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A molecular phylogeny of aquatic gastropods provides a new perspective on biogeographic history of the Snake River Region

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Abstract

Mitochondrial DNA sequences of aquatic gastropods of the subgenus *Pyrgulopsis* (*Natricola*) were analyzed to test a commonly accepted hypothesis concerning the early history of the Snake River in the northwestern US. Distributions of *Natricola* and other regional biota were previously used to infer that the Snake River flowed to the Pacific through southeastern Oregon and northern California during the Neogene prior to its capture by the Columbia River in the late Pliocene (2 Ma). A molecular phylogeny based on partial sequences of COI and NDI (1149 bp) indicates that the *Natricola* clade is restricted to the modern Snake-Columbia River Basin and the Oregon Lakes region whereas northern California populations previously assigned to this subgenus belong to other lineages. The *Natricola* clade is not deeply subdivided into Oregon Lakes and Snake River Basin units consistent with late Pliocene fragmentation of the hypothesized paleodrainage, but instead is shallowly structured and contains multiple transitions among these two geographic areas. The strongly supported sister relationship between *Natricola* and a species from northwest Nevada (*P. imperialis*) is consistent with a recent proposal that the ancestral Snake River did not flow through southeast Oregon but instead flowed south to the Humboldt River. Within the context of this hypothesis, the multiple transitions between the Snake River Basin and the Oregon Lakes region that occurred within *Natricola* may be attributed to a late Pleistocene connection between these areas that was unrelated to the early course of the Snake River.
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1. Introduction

The early history of the Snake River (northwestern US) has long intrigued both geologists and biogeographers. The lower reach of this river, which flows from the western Snake River Plain north along the Idaho–Oregon border before turning west to join the Columbia River, is a geologically recent (Quaternary) feature resulting from capture of the Snake by a northward flowing stream at the head of Hells Canyon (Wheeler and Cook, 1954). During the late Miocene and early Pliocene, prior to its integration with the Columbia River, drainage of the western Snake River Plain was impounded in a series of lakes ("Lake Idaho," *fide*

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Cope, 1883). This freshwater lacustrine system (Kimmel, 1982; Middleton et al., 1985) had an at least periodic outlet whose physical trace has not been found (Wood, 1994). Biotic distributions instead have been used to infer that the ancestral Snake River flowed through southeast Oregon to the California Pacific coast, via either the Klamath (Taylor, 1960, 1985) or Sacramento (Miller, 1965) Rivers (also see Wheeler and Cook, 1954). Although this hypothesis is well entrenched in the literature (Christiansen and Yeats, 1992; Malde, 1965, 1991; Smith et al., 2000), its supporting biogeographic evidence has never been rigorously evaluated.

The only reasonably well established geological benchmark that can be associated with this postulated paleodrainage is the capture of the Snake River by the Columbia River, which is generally thought to have occurred about 2.0 Ma (Malde, 1991; Othberg, 1998;

Sadler and Link, 1996) in conjunction with the draining of Lake Idaho and incision of Hells Canyon. [Note that some workers have suggested that this shift in drainage occurred earlier in the Pliocene (e.g., Link and Fanning, 1999; Smith, 1999; Wood, 2000) and thus we consider 2.0 Ma to be a minimal date for this event.] This would have severed a drainage outlet (of Lake Idaho) through southeast Oregon and separated biotas east and west of the modern divide between the Oregon Great Basin (referred to herein as the "Oregon Lakes" region) and Snake River Basin.

In this study, we evaluate the role that this stream capture event may have played in diversification of the nymphophiline gastropod subgenus Pyrgulopsis (Natricola) Gregg and Taylor, 1965, which was one of the taxa used to infer an early western route of the Snake paleodrainage (Taylor, 1982; Taylor and Smith, 1981; Fig. 1). [Note that Natricola, which was diagnosed by its large size and elongate penial accessory process (Gregg and Taylor, 1965), is currently treated as a junior synonym of Pyrgulopsis Call and Pilsbry, 1886 (fide Hershler and Thompson, 1987). In this paper, we use Natricola only as an informal collective name for its species, which are individually associated with their correct genus.] These small, gill-breathing snails are tightly linked with their permanent aquatic habitats (rivers, streams, lakes, and springs) and thus were considered highly suitable for inferring prior drainage relationships (fide Taylor, 1985; Taylor and Bright, 1987). Two species of Natricola (P. idahoensis, P. robusta) are restricted to the (modern) Snake River Basin. Taxonomically undescribed populations from three widely separated tributaries of the Snake River (Taylor and Smith, 1981, Fig. 5; Taylor, 1977, 1982) and from the Columbia River (Frest and Johannes, 1995) also have been assigned to the subgenus. Two other species of Natricola traverse the divide between the Snake River Basin and western areas. One (P. hendersoni) is distributed in the Malheur River (Snake River drainage) and portions of the Oregon Lakes region (Abert, Harney Basins), while the other (P. intermedia) ranges among the Owyhee River (Snake River Basin), Oregon Lakes region (Barren Valley), Pit River, and Klamath River Basins. A fossil species of Natricola was also described from Pliocene lacustrine deposits in northeast California (Taylor and Smith, 1981).

