Molecular phylogenetics of central Canadian Physidae (Pulmonata: Basommatophora)

E. Pip and J.P.C. Franck

Abstract: The phylogenetic relationships of four nominal south-central Canadian freshwater physids (Physa (sensu lato) skinneri Taylor, 1954, Physa integra (Haldeman, 1841), Physa gyrina (Say, 1821), and the endemic Physa winnipegensis Pip, 2004) were studied by analyses of combined partial sequences coding for mitochondrial 16S and cytochrome c oxidase (COI) genes. Maximum parsimony and neighbour joining analyses, as well as comparisons with published sequences, supported four major clades of physids, of which three are represented in central Canada. Physa skinneri and P. integra were placed within the Physa fontinalis (L., 1758) and Physa acuta Draparnaud, 1805 clades, respectively. Physa winnipegensis formed a distinct branch within the P. acuta group. An additional, previously unreported and unclassified, morph within the P. acuta group was identified from Lake Winnipeg.

Résumé: Des analyses des séquences partielles combinées codant pour les gènes mitochondriaux 16S et cytochrome c oxydase (COI) nous ont permis d’examiner les relations phylogénétiques de quatre espèces nominales de physidés d’eau douce du centre-sud du Canada (Physa (sensu lato) skinneri Taylor, 1954, Physa integra (Haldeman, 1841), Physa gyrina (Say, 1821) et l’espèce endémique Physa winnipegensis Pip, 2004). Des analyses de parcimonie maximale et du plus proche voisin et des comparaisons avec les séquences disponibles dans la littérature appuient l’existence de quatre clades de physidés, dont trois sont représentés dans le centre du Canada. Physa skinneri se retrouve dans le clade de Physa fontinalis (L., 1758) et P. integra dans celui de Physa acuta Draparnaud, 1805. Physa winnipegensis forme une branche distincte du groupe de P. acuta. Nous avons identifié une forme supplémentaire, encore inédite et non classifiée, dans le groupe P. acuta provenant du lac Winnipeg.

[Traduit par la Rédaction]

Introduction

The family Physidae encompasses a globally distributed family of sinistral freshwater snails that are particularly morphologically diversified in North America. The great variety of physical and chemical freshwater habitats occupied by members of this family, as well as environmental factors such as wide temperature ranges and predation, have contributed to the development of numerous ecophenotypes (Burnside 1998; DeWitt et al. 2000). Diversification has been further promoted by the isolation associated with island biogeography of discontiguous water bodies, particularly for organisms of limited mobility dependent on passive dispersal agents (e.g., Pip 1986; Jarne 1995), resulting in several very narrow endemics known only from a single site or a small cluster of related locations.

The status of distinct species vs. conspecific ecophenotypes (as well as subspecies) within the subfamily Physinae, and the relationships among closely allied nominal species, have been the subject of much disagreement and confusion. Aside from morphological characteristics of the shell, attributes such as radular structure (e.g., Baker 1928; Clarke 1981), anatomy of the penial complex (Baker 1928; Clampitt 1970; Te 1973, 1974, 1975; Taylor 1988, 2003; Wethington 2004; Wethington and Guralnick 2004), reproductive isolation (Wethington and Dillon 1997; Dillon et al. 2002, 2005; Dillon and Wethington 2004; Wethington 2004), and allozyme patterns (e.g., Te 1978; Buth and Suloway 1983; Liu 1993; Dillon and Wethington 2006) have been studied in an attempt to clarify phylogenetic relationships. However, the various individual parameters may often not be sufficiently diagnostic and may yield contradictory results when applied in combination (Te 1975; Davis 1978). Similar problems have been encountered in the pulmonate freshwater gastropod family Lymnaeidae (see Remigio and Blair 1997).

