



Molecular systematics and radiation of western North American nymphophiline gastropods

Hsiu-Ping Liu^a, Robert Hershler^{b,*}

^a Department of Biological Sciences, University of Denver, Denver, CO 80208, USA

^b Department of Zoology, Smithsonian Institution, P.O. Box 37012, NHB W-305, MRC 163, Washington, DC 20013-7012, USA

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Abstract

Three mitochondrial DNA (mtDNA) markers were used to infer the phylogenetic relationships of the morphologically diverse, species rich, and poorly understood western North American aquatic gastropod genus *Pyrgulopsis* (Hydrobiidae: Nymphophilinae). Sequences were obtained from 62 of 124 currently recognized species of *Pyrgulopsis* and representatives of four related genera of North American nymphophilines. Separate and combined analyses of the mtDNA datasets recovered a well supported clade composed of *Pyrgulopsis* and two other North American nymphophiline genera (*Floridobia*, *Nymphophilus*) consistent with the results of a prior study based on a single gene and with anatomical evidence suggesting that these taxa form a monophyletic group. Phylogenetic relationships among lineages of *Pyrgulopsis* were little resolved in our analyses and provided no obvious basis for splitting this large genus into multiple genera. The little differentiated Mexican genus *Nymphophilus* was consistently placed within *Pyrgulopsis* in our trees and is formally synonymized with it herein. *Pyrgulopsis* was also depicted as paraphyletic with respect to *Floridobia* in some of our trees while in others the latter was sister to a *Nymphophilus* + *Pyrgulopsis* clade. Based on these equivocal results and the morphological and geographical divergence of eastern North American *Floridobia* relative to *Pyrgulopsis*, we recommend that the former be maintained as a separate genus. The short, weakly supported branches within *Pyrgulopsis* and the noncongruence between our molecular phylogenetic hypotheses and geographical groupings of species are attributed to an early rapid diversification of the genus, perhaps triggered by the complex changes in western topography which occurred during the late Tertiary. Our results also indicate that penial morphologies used to define species groups of *Pyrgulopsis* have been subject to striking convergence throughout the West, suggesting another compelling facet of the radiation of these snails.

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1. Introduction

The hydrobiid gastropod subfamily Nymphophilinae is one of several groups of freshwater mollusks that have extensively diversified in North America (Burch, 1989; Martens, 1890–1901). Nine nymphophiline genera are restricted to the eastern part of the continent, seven of which contain one to three species (*Birgella*, *Cincinnatia*,

Notogillia, *Nymphophilus*, *Rhaphinema*, *Spilochlamys*, and *Stiobia*) while the others contain 13 (*Marstonia*) and 14 (*Floridobia*) species. A 10th genus, *Pyrgulopsis*, has a predominantly western distribution and is the largest genus of aquatic mollusks on the continent with 124 species. *Pyrgulopsis* lives in coastal lowlands to montane settings and ranges from the Pacific margin to slightly east of the continental divide. The tiny, gill-breathing species of *Pyrgulopsis* usually live in springs and small streams.

Pyrgulopsis has been a popular subject for biogeographic studies because of its limited vagility and tight

* Corresponding author. Fax: +1 202 257 2343.

E-mail address: hershler.robert@nmnh.si.edu (R. Hershler).

linkage with aquatic habitats (Hershler and Pratt, 1990; Hershler and Sada, 2002; Taylor, 1960, 1985, 1987; Taylor and Bright, 1987). The genus has undergone an extensive radiation in penial morphology, which ranges from simple, blade-like structures to lobate forms having complex glandular ornament (Hershler, 1994; Taylor, 1987). *Pyrgulopsis* has become a focus of conservation attention because of the typically narrow geographic ranges and fragile habitats of its species (e.g., Nevada Natural Heritage Program, 2000; New Mexico Department of Game and Fish, 2000; State of Utah Division of Wildlife Resources, 2003). In spite of these important features, the systematics of *Pyrgulopsis* remains in an early stage of refinement and its phylogenetic relationships are poorly understood. Most congeners (102/124, 82%) were described only subsequent to 1985. Early workers placed most of the few western nymphophilines then known in *Fontelicella* (Gregg and Taylor, 1965; Taylor, 1975, 1987) while *Pyrgulopsis* was utilized for a few unusually sculptured species scattered across the United States (Berry, 1947; Call and Pilsbry, 1886). Hershler and Thompson (1987) synonymized *Fontelicella* with *Pyrgulopsis* and also treated 11 species distributed well east of the continental divide as members of *Pyrgulopsis*, only to later transfer them to *Marstonia* (Thompson and Hershler, 2003), thus restricting *Pyrgulopsis* to western North America and closely proximal areas.

Pyrgulopsis is weakly diagnosed by a combination of several characters (Hershler, 1998). Although it is well differentiated from geographically proximal *Cincinnati* (Hershler and Thompson, 1996) and *Marstonia* (Thompson and Hershler, 2003), *Pyrgulopsis* differs little from sympatric *Nymphophilus* (Hershler, 1998). Inasmuch as several other western nymphophilines that were recently described as separate genera (*Mexistiobia*, Hershler, 1985; *Apachecoccus*, *Yaquicoccus*, Taylor, 1987) are now treated as synonyms of broadly inclusive *Pyrgulopsis* (Hershler, 1994), the continued recognition of *Nymphophilus* as distinct seems questionable.

The sole morphology-based phylogenetic analysis of *Pyrgulopsis* did not test its monophyly as it was restricted to 60 species then placed in the genus (but now assigned to *Pyrgulopsis* or *Marstonia*) and the type species of *Nymphophilus* (Hershler, 1994). The resulting, poorly resolved trees depicted *Pyrgulopsis* as paraphyletic with respect to *Marstonia*, which was then considered a synonym of the former. Hershler et al. (2003a) used partial COI mitochondrial DNA sequences to examine relationships among all but one (monotypic *Birgella*) of the North American nymphophiline genera. The 21 species of *Pyrgulopsis* included in this analysis were little resolved and the results were equivocal with respect to whether these form a clade or a paraphyletic assemblage within which *Nymphophilus* and *Floridobia* were nested. A close phylogenetic relationship between these three genera is consistent with the unique female reproductive

groundplan that they share among the North American nymphophilines (Hershler et al., 2003a).

Subsequent molecular analyses of *Pyrgulopsis* were restricted to small groups of species and focused on relationships within single geographic regions (Hershler et al., 2003b; Hershler and Liu, 2004b; Hurt, 2004; Liu et al., 2003). In this paper, we analyze mitochondrial DNA sequences from a much larger set of congeners. Our objectives are to further explore the relationships among *Floridobia*, *Nymphophilus*, and *Pyrgulopsis* and to test *Pyrgulopsis* monophyly. We also examine the relationships among species of *Pyrgulopsis* and explore the morphological and geographical diversification of the genus within the context of our molecular-based phylogenetic hypothesis.

2. Materials and methods

2.1. Specimens

Sixty-two species of *Pyrgulopsis* were sampled, representing half of the currently recognized members of the genus (Table 1). These included one or more representatives from 11 major physiographic regions in which the genus is distributed (Fig. 1), and multiple members of each of the morphological species groups recently delineated by Hershler and Sada (2002). Unfortunately, the type species of *Pyrgulopsis* (*P. nevadensis*) could not be included in this analysis because it has not been collected alive since the late 1800s and is likely extinct (Hershler, 1994). However, we did sample two other congeners (*P. archimedis*, *P. inopinata*) which also have carinate shells diagnostic of *Pyrgulopsis sensu lato*. Both species of *Nymphophilus* and representatives of both lineages of *Floridobia* (Hershler et al., 2003a, Figs. 2 and 3) were included as outgroups, along with three species of the genus *Marstonia*. *Cincinnati integra* was used to root trees based on our previous analysis (Hershler et al., 2003a) which showed this species (which belongs to a monotypic genus) to be closely related to the *Floridobia* + *Nymphophilus* + *Pyrgulopsis* clade. Inasmuch as our focus is not on species-level issues, single samples (usually from type localities) were used for all but one species (*P. robusta*). Although multiple specimens were generally sequenced from each sample, intra-specific variation was usually minimal and thus only one sequence from each was used in the final set of phylogenetic analyses. The total dataset consisted of 213 sequences, 169 of which are newly reported herein. Locality and museum voucher information are in Appendix A.

2.2. Laboratory methods

Total genomic DNA was extracted from individual snails preserved in 70% ethanol using a CTAB protocol

Table 1
List of species included in this study, with species group and geographical region codings, and GenBank Accession Nos. for sequences