The goal of this paper is to assess monophyly of Natricola and to determine whether it has diverged into eastern (modern Snake River Basin) and western (Oregon Lakes, Pit River Basin, Klamath River Basin) lineages consistent with late Pliocene capture of the Snake River and fragmentation of its ancestral western route.
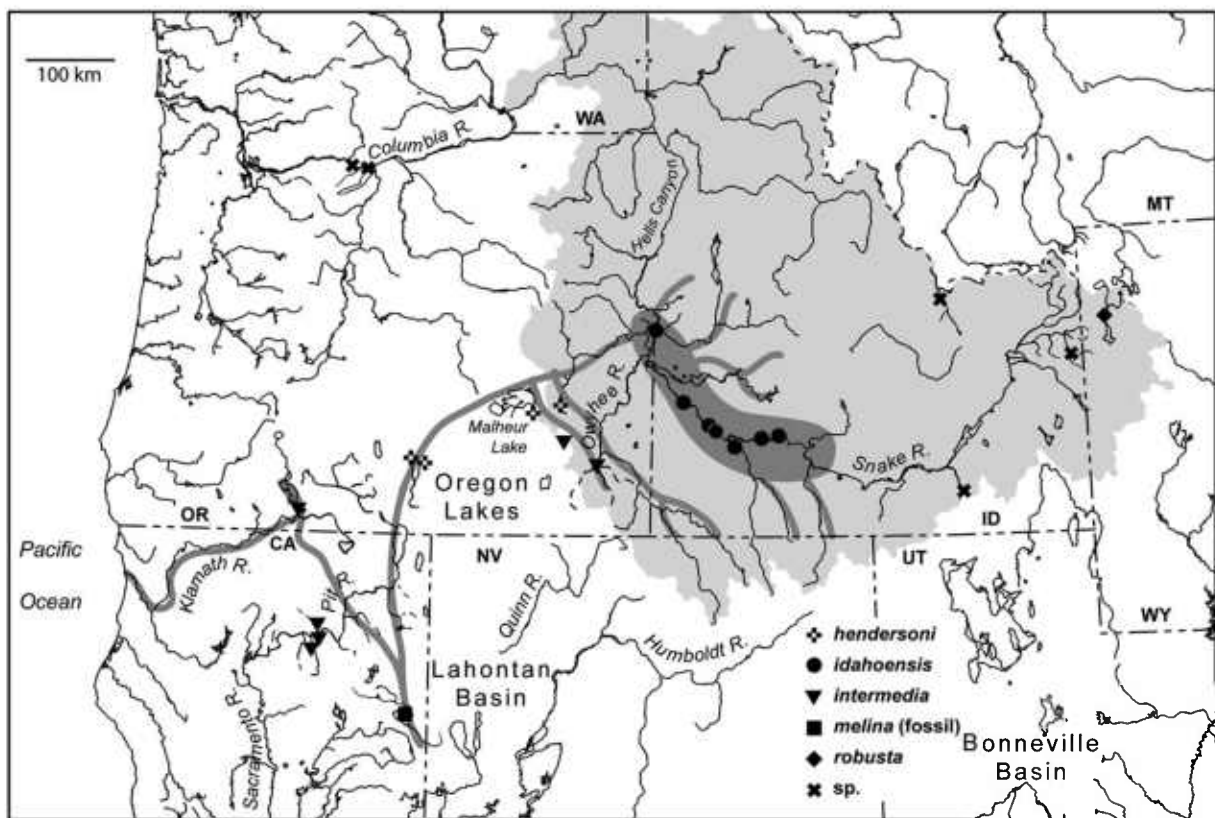

Fig. 1. Map showing location of sampled Pyrgulopsis (Natricola) populations and fossil P. melina. The lightly shaded area delineates the modern Snake River Basin while the hypothesized route of the ancestral Snake River (from Taylor and Bright, 1987, Fig. 5) is more darkly shaded.

If this vicariance hypothesis is correct, and subsequent dispersal across the Snake River–Oregon Lakes divide has not occurred, then one or both of these groups should be monophyletic. A previous, morphology-based analysis of 60 species of *Pyrgulopsis* (which included several species of *Natricola*) did not provide sufficient resolution (Hershler, 1994) to test this hypothesis. In the present study we used two mitochondrial markers, cytochrome *c* oxidase subunit I (COI) and NADH dehydrogenase subunit I (NDI), to infer phylogenetic relationships among species of *Natricola*.

2. Materials and methods

2.1. Specimens

We analyzed multiple samples of each nominal species of *Natricola*, spanning most or all of their geographic ranges. We also analyzed samples of undescribed populations from the Snake-Columbia River Basin which have been referred to *Natricola* in the literature. In the absence of a robust phylogenetic hypothesis for the genus *Pyrgulopsis*, we sampled other large-sized regional congeners (note that *Natricola* was diagnosed in part by its large size) to rigorously evaluate monophyly of *Natricola*. These outgroups consisted of eight nominal species and three undescribed populations from the Bonneville, Klamath River, Lahontan, and Sacramento River Basins. *Marstonia agarhecta*, a member of the eastern North American nymphophiline fauna which was previously shown to be sister to the clade containing *Pyrgulopsis* (Hershler et al., 2003a,b), was used to root all trees. We sequenced two specimens from 30 of the 33 samples. Vouchers for each sample were deposited in the National Museum of Natural History, which houses the collections of the former United States National Museum (USNM). Specimen data are summarized in Table 1.