A nomenclatural history of the Physidae is found in Te (1975) and Taylor (2003). Species within the subfamily Physinae have been grouped by various workers under the single genus Physa Draparnaud, 1801 for convenience and uncertainty of relationships (e.g., Clarke 1973, 1981; Te 1975), or divided into various, at times controversial, generic and subgeneric compartments (e.g., Te 1980; Bogatov and Zatravkin 1990; Remigio et al. 2001). The most comprehensive current reclassification is that of Taylor (2003), which is based on the anatomy of the male reproductive system. The latter worker recognized ~80 species grouped into 23 genera. Conversely, the discovery that different nominal species from widely separated geographic sources can interbreed under experimental conditions (e.g., Dillon et al. 2002), combined with allozyme analysis (Dillon and Wethington 2006), has supported a reduction in the number of recognized species, as well as a return to simplification of superspecific divisions, with possibly, once again, only a single genus Physa in this subfamily (Wethington 2004).

Elucidation and interpretation of inter- and intra-specific
relationships within this family need to be undertaken even as habitat destruction and degradation (Pip 2005), irreversible loss of populations, erosion of genetic diversity, and disappearance of unique gene pools proceed at an unprecedented rate (Pip 2000). The Physidae are particularly vulnerable in that significant numbers of nominal species are endemic with highly restricted areas of occurrence, for example hot springs (Clarke 1981; Te and Clarke 1985; Wethington and Guralnick 2004).

Nucleotide sequencing has proven to be a practical tool for the deduction of phylogenetic relationships in gastropods (Remigio and Hebert 2003). Differences not apparent in allozymes are evident in mitochondrial DNA. Using segments of mitochondrial ribosomal 16S and cytochrome c oxidase I (COI), Remigio et al. (2001) examined the Canadian species Physella johnsoni (Clench, 1926), Physella wrighti Te and Clarke, 1985, and Physella gyrina (Say, 1821). These workers found very little or no intraspecific sequence variation for both types of segments for the individuals examined. However, interspecific differences and their relative magnitudes were apparent. A more extensive study was carried out by Wethington (2004), and Wethington and Guralnick (2004), who examined a number of species from hot and cold springs. These workers found intraspecific genetic variation, as well as varying levels of contiguity, among nominal species and recognized four primary groups of genetically related physids.

In the present study, we amplified partial sequences from COI and 16S segments in four sympatric physids found in south-central Canada: Physa skinneri Taylor, 1954 (= Physa jemnessi skinneri of Clarke (1973, 1981)), Physa integra (Haldeman, 1841) (= Haitia integra of Taylor (2003)), Physella gyrina (Say, 1821), and the endemic Lake Winnipeg physid, Physa winnipegensis Pip, 2004. We also examined representatives of an additional Lake Winnipeg physid that could not be attributed to any current nominal taxon. Our objectives were to establish the relative phylogenetic positions of these central Canadian DNA sequences to published sequences for the same and other physid species from other geographical regions. Until a consensus can be reached regarding generic standings, all taxa in the present study are referred to as Physa (sensu lato).

**Materials and methods**

**DNA isolation, amplification, and sequencing**

Individuals used in this study were field-collected in Manitoba and Alberta (Table 1). Specimens were preserved in 95% ethanol, stored at 4 °C, and analyzed within 4 months of collection. Tissue samples consisted of the tips of the foot muscles, which were microscopically examined to verify that parasites were absent. Additional tissue samples were obtained from preserved specimens at the Manitoba Museum and the University of Guelph; however, none of these proved to be amplifiable because of the degradation of the DNA.

Sample preparation and sequencing analyses were performed by ATG Genetics (Vancouver, British Columbia). The preserved tissue samples were digested in proteinase K (EC 3.4.21.64) buffer, followed by organic extraction and precipitation with ethanol. A portion of the COI mtDNA gene was amplified from the total genomic DNA by polymerase chain reaction (PCR) using synthetic oligonucleotides COI(F) (defined as GGT CAA CAA ATC ATA AAG ATA TTG G) and COI(R) (defined as TAA ACT TCA GGG TGA CCA AAA AAT CA) (Folmer et al. 1994). For the 16S segment, the synthetic sequences were 16S(F) (defined as CGC CTG TTT ATC AAA AAC AT) and 16S(R) (defined as CCG GTC TGA ACT CAG ACG T) (Remigio et al. 2001). The PCR products were gel-purified and sequenced on both strands with the same primers used for amplification. Sequencing was carried out using an ABI 3730 Big Dye (version 3.1) automated sequencer at the NAPS Unit, University of British Columbia, Vancouver. The forward and reverse sequences were assembled using the CAP3 sequence assembly program (Huang and Madan 1999). No gaps were encountered in the completed sequences. Sequences were deposited in GenBank with consecutive primary accession numbers EF488670–EF488681 for the COI sequences and EU056582–EU056592 for the 16S sequences (Table 1).

