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BIOGEOGRAPHY OF A WIDESPREAD FRESHWATER CRUSTACEAN: PSEUDOCONGRUENCE AND CRYPTIC ENDEMISM IN THE NORTH AMERICAN *DAPHNIA LAEVIS* COMPLEX

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Abstract.—The lack of morphological variation in many freshwater invertebrates over vast distances has been cited as evidence for their frequent, long-distance dispersal. This scenario implies that vicariance will be an insignificant determinant of species distributions or diversity. We carried out a phylogeographic and population genetics study of one widespread crustacean group, the North American *Daphnia laevis* complex. Allozyme and sequence variation of two mtDNA genes (12S and 16S rRNA) clearly indicates the existence of five morphologically cryptic, largely allopatric groups (*Daphnia dubia, D. laevis laevis, D. laevis gessneri, D. magniceps magniceps*, and *D. magniceps pacifica* ssp. n.). Within each of these groups, there is weak or no genetic differentiation over large geographic areas suggesting their recent long-distance dispersal. The present-day distributions and phylogeography of the regional groups suggests the occurrence of both deep and shallow vicariance events. Although divergence times from mtDNA sequences do indicate both deep and shallow divergences, these estimates are incongruent with their proposed vicariance times. The results show that even within closely related freshwater invertebrates, a complex biogeography exists, whose analysis is made difficult by long-distance dispersal, cryptic endemism, and pseudocongruence.

Key words.—Allozymes, biogeography, Crustacea, *Daphnia*, dispersal, mtDNA.

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"We should not forget the probability of many fresh-water forms having formerly ranged continuously over immense areas and then having become extinct at intermediate points. But the wide distribution of fresh-water plants and of the lower animals . . . apparently depends in main part on the wide dispersal of their seeds and eggs by animals, more especially by fresh-water birds" Darwin (1859 p. 304).

Freshwater invertebrates have played a key role in the formation of biogeographical principles such as vicariance and dispersalism, but there are still few empirical studies beyond Darwin's original demonstrations and observations. Detailed species phylogenies are necessary to assess the importance and interactions of processes (e.g., Cracraft 1994), and these have been rare for freshwater invertebrates. For microcrustaceans the vast majority of studies have been ecological, and many groups are still regarded as species-poor with cosmopolitan distributions and little geographic structure. In this paper we examine the molecular biogeography of *Daphnia laevis* Birge 1878, a common and apparently widespread species of freshwater microcrustacean.

Many microcrustaceans seem like ideal candidates for the sort of long-distance dispersal envisaged by Lyell (1832) and Darwin (1859). Branchiopods, copepods, and ostracods, for example, possess resting stages that can survive desiccation—a requisite for any long voyage out of water. In addition, the eggs of some taxa have characteristics that both expose them to avian vectors and increase their hitchhiking ability. The eggs of branchiopod crustaceans are often covered by sticky envelopes, knobs, spines, or air-trapping dimples that impart buoyancy (Fryer 1996). These eggs also survive passage through avian guts (Proctor and Malone 1965) and are produced chiefly in the spring and autumn in temperate waters when waterfowl migration is at its peak. Finally, successful colonization of new habitats is aided by a clonal reproductive phase in the life cycle of some microcrustaceans—populations can be founded by a single propagule and finding a mate is never a limitation.

Even though a lack of geographic morphological variation and the potential for frequent long-distance dispersal are apparent in microcrustaceans, freshwater biogeographers have recently provided evidence of endemism and restricted gene flow. Part of this evidence comes from detailed biogeographical studies of recolonization after catastrophes like glaciation. Like other freshwater animals, microcrustaceans were trapped in ice-free refugia during the last glaciation. Yet, several thousand years after the glacial retreat, many microcrustaceans have failed to expand their ranges into apparently similar habitats much beyond the refugia, which suggests weak dispersal abilities (e.g., Hebert and Hann 1986; Stemberger 1995). Still other evidence of endemism comes from recent global comparisons of fauna based on modern taxonomies (Frey 1987; Hebert 1995).

Many crustacean groups exhibit the pattern that Darwin observed, namely that of geographically widespread, morphologically similar groups. Some genera, such as *Daphnia*, are notorious for their phenotypic plasticity; it is from studies of this genus that Woltereck (1909) first described the reaction norm concept. Later, Mayr (1942) noted that the extensive phenotypic plasticity of this genus made testing of biogeographic hypotheses by present-day distributional or morphological evidence intractable. Fortunately, molecular markers can aid critical tests of biogeographic and historical hypotheses. For the few species of *Daphnia* that have been examined over a broad geographic scale, there is weak to modest allozyme and mtDNA structure over distances of thousands of kilometers, even across potential geographic dispersal bar-

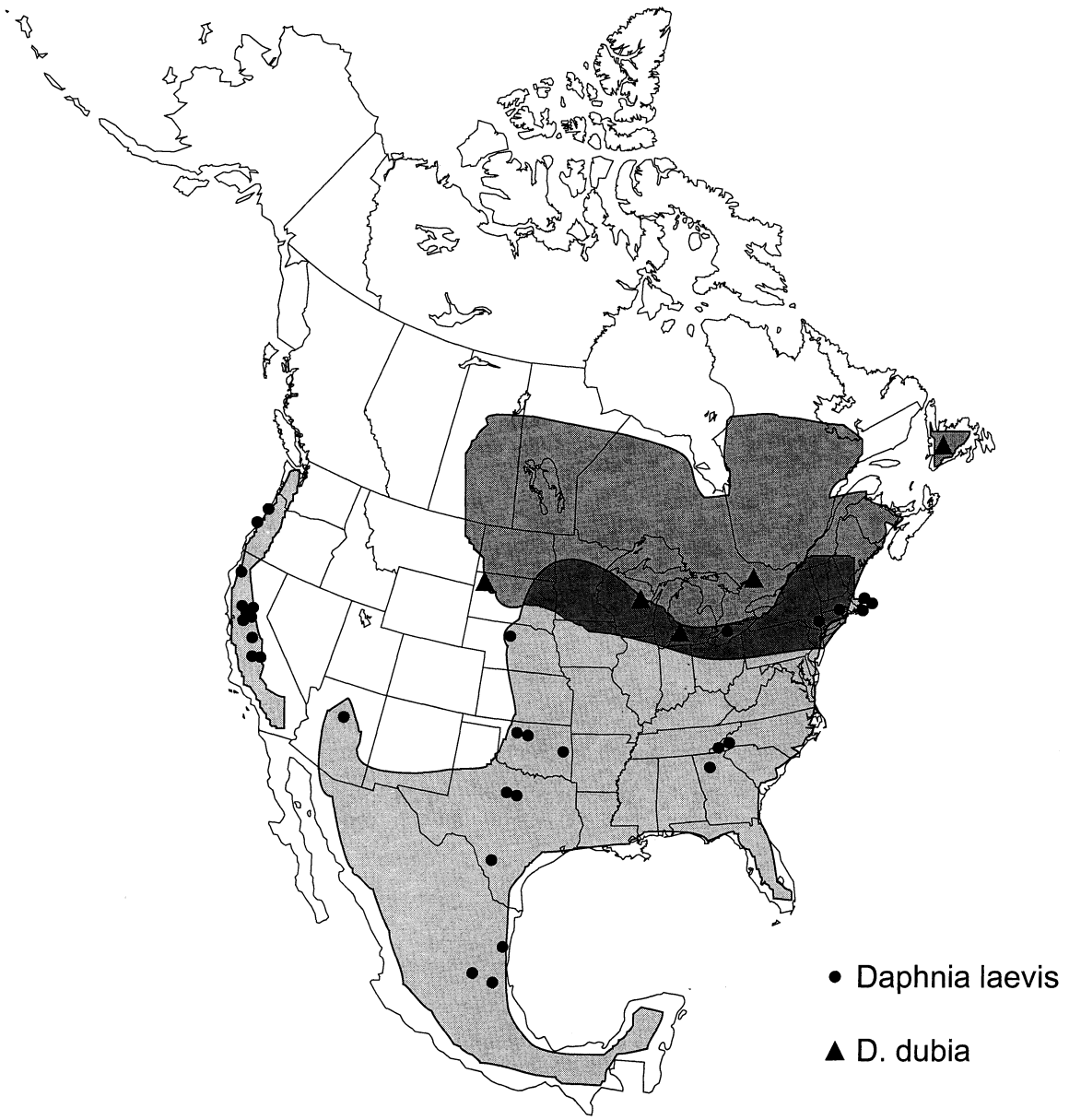

FIG. 1. Proposed geographic distributions of the two species commonly recognized in the North American *Daphnia laevis* complex (Brooks 1957; Keller and Pitbaldo 1989; Hebert 1995). Symbols represent populations examined for genetic variation in the present study.

riers like mountain ranges (Taylor and Hebert 1993; Cerny and Hebert 1993; Hebert and Wilson 1994; Hebert and Finston 1996; Taylor et al. 1996; Crease et al. 1997; Weider and Hobæk 1997). The main structuring that occurs seems to be related to the freshwater habitat continuum from temporary ponds to large lakes. That is there are numerous sister clades of *Daphnia* that are separated only by habitat and not geographic area (Brooks 1957; Lynch 1985; Taylor et al. 1996).

The *D. laevis* complex is an excellent group in which to examine the relative importance of biogeographic forces because its distribution traverses many geographic and ecological boundaries. Its geographic range includes the Nearctic, Neotropical, and Ethiopian regions (Brooks 1957; Green 1990; Taylor et al. 1996). According to Brooks (1957), one species (*D. dubia*) of this group is common in the glacial lakes of eastern North America, whereas the other (*D. laevis*) is common in ponds in the eastern half of the United States (Fig. 1). There is also a disjunct group of *D. laevis* populations along a coastal strip from California to British Columbia on the western side of the Cascade–Sierra Nevada range (Fig. 1) and another lacustrine type in the southeastern United States and Mexico that cannot be distinguished morphologically from the neotropical species *D. gessneri* (Herbst 1967).

Although *D. laevis* was the first species of *Daphnia* described from the New World (Birge 1878), the subject of the first experiments on the chemical requirements of *Daphnia* (see Hutchinson 1932), and the target of early evolutionary studies (Banta et al. 1939), its taxonomy remains confused

TABLE 1. Summary of allele frequencies observed in North American populations of the *Daphnia laevis* complex at seven allozyme loci. Alleles are labeled according to their relative mobility with a *Daphnia pulex* standard (the R_f of the rare allele *Gpi*^{d} was unmeasured). Population codes are explained in Appendix 1.

	Population													
Locus	ON1	NE1	OK1	TX1	TX2	TX3	OK2	AZ1	CA1	CA2	CA9	CA3	CA4	CA5
sAat														
(*n*)	22	39	19	46	40	40	20	20	40	40	20	38	38	39
0.85	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
0.92	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ao														
(*n*)	22	39	19	46	40	40	20	20	40	40	20	38	38	39
1.00	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
1.11	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Fumh														
(*n*)	22	39	19	46	40	40	20	20	40	40	20	38	38	39
0.81	.000	.000	.000	.000	.000	.000	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000
1.00	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000
1.15	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
Ldh														
(*n*)	22	39	19	46	40	40	20	20	40	40	20	38	38	39
1.03	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mpi														
(*n*)	22	39	19	46	40	40	20	20	40	40	20	38	38	39
1.12	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
1.14	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.013	.013	.525	.184	.868	.385
1.20	.000	.000	.000	.000	.000	.000	.000	.000	.988	.988	.475	.816	.132	.615
Gpi														
(*n*)	22	39	19	46	40	40	20	20	40	40	20	38	38	39
0.72	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.197	.000	.000
0.81	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.803	1.000	1.000
1.00	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
d	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
Pgm														
(*n*)	22	39	18	46	40	40	20	20	40	40	20	38	38	39
1.08	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
1.14	1.000	1.000	.444	1.000	.000	.538	.525	1.000	.250	.000	.000	.184	.197	.000
1.25	.000	.000	.556	.000	1.000	.463	.475	.000	.750	1.000	1.000	.816	.803	1.000

(e.g., Brooks 1957; Korinek 1984; Kraus 1986). In all, four species and one subspecies in the complex have been described: *Daphnia laevis* (Birge 1878; type locality Massachusetts); *Daphnia magniceps* (Herrick 1884; type locality Minnesota); *Daphnia dubia* (Herrick 1883; type locality Minnesota); *Daphnia gessneri* (Herbst 1967; type locality Brazil); and *Daphnia laevis tarasca* (Kraus 1986; type locality Mexico). The morphological differentiation among these taxa is based chiefly on length differences of a minor process on the postabdomen and notoriously plastic head shapes. The taxonomy has vacillated during this century from recognition of one species (*D. laevis*) to several, but most studies now follow Brooks (1957) who recognized only *D. laevis* and *D. dubia*. Brooks (1957) hypothesized that *D. dubia*, whose distribution is restricted to a segment of formerly glaciated portions of North America, was a post-Pleistocene derivative of the southern species, *D. laevis* (see Fig. 1).

This study aimed to determine, with multiple nuclear and mtDNA markers, the geographic structure and phylogenetic relationships of North American *D. laevis* populations. Furthermore, we tested two historical biogeographic hypotheses: (1) that formation of disjunct Pacific populations of *D. laevis* resulted from range disruption linked to formation of the Sierra Nevada and Cascade mountain ranges; and (2) that the glacial lake-dwelling species, *D. dubia*, is a post-Pleistocene derivative of the parapatric southern pond-dwelling species *D. laevis* (see Brooks 1957). Both of these hypotheses predict a spatial association of clades with a geographic boundary and an origin coincident with formation of the boundary. Finally, we aimed to develop a taxonomy for the *D. laevis* complex based on the concordance of independent, genetically based species-characters (Avise and Ball 1990).

MATERIALS AND METHODS

Specimen Collections

Populations of *Daphnia* were collected from 33 different sites in Canada, the United States, and Mexico (Appendix 1). Individuals belonging to the *D. laevis* complex (including *D. dubia*) were sorted from other species in the field using the key of Brooks (1957), and either flash frozen in liquid nitrogen, or ethanol preserved. Individuals of one population (Huzzy Lake, MI) could readily be assigned to

TABLE 1. Extended.

Locus	Population														
	CA6	CA7	CA8	OR2	MA1	MA3	CT1	MA2	NC2	NY1	MEX1	MEX3	MEX2	SD1	NFLD1
sAat															
(*n*)	21	20	40	37	20	20	4	20	20	4	18	7	32	40	8
0.85	.000	.000	.000	.108	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
0.92	1.000	1.000	1.000	.892	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Ao															
(*n*)	21	20	40	37	20	20	1	20	20	4	18	7	32	40	8
1.00	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	1.000
1.11	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.000	.000
Fumh															
(*n*)	21	20	40	37	20	20	4	20	20	4	18	7	32	40	8
0.81	1.000	1.000	1.000	1.000	.000	.000	.000	.000	.000	.000	.000	.357	.000	.000	.000
1.00	.000	.000	.000	.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.643	1.000	.000	.000
1.15	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	1.000
Ldh															
(*n*)	21	20	40	37	20	20	1	20	20	4	18	7	32	40	8
1.03	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
Mpi															
(*n*)	21	20	40	37	20	20	4	20	20	4	18	7	32	40	8
1.12	.000	.000	.000	.000	.000	.000	.125	.000	.000	.000	.000	.000	.000	.000	.000
1.14	.167	.625	1.000	.000	.000	.000	.000	.000	.000	1.000	.000	.000	.000	.000	.000
1.20	.833	.375	.000	1.000	1.000	1.000	.875	1.000	1.000	.000	1.000	1.000	1.000	1.000	1.000
Gpi															
(*n*)	21	20	40	37	20	20	1	40	20	4	18	7	32	40	8
0.72	.000	.000	.000	.000	.000	.000	.000	.125	.000	.000	.000	.000	.000	.000	.000
0.81	1.000	1.000	1.000	1.000	1.000	1.000	1.000	.875	1.000	1.000	1.000	1.000	1.000	.000	.000
1.00	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.475	1.000
d	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.525	.000
Pgm															
(*n*)	21	20	40	37	20	20	4	40	20	4	18	7	29	40	8
1.08	.000	.000	.000	.000	.000	.250	.625	.625	.000	.000	.000	.714	.328	.000	.000
1.14	.048	.025	.175	.149	1.000	.750	.375	.375	1.000	1.000	1.000	.286	.672	.000	.000
1.25	.952	.975	.825	.851	.000	.000	.000	.000	.000	.000	.000	.000	.000	1.000	1.000

D. dubia by their possession of sharply pointed, recurved head shapes. A comprehensive array of habitats was sampled throughout North America, with the sole gap being ponds from the southeastern United States (see Hebert et al. 1989a). We used two populations of *D. dubia* (Little Wren Lake, ON, and Stormy Lake , WI) and one population of *D. laevis* (Rondeau Park, ON), which agree well with the typical morphological and ecological descriptions (see Brooks 1957), as genetic references for the present study. These populations have been characterized both for allozyme and 12S rRNA sequence variation (Taylor et al. 1996; Genbank DLU34734, DDU34735, DDU34736). Four sites (GA1, NC1, MI1, and OR1) lack allozyme and ten sites lack mtDNA information (CA3,6; MEX2,3; NE1; NC2; OR2; SD2; and OK1,2) because all of the collected specimens were either used for one type of analysis, or preserved in a way that precluded doing both DNA and enzyme analysis (i.e., EtOH). For outgroups, we sequenced representative taxa from each of the three *Daphnia* subgenera: *Daphnia* (*Ctenodaphnia*) *longicephala* (Fleurieu Peninsula, South Australia), *Daphnia* (*Daphnia*) *ambigua* (Little Presa, Mexico), and *Daphnia* (*Hyalodaphnia*) *galeata mendotae* (Guelph Lake, Ontario).

