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Ancient vicariance and recent dispersal of springsnails (Hydrobiidae: Pyrgulopsis*) in the Death Valley system, California-Nevada*

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ABSTRACT

The Death Valley system (southeastern California and southwestern Nevada) contains a locally endemic aquatic biota that has long been the subject of compelling biogeographic speculation, yet it remains little studied phylogenetically. Springsnails (Hydrobiidae: *Pyrgulopsis*) are one of the most diverse elements of this fauna, and they are thought to have evolved in association with late Tertiary rearrangements of landscape and drainage. We assembled a molecular phylogeny for this fauna to investigate its evolutionary development in relation to regional geological history. Sequences for two mitochondrial genes were obtained from 80 populations representing 13 of the 14 Death Valley system springsnail species, and 31 extralimital congeners. Combined analyses of the 1188 base-pair data set consistently depicted the Death Valley system fauna as a polyphyletic assemblage of eight or nine lineages. Based on a molecular clock, the six lineages endemic to the Death Valley system were estimated to be minimally Pliocene in age, which is concordant with inception of regional topographic closure during this time period. The single endemic lineage with a well-resolved sister relationship was closest to a species from the upper Gila River basin, which also suggests an old divergence event. Three other lineages shared a pattern of shallow structuring (divergence events younger than 0.7 Ma) across multiple drainage basins, some of which have long been isolated. This suggests that, contrary to previous thought, regional springsnail biogeography has been shaped in part by geologically recent (Pleistocene) dispersal, and, in some places, it has occurred by means other than spread through continuous reaches of aquatic habitat.

Keywords: Death Valley region, biogeography, mtDNA, Gastropoda.

INTRODUCTION

The Death Valley system (Fig. 1) in southeastern California and southwestern Nevada is composed of a number of endorheic valleys and basins (including the Amargosa, Mojave, and Owens River basins) that were integrated by chains of spilling pluvial lakes during the late Quaternary; Death Valley forms the terminus of this extensive drainage (Miller, 1943). The Death Valley system contains a locally endemic aquatic fauna (Sada et al., 1995; Sada and Vinyard, 2002) that has long been the subject of compelling

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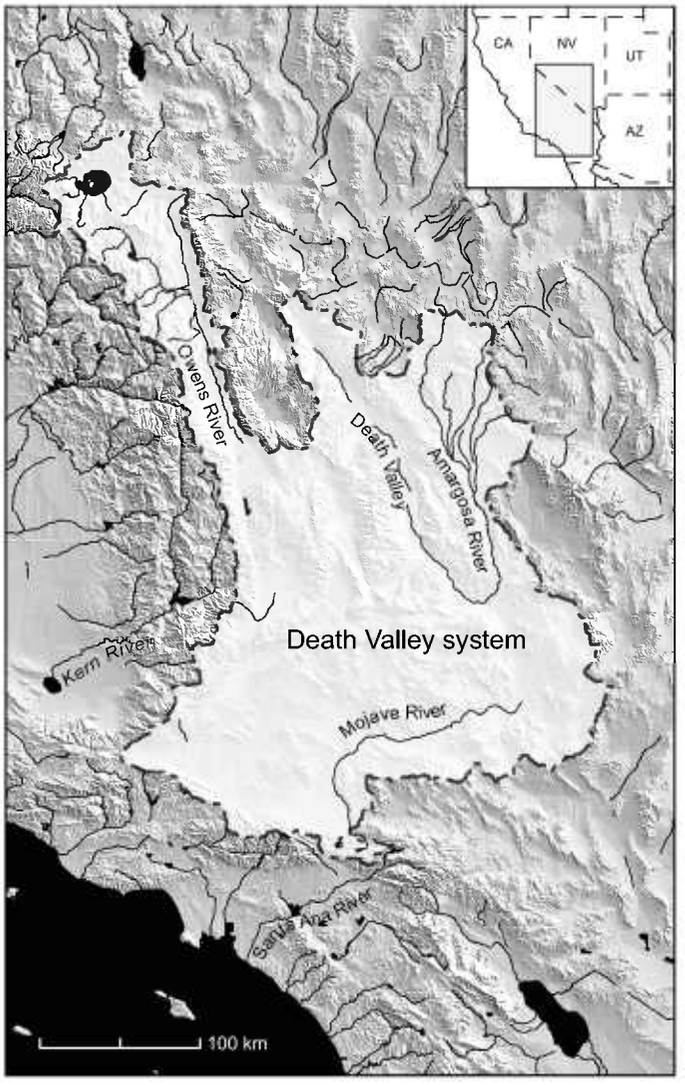

