Taxonomic and Morphological Evaluation of an Endemic Freshwater Gastropod

By

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Abstract

Mollusks are the second most diverse animal phylum in terms of number of described species and nonmarine mollusks are among the most imperiled groups of invertebrate taxa. In particular, freshwater and terrestrial mollusks have the highest number of documented extinctions of any major taxonomic group. Such extinctions are not without their consequences as nonmarine mollusks provide several ecosystem functions including regulation of rates of primary production, decomposition, water clarity, and nutrient cycling. Given this and the level of fluidity found within freshwater mollusk taxonomy, the proper identification of potentially endangered species is an issue of great concern.

The present study evaluates the taxonomic status of a small freshwater snail endemic to southeastern Oregon within the Owyhee River, referred to here as the Owyhee physa. Molecular, morphological, and environmental analyses were employed using specimens from both a sister species, "*Physa gyrina*", and another species with similar morphology and habitat conditions as the Owyhee physa, *Physa zionis*. Molecular analyses also incorporated supplemental sequences from other physid species to assess the status of the Owyhee physa within the Physidae family. Genetic analyses at the cytochrome oxidase 1 and ITS-1 and ITS-2 gene regions indicate that the Owyhee physa is a phylogenetically distinct species with "*Physa gyrina*" as its sister species. Using theoretical morphological techniques, the shell morphology of each of the three physid species was qualitatively described. Qualitative analyses of these morphological descriptions along with the phylogenetic tree and environmental data for each

species suggests that convergent evolution played an important role in influencing shell morphology within this taxonomic group. The present work has provided additional information about the current status of Physidae species richness, but additional studies must be done to both broaden and deepen our knowledge of freshwater gastropod diversity.

Chapter I: Introduction

Mollusks and the Physidae Family

Mollusks are the second most diverse animal phylum on the planet with an estimated 200,000 formally described and undescribed species (Lydeard *et al* 2004). Within this group, nonmarine mollusks make up 20% - 40% of the species richness and simultaneously represent one of the most imperiled groups of invertebrates on the planet (Lydeard *et al* 2004). Freshwater and terrestrial mollusks have the largest number of documented extinctions of any major taxonomic group; 42% of the 693 recorded species extinctions since the year 1500 have been mollusks (Lydeard *et al* 2004). These extinctions are not without consequence as nonmarine mollusks, particularly freshwater mollusks, provide several ecosystem functions and services, including regulating rates of primary production, decomposition, water clarity, and nutrient cycling. Freshwater mollusks therefore play an important role in ecosystem stability and often substantially affect human welfare (Strayer 2006).

One of the major factors contributing to the global decline of freshwater mollusks is human-induced stress on freshwater ecosystems; these impacts range from habitat degradation to competitive or predatory species introductions (Regnier *et al* 2009). In particular, dams are a primary cause of habitat loss and fragmentation for nonmarine mollusks (Strayer 2006). River systems that have been affected by dams cover nearly half of the world and cause several problems for freshwater mollusks, such as the alteration of flow,

temperature, and sediment supply, which may result in environmental conditions that are unsuitable for native species (Strayer 2006). Additionally, dams often act as barriers that can reduce the ability of invertebrate species to migrate across landscapes in response to environmental changes. In the face of increasing industrialization and urban sprawl, freshwater species face threats not only to their dispersal abilities, but also in their ability to adapt to biotic changes occurring in habitats where they are found.

The introduction of organisms across major geographic barriers as a result of globalization has irreparably impacted freshwater macroinvertebrates. For instance, the introduction of zebra mussels, *Dreissena polymorpha*, into North America has led to the extirpation of many populations of mussels through competitive exclusion (Lydeard *et al* 2004). As the number of introduced and invasive species continues to rise, freshwater mollusks face an increased risk of extinction due to their dependency on habitats that are becoming increasingly restricted and unsuitable.

As a result of these anthropogenic impacts and other ecological and evolutionary constraints, invertebrate species endemicity is very common. It has been suggested that many freshwater invertebrate species actually have large ranges, but recent taxonomic studies on morphological and molecular characteristics have shown that such wide-spread species actually consist of several distinct species, each with its own smaller range (Strayer 2006). High levels of endemism often occur in very old river systems, such as the Tennessee and Mobile basins in the southern United States, where time and isolation have supported the evolution of distinct molluscan species (Lydeard *et al* 2004). In Alabama alone, over 100 species of aquatic snails have never been collected outside of the state. This level of endemism coincides with very

restricted ranges, dispersal limitations, and habitat specialization, making these species especially vulnerable to extinction (Alabama Department of Conservation and Natural Resources 2008).

In stark contrast to this general trend of restriction and localization, the freshwater family Physidae has a broad Holarctic (i.e. Northern hemisphere) distribution which extends into Central and South America (Wethington and Lydeard 2007). Thought to be mainly New World snails, physids have been introduced worldwide and have a very large and diverse geographic range (Harrold and Guralnick 2010). Physidae is the most abundant and widespread gastropod group in North America (Wethington and Lydeard 2007) and members of this group are recognized by their high-spired, sinistral (left coiled) shells, and lack of an operculum (a structure that covers the aperture when the animal retracts into its shell) (Figure 1-1) (Burch 1982). Physids are hermaphroditic, capable of self-fertilization, and can produce a large number of offspring in a single year. Additionally, because many Physidae species have large reproduction and maturation rates, their abundances and densities tend to be very high wherever they are present (Wethington 2004).

Physid snails are known to display a wide variety of life history traits that are influenced by climate, type of habitat, presence or absence of predators or parasites, and many other ecological factors (Wethington 2004). This large variation in the composition of freshwater habitats occupied by physid snails has contributed to the development of a number of ecophenotypes within this taxonomic group. For example, physid snails reared in the presence of fish predators develops thickened, more rotund shells with wide apertures, while those reared in the absence of predators do not exhibit such shell modifications (West-Eberhard

2003). Physidae diversification has been further promoted by the geographic isolation of water bodies inhabited by these snails, particularly for species that depend on passive dispersal agents for mobility; this has resulted in several very restricted endemic species known only from a single site or a small cluster of related locations (Pip and Franck 2008).

Due to the high level of diversity within this group and the influence of environmental factors on morphology, our understanding of the taxonomy and status of these predominant members of freshwater communities of North America is significantly lacking (Wethington and Lydeard 2007). Even within this group of largely ubiquitous species, knowledge of the extent to which endemicity occurs is unknown. Given the large geographic range and the phenotypic plasticity of members of this family, there is the potential that highly restricted species are overlooked or misidentified. Therefore, the present study addresses the taxonomic status and environmental influence on morphology of a putative species of physid found in southeastern Oregon.

Originally discovered by Terrence J. Frest and Edward Johannes in a single stream complex of the Owyhee River (Malheur County, Oregon) in 1988, this physid snail (hereafter called the Owyhee physa) was rudimentarily described in a report of the Columbia Basin Mollusk Species of Special Concern (Frest and Johannes 1995). Frest and Johannes recognize that the Owyhee physa may be a distinct species, but did not formally describe it in their report. During later surveys, Dr. Robert Hershler of the American Museum of Natural History, David Hopper of the Idaho US Fish and Wildlife Service, and Dr. Don Sada of the University of Nevada – Reno all noted the Owyhee physa and found no other similar molluscan species in the Great Basin and the Owyhee Drainage region (D. Hopper, personal communication). In a family of

cosmopolitan species, the Owyhee physa represents a rare case of endemicity more similar to that of most freshwater mollusks than to other physids.

In their 1995 report, Frest and Johannes recommend that this species be considered sensitive by the Forest Service, the BLM, and other land management and wildlife agencies because of its highly restricted range and ongoing threats to its survival (Frest and Johannes 1995). However, because the Owyhee physa has not been formally recognized as a species and so little is known about the role it plays in its environment, little progress has been made towards protecting it. In light of this, the purpose of the present study is to determine the taxonomic status of the Owyhee physa. Additionally, because the Owyhee physa requires a very specialized habitat and is often the only mollusk found where it occurs, this study will also describe how environmental variables influence shell morphology within this taxonomic group and, therefore, how this affects the taxonomy of physids which largely depends on morphological characters. An assessment of this putative species will advance our understanding of patterns of biodiversity within this taxonomic group and provide a proper evaluation of its conservation needs.