2.2. Laboratory methods

Genomic DNA was extracted from 70% ethanol-preserved individual snails using a CTAB protocol (Bucklin, 1992). Partial sequences of mitochondrial cytochrome *c* oxidase subunit I (COI) and NADH dehydrogenase subunit I (NDI) were obtained. These regions were selected because they have proven useful in resolving relationships within *Pygulopsis* (Hershler and Liu, 2004; Hershler et al., 2003a,b; Liu et al., 2003) and among other members of the family Hydrobiidae (Wilke and Davis, 2000; Wilke et al., 2001). Seven hundred and ten base pairs of COI and 550 bp of NDI were amplified via the polymerase chain reaction using the primers LCO1490 and HCO2198 (Folmer et al., 1994) and ND43F and RND592F (Liu et al., 2003).

The amplification condition described in Liu et al. (2003) was used.

The amplified PCR product was cleaned using the Exo/SAP method. Double-stranded DNA templates were incubated at 37 °C for 30 min and then at 85 °C for another 15 min with five units of Exonuclease I (ExoI, Amersham) and 0.5 unit Shrimp Alkaline Phosphatase (SAP, Amersham). One to five microliters (20 ng) of the cleaned PCR product were used as a template for cycle sequencing reactions in a 10 μl total volume with the CEQ DTCS Quick Start Kit (Beckman Coulter). The following cycling conditions were used: 96 °C for 2 min, then 30 cycles of 96 °C for 20 s, 45 °C for 20 s, and 60 °C for 4 min. The cycle-sequenced products were purified using an ethanol precipitation method following the Beckman Coulter protocol and separated by electrophoresis using a Beckman Coulter CEQ8000 sequencer. Sequences were determined for both strands, and were edited and aligned using Sequencer 3.1.1 (Gene Codes, Ann Arbor, MI).

2.3. Data analyses

Mutational saturation for each codon was examined by plotting the absolute number of transitions and transversions against inferred genetic distance (HKY distance), and by plotting p-distance against inferred distance (HKY distance) (Berbee et al., 1995; Griffiths, 1997; Siemer et al., 1998). No mutational saturation was evident for all three codon positions.

Phylogenetic hypotheses based on distance, parsimony, maximum-likelihood methods were generated using PAUP* 4.0b10 (Swofford, 2002). A Bayesian analysis using MrBayes 3.04 (Huelsenbeck and Ronquiest, 2001) was conducted as another means of estimating phylogeny. Data from the two genes were analyzed separately using unweighted parsimony to detect possible areas of strongly supported incongruence (e.g., De Queiroz, 1993; Wiens, 1998). These were not found and therefore we combined the data for subsequent analysis. The partition homogeneity/incongruence-length difference test implemented in PAUP* further confirmed the absence of significant disagreement among these datasets ($P = 0.10$). The HKY model with some sites assumed to be invariable and with variable sites assumed to follow a discrete Γ distribution (e.g., HKY + I + Γ; Hasegawa et al., 1985) was selected as the best fit for the combined dataset (Modeltest 3.06; Posada and Crandall, 1998).

Maximum-parsimony (MP) analyses were conducted with equal weighting, using the heuristic search option with tree bisection reconnection branch-swapping and 10 random additions. Bootstrapping with 1000 replications (as implemented in PAUP*) was used to evaluate node support. HKY distances were used to generate a neighbor-joining (NJ) tree based on the clustering

Table 1
Locality and voucher information, and GenBank Accession numbers for specimens utilized in this study