Species	Group	Region	COI	ND1	16S
<i>Pyrgulopsis</i>					
<i>anatina</i>	5	North and Central Great Basin	AY627926	AY628048	AY627985
<i>archimedis</i>	5	E. Oregon-Snake River Plain	AY426355 ^e	AY426403 ^e	AY627983
<i>arizonae</i>	2	Transition Zone	AY627948	AY628072	AY628015
<i>avernalis</i>	2	North and Central Great Basin	AF520930 ^a	AY628042	AY627974
<i>bernardina</i>	2	Sonoran Desert	AY627951	AY628075	AY628018
<i>bifurcata</i>	2	North and Central Great Basin	AY627925	AY628047	AY627984
<i>breviloba</i>	3	North and Central Great Basin	AY627928	AY628050	AY627987
<i>brunaeuensis</i>	3	E. Oregon-Snake River Plain	AF520941 ^a	AY628038	AY627970
<i>bryantwalkeri</i>	3	North and Central Great Basin	AY627942	AY628066	AY628006
<i>californiensis</i>	0	Western California	AY627924	AY628046	AY627981
<i>carinifera</i>	3	North and Central Great Basin	AY627920	AY628041	AY627973
<i>conica</i>	3	Sonoran Desert	AY627958	AY628083	AY628029
<i>cruciglans</i>	8	North and Central Great Basin	AY627931	AY628054	AY627991
<i>davisi</i>	0	Rio Grande Rift	AY627950	AY628074	AY628017
<i>deaconi</i>	0	Walker Lane Belt	AY367483 ^c	AY367542 ^c	AY627994
<i>diablensis</i>	5	Great Valley	AY627922	AY628044	AY627979
<i>dixensis</i>	1	North and Central Great Basin	AY627946	AY628070	AY628012
<i>eremica</i>	1	Walker Lane Belt	AY627947	AY628071	AY628013
<i>erythropoma</i>	2	Walker Lane Belt	AY367484 ^c	AY367543 ^c	AY627976
<i>fairbanksensis</i>	3	Walker Lane Belt	AY627921	AY628043	AY627977
<i>gibba</i>	6	E. Oregon-Snake River Plain	AY197603 ^b	AY426413 ^e	AY628010
<i>gilae</i>	0	Transition Zone	AY627952	AY628076	AY628019
<i>giulianii</i>	6	Walker Lane Belt	AF520937 ^a	AY367545 ^c	AY628024
<i>glandulosa</i>	4	Transition Zone	AY627959	AY628084	AY628030
<i>greggi</i>	1	Great Valley	AF520943 ^a	AY367546 ^c	AY627978
<i>hubbsi</i>	3	North and Central Great Basin	AY627918	AY628039	AY627971
<i>inopinata</i>	4	Transition Zone	AY426360 ^e	AY426415 ^e	AY627998
<i>intermedia</i>	0	E. Oregon-Snake River Plain	AY379442 ^d	AY426385 ^e	AY627968
<i>kolobensis</i>	5	Transition Zone	AY627939	AY628063	AY628003
<i>landyei</i>	7	North and Central Great Basin	AY627930	AY628053	AY627990
<i>lata</i>	5	North and Central Great Basin	AY627927	AY628049	AY627986
<i>lentiglans</i>	3	North and Central Great Basin	AY627936	AY628059	AY627999
<i>lockensis</i>	1	North and Central Great Basin	AY627932	AY628055	AY627992
<i>manantiali</i>	3	Chihuahuan-Mexican Altiplano	AY627955	AY628079	AY628022
<i>merriami</i>	0	North and Central Great Basin	AY627919	AY628040	AY627972
<i>micrococcus</i>	3	Walker Lane Belt	AF520944 ^a	AY367492 ^c	AY628025
<i>militaris</i>	5	North and Central Great Basin	AY197596 ^b	AY426417 ^e	AY628014
<i>montana</i>	3	North and Central Great Basin	AY627940	AY628064	AY628004
<i>neritella</i>	7	North and Central Great Basin	AF520951 ^a	AY628052	AY627989
<i>owensensis</i>	6	Walker Lane Belt	AF520922 ^a	AY367549 ^c	AY627982
<i>pecosensis</i>	4	Rio Grande Rift ^f	AF520929 ^a	AY628081	AY628026
<i>peculiaris</i>	0	North and Central Great Basin	AF520912 ^a	AY628061	AY628001
<i>pellita</i>	4	North and Central Great Basin	AY627943	AY628067	AY628007
<i>pictilis</i>	5	North and Central Great Basin	AY627944	AY628068	AY628008
<i>plicata</i>	8	Transition Zone	AY627935	AY628058	AY627997
<i>robustal</i>	4	Middle Rocky Mountains	AF520949 ^a	AY426395 ^e	AY627960
<i>robusta2</i>	4	E. Oregon-Snake River Plain	AY197594 ^b	AY628037	AY627969
<i>sadai</i>	0	North and Central Great Basin	AY627945	AY628069	AY628011
<i>saxatilis</i>	5	North and Central Great Basin	AY627934	AY628057	AY627996
<i>simplex</i>	3	Colorado Plateau	AY627949	AY628073	AY628016
<i>sola</i>	3	Transition Zone	AY627957	AY628082	AY628028
<i>stearnsiana</i>	3	Western California	AF520925 ^a	AY367551 ^c	AY628027
<i>sterilis</i>	5	North and Central Great Basin	AY367491 ^c	AY367554 ^c	AY628009
<i>sublata</i>	4	North and Central Great Basin	AY627938	AY628062	AY628002
<i>sulcata</i>	7	North and Central Great Basin	AY627929	AY628051	AY627988
<i>taylori</i>	5	Western California	AY627923	AY628045	AY627980
<i>thermalis</i>	0	Transition Zone	AY627953	AY628077	AY628020
<i>trivialis</i>	5	Transition Zone	AY627941	AY628065	AY628005
<i>turbatrix</i>	5	Walker Lane Belt	AF520936 ^a	AY367555 ^e	AY627975
<i>variegata</i>	5	North and Central Great Basin	AY627937	AY628060	AY628000

Table 1(continued)

Species	Group	Region	COI	ND1	16S
<i>ventricosa</i>	0	Western California	AY426365 ^e	AY426423 ^e	AY627995
<i>villacampae</i>	0	North and Central Great Basin	AY627933	AY628056	AY627993
<i>wongi</i>	0	Walker Lane Belt	AY627956	AY628080	AY628023
Outgroups					
<i>Cincinnatia integra</i>	0	Chihuahuan-Mexican Altiplano	AY627916	AY628033	AY627964
<i>Floridobia floridana</i>	0	Eastern United States	AF520916 ^a	AY628035	AY627966
<i>Floridobia winkleyi</i>	0	Eastern United States	AF520917 ^a	AY628036	AY627967
<i>Marstonia agarhecta</i>	3	Eastern United States	AF520934 ^a	AY426433 ^e	AY627963
<i>Marstonia halcyon</i>	3	Eastern United States	AF520935 ^a	AY628031	AY627961
<i>Marstonia hershleri</i>	3	Eastern United States	AF520946 ^a	AY628032	AY627962
<i>Nymphophilis acarinatus</i>	3	Chihuahuan-Mexican Altiplano	AY627954	AY628078	AY628021
<i>Nymphophilis minckleyi</i>	3	Chihuahuan-Mexican Altiplano	AY627917	AY628034	AY627965

(Unnamed) Species groups are from Hershler and Sada (2002, appendix) and are numbered per their listing. Pertinent literature for species group assignment and geographical occurrence includes Davis and Mazurkiewicz (1985); Hershler (1985, 1990, 1994, 1995, 1998), Hershler and Landye (1988), Hershler and Liu (2004a), Hershler and Pratt (1990), Hershler and Sada (1987, 2000), Hershler and Thompson (1996), Taylor (1966b, 1987), and Thompson (1968, 1969, 1977, 1995).

^a Sequence reported by Hershler et al. (2003a).

^b Sequence reported by Hershler et al. (2003b).

^c Sequence reported by Liu et al. (2003).

^d Sequence reported by Hershler and Liu (2004a).

^e Sequence reported by Hershler and Liu (2004b).

^f For the sake of convenience this sample, which is from the upper Pecos River watershed (dot in the lower right side of Fig. 1), was coded as a member of the closely proximal Rio Grande Rift region.

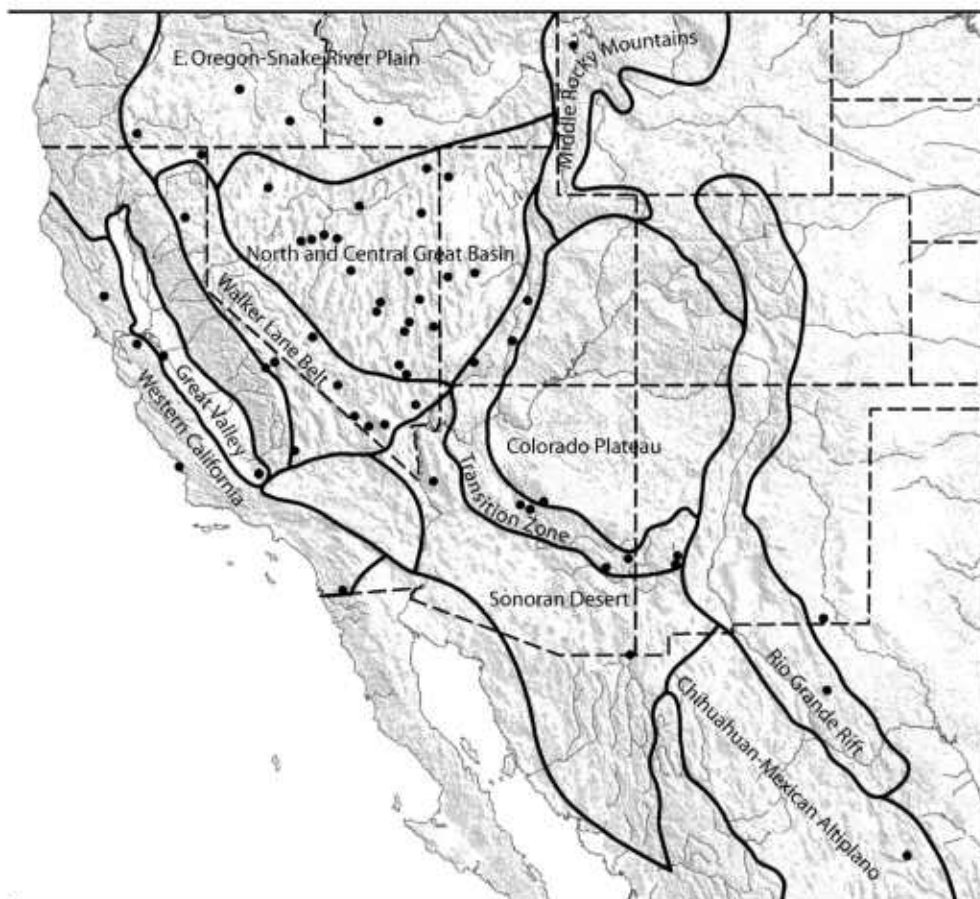


Fig. 1. Map showing sampling localities of *Pyrgulopsis* species in relation to western physiography. Regions and boundaries largely follow Stewart (1998, Fig. 1). Some dots represent two or more closely proximal localities.

