

Recognition of a highly restricted freshwater snail lineage (Physidae: *Physella*) in southeastern Oregon: convergent evolution, historical context, and conservation considerations

Alexandria C. Moore · John B. Burch ·
Thomas F. Duda Jr.

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Abstract Non-marine mollusks have the highest number of documented extinctions of any major taxonomic group. Given their conservation status and the numerous cases of taxonomic uncertainty concerning freshwater mollusks in particular, the recognition of potentially endangered species is critically important. Here, we evaluate the genetic distinctiveness and phylogenetic position of a freshwater snail restricted to a series of geothermal springs within the Owyhee River drainage in Oregon (the ‘Owyhee wet-rock physa’). Because these snails closely resemble *Physella* (*Petrophysa*) *zionis*, a wet-rock physa that occurs in a small area in Zion National Park (Utah), the Owyhee wet-rock physa is presumably either closely related to or represents a disjunct population of *P. zionis*. However, phylogenetic analyses of sequences of mitochondrial cytochrome oxidase I and nuclear first and second internal transcribed spacer gene regions indicate that the Owyhee wet-rock physa is genetically distinct from other physid species. Despite exhibiting similar morphologies, the Owyhee wet-rock physa and *P. zionis* are distantly related physids; instead, the Owyhee wet-rock physa is most closely related to a population of physids from central California. These results suggest that convergent evolution may be responsible for the similar shell morphologies of the Owyhee wet-rock physa and *P. zionis*. Furthermore, the close relationship between physid populations in southeastern Oregon

and central California suggests a historical connection between the Owyhee River and river drainages to the south. Finally, we recommend that the Owyhee wet-rock physa be considered critically endangered based on its extremely limited distribution.

Keywords Freshwater gastropods · Physidae · Convergent evolution · COI · ITS1 · ITS2

Introduction

The decline in population sizes and extinctions of numerous North American freshwater gastropod species during the past century are well established (Lydeard et al. 2004; Regnier et al. 2009; Johnson et al. 2013). For example, 55 % of Physidae species, 79 % of Pleuroceridae species, and 91 % of Hydrobiidae species of North America are imperiled (Johnson et al. 2013). Indeed, freshwater snails of North America exhibit the greatest imperilment rate of all freshwater taxa that have been evaluated to date (Johnson et al. 2013). Such potential and realized losses are not without their consequences as freshwater gastropods provide several important ecosystem functions, including regulating rates of primary production, decomposition, and nutrient cycling (Strayer 2006; Lysne et al. 2008; Johnson et al. 2013). Freshwater snails therefore play an important role in ecosystem stability and often substantially affect human welfare (Strayer 2006; Johnson et al. 2013). Thus, the recognition and proper identification of potentially imperiled species is especially important for freshwater gastropods. This is of the utmost concern here for a highly restricted population of a freshwater physid snail that occurs in southeastern Oregon.

A. C. Moore (✉) · J. B. Burch · T. F. Duda Jr.
Department of Ecology and Evolutionary Biology and Museum
of Zoology, University of Michigan, 1109 Geddes Avenue,
Ann Arbor, MI 48109, USA
e-mail: mooreale@umich.edu

T. F. Duda Jr.
Smithsonian Tropical Research Institute, Balboa, Ancón,
Republic of Panama

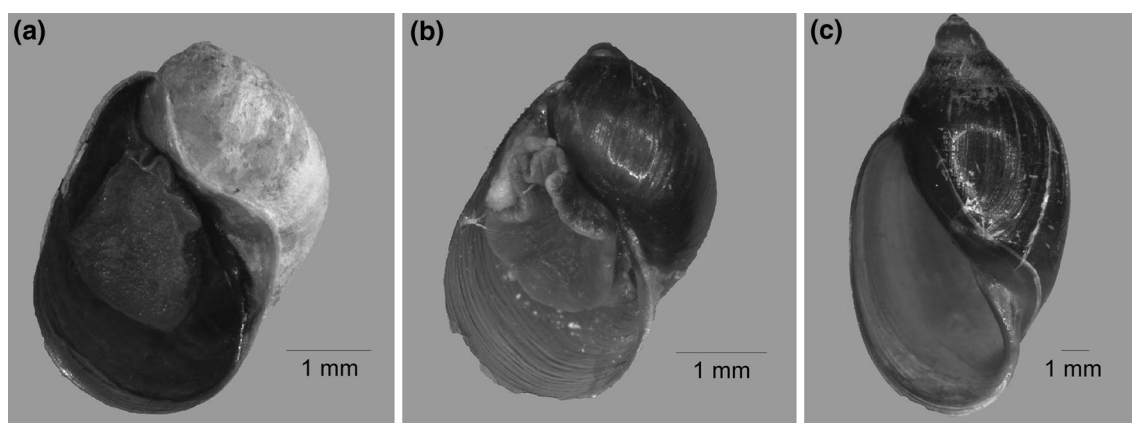


Fig. 1 Shell images of **a** the Owyhee wet-rock physa, **b** *Physella zionis* and **c** *Physella cf. gyrina*. Scale bars denote 1 mm

