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RESEARCH ARTICLE

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

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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.

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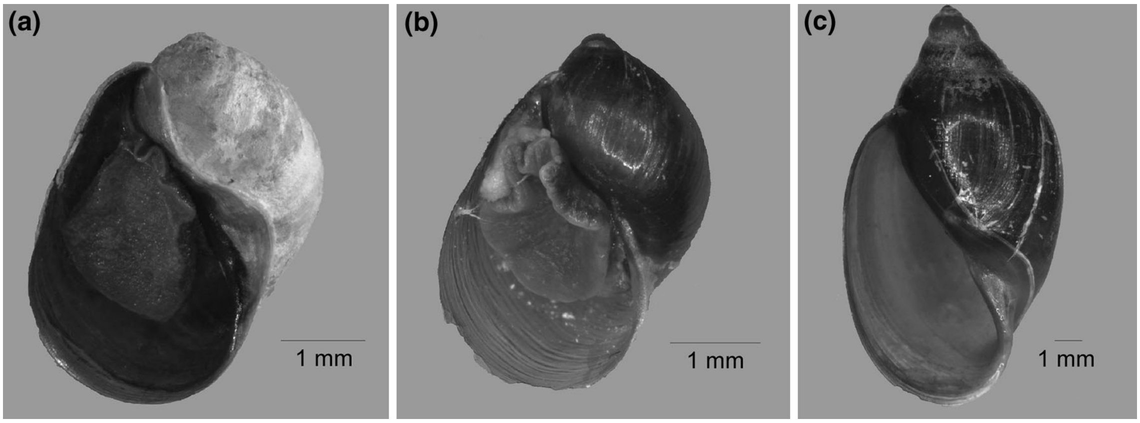

Fig. 1 Shell images of **a** the Owyhee wet-rock physa, **b** *Physella zionis* and **c** *Physella cf. gyrina. Scale bars* denote 1 mm

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 Go*Taq* polymerase MasterMix (Promega) with the following primer pairs: GGTCAACAAATCATAAAGATATTGG (LCO1490) and TAAACTTCAGGGTGACCAAAAAATCA (HCO2198) for COI (Folmer et al. 1994), TAACAAGGTTTCCGTAGGTG and AGCTRGCTGCGTTCTTCATCGA for ITS-1 (White et al. 1996), and GGGTCGATGAAGAACGCAG and GCTCTTCCCGCTTCACTCG 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 H_2O. 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. natricina*. 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
	COI	ITS-1	ITS-2	
Biomphalaria kuhniana	–	AY030380	AY030380	–
Biomphalaria pfeifferi	DQ084831	–	–	Senegal: Lake De Guirs
Biomphalaria pfeifferi	AF199099	–	–	–
Biomphalaria pfeifferi	AF199101	–	–	
Biomphalaria pfeifferi	AF199102	–	–	
Biomphalaria pfeifferi	AF199104	–	–	
Physa jennessi	GU680897	–	–	–
Physa jennessi	GU680896	–	–	
Physa jennessi	GU680892	–	–	
Physa skinneri	EF488673	–	–	–
Physa skinneri	EF488672	–	–	
Physella acuta	AY651188	–	–	USA: Hunter Hollow, Reynolds County, Missouri
Physella acuta	EU038368	–	–	France: The Rieutort Wadi in Saint Martin de Londres, 25 km north of Montpellier
Physella acuta	EU038356	–	–	Canada: Niagara River
Physella acuta	EU038367	–	–	Cuba: Santiago de Cuba
Physella acuta	EU038366	–	–	
Physella acuta	EU038357	–	–	
Physella acuta	AY651174	–	–	USA: creek south of Vega Reservoir, Mesa County, Colorado
Physella acuta	EU038360	–	–	Canada: Billings Bridge, near Ottawa, Ontario
Physella acuta	EU038361	–	–	
Physella acuta	AY651181	–	–	USA: Farmers Ditch, trib of Boulder Creek (Mt. Princeton Hot Springs), Chaffe County, Colorado
Physella acuta	AY282589	–	–	–
Physella acuta	AY651203	–	–	USA: Big Horn River near Lower Kane Cave, Wyoming
Physella acuta	GU247996	–	–	USA: Bosque del Apache, New Mexico
Physella acuta	GU247993	–	–	
Physella acuta	GU247995	–	–	
Physella acuta	FJ373016	–	–	–
Physella acuta	–	HQ283259	HQ283272	–
Physella anatina	AY651177	–	–	USA: Yellow creek, Rio Blanco County, Colorado
Physella anatina	AY651176	–	–	USA: Elk creek, Garfield County, Colorado
Physella anatina	AY651175	–	–	USA: Landsman Creek, Yuma County, Colorado
Physella ancillaria	EU038388	–	–	USA: Lake Michigan at Petosky, Emmet County, Michigan
Physella ancillaria	EU038387	–	–	USA: Delaware River near Easton, Pennsylvania
Physella ancillaria	EU038392	–	–	
Physella ancillaria	EU038358	–	–	Canada: Ottawa River, Ottawa, Ontario
Physella ancillaria	EU038359	–	–	
Physella ancillaria	EU038380	–	–	
Physella ancillaria	EU038385	–	–	USA: Higgins Lake, Roscommon County, Michigan
Physella ancillaria	EU038383	–	–	USA: Lake Michigan at Frankfort, Benzie County, Michigan