**Sequence and phylogenetic analyses**

The 16S and COI mtDNA sequences were aligned using the default settings of Clustal X (version 1.81; Thompson et al. 1997). The sequences were aligned to previously published phsyid sequences; GenBank accession numbers and localities for the latter, as well as the Pseudosuccinea columella (Say, 1817) (Lymnaeidae) outgroup, are given in Table 1. The Clustal X multiple sequence alignment was imported into the GeneDoc program (Nicholas and Nicholas 1997) to manually edit nonalignable sites from the 5' and 3' ends of the alignment. Previous studies have documented the high variation in length of 16S rRNA loops (Lydeard et al. 2000). The regions corresponding to the loops were deleted from the alignment, resulting in a final alignment with 878 sites.

The maximum parsimony tree reconstruction was performed using PAUP (beta version 4.0; Swofford 2001) bootstrap method heuristic search and tree bisection reconnection (TBR) branch swapping with stepwise addition and MULPARS in effect. Branches having maximum lengths of zero were collapsed to yield polytomies and no topological constraints were used. Characters were assigned equal weights.

A maximum likelihood tree was constructed using the TreePuzzle program (Schmidt et al. 2002). An optimum model of substitution was chosen using the MultiPhyl websolver (http://distributed.cs.nuim.ie/multiphy1.php [accessed 16 January 2007]; Keane et al. 2007) with code from the ModelGenerator program (Keane et al. 2006). The model selected for the physid data set under Akaike’s information criterion was the Kimura three-parameter model of base substitution with unequal base frequencies, a proportion of invariant sites, and a gamma-distributed site substitution rate (K81uf + I + G). The tree was viewed using TreeView (Page 1996).

**Results**

The physid combined 16S + COI multiple sequence alignments were phylogenetically analyzed using both maximum
parsimony (MP) and maximum likelihood (ML) methodologies. Both of the tree reconstruction techniques identified four strongly supported clades, namely the Physa acuta Draparnaud, 1805, Physa pomilia Conrad, 1834, Physa fontinalis (L., 1758), and P. gyrina clades (Figs. 1A, 1B). Nodes for the four clades were robustly supported by bootstrap analyses for the MP tree and by quartet puzzling support values for the ML tree. The Lake Winnipeg P. integra (diagnosed according to shell and penial morphology) and P. winnipegensis were located within the P. acuta clade, which also included published sequences for Physella anatina Lea, 1864 (Colorado), Physa acuta (Wyoming), Physa heterostropha Say, 1816 (Pennsylvania), and Physella virgata A. Gould, 1855 (Arizona). Both the COI and 16S sequences for the four clades were robustly supported by bootstrap values for the ML tree. The Lake Winnipeg analyses for the MP tree and by quartet puzzling support values for the four clades were robustly supported by bootstrap values for the ML tree (Fig. 1B), but with P. winnipegensis in the MP tree (Fig. 1A). These differences originated in the 16S portion of the combined sequences; when only COI data were analyzed (not shown), P. winnipegensis occupied its own unique branch in both MP and ML tree reconstructions (as in Fig. 1B), whereas co43906 was placed in the main body of the P. acuta group in both tree reconstructions.