Physidae is a family of freshwater gastropods that has been estimated to contain approximately 80 species that occur in a variety of habitat types throughout the world (Taylor 2003). In total, 47 physid species occur in the U.S. and Canada (Johnson et al. 2013) and several are highly endemic (Burch and Tottenham 1980; Taylor 2003). The ‘Owyhee wet-rock physa’ (sensu Frest and Johannes 1995) is a relatively small (<5 mm shell length) physid that is restricted to a small hot spring complex of the Owyhee River (Oregon) where it is often the dominant molluscan species (Frest and Johannes 1995). Unlike nearly all other physid species, which are known for their high-spired, elongate shell morphology, the Owyhee wet-rock physa has a wide-aperture and rotund shell, much like *Physella (Petrophysa) zionis* (Pilsbury 1925) of Zion National Park (Utah) (Fig. 1a–b). In addition to these morphological similarities, both physid species are found in similar landscapes that are characterized by vast canyons and river tributaries coursing through them. Within this landscape, the range of the Owyhee wet-rock physa is highly restricted; it has only been observed in a single stream complex of the river and has therefore been previously recommended for protection (Frest and Johannes 1995). Because of the unique shell morphology that it shares with *P. zionis* and their similar habitats, the Owyhee wet-rock physa is presumably either a closely related species or a geographically disjunct population of *P. zionis*.

As a freshwater mollusk, the Owyhee wet-rock physa may play an important role in the habitat in which it is found, potentially influencing both ecosystem functions and stability. However, because so little is known about this putative species, including its genetic distinctiveness, a formal evaluation is essential. Due to the current state of ambiguity concerning numerous groups of freshwater gastropods, including physids (see Rogers and Wethington 2007; Wethington and Lydeard 2007; Gates et al. 2012; Johnson et al. 2013), and the extremely limited distribution

of the Owyhee wet-rock physa, an assessment of this putative species advances our understanding of biodiversity patterns within the Physidae and evaluates the conservation needs of this highly restricted snail. Here we examined mitochondrial and nuclear gene sequences of the Owyhee wet-rock physa to determine its phylogenetic position within the Physidae and assessed whether or not it represents an evolutionarily significant unit that should be formally described and considered for conservation.

Materials and methods

Specimens

David Hopper of the U.S. Fish and Wildlife Service collected and provided specimens of the Owyhee wet-rock physa from a geothermal spring complex in the Three Forks area of the Owyhee River (Oregon), a tributary of the Snake River, in 2011. We received samples of the suspected sister species, *Physella zionis*, from the Orma J. Smith Museum of Natural History (Caldwell, Idaho). These specimens were originally collected from the Virgin River within Zion National Park (Utah) by Amy Wethington, Elliot Rogers and Christopher Rogers. Benoit Dayrat (Pennsylvania State University) collected and provided specimens of a population of physids from Agua Fria Creek (Mariposa County, California) of the San Joaquin River watershed that were tentatively identified as *Physella gyrina*; additional samples of this population were also obtained from the Invertebrate Zoology collections of the California Academy of Sciences (San Francisco). We sought these latter specimens after preliminary results showed that mitochondrial gene sequences of the Owyhee wet-rock physa were most similar to a sequence of *P. gyrina* that was previously published by Dayrat et al. (2011) [GenBank accession number HQ660033]. Specimens were preserved and maintained in 70–95 % ethanol.