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Figure 1-1. Close up of "*Physa gyrina*" individual at 1000 micrometer scale, exhibiting typical physid snail features. Shell is sinistral or "left-handed", lacks an operculum, with a relatively high spire.
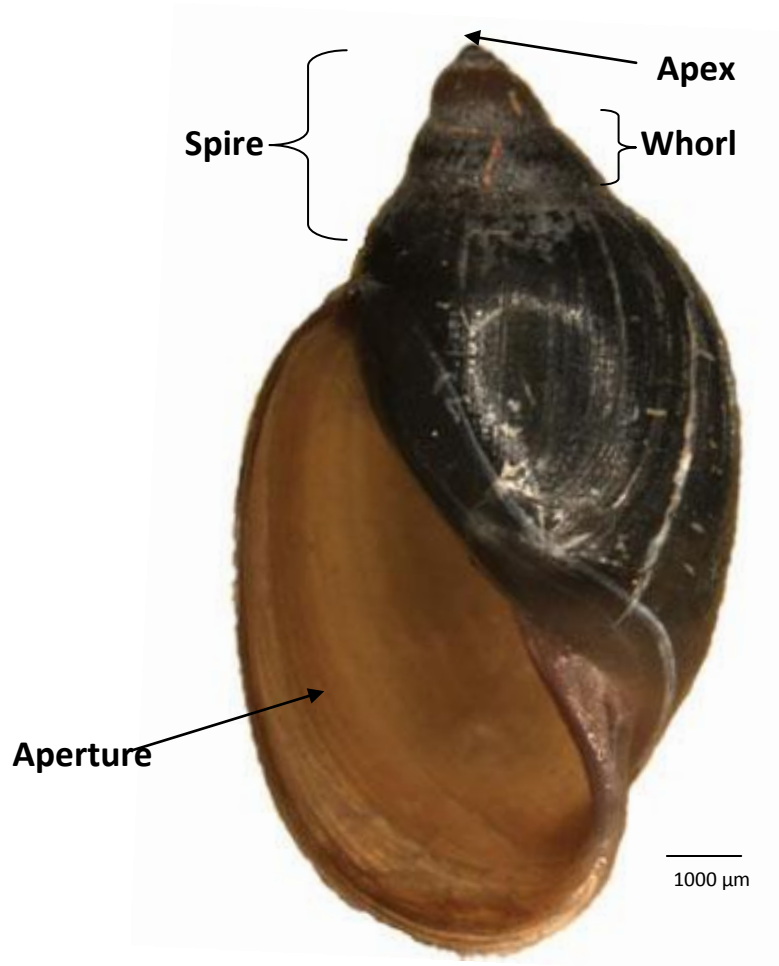
Chapter II: Physidae Classification and Molecular Analyses

Introduction

Throughout the 19th century, taxonomic classification systems divided the Physidae family into two genera, *Aplexa* and *Physa*, using characters of the organism's shell and mantle edge (Te 1978). Later, taxonomists split the family even further into multiple additional genera based on geographic location, morphological traits, and anatomical differences (Baker 1926, 1928; Starobogatov 1967; etc). However, because physid morphology is heavily influenced by environmental variables and anatomical information was too poorly known at the time to be decisive, early efforts to classify the Physidae family produced many inconsistent taxonomic representations.

In an attempt to consolidate these classification schemes, George Ang Te (then a PhD student at the University of Michigan) produced an unpublished doctoral dissertation in which he examined 85 species of physids (78 of which were nominal species, the remainder represented populations Te thought were unique) using 71 morphological characters, 34 anatomical traits and 37 shell traits (Te 1978). Through this work Te described a monophyletic classification of the Physidae where he suggested two subfamilies, Aplexinae and Physinae, the former with genera *Aplexa* and *Stenophysa*, and the latter with genera *Physa* and *Physella* based on analyses of the penial complex. However, despite the utility of Te's dissertation, including its use as an influential guide to the North American freshwater snails (Burch and

Tottenham 1980), the taxonomy of physids has continued to be modified over the last several years. For example, two separate studies provide contrasting views on the status of the thermal spring snails, *Physella johnsoni* and *Physella wrighti* (Remigio *et al* 2001; Wethington and Guralnick 2004). Based on molecular analyses of sequences of the mitochondrial cytochrome oxidase I (mtCOI) and 16S rRNA gene regions, Remigio and colleagues (2001) found *Physella wrighti* to be the sister taxon of *Physella johnsoni*, while Wethington and Guralknick (2004) conclude that they are indistinguishable. In an effort to extend the understanding of the evolutionary relationships of the Physidae, more recent classification schemes have been produced for the Physidae (e.g. Taylor 2003, Wethington and Lydeard 2007).

Taylor's (2003) reclassification of the Physidae, considered the best recent treatment of physids (J. Burch, personal communication), is based almost entirely on the terminal male reproductive system (i.e. the penial complex) where, unlike other morphological and anatomical traits, progressive characters are found consistently. Using five characters of the penial complex – form and composition of the penial sheath, proportions and structure of the penis, presence or absence of a penial stylet, location of the penial canal pore, and number and insertions of penial retractor muscles – Taylor (2003) concluded that the Physidae consists of 23 genera with about 80 species. However, the taxonomic representation of the Physidae of Wethington and Lydeard (2007), based on molecular analyses of two gene regions, penial morphology, and reproductive isolation, identified only six groups of physids supported by both penial morphology and molecular phylogenetic analyses. This level of taxonomic fluidity continues to plague a field where correctly identifying species, their ranges, abundance, and conservation status are essential.

The proper identification of potentially endangered species is especially important for freshwater mollusks. The decline in population sizes of North American freshwater gastropods has been well-documented for formally described species, but impacts on unrecognized species are particularly important to establish and currently lacking. This concern is recognized specifically for a putative species of freshwater snail found in southeastern Oregon, the Owyhee physa. This physid is an extremely small, warm-spring, wet-rock snail, bearing resemblance to another physid snail, *Physa zionis*, and is found in very shallow waters where it is often the dominant molluscan species (Frest 1995; see Figure 2-1). Its range has thus far been described as highly restricted; it has been observed in only a single stream complex along the Owyhee River and has been recommended for protection (Frest 1995). As a freshwater mollusk, the Owyhee physa may play an important role in the ecosystem in which it is found, potentially influencing both ecosystem functions and stability. Because so little is known about this putative species, including its taxonomic status and the specific role that it plays within its environment, a formal evaluation of this snail is essential. Based on morphological similarities, we hypothesized that the Owyhee physa is a distinct species that is closely related to *Physa zionis*, which it resembles. Due to the current atmosphere of taxonomic disarray within freshwater mollusks, an assessment of this putative species will advance our understanding of patterns of biodiversity within this taxonomic group and provide a proper evaluation of their conservation needs.

Materials and Methods

Specimen Sampling and Acquisition

David Hopper of the Idaho US Fish and Wildlife Service collected Owyhee physa specimens from natural populations in the Owyhee River, Oregon at the Three Forks warm springs in 2011. Specimens used for molecular and morphological comparisons with the Owyhee physa were acquired from the original authors with published molecular data in GenBank. The *P. zionis* samples were collected from the Zion National Park in Springdale, Utah by Amy Wethington and D. Christopher Rogers (2003).