Species	Locality (drainage), UTM coordinates, vouchers	Specimen	COI	ND1
Pyrgulopsis (*Natricola*)				
P. hendersoni	Hughet Spring, Harney Basin, Harney, OR (Oregon Lakes), 316730E,	P3B	AY379430[a]	AY426377
	4790580N, Zone 11, USNM 863508	P3D	AY379431[a]	AY426378
	South Fork Malheur River, Malheur Cave Road crossing, Harney, OR	P5B	AY379432[a]	AY426379
	(Snake River), 391960E, 4788860N, Zone 11, USNM 863509	P5C	AY379433[a]	AY426380
	XL Spring, XL Ranch, Abert Lake Basin, Lake, OR (Oregon Lakes),	D33C	AY426348	AY426381
	727710E, 4739300N, Zone 10, USNM 1010682	D33D	AY426349	AY426382
P. idahoensis	Snake River, river mile 538.1, Elmore, ID (Snake River), 637842E,	P179A	AY379426[a]	AY426373
	4755062N, Zone 11, USNM 1010689	P179B	AY379427[a]	AY426374
	Bruneau Arm of C.J. Strike Reservoir, (Bruneau) river mile 3.8,	P171A	AY379424[a]	AY426371
	Owyhee, ID (Snake River), 590156E, 4751733N, Zone 11, USNM	P171C	AY379425[a]	AY426372
	1004890			
	Snake River, river mile 446.8, Canyon, ID (Snake River), 536432E,	IdaG	AY426346	AY426375
	4794105N, Zone 11, USNM 1010691	IdaH	AY426347	AY426376
P. intermedia	Crooked Creek, US 95 crossing, Malheur, OR (Snake River), 440000E,	P1B	AY379442[a]	AY426385
	4739100N, Zone 11, USNM 863510	P1E	AY426351	AY426386
	Crooked Creek Spring, Malheur, OR (Snake River), 439020E, 4738580N,	P2B	AY426352	AY426387
	Zone 11, USNM 863511	P2C	AY426353	AY426388
	Skylight Spring, Barren Valley, Malheur, OR (Oregon Lakes), 411280E,	P4B	AY379444[a]	AY426389
	477880N, Zone 11, USNM 863512	P4C	AY379445[a]	AY426390
	Skylight Spring, Barren Valley, Malheur, OR (Oregon Great Basin),			
	USNM 863512			
	Fall River, Caltrout Public Fishing Access Area, Shasta, CA (Pit River),	D1A	AY197577[b]	AY426408
	626700E, 4549500N, Zone 10, USNM 894699	D1D	AY426357	AY426409
	Pit River, at CA 299 bridge near (upstream of) confluence of Hat	D7A	AY197580[b]	AY426410
	Creek, Shasta, CA (Pit River), 622340E, 4537400N, Zone 10, USNM	D7C	AY426358	AY426411
	1004548			
	Baum Lake (impoundment of Hat Creek), north of Cassel, Shasta, CA (Pit	D8B	AY197579[b]	AY426412
	River), 622400E, 4532260N, Zone 10, USNM 874369			
	Sucker Springs Creek, Shasta, CA (Pit River), 625320E, 4538180N, Zone	D3E	AY197591[b]	AY426421
	10, USNM 1004526	D3F	AY197591[b]	AY426422
	Spring, west side of Link River, west of Klamath Falls, Klamath, OR	D31A	AY197586[b]	AY426406
	(Klamath River), 599535E, 4674920N, Zone 10, USNM 1006053	D31B	AY426356	AY426407
P. robusta	Spring, tributary to Polecat Creek, Teton, WY (Snake River), 522796E,	P178A	AY379436[a]	AY426393
	4886926N, Zone 12, USNM 1009842	P178B	AY379437[a]	AY426394
	Polecat Creek, west of Flagg Ranch, Teton, WY (Snake River), 525321E,	P47B	AF520949[a]	AY426395
	4883884N, Zone 12, USNM 905297			
P. sp.	Teton River, Buxton Bridge crossing, Teton, ID (Snake River), 484753E,	P161A	AY379446[a]	AY426400
	4841148N, Zone 12, USNM 1003706	P161B	AY379447[a]	AY426401
P. sp.	Mud Creek, Birch Creek Valley, Lemnhi, ID (Snake River), 341716E,	P139B	AY426369	AY426429
	4901918N, Zone 12, USNM 905287	P139C	AY426370	AY426430
P. sp.	East Fork Rock Creek, Rockland Valley, Power, ID (Snake River),	P186A	AY426344	AY426431
	353261E, 4713604N, Zone 12, USNM 905313	P186D	AY426345	AY426432
P. sp.	Columbia River, East Mayer State Park, Wasco, OR (Columbia River),	D30A	AY379438[a]	AY426396
	635140E, 5059360N, Zone 10, USNM 1010683	D30B	AY379439[a]	AY426397
P. sp.	Columbia River, Celilo State Park, Wasco, OR (Columbia River),	P49C	AY379440[a]	AY426398
	658920E, 5057100N, Zone 10, USNM 894695	P49D	AY379441[a]	AY426399
Outgroups				
P. archimedis	Upper Klamath Lake, Hagelstein Park outlet, Klamath, OR (Klamath	P50A	AF520950[c]	AY426402
	River), 597740E, 4693000N, Zone 10, USNM 894697	P50C	AY426355	AY426403
P. gibba	Spring, west of Fee Reservoir, Surprise Valley, Lassen, CA	P134B	AY197603[b]	AY426413
	(Lahontan), 746112E, 4636572N, Zone 10, USNM 1002892	P134D	AY426359	AY426414
P. imperialis	Spring, Thacker Pass, Kings River Valley, Humboldt, NV	P140A	AY379450[a]	AY426383
	(Lahontan), 407808E, 4617216N, Zone 11, USNM 1002354	P140C	AY426350	AY426384

Table 1 (*continued*)

Species	Locality (drainage), UTM coordinates, vouchers	Specimen	COI	ND1
P. inopinata	Spring, Glenwood, Sevier River drainage, Sevier, UT (Bonneville), 414622E, 4291558N, Zone 12, USNM 894843	P100A P100C	AY426360 AY426361	AY426415 AY426416
P. kolobensis	Big Malad Spring, Malad Valley, Oneida, ID (Bonneville), 387402E, 4675204N, Zone 12, USNM 1003673	P162C P162D	AY379449[a] AY426354	AY426391 AY426392
P. militaris	Spring, Soldier Meadow, Humboldt, NV (Lahontan), 314127, 4585192, Zone 11, USNM 1002576	P147A P147C	AY197596[b] AY426362	AY426417 AY426418
P. pilsbryana	Spring, Saint Charles Creek, Bear Lake Valley, Bear Lake, ID (Bonneville), 463096E, 4662541N, Zone 12, USNM 905389	P137B P137C	AY426363 AY426364	AY426419 AY426420
P. ventricosa	Spring, Seigler Canyon, Clear Lake Basin, Lake, CA (Sacramento River), 528195E, 4303155N, Zone 10, USNM 894819	P87B P87C	AY426365 AY426366	AY426423 AY426424
P. sp.	Lost River, near Horsefly Irrigation District, Klamath, OR (Klamath River), 632150E, 4672660N, Zone 10, USNM 894698	P51A P51B	AY197584[b] AY197585[b]	AY426404 AY426405
P. sp.	Big Springs, Bonanza, Klamath, OR (Klamath River), 632150E, 4672820N, Zone 10, USNM 1016099	D23A D23C	AY197587[b] AY426367	AY426425 AY426426
P. sp.	Sprague River, north of Beatty, Lake, OR (Klamath River), 642220E, 4702100N, Zone 10, USNM 1016100	D25A D25B	AY197589[b] AY426368	AY426427 AY426428
M. agarhecta	Bluff Creek, Hwy 129 crossing, Pulaski, GA (Ocmulgee River), 271961E, 3557991N, Zone 17, USNM 894686	P37A	AF520934[c]	AY426433