Figure 1. Map showing location of the Death Valley system in southeastern California and southwestern Nevada.

biogeographic inquiries. In a series of classic biogeographic studies, Hubbs and Miller attempted to correlate the distributions of Death Valley system (and other western North American) fishes with the expansion and contraction of regional drainage during the late Quaternary (e.g., Miller, 1946; Hubbs and Miller, 1948). Subsequent investigators have instead emphasized the role of middle to late Tertiary rearrangements of topography and drainage in shaping the biogeographic history of Death Valley system aquatic fauna (e.g., Taylor, 1985; Minckley et al., 1986; Polhemus and Polhemus, 2002). Recent molecular phylogenetic studies of cyprinodontid pupfishes (*Cyprinodon*) (Echelle and Dowling, 1992; Echelle et al., 2005) and cochliopid gastropods (*Tryonia*) (Hershler et al., 1999a, 1999b) have documented deeply divergent Death Valley system lineages consistent with ancient origins; however, most of the regional fauna has yet to be studied phylogenetically.

The hydrobiid gastropod genus *Pyrgulopsis* (= *Fontelicella* of authors) is one of the most ubiquitous and diverse elements (14 species; Fig. 2) of the Death Valley system aquatic fauna (Hershler and Sada, 1987; Hershler, 1989, 1998; Hershler and Pratt, 1990). This large genus (126 species; Liu and Hershler, 2005) ranges from the Columbia River basin to the Mexican Altiplano and from just east of the continental divide to the Pacific margin (Hershler, 1994; Hershler and Gustafson, 2001). The tiny, gill-breathing species of *Pyrgulopsis,* which are commonly known as springsnails, have been popular subjects of western biogeographic studies owing to their antiquity and close linkage with aquatic systems (e.g., Taylor, 1985; Hershler and Pratt, 1990; Hershler and Sada, 2002). The fossil record of *Pyrgulopsis* extends from the middle to late Miocene (Hershler and Liu, 2004a), and diversification of the modern fauna is considered to be a product of late Tertiary tectonic and hydrographic events (Taylor, 1966, 1985). Congeners are restricted to perennial waters throughout their entire life cycle, do not tolerate desiccation well, and consequently are thought to be poor dispersers capable of spread only within their habitats (Taylor, 1985; Taylor and Bright, 1987). Springsnail species are distinguished largely on the basis of penial morphology, which displays great variation within the genus (Taylor, 1987; Hershler, 1994). However, phylogenetic relationships within the genus cannot be well resolved using morphological criteria because of extensive homoplasy (evolutionary convergence) and paucity of informative characters (Hershler, 1994), necessitating the use of a molecular approach.

In contrast with *Cyprinodon* and *Tryonia,* which live in highly mineralized, thermal springs and streams in Death Valley system lowlands (Miller, 1948; Hershler and Sada, 1987; Hershler, 1989), *Pyrgulopsis* ranges among both basinal and upland settings and inhabits diverse spring-fed waters within this region (Hershler and Sada, 1987; Hershler, 1989). Seven congeners are restricted to the Amargosa River basin (Fig. 1) (*P. amargosae, P. crystalis, P. erythropoma, P. fairbanksensis, P. isolata, P. nanus, P. pisteri*), and three are endemic to Owens Valley (*P. aardahli, P. owensensis, P. perturbata*). The remaining four range among the Death Valley system and one or more geographically proximal, extralimital basins (*P. giulianii, P. micrococcus, P. turbatrix, P. wongi*). One of these widely distributed species (*P. micrococcus*) was recently shown to be a composite of genetically divergent lineages (Liu et al., 2003), which suggests that the current taxonomy of this fauna underestimates species diversity and requires revision.