Extraction and Amplification of DNA

All samples were preserved in 95% ethanol (EtOH). I extracted genomic DNA from 11-13 individuals of each species using the E.Z.N.A.® Mollusc DNA Kit (Omega Bio-Tek). I then prepared DNA templates for amplification by removing a small sample of tissue from the foot of each specimen (approximately 10-25 mg) and incubating it at 60°C in ML1 buffer and proteinase K (from kit) for at least 1 hour. Following established protocol for mollusks, samples were homogenized and DNA was extracted with chloroform to remove polysaccharides, and then further purified to eliminate proteins and other contaminants. Where possible, the shells of specimens were minimally altered during tissue extraction; however, for smaller and more fragile individuals, original shell morphology was not maintained. For each individual, three markers were selected for sequencing: mitochondrial cytochrome oxidase I (COI), nuclear first internal transcribed spacer (ITS-1), and nuclear second internal transcribed spacer (ITS-2). Target fragments were amplified using standard polymerase chain reaction (PCR) procedure for each marker using Go*Taq* polymerase MasterMix (Promega) with the following primer pairs in

10 micromolar concentration: LCO1490 and HCO2198 for COI (Folmer *et al* 1994), ITS-1F and ITS-1R for ITS-1 (Schizas *et al* 1999), and 025 ITS-2F and 026 ITS-2R 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, all excluding primers. Annealing temperatures for each primer pair were as follows: 45°C for COI, 54°C for 16S, 55°C for ITS-1 and 50°C for ITS-2. Each run included a negative control (lacking DNA template) to check for contamination. For each PCR product, 4-microliter subsamples were run on a 1% agarose gel using 0.5X concentration of TBE buffer solution to determine the presence of target fragments.

Sequencing and Phylogenetic Analyses

The remaining aliquots of the amplified fragments were sequenced through the University of Michigan DNA Sequencing Core using the original amplification primers for each marker. For each gene region sent for sequencing, four microliters of the PCR product were diluted in 16 microliters of water. Amplification primers were used for sequencing and supplied to the University of Michigan sequencing facility at one-micromolar concentrations. I edited the chromatograms manually using Sequencher version 5.0 and converted the resulting sequences into an editable text file using MEGA version 5.05. For each gene region, supplementary sequences of other physid and outgroup species were downloaded from GenBank and incorporated into the aligned dataset. Supplementary sequences were chosen based on genetic similarity to the Owyhee physa sequences. Preliminary DNA datasets were pruned to remove redundant sequences. These additional sequences were incorporated into the analyses to establish the taxonomic relationship of the physa species of interest and other physid snails and were not used to infer any conclusions about the systematics of physids in general.

I used the model test function in MEGA version 5.05 to determine the best model of nucleotide substitution for each dataset. Phylogenetic trees were constructed using MrBayes® v. 3.2.0 Bayesian Inference with the following models: Tamura 3-parameter with Gamma distribution (T92+G) for COI; Tamura 3-parameter (T92) for ITS-1 and Jukes Cantor (JC) for ITS-2. I examined sequences from each gene region separately, but also examined combined sequence data of both internal transcribed spacer genes. For combined Bayesian analyses, sequences were partitioned and separate models of nucleotide substitution were used for each data partition. Due to the limited number of shared outgroup sequences for ITS-1 and ITS-2 genes in GenBank, only one outgroup sequence was used for this analysis. For both major gene regions (COI and ITS-combined), Bayesian Inference analysis was executed for a maximum of 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.

Preliminary phylogenetic analyses with sequences of other physids obtained from GenBank placed the Owyhee physa as a sister lineage to a specimen identified as "*P. gyrina*" by the collector (Dayrat *et al* 2011). This particular lineage of "*P. gyrina*", which was distinct from other clades recognized as *P. gyrina* was represented by only a single sequence and therefore additional data were needed to evaluate the relationships of these taxa. I obtained samples of "*P. gyrina*" from the California Academy of Science [Accession Numbers: HQ66033.1, HQ659967.1, and HQ659901.1] and additional individuals from the original collector (B. Dayrat, University of California – Merced) to supplement the preliminary dataset. All DNA extractions

and molecular analyses of these additional specimens were completed using identical protocols as described above for the Owyhee physa and *P. zionis*.

Finalizing Phylogenetic Trees

All phylogenetic trees were prepared using FigTree version 1.3.1 and edited with Canvas v. 14. For each gene region, trees were rooted to the outgroup sequences.

Results

Sequence Data

I obtained sequences of the COI gene region from 35 individuals (13 Owyhee physa, 11 "*Physa gyrina*", and 11 *Physa zionis*) and aligned these with 91 sequences of other physids from GenBank (GenBank accession numbers are provided in Appendix 2-1). I also obtained sequences of a region of both the ITS-1 and ITS-2 gene regions from 35 (12 Owyhee physa, 11 "*Physa gyrina*", and 12 *Physa zionis)* individuals and included these with two sequences of *Physa acuta* from GenBank (See Appendix 2-1 for accession numbers).

Individuals from the Owyhee physa population and those from the "*P. gyrina*" population have an average genetic distance of 4.5%, while the Owyhee physa have within population mean distance of 0.05% (Table 2-1 and 2-2). In contrast, the genetic distance between "*P. gyrina*" and Owyhee physa individuals in ITS-1 and ITS-2 gene regions was 1.8% (0.1% within Owyhee) and 0.8% (0.3% within Owyhee), respectively (Table 2-1 and 2-2).

Gene Trees

The T92+G model was determined to be the best model for the COI dataset. The tree resolved many clades with strong posterior probability (pp) support. Three main groups of the

sequences of interest are highlighted in the tree derived from the COI dataset. The first group (green, Figure 2-2; pp 1.0) contains all of the individuals identified as "P. gyrina" by Benoit Dayrat (2011) from California. The second group (red, Figure 2-2; pp 1.0) is recognized as the sister group to "*P. gyrina*" and contains all of the individuals of the Owyhee physa from Oregon. And the third group (blue, Figure 2-2; pp 1.0) contains all of the individuals of *P. zionis* that were sequenced for this study and a sequence downloaded from GenBank of the same taxonomic description (See Figure 2-2 and Appendix for GenBank accession number).

The T92 model was determined to be the best model for the ITS-1 dataset and the JC model was best for the ITS-2 dataset. Trees reconstructed from analysis of the individual datasets (not shown) exhibited similar topologies as those obtained from the combined dataset. Four main sets of sequences are present in the tree derived from analysis of the combined ITS-1 and ITS-2 data (Figure 2-3). The first group contains all 11 individuals pre-identified as "*Physa gyrina*" (Figure 2-3; pp 0.966). The second group contains all 12 of the Owyhee physa individuals (Figure 2-3; pp 0.875). The third group contains all of the individuals pre-identified as *Physa zionis* from Zion National Park, Utah (Figure 2-3; pp 1.0). Finally, the fourth group is a single sequence from the species *Physa acuta* from GenBank.

Discussion

The goal of this study was to determine the species status of the Owyhee physa within the Physidae family based on analyses of sequence data. Examination of 105 sequences from 35 individuals within the genus *Physa* revealed that the Owyhee specimens represent a monophyletic clade with strong support values in trees constructed from sequences of one

mitochondrial and two nuclear gene regions (Figures 2-2 and 2-3). Although the two gene trees differ in their support values (high posterior probability values in the COI tree and moderate values in the ITS tree), the Owyhee clade remained monophyletic and distinct among the trees that were constructed.

Dispersal of populations into unique habitats and their subsequent isolation have been suggested as mechanisms that can lead to speciation (Darwin 1859). Separated by a distance of roughly 600 miles, prolonged geographic isolation and habitat specialization may have resulted in the accumulation of significant genetic differences between the Owyhee physa and "*P. gyrina*".

While there are no standardized genetic benchmarks to determine what constitutes a valid species, the genetic distances between the Owyhee physa and "*P. gyrina*" at each of the gene regions suggests that they have a relatively recent history of divergence but are clearly distinct. The mean levels of genetic divergence between the Owyhee physa and its sister taxon were 4.9% (COI), 1.8% (ITS-1) and 0.8% (ITS-2), which are not uncommon values in distinguishing between physid species. In their analysis of relationships and evolutionary history of three species of the genus *Physella*, Remigio and colleagues (2001) found that the ranges of molecular distances separating *Physa wrighti* from *Physa johnsoni* were 0.6%-1.6% at 16S and 1.4-1.9% at COI, and the distance separating *Physa wrighti* and *Physa gyrina* were 0.6%-1.4% at 16S and 0.5-1.2% at COI. Similarly, in the most recent taxonomic evaluation of the taxonomically contentious physid species *Physa natricina* using *Physa acuta* and *Physa anatina* as reference taxa, Gates *et al* (2013) found that the genetic divergence between the two reference taxa was 4.1% using combined COI and 16S sequences. Because morphological and

anatomical characteristics are not often useful in distinguishing between physid species due to the fact that these characteristics can be influenced by environmental conditions, these traits have not been included here as species discriminators. Nonetheless, based on the genetic distances and the strong posterior probability values of the reciprocally monophyletic clades, the status of the Owyhee physa as a distinct species is supported.