[a] Sequence reported by Hershler and Liu (2004).
[b] Sequence reported by Hershler et al. (2003a,b).
[c] Sequence reported by Hershler et al. (2003a,b).

method of Saitou and Nei (1987). Node support was assessed by completion of 1000 bootstrap replications (Felsenstein, 1985) in PAUP*, using the fast-search option. Maximum-likelihood (ML) analyses were based on the HKY + I + Γ model with empirical base frequencies using the heuristic search algorithm. A neighbor-joining tree (HKY distances) was used as the initial topology for branch-swapping. Node support was evaluated by 100 bootstrap pseudoreplicates. Bayesian analyses were performed based on the HKY model with invariable and variable sites with a discrete gamma distribution (e.g., HKY + I + Γ; Hasegawa et al., 1985) model of evolution. Several short runs were first conducted using the default random tree option to determine when the log likelihood sum reached a stable value (by plotting the log-likelihood scores of sample points against generation time). The log likelihood sum started at approximately −14,500 and reached a stable equilibrium at approximately −4665 after about 20,000 generations. Then metropolis-coupled MCMC simulations were run with four chains using the default random tree option for 1,000,000 generations and Markov chains were sampled at intervals of 10 generations to obtain 100,000 sample points. The last 97,000 sampled trees with branch lengths (the first 3000 trees having been removed as "burn-in") were used to generate a 50% majority rule consensus tree. The percentage of samples that recovered specific clades on this topology represents that clade's posterior probability; these are the P values,

and $P \geqslant 95\%$ was considered evidence of significant support for a clade (Huelsenbeck and Ronquiest, 2001).

Sequence divergence (average number of nucleotide differences per site between groups) and standard error were calculated using MEGA 2 (Kumar et al., 2001). An a priori hypothesis of *Natricola* monophyly was explicitly tested using the Kishino–Hasegawa (Kishino and Hasegawa, 1989) and Templeton tests (Templeton, 1983) implemented in PAUP*. A hypothesis of constant evolutionary rate (molecular clock) was tested using a standard likelihood ratio test. The likelihood of the constrained tree was compared to the likelihood of the same tree with the molecular clock enforced using a χ^2 distribution with the degrees of freedom equal to the number of taxa−2. This test indicated that our data do not support a molecular clock null hypothesis ($P < 0.0001$).

Drainage basin occurrence was treated as an unordered character and mapped onto the preferred phylogenetic hypothesis using MacClade 3.0 (Maddison and Maddison, 1992) in order to evaluate the hypothesis of vicariance associated with fragmentation of the Snake paleodrainage.

3. Results

Ninety of 126 sequences utilized in our analyses are newly reported herein (Table 1) and have been deposited

in GenBank under Accession Nos. AY426344–AY426433. The alignment of COI sequences yielded 619 bp, of which 162 sites were variable (26.2%) and 116 were parsimony informative (18.7%). For NDI, 530 bp were sequenced, of which 179 sites were variable (33.8%) and 138 were parsimony informative (26%).