Herein we provide a molecular phylogenetic perspective on the biogeographic history of the Death Valley system springsnails. Our phylogenetic analysis is based on a large body of recently published and newly obtained deoxyribonucleic acid (DNA) sequence data from two mitochondrial genes, and analyses included representatives of all of the Death Valley system springsnail species except *P. aardahli,* as well as 31 other congeners from extralimital drainages. Our specific goals were to delineate the evolutionarily distinct lineages of Death Valley system springsnails, establish their sister relationships and levels of divergence, and investigate the relative importance of late Tertiary and Quaternary events in structuring the biogeographic history of this diverse assemblage of presumably poorly dispersing animals.

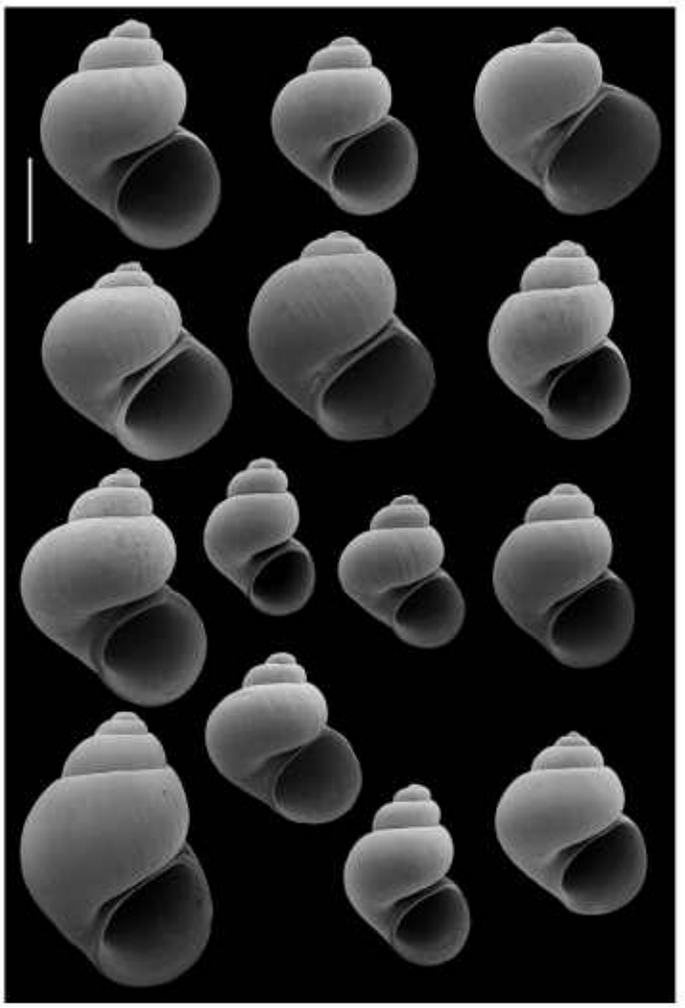

Figure 2. Scanning electron micrographs of shells of the Death Valley system springsnails. Top row (left to right), *P. aardahli, P. amargosae, P. crystalis;* second row, *P. erythropoma, P. fairbanksensis, P. giulianii;* third row, *P. isolata, P. micrococcus, P. nanus, P. owensensis;* bottom row, *P. perturbata, P. pisteri, P. turbatrix, P. wongi.* Scale = 1.0 mm.