The molecular data analyzed for this study illustrates that there are still many gaps to fill in our understanding of Physidae taxonomy. The most recent investigations of the Physidae have focused on clarifying the relationships among already recognized species (e.g. Gates *et al* 2013, Rogers and Wethington 2007), but few to date have conducted thorough surveys to help determine the overall species richness of this taxonomic group. The Owyhee physa represents one instance of a species discovery, but it also provides evidence that numerous other species are yet to be identified. For example, the sister group of the Owyhee physa, "*P. gyrina*", was given its preliminary taxonomic name based on morphological similarity to the recognized cosmopolitan species *Physa gyrina*. However, based on the mitochondrial COI gene tree produced here (Figure 2-2), a clade consisting of sequences of "*P. gyrina*" falls out quite distinctly from those that consist entirely of sequences from Physa gyrina.

In addition, due to their morphological similarities, we expected that the Owyhee physa is more closely related to *Physa zionis* than the molecular data show. This suggests that the morphological similarity of these two species may be a result of similar environmental pressures and convergent evolution. Although we have not collected data to rigorously analyze how convergent evolution may be influencing the morphological similarity of the Owyhee

physa and *Physa zionis*, Chapter 3 of this study focuses on their morphology in the context of their environments to qualitatively compare them.

Our understanding of the relationships between species is contingent upon correctly identifying them. Morphological data alone are often too variable to be used as species discrimination tools, causing taxonomic redundancies where in fact distinct species should be listed. Thus, it is essential that future studies incorporate thorough regional surveys and genetic analyses to develop a more complete history of the Physidae family in North America.

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Table 2-1. Average genetic distances between pairs of species with standard error calculations and the evolutionary model used. Models used for these analyses were Tamura 3-Parameter with Gamma distribution (T92+G), Tamura 3-Parameter (T92), and Jukes-Cantor (JC).

Gene Region	Species 1	Species 2	Distance	Std Error	Model
COI	Owyhee	"*P. gyrina*"	0.049	0.009	T92+G
COI	Owyhee	*P. zionis*	0.205	0.023	T92+G
COI	"*P. gyrina*"	*P. zionis*	0.200	0.023	T92+G
ITS1	Owyhee	"*P. gyrina*"	0.018	0.006	T92
ITS1	Owyhee	*P. zionis*	0.102	0.014	T92
ITS1	"*P. gyrina*"	*P. zionis*	0.084	0.012	T92
ITS2	Owyhee	"*P. gyrina*"	0.008	0.004	JC
ITS2	Owyhee	*P. zionis*	0.021	0.006	JC
ITS2	"*P. gyrina*"	*P. zionis*	0.025	0.007	JC

Table 2-2. Average genetic distances within the Owyhee physa with standard error calculations and the evolutionary model used. Models used for these analyses were Tamura 3-Parameter with Gamma distribution (T92+G), Tamura 3-Parameter (T92), and Jukes-Cantor (JC).

Gene Region	Distance	Std Error	Model
COI	0.000502	0.000353	T92+G
ITS-1	0.000996	0.000538	T92
ITS-2	0.00033	0.000315	JC