All analyses of the combined mitochondrial gene sequences strongly supported a *Natricola* clade composed of *P. hendersoni*, *P. idahoensis*, *P. robusta* (the type species of the subgenus), *P. intermedia* populations from the Oregon Lakes region and Snake River drainage, and undescribed populations from the Columbia River Basin (Fig. 2, a Bayesian 50% majority rule topology with branch lengths). The remaining taxa that were previously assigned to *Natricola*, consisting of *P. intermedia* populations from the Pit and Klamath Basins, and undescribed populations from Snake River tributaries in Idaho, were excluded from this clade in all cases (Fig. 2).
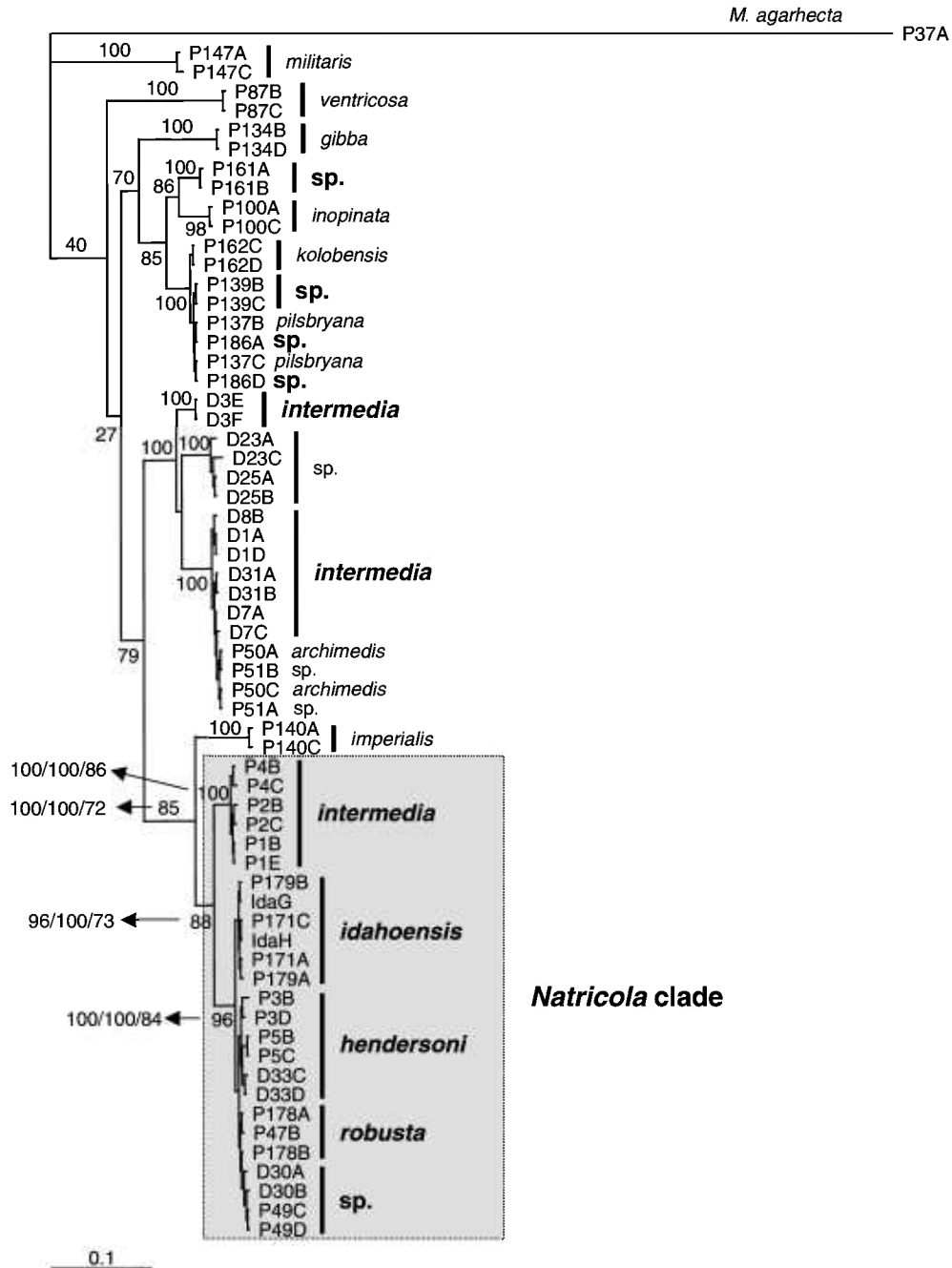

Fig. 2. A Bayesian 50% majority rule topology with branch lengths, based on combined COI and NDI mitochondrial DNA sequences, depicting phylogenetic relationships among populations and species of the subgenus *Pyrgulopsis* (*Natricola*) (boldfaced, larger font) and other congeners from the northwestern United States. Posterior probabilities associated with individual nodes are shown, and bootstrap values for the MP, NJ, and ML trees, respectively are also provided for clades discussed in the text.

A sister relationship between the *Natricola* clade and *P. imperialis* (from the Quinn River drainage, northwest Nevada; Fig. 1) was strongly supported in all analyses. All analyses also strongly supported division of *Natricola* into two subclades, one composed of populations of *P. intermedia* from southeastern Oregon, and the other containing *P. hendersoni*, *P. idahoensis*, *P. robusta*, and undescribed populations from the Columbia River.

The MP analysis yielded 1152 equally parsimonious trees (TL = 649) whose topologies varied in placement of the clade composed of D3E-F (*P. intermedia*), which was as in Fig. 2 or alternatively as sister to a clade composed of *P. archimedis*, *P. intermedia*, and *P.* sp. (P51). In addition, trees differed in the relative positions of terminals within each of the well supported subclades. The NJ trees were identical to the Bayesian topology. The ML analysis yielded results that differed from the Bayesian topology only in terms of the clade containing *P. pilsbryana*, *P.* sp. (P139, P161, and P186), *P. kolobensis*, and *P. inopinata*, which was positioned basally instead of as in Fig. 2.

When monophyly of *Natricola* (sensu lato, see Section 1 above) was forced using the CONSTRAINT function in PAUP*, the shortest trees were 758 steps. The resulting topology was significantly different from the most parsimonious trees based on the Kishino–Hasegawa ($t = 9.76, P < 0.0001$) and Templeton tests ($Z = 9.30, P < 0.0001$), providing additional justification for rejecting a hypothesis of monophyly of this group.

4. Discussion

4.1. Taxonomic implications

Our finding that *Natricola* is polyphyletic is not altogether surprising given that this subgenus was but loosely diagnosed by two non-unique characters (see Hershler, 1994). Our ongoing study of the molecular systematics of *Pyrgulopsis* has determined that other traditional groupings of these snails also appear to be composites of divergent lineages and need to be re-evaluated with the aid of more robust morphological datasets (in preparation; also see Liu et al., 2003).