Allozyme Electrophoresis

Individuals from 29 populations were scored for enzyme variability at seven commonly polymorphic and well-resolved loci: aldehyde oxidase (*Ao*, EC 1.2.3.1), supernatant aspartate amino transferase (*sAat*, EC 2.6.1.1), fumarate hydratase (*Fumh*, EC 4.2.1.2), glucose-6-phosphate isomerase (*Gpi*, EC 5.3.1.9), lactate dehydrogenase (*Ldh*, EC 1.1.1.27), mannose-6-phosphate isomerase (*Mpi*, EC 5.3.1.8), and phosphoglucomutase (*Pgm*, EC 5.4.2.2). Electrophoresis was conducted by standard methods (Hebert and Beaton 1993), using a Tris-glycine buffer (pH = 8.5) for all enzyme systems. Specimens of *D. pulex* from Windsor, Ontario (W2-8), and *D. laevis* from Rondeau Park, Ontario (ON1), were included in assays for mobility standards. Direct side-by-side comparisons of all putative novel alleles were later conducted at *Fumh, Pgm*, and *Mpi*. Alleles in the W2-8 standard were assigned an R_f value of 1.0, and all other alleles were scored with respect to the standard.

Analysis of allozyme data was carried out with BIOSYS-1 (Swofford and Selander 1981). Two populations (NY1 and CT1) were omitted from statistical analyses because of sample sizes less than five. Genotypic frequencies for each pop-

ulation were compared with Hardy-Weinberg (HW) expectations, using Fisher's exact test. Pairwise genetic distances among populations were calculated using Cavalli-Sforza and Edwards (1967) chord distance and these were exposed to multidimensional scaling (MDS) to explore spatial patterning of genetic variation. MDS is a graphical tool for examining spatial relationships of data from a dissimilarity matrix (here, a genetic distance matrix of populations) in Euclidean space. The approach permits genetically intermediate taxa to remain spatially intermediate instead of forcing them to cluster into a pseudogroup as in hierarchical methods (Lessa 1990).

Mitochondrial DNA Analysis

Total DNA was extracted from live or previously frozen individuals using a CTAB protocol (Doyle and Doyle 1987). Each 50 μl PCR reaction consisted of 2 μl of DNA template, buffer (Boehringer-Mannheim), 1.5 mM $MgCl_2$, 2 mM of each dNTP, 1 μM of each primer, 0.5 to 1 units of *Taq* DNA polymerase. The 12S primers (from Taylor et al. 1996) were designed from conserved regions between *Daphnia pulex* and *Drosophila yakuba* within the 12S rRNA mitochondrial gene (5′-ATGCACTTTCCAGTACATCTAC-3′,5′-AAATCGTG CCAGCCGTCGC-3′) whereas the 16Sar (5′-CGCCTGTTT ATCAAAAACAT-3′) and 16Sbr (5′-CCGGTCTGAACTCA GATCACGT-3′) primers are from Palumbi et al. (1991). The PCR conditions for both primer pairs consisted of 40 cycles of 30 sec at 94°C; 30 sec at 50°C and 1 min at 72°C; followed by 1 cycle of 5 min at 72°C. The products were purified using the Qiaex II (Qiagen) kit and sequenced directly using dye-labeled terminators and cycle sequencing (Amplitaq FS). Electrophoresis was carried out on an ABI 377 automated sequencer. Both strands of every unique haplotype were completely sequenced. The DNA sequences have been submitted to the Genbank/EMBL database (Accession nos. AFO64152-AFO64189).

Sequence electrophoregrams of the two strands were compared by Sequence Navigator (ABI) and the consensus sequences were aligned by the Clustal algorithm (Higgins and Sharp 1988) with default conditions and adjusted by eye using the SeqApp 1.9a sequence editor (Gilbert 1992). Secondary structure models (de Rijk et al. 1997; van de Peer et al. 1997) of arthropods were used to identify conserved stems and the alignment strategy and nomenclature followed Kjer (1995). Maximum-likelihood (ML) estimates and likelihood mapping were carried out by Puzzle 3.1 (Strimmer and von Haeseler 1997). All remaining phylogenetic analyses including maximum-parsimony (MP) analyses were carried out using PAUP* 4.0 (Swofford 1998).

Results

Nuclear Genetic Variation

A summary of the allozyme variation detected with the individual population allele frequencies is presented in Table 1. Within *D. laevis*, two loci were invariant (*Ao* and *Ldh*), two were variable in two or less populations (*sAat, Gpi*), and three showed substantial variation among populations (*Fumh, Mpi, Pgm*). When interspecific hybrids are common or there is a lack of sexual recruitment, HW deviations are frequent even with small sample sizes in *Daphnia* (e.g., Hebert et al. 1989b). We were able to reject the null hypothesis of HW equilibrium in only one test (*Pgm* in MA2; Fisher's exact test; $P = 0.039$). In this case heterozygote excess was detected but this significant result disappears upon adjusting the alpha-level for multiple tests. These tests suggest that sexual recruitment occurs and that hybrid clones in the *D. laevis* complex are rare in the areas that we sampled.

Marked geographic structuring was present at the three most variable loci: *Fumh, Mpi*, and *Pgm. Daphnia dubia* was fixed for the $Fumh^{115}$ allele; Pacific *Daphnia laevis* was fixed for the $Fumh^{81}$ allele, whereas the remaining populations were fixed, in all but one case (MEX2) for the $Fumh^{100}$ allele (Figs. 2a, 3a). At *Mpi* three alleles were detected, but only Mpi^{114} and Mpi^{120} were common (Fig. 2b). Seven of the nine pacific populations were polymorphic for the two common alleles, whereas all but one of the other populations was fixed for either Mpi^{114} or Mpi^{120} (Fig. 3b). The populations from sites along the east coast of North America, including lake-dwelling *D. laevis* from Mexico and North Carolina and pond populations from the northeastern states, were fixed for Mpi^{120}, whereas the inland populations were fixed for Mpi^{114}. Although there was greater within-population variation, a similar geographic pattern was found at *Pgm* where three alleles were common (Fig. 3c). In the Pacific *D. laevis*, two alleles were detected with Pgm^{125} more common than Pgm^{114} in all populations; in the Atlantic areas Pgm^{108} and Pgm^{114} were common, but Pgm^{125} was undetected. The central populations possessed only the Pgm^{114} and Pgm^{125}alleles, with Pgm^{114} fixed in six of 10 populations.

Multidimensional scaling of the genetic distances based on all detected allozyme variation in *D. laevis* sensu Brooks (1957) revealed a clear geographic pattern of genetic structure (Fig. 4). Three groups of populations were apparent: Pacific, Central, and Atlantic (comprised of southern lake-dwelling populations and northeastern pond-dwelling populations). Among each of the *D. laevis* groups, there was about a three-fold increase in the mean genetic distances over that found within a group (Table 2).

Mitochondrial DNA Sequence Variation

One goal of molecular phylogenetics is to optimize the signal to noise ratio for the taxa examined—too much variation may lead to alignment uncertainty and saturation that increases homoplasy; too little variation impedes phylogenetic resolution. The 12S and 16S sequences appear to provide a useful phylogenetic window for the *D. laevis* complex (Table 3). The alignment of sequences was unambiguous for the ingroups because only three single gaps were needed in the 12S data and a single gap in the 16S data (Appendixes 2,3). When the three outgroups are included there is a range of five to seven gaps per taxon in the 12S data and two to four gaps in the 16S data. The total length of the 12S and 16S alignments was 569 and 491 base pairs, respectively. Of these sites, 23% for the 12S and 15% for the 16S were potentially informative according to the parsimony criterion. A plot of genetic distances involving the same taxa for the 12S and 16S genes (Fig. 5) indicated that the 12S gene is evolving

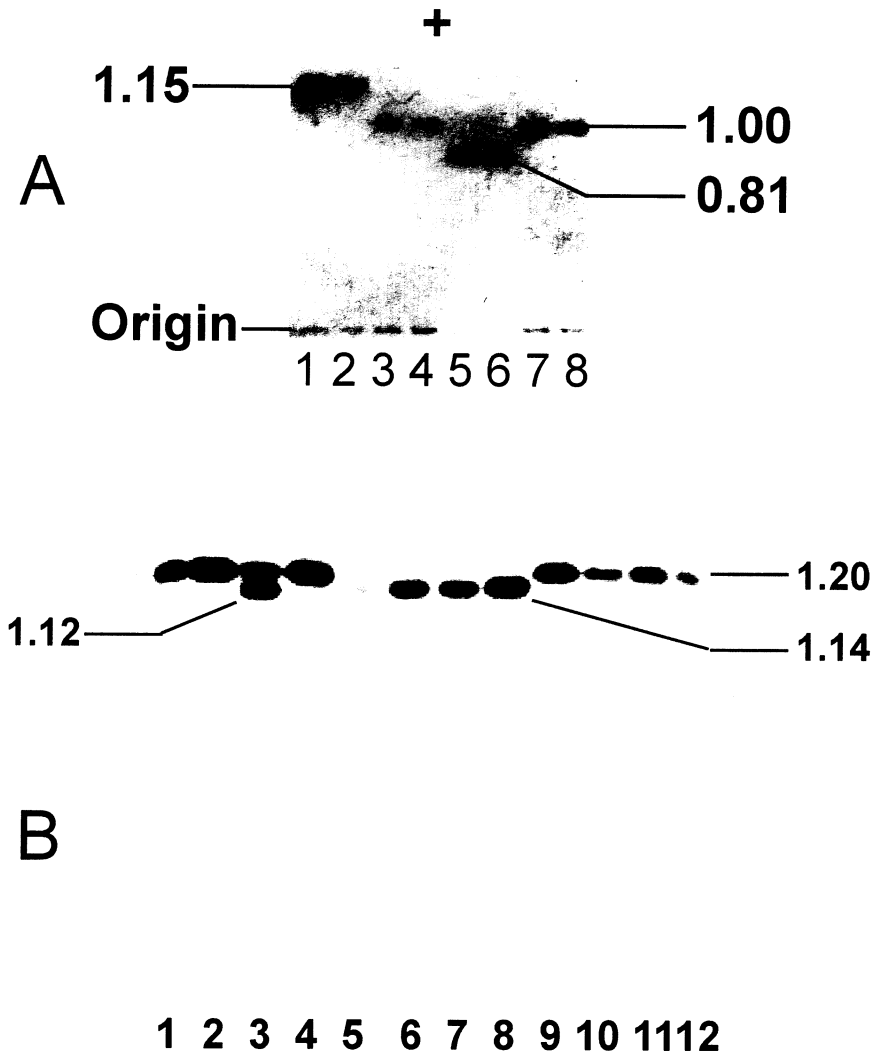

FIG. 2. Electromorphs detected at two diagnostic allozyme loci (A, *Fumh*; B, *Mpi*) in the *Daphnia laevis* complex. The + symbol indicates the anodal end of the gel. Lane designations are for gel A: 1–2 *Daphnia dubia* NFLD 1, 3–4 *D. laevis* Central group (NY1), 5–6 *D. laevis* Pacific Group (CA9), 7–8 *D. laevis* Atlantic group (CT1); for gel B: 1–4 Atlantic group (CT1), 5–8 Central group (NY1), and 9–12 Atlantic group (MA2).

at approximately 1.5 times the rate of the 16S gene (Y = 1.495 [LSU] + 0.005). The slower divergence rate of the 16S fragment may be partially due to fewer sites being able to vary (Simon et al. 1994), because there are relatively more invariant sites for this gene (365, 74.3%) compared to 12S (337, 59.0%) and the Ti/Tv ratio becomes asymptotic more rapidly (i.e., at > 10% sequence divergence for 16S but > 20% for 12S; Table 3; Taylor, unpubl. data). Nevertheless, transitional saturation is unapparent within the ingroup taxa and in some of the outgroup taxa. Base composition was similar among taxa and even between the two rRNA genes. For 12S, the mean base frequencies were A, 0.34; C, 0.13; G, 0.19; T, 0.34 (test for homogeneity, $\chi^2 = 18.87$, df = 81, $P > 0.9$), and for 16S the means were A, 0.32; C, 0.13; G, 0.22; T, 0.33 (test for homogeneity, $\chi^2 = 3.87$, df = 36, $P > 0.9$). A sense of substitutional saturation may be obtained by examining the number of different bases at each site. The number of parsimony informative sites having three or fewer bases was 125 of 131 (95.4%) for the 12S rRNA, and 69 of 74 (93.2%) for 16S rRNA. There were no sites with four bases among ingroup taxa. The maximum genetic distance among taxa at < 0.25 (< 0.15 in the ingroup) was within the range of optimal signal for rRNA studies (Hillis and Dixon 1991). Tests of skewness from random tree distributions yielded highly significant results, indicating signal somewhere in the data (Hillis and Huelsenbeck 1992). As local signal and replicate samples can exert undue influence on this statistic, we trimmed the dataset down to 10 ingroup representatives before the analysis. Another approach to measure the a priori signal of a sequence dataset is likelihood mapping (Strimmer and von Haeseler 1997). This graphical tool plots likelihood quartets from a test set onto basins of attraction of which there are three types—starlike, netlike, and treelike. In our analyses, the 12S and combined data had a very high proportion of quartets in the treelike areas, indicating excellent resolution potential. The 16S dataset had only moderate treelikeness leading to the prediction that parts of the trees resulting from its analyses will be unresolved.

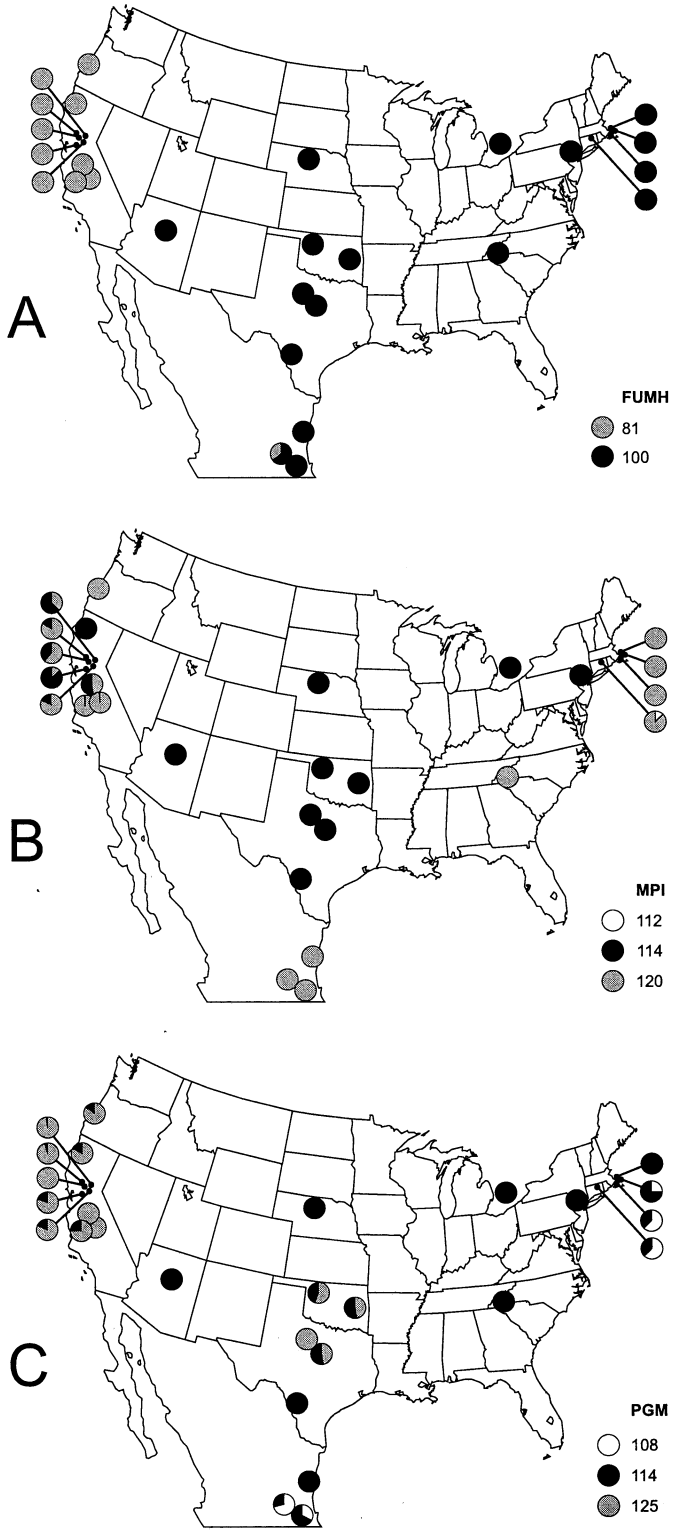

FIG. 3. Geographic distributions of allele frequencies at three polymorphic allozyme loci (*Fumh, Mpi, Pgm*) in North American *Daphnia laevis*. See Appendix for sampling sites.

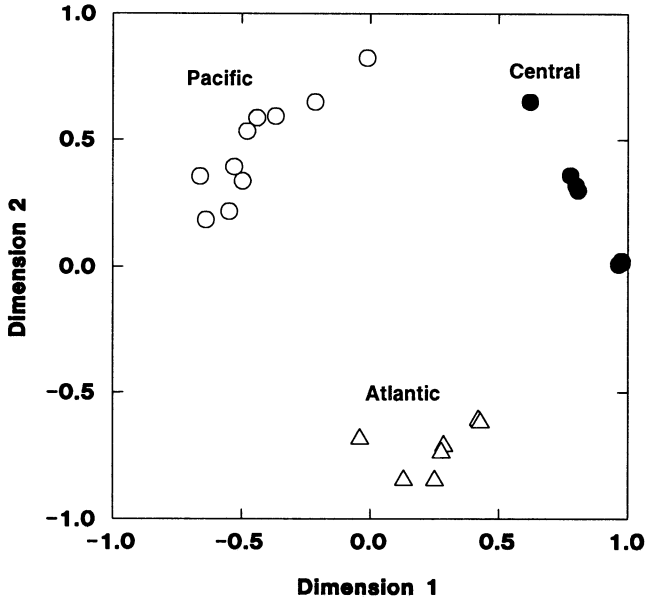

FIG. 4. Multidimensional scaling of a genetic distance matrix (chord) derived from 7 allozyme loci in the North American *Daphnia laevis* complex (R^2 = 0.999). The stimuli represent 24 populations of *Daphnia laevis* whose geographic locations are shown on the plot. Only populations that had a sample size of > 15 individuals per locus were included in this analysis. For population names see Appendix 1.

An increase in treelikeness with length of sequence (e.g., Strimmer and von Haeseler 1997) was unapparent in our combined data, revealing that reliability is a function of both quantity and quality of signal. Overall, the tests and heuristic analyses of signal indicate that the resolution potential for the mitochondrial rRNA genes in the *D. laevis* complex is strong.