Figure 2-1. Shell images of (A) the Owyhee physa, (B) *Physa zionis* and (C) "*Physa gyrina*".
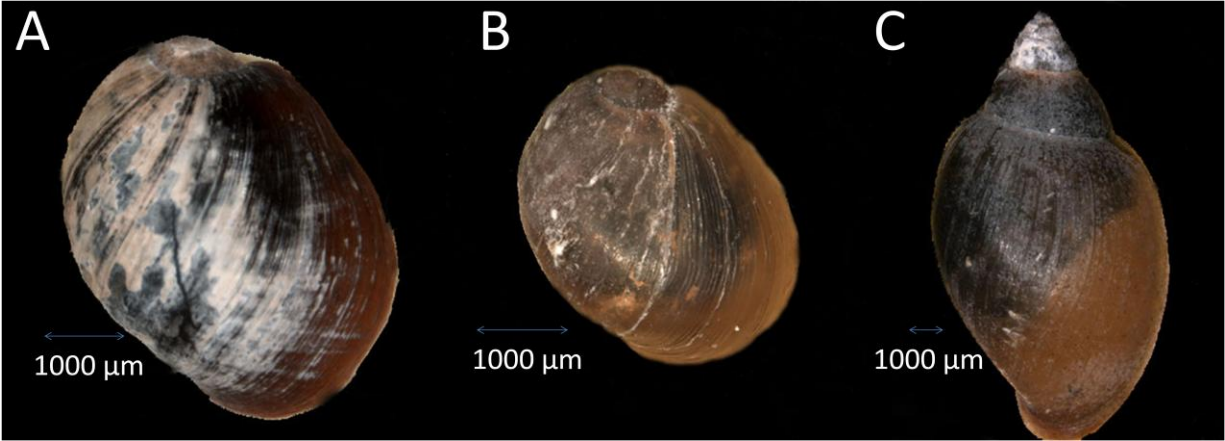
Figure 2-2. Consensus tree recovered from Bayesian analyses of the mitochondrial COI gene region. Numbers on branches indicate Bayesian posterior probabilities. Branch length scale is on the lower left.
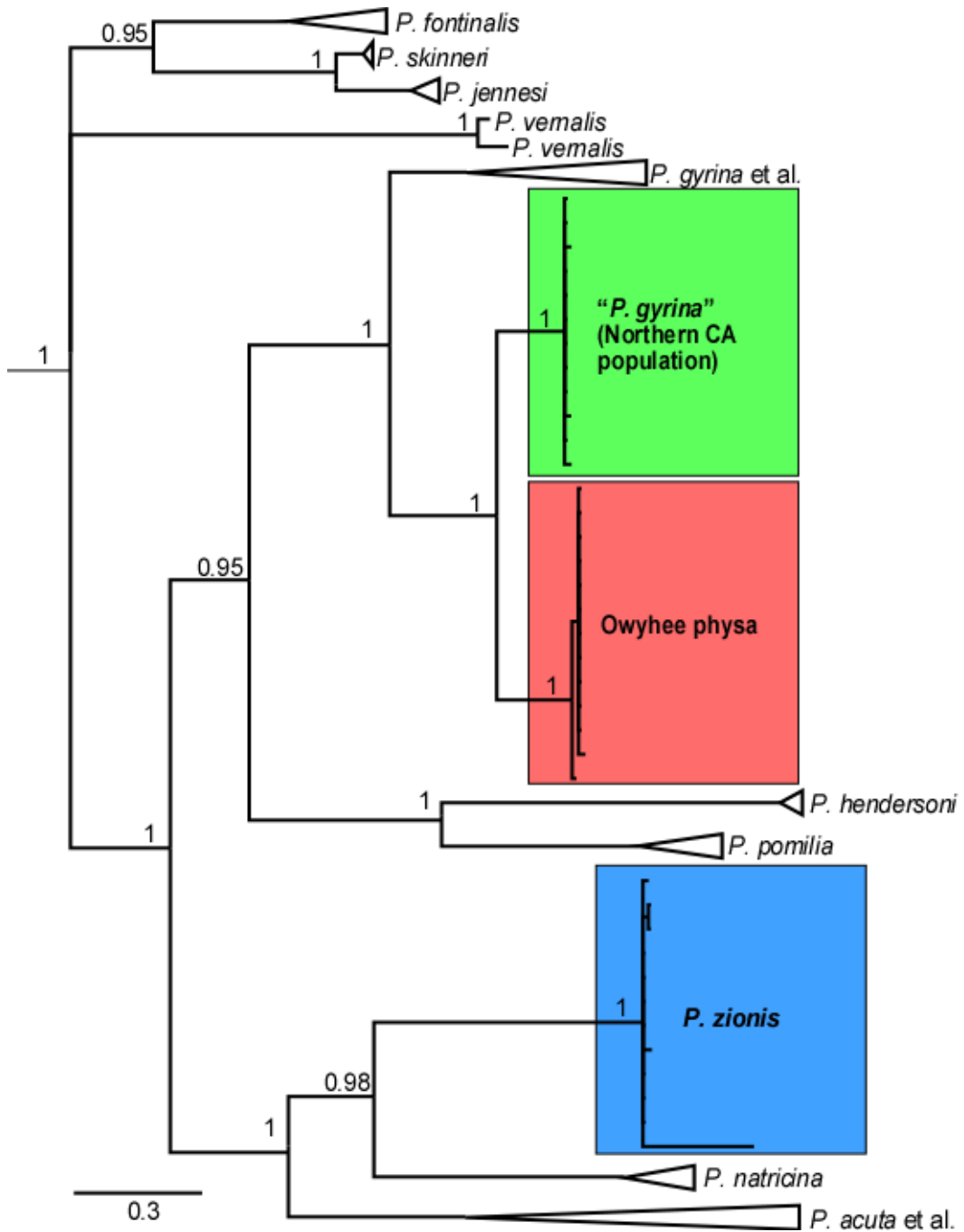
Figure 2-3. Consensus tree recovered from Bayesian analyses of the combined nuclear ITS-1 and ITS-2 gene regions. Numbers on branches indicate Bayesian posterior probabilities. Branch length scale is on the lower left. Sequenced specimens are denoted by the following: the Owyhee physa individuals are represented by Physa001 – Physa014, "*Physa gyrina*" individuals are Pgyrina1 – Pgyrina12, and *Physa zionis* individuals are PzUtah001 – PzUtah012
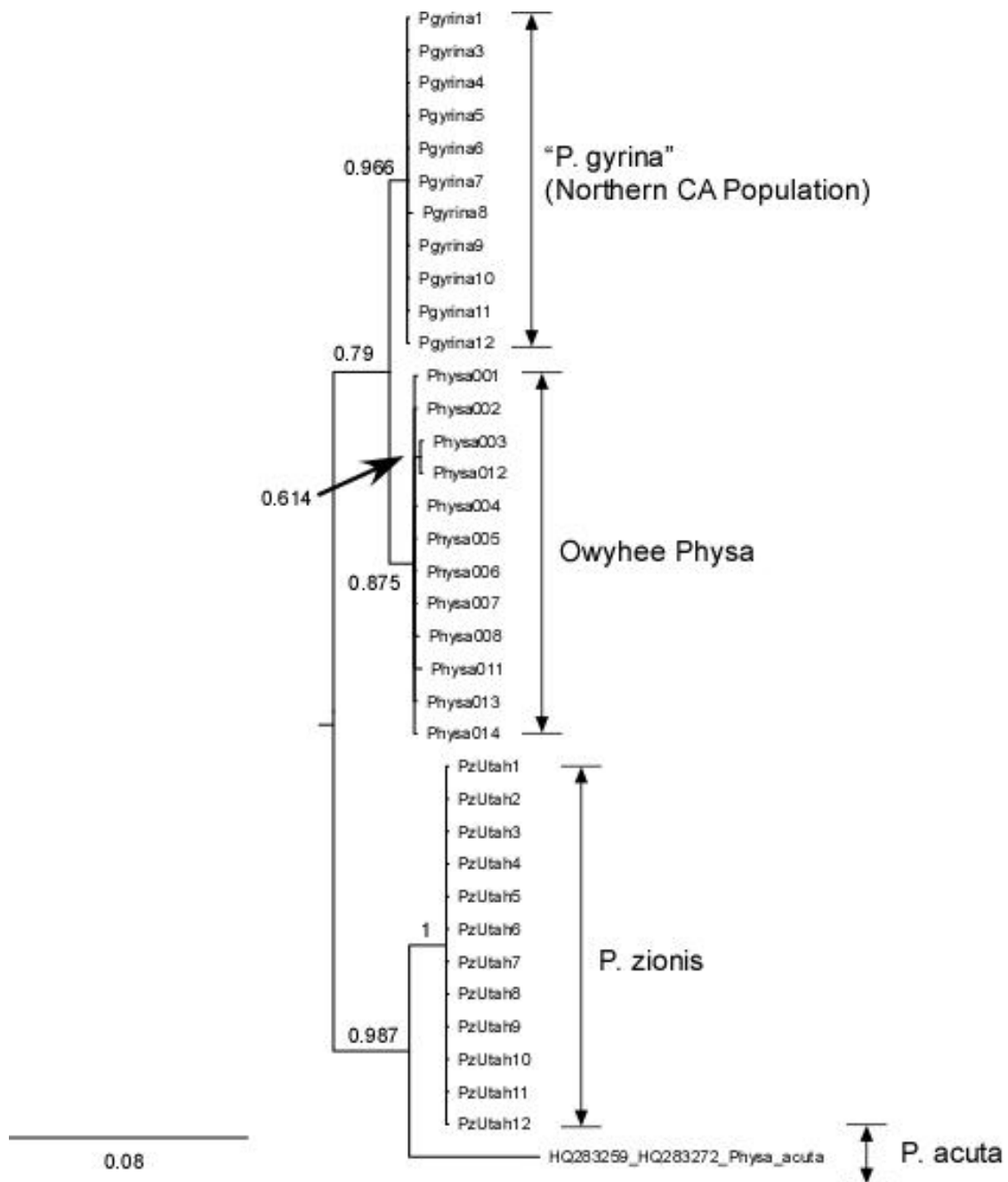
Appendix 2-1. Morphological ID (Genus species) and GenBank accession numbers of individuals used in molecular analyses.

Morphological ID	GenBank Accession Numbers		
	<u>COI</u>	<u>ITS-1</u>	<u>ITS-2</u>
Physa acuta	AY651188	-	-
Physa acuta	EU038368	-	-
Physa acuta	EU038356	-	-
Physa acuta	EU038367	-	-
Physa acuta	EU038366	-	-
Physa acuta	EU038357	-	-
Physa acuta	AY651174	-	-
Physa acuta	EU038360	-	-
Physa acuta	AY651181	-	-
Physa acuta	AY282589	-	-
Physa acuta	AY651203	-	-
Physa acuta	EU038361	-	-
Physa acuta	GU247996	-	-
Physa acuta	GU247993	-	-
Physa acuta	GU247995	-	-
Physa acuta	FJ373016	-	-
Physa acuta	-	HQ283259	HQ283272
Physella anatina	AY651177	-	-
Physella anatina	AY651176	-	-
Physella anatina	AY651175	-	-
Physa ancillaria	EU038388	-	-
Physa ancillaria	EU038392	-	-
Physa ancillaria	EU038385	-	-
Physa ancillaria	EU038383	-	-
Physa ancillaria	EU038382	-	-
Physa ancillaria	EU038358	-	-
Physa ancillaria	EU038387	-	-
Physa ancillaria	EU038359	-	-
Physa ancillaria	EU038381	-	-
Physa ancillaria	EU038380	-	-
Physella virgata	AY651171	-	-
Physella virgata	AY651170	-	-
Physa integra	EF488674	-	-