Based on the results of this study and accompanying morphological analysis, elsewhere we are transferring the Klamath and Pit River populations of *P. intermedia* to another species (Hershler et al., 2003a,b), and placing *P. hendersoni*, *P. idahoensis*, and Columbia River populations in synonymy with *P. robusta* (Hershler and Liu, 2004).

4.2. Biogeography and regional drainage history

Our findings indicate that the distribution of *Natricola* does not well "trace" a previously hypothesized early route of the Snake River through southeast Oregon and northern California to the Pacific. *Natricola* instead is restricted to the (modern) Snake-Columbia River Basin and the Oregon Lakes region, whereas populations (assigned to this subgenus) from more downflow segments of the hypothesized paleodrainage (Klamath, Pit River Basins) belong to other *Pyrgulopsis* lineages. (Note that fossil "*Natricola*" from the Lahontan Basin in northeastern California cannot be confidently assigned to the *Natricola* clade based on its shell.) Our findings also indicate that neither the Snake-Columbia River Basin nor Oregon Lakes members of the *Natricola* clade form monophyletic groups in accordance with the Snake paleodrainage hypothesis (Fig. 3). Our data imply that this clade originated within the confines of the modern Snake River Basin and subsequently invaded the Oregon Lakes region at least twice, e.g., within *P. hendersoni* and *P. intermedia* (Fig. 3). The shallow geographic structuring of these two species does not suggest vicariance associated with late Pliocene severance of the postulated drainage outlet from the western Snake River Plain to the Oregon Lakes region. The average COI sequence divergence between Snake River Basin (P1, P2) and Oregon Lakes (P4) populations of *P. intermedia* is 0.00081 with a SE of 0.00076 while populations of *P. hendersoni* from these drainages (P3, D33 vs. P5, respectively) differ by 0.0004 with a SE of 0.00039. If divergence of these two sets of populations was initiated by capture of the Snake River (by the Columbia River) 2.0 Ma, then a COI clock rate of 0.02–0.04% per million years is implied. This is a much slower COI clock rate than has been estimated for other hydrobiid snails (Wilke, 2003) and for invertebrates generally (Brown et al., 1979). Furthermore this slow COI clock rate is not consistent with the fossil record. The COI sequence divergence between species of *Pyrgulopsis* and closely related eastern North American *Floridobia* (Hershler et al., 2003a,b) ranged from 8.54 to 12.34%. If we assume an evolutionary rate of 0.02–0.04% per million years (per the above), then 617–213 Ma divergence of these two genera is implied! However, the oldest fossils belonging to either of these genera are only 15–11 Ma (*Pyrgulopsis truckeensis*, lower member of Truckee Formation, northwest Nevada; age estimated from Firby, 1993; Stewart and Perkins, 1999).

The traditional model for regional drainage history was recently criticized by Repenning et al. (1995), who suggested that southeast Oregon has been an uplifted highland since the mid-Miocene (15 Ma) and thus could not have been crossed by the late Neogene Snake paleoriver. They argued, on the basis of mammalian biogeography and distribution of basinal deposits roughly contemporaneous with the inferred Snake paleoriver, that this drainage instead flowed south from the western Snake River Plain into northern Nevada before assuming

its westward route to the Pacific (also see Link and Fanning, 1999; Link et al., 2003; Wagner et al., 1997). This hypothesis (which was dismissed by Smith et al., 2000) is congruent with the (well supported) sister relationship between the *Natricola* clade and *P. imperialis* (Fig. 2), and suggests that fragmentation of the Snake paleoriver, which Repenning et al. (1995) attributed to 4.75–4.5 Ma inception of regional basin and range faulting, was associated with the origin of *Natricola*

