline lake	8000/-	43°31'53"	70°38'24"	AY323059
99	<i>D. dadayana</i> (B)	Rt. 62, CH (S side of Rt. 62, just W of intersection with 63)	farm dam	2700/-	43°44'04"	70°01'56"	AY323085

100	<i>D. obtusa</i> 1 (B)	Tecka, CH (E side of Rt. 40, ~10 km N of town, near I594 km sign)	pond	310/weakly turbid	43°22'44"	70°52'50"	–
101	<i>D. obtusa</i> 2 (D)	Rt. 62, CH (N side of Rt. 62, 44.9 km E of intersection with 63)	shallow, weakly saline lake	10 800/–	43°44'57"	69°29'52"	AY323060
102	<i>D. spinulata</i> (B)	Rt. 62, CH (S side of Rt. 62, ~85 km E of Los Altares)	roadside pond	4150/–	43°43'22"	67°22'17"	AY323112
103	<i>D. spinulata</i> (B)	Las Plumas, CH (N side of Rt. 25, 32 km W of town)	excavated pond	2020/weakly turbid	43°42'38"	66°54'44"	same as 102
105	<i>D. dadayana</i> (B) <i>D. obtusa</i> 1 (B)	Rawson, CH (E side of Rt. 1, 88.1 km N of Punta Tombo)	grassy pond	270/–	43°25'53"	65°17'58"	AY323086 AY323055
108	<i>D. spinulata</i> (B) <i>D. dadayana</i> (B)	Península Valdés, CH (W side of Rt. 47, 6.7 km N of intersection with 52)	mud pan	1000/extremely turbid	42°31'37"	64°09'52"	AY323113 AY323087
111	<i>D. spinulata</i> (B)	Puerto Madryn, CH (W side of Rt. 3, ~35 km N of intersection with Rt. 2)	large playa lake	1710/–	42°19'12"	65°11'42"	–
112	<i>D. spinulata</i> (B)	Sierra Grande, RN (Rt. 3, ~11 km S of town)	playa pond	300/–	41°50'16"	65°17'16"	same as 112
113	<i>D. spinulata</i> (B)	Sierra Grande, RN (W side of Rt. 3, ~19 km N of town)	excavated pond	600/–	41°21'15"	65°21'58"	AY323115
116	<i>D. spinulata</i> (A)	Sierra Grande, RN (E side of Rt. 3, ~57 km N of town)	excavated pond	360/–	41°02'28"	65°23'41"	–
118	<i>D. obtusa</i> 2 (B)	Sierra Grande, RN (W side of Rt. 3, ~42 km N of Sierra Grande)	excavated pond	420/weakly turbid	41°10'35"	65°22'57"	AY323061
122	<i>D. obtusa</i> 1 (D)	Dufaur, BA (back road, ~20 km W of town, between 2 lakes)	roadside ditch	3600/–	37°54'38"	62°33'28"	same as 1
123	<i>D. spinulata</i> (D)	Dufaur, BA (back road, ~20 km W of town, between 2 lakes)	muddy pond	3000/–	37°54'38"	62°33'28"	AY323116
124	<i>D. menucoensis</i> (B)	Laguna Las Encadenadas de Tornquist, BA (W of Dufaur)	saline playa lake	16 000/–	37°53'37"	62°32'44"	AY323078
125	<i>D. similis</i> (B)	Laguna Puán, BA	eutrophic lake	6100/–	37°33'00"	62°46'29"	AY323121
126	<i>D. spinulata</i> (A)	Puán, BA (dirt road, ~7 km N of town)	playa lake	2000/–	37°29'07"	62°44'33"	–
128	<i>D. spinulata</i> (B)	Laguna de Lobos, BA	eutrophic lake	4480/–	35°16'32"	59°07'45"	same as 63