Morphological ID	GenBank Accession Numbers		
	COI	**ITS-1**	**ITS-2**
Physa heterostropha	AY651192	**-**	**-**
Physa heterostropha	AY651193	-	-
Physa winnipegensis	EF488681	-	-
Physella spelunca	AY651205	-	-
Physella cupreonitens	AY651183	-	-
Physella wolfiana	AY651179	-	-
Physella wrighti	AF419323	-	-
Physella wrighti	AF346745	-	-
Physella aurea	AY651202	-	-
Physella aurea	AY651201	-	-
Physa gyrina	EU038398	-	-
Physa gyrina	HQ660033	-	-
Physa gyrina	EU038373	-	-
Physa gyrina	EF488671	-	-
Physa gyrina	EU038374	-	-
Physa gyrina	EF488670	-	-
Physella gyrina	AY651178	-	-
Physella gyrina	JF806435	-	-
Physella johnsoni	AF346739	-	-
Physella johnsoni	AY651172	-	-
Physella johnsoni	AF346737	-	-
Physella johnsoni	AY651173	-	-
Physella johnsoni	AF346736	-	-
Petrophysa zionis	AY651198	-	-
Physa natricina	GU830944	-	-
Physa natricina	GU830951	-	-
Physa natricina	GU830950	-	-
Physa natricina	GU830949	-	-
Physa natricina	GU830942	-	-
Physa natricina	GU830947	-	-
Physa natricina	GU830946	-	-
Physa fontinalis	AY651189	-	-
Physa fontinalis	FJ373018	-	-
Physa fontinalis	EU818796	-	-
Physa fontinalis	AY651190	-	-
Physella hendersoni	AY651196	-	-
Physella hendersoni	AY651194	-	-
Physa skinneri	EF488673	-	-
Physa skinneri	EF488672	-	-

Morphological ID	GenBank Accession Numbers		
	COI	**ITS-1**	**ITS-2**
Physa jennessi	GU680897	-	-
Physa jennessi	GU680896	-	-
Physa jennessi	GU680892	**-**	**-**
Physa pomilia	EU038353	-	-
Physa pomilia	EU038363	-	-
Physa vernalis	EU038375	-	-
Physa vernalis	EU038376	-	-
Biomphalaria pfeifferi	DQ084831	-	-
Biomphalaria pfeifferi	AF199099	-	-
Biomphalaria pfeifferi	AF199101	-	-
Biomphalaria pfeifferi	AF199102	-	-
Biomphalaria pfeifferi	AF199104	-	-
Biomphalaria kuhniana	AY030380	-	-

Chapter III: Morphology, Habitat, and Convergent Evolution

Introduction

In many gastropod species, shell morphology is a function of several variables, including mechanical stability, predator defense, sexual selection, and climate selection (Britton 1995; Wilson, Glaubrecht, and Meyer 2004; Goodfriend 1986). However, some shell shapes are found more often in certain habitat types. For example, environments that are high-intensity and wave-washed, such as rocky intertidal zones, are often inhabited by snails with wide-aperture shells and relatively low surface areas (Urabe 1998). Snails with high spires and highly sculpted forms, on the other hand, are commonly found in environments with quieter, calmer waters (Urabe 1998). Although many exceptions exist, the large influence of the environment and ecological pressures on shell morphology suggests that convergent evolution may play an important role in the development of shell shape in molluscan species.

The present study aims to compare shell morphologies of the Owyhee physa, *Physa zionis*, and "*Physa gyrina*" in the context of the environments in which each species is found. To address these phenotypic differences, high-resolution images and three-dimensional models of shell morphology were used to describe the shell structures of the three species, both qualitatively and quantitatively. Morphological analyses that utilize mathematical methods are variable and include morphometrics, theoretical morphology, constructional morphology, and

functional morphology. Although the focus of each discipline is different, what unites them is their use of models or statistical tools to study biological shape and form.

The geometric form of invertebrate shells in particular has been a prevalent system for morphological studies since the early 1900s. David Raup, considered the founder of theoretical morphology, was the first to use digital-computer simulations to study invertebrate shell morphology and morphogenesis (Raup 1961, 1962). In these early studies, Raup focused primarily on analyzing the geometry of shell coiling in existent and non-existent shell shapes (Raup 1966, 1967) and examining echinoid (sea urchin) shell growth (Raup 1968). Later, others such as Okamoto (1988a, 1988b), Ackerly (1989), and Savazzi (1990) expanded on these previous discussions of shell growth patterns and the models used to construct them. Because invertebrate shells come in a wide variety of shapes and display varying patterns of morphogenesis, they lend themselves well to evolutionary and ecological studies of diversity.

Here, we assess morphological variation using modeling approaches and qualitative descriptions and use this information in tandem with environmental data to address how these variables influence phenotype among the three physid species. The use of these morphological descriptions has the potential to provide valuable information about the impact their evolutionary histories have had on shell shape. When assessed in conjunction with environmental data for each species' habitat, these morphological descriptions may allow us to better understand the influence that convergent evolution has on shell morphology within this highly diverse and widespread family.

Materials and Methods

Morphological Descriptions

Using a Leica MZ16 microscope and Image Pro Discover v.5 camera software, photographs were taken of 12 individuals of Owyhee physa, "*Physa gyrina*", and *Physa zionis* specimens. Each shell was placed on a glass slide, held in place using a small piece of modeling clay, and photographed in two different positions: aperture-side up with the spire aligned vertically on the slide; aperture-side down with the spire aligned vertically on the slide. For each photograph, a scale bar indicating 1000 micrometers was used.

Models of shell morphology were produced with the help of Janice Pappas, research scientist at the University of Michigan Museum of Paleontology. *Physa* shell morphology was modeled using the methods of Pappas and Miller (Accepted) and is based on parametric three-dimensional (3D) equations (Pappas 2005a, b, 2008). The idea in using such methods is to take basic geometric forms, combine them and change their shape in a three-dimensional fashion to produce a 3D surface. Three-dimensionality is represented as variables in the x-, y-, and z-directions where the parameters u and v represent whorl and aperture attributes, respectively. Typically, gastropod shell morphology is modeled with helical curves as a basis (e.g., Moseley 1838, 1842; Thompson 1917; Raup 1961, 1966; Raup and Michelson 1965; Okamoto 1988; Savazzi 1990; Checa 1991; Prusinkiewicz and Fowler 1995). Instead, we start with the 3D surface of a basic geometric form and create morphological variation by changing the functions and their values within each set of parametric equations (Pappas and Miller, Accepted). The 3D form that results is a model representing the notable attributes of the actual shell surface of study (Pappas, personal communication).

Habitat Data

Using ISI Web of Science and public government agency resources, I conducted a literature review for environmental data and descriptions for the locations where each of the three physid species are found: the Owyhee River at Three Forks in Malheur County, Oregon for the Owyhee physa; Zion National Park in Hurricane, Utah for *Physa zionis*; and the Sierra Nevada foothills, California for "*Physa gyrina*". For each location, data on the following environmental variables were collected to describe their respective habitats: habitat type (temporary or permanent), climate classification, mean annual precipitation, regional elevation range, and geography type.

<div align="center">

Results

</div>

Morphological Descriptions

Three-dimensional shell models and high-resolution photographs of each species can be found in Figure 3-1. Using these model renderings and images, qualitative shell descriptions are as follows:

Owyhee physa. The shell is thin, brown to dark brown in color, and semiovate shaped. The spire is very small and low, unlike that of most physid species. The aperture is nearly as long as the shell and is wide, with no operculum. The coil of the shell is sinistral or "left-handed" and the outer lip leading into the aperture is thin and sharp. The length of the shell ranged from 3.0 mm – 4.5 mm for the individuals used for sequencing. The spire of each shell contained two complete whorls.

"*Physa gyrina*". The shell is elliptical to elliptically oval shaped and can be found in colors that range from tan to dark brown. The spire is high with rounded whorls. The aperture is

considerably longer than the spire and is elongately oval. The outer lip is sharp and curved and the coil of the shell is sinistral. The length of the shell measured between 6 mm – 12 mm for the individuals used for sequencing. The spire of each shell contained four complete whorls.

Physa zionis. The shell is thin, tan to cinnamon colored, and semiovate in shape (Pilsbury 1925). As stated in the original description of the species, there is a very small and low spire containing small growth lines along the last whorl. The aperture is almost as long as the shell and is very broadly open, with no operculum. The outer lip is thin and sharp and the coil of the shell is sinistral (Pilsbury 1925). The length of the shell ranged from 3.5 mm – 4 mm for individuals used for sequencing. The spire of each shell contained two complete whorls.