rather than with subsequent branching events within this clade. [Note that this phylogenetic relationship is conserved in a sampling of almost all species of *Pyrgulopsis* (in preparation).] Within the context of this model the (subsequent) transition between the Snake River and Oregon Lakes region that occurred within the *Natricola* clade may be attributed to a geologically recent connection between these areas that was unrelated to the ancestral route of the Snake River. Shoreline
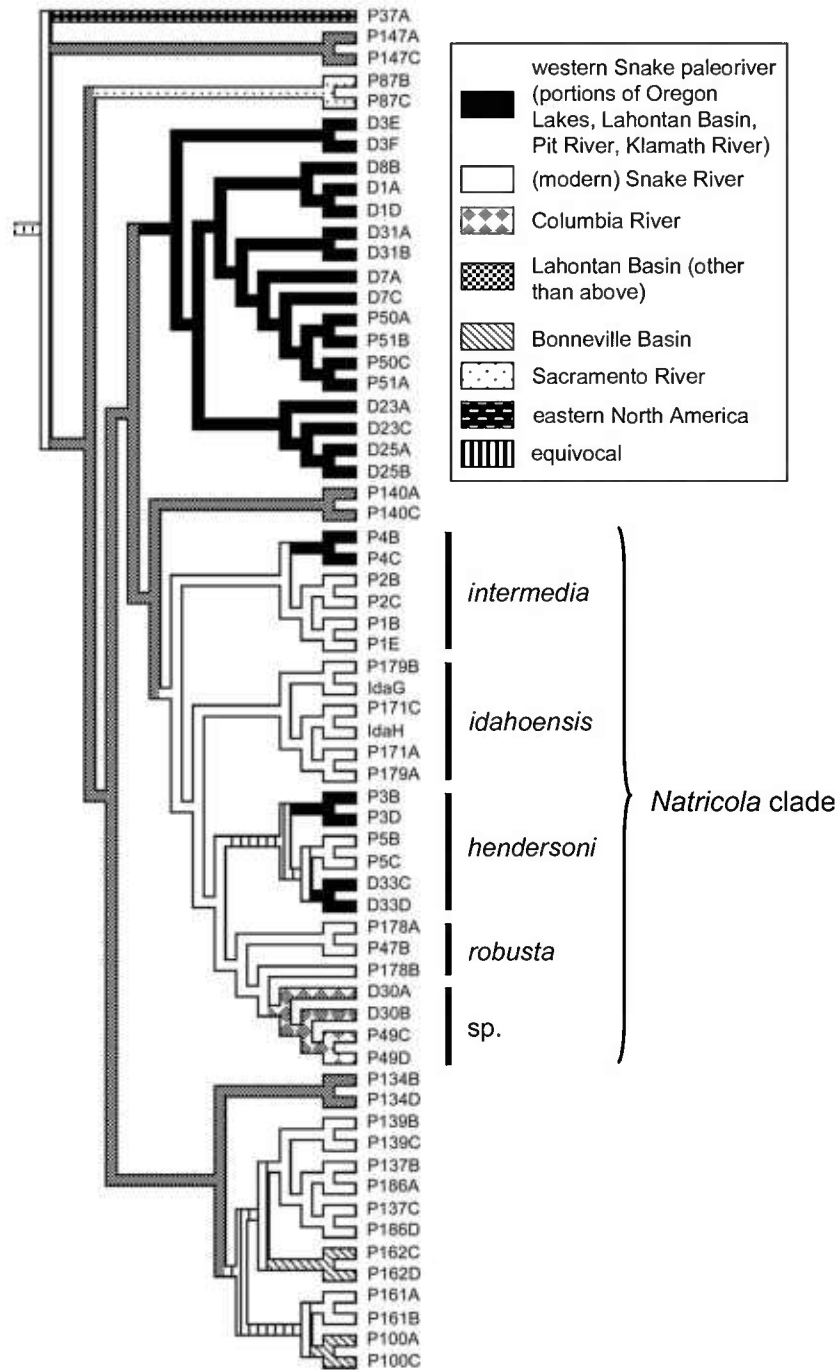

Fig. 3. Phylogenetic reconstruction of drainage occurrences (based on Fig. 2). Note the presence of multiple transitions between eastern (modern Snake River Basin) and western segments of the Snake paleoriver within the *Natricola* clade.

evidence indicates that Pleistocene Lake Malheur (Oregon Lakes region, Fig. 1) periodically spilled through the Malheur Gap to the Snake River Basin until becoming blocked by the Voltage basalt flow ca. 32 Ka (Camp et al., 2003; Gehr and Newman, 1978) and also subsequently overflowed its sill during several Holocene high stands (Dugas, 1998). This prior zone of aquatic continuity would have provided ample opportunities for inter-basinal dispersal of these snail species, both of which currently straddle the local divide between these areas (Fig. 1). Wilke (2003) recently demonstrated that the Folmer COI fragment cannot resolve soft polytomies within a time frame of <250 Ka, and thus this short interval cannot be used to confidently calibrate a molecular clock. However, if a 32 Ka date for the divergence of populations of these two areas is used for calibration, a COI clock rate of 1.25–2.5% per million years is obtained, which well conforms to other estimates for this gene (Wilke, 2003). This clock rate implies 9.8–3.4 Ma divergence between *Pyrgulopsis* and *Floridobia*, which is fairly consistent with the known fossil record (see above paragraph). [Note that since most members of *Pyrgulopsis* have closely similar shells the fossil record of these snails is little informative at the species level (Hershler and Sada, 2002) and cannot be used to delineate the actual dates of these divergences.]

Our findings also imply geologically recent dispersal of *Natricola* within the Snake-Columbia River Basin. As discussed above, the Columbia River did not become integrated with the Snake River until the late Pliocene. The extant populations in the lower reach of this river (*Pyrgulopsis* sp., D30, P49) presumably were founded by invasion from the Snake River following its capture (by a tributary of the Columbia River). This downflow migration of snails perhaps was facilitated by the regionally extensive late Pleistocene flooding (Baker and Bunker, 1985; O'Connor, 1993). The Wyoming headwaters of the Snake River inhabited by *P. robusta* are also recent additions to the main river (Taylor, 1985). This area drained eastward before becoming captured by the Snake River in the Pliocene (Link et al., 2003; Pierce and Morgan, 1999) and presumably was subsequently colonized by *Natricola* as a result of migration upflow. The broadly disjunct distribution of *Natricola* within the Snake-Columbia River Basin (Fig. 1) is surprising given the minimal genetic differentiation and presumed dispersal ability of these snails. We can but speculate that the modern distribution of these snails may be constrained by ecological factors such as a requirement for clean, spring-enriched waters.

Acknowledgments

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