12S rRNA Gene Trees

Maximum-parsimony analysis of the 12S rRNA data using the heuristic search algorithm with random addition of sequences (40 replicates) yielded 12 trees of length 367 (CI = 0.796, RI = 0.877). A strict consensus of these trees is shown in Figure 6. The 12S tree revealed four major groups: *D. dubia* and the three geographic groups of *D. laevis* (Pacific, Central, and Atlantic). There was strong support (100% bootstrap value; 5–17 decay index) for three of these groupings, whereas the Central grouping had modest support (78% bootstrap; 2 decay index). There was also strong support for *D.*

TABLE 2. Chord genetic distances for seven allozyme loci in the *Daphnia laevis* complex. Groups were identified by multidimensional scaling (MDS).

Taxon	Mean D_{chord} (range)
Daphnia laevis (Atlantic)	0.16 (0.00–0.28)
D. laevis (Pacific)	0.18 (0.03–0.35)
D. laevis (Central)	0.15 (0.00–0.34)
D. dubia	0.19 (one comparison)
Central vs. Pacific	0.47 (0.35–0.58)
Central vs. Atlantic	0.41 (0.34–0.51)
Pacific vs. Atlantic	0.48 (0.36–0.57)

TABLE 3. A priori heuristics for phylogenetic reliability of three mtDNA sequence datasets (SSU, LSU, and combined) from the North American *Daphnia laevis* group.

Reliability estimate	12S rRNA	16S rRNA	12S + 16S rRNA
Skewness of tree length distributions (g_1 from 10,000 random trees)	−0.994**	−0.946**	−0.930**
Likelihood mapping (frequency of quartets in treelike regions)	0.954	0.779	0.936
Potential parsimony informative sites	131	74	205
Informative sites with < three different bases across alignment	125	69	194
Maximum genetic distances			
ingroup	0.134	0.091	0.109
outgroup	0.240	0.192	0.216
Range of transition/transversion ratios			
ingroup	> 5.0–1.8	> 4.0–1.0	> 9.0–1.2
outgroup	1.2–0.7	1.2–0.7	1.1–0.7

** $P < 0.01$.

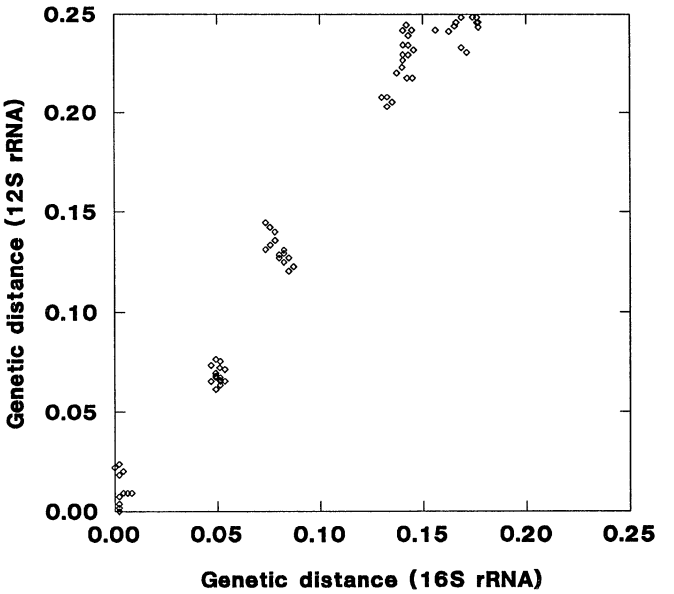

FIG. 5. Plot of pairwise sequence divergences (Kimura 1980) for 12S rDNA and 16S rDNA fragments for the same specimens in the *Daphnia laevis* complex and outgroups.

dubia being basal to any of the other members of the *D. laevis* complex. The populations on either side of the western Cordillera (Pacific and Central) formed a strong clade. Within each of the four major groups there was little structure, with the exception of the separation of the Atlantic clade into northeastern and southeastern/Mexican subgroups (96% bootstrap; 3 decay index).

Maximum-likelihood analysis of the 12S data produced a tree with the same topology as the MP tree (Fig. 7). To minimize arbitrariness and the inclusion of superfluous parameters, we conducted a series of likelihood ratio tests (LRTs) of nested models (Huelsenbeck and Rannala 1997). This approach aims to include only those parameters that significantly increase the likelihood of the resulting tree. In our tests (Table 4), the likelihood of the tree was significantly improved by a model that included unequal transition/transversion rates (Kimura 1980), unequal base composition (Hasegawa et al. 1985), and unequal rates of change among sites (Yang 1993). All of these parameters were estimated from the data and incorporated into the model. Parameters that failed to significantly improve the model were the inclusion of two transition classes (Tamura and Nei 1993) and the assumption of a molecular clock or equal rates among lineages (16S data only). Although the optimization of parameters increased the likelihood of the tree, the topologies of the 12S and 16S trees were unaffected by the models examined.

16S rRNA and Combined Trees

Maximum parsimony of the 16S rDNA sequence data using a branch and bound search algorithm found six shortest trees of 207 steps long (CI = 0.821, RI = 0.799). A strict consensus of these trees is shown in Figure 8 and again, the ML topologies (α = 0.12, Ti/Tv = 2.25, Log L = −1539.17) were identical in topology to the MP trees. The major groups

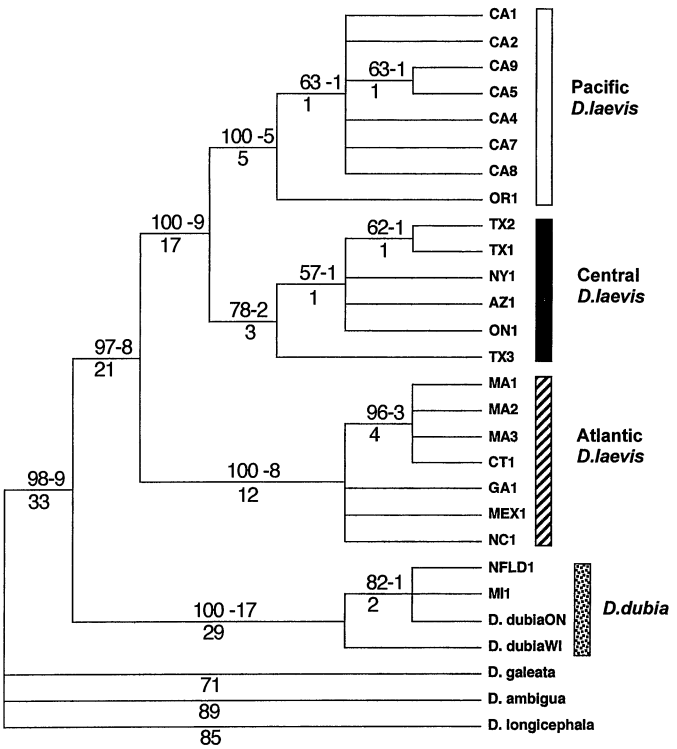

FIG. 6. Strict consensus of 12 shortest trees found using the parsimony criterion (length = 367) based on sequence variation from a fragment of the mitochondrial 12S rRNA gene (see Appendix 2). The numbers above the branches are indications of support—bootstrap values (1000 replications) and decay indices (after the dash). The numbers below the branches show the branch length in steps. Major geographic associations are indicated by labeled and patterned blocks. For population and abbreviation details see Appendix 1.

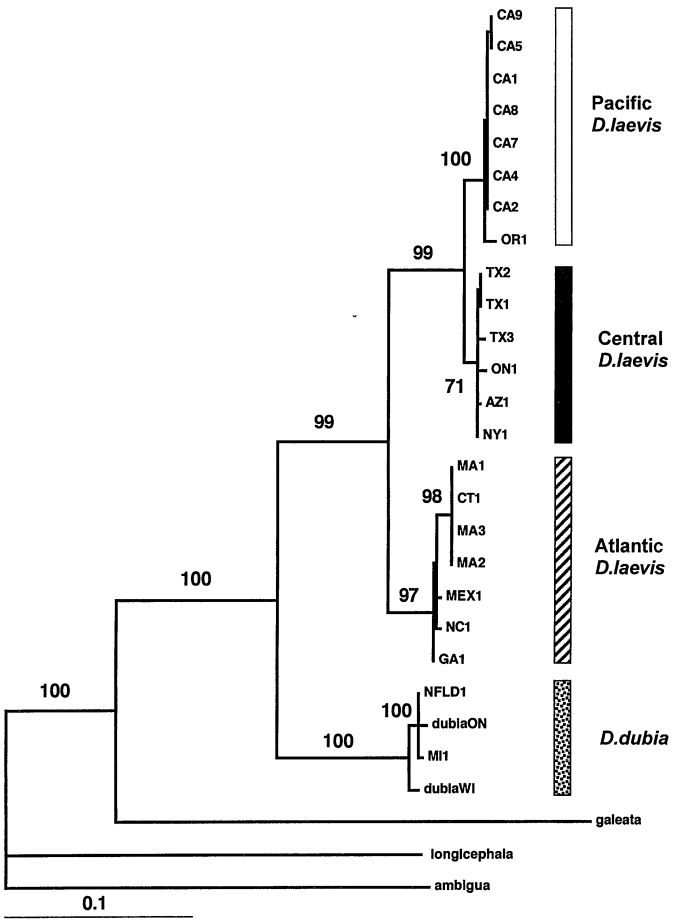

FIG. 7. Maximum-likelihood (ML) tree of the 12S rDNA sequences in the North American *Daphnia laevis* complex. Branch lengths are proportional to ML distances. Numbers above the branches are quartet puzzle values. The model used was the HKY85 with among-site rate heterogeneity (Hasegawa et al. 1985; $\alpha = 0.28$, Ti/Tv = 2.38, log $L = -2299.04$).

are similar to those indicated by allozyme and the 12S rRNA data, but there was no divergence apparent between the Central and Pacific populations of *D. laevis*. A partition-homogeneity test (Swofford 1998) using a branch-and-bound search algorithm and 100 replicates, found no differing trees (0/100, $P = 1.00$) between the genes, indicating that with the parsimony criterion the same tree is reconstructed with each gene. Combining the 12S and 16S data produced two trees of 562 steps in length (CI = 0.806, RI = 0.775) after a branch- and-bound search under the parsimony criterion. The bootstrap and decay index support for most groups increases, but the topology from the 12S data alone was unchanged (Fig. 9b). The ML tree of the combined data ($\alpha = 0.19$, Ti/Tv = 2.42, Log $L = -3768.81$) was identical to the MP trees.

If the Pacific group of *D. laevis* was isolated from its Central counterpart by the formation of mountain ranges, these two groups should be sister taxa. All of the trees found support this relationship. The best MP trees found under the nonmonophyly constraint of Pacific and Central populations are significantly longer than those of the monophyly hypothesis (Kishino-Hasegawa test: length increase = 19; $t_1 = 4.18$, $SD_1 = 4.547$, $P1 < 0.0001$; $t_2 = 3.9895$, $SD_2 = 4.76$, $P_2 < 0.0001$). The last stage of lineage sorting after a vicariance event should yield two groups that are reciprocally monophyletic (Neigel and Avise 1986). The tree showing reciprocal monophyly was found in the combined analysis but the shortest tree found under the nonreciprocal monophyly hypothesis is not significantly longer (Kishino-Hasegawa test: length increase = 3, $P > 0.05$).

TABLE 4. Likelihood ratio tests of nested parameters for maximum-likelihood estimates of trees based on mtDNA sequences from the *Daphnia laevis* complex.

Parameter null hypothesis	rDNA gene	log L_0	log L_1	$-2 \log\Lambda$
Equal transition/transversion rate	12S	−2478.41	−2446.98	62.86**
	16S	−1673.44	−1650.43	46.02**
Equal base composition	12S	−2446.98	−2372.58	148.80**
	16S	−1650.43	−1610.70	79.46**
One category of transitions	12S	−2372.58	−2372.50	0.16
	16S	−1610.70	−1610.61	0.18
Equal rate change among sites	12S	−2372.58	−2299.04	147.08**
	16S	−1610.70	−1539.17	143.06**
Unequal rate change among lineages (no molecular clock)[1]	16S	−1615.00	−1610.70	8.60

** $P < 0.05$.
[1] Test of a molecular clock for a parameter-rich model was computationally prohibitive for the full 12S dataset.

One a priori scenario examined in this study involved the derivation of *D. dubia* from *D. laevis*. None of the tree topologies found supported this scenario, instead they placed *D. dubia* basal to *D. laevis*. The observed MP tree from the combined data was significantly shorter than the trees found under a constraint of *D. dubia* being derived from the Pacific/Central *D. laevis* (Kishino-Hasegawa test: length increase = 11; $t_1 = 2.53$, $SD_1 = 4.35$, $P_1 = 0.012$; $t_2 = 2.41$, $SD_2 = 4.57$, $P_2 = 0.016$). However the observed MP was not significantly shorter from trees found under a constraint of *D. dubia* being derived from Atlantic *D. laevis* (Kishino-Hasegawa test: length increase = 8; $t_1 = 1.71$, $SD_1 = 4.69$, $P_1 = 0.088$; $t_2 = 1.63$, $SD_2 = 4.90$, $P_2 = 0.103$).

DISCUSSION

Biogeography of Daphnia laevis

Darwin's (1859) biogeographic paradox of freshwater invertebrates remains unresolved for the vast majority of taxa. He observed that freshwater invertebrates are surprisingly similar in appearance among continents, whereas the terrestrial faunas are very dissimilar. How can groups lacking an active dispersal stage and living in fragmented habitats maintain apparently uniform geographic structure over vast distances? Our evidence from the freshwater crustacean *D. laevis* clearly indicates that uniform geographic structure may be more apparent than real because of the existence of cryptic endemic groups. Both allozyme and mtDNA variation reveal marked regionalism in the *D. laevis* complex despite subtle morphological differentiation. Genetic evidence from nuclear loci (*Fumh, Pgm*, and *Mpi*) and two mtDNA genes supports or is consistent with the existence of four trenchant mono-

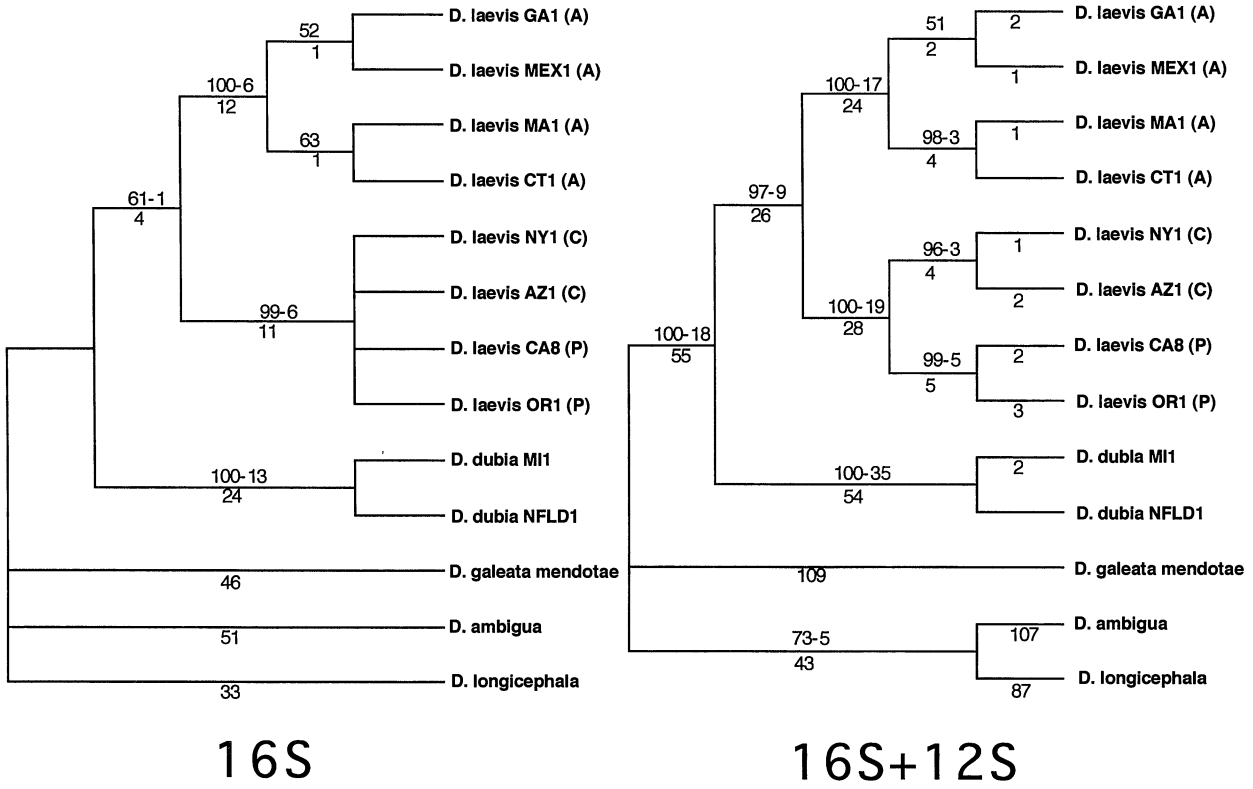

FIG. 8. Strict consensus of shortest trees found using the parsimony criterion based on sequence variation from the mitochondrial 16S rRNA gene and combined 12S and 16S rRNA genes (see Appendix 3). The numbers above the branches are indications of support—bootstrap values (1000 replications) and decay indices (after the dash). The numbers below the branches show the branch length in steps. Parentheses indicate region designations from allozyme variation: Atlantic (A), Central (C), and Pacific (P). For other population and abbreviation details see Appendix 1.

phyletic groups (Pacific, Central, Atlantic, and Northern) in the *D. laevis* complex of North America (Fig. 9). The hypothesis of cosmopolitanism for *D. laevis* (i.e., the existence of one species over the range of Africa, South America, and North America) is rejected because even in North America there are at least four endemic groups.