APPENDIX 1 Continued

Site	<i>Daphnia</i> species present (data type)	Sampling locality (directions)	Habitat type	Conductivity (in µS)/Turbidity	Lat. (S)	Long. (W)	COI GenBank No
132	<i>D. obtusa</i> 1 (D)	Valle del Río Deseado, SC (W side of Rt. 12 [aka 501], S of Pico Truncado)	stream-bed pond	4270/clear	46°53'05"	68°08'43"	AY323056
133	<i>D. menucoensis</i> (B)	Rt. 3, SC (W side of Rt. 3, near km 2193 of this highway)	shallow saline lake	35 600/moderately turbid	48°48'43"	67°40'56"	AY323079
135	<i>D. obtusa</i> 2 (D)	Rt. 3, SC (W side of Rt. 3, km 2202 of this highway, near duct)	weakly saline pond	8200/–	48°53'33"	67°40'29"	AY323062
	<i>D. 'pulicaria'</i> (B)						same as 205
	<i>D. menucoensis</i> (D)						same as 133
136a	<i>D. menucoensis</i> (B)	Rt. 3, SC (W side, near km 2202)	shallow weakly saline lake	8200/weakly turbid	48°53'33"	67°40'29"	AY323080
136b	<i>D. obtusa</i> 2 (B)	Rt. 3, SC (E side, near km 2202)	pond	2620/–	48°53'33"	67°40'29"	same as 135
137a	<i>D. menucoensis</i> (B)	Rt. 3, SC (E side, near km 2216)	shallow lake	5200/weakly turbid	48°59'58"	67°39'26"	AY323081
137b	<i>D. menucoensis</i> (B)	Rt. 3, SC (E side, near km 2250)	shallow saline lake	16 800/weakly turbid	49°17'23"	67°46'53"	same as 133
138	<i>D. dadayana</i> (B)	Rt. 3, SC (E side, near km 2246)	small shallow lake	385/weakly turbid	49°15'00"	67°46'44"	AY323088
139	<i>D. dadayana</i> (B)	Rt. 3, SC (W side, near km 2245)	small lake	309/moderately turbid	49°14'05"	67°46'33"	AY323089
141	<i>D. obtusa</i> 2 (A)	Rt. 3, SC (E side, just S of Río Chico)	roadside ditch	2010/turbid	49°47'25"	68°38'58"	–
	<i>D. dadayana</i> (A)						–
142	<i>D. dadayana</i> (B)	Cmte. Luis Piedra Buena, SC (S side of Rt. 2605 [a.k.a. 17], N side of Río Santa Cruz)	small shallow lake	1520/turbid	50°00'57"	69°01'43"	AY323090
144	<i>D. dadayana</i> (B)	city of Río Gallegos, SC (S side of Rt. 1, 7 km E of turn-off from Rt. 3)	pond	500/turbid	51°47'55"	69°16'08"	AY323091
147	<i>D. 'pulicaria'</i> (A)	Chorrillo de los Frailes, SC (S side of Rt. 1, 24 km E of turn-off from Rt. 3)	river-bed pond	2800/turbid	51°54'22"	69°07'43"	–
148	<i>D. dadayana</i> (B)	Cabo Virgenes, SC (S side of Rt. 1, 89 km E of turn-off from Rt. 3)	large excavated pond	381/clear, tannic	52°16'46"	68°38'56"	AY323093
149	<i>D. dadayana</i> (A)	Cabo Virgenes, SC (S side of Rt. 1, 96 km E of turn-off from Rt. 3)	pond	580/turbid	52°17'51"	68°34'21"	–
150	<i>D. dadayana</i> (B)	Cabo Virgenes, SC (S side of Rt. 1, 104 km E of turn-off from Rt. 3)	pond	580/very turbid	52°19'16"	68°28'45"	same as 148
151	<i>D. dadayana</i> (B)	Cabo Virgenes, SC (S side of Rt. 1, 82 km E of turn-off from Rt. 3)	shallow lake	580/turbid	52°14'22"	68°42'19"	same as 148