Because the construction of morphological models is mathematically intensive, details on the specific quantitative values included in each model will not be presented here. However, shell models can be compared in terms of how similar the parameter values in the x-, y-, and z-directions are relative to the other species. This information is described as follows:

For each *Physa* model, changes in the *x*-, *y*-, and *z*-directions indicate particular shell morphological attributes from solutions to the first partial derivatives of the parametric 3D equations (Figure 3-1). In the *x*-direction with respect to parameter *v*, the change among the three *Physa* species is relatively small, but the order of change in minimum dimension of aperture size and shape is the Owyhee physa, *Physa zionis*, followed by *"Physa gyrina"*. Specifically, the Owyhee physa and *Physa zionis* are closer in value to each other than they are to *"Physa gyrina"*. Change in the *y*-direction with respect to parameter *v* reflects change in maximum dimension of size and shape of the aperture. *"Physa gyrina"* has the largest value followed by *Physa zionis*, then the Owyhee physa. However, the values for *Physa zionis* and the

Owyhee physa are much closer to each other than they are to *"Physa gyrina"*. Changes in the *z*-direction with respect to parameter *u* define the change in maximum dimension of whorl size and shape. *Physa zionis* has the largest value followed closely by *"Physa gyrina"*, then the Owyhee physa. In this case, *"Physa gyrina"* and the Owyhee physa are closer together in value than either one is to *Physa zionis*. This reflects the complexity of the interrelationship between changes in aperture shape with changes in each whorl diameter from top to bottom of the shell (Pappas, personal communication).

Habitat Description

The Owyhee physa is a warm-spring snail found in very shallow small springs and seeps within rock cliff faces along the Owyhee River, Oregon (Frest and Johannes 1995). Substrates within the habitat vary from basalt bedrock to sand, gravel, and cobbles. The area is in a major river canyon with exposed volcanic rock and open, dry sage scrub (Frest and Johannes 1995). Similarly, *Physa zionis* is found within a major river canyon along the north fork of the Virgin River in Zion National Park, Utah (Oliver and Bosworth 1999). This species is located in places where water seeps from joints within the rock cliffs along canyon walls and where green algae forms on the wet rock faces below (Pilsbury 1925). Finally, *"Physa gyrina"* is found in temporary creeks of the Sierra Nevada Foothills, California. Where individuals are present, the water is calm, clear, and shallow with a maximum depth of one foot (B. Dayrat, personal communication, 2013).

A summary of the environmental characteristics for each species can be found in Table 3-1.

Discussion

Snail shells are an important determinant of fitness, particularly for aquatic snails, and are known for exhibiting considerable phenotypic plasticity (Kemp and Bertness 1984). Specific shell morphologies are favored depending on different environmental conditions, such as current velocity, predator presence and abundance, and temperature (Raffaelli 1978; Hunter 1989; Crowl and Schnell 1990; Vermeij 1993; Johnson and Brown 1997; Minton *et al*. 2008). Because shell shape and size play important roles in an organism's ability to not only defend itself against predators and acquire resources, but also to stay level within the flow regime of its habitat, species with similar selection pressures often exhibit similar morphologies. Since snail shells show such a high level of ecophenotypic plasticity, convergent evolution is a prevalent mechanism in explaining the evolutionary history of shell shape among species (Serb *et al* 2011).

The endemic gastropod fauna of Lake Tanganyika in East Africa, for example, garnered considerable attention from early malacologists who incorrectly placed them in marine gastropod families (Bourguignat 1890; Moore 1898, 1899, 1903). Studies of the internal anatomy of the Tanganyika snails confirmed that they belong in freshwater families and are not closely related to the marine snails that they so resemble (West and Cohen 1996). With heavily calcified shells and displaying coarse ribbing, spines, and apertural lip thickening, Tanganyika and marine snails share several environmental pressures that led many to believe that their strikingly similar morphologies are a result of convergent evolution (West and Cohen 1996). Although many snail morphologies do not follow general environmentally adaptive

explanations, convergence of shell morphology can often be explained, at least in part, by environmental similarities.

The family Physidae is known for being geographically widespread relative to other freshwater molluscan taxa, and for having species with great environmental tolerances and exhibiting ecophenotypic plasticity. These traits have allowed species within this family to exploit a wide range of habitats, from ditches and ponds to streams and rivers (Burch 1982). In an example of this phenotypic plasticity, during a laboratory experiment physid snails reared in the presence of a fish (a shell-crushing predator), produced rotund shells, while those reared in the presence of a crayfish (a shell-entry predator), produced elongate shells (DeWitt 1995). These observations support functional predictions that rotund shells are more crush-resistant because the crushing force is spread across a larger surface area (Palmer 1979), while elongate shells are more entry-resistant because the shell entryway is narrowed (Snyder 1966; Osenberg 1988). At this level of analysis (i.e. within a species), phenotypic plasticity is not evidence of diverging populations, but rather represents the diversity of life-history strategies present within a species. This morphological diversity has the potential to confer fitness advantages given different environmental variables and explains, to some degree, the high tolerance for environmental variance found in the Physidae.

It is important to note here that because the purpose of this study was not to test convergent evolution within the Physidae family, but rather to exhibit their morphological similarities in the context of their environments, extensive details of their evolutionary history in their environments will not be presented. However, here we have shown that although the

Owyhee physa and *Physa zionis* are members of distinct clades, they share morphological traits that may be a result of the similar habitats in which they are found.

The shells of the Owyhee physa and *Physa zionis* ranged from 3 mm – 4.5 mm in length with wide apertures that contain a large, broad-sized foot. As described by Pilsbury (1925) when referencing *Physa zionis*, both species have hemispherical shells and no projecting spire. Conversely, "*Physa gyrina*" exhibits typical physid shell characteristics with a long, large aperture and a high spire. As seen in Figure 3-1 and Figure 3-2, the Owyhee physa is far more similar morphologically to *Physa zionis* than to "*Physa gyrina*", despite their genetic relatedness. Such similarities suggest that the shared morphological traits between these two species may be influenced, at least in part, by similar selective pressures in their environments. Although the present data do not address these environmental pressures in detail, they do highlight some general trends in the composition each habitats.

The Owyhee physa and *Physa zionis* are found in habitats that share at least two major environmental features. Both species are established in regions characterized by large, canyon landscapes and semi-arid steppe climate. While many ecological and evolutionary factors influence morphology, these broad-scale environmental similarities may indicate that the Owyhee physa and *Physa zionis* share other habitat characteristics that led to their similar morphologies. To adequately assess the impact that convergent evolution has had on species within this taxonomic group, future studies should collect detailed historical and contemporary ecological and environmental variables for each species of interest and compare those variables with morphological characteristics.

The Physidae family is the most diverse group of freshwater mollusks in North America, but currently our understanding of the relationships among species and the mechanisms that led to this diversity is significantly lacking. The present study has provided evidence to suggest that convergent evolution may play an important role in influencing the level of diversity seen in this freshwater family and be a contributing factor in the current level of taxonomic fluidity found within this and other groups of freshwater mollusks.

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Table 3-1. Regional habitat data for the Owyhee physa, *Physa zionis*, and "*Physa gyrina*".

	Owyhee physa	*Physa zionis*	"*Physa gyrina*"
Location	Oregon	Utah	California
Habitat Type	Permanent	Temporary	Temporary
Climate	Semi-Arid Steppe Climate	Midlatitude Desert Climate/Semi-Arid Steppe Climate	Mediterranean/Boreal Climate
Mean Annual Precipitation	15-20 in	14 in	20 - 80 in
Regional Elevation	2,100-6,800 ft	3,600-8,700 ft	1,000-14,500 ft
Geography Type	Canyons	Canyons	Mountains/Valleys

Figure 3-1. Shell photos and models of the Owyhee physa (top row), *P. zionis* (middle row), and "*P. gyrina*" (bottom row). (Shell photos: Alex Moore; models: Janice L. Pappas).