Our genetic and biogeographic evidence suggests that the crux of the freshwater invertebrate paradox is morphological stasis rather than biogeographic processes. Given the North American pattern of endemism, it is likely that the African and South American *D. laevis* are also endemic. A reasonable inference, then, is that morphological evolution has been negligible in the *D. laevis* complex since the breakup of Gondwana during the Cretaceous. We know that morphological evolution of *D. laevis* has been weak among continents because some of the world's leading *Daphnia* morphologists (e.g., Brooks 1957; Korinek 1984) have compared specimens across continents and found that even the extremely subtle differences that usually characterize species in this genus are unapparent. The extent of the discrepancy between morphological and molecular evolution in *D. laevis* is at least on par with that of classic "living fossil" species. Avise et al. (1994), for example, observed a 6% sequence divergence for morphologically similar species of the genus *Tachypleus*, while we found a 5–8% divergence using the homologous 16S rRNA sequence just within the North American *D. laevis* complex. Stasis is the Gordian knot of evolutionary biology. The mystery is great for *D. laevis* when one considers the detailed studies of Banta et al. (1939) showing that spontaneous genetic mutations affecting morphology (e.g., excavated head and dish-face) occur at high frequency (12 of 1545 offspring) in cultures.

Although we have identified endemism, each of the major groups is distributed over a vast portion of the continent and there is little evidence of increased isolation by distance. For each of the four groups, allozyme structure was either absent or only marginally greater than zero. Moreover, the mtDNA gene trees within regions were unresolved or had no variation. Still, we avoid accepting the null hypothesis of no geographic structure within major genetic groups because we may have sampled genes that show no structure over these distances. If we had examined only 16S, for example, the genetic split between the central and pacific lineages would have been unapparent. Nevertheless, our demonstration of weak geographic variation at allozyme and mtDNA loci does indicate recent sharing of gene pools among populations that are now separated by large distances (e.g., New York and Arizona, Mexico and Georgia, Newfoundland and Wisconsin). This suggests that members of this complex are capable of dispersing over long distances in short amounts of time.

The mode of this dispersal is unknown, but our genetic data are consistent with the hypothesis that avian dispersal is important in microcrustaceans. In lake-dwelling species, direct evidence of dispersal among water bodies is provided

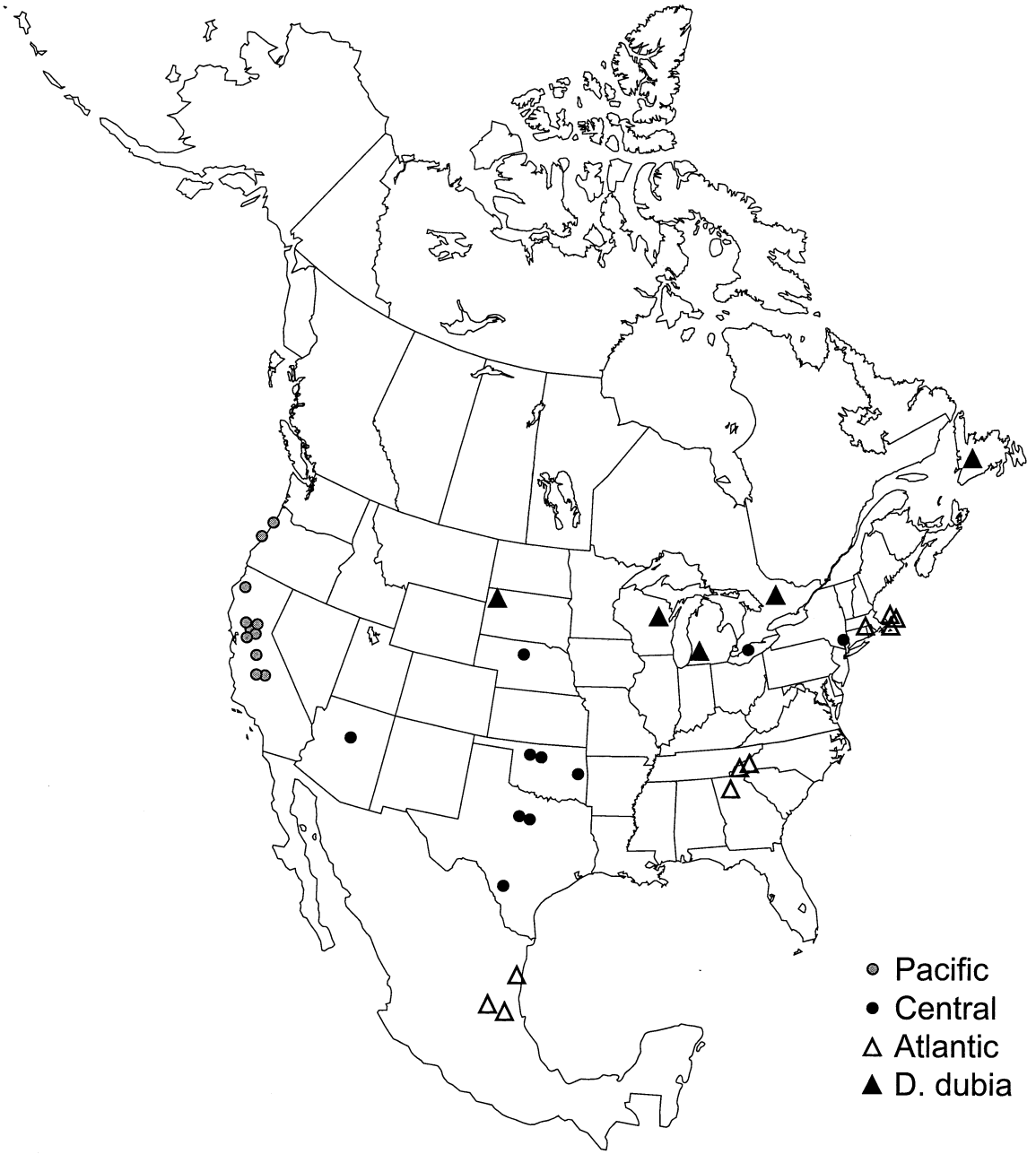

FIG. 9. Geographic distribution of major genetic groups in the North American *Daphnia laevis* complex identified by mtDNA sequence variation and allozymes. For site details see Appendix 1.

by the frequent observation of waterfleas in samples from connecting waterways (e.g., Selgeby 1975) and indirect evidence of dispersal comes from the observation of greater gene frequency similarities among populations from connected water bodies compared to those from isolated water bodies (reviewed in DeMeester 1996; Taylor, unpubl. data). However, there is no evidence that distant populations of any of the major *D. laevis* groups have been recently interconnected. Instead, their broad distribution must reflect the passive dispersal of resting eggs via biotic or abiotic agents. The popular hypothesis of avian dispersal of *Daphnia*, predicts concordance between genetic structure and major flyways. For *D. laevis*, this prediction is realized as the distribution of each genetic lineage roughly coincides with a major flyway for waterfowl, that is, Western, Mississippi, and Atlantic flyways. This hypothesis may also explain the weakness of geographic structure within the major groups, which is otherwise surprising given the geomorphological complexities of these areas and the numerous endemic areas found for other biota within the flyways (e.g., Riddle 1996). Wind dispersal does not predict the observed pattern nor can it explain the timing of resting egg production and the egg-case modifications of daphniids. *D. laevis* also has a habitat preference that increases exposure to migratory waterfowl—they most often

live in large, shallow, permanent ponds (Brooks 1957). Experimental manipulations are necessary to examine the importance of animal movements to the observed geographic structure of *Daphnia*. Also, the flyways and the observed geographic structure could be of very different ages, which might indicate that avian dispersal helps to maintain rather than create the geographic structure of *D. laevis*.

There are several recent findings of within-continent geographic structure and endemism in *Daphnia* (e.g., Taylor and Hebert 1993; Taylor and Hebert 1994; Hebert and Wilson 1994; Hebert and Finston 1996; Crease et al. 1997; Weider and Hobæk 1997). At present there are few phylogenetic studies that examine the relationships of these endemic groups. Yet, even now it is clear that universal areas of endemism for freshwater zooplankton are nonexistent and that their biogeography is more complex than freshwater vertebrates (e.g., Bernatchez and Wilson 1998). For example, there is weak geographic structure in *D. galeata mendotae, D. dentifera*, and *D. pulicaria* over the scale of North America using allozymes and mtrRNA variation (Cerny and Hebert 1993; Taylor and Hebert 1994; Taylor et al. 1996; Crease et al. 1997), whereas other *Daphnia* species are restricted to one or two water bodies (e.g., Hebert 1995). The complexity of biogeographic patterns is likely due to the potential for extreme differences in vagility for crustaceans.

Gene Trees and Taxonomic Trees

Although molecular markers have advantages over morphological markers (e.g. independence, known genetic basis, abundance), species boundaries can be muddied by shared ancestral alleles, introgression, hybridization, and gene duplications (Moritz and Hillis 1996). Past genetic studies on *Daphnia* biogeography have focused on syngameons such as the *longispina, pulex*, and *carinata* complexes. In these groups, there is incomplete reproductive isolation, resulting in regionally dominant hybrid clones and introgression. These findings have led some to favor the old hypothesis that few discrete species occur in *Daphnia* and that determining species boundaries may be intractable (Banta et al. 1939; Lehman et al. 1995). Our present study reveals the existence of a *Daphnia* species group that appears to lack genetic fluidity. We found no evidence for hybridization among taxa, but we did find reciprocal monophyly for multiple, independent, genetic markers in at least four major groups within the *D. laevis* complex. This scenario makes the genetic determination of sympatric species boundaries straightforward (Avise and Ball 1990).

Our findings agree with Brooks (1957) who resurrected *D. dubia* as a valid species for the northern, lake-dwelling form. We expanded the sampling from a previous study (Taylor et al. 1996) to include another gene, 16S rDNA, and specimens from the extremes of *D. dubia*'s distribution (South Dakota and Newfoundland). The presence of numerous fixed allozyme differences and marked mtDNA differentiation from sympatric populations of the Central and Atlantic groups constitutes strong evidence for its reproductive isolation from these taxa. The nonmolecular diagnostic characters for *D. dubia* are its pointed head, usually with a recurved shape (Fig. 10A), and a preference for lacustrine habitats (Brooks 1957). These characters are true for the vast majority of *D. dubia* populations, but we caution that its head shape converges in the spring to a *D. laevis*–type shape, and that *D. dubia* can live in remarkably small bodies of water (e.g., NFLD1). Moreover, southern populations of the *laevis* complex commonly dwell in lakes and possess a pointed head shape (Fig. 10C). Thus, there is more morphological and ecological overlap between *D. laevis* and *D. dubia* than recognized by Brooks (1957).

Ironically, the original type population of *D. laevis*, a proposed exemplar of a widely distributed zooplankter, probably belongs to the most geographically restricted taxon in the species complex. The type population belongs to a taxon, *D. laevis laevis*, that we found only in the former Atlantic or Georges Bank glacial refugium. We conservatively propose that the sister clade of *D. l. laevis*, found in lakes and reservoirs of the southeast United States and in Mexico (Fig. 10B,C), be designated as *D. laevis gessneri. Daphnia l. gessneri* is a lacustrine taxon that possesses a reduced second postabdominal process and an angulated head shape (see Fig. 10C; Herbst 1967). More sampling (particularly in South America) is required to clearly demarcate the geographic ranges of *D. laevis* and its subspecies.

The evidence presented here is the first to clearly show that the Central-Pacific clade is a separate species from *D. laevis* sensu stricto and we have therefore resurrected Herrick's (1884) *Daphnia magniceps* as valid species (Fig. 10D). Herrick (1884) erected this new species from specimens collected in Minnesota, partly as Birge's (1878) type description of *D. laevis* in New England showed a markedly pointed helmet in juveniles. However, it is difficult to discriminate adults of these two taxa, on the basis of morphology. *Daphnia magniceps* occupies fish-free, permanent ponds and swamps throughout much of the central United States, but further sampling is necessary to rule out its occurrence in southeastern North America where a *D. laevis*–like species is common (Hebert et al. 1989a).

The most closely related taxon to *D. magniceps* occurs on the Pacific Coast, west of the Sierra Nevada–Cascades range. Because the Pacific taxon is markedly allopatric from the other forms, its reproductive isolation from them is difficult to ascertain. Nevertheless, reciprocal monophyly exists between this taxon and *D. magniceps* at 12S rDNA, and *Fumh*, thus meeting the taxonomic criterion of a concordance among independent genetic markers (Avise and Ball 1990). We recommend that this form (Fig. 10E) be designated with the subspecies name *Daphnia magniceps pacifica*. The demonstration of cryptic endemic groups provides an empirical rationale for conserving their habitat—the permanent, fish-free ponds containing *D. magniceps pacifica* are rapidly disappearing.

Phylogeographic Pseudocongruence

The glacial ice sheets advanced and retreated for the last 2.5 million years, profoundly impacting biotic distributions by causing cycles of vicariance and dispersal. For freshwater organisms, the primacy of glacial cycles as a biogeographic machine has been the favored hypothesis. Indeed, genetic evidence from vertebrates is concordant over a wide array of

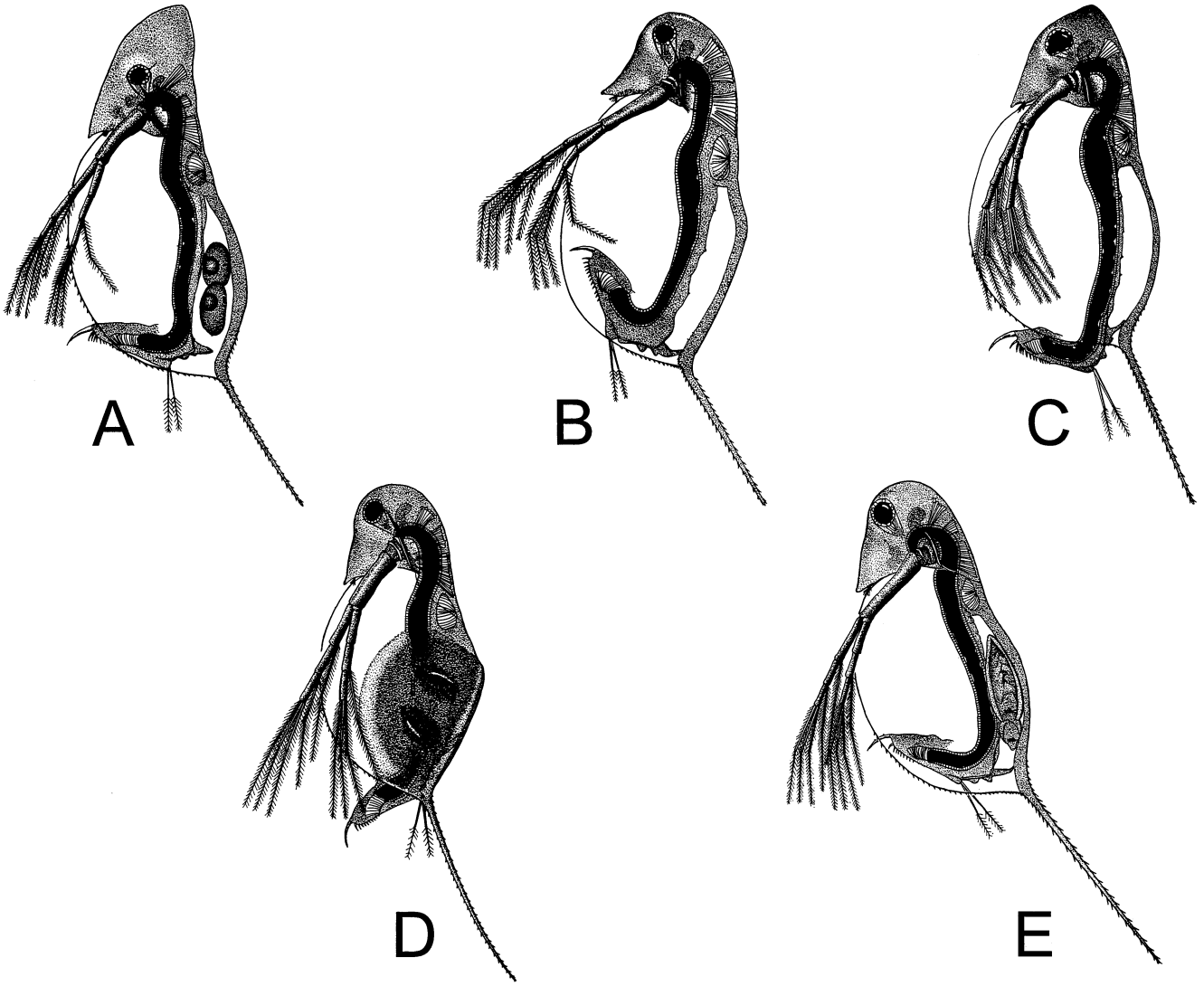

FIG. 10. Camera lucida drawings of representative phenotypes in the North American *Daphnia laevis* complex. All drawings are lateral views of mature females and each population possessed more morphological variation than shown here. A. *Daphnia dubia* from Little Wren Lake, Ontario; B. *Daphnia laevis gessneri*, La Presa Esperanza, Mexico MEX2; C. *D. laevis gessneri* from Santeetlah Lake, N. C. (NC2); D. *Daphnia magniceps magniceps* with resting eggs, Rondeau Park, Ontario (ON1); and E. *Daphnia magniceps pacifica* ssp. n., pond near Glenada, Oregon (OR1).

taxa in showing that shallow historical events, such as glaciation, strongly affected distributions (Avise 1992; Bernatchez and Wilson 1998). Yet, present-day distributions of phylogenetic groups can be misleading with respect to inference of biogeographic processes. Even in the simplest case where different clades exist on opposite sides of a geographic barrier, discerning between recent dispersal and vicariance is difficult and may lead to a misdiagnosis termed "pseudocongruence" if the null model of geographical vicariance is assumed (Page 1990; Cunningham and Collins 1994). In these situations, historical information is invaluable but generally unavailable for invertebrates, which often lack detailed fossil records (Avise et al. 1994).

TABLE 5. Estimated divergence times of selected clades in the *Daphnia laevis* complex from the calibrations of Cunningham et al. (1992) for the same 16S mtDNA fragment in hermit crabs.