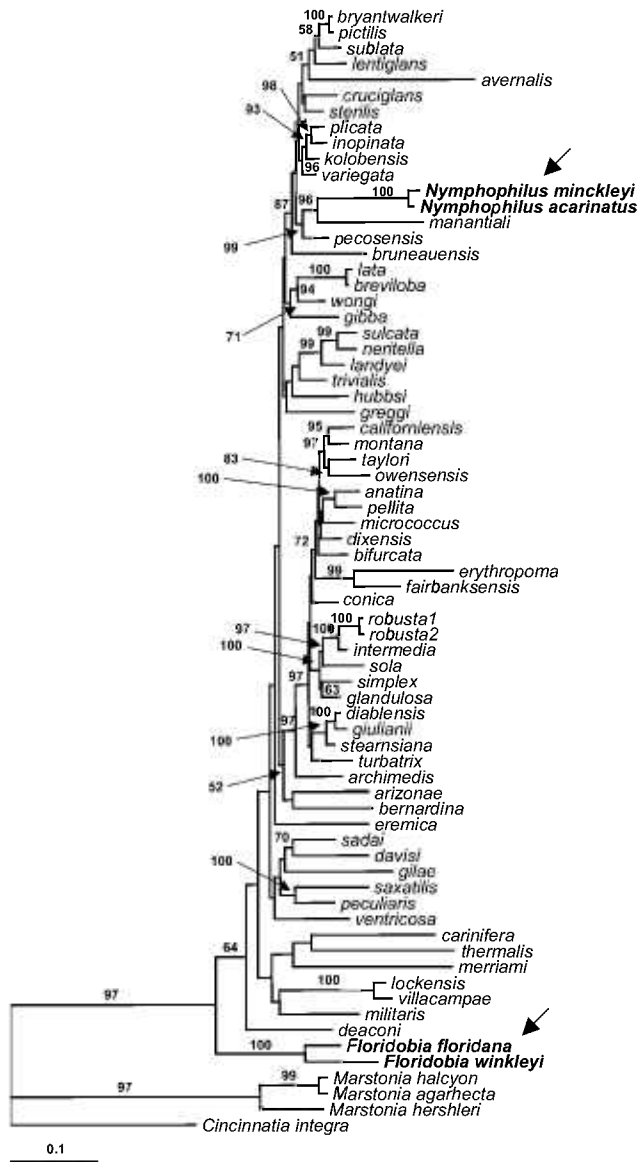
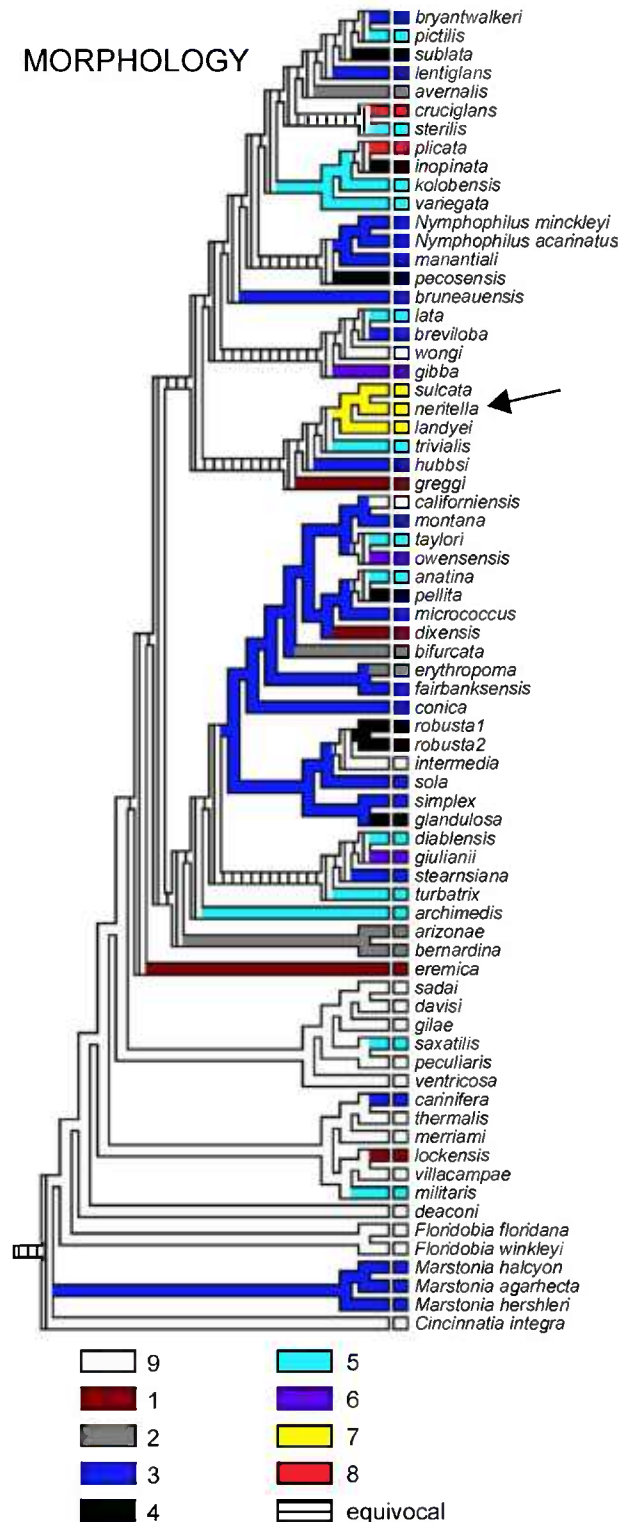


Fig. 2. Bayesian tree for the combined dataset (COI, ND1, 16S). One of two resulting trees based on the HKY + I + Γ model with base frequencies of A = 0.3540, C = 0.1956, G = 0.1392, T = 0.3112; a proportion of invariable sites of 0.5696; and a gamma distribution shape parameter of 0.7557. Bayesian posterior probability values are given when greater than 50%.

Fig. 3. Phylogenetic reconstruction of morphological species groups. The topology is that of Fig. 2 (Bayesian analysis of combined dataset). The arrow identifies the only group depicted as monophyletic. Diagnostic features (from Hershler and Sada, 2002) of these unnamed groups (numbered 1–9) are as follows: (1) penis blade-like, without glands; (2) penis with circular ventral gland; (3) penis with terminal gland; (4) penis with elongate gland along outer edge proximal to filament; (5) penis with terminal gland and small gland on base of filament; (6) penis with terminal gland, narrow ventral gland, and small dorsal gland proximal to filament; (7) central radular teeth with weakly developed or no basal cusps, fused lateral cusps, and elongate basal tongue; (8) penis with large, raised, proximally positioned dorsal gland; and (9) penis with long gland on filament, additional dorsal glands, well developed terminal gland, and one or more ventral glands.

(Bucklin, 1992). Partial sequences of the cytochrome *c* oxidase subunit I (COI), NADH dehydrogenase subunit I (ND1), and 16S rRNA (16S) genes were amplified via polymerase chain reaction (PCR) using the primers LCO1490 and HCO2198 (Folmer et al., 1994) or COIL1492 and COIH2390 (Liu et al., 2001), ND43F and RND592F (Liu et al., 2003), and 16Sar-L and 16Sbr-H

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(Palumbi et al., 1991), respectively. Amplifications were conducted in a 25 μ l total volume, containing 5 μ l of Invitrogen optimizer buffer F (10 mM MgCl₂, pH 9.0) (Invitrogen) for COI and buffer D (17.5 mM MgCl₂, pH 8.5) for ND1 and 16S, 2.5 μ l dNTPs (2.5 mM each), 1.25 μ l of each primer (10 μ M), 1 U *Taq* polymerase, 1 μ l of template (ca. 50–100 ng double-stranded DNA), and 13.8 μ l of sterile water. Thermal cycling was performed with an initial denaturation for 2 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at gene-specific annealing temperature, 2 min at 72 °C, with a final extension of 10 min at 72 °C. Annealing temperatures were 45–50 °C for COI, 55 °C for ND1, and 50 °C for 16S.

The amplified PCR product was cleaned using the Exo/SAP method. Double-stranded DNA templates were incubated at 37 °C for 30 min and then at 85 °C for another 15 min with 5 U of Exonuclease I (ExoI, Amersham) and 0.5 U Shrimp Alkaline Phosphatase (SAP, Amersham). One to five microliters (ca. 10–20 ng) of the cleaned PCR product was used as a template for cycle sequencing reactions in a 10 μ l total volume with the CEQ DTCS Quick Start Kit (Beckman–Coulter). The following cycling conditions were used: 96 °C for 2 min, then 35 cycles of 96 °C for 20 s, 45–50 °C for 20 s, and 60 °C for 4 min. The cycle-sequenced products were purified using an ethanol precipitation method following the Beckman–Coulter protocol and separated by electrophoresis using a Beckman–Coulter CEQ8000 sequencer. Sequences were determined for both strands.

2.3. Data analysis

Sequences were edited and aligned using Sequencher 3.1.1 (Gene Codes, Ann Arbor, MI). The protein-coding COI and ND1 sequences were aligned unambiguously. The 16S sequences contained nine insertion/deletion sites. An initial alignment of the 16S sequences (which contained nine insertion/deletion sites) based on Sequencher was revised manually. Alignment of 16S was done without reference to secondary structure models owing to the high levels of primary sequence conservation. Base compositional differences were evaluated with the χ^2 test. Nucleotide substitution patterns were assessed by plotting transition/transversion ratios for pairs of sequences against inferred genetic distance and plotting the absolute number of transitions and transversions against inferred distance.

Sequences for each gene were initially analyzed separately. The partition homogeneity/incongruence-length difference test (Farris et al., 1994; ILD) implemented in PAUP* was used to determine whether the datasets were consistent and could be combined for phylogenetic analysis. For each of the pairwise data partitions, the test was implemented using parsimony-informative sites only and 100 replicates.

The best models of sequence evolution were selected using likelihood ratio tests implemented in Modeltest 3.06 (Posada and Crandall, 1998). The HKY + I + Γ substitution model (Hasegawa et al., 1985) was chosen for the COI and combined datasets while the TrN + I + Γ model (Tamura and Nei, 1993) was chosen for the 16S and ND1 datasets.