Extraction and DNA amplification

We extracted genomic DNA from multiple individuals of the Owyhee wet-rock physa, *Physella zionis*, and *Physella gyrina* with the E.Z.N.A.[®] Mollusk DNA Kit (Omega Bio-Tek). We targeted three gene regions for sequencing: mitochondrial cytochrome oxidase I (COI), nuclear first internal transcribed spacer (ITS-1), and nuclear second internal transcribed spacer (ITS-2). For the COI gene region, we amplified this fragment from DNA of 35 individuals: 13 Owyhee wet-rock physa, 11 *Physella zionis*, and 11 *Physella gyrina*. We also amplified both nuclear gene regions from DNA of 35 individuals: 12 Owyhee wet-rock physa, 12 *P. zionis*, and 11 *P. gyrina*. We performed amplifications using GoTaq polymerase MasterMix (Promega) with the following primer pairs: GGTCACAAATCATAAAGATATTGG (LCO1490) and TAAACTTCAGGGTGACCAAAAAA TCA (HCO2198) for COI (Folmer et al. 1994), TAA CAAGGTTTCCGTAGGTG and AGCTRGCTGCGT TCTTCATCGA for ITS-1 (White et al. 1996), and GGGTCGATGAAGAACGCAG and GCTCTTCCCGCT TCACTCG for ITS-2 (Xu et al. 2001). These primers amplify approximately 650 base pairs (bp) of the COI gene, 550 bp of the ITS-1 gene, and 500 bp of the ITS-2 gene, excluding primer regions. Annealing temperatures for each primer pair were as follows: 45 °C for COI, 55 °C for ITS-1 and 50 °C for ITS-2.

Sequencing and phylogenetic analyses

We sequenced amplicons in both directions at the University of Michigan DNA Sequencing Core using the original amplification primers for each gene region and prepared templates for sequencing by diluting amplification products 1:5 in double-distilled H₂O. We then evaluated chromatograms and edited sequences using Sequencher version 5.0 (Gene Codes Corporation), and manually aligned sequences for each gene region with Se-Al version 2.0a11 (Rambaut 1996).

We obtained sequences of other physid species and potential outgroups from GenBank based on best matches of BLAST searches of COI, ITS-1, and ITS-2 sequences of the Owyhee wet-rock physa. We specifically included previously published sequences of various physids (Remigio et al. 2001; Albrecht et al. 2004; Wethington and Guralnick 2004; Wethington and Lydeard 2007; Albrecht et al. 2008; Pip and Franck 2008; Albrecht et al. 2009; Dayrat et al. 2011; Gates et al. 2012) and two *Biomphalaria* species (DeJong et al. 2001; Jorgensen et al. 2007) as outgroups. Preliminary DNA datasets were pruned to remove redundant sequences.

We used the model test function of MEGA version 5.05 (Tamura et al. 2011) to determine the best model of

nucleotide substitution for each dataset. Phylogenetic trees were constructed using MrBayes[®] version 3.2.0 with appropriate models. We examined each dataset separately as well as a combined dataset that included both ITS regions (sequences were partitioned and separate models were used for each data partition). For each gene region, runs were executed for 10,000,000 generations; current trees and parameter values were recorded every 100 generations; the first 25 % of the trees were discarded; convergence diagnostics were calculated every 1,000 generations. Phylogenetic trees were prepared with FigTree version 1.3.1 (Rambaut 2009) and rooted to non-physid outgroup sequences of *B. kuhniana* or *B. Pfeifferi* (Table 1).

Results

Phylogenetic trees

The model test function in MEGA determined that the Tamura 3-parameter with gamma distribution (T92 + G) model was the best model for the COI dataset. The resultant COI tree contains many well-resolved clades with very strong posterior probability support (Fig. 2a). All sequences recovered from specimens of the Owyhee wet-rock physa occur in a single clade. This clade exhibits a strongly supported sister group relationship with a clade containing all sequences recovered from individuals from Agua Fria Creek (Mariposa County, California) that were identified as *Physella gyrina* by the original collector. Because sequences of these latter specimens are clearly distinct from sequences of numerous individuals from varied geographic locations that were also identified as *P. gyrina*, we suspect that the population from Agua Fria Creek represents a previously unrecognized physid species; we subsequently refer to this population as *P. cf. gyrina*. Sequences of *P. zionis* occur in a clade that is quite distant from the Owyhee wet-rock physa clade and shows closer affinity to a clade containing sequences of *P. naticina*. The average genetic distance (T92 + G) among COI sequences of the Owyhee wet-rock physa and *P. cf. gyrina* is 0.049. In contrast, COI sequences of the Owyhee wet-rock physa and *P. zionis* show an average genetic distance among groups of 0.205.