Clade	Kimura's genetic distance	Estimated divergence times in million years (95% CL)
Central/Pacific	0.002	0.6 (0–2)
Southeast/Northeast	0.006	1.6 (0–3.6)
Central-Pacific/Atlantic	0.048	12.6 (5.2–19.7)
dubia/laevis	0.076	20.0 (9.1–30.5)

With the present data, some insights may be afforded by inferences of divergence times derived from mtDNA sequence comparisons (Table 5). Although such inferences possess inherently wide confidence limits and are generally unreliable when based on calibrations from distantly related taxa and nonhomologous DNA (e.g., Hillis et al. 1996; Ayala 1997), one can reliably distinguish between recent gene flow and ancient subdivisions (e.g., Cunningham and Collins

1994). In our present analysis we made divergence time estimates based on the calibrations for another crustacean group (Cunningham et al. 1992), which used the homologous 16S fragment to our comparison. A constraint for a molecular clock for the 16S data (Table 4) did not significantly improve the ML model, indicating that the assumption of equal rates of evolution among lineages is reasonable for our data.

Although previous phylogeographic studies have revealed endemic species in *Daphnia* species complexes (e.g., Hebert and Wilson 1994; Taylor and Hebert 1994; Crease et al. 1997), the present study is the first to clearly demonstrate the separation of sister taxa of *Daphnia* by a geographic boundary on a continent (Central and Pacific groups). Thus the geographic and phylogenetic predictions of vicariance are satisfied. For the groups on either side of the Western Cordillera, the temporal prediction of vicariance is a deep split. Yet, the estimated divergence times are very recent. The mean estimated divergence time is 0.6 M.Y.B.P, but some haplotypes are shared between regions. With the 12S gene reciprocal monophyly is achieved, but the divergence is also small at 1.8%. Using the general arthropod clock of Brower (1994), the divergence time is still < 1 M.Y.B.P. The timing of the rise of the Sierra Nevada range is incongruent with this estimate as the rise of much of the Western Cordillera probably occurred before 10 M.Y.B.P. (Small and Anderson 1995). A rate variation of 10-fold within crustaceans is required for congruence, but the published mtDNA rRNA calibrations for crustaceans (Cunningham et al. 1992; Sturmbauer et al. 1996) and vertebrates (endotherms and ectotherms) are between 0.38%–1% per million years, less than a threefold range (Caccone et al. 1997 and references therein). Therefore, it is unlikely that the rise of the Sierras lead to a vicariance event and the biogeographic pattern we observed; instead it is probable that shallow-history dispersal has occurred across this mountain range. This dispersal apparently ceased during the glacial-interglacial cycles and may have been affected by avian flyway modifications at that time.

Another contrast of deep versus shallow divergences is provided by the *D. laevis–D. dubia* split. Taylor et al. (1996) provided estimates that this was a deep split using 12S rDNA and allozymes. Here we examined the 16S rDNA, which has crustacean calibrations, and a more comprehensive geographic range of specimens and find a similar deep split. The estimated divergence time of 20 M.Y.B.P. is 2000-fold greater divergence than that expected from post- Wisconsinan glaciation divergence hypothesized by Brooks (1957) and eightfold difference from the beginning of the glacial-interglacial cycles in the Pliocene. Again, we conclude that the time of divergence is incongruent with the expectations based on geographic distributions and phylogenies. We have found no close relative of *D. dubia* even after examining morphologically similar, lake-dwelling forms from Mexico. We therefore propose that lineages closely related to *D. dubia* are extinct in North America and that further insight concerning its origins requires the sampling of allied forms from South America and Africa.

In addition to challenging a priori hypotheses, our work has revealed an unexpectedly deep divergence between the Atlantic clade and the Central clade. The divergence for both 16S (5%) and the 12S (7%) genes indicate a split about 12 M.Y.B.P. If one were to use only phylogenetic estimates and present-day distributions, the expectations would be that the Central group and the Atlantic group diverged as a result of occupying different well-established glacial refugia, that is, the Mississippian and Missourian refugia versus the Atlantic refugium (Avise 1992). This scenario requires a recent divergence, but the observed divergence is clearly deep.

An emerging biogeographic theme, from this and other recent studies on water fleas (e.g., Taylor and Hebert 1994; Taylor et al. 1996), is that of pseudocongruence. Genetic divergences turn out to be shallow in several cases where proposed vicariance events are ancient (e.g., continental drift and orogenesis) and deep where proposed vicariance events are shallow (e.g., glaciation). To explain such results, ad hoc vicariance scenarios or other forces such as dispersion, extinction, and ecological factors must be invoked. Yet, for some areas in North America, vicariance events are numerous and separated by little time (Riddle 1996). The result is that identifying sources of vicariance, if they are responsible for the present-day distributions, may be very difficult for microcrustaceans. Still, our phylogeographic study indicates that within-continent endemic groups do exist in apparently widespread freshwater crustaceans. Detailed phylogenies of other freshwater invertebrate taxa are necessary to apply the methods of historical biogeography (Platnick and Nelson 1978) and identify endemic areas. This kind of analysis should provide insights both into the relative importance of biogeographic forces and the efficacy of proposed adaptations for passive dispersal.

We did find some evidence of temporal and phylogeographic congruence in the Atlantic group of *D. laevis*. The northeastern clade is found only in the vicinity of the Georges Bank refugium, a proposed freshwater refugium for many groups (Stemberger 1995; Bernatchez and Wilson 1998). The estimated time of divergence between the southeast and northeast clades is approximately 1.6 M.Y.B.P., which meets the shallow divergence requirement of the glacial cycles hypothesis. Although molecular phylogenies have yet to be carried out, allozyme studies of other cladocerans suggest that this Atlantic refugium may be a general area of endemism (Taylor and Hebert 1993; Hebert and Finston 1996, 1997). If *D. dubia* and *D. laevis* were both trapped in the Atlantic refugium (our phylogeographic evidence is consistent with this idea), then *D. dubia* has been more successful in recolonization. This lake-dwelling species is now found from Newfoundland to the prairies, whereas the northeast clade of *D. laevis* has failed to successfully disperse. This result seems unexpected because resting eggs are rare in *D. dubia* but common in *D. laevis* (Brooks 1957). It is possible that an ability to colonize proglacial lakes may have rapidly expanded the range of *D. dubia* compared to the pond-dwelling *D. laevis*.

Conclusions

The present study used allozyme-based population genetics and sequence-based phylogenies of two mtDNA genes to study crustacean phylogeography on a continental scale. Much as the founders of the discipline had predicted for freshwater invertebrates, dispersion has probably been the

major determinant of biogeography for the *D. laevis* complex, but other forces such as vicariance, extinction, and ecology likely play important roles. Our finding that the *D. laevis* complex is distributed in at least five cryptic, largely allopatric groups in North America opens the possibility that hidden endemic areas may exist even for apparently widespread freshwater invertebrates. Nevertheless, we found that molecular estimates of divergence times often greatly disagree with predicted vicariance times based on phylogeographic patterns (i.e., there is pseudocongruence) in *Daphnia*. Empirical studies from several other taxa are needed to construct area cladograms for freshwater invertebrates to assess the generality of our results and to further test biogeographic hypotheses.

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Literature Cited

Avise, J. C. 1992. Molecular population structure and the biogeographic history of a regional fauna: a case history with lessons for conservation biology. Oikos 63:62–76.

Avise, J. C., and R. M. Ball. 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxf. Surv. Evol. Biol. 7:45–67.

Avise, J. C., W. S. Nelson, and H. Sugita. 1994. A speciational history of "living fossils": molecular evolutionary patterns in horseshoe crabs. Evolution 48:1986–2001.

Ayala, F. J. 1997. Vagaries of the molecular clock. Proc. Natl. Acad. Sci. USA 94:7776–7783.

Banta, A. M., T. R. Wood, L. A. Brown, and L. Ingle. 1939. Studies on the physiology, genetics, and evolution of some Cladocera. Carnegie Institution of Washington, Washington, DC.

Bernatchez, L., and C. C. Wilson. 1998. Comparative phylogeography of Nearctic and Palearctic fishes. Mol. Ecol. 7:431–452.

Birge, E. A. 1878. Notes on Cladocera. Trans. Wisc. Acad. Sci. 4:77–110.

Brooks, J. L. 1957. The systematics of North American *Daphnia*. Mem. Conn. Acad. Arts Sci. 13:1–180.

Brower, A. V. Z. 1994. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. Proc. Natl. Acad. Sci. USA. 91:6491–6495.

Caccone, A., M. C. Milinkovitch, V. Sbordoni, and J. R. Powell. 1997. Mitochondrial DNA rates and biogeography in European newts (Genus *Euproctus*). Syst. Biol. 46:126–144.

Cavalli-Sforza, L. L., and A. F. W. Edwards. 1967. Phylogenetic analysis and estimation procedures. Evolution 21:550–570.

Cerny, M., and P. D. N. Hebert. 1993. Genetic diversity and breeding system variation in *Daphnia pulicaria* from North American lakes. Heredity 71:497–507.

Cracraft, J. 1994. Species diversity, biogeography, and the evolution of biotas. Am. Zool. 34:33–47.

Crease, T. J., S. K. Lee, S. L. Yu, K. Spitze, N. Lehman, and M. Lynch. 1997. Allozyme and mtDNA variation in populations of the *Daphnia pulex* complex from both sides of the Rocky Mountains. Heredity 79:242–251.

Cunningham, C. W., and T. M. Collins. 1994. Developing model systems for molecular biogeography: vicariance and interchange in marine invertebrates. Pp. 405–433 *in* B. Schierwater, B. Streit, G. P. Wagner, and R. DeSalle, eds. Molecular ecology and evolution: approaches and applications. Birkhauser Verlag, Basel, Switzerland.

Cunningham, C. W., N. W. Blackstone, and L. W. Buss. 1992. Evolution of king crabs from hermit crab ancestors. Nature 355: 539–542.

Darwin, C. 1859. On the origin of species by means of natural selection. John Murray, London.

De Rijk, P., Y. Van de Peer, and R. De Wachter. 1997. Database on the structure of large ribosomal subunit RNA. Nucl. Acids Res. 25:117–122.

DeMeester, L. 1996. Local genetic differentiation and adaptation in freshwater zooplankton populations: patterns and processes. Ecoscience 3:385–399.

Doyle, J. J., and J. L Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19:11–15.

Frey, D. G. 1987. The taxonomy and biogeography of the Cladocera. Hydrobiologia 145:5–17.

Fryer, G. 1996. Diapause, a potent force in the evolution of freshwater crustaceans. Hydrobiologia 320:1–14.

Gilbert, D. 1992. SeqApp. Ver. 1.9a. A multiple sequence editor for Macintosh computers. Published electronically on the Internet at fly.bio.indiana.edu.

Green, J. 1990. Zooplankton associations in Zimbabwe. J. Zool. Lond. 222:259–283.

Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 21:160–174.

Hebert, P. D. N. 1995. The *Daphnia* of North America: an illustrated Fauna. CD-ROM. University of Guelph, Guelph, Ontario, Canada.

Hebert, P. D. N., and M. J. Beaton. 1993. Methodologies for allozyme analysis using cellulose acetate electrophoresis. Helena Laboratories, Beaumont, TX.

Hebert, P. D. N., and T. L. Finston. 1996. Genetic differentiation in *Daphnia obtusa*: a continental perspective. Fresh. Biol. 35: 311–321.

———. 1997. A taxonomic reevaluation of North American *Daphnia* (Crustacea, Cladocera). 3. The *D. catawba* complex. Can. J. Zool. 75:1254–1261.

Hebert, P. D. N., and B. J. Hann. 1986. Patterns in the diversity of arctic zooplankton communities. Can. J. Aquat. Sci. 43:1416–1425.

Hebert, P. D. N., and C. C. Wilson. 1994. Provincialism in plankton: endemism and allopatric speciation in Australian *Daphnia*. Evolution 48:1333–1349.

Hebert, P. D. N., S. S. Schwartz, and L. J. Weider. 1989a. Geographic patterns in genetic diversity and parthenogenesis within the *Daphnia pulex* group from the southern United States. Am. Mid. Natl. 122:59–65.

Hebert, P. D. N., S. S. Schwartz, and J. Hrbácek. 1989b. Patterns of genotypic diversity in Czechoslovakian *Daphnia*. Heredity 62:207–216.

Herbst, V. H. Y. 1967. Copepoda und Cladocera (Crustacea) aus Südamerika. Gewäss. Abwass. 44/45:96–108.

Herrick, C. L. 1883. Heterogenetic development in Diaptomus. Am. Nat. 17:381–387, 499–505.

———. 1884. A final report on the Crustacea of Minnesota included in the orders Cladocera and Copepoda. The geological and natural history survey of Minnesota. Johnson, Smith, and Harrison, Minneapolis, MN.

Higgins, D. G., and P. M. Sharp. 1988. CLUSTAL: a package for performing multiple sequence alignment on a microcomputer. Gene 73:237–244.

Hillis, D. M., and M. T. Dixon. 1991. Ribosomal DNA: molecular evolution and phylogenetic inference. Q. Rev. Biol. 66:411–453.

Hillis, D. M., and J. P. Huelsenbeck. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. J. Hered. 83:189–195.

Hillis, D. M., B. K. Mable, and C. Moritz. 1996. Applications of molecular systematics: the state of the field and a look to the future. Pp. 515–543 *in* D. M. Hillis, C. Moritz and B. K. Mable, eds. Molecular systematics. Sinauer, Sunderland, MA.

HUELSENBECK, J. P., AND B. RANNALA. 1997. Phylogenetic methods come of age: testing hypotheses in an evolutionary context. Science 276:227–232.

HUTCHINSON, G. E. 1932. Experimental studies in ecology. I. The magnesium tolerance of Daphniidae and its ecological significance. Int. Rev. Ges. Hydrobiol. Hydrog. 28:90–108.

KELLER, W., AND J. R. PITBALDO. 1989. The distribution of crustacean zooplankton in northern Ontario, Can. J. Biogeogr. 16: 249–259.

KIMURA, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111–120.

KJER, K. M. 1995. Use of rRNA secondary structure in phylogenetic studies to identify homologous positions: an example of alignment and data presentation from the frogs. Mol. Phylogenet. Evol. 4:314–330.

KORINEK, V. 1984. Cladocera: hydrobiological survey of Lake Bangweulu Luapula River basin. Bruxelles 13:1–117.

KRAUS, K. 1986. *Daphnia laevis tarasca* ssp. nov., a lake-dwelling subspecies of *Daphnia laevis* Birge 1878 (Cladocera: Daphniidae) from Mexico. Crustaceana 50:225–230.

LEHMAN, N., M. E. PFRENDER, P. A. MORIN, T. J. CREASE, AND M. LYNCH. 1995. A hierarchical molecular phylogeny within the genus *Daphnia*. Mol. Phylogen. Evol. 4:395–407.

LESSA, E. P. 1990. Multidimensional analysis of geographic genetic structure. Syst. Zool. 39:242–252.

LYELL, C. 1832. Principles of geology. Vol. 2. John Murray, London.

LYNCH, M. 1985. Speciation in the Cladocera. Verh. Int. Verein. Limnol. 22:3116–3123.

MAYR, E. 1942. Systematics and the origin of species. Reprinted 1982, Columbia University Press, New York.

MORITZ, C., AND D. M. HILLIS. 1996. Molecular systematics: context and controversies. Pp. 1–13 *in* D. M. Hillis, C. Moritz, and B. K. Mable, eds. Molecular systematics. Sinauer, Sunderland, MA.

NEIGEL, J. E., AND J. C. AVISE. 1986. Phylogenetic relationships of mitochondrial DNA under various models of speciation. Pp. 515–534 *in* E. Nevo and S. Karlin, eds. Evolutionary processes and theory. Academic Press, New York.

PAGE, R. D. M. 1990. Temporal congruence and cladistic analysis of biogeography and cospeciation. Syst. Zool. 39:205–226.

PALUMBI, S. R., A. P. MARTIN, S. ROMANO, W. O. MCMILLAN, L. STICE, AND G. GRABOWSKI. 1991. The simple fool's guide to PCR. Special Publ. Dept. Zoology, University of Hawaii, Honolulu, HI

PLATNICK, N. I., AND G. NELSON. 1978. A method of analysis for historical biogeography. Syst. Zool. 27:1–16.

PROCTOR, V. W., AND C. MALONE. 1965. Further evidence of the passive dispersal of small aquatic organisms via the intestinal tract of birds. Ecology 46:728–729.

RIDDLE, B. R. 1996. The molecular phylogeographic bridge between deep and shallow history in continental biotas. Trends Ecol. Evol. 11:207–211.

SELGEBY, J. H. 1975. Life histories and abundance of crustacean zooplankton in the outlet of Lake Superior, 1971–1972. J. Fish. Res. Bd. Can. 32:461–470.

SIMON, C., F. FRATI, A. BECKENBACH, B. CRESPI, H. LIU, AND P. FLOOK. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Ent. Soc. Am. 87:651–701.

SMALL, E. E., AND R. S. ANDERSON. 1995. Geomorphically driven late Cenozoic rock uplift in the Sierra Nevada, California. Science 270:277–280.

STEMBERGER, R. 1995. Pleistocene refuge areas and postglacial dispersal of copepods of the northeastern United States. Can. J. Fish. Aquat. Sci. 52:2197–2210.

STRIMMER, K., AND A. VON HAESELER. 1997. Likelihood-mapping: a simple method to visualize phylogenetic content of a sequence alignment. Proc. Natl. Acad. Sci. USA 94:6815–6819.

STURMBAUER, C., J. S. LEVINTON, AND J. CHRISTY. 1996. Molecular phylogeny analysis of fiddler crabs: test of the hypothesis of increasing behavioral complexity in evolution. Proc. Natl. Acad. Sci. USA 93:10855–10857.

SWOFFORD, D. L. 1998. PAUP*: Phylogenetic analysis using parsimony (and other methods). Ver. 4.0. Sinauer, Sunderland, MA.

SWOFFORD, D. L., AND R. B. SELANDER. 1981. BIOSYS-1: a FORTRAN program for comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281–283.

TAMURA, K., AND M. NEI. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10:512–526.

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

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

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

VAN DE PEER, Y., J. JANSEN, P. DE RIJK, AND R. DE WACHTER. 1997. Database on the structure of small ribosomal subunit RNA. Nucl. Acids Res. 25:111–116.

WEIDER, L. J., AND A. HOBÆK. 1997. Postglacial dispersal, glacial refugia, and clonal structure in Russian/Siberian populations of the arctic *Daphnia pulex* complex. Heredity 78:363–372.

WOLTERECK, R. 1909. Weitere experimentelle Untersuchungen über Artveränderung, speziell über das Wesen quantitativer Artunterschiede bei Daphniden. Verh. Dtsch. Zool. Ges. 1909:110–172.