Phylogenetic analyses based on distance, parsimony, and maximum-likelihood methods were conducted using PAUP* 4.0b10 (Swofford, 2002). A Bayesian analysis using MrBayes 3.04 (Huelsenbeck and Ronquist, 2001) was also performed as another means of estimating phylogeny. Appropriate inferred distances (e.g., HKY distance for the COI and combined datasets; TrN distance for the 16S and ND1 datasets) were used to generate neighbor-joining (NJ) trees based on the clustering method of Saitou and Nei (1987). Node support was assessed by completion of 1000 bootstrap replications (Felsenstein, 1985) in PAUP*, using the fast-search option. Since no mutational saturation was evident, maximum-parsimony (MP) analyses were conducted with equal weighting, using the heuristic search option with tree bisection reconnection branch-swapping, 100 replications of random stepwise additions, gaps treated as missing, and MAXTREES set to 10,000. Bootstrapping with 1000 replications (as implemented in PAUP*) was used to evaluate node support. Maximum-likelihood (ML) analyses were based on the HKY + I + Γ model for the COI and combined datasets and the TrN + I + Γ model for the 16S and ND1 datasets. A neighbor-joining tree with Juke–Cantor distance was used as the initial topology for branch-swapping. Node support was evaluated by 100 bootstrap pseudoreplicates.

Bayesian analyses were performed based on the HKY + I + Γ model for the COI and combined datasets and TrN + I + Γ model for the 16S and ND1 datasets. Several short runs were first conducted using the default random tree option to determine when the log likelihood sum reached a stable value (by plotting the log-likelihood scores of sample points against generation time). Metropolis-coupled Markov chain Monte Carlo simulations were then run with four chains using the default random tree option for 1,000,000 generations, and Markov chains were sampled at intervals of 10 generations to obtain 100,000 sample points. The last 95,000 sampled trees with branch lengths (the first 5000 trees having been removed as “burn-in”) were used to generate a 50% majority rule consensus tree. The percentage of samples that recovered a specific clade on this topology represents the clade’s posterior probability; these are the *P* values, and *P* \geq 95% was considered evidence of significant support (Huelsenbeck and Ronquist, 2001).

A hypothesis of *Pyrgulopsis* monophyly was explicitly tested by comparing constrained (monophyletic) and

unconstrained most parsimonious trees. We similarly tested a hypothesis of monophyly of *Floridobia* and monophyly of *Pyrgulopsis* + *Nymphophilus*. Alternative topologies were statistically compared using the Templeton and winning-sites, and Kishino–Hasegawa tests implemented in PAUP* (Prager and Wilson, 1988; Kishino and Hasegawa, 1989). Goldman et al. (2000) suggested that using the Kishino and Hasegawa (1989) test is inappropriate when an optimal tree(s) is compared a posteriori with other trees. However, due to the size of our dataset, the SOWH test recommended by Goldman et al. (2000) is prohibitively time consuming. A molecular clock hypothesis for individual datasets was tested using the likelihood ratio test (Felsenstein, 1981), based on the ML topology under the best model selected with and without the constraint of a molecular clock.

Species of *Pyrgulopsis* (and outgroups) were assigned to the informal morphological groups described by Hershler and Sada (2002) based on the literature and personal observation (Table 1). These groups were treated as unordered character states and mapped onto the preferred phylogenetic hypothesis using MacClade 3.0 (Maddison and Maddison, 1992) as a means of reconstructing their evolution. Occurrence of sampling localities among major western physiographic regions (Fig. 1, Table 1) was similarly mapped onto the tree to evaluate geographical diversification of *Pyrgulopsis*. Geographical regions, whose characteristic physiography and structure generally date to the late Tertiary (Stewart, 1998), were chosen as the unit of analysis rather than major western drainage basins, which have been shown to be complex composites (e.g., Belcher, 1975; Malde, 1991; Young, 2004).

3. Results

New sequences were deposited in GenBank under Accession Nos. AY627916–AY628084 (Table 1). The alignment of COI sequences yielded 658 bp, of which 251 sites were variable (38.1%) and 223 were parsimony informative (33.9%). Overall nucleotide composition was biased towards thymine (T) (36.9%) and adenine (A) (25.1%), followed by cytosine (C) (19.4%) and guanine (G) (18.6%). A total of 530 bp of ND1 was analyzed, of which 251 sites were variable (47.4%) and

214 were parsimony informative (40.4%). Average base frequencies for this gene were 29.0% A, 36.5% T, 18.9% C, and 15.5% G. The total length of 16S varied from 467 bp in *Floridobia winkleyi* to 475 bp in *Pyrgulopsis deaconi*. The total aligned data matrix including indels was 476 bp, of which 112 sites were variable (23.5%) and 67 were parsimony informative (14.1%). Average base frequencies for 16S were 34.2% A, 30.0% T, 16.0% C, and 19.8% G. Base frequencies were homogeneous ($\chi^2 = 138.92$, $df = 210$, $P = 0.99$). All three genes showed no evidence of mutational saturation. Sequence divergence values within and among *Floridobia*, *Marstonia*, *Nymphophilus*, and *Pyrgulopsis* are summarized in Table 2. *Floridobia*, *Nymphophilus*, and *Pyrgulopsis* were well differentiated from the outgroup, *Marstonia* (e.g., COI and ND1 divergences >15%). For all three genes *Floridobia* was slightly more differentiated from *Pyrgulopsis* than was *Nymphophilus* (Table 2). Aside from two pairs of little divergent congeners (*P. breviloba* and *P. lata*, *P. bryantwalker* and *P. pictilis*), species of *Pyrgulopsis* differed from one another by 1.1–13.1% for COI, and 1.7–15.8% for ND1. Three pairs of *Pyrgulopsis* species had identical 16S sequences (*P. bryantwalker* and *P. pictilis*, *P. plicata* and *P. inopinata*, *P. sterilis* and *P. glandulosa*).

The ILD tests indicated no significant incongruence between COI and 16S ($P = 0.6$), COI and ND1 ($P = 0.59$), and 16S and ND1 ($P = 1.0$) and thus we performed a set of analyses based on the combined dataset of 1664 bp, one of which (a Bayesian tree) is shown in Fig. 2. Monophyly of the genera *Floridobia*, *Marstonia*, *Nymphophilus*, and the *Floridobia* + *Nymphophilus* + *Pyrgulopsis* clade was strongly supported. *Nymphophilus* was always nested within *Pyrgulopsis* while *Floridobia* was sister to the *Nymphophilus* + *Pyrgulopsis* clade in all but the maximum likelihood analysis (not shown), in which it was instead nested within *Pyrgulopsis* (sister to *P. deaconi*). *Nymphophilus* was depicted as sister to *P. manantiali* in all but the maximum parsimony analysis, and this relationship was strongly supported in the Bayesian trees (96%, Fig. 2). Placement of individual species of *Pyrgulopsis* varied substantially in the resulting trees. *Pyrgulopsis* lineages were poorly resolved and internal nodes were generally connected by very short branches (Fig. 2); e.g., 48 out of 63 interior branches within *Pyrgulopsis* have less than 10 changes. Only the

Table 2

Percent pairwise divergence (P values) ranges for COI, ND1, and 16S sequences for *Floridobia* (2 species), *Marstonia* (3), *Nymphophilus* (2), and *Pyrgulopsis* (62)

	<i>Floridobia</i>			<i>Marstonia</i>			<i>Nymphophilus</i>			<i>Pyrgulopsis</i>		
	COI	ND1	16S	COI	ND1	16S	COI	ND1	16S	COI	ND1	16S
<i>Floridobia</i>	5.8	7.4	2.4									
<i>Marstonia</i>	16.8–17.5	17.5–20.4	4.7–6.4	1.1–6.7	1.4–8.9	0.2–1.1						
<i>Nymphophilus</i>	10.0–10.9	11.7–14.9	3.6–5.4	15.9–18.6	18.1–19.2	5.9–6.6	1.2	0.8	1.1			
<i>Pyrgulopsis</i>	8.1–12.9	11.3–15.7	2.1–6.0	13.7–18.9	17.4–21.5	4.5–7.5	5.1–11.1	8.7–13.2	1.9–5.1	0.3–13.1	0.6–15.8	0.0–5.3

Table 3
Summary of phylogenetic analyses

Dataset	Analysis	# Trees	<i>Floridobia</i> + <i>Nymphophilus</i> + <i>Pyrgulopsis</i> clade Support	<i>Floridobia</i> clade		<i>Nymphophilus</i> clade		<i>Marstonia</i> clade Support
				Support	Sister relationship	Support	Sister	
COI	MP	1323 ^a	83	97	<i>P. avernalis</i> + <i>P. merriami</i>	100	<i>P. manantiali</i>	100
	NJ	1	94	98	<i>P. avernalis</i> + <i>P. merriami</i>	100	<i>P. manantiali</i>	100
	ML	1	84	98	<i>P. eremica</i>	96	<i>P. manantiali</i>	100
NDI	MP	492 ^b	80	93	<i>Nymphophilus</i> + <i>Pyrgulopsis</i>	100	<i>P. eremica</i>	100
	NJ	1	70	89	<i>Nymphophilus</i> + <i>Pyrgulopsis</i>	100	<i>P. eremica</i>	100
	ML	2	76	98	<i>P. deaconi</i>	99	unresolved	100
16S	MP	>10,000 ^c	96	82	<i>Nymphophilus</i> + <i>Pyrgulopsis</i>	95	unresolved	97
	NJ	1	98	77	<i>Nymphophilus</i> + <i>Pyrgulopsis</i> (exclusive of <i>P. merriami</i>)	95	<i>P. manantiali</i>	99
	ML	2	88	84	<i>Nymphophilus</i> + <i>Pyrgulopsis</i>	95	unresolved	91
Combined	MP	357 ^d	100	100	<i>Nymphophilus</i> + <i>Pyrgulopsis</i>	100	<i>Pyrgulopsis</i>	100
	NJ	1	100	100	<i>Nymphophilus</i> + <i>Pyrgulopsis</i>	100	<i>P. manantiali</i>	100
	ML	1	99	100	<i>P. deaconi</i>	100	<i>P. manantiali</i>	100
	Bayesian	2	97	100	<i>Nymphophilus</i> + <i>Pyrgulopsis</i>	100	<i>P. manantiali</i>	97

^a 1453 steps.