The Tamura 3-parameter (T92) model was the best model for the ITS-1 dataset and the Jukes-Cantor (JC) model was best for the ITS-2 dataset. Trees reconstructed from analyses of the individual datasets (not shown) exhibited identical topologies as those obtained from the combined nuclear dataset (Fig. 2b). As revealed in the COI tree, ITS-1 and ITS-2 sequences of the Owyhee wet-rock physa are more similar to sequences from *P. cf. gyrina* than to sequences of

Table 1 Morphological ID (*Genus species*), GenBank accession numbers, and sampling locations of specimens used in molecular analyses

Morphological ID	GenBank accession numbers			Location
	<u>COI</u>	<u>ITS-1</u>	<u>ITS-2</u>	
<i>Biomphalaria kuhniana</i>	–	AY030380	AY030380	–
<i>Biomphalaria pfeifferi</i>	DQ084831	–	–	Senegal: Lake De Guirs
<i>Biomphalaria pfeifferi</i>	AF199099	–	–	–
<i>Biomphalaria pfeifferi</i>	AF199101	–	–	–
<i>Biomphalaria pfeifferi</i>	AF199102	–	–	–
<i>Biomphalaria pfeifferi</i>	AF199104	–	–	–
<i>Physa jennessi</i>	GU680897	–	–	–
<i>Physa jennessi</i>	GU680896	–	–	–
<i>Physa jennessi</i>	GU680892	–	–	–
<i>Physa skinneri</i>	EF488673	–	–	–
<i>Physa skinneri</i>	EF488672	–	–	–
<i>Physella acuta</i>	AY651188	–	–	USA: Hunter Hollow, Reynolds County, Missouri
<i>Physella acuta</i>	EU038368	–	–	France: The Rieutort Wadi in Saint Martin de Londres, 25 km north of Montpellier
<i>Physella acuta</i>	EU038356	–	–	Canada: Niagara River
<i>Physella acuta</i>	EU038367	–	–	Cuba: Santiago de Cuba
<i>Physella acuta</i>	EU038366	–	–	–
<i>Physella acuta</i>	EU038357	–	–	–
<i>Physella acuta</i>	AY651174	–	–	USA: creek south of Vega Reservoir, Mesa County, Colorado
<i>Physella acuta</i>	EU038360	–	–	Canada: Billings Bridge, near Ottawa, Ontario
<i>Physella acuta</i>	EU038361	–	–	–
<i>Physella acuta</i>	AY651181	–	–	USA: Farmers Ditch, trib of Boulder Creek (Mt. Princeton Hot Springs), Chaffe County, Colorado
<i>Physella acuta</i>	AY282589	–	–	–
<i>Physella acuta</i>	AY651203	–	–	USA: Big Horn River near Lower Kane Cave, Wyoming
<i>Physella acuta</i>	GU247996	–	–	USA: Bosque del Apache, New Mexico
<i>Physella acuta</i>	GU247993	–	–	–
<i>Physella acuta</i>	GU247995	–	–	–
<i>Physella acuta</i>	FJ373016	–	–	–
<i>Physella acuta</i>	–	HQ283259	HQ283272	–
<i>Physella anatina</i>	AY651177	–	–	USA: Yellow creek, Rio Blanco County, Colorado
<i>Physella anatina</i>	AY651176	–	–	USA: Elk creek, Garfield County, Colorado
<i>Physella anatina</i>	AY651175	–	–	USA: Landsman Creek, Yuma County, Colorado
<i>Physella ancillaria</i>	EU038388	–	–	USA: Lake Michigan at Petosky, Emmet County, Michigan
<i>Physella ancillaria</i>	EU038387	–	–	USA: Delaware River near Easton, Pennsylvania
<i>Physella ancillaria</i>	EU038392	–	–	–
<i>Physella ancillaria</i>	EU038358	–	–	Canada: Ottawa River, Ottawa, Ontario
<i>Physella ancillaria</i>	EU038359	–	–	–
<i>Physella ancillaria</i>	EU038380	–	–	–
<i>Physella ancillaria</i>	EU038385	–	–	USA: Higgins Lake, Roscommon County, Michigan
<i>Physella ancillaria</i>	EU038383	–	–	USA: Lake Michigan at Frankfort, Benzie County, Michigan