YANG, Z. 1993. Maximum likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. Mol. Biol. Evol. 10:1396–1401.

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APPENDIX 1

Sampling site information and head shapes (see Brooks 1957) for members of the North American *Daphnia laevis* complex.

Site codes	Sampling sites	Water body	Collection date	Head morphology
AZ1	7.5 km N of Happy Jack on Hwy 3, Coconino Co., AZ	shallow farm pond	17 Apr. 1990	rounded
CA1	S side of Hwy 180, 54.9 km W of Hume Lake, near Squaw Valley, Fresno Co., CA	farm pond	12 Apr. 1990	rounded
CA2	S side of Hwy 180, 59.4 km W of Hume Lake, near Squaw Valley, Fresno Co., CA	farm pond	12 Apr. 1990	rounded
CA3	S side of Hwy 140, 35.8 km E from Hwy 99, near Mariposa, Merced Co., CA	farm pond	13 Apr. 1990	rounded
CA4	N side of Hwy 104, 15.2 km E from Hwy 99, near Clay, Sacramento Co., CA	farm dam	13 Apr. 1990	rounded
CA5	S side of Hwy 104, 18.4 km E from Hwy 99, Sacramento Co., CA	pond	13 Apr. 1990	rounded
CA6	N side of Hwy 104, 18.8 km E from Hwy 99, Sacramento Co., CA	ditch-pond	13 Apr. 1990	rounded
CA7	N side of Hwy 104, 27.2 km E from Hwy 99, Sacramento Co., CA	shallow pond	13 Apr. 1990	rounded
CA8	N side of Hwy 104, 29.8 km E from Hwy 99, Sacramento Co., CA	farm pond	13 Apr. 1990	rounded
CA9	S side of Hwy 36, 50 km E from Hwy 3, Tehama Co., CA	large farm pond	17 Apr. 1991	rounded
CT1	S side of Hwy 44, 12 km E from Hwy 74, NE CT	large beaver pond	17 Apr. 1991	rounded
GA1	Blue Ridge Lake, Fannin Co., GA	reservoir	15 Dec. 1992	angulated
MA1	Truro on E side of Hwy 6, Cape Cod, MA	shallow pond	17 Apr. 1991	angulated
MA2	2.1 km from Race Point, S side of Hwy 6, Cape Cod, MA	pond	17 Apr. 1991	rounded
MA3	0.7 km from Race Point, S side of Hwy 6, Cape Cod, MA	shallow pond	17 Apr. 1991	rounded
MEX1	Laguna Champayáan, SE Tamaulipas, Mexico (25′22°, 5′98°)	lake	22 Feb. 1992	rounded
MEX2	La Presa Esperanza, Hidalgo, Mexico (3′20°, 20′98°)	shallow reservoir	24 Feb. 1992	rounded
MEX3	Presa in Huichapan, Hidalgo, Mexico (22′20°, 39′99°)	reservoir	25 Feb. 1992	rounded
MI1	Huzzy Lake, Van Buren Co., MI	lake	4 Jul. 1991	pointed
NE1	on W side of Hwy 83, 18.4 km S of Hwy S16B, Cherry Co., NE	man-made pond	9 May 1989	rounded
NC1	Hiwassee Lake, Cherokee Co., NC	reservoir	15 Dec. 1992	angulated
NC2	Santeetlah Lake, Graham Co., NC	reservoir	14 Dec. 1992	rounded
NFLD1	Pond W side Hwy 1, 5.7 km south of Cornerbrook, Newfoundland	Pond	8 Jul. 1990	rounded/pointed
NY1	N side of Hwy 84, 2.8 km E of Hwy 208, Orange Co., NY	ditch-pond	19 Apr 1991	rounded
OK1	E side of Hwy 8, 14 km N of Hwy 64, near Cherokee, Alfalfa Co., OK	pond	20 Apr. 1990	rounded
OK2	E side of Hwy 132, 7.9 km N of Hwy 51, Garfield Co., OK	pond	20 Apr. 1990	rounded
ON1	Rondeau Provincial Park, on N shore of Lake Erie, Ontario (16′42°, 52′81°)	forest pond	April 1990–93	rounded
OR1	South Jetty access road, near Glenada, OR (57′43°, 7′124°)	pond	16 Apr. 1993	rounded
OR2	near estuary of the Siltcoos River W, OR (53′43°, 8′124°)	pond	16 Apr. 1993	rounded
SD1	10 km E of Reva SD (32′45°, 5′103°)	pond	22 May 1989	rounded
TX1	On Hwy 85, 1.7 km E of Hwy 277, near Carrizo Springs, Dimmit Co., TX	shallow pond	8 Apr. 1990	rounded
TX2	W side of Hwy 183, 5.8 km S of Hwy 20 near Cisco, Eastland Co., TX	small farm dam	6 Apr. 1990	rounded
TX3	W side of Rd. 1853, 4.5 km N of Hwy 6, Eastland Co., TX	pond	6 Apr 1990	rounded

APPENDIX 2.

Aligned sequences and proposed secondary structures for the mitochondrial small subunit (12S rRNA) fragment used in this study. Nomenclature follows Kjer (1995) and stem designations follow Van de Peer et al. (1997). Nucleotides paired within the fragment are underlined, simple hairpins are delineated by round brackets, and interrupted stem structures are marked by square brackets. See Appendix 1 for full population designations. Sequences ON1, *dubia*ON, and *dubia*WI are from Taylor et al. (1996).

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                    21'    19'       3'          22          23                    24                  25

CA1          GGUUAAA[CGGAG][AGUUC]A[AGUAGAAUGCA][U GUU]UAGA[CUAGUU AACA]-AUGGAUUAG[AUUAG]UUAUAUUU(A UAUC UUU
CA2          GGUUAAA[CGGAG][AGUUC]A[AGUAGAAUGCA][U GUU]UAGA[CUAGUU AACA]-AUGGAUUAG[AUUAG]UUAUAUUU(A UAUC UUU
CA9          GGUUAAA[CGGAG][AGUUC]A[AGUAGAAUGCA][U GUU]UAGA[CUAGUU AACA]-AUGGAUUAG[AUUAG]UUACAUUU(A UAUC UUU
CA4          GGUUAAA[CGGAG][AGUUC]A[AGUAGAAUGCA][U GUU]UAGA[CUAGUU AACA]-AUGGAUUAG[AUUAG]UUAUAUUU(A UAUC UUU
CA5          GGUUAAA[CGGAG][AGUUC]A[AGUAGAAUGCA][U GUU]UAGA[CUAGUU AACA]-AUGGAUUAG[AUUAG]UUACAUUU(A UAUC UUU
CA7          GGUUAAA[CGGAG][AGUUC]A[AGUAGAAUGCA][U GUU]UAGA[CUAGUU AACA]-AUGGAUUAG[AUUAG]UUAUAUUU(A UAUC UUU
CA8          GGUUAAA[CGGAG][AGUUC]A[AGUAGAAUGCA][U GUU]UAGA[CUAGUU AACA]-AUGGAUUAG[AUUAG]UUAUAUUU(A UAUC UUU
OR1          GGUUAAA[CGGAG][AGUUC]A[AGUAGAAUGCA][U GUU]UAGA[CUAGUU AACA]-AUGGAUUAG[AUUAG]UUAUAUUU(G UAUC UUU
TX2          GGUUAAA[CGAAG][AGUUC]A[AGUAGAAUACA][U GUU]UAGA[CUAGUU AACA]-AUGGAUUAG[AUUAG]UUAUAUUU(A UAUC UUU
TX3          GGUUAAA[CGAAG][AGUUC]A[AGUAGAAUACC][U GUU]UAGA[CUAGUU AACA]-AUGGAUUAG[AUUAG]UUAUAUUU(A UAUC UUU
NY1          GGUUAAA[CGAAG][AGUUC]A[AGUAGAAUACA][U GUU]UAGA[CUAGUU AACA]-AUGGAUUAG[AUUAG]UUAUAUUU(A UAUC UUU
AZ1          GGUUAAA[CGAAG][AGUUC]A[AGUAGAAUACA][U GUU]UAGA[CUAGUU AACA]-AUGGAUUAG[AUUAG]UUAUAUUU(A UAUC UUU
TX1          GGUUAAA[CGAAG][AGUUC]A[AGUAGAAUACA][U GUU]UAGA[CUAGUU AACA]-AUGGAUUAG[AUUAG]UUAUAUUU(A UAUC UUU
MA1          GGUUAGA[CGAAG][AGUUC]G[AGUAGAAUAUG][U GUU]UAGA[CUAGUU AACA]-UUAAAUUGG[UUUAG]UUAUAUUC(A UAUU UUU
MA2          GGUUAGA[CGAAG][AGUUC]G[AGUAGAAUAUG][U GUU]UAGA[CUAGUU AACA]-UUAAAUUGG[UUUAG]UUAUAUUC(A UAUU UUU
MA3          GGUUAGA[CGAAG][AGUUC]G[AGUAGAAUAUG][U GUU]UAGA[CUAGUU AACA]-UUAAAUUGG[UUUAG]UUAUAUUC(A UAUU UUU
CT1          GGUUAGA[CGAAG][AGUUC]G[AGUAGAAUAUG][U GUU]UAGA[CUAGUU AACA]-UUAAAUUGG[UUUAG]UUAUAUUC(A UAUU UUU
GA1          GGUUAGA[CGAAG][AGUUC]G[AGUAAAAUAUG][U GUU]UAGA[CUAGUU AACA]-UUAAAUUGG[UUUAG]UUAUAUUC(A UAUU UUU
MEX1         GGUUAGA[CGAAG][AGUUC]G[AGUAAAAUAUG][U GUU]UAGA[CUAGUU GACA]-UUAAAUUGG[UUUAG]UUAUAUUC(A UAUU UUU
NC1          GGUUAGA[CGAAG][AGUUC]G[AGUAAAAUAUG][U GUU]UAGA[CUAGUU AACA]-UUAAAUUGG[UUUAG]CUAUAUUC(A UAUU UUU
NFLD1        GGUUAGA[CGAAG][AACUC]A[AAUGGAAUACG][U GUU]UGGU[CUAGUU AACG]-UUAGAUUAA[UAUAG]UUGUAUUU(U UAUU UUU
MI1          GGUUAGA[CGAAG][AACUC]A[AAUGGAAUGCG][U GUU]UGGU[CUAGUU AACG]-UUAGAUUAA[UAUAG]UUGUAUUU(U UAUU UUU
ON1          ???????[?????][?????]?[???????????][? ???]????[?????? ????]-??GGAUUAG[AUUAG]UUAUA?UU(A UAUC UUU
dubiaON      ???????[?????][?????]?[???????????][? ???]????[???GUU ???G]-UUAUAUUAA[UAUAG]UUGUA?UU(U UAUU UUU
dubiaWI      ???????[?????][?????]?[???????????][? ???]????[??AGUU AACG]-UUAGAUUAA[UAUAG]UUGUAU?A(U UAUU UUU
galeata      GGUUAGA[CGAAG][AAUCC]A[UAUAGAAUGAA][U GUU]UGCG[UUAGUU AACG]-UUCAAUUUU[UGAAG]UUUUUCUA(G CUUU UUU
ambigua      GGUUAGA[CGAAA][AAUUC]U[AGUAGAGUAGA][U GUU]UGUU[GUAGUU]AACA -UAAAAUUAC[UAAAG]AUAACAAA C(UUUA UUU
longiceph.   GGUUAGA[CGAAG][AAUCU]G[AAUGGAAUAAU] U[UAU]UAUU[UUAAUU AACA]GUAAAUAAUU[UUAAG]UAAAUUUA U UCUU(UUU

                    25'          26    26'      24'          23'          27

CA1          UUUAGGUGAAA UUUAU)-AUAAGA(UACUAUUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(GUUUAGAGGAUAGACCAG
CA2          UUUAGGUGAAA UUUAU)-AUAAGA(UACUAUUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(GUUUAGAGGAUAGACCAG
CA9          UUUAGGUGAAA UUUAU)-AUAAGA(UACUAUUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(GUUUAGAGGAUAGACCAG
CA4          UUUAGGUGAAA UUUAU)-AUAAGA(UACUAUUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(GUUUAGAGGAUAGACCAG
CA5          UUUAGGUGAAA UUUAU)-AUAAGA(UACUAUUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(GUUUAGAGGAUAGACCAG
CA7          UUUAGGUGAAA UUUAU)-AUAAGA(UACUAUUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(GUUUAGAGGAUAGACCAG
CA8          UUUAGGUGAAA UUUAU)-AUAAGA(UACUAUUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(GUUUAGAGGAUAGACCAG
OR1          UUUGGGUGAAA UUUAU)-AUAAGA(UACUAUUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(GUUUAGAGGAUAGACCAG
TX2          UUUAGGUGAAA UUUAU)-AUAAGA(UACUAGUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(GUUUAGAGGAUAAACCAG
TX3          UUUAGGUGAAA UUUAU)-AUAAGA(UACUAAUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(GUUUAGAGGAUAAACCAG
NY1          UUUAGGUGAAA UUUAU)-AUAAGA(UACUAAUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(GUUUAGAGGAUAAACCAG
AZ1          UUUAGGUGAAA UUUAU)-AUAAGA(UACUAAUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(GUUUAGAGGAUAAACCAG
TX1          UUUAGGUGAAA UUUAU)-AUAAGA(UACUAGUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(GUUUAGAGGAUAAACCAG
MA1          UUUAGGUGAAA UUUAU)-AUAAAA(UAUUAAUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(AUUUAGGGGAAAAACCAG
MA2          UUUAGGUGAAA UUUAU)-AUAAAA(UAUUAAUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(AUUUAGGGGAAAAACCAG
MA3          UUUAGGUGAAA UUUAU)-AUAAAA(UAUUAAUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(AUUUAGGGGAAAAACCAG
CT1          UUUAGGUGAAA UUUAU)-AUAAAA(UAUUAAUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(AUUUAGGGGAAAAACCAG
GA1          UUUAGGUGAAA UUUAU)-AUAAAA(UAUUAAUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(AUUUAGGGGAAAAACCAG
MEX1         UUUAGGUGAAA UUUAU)-AUAAAA(UAUUAAUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(AUUUAGGGGAAAAACCAG
NC1          UUUAGGUGAAA UUUAU)-AUAAAA(UAUUAAUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(AUUUAGGGGAAAAACCAG
NFLD1        CAAAGGUGAAA UUUAU)-AUAAAA(UGUUAAUA)UUUC[UUCUA]UAAUCUA[UUGG AAUUAG]AAA(GUUUAGGUAAUAAACCAG
MI1          CAAAGGUGAAA UUUAU)-AUAAAA(UGUUAAUA)UUUC[UUCUA]UAAUCUA[UUGG AAUUAG]AAA(GUUUAGGUAAUAAACCAG
ON1          UUUAGGUGAAA UUUAU)-AUAAGA(UACUAAUA)AUUC[UUUUA]UAAUUUA[UAGG AACUAG]AAA(GUUUAGAGGAUAAACCAG
dubiaON      CAAAGGUGAAA UUUAU)-AUAAAA(UGUUAAUA)UUUC[UUCUA]UAAUCUA[UUGG AAUUAG]AAA(GUUUAGGUAAUAAACCAG
dubiaWI      CAAAGGUGAAA UUUAU)-AUAAAA(UGUUAAUA)UUUC[UUCUA]UAAUCUA[UUGG AAUUAG]AAA(GUUUAGGUAAUAAACCAG
galeata      GUUGGGUGAAA UCAGU)-AUAAGA(AGCUCUUU)CCUC[UUUAA]AAAUUAG[UAGG AACUAA]AAA(ACUUUGAUUUAAAACCAG
ambigua      UAAAGGUGAAA UUUGA)UAAUAGA(GGAUCUAU)UUCU[UUAAU]UAAUUUU AAGG[AACUAG]AAA(GUUUAGAUCUAAAACCAG
longiceph.   UUUGGGUGAAA)UUUA- UAUAAAA(GAACAAAU)AAUC[UUGUA]UUAUUUA[UAGG AAUUAA]UAA(GUGAAGAUAAAAAACCAG
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APPENDIX 2. Continued.