153	<i>D. 'pulicaria'</i> (A)	Chorrillo de los Frailes, SC (N side of Rt. 1, 24 km E of turn-off from Rt. 3)	pond	1400/clear	51°54'22"	69°07'43"	—
156	<i>D. 'pulicaria'</i> (B)	city of Río Grande, TF (E side of Rt. 3, ~8 km N of town)	quarry pond	382/clear, darkly tannic	53°43'47"	67°47'08"	same as 205
158	<i>D. dadayana</i> (B)	Ea. María Behety, TF (NE side of Rt. C, 7 km NW of estancia)	roadside pond	50/turbid	53°44'45"	68°00'53"	same as 148
159	<i>D. obtusa</i> 1 (A)	city of Río Grande, TF (N side of Rt. C, just W of turn-off from Rt. 3)	large excavated pond	970/moderately	53°47'15"	67°47'46"	—
160	<i>D. obtusa</i> 3 (A)						—
160	<i>D. 'pulicaria'</i> (B)						AF489524
160	<i>D. 'pulicaria'</i> (A)	city of Río Grande, TF (N side of Rt. C, just beside Laguna de los Cisnes)	excavated pond	62/moderately turbid	53°47'32"	67°48'57"	—
161	<i>D. dadayana</i> (B)						AY323092
161	<i>D. dadayana</i> (B)	Ea. María Behety, TF (S side of Rt. C, between Ea. M. Behety and Ea. Los Flamings)	roadside pond	250/nearly clear	53°44'45"	68°00'53"	same as 148
162	<i>D. obtusa</i> 3 (B)	Laguna San Luis, TF (sampled from Ea. San Luis)	holding tank with lake water	600/clear	53°54'49"	67°36'03"	—
163	<i>D. obtusa</i> 1 (A)	Río Grande, TF (W side of Rt. 3, just N of 2885 km marker)	roadside pond	92/nearly clear	54°08'57"	67°15'45"	—
164	<i>D. dadayana</i> (B)	Laguna San Luis, TF	lake	580/slightly	53°54'49"	67°36'03"	same as 148
165	<i>D. obtusa</i> 3 (A)	Río Julio, TF (beside river valley, S side of Rt. 3, near km 2893)	pond	100/nearly clear	54°12'32"	67°12'56"	—
167	<i>D. obtusa</i> 1 (A)	Río Grande, TF (W side of Rt. 3, just N of 2915 km marker)	pond in bog	139/clear, darkly tannic	54°22'38"	67°14'59"	—
169	<i>D. obtusa</i> 1 (D)	Laguna Victoria, TF (N side of Rt. J, 12 km E of turn-off from Rt. 3)	small lake	34/clear, tannic	54°46'35"	67°42'03"	same as 132
170	<i>D. 'pulicaria'</i> (A)	Ea. Harberton, TF (Rt. J, ~2 km along NW of estancia)	grass plain pond	33/tannic	54°52'37"	67°18'22"	—
171	<i>D. 'pulicaria'</i> (B)	Ea. Harberton, TF (Rt. J, just W of estancia)	large pond	3550/tannic	54°52'23"	67°20'06"	same as 205
172	<i>D. obtusa</i> 1 (B)	Ea. Harberton, TF (N side of Rt. J, 4 km W of estancia)	grassy plain pond	170/clear	54°52'23"	67°23'43"	same as 132
173	<i>D. obtusa</i> 3 (A)	Rt. J, TF (S side, 33 km E of turn-off from Rt. 3)	pond in bog	159/clear, tannic	54°51'31"	67°27'48"	—
174	<i>D. 'pulicaria'</i> (A)						—
174	<i>D. obtusa</i> 3 (D)	near Laguna Bombilla, TF (left side of dirt road, toward lake, several km off Rt. 3)	excavated pond	62/clear	54°36'29"	67°49'31"	—
	<i>D. 'pulicaria'</i> (A)						—