MATERIALS AND METHODS

Sampling

We analyzed a combination of 42 new and 118 previously published sequences of mitochondrial cytochrome *c* oxidase subunit I (COI) and reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase subunit I (NDI). Sequences were obtained from 39 populations representing all previously described members of the Death Valley system springsnail fauna except *P. aardahli,* the single known population (Hershler, 1989) of which is on private land and was not accessible to us. One or two populations were sampled for each of nine narrowly distributed species, and 2 to 15 populations were sampled for the four congeners that range among multiple basins. We also sampled three undescribed species that were recently discovered in the Death Valley system.

In order to evaluate the phylogenetic relationships of the diverse Death Valley system fauna, we also included in our analyses sequences from 37 other populations from western California (five species) and the Bonneville (northwestern Utah; one species), lower Colorado River (Arizona-California border; 24 species), and Lahontan (northwestern Nevada; two species) basins (Fig. 1). Our extralimital sampling focused on congeners that were geographically proximal to the Death Valley system and (or) have been previously shown to be closely related to Death Valley system taxa (Liu et al., 2003; Liu and Hershler, 2005). The type species of the eastern North American genus *Floridobia* (*F. floridana*), which was previously shown to be closely related to *Pyrgulopsis* (Liu and Hershler, 2005), was used to root all trees.

Although multiple specimens were sequenced from each sample and included in preliminary analyses, only single exemplars were utilized in the preparation of final trees owing to the large size of the data set (80 total samples). Samples newly obtained for this study were collected with a fine hand sieve and preserved in concentrated (90%) ethanol. Voucher material for all sequences utilized in this study was reposited in the collections of the National Museum of Natural History, Smithsonian Institution (USNM). Collection localities and Genbank accession numbers are listed in Table 1.

DNA Extraction, PCR Amplification, and DNA Sequencing

Genomic DNA was isolated from individual snails using a CTAB (cetyl trimethyl ammonium bromide) protocol (Bucklin, 1992). For the COI gene, COIL1490 and COIH2198 (Folmer et al., 1994) were used to amplify a 710 base-pair (bp) fragment via polymerase chain reaction (PCR). For the NDI gene, ND43F and RND592F (Liu et al., 2003) were used to amplify an ~570 bp fragment. The amplification and cycle sequencing conditions were those described in Liu et al. (2003). These two genes were used because they have provided satisfactory phylogenetic resolution in previous population- and species-level studies of *Pyrgulopsis* (Hershler et al., 2003a, 2003b; Liu et al., 2003; Hurt, 2004; Hershler and Liu, 2004a, 2004b; Liu and Hershler, 2005).

Data Analysis

Sequences were determined for both strands and then edited and aligned using Sequencher™ version 3.1.1. Since the COI and NDI genes belong to a single linkage group (mitochondrial genome) and have been found to evolve similarly (Liu and Hershler, 2005), they were combined and analyzed together. Base compositional differences were evaluated using the χ^2 test. Modeltest 3.7 (Posada and Crandall, 1998) was used to obtain the most appropriate substitution model (using the Akaike information criterion) and parameter values for data sets.

Phylogenetic relationships were inferred using four different methodologies of phylogenetic reconstruction: genetic distance, maximum parsimony (MP), maximum likelihood (ML), and Bayesian inference. The distance, MP, and ML analyses were