Table 1 continued

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

Table 1 continued

Morphological ID	GenBank accession numbers			Location
	COI	ITS-1	ITS-2	
Physella pomilia	EU038353	–	–	USA: Randons Creek, near Claiborne, Monroe County, Alabama
Physella pomilia	EU038363	–	–	USA: Beaver Brook State Park, Windham County, Connecticut
Physella spelunca	AY651205	–	–	USA: Lower Kane Cave, Wyoming
Physella vernalis	EU038375	–	–	USA: Bristol County, Massachusetts
Physella vernalis	EU038376	–	–	
Physella virgata	AY651171	–	–	USA: Gila River, Arizona
Physella virgata	AY651170	–	–	
Physella winnipegensis	EF488681	–	–	–
Physella wolfiana	AY651179	–	–	USA: Colorado River near Hot Sulphur Springs, Colorado
Physella wrighti	AF419323	–	–	–
Physella wrighti	AF346745	–	–	
Physella zionis	AY651198	–	–	USA: The Narrows, Zion National Park, Utah
Physella zionis	**KF305417**	**KF305381**	**KF305346**	USA: The Narrows, Zion National Park, Washington County, Utah
Physella zionis	**KF305418**	**KF305382**	**KF305347**	
Physella zionis	**KF305419**	**KF305383**	**KF305348**	
Physella zionis	**KF305420**	**KF305384**	**KF305349**	
Physella zionis	**KF305421**	**KF305385**	**KF305350**	
Physella zionis	**KF305422**	**KF305386**	**KF305351**	
Physella zionis	–	**KF305387**	**KF305352**	
Physella zionis	**KF305423**	**KF305388**	**KF305353**	
Physella zionis	**KF305424**	**KF305389**	**KF305354**	
Physella zionis	**KF305425**	**KF305390**	**KF305355**	
Physella zionis	**KF305426**	**KF305391**	**KF305356**	
Physella zionis	**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.

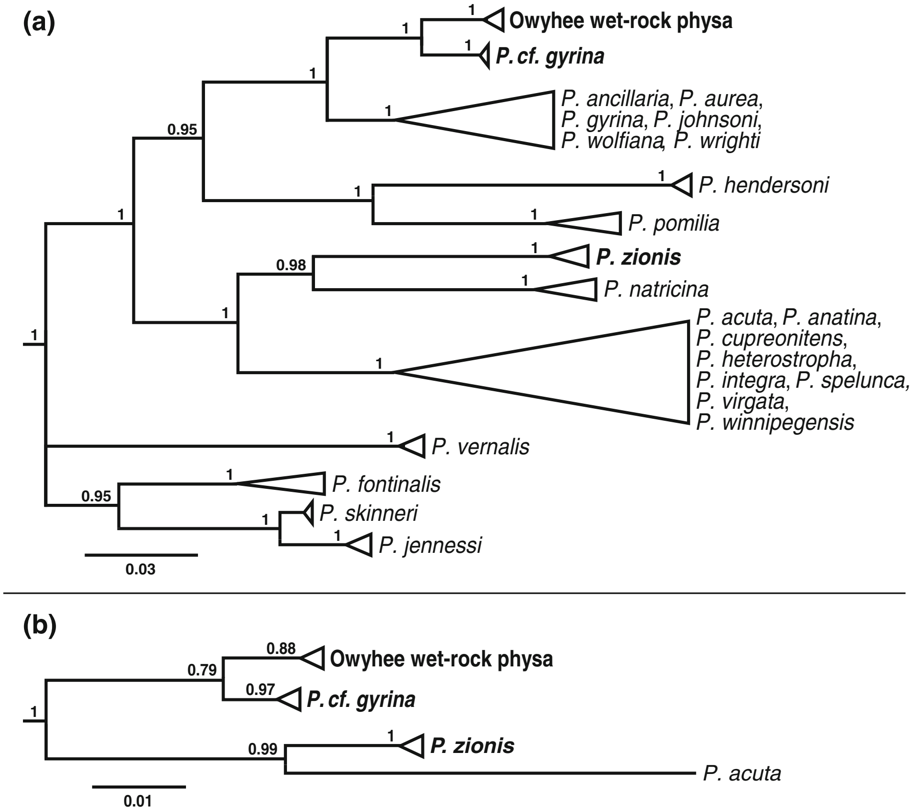

Fig. 2 **a** Consensus trees recovered from Bayesian analyses of the mitochondrial COI gene region and **b** the combined nuclear ITS-1 and ITS-2 gene regions. **a**, **b** Clades were collapsed for legibility and numbers on branches indicate Bayesian posterior probabilities. Species of interest are 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 km^2. 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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