APPENDIX 1 Continued

Site	<i>Daphnia</i> species present (data type)	Sampling locality (directions)	Habitat type	Conductivity (in μS)/Turbidity	Lat. (S)	Long. (W)	COI GenBank No
175	<i>D. pulicaria</i> ' (A)	near Laguna Bombilla, TF (left side of dirt road, heading to lake, several km off Rt. 3)	pond	58/moderately turbid	54°36'20"	67°51'46"	–
178	<i>D. obtusa</i> 1 (A)	Tolhuin, TF (S side of Rt. 3, between La Prefectura and Tolhuin)	grassy pond	115/clear	54°35'38"	67°35'13"	–
183	<i>D. pulicaria</i> ' (A) <i>D. obtusa</i> 1 (D)	Tolhuin, TF (2nd pond, same site as 178)	grassy pond	125/clear	54°35'38"	67°35'13"	– same as 132
186	<i>D. dadayana</i> (A)	Rt. 5, SC (N side of Rt. 5, near 197 km of this highway)	large shallow pond	160/very turbid	50°31'26"	71°27'03"	–
188	<i>D. obtusa</i> 3 (B)	Rt. 5, SC (near km 58)	muddy pond	700/clear	51°21'59"	70°15'30"	–
189	<i>D. pulicaria</i> ' (B)	Rt. 5, SC (N side, near km 188)	pond	3600/turbid	51°01'11"	70°50'43"	–
191	<i>D. dadayana</i> (A)	Rt. 5, SC (near km 58)	pond	700/clear	51°21'59"	70°15'30"	AY323066
193	<i>D. obtusa</i> 3 (D) <i>D. obtusa</i> 1 (B)	near Río La Leona, SC (NW side of Rt. 40, by bridge, ~20 km N of of Rt. 11)	pond by river	230/clear	50°09'30"	71°59'18"	– same as 132
194	<i>D. obtusa</i> 1 (D)	near Río La Leona, SC (SE side of Rt. 40, by bridge, pond with macrophytes)	pond by river	305/clear	50°09'30"	71°59'18"	– same as 132
195	<i>D. obtusa</i> 1 (B)	near Río La Leona, SC (SE side of Rt. 40, by bridge, one of several ponds)	pond by river	280/clear	50°09'30"	71°59'18"	– same as 132
196	<i>D. dadayana</i> (A)	Rt. 40, SC (W side of Rt. 40, ~59 km N of Tres Lagos)	large pond	600/turbid	49°13'14"	71°21'18"	–
197	<i>D. dadayana</i> (B)	Rt. 40, SC (E side of Rt. 40, ~66 km N of Tres Lagos)	pond on volcanic rock substrate	220/transparent	49°10'02"	71°18'33"	AY323094
199	<i>D. obtusa</i> 3 (B)	Rt. 40, SC (E side of rt. 40, by bridge ~135 km N of Ea. La Angostura)	culvert pond	131/slightly turbid	47°49'20"	70°48'39"	–
201	<i>D. pulicaria</i> ' (B)	Las Heras, SC ('Cargadero Sur 07', S side of Rt. 43, 5 km E of town)	1st quarry pond	4780/clear	46°33'00"	68°46'12"	AF489523
202	<i>D. pulicaria</i> ' (B)	Las Heras, SC ('Cargadero Sur 07', S side of Rt. 43, 5 km E of town)	2nd quarry pond	2000/clear	46°33'00"	68°46'12"	– same as 205
203	<i>D. dadayana</i> (B)	Sarmiento, CH (NE side of Rt. 20, 32 km E of town)	pond	1900/very turbid	45°42'46"	68°52'08"	AY323095
204	<i>D. obtusa</i> 1 (B) <i>D. notacantha</i> (B)	Sarmiento, CH (SW side of Rt. 20, 16 km E of town)	pond	218/slightly turbid	45°35'57"	69°00'07"	AY323057 – same as 209