Table 1 continued

Morphological ID	GenBank accession numbers			Location
	<u>COI</u>	<u>ITS-1</u>	<u>ITS-2</u>	
<i>Physella ancillaria</i>	EU038382	–	–	USA: North Fish Tail Bay, Douglas Lake, Cheboygan County, Michigan
<i>Physella ancillaria</i>	EU038381	–	–	
<i>Physella aurea</i>	AY651202	–	–	USA: Hot Springs, Bath, Virginia
<i>Physella aurea</i>	AY651201	–	–	
<i>Physella cupreonitens</i>	AY651183	–	–	USA: Hot Springs in Wellesville, Fremont County, Colorado
<i>Physella fontinalis</i>	AY651189	–	–	Netherlands: Nooredemeek
<i>Physella fontinalis</i>	AY651190	–	–	
<i>Physella fontinalis</i>	FJ373018	–	–	–
<i>Physella fontinalis</i>	EU818796	–	–	Germany: Brandenburg, Obersdorf, Lake Vordersee
<i>Physella gyrina</i>	EU038398	–	–	USA: Fish Lake, Sevier County, Utah
<i>Physella gyrina</i>	HQ660033	–	–	–
<i>Physella gyrina</i>	EU038373	–	–	USA: Silver Creek, 1.5 mi down Arbuckle Rd., Madison County, Kentucky
<i>Physella gyrina</i>	EU038374	–	–	
<i>Physella gyrina</i>	EF488671	–	–	–
<i>Physella gyrina</i>	EF488670	–	–	–
<i>Physella gyrina</i>	AY651178	–	–	USA: Garfield Creek, Garfield County, Colorado
<i>Physella gyrina</i>	JF806435	–	–	–
<i>Physella cf. gyrina</i>	KF305406	KF305358	KF305324	USA: Agua Fria Creek, Mariposa County, California
<i>Physella cf. gyrina</i>	KF305407	–	–	
<i>Physella cf. gyrina</i>	KF305408	KF305359	KF305325	
<i>Physella cf. gyrina</i>	KF305409	KF305360	KF305326	
<i>Physella cf. gyrina</i>	KF305410	KF305361	KF305327	
<i>Physella cf. gyrina</i>	–	KF305362	KF305328	
<i>Physella cf. gyrina</i>	KF305411	KF305363	KF305329	
<i>Physella cf. gyrina</i>	KF305412	KF305364	KF305330	
<i>Physella cf. gyrina</i>	KF305413	KF305365	KF305331	
<i>Physella cf. gyrina</i>	KF305414	KF305366	KF305332	
<i>Physella cf. gyrina</i>	KF305415	KF305367	KF305333	
<i>Physella cf. gyrina</i>	KF305416	KF305368	KF305334	
<i>Physella hendersoni</i>	AY651196	–	–	USA: Salkehatchi River off 17A, Yemassee, South Carolina
<i>Physella hendersoni</i>	AY651194	–	–	
<i>Physella heterostropha</i>	AY651192	–	–	USA: Schuylkill River at Fairmont Park, Philadelphia, Pennsylvania
<i>Physella heterostropha</i>	AY651193	–	–	
<i>Physella integra</i>	EF488674	–	–	–
<i>Physella johnsoni</i>	AF346739	–	–	–
<i>Physella johnsoni</i>	AY651172	–	–	Canada: Middle Spring, Banff, Alberta
<i>Physella johnsoni</i>	AY651173	–	–	
<i>Physella johnsoni</i>	AF346737	–	–	–
<i>Physella johnsoni</i>	AF346736	–	–	
<i>Physella natricina</i>	GU830944	–	–	–
<i>Physella natricina</i>	GU830951	–	–	
<i>Physella natricina</i>	GU830950	–	–	
<i>Physella natricina</i>	GU830949	–	–	
<i>Physella natricina</i>	GU830942	–	–	
<i>Physella natricina</i>	GU830947	–	–	
<i>Physella natricina</i>	GU830946	–	–	