However, an inconsistency was evident in the placement of co43906 (a published P. anatina sample from Colorado), which was grouped in the main body of the P. acuta group in the ML tree (Fig. 1B), but with P. winnipegensis in the MP tree (Fig. 1A). These differences originated in the 16S portion of the combined sequences; when only COI data were analyzed (not shown), P. winnipegensis occupied its own unique branch in both MP and ML tree reconstructions (as in Fig. 1B), whereas co43906 was placed in the main P. acuta group in both trees. Otherwise, trees using only COI data produced results similar to trees using the combined data.

The five individuals identified as Physa sp. from Lake Winnipeg could not be assigned to a nominal species in terms of shell morphology (e.g., greater width to length ratio and a more compressed spire than in P. integra). Reproduc-

<table>
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<th>Identity</th>
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<th>16S Accession No.</th>
<th>Locality</th>
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Note: The individuals collected for this study are in boldface type. Binoms are based on those reported in GenBank.

*No 16S sequence available for this sample.
Fig. 1. Phylogenetic relationships of the Manitoba physids based on maximum parsimony (MP) and maximum likelihood (ML) analyses of the combined 16S and COI sequences. The MP tree (A) was constructed using the heuristic search method with tree bisection reconnection (TBR) branch swapping with stepwise addition. Majority rule bootstrap values based on 100 bootstrap replicates are indicated on the major nodes. The ML tree (B) was constructed using the Kimura three-parameter model of base substitution with unequal base frequencies, a proportion of invariant sites, and a gamma-distributed site substitution rate (K81uf + I + G). The proportion of invariant sites is 0.30 and the gamma distribution alpha parameter is 0.57. Base frequencies are as follows: A = 0.330, C = 0.12410, G = 0.15199, and T = 0.39382. Quartet puzzling support values generated by the TreePuzzle program are indicated at the major nodes of the tree. The taxon labels for all species included in the phylogenetic analyses are given in Table 1.

(A) 16S + COI Maximum Parsimony Tree (MP)

(B) 16S + COI Maximum Likelihood Tree (ML)

Discussion

The four major clades of P. acuta, P. gyrina, P. fontinalis, and P. pomilia identified in both the MP and ML trees were consistent with the physid phylogeny described by Wethington and Guralnick (2004). Manitoba physids were represented within the first three clades; the fourth group, represented by P. pomilia (Wethington 2004), appears to be a small group with no northern representatives identified in our survey.

Physa gyrina has a wide geographical distribution in Canada (Clarke 1973, 1981; Pip 1992), as well as a very wide ecological tolerance range for water chemistry parameters (Pip 1988a). The wide distribution of this species is reflected in its morphological and molecular diversity. It is an efficient colonizer of new habitats (Pip 1986) and is the largest and most common physid in the southern parts of
the prairie provinces including Manitoba (Pip 1978, 2000). This snail is highly adaptable (Clampitt 1970; Rollo and Harylk 1988) and can adjust its microhabitat preferences and microdistribution to reduce interspecific competition to take advantage of optimal nutritional value of food (Pip and Stewart 1976). Like most pulmonates, it occurs primarily in shallow water, although it has been reported from depths >5 m (Pip 1991), where it harvests oxygen bubbles released by submerged macrophytes and algae (E. Pip, unpublished data). While the life span of most individuals is 1 year, some large individuals may survive longer (Pip and Stewart 1976).

In the present study, the Manitoba (MBpogy1) specimen was grouped with its conspecific relatives from the US (Figs. 1A, 1B), as well as P. johnsoni from Alberta and a Physella aurea Lea, 1838 individual from Virginia. The latter two species and P. gyrina at present form an unresolved polytomy. While Te (1978), Remigio and Hebert (1998), and Lepitzki (2002) concluded on the basis of reproductive characters, allozymes, and DNA evidence that P. johnsoni is a distinct species, Taylor (2003), Wethington (2004), and Wethington and Guralnick (2004) did not recognize it as sufficiently distinct from P. gyrina. Thus, reconciliation of DNA sequence data with traditional morphological, anatomical, and biochemical parameters may require comparisons of larger or other genome sections.