205	<i>D. 'pullicaria'</i> (B)	Las Heras, SC ('Cargadero Sur 07', S side of Rt. 43, 5 km E of town)	3rd quarry pond	5100/slightly turbid	46°33'00"	68°46'12"	AF489525
206	<i>D. obtusa</i> 1 (B)	Sarmiento, CH (SW side of Rt. 20, ~8 km E of town)	pond	1100/nearly clear	45°37'04"	68°58'36"	same as 204
207	<i>D. notacantha</i> (D)						same as 209
	<i>D. dadayana</i> (B)	Sarmiento, CH (W side of Rt. 24, ~7 km N of petrified forest reserve)	pond	780/turbid	45°46'38"	69°04'51"	AY323096
209	<i>D. spinulata</i> (B)	Sarmiento, CH (left side of dirt road heading from town to Lago Musters, at sharp corner, ~4 km from town)	pond	7500/very turbid	45°34'50"	69°05'08"	AY323118
	<i>Ctenodaphnia</i> sp. #1 (B)						AY323122
210	<i>D. obtusa</i> 1 (B)	Sarmiento, CH (W side of Rt. 24, between town and petrified forest park)	farm dam	1600/clear	45°38'13"	69°04'21"	same as 204
212	<i>D. spinulata</i> (B)						same as 209
	<i>D. obtusa</i> 1 (B)	Sarmiento, CH (W side of Rt. 24, ~12 km S of town)	large pond	200/moderately turbid	45°41'08"	69°05'00"	same as 204
213	<i>D. spinulata</i> (B)	Sarmiento, CH (left side of dirt road heading from town to Lago Musters, ~6 km from town)	ditch	550/turbid	45°34'45"	69°05'27"	same as 209
225	<i>D. obtusa</i> 1 (A)	Pampa de Achala, CO (left side of Rt. 20 from Villa Carlos Paz heading towards Mina Clavero, past El Condor)	sluggish stream pond	77/clear	31°36'42"	64°46'43"	–
226	<i>D. gessneri</i> (B)	Lago San Roque, CO (sample taken near dam)	reservoir	250/nearly clear	31°22'30"	64°26'06"	same as 30
229	<i>D. notacantha</i> (B)	Ulapes, LR (E side of Rt. 79, 5 km S of town)	farm dam	260/turbid	31°34'46"	66°12'06"	AY323119
231	<i>D. ornithocephala</i> (B)						AY323123
	<i>D. ornithocephala</i> (D)	Candelaria, SL (E side of Rt. 79, ~4 km S of exit for town)	ditch (puddle)	145/turbid	32°04'49"	65°49'31"	AY323124
233	<i>D. ornithocephala</i> (B)	Ulapes, LR (E side of Rt. 79, at fork with exit to town)	pond	690/turbid	31°34'11"	66°13'37"	same as 229
234	<i>D. ornithocephala</i> (B)	Ulapes, LR (ranch on E side of Rt. 79, ~8 km N of town)	farm dam	234/fairly clear	31°30'05"	66°13'19"	AY323125