Chapter IV: Implications and Future Work

Discussion

Implications

Within the last century, the rate of biodiversity loss has grown to exceed that of historical background rates by a factor of at least 100 (Pimm *et al* 1995). Additionally, the projected loss of species in the coming century has a median estimate of 30% (Barnosky *et al* 2011). Anthropogenic impacts, such as resource depletion, habitat fragmentation, non-native species and pathogen introductions, overexploitation, and global climate change are the primary causes of these losses (e.g. Tilman *et al* 1994; Jackson *et al* 2001; Thomas *et al* 2004). These and other human activities have been documented as directly causing several recent extinctions. For example, the Caribbean monk seal was officially declared extinct in 2008 by the IUCN as a result of overhunting for food and oil (Kovaks 2008). Similarly, the last known individual of the Pyrenean ibex was found dead in 2000 of unknown causes. The species had once been very abundant in the Spanish Pyrenees Mountains but their populations drastically declined in the 19th and 20th centuries due to hunting pressures and competition with domestic and wild ungulates (Folch *et al* 2009).

The current rate of reduction in species richness and undisturbed habitat substantially affects not only the markets and local communities that rely on these goods, but also the ecosystem services and functions that they provide. In the last 20 years, a number of studies

have been conducted to determine the impacts that biodiversity loss has on community structure, ecosystem services and functions (Cardinale *et al* 2011; Steinbeiss *et al* 2008; Ruiz-Jaen and Potvin 2010; etc). Meta-analyses of these studies have shown that a decline in biodiversity at all scales (i.e. genes, species, and functional groups) reduces the efficiency by which communities capture essential resources and convert them into biomass (Cardinale *et al* 2011; Balvanera *et al* 2006; Cardinale *et al* 2006; Worm *et al* 2006; Cardinale *et al* 2007; Stachowicz *et al* 2007). Furthermore, there is an increasing amount of evidence to suggest that biodiversity is correlated with certain provisioning and regulating ecosystem services, such as biocontrol (e.g. disease prevalence, resistance to plant invasion, abundance of herbivorous pests, etc) and carbon sequestration (Cardinale *et al* 2012). As species richness declines, the services that they provide become unable to meet the needs of both natural and human communities.

There no longer exists any natural areas that have not been affected by humans, but relative to other systems, freshwater habitats are considered the most endangered (Allan and Flecker 1993; Malmqvist and Rundle 2002). Freshwater regions are naturally scarce; the total surface area of fresh waters of all kinds is only 5-10 million km^2 (Shiklomanov 1993; Cole *et al*. 2006), which is less than the area of Europe (Strayer *et al* 2006). Furthermore, because freshwater habitats are embedded in and are downhill of terrestrial regions, they are unavoidably impacted by human activities that are concentrated around their drainage basins (Strayer *et al* 2006). The disproportionate impact that freshwater habitats experience due to human activities places freshwater biota at an increased risk of decline and extinction.

Although it is well known that freshwater habitats are at enormous risk due to human influence, knowledge of the groups of species that are most at risk within these habitats is highly variable. For instance, the best studied groups of freshwater invertebrates have roughly the same number of described species as freshwater fish, but have received approximately one-tenth of the attention from scientists (Strayer *et al* 2006; Dudgeon *et al* 2005). The biased focus of taxonomic studies on charismatic megafauna and economically important biota has left our understanding of freshwater invertebrate taxonomy highly fragmented and incomplete. Moreover, like the habitats in which they are found, freshwater species are in rapid decline worldwide, due to five main factors: overexploitation, water pollution, flow modification, degradation of habitats, and invasion by exotic species (Dudgeon *et al* 2005). But because of the taxonomic bias present in studies of freshwater taxa, it is difficult to assess how these factors may be impacting groups that have historically been studied less.

The present study has provided some much needed additional information about the taxonomy of the freshwater mollusk family Physidae. Based primarily on molecular data, this study supports the inclusion of the Owyhee physa as a distinct species within this family. Found only in one stream complex of the Owyhee River (Oregon) and nowhere else, the Owyhee physa is a highly endemic species with a very restricted range. Much like all other freshwater habitats, the Owyhee River has historically been affected by human use. Flowing from northeastern Nevada through southeastern Oregon, the Owyhee River was drastically modified in 1933 when the Owyhee Dam was built in northern Malheur County, Oregon (Bureau of Reclamation 2006). Constructed to provide agricultural irrigation to the region surrounding the Snake River, this impoundment caused the extirpation of migrating fish, such as salmon, from

the Owyhee River Basin (Oregon Environmental Council 2009). The Owyhee River and surrounding areas have also experienced anthropogenic impacts as a result of the recreational uses that are permitted in the region, including camping, fishing, kayaking and other boating activities.

Without a clear understanding of the impacts that these activities have on the habitat and the organisms found therein, and other species remain at risk for population decline and extinction. Currently, portions of the Owyhee River are protected under the Wild and Scenic Rivers Act of 1968 and the Omnibus Public Land Management Act of 2009 (Owyhee Initiative 2011), but much of this protection focuses on the conservation of habitats for large mammals and wildlife. To adequately conserve those species that are at most risk, it is essential to know what species are present and their status within their habitat.

The present study has called into focus how incomplete our knowledge of freshwater invertebrate taxonomy is, especially within this region of the United States. Through this work, we were able to not only identify the Owyhee physa as a distinct, endemic species, but our data also suggests that "*Physa gyrina*", the sister taxon of , may also be a new and currently undescribed species. Because much of what is known about freshwater gastropod diversity in the United States has focused heavily on the Mobile Bay basin and the American southwest (Strong *et al* 2008), the present work provides evidence to support additional studies in regions that have historically received far less attention.

New species discoveries contribute not only to our understanding of regional diversity and the evolutionary relationships among species, but also how we view conservation of this biological diversity. Without a proper evaluation of the species that exist and the roles that they

play in their environment, our ability to effectively protect them is severely limited. This study has provided additional information about the current status of Physidae species richness, but additional studies must be done to both broaden and deepen our knowledge of freshwater gastropod diversity.

Future Work

The establishment of the Owyhee physa as a new species along with evidence to support the eventual species status of "*Physa gyrina*" contributes substantially to our knowledge of freshwater invertebrate taxonomy, particularly freshwater gastropods. However, because an estimated 10,000 species of freshwater mollusks are predicted to be currently undescribed, additional work is essential (Lydeard *et al* 2004). To further our taxonomic and biological understanding of this understudied group, future investigations should focus on increasing the number of regional surveys, particularly in the western and midwestern United States, and incorporate modern molecular analyses in taxonomic evaluations. Because freshwater taxonomic classifications are both incomplete and highly variable, evaluating putative species on the basis of morphological and anatomical characters alone is insufficient for species discrimination efforts. Including molecular techniques in the analyses of putative species will provide information not only on the evolutionary history of populations based on genetic data, but also on how morphology and anatomical characters may change under different environmental pressures and relative to genetic distances.

Conducting formal evaluations of potentially new species is a crucial first step in gaining a better understanding of the biological diversity present in freshwater taxa. However, because

of the current rate at which freshwater habitats are being modified and lost, future studies should do more than solely determine the taxonomic status of putative species. is found in a region of the Owyhee River that has seen increased foot-traffic and human influence in recent years, primarily due to the remote location that affords beautiful scenic views, fishing and boating, and the presence natural hot springs (D. Hopper, personal communication). Although this study has presented data to support the species status of the Owyhee physa, little more than this is known about the species. In light of the current status of freshwater ecosystems, future studies of and other freshwater species should investigate their ecology and the roles that they play in their respective habitats. Freshwater ecosystems contain many poorly understood and unrecognized ecological networks as a result of the limited research that has been done within these systems. Increased efforts to investigate these understudied regions will not only provide basic biological knowledge of the organisms found within these habitats, but will also help to uncover how global change and species losses will alter the way that these ecosystems function and persist.

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