^b 1547 steps.

^c 297 steps.

^d 3422 steps.

following clades were well supported: *P. breviloba* + *P. lata*, *P. bryantwalkeri* + *P. pictilis*, *P. erythropoma* + *P. fairbanksensis*, *P. intermedia* + *P. robusta*, *P. lockensis* + *P. villacampae*, and *P. diablensis* + *P. giulianii* + *P. stearnsiana*.

When monophyly of *Pyrgulopsis* was forced using the CONSTRAINT function in PAUP*, the shortest trees (3475 steps) were significantly different from the unconstrained, most parsimonious trees (TL = 3422, combined MP dataset) based on the Templeton and winning-sites ($P < 0.001$) and the Kishino–Hasegawa ($P < 0.001$) tests. When monophyly of *Floridobia* and monophyly of *Pyrgulopsis* + *Nymphophilus* were similarly forced, the resulting trees (TL = 3469) were also significantly different from unconstrained topologies ($P < 0.05$ for both tests).

The results of the combined analyses were highly congruent with those based on the separate datasets (Table 3). Representative (maximum likelihood) trees for the COI, NDI, and 16S datasets are shown in Appendix B. As was the case with the combined dataset, *Nymphophilus* was most frequently resolved as sister to *P. manantiali* [e.g., Appendix B (A)], although in other topologies it was either sister to *P. eremica* (Table 3) or depicted as an unresolved long branch [Appendix B (B)]. The position of *Floridobia* when nested within *Pyrgulopsis* varied and was never strongly supported. Likelihood ratio tests rejected clock-like behavior of sequences for the individual datasets ($P < 0.001$).

Our preferred phylogenetic hypothesis (Bayesian tree based on the combined dataset) is little congruent with previously proposed morphological groupings of *Pyrgulopsis* species (Fig. 2). One small, geographically localized

group was depicted as monophyletic (character state 7, Fig. 3) while each of the other eight groups was shown to be polyphyletic. Our molecular phylogenetic hypotheses similarly conflicts with geographical groupings of *Pyrgulopsis* species as all eight regions which contain two or more congeners were depicted as polyphyletic (Fig. 4).

4. Discussion

A main goal of this study was to rigorously test whether *Pyrgulopsis* is a monophyletic group. Our phylogenetic analyses of separate and combined datasets from three mitochondrial genes did not support monophyly of *Pyrgulopsis* and are congruent with a prior study based on COI sequences (Hershler et al., 2003a). These analyses also confirmed a close relationship between *Floridobia*, *Nymphophilus*, and *Pyrgulopsis*, which were previously shown to form a clade based on COI sequences (Hershler et al., 2003a).

4.1. Implications for systematics

Our results provide no basis for splitting paraphyletic *Pyrgulopsis* as relationships within this genus were poorly resolved and little congruent with morphological groupings (Fig. 3). In our view, all of the western North American nymphophilines should continue to be placed in *Pyrgulopsis*.

The taxonomic distinction between *Pyrgulopsis* and both *Floridobia* and *Nymphophilus* needs to be reassessed based on our results. *Nymphophilus*, which lives in large thermal springs in the Cuatro Ciénegas

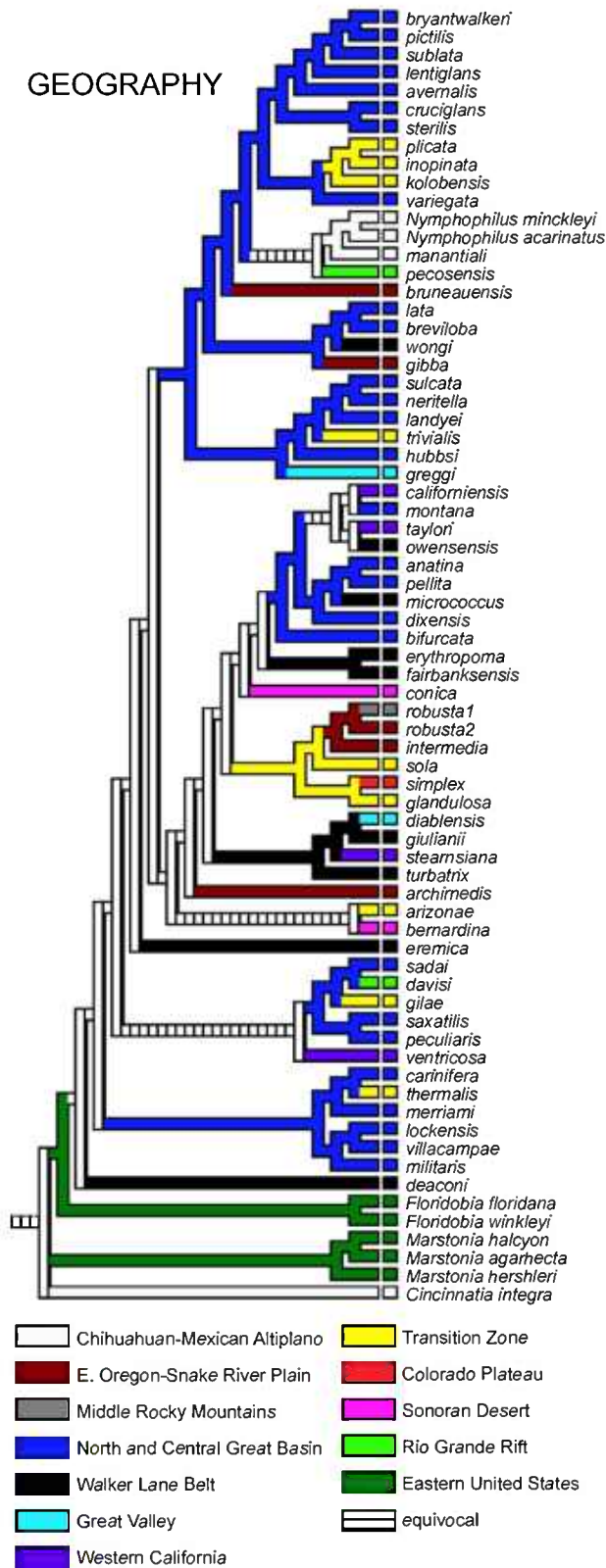


Fig. 4. Phylogenetic reconstruction of physiographic region occurrences based on the topology in Fig. 2.

basin (northeast Mexico), was originally diagnosed by the large, carinate, trochoid shell; multispiral operculum; and raised pallial vas deferens of its single species

(Taylor, 1966b). Hershler (1985) later described a smaller, smooth-shelled congener. Recent studies indicate that *Nymphophilus* is little differentiated from *Pyrgulopsis* apart from the unusual shell of its type species (Hershler, 1994; Thompson, 1979). The close morphological and genetic (Table 2) similarity of these sympatric genera, together with the consistent placement of *Nymphophilus* within *Pyrgulopsis* in our trees, provides strong justification for treating them as synonyms, which we formalize in Appendix C.

If *Floridobia* is also placed in synonymy with *Pyrgulopsis* the latter would be rendered monophyletic, thereby “solving” the systematic problem posed by our molecular phylogenies. However, there are good reasons not to perform this action. *Floridobia*, which is distributed in close proximity to the Atlantic margin of the United States (Davis and Mazurkiewicz, 1985; Thompson, 1968, 2000), is widely separated geographically relative to *Pyrgulopsis*. *Floridobia* is differentiated from all other North American nymphophilines by a significant modification of female reproductive groundplan, the presence of a second seminal receptacle (Hershler et al., 2003a). The mtDNA sequences of *Floridobia* are slightly more divergent relative to *Pyrgulopsis* than are those of *Nymphophilus* (Table 2) and the genus was frequently positioned as sister to a clade composed of the latter two taxa. Based upon this evidence, we continue to recognize *Floridobia* as a separate genus, pending additional study of the interesting systematic and biogeographic (Hershler et al., 2003a) issues posed by its close relationship to *Pyrgulopsis*.

4.2. The western North American nymphophiline radiation

Our molecular-based phylogeny of the western nymphophilines contained generally short and weakly supported interior branches and was little congruent with morphological and geographical groupings of species. The presence of short interior branches on inferred phylogenies is commonly attributed to an episode of rapid evolution (Malcomber, 2002; Megens et al., 2004; Morrison et al., 2004; Rüber et al., 2003), although this could also represent an artifact of the dataset or analytical methodology (Fishbein et al., 2001). Since this pattern is replicated in various analyses of three mitochondrial genes, we are confident that our results approximate “biological reality.” Fossil evidence suggests that *Pyrgulopsis* minimally originated during the late Miocene (Hershler and Liu, 2004b), a turbulent period in which the subdued mid-Tertiary western landscape (Gresens, 1978, 1981; Potochnik and Damon, 1986) was disrupted by tectonic activity and the complex, mountainous physiography of the present day region began to take shape (Christiansen and Yeats, 1992). This dynamic setting could have provided the stage for rapid radiation of the western nymphophilines into diverse lineages which were jointly splintered by range uplift and other tectonic

events, resulting in the phylogenetic mosaic that is now found in individual physiographic regions. This scenario is consistent with a previously proposed correlation between rapid diversification of western North American freshwater mollusks (inferred from the fossil record) and regional tectonism (Taylor, 1965, 1966a, 1985) and can be further explored using a well calibrated molecular clock for the western nymphophilines.