APPENDIX 1 Continued

Site	<i>Daphnia</i> species present (data type)	Sampling locality (directions)	Habitat type	Conductivity (in μ S)/Turbidity	Lat. (S)	Long. (W)	COI GenBank No
235	<i>D. spinulata</i> (A)	Chepes, LR (S side of Rt. 141, near 60 km marker)	farm dam	510/turbid	31°22'22"	66°52'36"	–
237	<i>D. ambigua</i> (B)	Embalse Paso de las Carretas, SL (sample taken near dam)	reservoir	590/nearly clear	33°19'39"	65°52'18"	AF523691
242	<i>D. ambigua</i> (B)	Embalse La Florida, SL (sample taken near dam)	reservoir	200/clear	33°11'	66°01'	same as 237
243	<i>D. obtusa</i> 1 (D)	Parque General Paz, city of Buenos Aires, BA	pond	–/clear	34°34'12"	58°30'36"	same as 1
244	<i>D. obtusa</i> 2 (D)	Rt. 3, RN (km 1119, N of San Antonio Oeste, RN)	pond	–/very turbid	40°43'	64°59'	AY323063
245	<i>D. obtusa</i> 1 (D)	Reserva Ecológica Costanera Sur, city of Buenos Aires, BA	pond	–/clear	34°36'36"	58°21'36"	AY323058
246	<i>D. spinulata</i> (D)	Laguna de Gómez, BA	pond	–/clear	34°37'	61°07'	same as 123
247	<i>D. obtusa</i> 3 (D)	Laguna Jabón, NE	shallow lake	1555/–	38°58'41"	70°22'28"	AY323067
248	<i>D. obtusa</i> 2 (D)	Laguna Toro, Ea. Providencia, CH	shallow weakly saline lake	14 600/–	44°22'47"	70°35'56"	AY323064
249	<i>D. obtusa</i> 1 (D)	Parque Paseo de las Américas, city of Buenos Aires, BA	puddle	–/clear	34°33'00"	58°26'24"	same as 1
250	<i>D. obtusa</i> 1 (D)	Laguna El Carbón, BA	shallow lake	–/clear	36°59'	57°00'	same as 1
251	<i>D. obtusa</i> 3 (D)	Laguna Cronómetro, CH	shallow lake	–/clear	43°15'	71°02'	AY323068
252	<i>D. spinulata</i> (D)	near Los Horcones, BA	pond	–/clear	37°00'	56°56'	AY323120
253	<i>D. obtusa</i> 3 (D)	Lago El Martillo, CH	foothill lake	–/transparent	42°53'	71°31'	AY323069
254	<i>D. dadayana</i> (D)	Laguna Nahuel Pan, CH	shallow lake	–/turbid	42°57'38"	71°11'11"	AY323097
255	<i>D. ambigua</i> (D)	Lago Rosario, CH	foothill lake	–/clear	43°15"	71°22'	AF523692
256	<i>D. obtusa</i> 1 (D)	dirt road near Laguna Salada Grande de General Madariaga, BA	roadside pool	–/turbid	36°55'	56°58'	same as 1
257	<i>D. dadayana</i> (D)	Lago Stúñica, CH	shallow lake	–/turbid	43°07'	71°04'	AY323098
258	<i>D. spinulata</i> (D)	Laguna El Tigre, BA	shallow lake	–/weakly turbid	36°59'	57°02'	same as 42
259	<i>D. menucoensis</i> (D)	Laguna Escondida, TU (Cumbres Calchaquites)	Andean shallow lake (4350 m asl)	–/clear	26°40'	65°44'	AY323082
	<i>D. peruviana</i> (D)						AY323070

APPENDIX 2

COLLECTION SITES FOR NORTH AMERICAN *DAPHNIA* SPECIES SEQUENCED FOR COI

Species are listed by subgenus and by species complex, following the groupings proposed by Colbourne & Hebert (1996). J. Colbourne provided most of the COI sequences, which he mainly sequenced from the same individuals used for the phylogenetic analyses based on the 12S gene (Colbourne & Hebert, 1996). The remaining species were sequenced for the present study from the archived collections of P.D.N.H., or from DNA or samples provided by F. M. Jerónimo, C. Prokopench, or S. Schwartz. Site codes are provided for populations of *D. exilis*.