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                27'                          22'          31           31'          2'       32

CA1        GAUUAGAUACCCUGUUAUUCUAAAU)CAUA-CUAAA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
CA2        GAUUAGAUACCCUGUUAUUCUAAAU)CAUA-CUAAA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
CA9        GAUUAGAUACCCUGUUAUUCUAAAU)CAUA-CUAAA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
CA4        GAUUAGAUACCCUGUUAUUCUAAAU)CAUA-CUAAA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
CA5        GAUUAGAUACCCUGUUAUUCUAAAU)CAUA-CUAAA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
CA7        GAUUAGAUACCCUGUUAUUCUAAAU)CAUA-CUAAA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
CA8        GAUUAGAUACCCUGUUAUUCUAAAU)CAUA-CUAAA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
OR1        GAUUAGAUACCCUGUUAUUCUAAAU)CAUA-CUAAA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
TX2        GAUUAGAUACCCUGUUAUUCUAAAU)CAUA-CUUAA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
TX3        GAUUAGAUACCCUGUUAUUCUAAAU)CAUA-CUUAA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
NY1        GAUUAGAUACCCUGUUAUUCUAAAU)CAUA-CUUAA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
AZ1        GAUUAGAUACCCUGUUAUUCUAAAU)CAUA-CUUAA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
TX1        GAUUAGAUACCCUGUUAUUCUAAAU)CAUA-CUUAA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
MA1        GAUUAGAUACCCUGUUAUUCUAAAU)UAUA-UCAUA[AAUA]GGGUA(GUAAAAGUUAUUCUUCAAAC)CCA[AAGA]UU[UUGGC
MA2        GAUUAGAUACCCUGUUAUUCUAAAU)UAUA-UCAUA[AAUA]GGGUA(GUAAAAGUUAUUCUUCAAAC)CCA[AAGA]UU[UUGGC
MA3        GAUUAGAUACCCUGUUAUUCUAAAU)UAUA-UCAUA[AAUA]GGGUA(GUAAAAGUUAUUCUUCAAAC)CCA[AAGA]UU[UUGGC
CT1        GAUUAGAUACCCUGUUAUUCUAAAU)UAUA-UCAUA[AAUA]GGGUA(GUAAAAGUUAUUCUUCAAAC)CCA[AAGA]UU[UUGGC
GA1        GAUUAGAUACCCUGUUAUUCUAAAU)UAUA-UUAUA[AAUA]GGGUA(GUAAAAGUUAUUCUUCAAAC)CCA[AAGA]UU[UUGGC
MEX1       GAUUAGAUACCCUGUUAUUCUAAAU)UAUA-UCAUA[AAUA]GGGUA(GUAAAAGUUAUUCUUCAAAC)CCA[AAGA]UU[UUGGC
NC1        GAUUAGAUACCCUGUUAUUCUAAAU)UAUA-UCAUA[AAUA]GGGUA(GUAAAAGUUAUUCUUCAAAC)CCA[AAGA]UU[UUGGC
NFLD1      GAUUAGAUACCCUGCUAUUCUAAAU)UAUGUUUUUA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
MI1        GAUUAGAUACCCUGCUAUUCUAAAU)UAUGUUUUUA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
ON1        GAUUAGAUACCCUGUUAUUCUAAAU)CAUA-CUUAA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
dubiaON    GAUUAGAUACCCUGCUAUUCUAAAU)UAUGUUUUUA[AAUA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
dubiaWI    GAUUAGAUACCCUGCUAUUCUAAAU)UAUGUUUUUA[AAUA]GGGUA(GUAAAAGUCGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
galeata    GAUUAGAUACCCUGUUAUUCUCAGU)UAU--UCUUA[AACA]GGGUA(GUAAAAGUUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
ambigua    GAUUAGAUACCCUGUUAUUCUAAAU)GA-GAGUUUA[AAUA]GGGUA(GUAAAAGCUGUUCUUCAAAC)CCA[AAGA]UU[UUGGC
longiceph. GAUUAGAUACCCUGUUAUUCUUUAU)-A-AUGUUUA[-GUA]GGGUA(GUAAAAGUUAUUCUUCAAAC)CCA[AAGA]UU[UUGGC

                32           33          34           35          35'          36              38

CA1        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]AUA(AUCGAUAGACCUCGU)UUAAUCUUA[CCUUAAC]UUGUAUA[GCUUGU
CA2        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]AUA(AUCGAUAGACCUCGU)UUAAUCUUA[CCUUAAC]UUGUAUA[GCUUGU
CA9        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]AUA(AUCGAUAGACCUCGU)UUAAUCUUA[CCUUAAC]UUGUAUA[GCUUGU
CA4        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]AUA(AUCGAUAGACCUCGU)UUAAUCUUA[CCUUAAC]UUGUAUA[GCUUGU
CA5        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]AUA(AUCGAUAGACCUCGU)UUAAUCUUA[CCUUAAC]UUGUAUA[GCUUGU
CA7        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]AUA(AUCGAUAGACCUCGU)UUAAUCUUA[CCUUAAC]UUGUAUA[GCUUGU
CA8        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]AUA(AUCGAUAGACCUCGU)UUAAUCUUA[CCUUAAC]UUGUAUA[GCUUGU
OR1        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]AUA(AUCGAUAGACCUCGU)UUAAUCUUA[CCUUAAC]UUGUAUA[GCUUGU
TX2        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]AUA(AUCGAUAGACCUCGU)UUAAUCUUA[CCUUAAC]UAGUAUA[GCUUGU
TX3        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]AUA(AUCGAUAGACCUCGU)UUAAUCUUA[CCUUAAC]UUGUAUA[GCUUGU
NY1        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]AUA(AUCGAUAGACCUCGU)UUAAUCUUA[CCUUAAC]UAGUAUA[GCUUGU
AZ1        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]AUA(AUCGAUAGACCUCGU)UUAAUCUUA[CCUUAAC]UAGUAUA[GCUUGU
TX1        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]AUA(AUCGAUAGACCUCGU)UUAAUCUUA[CCUUAAC]UAGUAUA[GCUUGU
MA1        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]UUA(AUCGAUAGACCUCGU)UUUAUCUUA[CCUUAAC]UUGUUUA[GCUUGU
MA2        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]UUA(AUCGAUAGACCUCGU)UUUAUCUUA[CCUUAAC]UUGUUUA[GCUUGU
MA3        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]UUA(AUCGAUAGACCUCGU)UUUAUCUUA[CCUUAAC]UUGUUUA[GCUUGU
CT1        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]UUA(AUCGAUAGACCUCGU)UUUAUCUUA[CCUUAAC]UUGUUUA[GCUUGU
GA1        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]UUA(AUCGAUAGACCUCGU)UUUAUCUUA[CCUUAAC]UUGUUUA[GCUUGU
MEX1       GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]UUA(AUCGAUAGACCUCGU)UUUAUCUUA[CCUUAAC]UUGUUUA[GCUUGU
NC1        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]UUA(AUCGAUAGACCUCGU)UUUAUCUUA[CCUUAAC]UUGUUUA[GCUUGU
NFLD1      GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCU]AUA(AUCGAUAGGCCUCGU)UUUAUCUUA[CCUUUAC]UCGUUGA[GCUUGU
MI1        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCU]AUA(AUCGAUAGGCCUCGU)UUUAUCUUA[CCUUUAC]UCGUUGA[GCUUGU
ON1        GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCC]AUA(AUCGAUAGACCUCGU)UUUAUCUUA[CCUUAAC]UAGUAUA[GCUUGU
dubiaON    GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCU]AUA(AUCGAUAGGCCUCGU)UUUAUCUUA[CCUUUAC]UCGUUGA[GCUUGU
dubiaWI    GGCACUUUA]ACCAA[AUAGA]GG[AGCUUGCCCU]AUA(AUCGAUAGGCCUCGU)UUUAUCUUA[CCUUUAC]UCGUUGA[GCUUGU
galeata    GGCACUUUA]UCCAA[AUAGA]GG[AGCUUGUCCC]UUA(AUCGAUAGUCCUCGU)UUUAUCUUU[CCUUCAU]UUGUAAA[GCUUGU
ambigua    GGCAUUUCA]UCCUA[AUAGA]GG[AGCUUGUCCU]GUA(AUUGAUAAUCCUCGU)UUAAUCUUA[CCCUUUU]UUGUAGA[GCUUGU
longiceph. GGCAUUUUA]UCCAA[ACAGA]GG[AGCUUGUCUU]AUA(AUCGAUAAUCCCCGU)UUUAUCUUA[CCUUUAU]UUGUAUA[GCUUGU
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APPENDIX 2. Continued.

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                38            39      40     40'     41   41'    42    42'              38'

CA1          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]A
CA2          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]A
CA9          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]A
CA4          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]A
CA5          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]A
CA7          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]A
CA8          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]A
OR1          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAA)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]A
TX2          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]A
TX3          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]A
NY1          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)AAA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]A
AZ1          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]A
TX1          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]A
MA1          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAGG)-AA(AAAAAAUUU)UUCAACUU[CAGGUCAAGGUGCAGU]U
MA2          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAGG)-AA(AAAAAAUUU)UUCAACUU[CAGGUCAAGGUGCAGU]U
MA3          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAGG)-AA(AAAAAAUUU)UUCAACUU[CAGGUCAAGGUGCAGU]U
CT1          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAGG)-AA(AAAAAAUUU)UUCAACUU[CAGGUCAAGGUGCAGU]U
GA1          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]U
MEX1         AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]U
NC1          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]U
NFLD1        AUACCGCCG]UUGUCA[GA]UUAC(UCUAUAAGGA)UG(UUUUCAAG)-AA(AAAUAAUUU)UCUAACUU[CAGGUCAAGGUGCAGU]G
MI1          AUACCGCCG]UUGUCA[GA]UUAC(UCUAUAAGGA)UG(UUUUCAAG)-AA(AAAUAAUUU)UCUAACUU[CAGGUCAAGGUGCAGU]G
ON1          AUACCGCCG]UUGUCA[GA]UUAC(UCUAAAAGGA)UG(UUUUCAAG)-AA(AAAAAAUUU)UUUAACUU[CAGGUCAAGGUGCAGU]A
dubiaON      AUACCGCCG]UUGUCA[GA]UUAC(UCUAUAAGGA)UG(UUUUCAAG)-AA(AAAUAAUUU)UCUAACUU[CAGGUCAAGGUGCAGU]G
dubiaWI      AUACCGCCG]UUGUCA[GA]UUAC(UCUAUAAGGA)UG(UUUUCAAG)-AA(AAAUAAUUU)UCUAACUU[CAGGUCAAGGUGCAGU]G
galeata      AUACCGCCG]UUGUCA[GA]UUAC(UUUUAGAGAA)UG(UUUUCAAG)-UU(AAAAGACUU)UAUAACUU[CAGGUCAAGGUGCAGU]U
ambigua      AUACCGCCG]UUGUCA[GA]UUAC(UUCUAAAGAA)UG(UUUUCAAG)-AA(AAUUUUUUU)UCUUACUU[CAGGUCAAGGUGCAGU]U
longiceph.   AUACCUCCG]UUGACA[GA]UUAC(UCUAAAAGGA)UG(UUUUCUAG)-AA(AAAUUAUUU)UUUUACUU[CAGGUCAAGGUGCAGU]U

                36'          34'             45       45'               47          47'                33'

CA1          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UCUAAUUUA-AAAUUAG U)GGAAGGUGGA[UU
CA2          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UCUAAUUUA-AAAUUAG U)GGAAGGUGGA[UU
CA9          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UCUAAUUUA-AAAUUAG U)GGAAGGUGGA[UU
CA4          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UCUAAUUUA-AAAUUAG U)GGAAGGUGGA[UU
CA5          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UCUAAUUUA-AAAUUAG U)GGAAGGUGGA[UU
CA7          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UCUAAUUUA-AAAUUAG U)GGAAGGUGGA[UU
CA8          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UCUAAUUUA-AAAUUAG U)GGAAGGUGGA[UU
OR1          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UCUAAUUUA-AAAUUAG U)GGAAGGUGGA[UU
TX2          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUAUUAAGAA)A--ACGGAU(A CUUAAUUUA-AAAUUAG U)GGAAGGUGGA[UU
TX3          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUAUUAAGAA)A--ACGGAU(A CUUAAUUUA-AAAUUAG U)GGAAGGUGGA[UU
NY1          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUAUUAAGAA)A--ACGGAU(A CUUAAUUUA-AAAUUAG U)GGAAGGUGGA[UU
AZ1          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUAUUAAGAA)A--ACGGAU(A CUUAAUUUA-AAAUUAG U)GGAAGGUGGA[UU
TX1          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUAUUAAGAA)A--ACGGAU(A CUUAAUUUA-AAAUUAG U)GGAAGGUGGA[UU
MA1          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UUUAUUUUA-AAAUUAA U)GGAAGGUGGA[UU
MA2          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UUUAUUUUA-AAAUUAA U)GGAAGGUGGA[UU
MA3          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UUUAUUUUA-AAAUUAA U)GGAAGGUGGA[UU
CT1          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UUUAUUUUA-AAAUUAA U)GGAAGGUGGA[UU
GA1          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UUUAUUUUA-AAAUUAA U)GGAAGGUGGA[UU
MEX1         UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UUUAUUUUA-AAAUUAA U)GGAAGGUGGA[UU
NC1          UAU[GUUAAGG]U[GGAGGUGAGCU]ACAA(UUCUGUUAAGAA)A--ACGGAU(A UUUAUUUUA-AAAUUAA U)GGAAGGUGGA[UU
NFLD1        CAU[GUAAGGG]U[GGCGGUGAGCU]ACAA(UUCUGAUAAGAA)U--ACGAAU(G UUUAAUUUAUAAGUUGA U)UGAAGGUGGA[UU
MI1          CAU[GUAAGGG]U[GGCGGUGAGCU]ACAA(UUCUGAUAAGAA)U--ACGAAU(G UUUAAUUUAUAAGUUGA U)UGAAGGUGGA[UU
ON1          UAU[GUUAAGG]U[GGAGGUGAGCU]?CAA(UUCUAUUAAGAA)A--ACGGAU(A CUUAAUUUA-AAAUUAG U)UGAAGGUGGA[UU
dubiaON      CAU[GUAAGGG]U[GGCGGUGAGC?]ACAA(UUCUGAUAAGAA)U--ACGAAU(G UUUAAUUUAUAAGUUGA U)UGAAGGUGGA[UU
dubiaWI      UAU[GUAAAGG]U[GGCGGUGAGCU]ACAA(UUCUGAUAAGAA)C--ACGAAU(G UUUAAUUUAUAAGUUGA U)UGAAGGUGGA[UU
galeata      UAU[AUGGGGG]U[GGAGGUGAGCU]ACAG(UUUUCUUUAGAA)AAUACGGAU U(CUAAGCUUUGAAGCUAG)C UGAAGGUGGA[UU
ambigua      UAU[GGAAGGG]C[AGUGAUGAGCU]ACAA(UUUAUACUAAAA)A-UACGGAU(A CUAGAUUUUAAUAUUGG U)GGAAGGUGGA[UU
longiceph.   UAU[GUAAAGG]A[AAUGAUGAGCU]ACAA(UUUUUUAAAAAA)A--ACGGAU(U UC-AAUUUAAAAUCUGA)U UGAAGGUGGA[UU
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APPENDIX 2. Continued.

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                33'          48          48'                  32'                        49

CA1         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUUACU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUG
CA2         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUUACU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUG
CA9         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUUACU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUG
CA4         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUUACU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUG
CA5         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUUACU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUG
CA7         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUUACU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUG
CA8         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUUACU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUG
OR1         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUUACU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUU
TX2         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUUACU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUU
TX3         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUUACU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUU
NY1         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUUACU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUU
AZ1         UA U]UAGUA(AGUUA-AUUUAAGUAAAGUUUACU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUU
TX1         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUUACU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUU
MA1         UA U]UAGUA(AGUUA-AUUUAAGUAAAGUUAGCU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUU
MA2         UA U]UAGUA(AGUUA-AUUUAAGUAAAGUUAGCU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUU
MA3         UA U]UAGUA(AGUUA-AUUUAAGUAAAGUUAGCU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUU
CT1         UA U]UAGUA(AGUUA-AUUUAAGUAAAGUUAGCU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUU
GA1         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUAGCU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUU
MEX1        UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUAGCU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUU
NC1         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUAGCU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCACU(CUCCUCUU
NFLD1       UA U]UAGUA(AGUUA-GUUUAAGUAAAAUUAACU)GAAUAAGGUAA[UGAAGUGUGCACA]UAUCGCCCGUCACU(CUCCCCUU
MI1         UA U]UAGUA(AGUUA-GUUUAAGUAAAAUUAACU)GAAUAAGGUAA[UGAAGUGUGCACA]UAUCGCCCGUCACU(CUCCCCUU
ON1         UA U]UAGUA(AGUUA-AUUUAAGUAAAAUUUACU)GAAUAAGGUAA[UAAAGUGUGCACA]UAUCGCCCGUCA?U(C?????UU
dubiaON     UA U]UAGUA(AGUUA-GUUUAAGUAAAAUUAACU)GAAUAAGGUAA[UGAAGUGUGCACA]UAUCGCCCGUCAC?(C?????UU
dubiaWI     UA U]UAGUA(AGUUA-GUUUAAGUAAAAUUAACU)GAAUAAGGUAA[UGAAGUGUGCACA]UAUCGCCCG?????(????CCUU
galeata     UA]C UAGUA(AAGUA-AUUUAAGUAAAAUUACUU)GAAUAAGGCAA[UAAAGUGUGUACA]UAUCGCCCGUCACU(CUCUCCUU
ambigua     UA U]UAGUA(AGUUU-ACUUAAGUAUAGUAAAUU)GAAUAAGGCAA[UGAGAUGUGCACA]UAUCGCCCGUCACU(CUCUUCUU
longiceph.  UG U]UAGUA(AGCUUUACUAAAGAAAAGUAAGCU)GAAUUAGGCAA[UAGAAUGUGCACA]UAUCGCCCGUCACU(CUCCUCUU

                49'

CA1         AAGAGGAG)AUAAGUCGUAACAAA
CA2         AAGAGGAG)AUAAGUCGUAACAAA
CA9         AAGAGGAG)AUAAGUCGUAACAAA
CA4         AAGAGGAG)AUAAGUCGUAACAAA
CA5         AAGAGGAG)AUAAGUCGUAACAAA
CA7         AAGAGGAG)AUAAGUCGUAACAAA
CA8         AAGAGGAG)AUAAGUCGUAACAAA
OR1         AAGAGGAG)AUAAGUCGUAACAAA
TX2         AAGAGGAG)AUAAGUCGUAACAAA
TX3         AAGAGGAG)AUAAGUCGUAACAAA
NY1         AAGAGGAG)AUAAGUCGUAACAAA
AZ1         AAGAGGAG)AUAAGUCGUAACAAA
TX1         AAGAGGAG)AUAAGUCGUAACAAA
MA1         AAGAGGAG)AUAAGUCGUAACAAA
MA2         AAGAGGAG)AUAAGUCGUAAC???
MA3         AAGAGGAG)AUAAGUCGUAACAAA
CT1         AAGAGGAG)AUAAGUCGU??????
GA1         AAGAGGAG)AUAAGUCGUAACAAA
MEX1        AAGAGGAG)AUAAGUCGUAACAAA
NC1         AAGAGGAG)AUAAGUCGUAACAAA
NFLD1       AAGGGGAG)AUAAGUCGUAACAAA
MI1         AAGGGGAG)AUAAGUCGUAACAAA
ON1         ????????)????????????????
dubiaON     AAG?????)????????????????
dubiaWI     AAG?????)????????????????
galeata     AAGGAGAG)AUAAGUCGUAACAAA
ambigua     AAGAGGAG)AUAAGUCGU??????
longiceph.  AAGAGGAG)AUAAGUCGUAACAAA
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APPENDIX 3.

Aligned sequences and proposed secondary structures for the mitochondrial large subunit (16S rRNA) fragment used in this study. Nomenclature follows Kjer (1995) and stem designations follow De Rijk et al. (1997). Nucleotides paired within the fragment are underlined, simple hairpins are delineated by round brackets, and interrupted stem structures are marked by square brackets. See Appendix 1 for full population designations.