We attribute the haphazard geographic distribution of morphological groups of western nymphophilines to homoplasy of penial structure. Note that each of the eight groups that were depicted in our trees as polyphyletic is solely defined by penis type (Hershler and Sada, 2002), while the only group which formed a well supported clade (group 7) was defined by unique radular characters and contains diverse penial morphologies (Hershler and Sada, 2002). This result is consistent with a recent phylogenetic analysis based on COI sequences which showed that even on a relatively small geographic scale (Death Valley region), penial morphology did not well delineate monophyletic groups (Liu et al., 2003). Some of the penial types used to define groups in *Pyrgulopsis* are also found in other nymphophiline genera [group 2, *Birgella* (Thompson, 1984); group 3, *Marstonia* (Thompson, 1977); group 9, *Cincinnatia* (Hershler and Thompson, 1996), *Floridobia*, (Thompson, 1968)], casting additional doubt on their phylogenetic utility. The apparently iterative evolution of similar penial morphologies among western nymphophilines merits additional study, as does the possible adaptive significance of the remarkable diversity of these structures.

Note that our analyses (Fig. 2, Appendix B) recovered several well supported, locally endemic clades of robust-shelled *Pyrgulopsis* species which live in large warm springs (*P. erythropoma* + *P. fairbanksensis*, Ash Meadows; *P. lockensis* + *P. villacampae*, Railroad Valley). Based on this result the Cuatro Ciénegas fauna (*Nymphophilus sensu lato*) may be viewed as one of the multiple independent radiations in thermal ecosystems that occurred within the evolutionary history of *Pyrgulopsis*. Perhaps the occurrence of large, squat shells in these separate clades represents a parallel evolutionary response (*vide* Vermeij, 1978; Vermeij and Covich, 1978) to the snail-eating fishes (Deacon and Minckley, 1974) and other predators (e.g., crayfish) which commonly live in these habitats.

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Appendix A

Localities and museum voucher numbers for species included in this study. Abbreviations: UF, Florida Museum of Natural History, University of Florida; USNM, former United States National Museum, now National Museum of Natural History, Smithsonian Institution.

A.1. *Pyrgulopsis*

anatina, spring southeast of Old Collins Spring, Railroad Valley, Nye Co., NV, USNM 894713; *archimedis*, Upper Klamath Lake at Hagelstein Park, Klamath Co., OR, USNM 894697; *arizonae*, Medicine Spring, Bylas, Gila River drainage, Graham Co., AZ, USNM 1003881; *avernalis*, Muddy Spring, Moapa Valley, Lincoln Co., NV, USNM 903988; *bernardina*, spring, El Chorro, Rio de Bavispe drainage, Sonora, Mexico, USNM 1010780; *bifurcata*, springs west of Carico Lake, Carico Lake basin, Lander Co., NV, USNM 894715; *breviloba*, Flag Springs, White River Valley, Nye Co., NV, USNM 894708; *bruneauensis*, springs tributary to Hot Creek, Bruneau River drainage, Owyhee Co., ID, DNA only; *bryantwalkerii*, Warm Spring, Humboldt River drainage, Elko Co., NV, USNM 1002337; *californiensis*, spring tributary to Campo Creek, Laguna-San Diego coastal drainage, San Diego Co., CA, USNM 899093; *carinifera*, Muddy Spring, Moapa Valley, Lincoln Co., NV, USNM 903991; *conica*, Dripping Spring, Sacramento Wash, Mohave Co., AZ, DNA only; *cruciglans*, Flat Spring, Steptoe Valley, White Pine Co., NV, USNM 894717; *davisi*, spring tributary to Limpia Creek, Pecos River drainage, Jeff Davis Co., TX, USNM 1001932; *deaconi*, Red Spring, Las Vegas Wash, Clark Co., NV, USNM 894826; *diablensis*, stream, Del Puerto Canyon, San Joaquin River drainage, Stanislaus Co., CA, USNM 903985; *dixensis*, spring west of Cain Mountain, Dixie Valley, Pershing Co., NV, USNM 1002470; *eremica*, spring, tributary to Willow Creek, Eagle Lake basin, Lassen Co., CA, USNM 1002473; *erythropoma*, Kings Pool, Ash Meadows, Amargosa River drainage, Nye Co., NV, USNM 903983; *fairbanksensis*, Fairbanks Spring, Ash Meadows, Amargosa River drainage, Nye Co., NV, USNM 903995; *gibba*, springs west of Fee

Reservoir, Surprise Valley, Lassen Co., CA, USNM 1002892; *gilae*, spring tributary to East Fork Gila River, Grant Co., NM, USNM 1004620; *giulianii*, stream, Sand Canyon, Indian Wells Valley, Kern Co., CA, USNM 894352; *glandulosa*, Nelson Place Spring, Verde River, Yavapai Co., AZ, DNA only; *greggi*, Grapevine Creek, Kern River, Kern Co., CA, USNM 903984; *hubbsi*, Crystal Spring, Pahranaagat Valley, Lincoln Co., NV, USNM 903992; *inopinata*, Spring, Glenwood, Sevier River drainage, Sevier Co., UT, USNM 894843; *intermedia*, Crooked Creek, Owyhee River drainage, Malheur Co., OR, USNM 863510; *kolobensis*, Toquerville Springs, Virgin River drainage, Washington Co., UT, USNM 894877; *landyei*, “Middle Spring,” Steptoe Ranch, Steptoe Valley, White Pine Co., NV, USNM 894719; *lata*, Butterfield Springs, White River Valley, Nye Co., NV, USNM 894710; *lentiglans*, Crittenden Springs, Thousand Springs Creek drainage, Elko Co., NV, USNM 894837; *lockensis*, Big Spring, Locke’s Ranch, Railroad Valley, Nye Co., NV, USNM 894824; *manantiali*, Santa Tecla canal at Puerto Salado, Rio Salado drainage, Coahuila, Mexico, USNM 1010509; *merriami*, Ash Spring, Pahranaagat Valley, Lincoln Co., NV, USNM 903996; *micrococcus*, spring near Springdale, Amargosa River, Nye Co., NV, USNM 894330; *militaris*, spring, Soldier Meadow, Black Rock Desert, Humboldt Co., NV, USNM 1002361; *montana*, spring, upper Camp Valley, Meadow Valley Wash, Lincoln Co., NV, USNM 894876; *neritella*, Big Spring, Steptoe Ranch, Steptoe Valley, White Pine Co., NV, USNM 894720; *owensensis*, stream, canyon south of Piute Creek, Owens Valley, Mono Co., CA, USNM 899099; *pecosensis*, spring, Bound’s Ranch, Pecos River, Eddy Co., NM, USNM 892588; *peculiaris*, spring near Swasey Spring, Whirlwind Valley, Millard Co., UT, USNM 894883; *pellita*, Sullivan Spring, Antelope Valley, Eureka Co., NV, USNM 1002339; *pictilis*, Cain Spring, Antelope Valley, Lander Co., NV, USNM 1002341; *plicata*, spring, Black Canyon, Sevier River drainage, Garfield Co., UT, USNM 894844; *robusta1*, Polecat Creek, Snake River drainage, Teton Co. WY, USNM 905297; *robusta2*, Hughet Spring, Harney Lake basin, Harney Co., OR, USNM 863508; *sadai*, spring, Moss Creek, Reese River Valley, Lander Co., NV, USNM 1002355; *saxatilis*, Warm Springs, Snake Valley, Millard Co., UT, USNM 894848; *simplex*, spring near Strawberry, Verde River drainage, Gila Co., AZ, USNM 1003862; *sola*, Brown Springs, Verde River drainage, Yavapai Co., AZ, DNA only; *stearnsiana*, springs, Wildcat Canyon, San Francisco Bay drainage, Contra Costa Co., CA, USNM 894694; *sterilis*, spring, Hunts Canyon Ranch, Ralston Valley, Nye Co., NV, USNM 1002346; *sublata*, Wambolt Springs, Lake Valley, Lincoln Co., NV, USNM 894888; *sulcata*, spring northwest of Clark Spring, Steptoe Valley, White Pine Co., NV, USNM 894721; *taylori*, spring tributary to San Luis Obispo Creek, Central Cal-

ifornia coastal drainage, San Luis Obispo Co., CA, USNM 903986; *thermalis*, Hot Spring, Gila River drainage, Grant Co., NM, USNM 1004621; *trivialis*, Spring, Three Forks, Gila River drainage, Apache Co., AZ, USNM 894881; *turbatrix*, Horseshutem Springs, Pahrump Valley, Nye Co., NV, USNM 903989; *variiegata*, spring south of South Patterson Spring, Pilot Valley, Box Elder Co., UT, USNM 894835; *ventricosa*, spring, Seigler Canyon, Clear Lake basin, Lake Co., CA, USNM 894696; *villacampae*, Little Warm Spring, Railroad Valley, Nye Co., NV, USNM 894828; and *wongi*, spring, Birchim Canyon, Owens Valley, Inyo Co., CA, USNM 1011869.

A.2. Outgroups

Cincinnatia integra, Rio La Cienega, Veinte de Noviembre, Rio Moctezuma drainage, San Luis Potosi, Mexico, UF 283792; *Floridobia floridana*, Juniper Springs, St. Johns River drainage, Marion Co., FL, USNM 894755; *Floridobia winkleyi*, Salt marsh, Scarborough, Saco River drainage, Cumberland Co., ME, USNM 883964; *Marstonia agarhecta*, Bluff Creek, Ocmulgee River drainage, Pulaski Co., GA, USNM 894686; *Marstonia halcyon*, Ogeechee River, southwest of Rocky Ford, Screven Co., GA, USNM 905285; *Marstonia hershleri*, Coosa River, north of Wetumpka, Elmore Co., AL, USNM 894681; *Nymphophilis acarinatus*, La Tecla Vieja, Cuatro Ciénegas basin, Coahuila, Mexico, USNM 1010512; and *Nymphophilus minckleyi*, East Cold Spring, Cuatro Ciénegas basin, Coahuila, Mexico, USNM 1010516.