Species complex	Species	Locality	Source
SUBGENUS <i>CTENODAPHNIA</i>			
<i>atkinsoni</i>	<i>D. salina</i>	Shoe Lake, Saskatchewan	J.C.
<i>ephemeralis</i>	<i>D. ephemeralis</i>	pond near Guelph, Ontario	J.C.
<i>lumholtzi</i>	<i>D. lumholtzi</i>	Pomme de Terre Lake, Missouri	J.C.
<i>magna</i>	<i>D. magna</i>	pond near Crescent Lake, Nebraska	J.C.
<i>similis</i>	<i>D. exilis</i>	pond near Allende, Mexico (mex1)	P.H.
	<i>D. exilis</i>	second pond near Allende, Mexico (mex2)	P.H.
	<i>D. exilis</i>	pond near Mata, Mexico (mex3)	P.H.
	<i>D. exilis</i>	pond near Mexico City (mex4)	P.H.
	<i>D. exilis</i>	pond near Santo Domingo, Mexico (mex5)	P.H.
	<i>D. exilis</i>	pond near Ordway, Colorado (co)	P.H.
	<i>D. exilis</i>	pond near Storrie Lake, New Mexico (nm)	P.H.
	<i>D. exilis</i>	pond near Chandler, Oklahoma (ok1)	P.H.
	<i>D. exilis</i>	pond, Oklahoma (ok2)	S.S.
	<i>D. exilis</i>	pond near Watkins, Oklahoma (ok3)	S.S.
	<i>D. exilis</i>	pond near Amarillo, Texas (tx)	J.C.
	<i>D. similis</i>	pond near Soap Lake, Washington state	J.C.
	<i>D. similis</i>	pond near Fernley, Nevada	P.H.
SUBGENUS <i>DAPHNIA</i>			
<i>ambigua</i>	<i>D. ambigua</i>	pond, Florida	J.C.
<i>catawba</i>	<i>D. catawba</i>	Wren Lake near Dorset, Ontario	J.C.
	<i>D. minnehaha</i>	pond near Sault St. Marie, Ontario	P.H.
<i>obtusa</i>	<i>D. cheraphila</i>	pond near Buffalo, South Dakota	P.H.
	<i>D. neo-obtusa</i>	pond near Bend, Oregon	J.C.
	<i>D. obtusa</i>	pond near Chandler, Oklahoma	J.C.
	<i>D. obtusa</i>	pond at Coal Tipple, Portsmouth, Ohio	C.P.
	<i>D. pileata</i>	pond near Mesa, Mexico	P.H.
	<i>D. prolata</i>	pond near Amarillo, Texas	P.H.
<i>pulex</i>	<i>D. middendorffiana</i>	pond on Longstaff Bluff, Baffin Island, Nunavut	J.C.
	<i>D. pulex</i>	pond near Windsor, Ontario	J.C.
	<i>D. pulicaria</i>	Guelph Lake, Ontario	J.C.
	<i>D. tenebrosa</i>	tundra pond near Churchill, Manitoba	J.C.
<i>retrocurva</i>	<i>D. parvula</i>	Little Presa, Mexico	P.H.
<i>villosa</i>	<i>D. oregonensis</i>	pond near Cow Creek, Oregon	P.H.
	<i>D. villosa</i>	pond near Soap Lake, Washington state	P.H.
SUBGENUS <i>HYALODAPHNIA</i>			
<i>curvirostris</i>	<i>D. curvirostris</i>	pond near Tuktoyaktuk, N.W.T.	J.C.
<i>laevis</i>	<i>D. dubia</i>	Wren Lake near Dorset, Ontario	J.C.
	<i>D. magniceps</i>	pond in Rondeau Park, Ontario (formerly called <i>D. laevis</i> until the analyses of Taylor <i>et al.</i> , 1998)	J.C.
	<i>D. laevis</i>	reservoir near Mexico City	F.J.
<i>longiremis</i>	<i>D. longiremis</i>	lake on Melville Peninsula, Nunavut	J.C.
<i>longispina</i>	<i>D. dentifera</i>	Old Lake, Indiana	J.C.
	<i>D. mendotae</i>	Center Lake, Indiana	J.C.
	<i>D. thorata</i>	Flathead Lake, Montana	J.C.
	<i>D. umbra</i>	pond near Richards Bay, N.W.T.	J.C.