Table 1 continued

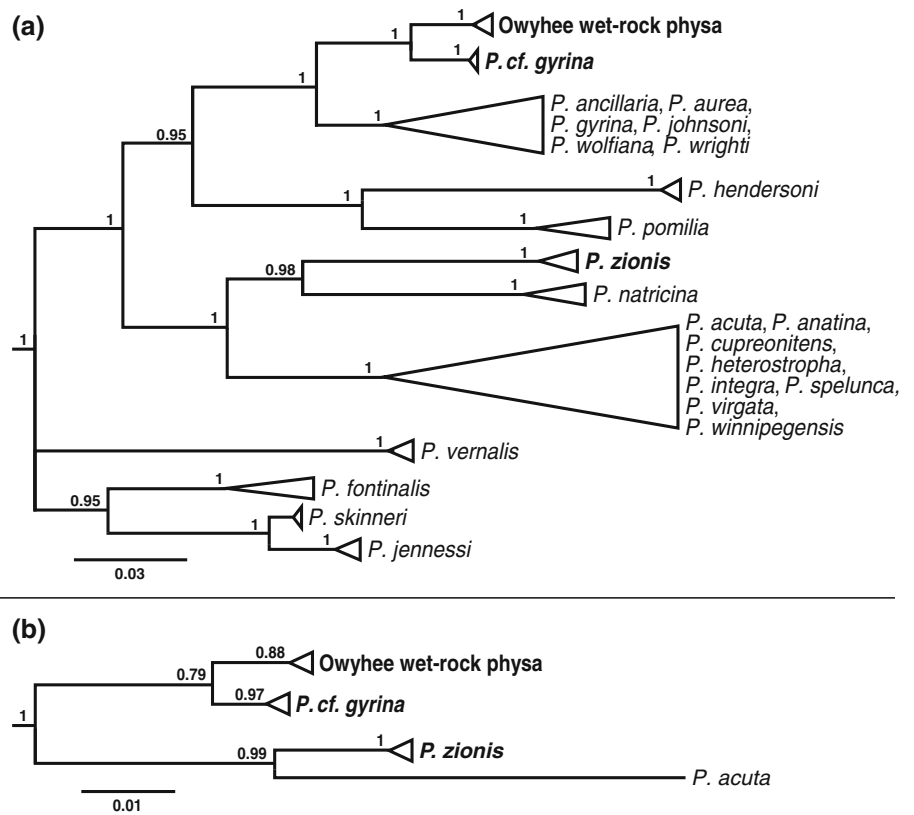
Morphological ID	GenBank accession numbers			Location
	COI	ITS-1	ITS-2	
<i>Physella pomilia</i>	EU038353	–	–	USA: Randons Creek, near Claiborne, Monroe County, Alabama
<i>Physella pomilia</i>	EU038363	–	–	USA: Beaver Brook State Park, Windham County, Connecticut
<i>Physella spelunca</i>	AY651205	–	–	USA: Lower Kane Cave, Wyoming
<i>Physella vernalis</i>	EU038375	–	–	USA: Bristol County, Massachusetts
<i>Physella vernalis</i>	EU038376	–	–	
<i>Physella virgata</i>	AY651171	–	–	USA: Gila River, Arizona
<i>Physella virgata</i>	AY651170	–	–	
<i>Physella winnipegensis</i>	EF488681	–	–	–
<i>Physella wolfiana</i>	AY651179	–	–	USA: Colorado River near Hot Sulphur Springs, Colorado
<i>Physella wrighti</i>	AF419323	–	–	–
<i>Physella wrighti</i>	AF346745	–	–	
<i>Physella zionis</i>	AY651198	–	–	USA: The Narrows, Zion National Park, Utah
<i>Physella zionis</i>	KF305417	KF305381	KF305346	USA: The Narrows, Zion National Park, Washington County, Utah
<i>Physella zionis</i>	KF305418	KF305382	KF305347	
<i>Physella zionis</i>	KF305419	KF305383	KF305348	
<i>Physella zionis</i>	KF305420	KF305384	KF305349	
<i>Physella zionis</i>	KF305421	KF305385	KF305350	
<i>Physella zionis</i>	KF305422	KF305386	KF305351	
<i>Physella zionis</i>	–	KF305387	KF305352	
<i>Physella zionis</i>	KF305423	KF305388	KF305353	
<i>Physella zionis</i>	KF305424	KF305389	KF305354	
<i>Physella zionis</i>	KF305425	KF305390	KF305355	
<i>Physella zionis</i>	KF305426	KF305391	KF305356	
<i>Physella zionis</i>	KF305427	KF305392	KF305357	
Owyhee wet-rock physa	KF305393	KF305369	KF305335	USA: Warm springs near Three Forks, Owyhee River, Malheur County, Oregon
Owyhee wet-rock physa	KF305394	KF305370	KF305336	
Owyhee wet-rock physa	KF305395	KF305371	KF305337	
Owyhee wet-rock physa	KF305396	KF305372	KF305338	
Owyhee wet-rock physa	KF305397	KF305373	KF305339	
Owyhee wet-rock physa	KF305398	KF305374	KF305340	
Owyhee wet-rock physa	KF305399	KF305375	KF305341	
Owyhee wet-rock physa	KF305400	KF305376	–	
Owyhee wet-rock physa	KF305401	–	–	
Owyhee wet-rock physa	KF305402	KF305377	KF305342	
Owyhee wet-rock physa	KF305403	KF305378	KF305343	
Owyhee wet-rock physa	KF305404	KF305379	KF305344	
Owyhee wet-rock physa	KF305405	KF305380	KF305345	

Bold lettering indicates newly generated sequences

P. zionis. The average genetic distances between the Owyhee wet-rock physa and *P. cf. gyrina* at ITS-1 (T92 distance) and ITS-2 (JC distance) are 0.018 and 0.008, respectively. In

contrast, the Owyhee wet-rock physa and *P. zionis* show average genetic distances (same models as specified above) of 0.102 and 0.021 at ITS-1 and ITS-2, respectively.