Appendix B

ML trees based on single molecular markers. Bootstrap values are given when greater than 50%. (A) Single resulting COI tree with log likelihood score of 7048.22 obtained using HKY + I + Γ model with base frequencies of A = 0.3056, C = 0.1965, G = 0.1605, T = 0.3374; a proportion of invariable sites of 0.5971; and a gamma distribution shape parameter of 1.1855. (B) One of two ND1 trees with log likelihood score of 7001.28 based on the TrN + I + Γ model with base frequencies of A = 0.3524, C = 0.2281, G = 0.1084, T = 0.3111; a rate matrix of [A – C] = 1.0, [A – G] = 31.9382, [A – T] = 1.0, [C – G] = 1.0, [C – T] = 17.8743; a proportion of invariable sites of 0.4806; and a gamma distribution shape parameter of 0.8363. C. One of two 16S trees with log likelihood score of 7711.06 based on the TrN + I + G model with base frequencies of A = 0.3608, C = 0.1586, G = 0.1846, T = 0.2960; a rate matrix of [A – C] = 1.0, [A – G] = 10.2831, [A – T] = 1.0, [C – G] = 1.0, [C – T] = 20.1318; a proportion of invariable sites of 0.6065; and a gamma distribution shape parameter of 0.6139.

Appendix C

Emended taxonomic status of *Nymphophilus* Taylor, 1966b. This genus is newly placed into synonymy with *Pyrgulopsis* and a revised diagnosis of the latter is given.

C.1. Genus *Pyrgulopsis* Call and Pilsbry, 1886

Pyrgulopsis Call and Pilsbry, 1886: 9 (type species by original designation, *Pyrgula nevadensis* Stearns, 1883). *Fontelicella* Gregg and Taylor, 1965: 103 (type species by original designation, *Fontelicella californiensis* Gregg and Taylor, 1965). *Natricola* Gregg and Taylor, 1965: 108 (type species by original designation, *Pomatiopsis robusta* Walker, 1908). *Microamnicola* Gregg and Taylor, 1965: 109 (type species by original designation, *Amnicola micrococcus* Pilsbry in Stewart, 1893). *Savaginius* Taylor, 1966a: 130 (type species by original designation, *Paludestrina nanna* Chamberlin, 1933). *Nymphophilus* Taylor, 1966b: 199 (type species by original designation, *Nymphophilus minckleyi* Taylor, 1966b). *Mexistiobia* Hershler, 1985: 46 (type species by original designation, *Mexistiobia manantiali* Hershler, 1985). *Apachecoccus* Taylor, 1987: 32 (type species by original designation, *Apachecoccus arizonae* Taylor, 1987). *Yaquicoccus* Taylor, 1987: 34 (type species by original designation, *Yaquicoccus bernardinus* Taylor, 1987).

C.1.1. Diagnosis

Shell globose to narrow-conic, smooth or spirally sculptured, usually umbilicate, clear or white. Protoconch flat or dome-like, smooth, wrinkled or sculptured with weak spiral striae. Body usually darkly pigmented; pallial roof without distinctive pigment bands. Cephalic tentacles extending beyond tip of snout, variably ciliated. Ctenidium usually well developed, kidney usually protruding into pallial cavity. Penis blade-like or distally bifurcate, usually ornamented with glandular units which are superficially positioned or borne on lobules. Bursa copulatrix and its duct usually superficially positioned on albumen gland, sometimes partly overlapped by gland. Bursal duct opens to coiled oviduct just behind posterior pallial wall. Single seminal receptacle opening to distal arm of coiled oviduct.

C.1.2. Content

Pyrgulopsis, as emended herein, contains 126 species. These include species listed as *Pyrgulopsis* in the recent literature (Hershler, 1994, 1995, 1998; Hershler and Sada, 2000; Hershler and Gustafson, 2001; Hershler et al., 2003b; Hershler and Liu, 2004b) and two species formerly allocated to *Nymphophilus* (Hershler, 1985; Taylor, 1966b).

C.1.3. Distribution

A large portion of western North America, from the Rio Grande basin to the Pacific coast, and from the Columbia River basin through portions of the Mexican Plateau.

C.1.4. Comment

Pyrgulopsis and closely related *Floridobia* are distinguished from all other North American nymphophilines by the superficial position of the bursa copulatrix and bursal duct on the albumen gland. *Pyrgulopsis* differs from *Floridobia* in having diffuse (rather than banded) pigmentation on the pallial roof and in having a single (as opposed to two) female seminal receptacle.

References

- Belcher, R.C., 1975. The geomorphic evolution of the Rio Grande. *Baylor Geol. Stud. Bull.* 29, 1–64.
- Berry, S.S., 1947. A new *Pyrgulopsis* from Oregon. *Nautilus* 60, 76–78.
- Bucklin, A., 1992. Use of formalin-preserved samples for molecular analysis. *Newsl. Crustacean Mol. Technol.* 2, 3.
- Burch, J.B., 1989. *North American Freshwater Snails*. Malacological Publications, Hamburg, MI.
- Call, R., Pilsbry, H.A., 1886. On *Pyrgulopsis*, a new genus of rissoid mollusk, with descriptions of two new forms. *Proc. Davenport Acad. Sci.* 5, 9–14.
- Chamberlin, R.V., Berry, E., 1933. Mollusks of the Pliocene deposits at Collinston. *Utah. Nautilus* 47, 25–29.
- Christiansen, R.L., Yeats, R.S., 1992. Post-Laramide geology of the U.S. Cordilleran region. In: Burchfiel, B.C., Lipman, P.W., Zoback, M.L. (Eds.), *The Cordilleran Orogen: Conterminous U.S. Geol. Soc. Am., Boulder*, pp. 261–406.
- Davis, G.M., Mazurkiewicz, M., 1985. Systematics of *Cincinnatiella winkleyi*. *Proc. Acad. Nat. Sci. Philadelphia* 137, 28–47.
- Deacon, J.E., Minckley, W.L., 1974. Desert fishes. In: Brown, Jr., G.W. (Ed.), *Desert Biology*. Academic Press, New York, pp. 385–488.
- Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1994. Testing significance of incongruence. *Cladistics* 10, 315–319.
- Felsenstein, J., 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* 17, 368–376.
- Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791.
- Fishbein, M., Hirsch-Jetter, C., Soltis, D.E., Hufford, L., 2001. Phylogeny of Saxifragales (Angiosperms, Eudicots): analysis of a rapid, ancient radiation. *Syst. Biol.* 50, 817–847.
- Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotech.* 3, 294–299.
- Goldman, N., Anderson, J.P., Rodrigo, A.G., 2000. Likelihood-based tests of topologies in phylogenetics. *Syst. Biol.* 49, 652–670.
- Gregg, W.O., Taylor, D.W., 1965. *Fontelicella* (Prosobranchia: Hydrobiidae), a new genus of West American freshwater snails. *Malacologia* 3, 103–110.
- Gresens, R.L., 1978. Regional significance of a late Eocene/early Oligocene erosion surface exposed in central Washington State. *Abstr. Geol. Soc. Am.* 10 (3), 107.
- Gresens, R.L., 1981. Extension of the Telluride erosion surface to Washington State, and its regional and tectonic significance. *Tectonophysics* 79, 145–164.