In central Canada, the broadest ranges of environmental chemical conditions are occupied by P. skinneri (= P. jenesissi skinneri of Clarke (1973, 1981)), which can tolerate habitats with the highest values for inorganic parameters (Pip 1988a) and with the warmest temperatures (Pip 1993), although it is also common in subarctic regions (Pip 1992). This delicate species prefers habitats with minimal disturbance and is only rarely found in lakes, where it is restricted to quiet backwaters. While fossil evidence of Canadian physids is limited because of taphonomic factors that result in higher attrition rates of thin-shelled species in bottom sediments (Pip 1988b, 1990), both P. skinneri and P. gyrina are known from late-glacial deposits at least 10 000 years old in southern Alberta (Harris and Pip 1973). These individuals were placed in the P. fontinalis clade (Figs. 1A, 1B). However, intraspecific variation was evident in that the two individuals drawn from the same population were not identical for either COI or 16S sequence.

While Clarke (1981) confined the distribution of P. integra to the Great Lakes and St. Lawrence regions, its occurrence in Manitoba (primarily in large lakes) (Pip 1978, 2000) has been confirmed by anatomical study by D.W. Taylor (personal communication). The Lake Winnipeg individual identified as P. integra (according to reproductive anatomy) appeared in the P. acuta clade (Figs. 1A, 1B). Recent reproductive isolation studies have suggested that P. integra from the American Midwest may be able to interbreed under experimental conditions with P. heterostropha from the southeastern US and P. acuta from Europe (Dillon et al. 2002), as well as with P. virgata from the southeastern US (Dillon et al. 2005).

Physa winnipegensis is a rare snail that is now known only from a few very small disjunct populations in Lake Winnipeg. Since its abundance in this lake 30–40 years ago, it has suffered a catastrophic decline as habitat destruction and eutrophication of the lake have progressed; it is currently estimated at <500 individuals (E. Pip, unpublished data). This species lives on wave-lashed rocks, feeds on periphyton, and is sensitive to heavy metals (Pip 2004). The two individuals drawn from the same population were identical for both COI and 16S sequences and formed an offshoot within the P. acuta clade, suggesting common ancestry with P. anatina, P. acuta, P. heterostropha, P. virgata, and P. integra. Of these, only P. integra occurs in central Canada. The inter-relationships among these species and their taxonomic status are controversial. On the basis of anatomy, Taylor (2003) has recognized them as distinct (although he interpreted P. virgata as Physella mexicana (Philippi, 1841)), while Wethington (2004), Wethington and Guralnick (2004), Dillon et al. (2002, 2005), and Dillon and Wethington (2006) have suggested that these species should all be referred to as P. acuta.

Five physid individuals (Physa sp. 1–5) (Figs. 1A, 1B) from two subpopulations in Lake Winnipeg constitute a sub-group within the P. acuta clade. These individuals could not be assigned to any current nominal species based on either shell morphology or reproductive anatomy, and may represent another undescribed taxon that is also endemic to Lake Winnipeg. Although these individuals formed a distinct sub-group, they were only 98%–99% identical for both COI and 16S.

In conclusion, based on partial sequence evidence, the relationships of central Canadian physids appear to be consistent with the finding of Wethington (2004) that physids from widely separated geographical areas can be grouped into a small number of clades, composed of variously related taxa issuing from different hierarchical levels of ancestry. It is evident that varying durations of geographic and reproductive isolations, and different selective pressures, have contributed to a continuum of genetic diversity within those taxa that have wide, even transcontinental, distributions. The degree of DNA variation within and among populations of nominal taxa requires more extensive elucidation, and, as suggested by Remigio and Hebert (2003), analyses of additional genes are probably necessary to obtain a more detailed understanding of delineations at the specific level, as well as reconciliation of molecular data with morphological, anatomical, and biochemical differences.

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References


Bogatov, V.V., and Zatratkin, M.N. 1990. Freshwater and brackish water gastropod molluscs of the far eastern USSR. USSR Academy of Sciences, Vladivostok.