Fig. 2 **a** Consensus trees recovered from Bayesian analyses of the mitochondrial COI gene region and **b** the combined nuclear ITS-1 and ITS-2 gene regions. **a, b** Clades were collapsed for legibility and numbers on branches indicate Bayesian posterior probabilities. Species of interest are denoted by **bold lettering**. Branch length scale is on the *lower left*



Discussion

The Owyhee wet-rock physa is genetically distinct from all other physid species (at least those with described COI sequences) (Fig. 2). This lineage also shows greater affinity at mitochondrial and nuclear gene sequences to a population of physids from Agua Fria Creek in Mariposa County, California (*Physella cf. gyrina*), a species with a more typical physid shell morphology (i.e. relatively high-spired shell) than to *Physella zionis*, another small-sized wet-rock physid (Fig. 1). The shared shell characteristics and general similarities in habitats of the Owyhee wet-rock physa and *P. zionis* imply that similar selective pressures may have caused the convergent evolution of shell morphologies of these lineages. Our results also suggest a potential vicariant split or past dispersal event between river drainage systems in southeastern Oregon and central California. Because physids tend to be easily dispersed, the latter scenario may be the more likely explanation. Finally, the highly restricted distribution of the Owyhee wet-rock physa and genetic distinctiveness of this form necessitates a formal description and consideration of this lineage as critically endangered, according to the conventions of the International Union for Conservation of Nature (IUCN).

Genetic distinctiveness of the Owyhee wet-rock physa

Examination of COI sequences from numerous members of the family Physidae revealed that sequences of the Owyhee wet-rock physa occur uniquely together in a strongly supported monophyletic clade (Fig. 2). Although sequences of all extant physid species are not currently available, this result supports recognition of the Owyhee River population as an evolutionarily distinct unit. Indeed, except for *Physella zionis*, no other North American physid populations are known to exhibit similar shell morphologies as the Owyhee wet-rock physa (Burch and Tottenham 1980).

While there are no standard genetic benchmarks to determine what constitutes a valid species, genetic distances between the Owyhee wet-rock physa and its sister taxon, *Physella cf. gyrina*, at each of the gene regions support the genetic distinctiveness of these two lineages. Mean levels of genetic divergence between the Owyhee wet-rock physa and its sister taxon were 0.049 (COI), 0.018 (ITS-1) and 0.008 (ITS-2). In their analysis of relationships and evolutionary history of three physid species (*P. gyrina*, *P. johnsoni*, and *P. wrighti*), Remigio et al. (2001) found that genetic distances among these nominal species were as low as 0.005 at COI. Similarly, in the most recent taxonomic evaluation of *P. natricina* using *P. acuta* and

Fig. 3 Map of the Owyhee River, Oregon and other drainage basins with which it once may have shared a geographical connection



P. anatina as reference taxa, Gates et al. (2012) found that the genetic divergence between two reference taxa was 0.041 based on combined COI and 16S sequences.

Geological history

Much of the geographic history of North America can be inferred from the historical and present distribution of organisms (Smith et al. 2000). This is especially true for regions of the western United States where the Owyhee wet-rock physa and *Physella cf. gyrina* are found (see Miller 1965; Miller and Smith 1967; Taylor 1985; Hershler et al. 2002, etc.).

The Owyhee River is a Snake River tributary, the latter of which presently runs from western Wyoming and south-central Idaho, along the northeastern border of Oregon and into southeastern Washington where it is transformed into a series of reservoirs (Fig. 3). Recent and fossil distributions of the freshwater clam *Pisidium ultramontanum*—found in the northern Great Basin, the Snake River drainage, and the Klamath River drainage—suggest that there was a series of lakes and rivers connecting the Snake River to watersheds in northern California (Taylor 1960). The distribution of *P. ultramontanum* links the Snake River to Klamath Lake and the Malheur basin in Oregon, along with Eagle Lake and the upper Pit River in California (Taylor 1960).

Additionally, similarities between Pliocene fish fauna of Modoc County, California (*Oncorhynchus*, *Klamathella*, *Ptychocheilus*, *Lavinia*, *Chasmistes*, etc.) and the Snake River Plain is further evidence suggesting historical hydrographic connections among the Pit River, the Snake River, and the Klamath Basin (Wheeler and Cook 1954; Miller and Smith 1981; Wagner et al. 1997). Taylor (1960) contends that these distribution patterns are likely as old as the Pliocene (5.3 to 2.6 MYA) and at least as old as the early Pleistocene (2.6 MYA to 11,700 years ago).

The sister group relationship of the Owyhee wet-rock physa and *P. cf. gyrina* suggests that these lineages have a relatively recent history of divergence. If we assume a substitution rate of 1.5 % per million years for COI (see Wilke et al. 2009), the divergence of the Owyhee wet-rock physa and *P. cf. gyrina* occurred around 1.7 million years ago. This timing of separation is consistent with a Pleistocene connection between the Owyhee River and drainages to the south (Taylor 1960).