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                E23          E23'        E24       E25             E25'       E26          E26'      E27

GA1             CU(CUUUCUGA--UUAUAGAAAG)UAC[CGCC]U(GCUCAAUGCUUUUUGUAAAUAGC)(CGCAGUAUCCUGACUGUG)CUA[AG]GU
MEX1            CU(CUUUCUGA--UUAUAGAAAG)UAC[CGCC]U(GCUCAAUGCUUUUUGUAAAUAGC)(CGCAGUAUCCUGACUGUG)CUA[AG]GU
MA1             CU(CUUUCUGA--UUAUAGAAAG)UAC[CGCC]U(GCUCAAUGCUUUUUGUAAAUAGC)(CGCAGUAUCCUGACUGUG)CUA[AG]GU
CT1             CU(CUUUCUGA--UUAUAGAAAG)UAC[CGCC]U(GCUCAAUGCUUUUUGUAAAUAGC)(CGCAGUAUCCUGACUGUG)CUA[AG]GU
MI1             CU(CUUUCUGA--UUAUAGAAAG)UAC[CGCC]U(GCUCAAUGCUUUAUGUAAAUAGC)(CGCAGUAUCCUGACUGUG)CUA[AG]GU
NFLD1           CU(CUUUCUGA--UUAUAGAAAG)UAC[CGCC]U(GCUCAAUGCUUUAUGUAAAUAGC)(CGCAGUAUCCUGACUGUG)CUA[AG]GU
NY1             CU(CUUUCUGA--UUAUAGAAAG)UAC[CGCC]U(GCUCAAUGCUUU-UGUAAAUAGC)(CGCAGUAUCCUGACUGUG)CUA[AG]GU
AZ1             CU(CUUUCUGA--UUAUAGAAAG)UAC[CGCC]U(GCUCAAUGCUUU-UGUAAAUAGC)(CGCAGUAUCCUGACUGUG)CUA[AG]GU
CA8             CU(CUUUCUGA--UUAUAGAAAG)UAC[CGCC]U(GCUCAAUGCUUU-UGUAAAUAGC)(CGCAGUAUCCUGACUGUG)CUA[AG]GU
OR1             CU(CUUUCUGA--UUAUAGAAAG)UAC[CGCC]U(GCUCAAUGCUUU-UGUAAAUAGC)(CGCAGUAUCCUGACUGUG)CUA[AG]GU
galeata         CU(CUUUUUGAAUAAAUAAAAAG)UAC[CGCC]U(GCUCAAUGCAUUUUGUAAAUGGC)(CGCAGUAUUUUGACUGUG)CUA[AG]GU
ambigua         CU(CCCCCUGAA-UUAUAAGGGG)UAU[CGCC]U(GCUCAAUGCAUU--GUAAAUAGC)(CGCAGUAUCCUGACUGUG)CUA[AG]GU
longiceph.      CU(CCUCUUGAA-AUAUAGGAGG)UAC[CGUC]U(GCUCAAUGCAAUUUGUAAAUAGC)(CGCAGUAUUCUGACUGUG)CUA[AG]GU

                     E27'    E28       E28'              E24'       E21'                  E18'          E1'

GA1             AGCAUAAUCA[UU]A(GUCUUUUAAUUGAAGGC)UGGUAUGAAC[GGCG]AGA[CGAGAAAAAA]GC[UGUCUCUAAUAUAAUAA][UU]
MEX1            AGCAUAAUCA[UU]A(GUCUUUUAAUUGAAGGC)UGGUAUGAAC[GGCG]AGA[CGAGAAAAAA]GC[UGUCUCUAAUAUAAUAA][UU]
MA1             AGCAUAAUCA[UU]A(GUCUUUUAAUUGAAGGC)UGGUAUGAAC[GGCG]AGA[CGAGAAAAAA]GC[UGUCUCUAAUAUAAUAA][UU]
CT1             AGCAUAAUCA[UU]A(GUCUUUUAAUUGAAGGC)UGGUAUGAAC[GGCG]AGA[CGAGAAAAAA]GC[UGUCUCUAAUAUAAUAA][UU]
MI1             AGCAUAAUCA[UU]A(GUCUUUUAAUUGAAGGC)UGGUAUGAAC[GGCG]AGA[CGAGAAAAAA]GC[UGUCUCUUAGAUAUUAG][UU]
NFLD1           AGCAUAAUCA[UU]A(GUCUUUUAAUUGAAGGC)UGGUAUGAAC[GGCG]AGA[CGAGAAAAAA]GC[UGUCUCUUAGAUAUUAG][UU]
NY1             AGCAUAAUCA[UU]A(GUCUUUUAAUUGAAGGC)UGGUAUGAAC[GGCG]AGA[CGAGAAAAAA]GC[UGUCUCUAAAAUAAUUA][UU]
AZ1             AGCAUAAUCA[UU]A(GUCUUUUAAUUGAAGGC)UGGUAUGAAC[GGCG]AGA[CGAGAAAAAA]GC[UGUCUCUAAAAUAAUUA][UU]
CA8             AGCAUAAUCA[UU]A(GUCUUUUAAUUGAAGGC)UGGUAUGAAC[GGCG]AGA[CGAGAAAAAA]GC[UGUCUCUAAAAUAAUUA][UU]
OR1             AGCAUAAUCA[UU]A(GUCUUUUAAUUGAAGGC)UGGUAUGAAC[GGCG]AGA[CGAGAAAAAA]GC[UGUCUCUAAAAUAAUUA][UU]
galeata         AGCAUAAUCA[UU]A(GUCUUUUAAUUGAAGGC)UGGUAUGAAU[GGUG]AGA[CGAGAAAAUA]GC[UGUCUCUGAAAUAGAUU][UU]
ambigua         AGCAUAAUCA[UU]A(GUCUCUUAAUUAGAGGC)UGGUAUGAAU[GGUG]AGA[CGAGAAAAAA]GC[UGUCUCUAAAGUAGCUA][UC]
longiceph.      AGCAUAAUCA[UU]A(GUCUUUUAAUUGAAGGC)UGGUAUGAAU[GACG]AGA[CGAGAAAAUA]GC[UGUCUCUACAAUAAAUU][UU]

                          F1          F1'          G1                  G2          G3'

GA1             GAAUUUUAUU(UUUAAGUGAAAAAGCUUAAA)UUUG[UUUGGGGGACGA]UCAGA[CCCUUUGGAGCUU]U(AUUACCUUAUAAG
MEX1            GAAUUUUAUU(UUUAAGUGAAAAAGCUUAAA)UUUG[UUUGGGGGACGA]UCAGA[CCCUUUGGAGCUU]U(AUUACCUUAUAAG
MA1             GAAUUUUAUU(UUUAAGUGAAAAAGCUUAAA)UUUG[UUUGGGGGACGA]UCAGA[CCCUUUGGAGCUU]U(AUUACCUCAUAAG
CT1             GAAUUUUAUU(UUUAAGUGAAAAAGCUUAAA)UUUG[UUUGGGGGACGA]UCAGA[CCCUUUGGAGCUU]U(AUUACCUCAUAAG
MI1             GAAUUUUAUU(UUUAAGUGAAAAAGCUUAAA)UUUA[UUUGGGGGACGA]UCAGA[CCCUUUGGAGCUU]U(AUUCUUUAGUGAG
NFLD1           GAAUUUUAUU(UUUAAGUGAAAAAGCUUAAA)UUUA[UUUGGGGGACGA]UCAGA[CCCUUUGGAGCUU]U(AUUCUUUAGUGAG
NY1             GAAUUUUAUU(UUUAAGUGAAAAAGCUUAAA)UUUG[UUUGGGGGACGA]UCAGA[CCCUUUGGAGCUU]U(AUUGUUUAACAAA
AZ1             GAAUUUUAUU(UUUAAGUGAAAAAGCUUAGA)UUUG[UUUGGGGGACGA]UCAGA[CCCUUUGGAGCUU]U(AUUGUUUAACAAA
CA8             GAAUUUUAUU(UUUAAGUGAAAAAGCUUAAA)UUUG[UUUGGGGGACGA]UCAGA[CCCUUUGGAGCUU]U(AUUGUUUAACAAA
OR1             GAAUUUUAUU(UUUAAGUGAAAAAGCUUAAA)UUUG[UUUGGGGGACGA]UCAGA[CCCUUUGGAGCUU]U(AUUGUUUAACAAA
galeata         GAAUUUUAUU(UUUAAGUGAAAAAGCUUAAA)UGCG[UUUGGGGGACGA]UCAGA[CCCUCUGGAGCUU]U(AUUUAUCCUCUUG
ambigua         GAAUUUCAUU(UUUAAGUGAAAAAGCUUAAA)UAUU[UUUGGAGGACGA]UCAGA[CCCUUUGGAGCUU]U(AUUUUGUUUUGAU
longiceph.      GAAUUUUAUU(UUUAAGUGAAAAAGCUUAAA)UAAU[UUUGAAGGACGA]UCAGA[CCCUUUGGAGCUU]U(AUUUAUUUUAAAG
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APPENDIX 3. Continued.

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                                        G3'          G6       G6'    G7                 G7'

GA1          UUUUUAUUAGUGAUAGAUAGUAGGAUAAAUUAGGUAAU)UUU(GUUGGGGCGAC)A(GGAAGUAAAAAAUAA-CACUUCU)UU
MEX1         UUUUUAUUAGUGAUAGAUAGUAGGAUAAAUUAGGUAAU)UUU(GUUGGGGCGAC)A(GGAAGUAAAAAAUAA-CACUUCU)UU
MA1          UUUUUAUUAGUGAUAGAUAGUAGGAUAAAUUAGGUAAU)UUU(GUUGGGGCGAC)A(GGAAGUAAAAAAUAA-CACUUCU)UU
CT1          UUUUUAUUAGUGAUAGAUAGUAGGAUAAAUUAGGUAAU)UUU(GUUGGGGCGAC)A(GGAAGUAAAAAAUAA-CACUUCU)UU
MI1          UUUUUACUGGUGUGAGAUAGUCAAAUAAAUUAAAGAAU)UUU(GUUGGGGCGAC)A(AGAAGAAUAAAAAUAA-CACUUCU)UU
NFLD1        UUUUUACUGGUGUGAGAUAGUCAAAUAAAUUAAAGAAU)UUU(GUUGGGGCGAC)A(AGAAGAAUAAAAAUAA-CACUUCU)UU
NY1          UUUUUACUAGUGAGAGAUAGGAGGAUAAGUUAAACGAU)UUU(GUUGGGGCGAC)A(GGAAGUAAAAAGUAA-CACUUCU)UU
AZ1          UUUUUACUAGUGAGAGAUAGGAGGAUAAGUUAAACGAU)UUU(GUUGGGGCGAC)A(GGAAGUAAAAAGUAA-CACUUCU)UU
CA8          UUUUUACUAGUGAGAGAUAGGAGGAUAAGUAAAACGAU)UUU(GUUGGGGCGAC)A(GGAAGUAAAAAGUAA-CACUUCU)UU
OR1          UUUUUACUAGUGAGAGAUAGGAGGAUAAGUUAAACGAU)UUU(GUUGGGGCGAC)A(GGAAGUAAAAAGUAA-CACUUCU)UU
galeata      UUUACACU-UUUUAAGAUUAUGAAAUAGUCGGGUAAAU)UUU(GUUGGGGCGAC)A(GGAAGAUUAAAAUAA-CACUUCU)UU
ambigua      UUUAGGUUAUUUUUAAAUAACAAAAGUUUUAGCAAAAU)UUU(GUUGGGGCGAC)A(GAAAGUUAUUAAGAAACACUUUU)UU
longiceph.   UUUAAAUU-GUUUUAGAUUAUUGAGU-GAAAAAUAAAU)UUU(GUUGGGGCGAC)A(GGGAGUGAAAAAUAA-CACUCUU)UU

             G9                    G9'                                          G2'                  G16

GA1          [UA]UUAAACACAUAUAGGUG[AA]AAAUUGAUCCUUAAGGAAAGAUUAAAAGAUU[AAGUUACCCUAGGG]AUAACAG(CGUAA
MEX1         [UA]UUAAACACAUAUAGGUG[AA]AAAUUGAUCCUUAAGGAAAGAUUAAAAGAUU[AAGUUACCCUAGGG]AUAACAG(CGUAA
MA1          [UA]UUAAACACGUAUAGGUG[AA]AAAUUGAUCCUUAGGGAAAGAUUAAAAGAUU[AAGUUACCCUAGGG]AUAACAG(CGUAA
CT1          [UA]UUAAACACGUAUAGGUG[AA]AAAUUGAUCCUUAAGGAAAGAUUAAAAGAUU[AAGUUACCCUAGGG]AUAACAG(CGUAA
MI1          [UA]CUUAACACGAAUUAGUG[AA]AAAUUGAUCCUCUUAGCGAGAUUAAAAGAUU[AAGUUACCCUAGGG]AUAACAG(CGUAA
NFLD1        [UA]UUUAACACGAAUUAGUG[AA]AAAUUGAUCCUCUUAGCGAGAUUAAAAGAUU[AAGUUACCCUAGGG]AUAACAG(CGUAA
NY1          [UA]UUAAACACACAUGAGUG[AA]AAAUUGAUCCUUAAGGAGAGAUUAAAAGAUU[AAGUUACCCUAGGG]AUAACAG(CGUAA
AZ1          [UA]UUAAACACACAUGAGUG[AA]AAAUUGAUCCUUAAGGAGAGAUUAAAAGAUU[AAGUUACCCUAGGG]AUAACAG(CGUAA
CA8          [UA]UUAAACACACAUGAGUG[AA]AAAUUGAUCCUUAAGGAGAGAUUAAAAGAUU[AAGUUACCCUAGGG]AUAACAG(CGUAA
OR1          [UA]UUAAACACACAUGAGUG[AA]AAAUUGAUCCUUAAGGAGAGAUUAAAAGAUU[AAGUUACCCUAGGG]AUAACAG(CGUAA
galeata      [UU]UAAAACGCUUUUAAGCG[AA]AUAUUGAUCCUUAGGGAGAGAUUAAAAGAUU[AAGUUACCCUAGGG]AUAACAG(CGUAA
ambigua      [UU]AAAACCACUCAUUAGUG[AA]AGAUUGAUCCCCGCAGAGGGAUUAAAAGACU[AAGUUACCCUAGGG]AUAACAG(CGUAA
longiceph.   [UU]UAAAACAAUUUUAAUUG[AA]AAAUUGAUCCUUAAAGAGAGAUUAAAAGACU[AAGUUACCCUAGGG]AUAACAG(CGUAA

                                  G16'                                 G17          G18

GA1          UCUUUUUGGAGAGUUCUAAUCGAUAAAAGGGUUUGCG)ACCUCGAUG[UUGGAUUAAGAA]AU(UAGCAAGGUGCAGAAGUU
MEX1         UCUUUUUGGAGAGUUCUAAUCGAUAAAAAGGUUUGCG)ACCUCGAUG[UUGGAUUAAGAA]AU(UAGCAAGGUGCAGAAGUU
MA1          UCUUUUUGGAGAGUUCUAAUCGAUAAAAAGGUUUGCG)ACCUCGAUG[UUGGAUUAAGAA]AU(UAGCAAGGUGCAGAAGUU
CT1          UCUUUUUGGAGAGUUCUAAUCGAUAAAAAGGUUUGCG)ACCUCGAUG[UUGGAUUAAGAA]AU(UAGCAAGGUGCAGAAGUU
MI1          UCUUUUUGGAGAGUUCUAAUCGAUAAAAAGGUUUGCG)ACCUCGAUG[UUGGAUUAAGAA]AU(UGGCAAGGUGCAGAAGUU
NFLD1        UCUUUUUGGAGAGUUCUAAUCGAUAAAAAGGUUUGCG)ACCUCGAUG[UUGGAUUAAGAA]AU(UGGCAAGGUGCAGAAGUU
NY1          UCUUUUUGGAGAGUUCUAAUCGAUAAAAAGGUUUGCG)ACCUCGAUG[UUGGAUUAAGAA]AU(UAGCAAGGUGCAGAAGUU
AZ1          UCUUUUUGGAGAGUUCUAAUCGAUAAAAAGGUUUGCG)ACCUCGAUG[UUGGAUUAAGAA]AU(UAGCAAGGUGCAGAAGUU
CA8          UCUUUUUGGAGAGUUCUAAUCGAUAAAAAGGUUUGCG)ACCUCGAUG[UUGGAUUAAGAA]AU(UAGCAAGGUGCAGAAGUU
OR1          UCUUUUUGGAGAGUUCUAAUCGAUAAAAAGGUUUGCG)ACCUCGAUG[UUGGAUUAAGAA]AU(UAGCAAGGUGCAGAAGUU
galeata      UCUUUUUGGAGAGUUCUAAUCGAUAAAAAGGUUUGCG)ACCUCGAUG[UUGGAUUAAGAA]AU(UGGCAAGGUGCAGAAGUU
ambigua      UCUUUUUGGAGAGUUCAUAUCGAUAAAAAGGUUUGCG)ACCUCGAUG[UUGGAUUAAGAA]AU(UAGCAAGGUGCAGAAGUU
longiceph.   UCUUUUUGGAGAGUUCAAAUCGAUAAAAAGGUUUGCG)ACCUCGAUG[UUGGAUUAAGAA]AU(UAGCAAGGUGCAGAAGUU

             G18'       G19        G19'

GA1          UUGCUG)GA(GAGUCUGUUCGACUU)U
MEX1         UUGCUG)GA(GAGUCUGUUCGACUU)U
MA1          UUGCUG)GA(GAGUCUGUUCGACUU)U
CT1          UUGCUG)GA(GAGUCUGUUCGACUU)U
MI1          UUGCUG)GA(AAGUCUGUUCGACUU)U
NFLD1        UUGCUG)GA(AAGUCUGUUCGACUU)U
NY1          UUGCUG)GA(AAGUCUGUUCGACUU)U
AZ1          UUGCUG)GA(AAGUCUGUUCGACUU)U
CA8          UUGCUG)GA(AAGUCUGUUCGACUU)U
OR1          UUGCUG)GA(AAGUCUGUUCGACUU)U
galeata      UUGUUG)GA(AAGUCUGUUCGACUU)U
ambigua      UUGCUG)GA(AAGUCUGUUCGACUU)U
longiceph.   UUGCUG)GA(GAGUCUGUUCGACUC)U
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