- Hasegawa, M., Kishino, H., Yano, T., 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *J. Mol. Evol.* 22, 160–174.
- Hershler, R., 1985. Systematic revision of the Hydrobiidae (Gastropoda: Rissooidea) of the Cuatro Ciénegas Basin, Coahuila, Mexico. *Malacologia* 26, 31–123.
- Hershler, R., 1990. *Pyrgulopsis bruneauensis*, a new springsnail (Gastropoda: Hydrobiidae) from the Snake River Plain, southern Idaho. *Proc. Biol. Soc. Wash.* 103, 803–814.
- Hershler, R., 1994. A review of the North American freshwater snail genus *Pyrgulopsis* (Hydrobiidae). *Smithson. Contrib. Zool.* 554, 1–115.
- Hershler, R., 1995. New freshwater snails of the genus *Pyrgulopsis* (Rissooidea: Hydrobiidae) from California. *Veliger* 38, 343–373.
- Hershler, R., 1998. A systematic review of the hydrobiid snails (Gastropoda: Rissooidea) of the Great Basin, western United States. Part I. Genus *Pyrgulopsis*. *Veliger* 41, 1–132.
- Hershler, R., Gustafson, D.L., 2001. First record for springsnails (Mollusca: Hydrobiidae: *Pyrgulopsis*) from the northern Rocky Mountains. *Proc. Biol. Soc. Wash.* 114, 297–308.
- Hershler, R., Landye, J.J., 1988. Arizona Hydrobiidae. *Smithson. Contrib. Zool.* 459, 1–63.
- Hershler, R., Liu, H.-P., 2004a. Taxonomic reappraisal of species assigned to the North American freshwater gastropod subgenus *Natricola* (Rissooidea: Hydrobiidae). *Veliger* 47, 66–81.
- Hershler, R., Liu, H.-P., 2004b. A molecular phylogeny of aquatic gastropods provides a new perspective on biogeographic history of the Snake River region. *Mol. Phylogenet. Evol.* 32, 927–937.
- Hershler, R., Pratt, W.L., 1990. A new *Pyrgulopsis* (Gastropoda: Hydrobiidae) from southeastern California, with a model for historical development of the Death Valley hydrographic system. *Proc. Biol. Soc. Wash.* 103, 279–299.
- Hershler, R., Sada, D.W., 1987. Springsnails (Gastropoda: Hydrobiidae) of Ash Meadows, Amargosa Basin, California-Nevada. *Proc. Biol. Soc. Wash.* 100, 776–843.
- Hershler, R., Sada, D.W., 2000. A new species of hydrobiid snail of the genus *Pyrgulopsis* from northwestern Nevada. *Veliger* 43, 367–375.
- Hershler, R., Sada, D.W., 2002. Biogeography of Great Basin aquatic snails of the genus *Pyrgulopsis*. In: Hershler, R., Madsen, D.B., Currey, D.R. (Eds.), *Great Basin Aquatic Systems History*. *Smithson. Contrib. Earth Sci.*, 33, pp. 255–276.
- Hershler, R., Thompson, F.G., 1987. North American Hydrobiidae (Gastropoda: Rissooidea): redescription and systematics relationships of *Tryonia* Stimpson, 1865 and *Pyrgulopsis* Call and Pilsbry, 1886. *Nautilus* 101, 25–32.
- Hershler, R., Thompson, F.G., 1996. Redescription of *Paludina integra* Say, 1821, type species of the genus *Cincinnatia* (Gastropoda: Hydrobiidae). *J. Moll. Stud.* 62, 33–55.
- Hershler, R., Liu, H.-P., Thompson, F.G., 2003a. Phylogenetic relationships of North American nymphophiline gastropods based on mitochondrial DNA sequences. *Zool. Scripta* 32, 357–366.
- Hershler, R., Frest, T.J., Liu, H.-P., Johannes, E.J., 2003b. Rissooidean snails from the Pit River Basin, California. *Veliger* 46, 275–304.
- Huelsensbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* 17, 754–755.
- Hurt, C.R., 2004. Genetic divergence, population structure and historical demography of rare springsnails (*Pyrgulopsis*) in the lower Colorado River basin. *Mol. Ecol.* 13, 1173–1187.
- Kishino, H., Hasegawa, M., 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Mol. Evol.* 29, 170–179.
- Liu, H.-P., Hershler, R., Thompson, F.G., 2001. Phylogenetic relationships of the Cochliopinae (Rissooidea: Hydrobiidae): an enigmatic group of aquatic gastropods. *Mol. Phylogenet. Evol.* 21, 17–25.
- Liu, H.-P., Hershler, R., Clift, K., 2003. Mitochondrial DNA sequences reveal extensive cryptic diversity within a western American springsnail. *Mol. Ecol.* 12, 2771–2782.
- Maddison, W.P., Maddison, D.R., 1992. *MacClade*, version 3.0. Sinauer Associates, Sunderland, MA.
- Malcomber, S.T., 2002. Phylogeny of *Gaertnera* Lam. (Rubiaceae) based on multiple DNA markers: evidence of a rapid radiation in a widespread, morphologically diverse genus. *Evolution* 56, 42–57.
- Malde, H.E., 1991. Quaternary geology and structural history of the Snake River Plain, Idaho and Oregon. In: Morrison, R.B. (Ed.), *The Geology of North America*. vol. K-2, Quaternary Nonglacial Geology: Conterminous US. *Geol. Soc. Am., Boulder*, pp. 251–281.
- von Martens, K.E., 1890–1901. Land and freshwater Mollusca. In: Godman, F.G., Salvin, O. (Eds.), *Biologia Centrali-Americana*. R.H. Porter, London, pp. 1–706.
- Megens, H.-J., van Moorsel, C.H.M., Piel, W.H., Pierce, N.E., de Jong, R., 2004. Tempo of speciation in a butterfly genus from the Southeast Asian tropics, inferred from mitochondrial and nuclear DNA sequence data. *Mol. Phylogenet. Evol.* 31, 1181–1196.
- Morrison, C.L., Ríos, R., Duffy, J.E., 2004. Phylogenetic evidence for an ancient rapid radiation of Caribbean sponge-dwelling snapping shrimps (*Synalpheus*). *Mol. Phylogenet. Evol.* 30, 563–581.
- Nevada Natural Heritage Program, 2000. Scorecard 2000: highest priority conservation sites. Available from: <[http://heritage.nv.gov/reports/scor2000.pdf\(6/9/04\)](http://heritage.nv.gov/reports/scor2000.pdf(6/9/04))>.
- New Mexico Department of Game and Fish, 2000. Threatened and endangered species of New Mexico. Biennial review and recommendations. Available from: <[http://www.wildlife.state.nm.us/PageMill/Images/NonGame/%20T%26E%20BR%20Booklet.pdf\(6/9/04\)](http://www.wildlife.state.nm.us/PageMill/Images/NonGame/%20T%26E%20BR%20Booklet.pdf(6/9/04))>.
- Palumbi, S., Martin, A., Romano, S., McMillan, W.O., Stice, L., Grabowski, G., 1991. The simple fool's guide to PCR. Privately distributed, Honolulu.
- Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14, 817–818.
- Potochnik, A.R., Damon, P.E., 1986. Tectonic and geomorphic implications of post-Laramide erosion. *Abstr. Geol. Soc. Am.* 18 (5), 403.
- Prager, E.M., Wilson, A.C., 1988. Ancient origin of lactalbumin from lysozyme: Analysis of DNA and amino acid sequences. *J. Mol. Evol.* 27, 326–335.
- Rüber, L., Van Tassell, J.L., Zardoya, R., 2003. Rapid speciation and ecological divergence in the American seven-spined gobies (Gobiidae, Gobiosomatini) inferred from a molecular phylogeny. *Evolution* 57, 1584–1598.
- Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406–425.
- State of Utah Division of Wildlife Resources, 2003. Utah sensitive species list. Available from: <[http://dwrcdc.nr.utah.gov/ucdc/ViewReports/SSL&Appendices121803.pdf\(6/9/04\)](http://dwrcdc.nr.utah.gov/ucdc/ViewReports/SSL&Appendices121803.pdf(6/9/04))>.
- Stearns, R.E.C., 1883. Description of a new hydrobiinoid gastropod from the mountain lakes of the Sierra Nevada, with remarks on allied species and the physiographical features of said region. *Proc. Acad. Nat. Sci. Philadelphia* 35, 171–176.
- Stearns, R.E.C., 1893. Report on the land and fresh-water shells collected in California and Nevada by the Death Valley Expedition, including a few additional species obtained by Dr. C. Hart Merriam and assistants in parts of the southwestern United States. *North Am. Fauna* 7, 269–283.
- Stewart, J.H., 1998. Regional characteristics, tilt domains, and extensional history of the late Cenozoic Basin and Range province, western North America. In: Faulds, J.E., Stewart, J.H. (Eds.), *Accommodation Zones and Transfer Zones: The Regional Segmentation of the Basin and Range Province*. *Geol. Soc. Am. Spec. Pap.* 323, pp. 47–74.

- Swofford, D.L., 2002. PAUP*: Phylogenetic Analysis Using Parsimony (and other methods). Version 4.0b10. Sinauer Associates, Sunderland, MA.
- Tamura, K., Nei, M., 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.W., 1960. Distribution of the freshwater clam *Pisidium ultramontanum*; a zoogeographic inquiry. *Am. J. Sci.* 258-A, 325–334.
- Taylor, D.W., 1965. Historical analysis of distribution of western American freshwater molluscs. *Am. Zoologist* 4, 436–437 [abstract].
- Taylor, D.W., 1966a. Summary of North American Blancan nonmarine mollusks. *Malacologia* 4, 1–172.
- Taylor, D.W., 1966b. A remarkable snail fauna from Coahuila, México. *Veliger* 9, 152–228.
- Taylor, D.W., 1975. Index and bibliography of late Cenozoic freshwater Mollusca of western North America. *Univ. Michigan Pap. Paleontol.* 10, 1–384.
- Taylor, D.W., 1985. Evolution of freshwater drainages and molluscs in western North America. In: Smiley, C.J., Leviton, A.J. (Eds.), *Late Cenozoic History of the Pacific Northwest, Interdisciplinary Studies on the Clarkia Fossil Beds of Northern Idaho*. Am. Assoc. Adv. Sci., San Francisco, pp. 265–321.
- Taylor, D.W., 1987. Fresh-water molluscs from New Mexico and vicinity. *New Mexico Bur. Mines Miner. Resour. Bull.* 116, 1–50.
- Taylor, D.W., Bright, R.C., 1987. Drainage history of the Bonneville Basin. In: Kopp, R.S., Cohenour, R.E. (Eds.), *Cenozoic Geology of Western Utah. Sites for Precious Metal and Hydrocarbon Accumulations*. Utah Geol. Assoc. Publ. 16, pp. 239–256.
- Thompson, F.G., 1968. *The Aquatic Snails of the Family Hydrobiidae of Peninsular Florida*. University of Florida Press, Gainesville.
- Thompson, F.G., 1969. Some hydrobiid snails from Georgia and Florida. *Q. J. Fla. Acad. Sci.* 32, 241–265.
- Thompson, F.G., 1977. The hydrobiid snail genus *Marstonia*. *Fla. State Mus. Biol. Sci. Bull.* 21, 113–158.
- Thompson, F.G., 1979. The systematic relationships of the hydrobioid snail genus *Nymphophilis* Taylor, 1966, and the status of the subfamily Nymphophilinae. *Malacol. Rev.* 12, 41–49.
- Thompson, F.G., 1984. North American freshwater snail genera of the hydrobiid subfamily Lithoglyphinae. *Malacologia* 25, 109–141.
- Thompson, F.G., 1995. A new freshwater snail from the Coosa River, Alabama (Gastropoda: Prosobranchia: Hydrobiidae). *Proc. Biol. Soc. Wash.* 108, 502–507.
- Thompson, F.G., 2000. Three new freshwater snails of the genus *Cincinnatia* from Peninsular Florida (Prosobranchia, Hydrobiidae). *Walkerana* 11, 55–73.
- Thompson, F.G., Hershler, R., 2003. Two genera of North American freshwater snails: *Marstonia* Baker, 1926, resurrected to generic status, and *Floridobia*, new genus (Prosobranchia: Hydrobiidae: Nymphophilinae). *Veliger* 45, 269–271.
- Vermeij, G.J., 1978. *Biogeography and Adaptation. Patterns of Marine Life*. Harvard University Press, Cambridge (MA).
- Vermeij, G.J., Covich, A.P., 1978. Coevolution of freshwater gastropods and their predators. *Am. Naturalist* 112, 833–843.
- Walker, B., 1908. *Pomatiopsis robusta*, n. sp. *Nautilus* 21, 97.
- Young, R.A., 2004. Abstracts for a working conference on the Cenozoic geologic evolution of the Colorado River system and the erosional chronology of the Grand Canyon region. Available from: <<http://www.flag.wr.usgs.gov/GCSymposium/abstract/index.pdf> (6/14/04)>.