The Klamath River drainage is thought to be a particularly important intermediate connection between the Owyhee River and central California (G.R. Smith, University of Michigan, personal communication). Nonetheless, at present there is little evidence to support this hypothesis other than the historical distribution of *P. ultramontanum* (Taylor 1960) and the similarities between the freshwater snail *Pyrgulopsis intermedia* of the Owyhee River and snail populations from the Pit River and Klamath Lake (Hershler et al. 2002). Future studies within the Klamath River and surrounding basins should therefore incorporate more gastropod surveys and molecular analyses to evaluate the biogeographic history of snails in this region. This work will further our understanding of the historical biogeography of the region as well as patterns of diversity and physid species distributions.

Convergent evolution of shell shape of the Owyhee wet-rock physa and *P. zionis*

Based on their similar shell morphologies, we hypothesized that the Owyhee wet-rock physa is more closely related to or possibly a disjunct population of *Physella zionis*. Shells of these lineages range between 3 and 4.5 mm in length and exhibit wide apertures that contain a large, broad-sized foot (Fig. 1a–b). As described by Pilsbury (1925) when referencing *P. zionis*, both species have hemispherical shells and no projecting spire. Conversely, *P. cf. gyrina*, the apparent sister species of the Owyhee wet-rock physa, exhibits typical physid shell characteristics with a relatively long aperture and high spire (Fig. 1c). The similar shell morphological traits of the Owyhee wet-rock physa and *P. zionis* may be influenced, at least in part, by similar selective pressures in their similar environments. Indeed, a

number of physids are known or suspected to exhibit variation in shell morphology in response to environment conditions (DeWitt 1995; Britton and McMahon 2004; DeWitt and Scheiner 2004; Wethington and Guralnick 2004).

The Owyhee wet-rock physa and *P. zionis* are both ‘wet-rock’ physids that occur in small tributaries of western river canyons (Pilsbury 1925; Frest and Johannes 1995) and occupy rocky substrates in swift moving, but low volume spring seeps and creeks. Although detailed environmental data for each area are not currently available, the Owyhee River and the Virgin River (where *P. zionis* dwells) flow through arid plateau regions characterized by exposed volcanic lithologies (in Oregon) and sandstone (in Utah), as well as open, dry sage scrub (Frest and Johannes 1995). Both river systems discharge relatively low quantities of water annually (USGS 2010, 2013), but are primarily unregulated and subject to seasonal flooding. While these desert rivers may experience major flow changes seasonally, the creeks and seeps in which these two species occur are spring-fed and subject to higher frequencies of low volume discharge (D. Hopper, U.S. Fish and Wildlife Service, personal communication).

While many ecological and evolutionary factors influence morphology, these broad-scale environmental similarities suggest that the Owyhee wet-rock physa and *P. zionis* may share other habitat characteristics that led to their similar morphologies. To adequately assess the impact that convergent evolution has had on these species, additional studies should analyze historical and contemporary ecological and environmental variables in comparison with these with morphological characteristics.

Conservation implications

The currently known range of the Owyhee wet-rock physa includes a small stretch of the Owyhee River that is considerably less than 0.05 km². The IUCN recommends that species with range sizes this small be considered ‘critically endangered’ as anthropogenic or stochastic influences could rapidly drive species with such extremely limited ranges to extinction (IUCN 2012). In addition, the Owyhee wet-rock physa, like few other physid species, appears to be restricted to warm springs and immediately adjacent aquatic areas. The quite limited distribution and specific habitat requirements of the Owyhee wet-rock physa place this lineage in an extremely precarious position. Although the IUCN may list undescribed species under exceptional circumstances (IUCN 2010), we of course recognize the need and our responsibility to describe the Owyhee wet-rock physa as a new species and we are currently in the process of preparing a formal description of this genetically distinct physid lineage.

Finally, our understanding of the taxonomy, phylogenetic relationships, and the evolutionary history of the family Physidae is significantly lacking (Wethington and Lydeard 2007). Given the globally abundant nature of this freshwater family and the array of taxonomic ambiguities found within it, a full taxonomic resolution is essential. Indeed, the limited scope of the present work revealed not just one by two genetically distinct physid lineages: the Owyhee wet-rock physa and a cryptic lineage of physids from Agua Fria Creek in central California that resembles the widespread physid, *P. gyrina*. Future work will likely uncover additional previously unrecognized physid lineages and contribute to our understanding of the mechanisms that led to the diversity of physids in North America, and the evolutionary history and patterns of diversification of freshwater taxa.

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