TABLE 1. SAMPLES, CODES, LOCALITIES, AND GENBANK ACCESSION NUMBERS

Pyrgulopsis species	Specimen code	Locality	COI	NDI
Death Valley system				
amargosae	Pamer2	Saratoga Spring, Death Valley, Amargosa River basin, San Bernardino Co., California	AY367479*	AY367537*
crystalis	–	Crystal Pool, Ash Meadows, Amargosa River basin, Nye Co., Nevada	AY367482*	AY367541*
erythropoma	–	Kings Pool (outflow), Point of Rocks, Ash Meadows, Amargosa River basin, Nye Co., Nevada	AY367484*	AY367543*
fairbanksensis	–	Fairbanks Spring, Ash Meadows, Amargosa River basin, Nye Co., Nevada	AY627921†	AY628043*
giulianii	M23A	Stream, Sand Canyon, Indian Wells Valley, Kern Co., California	AF520937§	AY367545*
giulianii	P21A	Dougherty Creek, Hwy 178 crossing, Kern River basin, Kern Co., California	DQ364018	DQ364039
isolata	–	Spring south of Clay Pits, Ash Meadows, Amargosa River basin, Nye Co., Nevada	AY367486*	AY367547*
micrococcus	M13B	Stream, Hanaupah Canyon, Death Valley, Amargosa River basin, Inyo Co., California	AY367449*	AY367513*
micrococcus	M15M	Stream, Hall Canyon, Panamint Valley, Inyo Co., California	AY367456*	AY367519*
micrococcus	M16M	Spring, Snow Canyon, Panamint Valley, Inyo Co., California	AY367458*	AY367520*
micrococcus	M19O	Cushenbury Springs, Southern Mojave basin, San Bernardino Co., California	AY367461*	AY367523*
micrococcus	M1A	Spring, Fleur de Lis Ranch, Oasis Valley, Amargosa River basin, Nye Co., Nevada	AF520944§	AY367492*
micrococcus	M20N	Springs at Big Bear Ranger Station, Santa Ana River basin, San Bernardino Co., California	AY367462*	AY367524*
micrococcus	M24N	Tennessee Spring, Panamint Valley, Inyo Co., California	AY367468*	AY367527*
micrococcus	M25A	Spring north of Tecopa Hot Springs, Amargosa River basin, Inyo Co., California	AY367469*	AY367529*
micrococcus	M26C	Shoshone Spring, Amargosa River basin, Inyo Co., California	AY367473*	AY367532*
micrococcus	M2N	Grapevine Springs, Death Valley, Amargosa River basin, Inyo Co., California	AY367430*	AY367494*
micrococcus	M30C	Spring south of Clay Pits, Ash Meadows, Amargosa River basin, Nye Co., Nevada	AY367477*	AY367536*
micrococcus	M3O	Spring east of Scottys Castle, Death Valley, Amargosa River basin, Inyo Co., California	AY367434*	AY367499*
micrococcus	P197A	Purgatory Spring, Ash Meadows, Amargosa River basin, Nye Co., Nevada	DQ364001	DQ364022
micrococcus	M18O	Saline Marsh, Saline Valley, Inyo Co., California	AY367460*	AY367522*
micrococcus	M21M	Springs, Thurman Flats, Mill Creek Canyon, Santa Ana River basin, San Bernardino Co., California	AY367463*	AY367525*
nanus	–	Five Springs, Ash Meadows, Amargosa River basin, Nye Co., Nevada	AY367487*	AY367548*
owensensis	–	Spring in canyon south of Piute Creek, Owens River basin, Mono Co., California	AF520922§	AY367549*
perturbata	–	"Northeast Spring," Fish Slough, Owens River basin, Mono Co., California	AY367488*	AY367550*
pisteri	–	Marsh Spring, Ash Meadows, Amargosa River basin, Nye Co., Nevada	DQ364004	DQ364025
turbatrix	P196D	Grapevine Springs, Nye County, Amargosa River basin, Nevada	DQ364000	DQ364021
turbatrix	P11A	Horseshutem Springs, Pahrump Valley, Nye Co., Nevada	AF520936§	AY367555*
turbatrix	P84A	Cold Creek Spring, Indian Springs Valley, Clark Co., Nevada	DQ364014	DQ364035
turbatrix	P86A	Spring, Lost Creek Canyon, Las Vegas Wash, Clark Co., Nevada	DQ364015	DQ364036
wongi	P185C	Spring along lower Pine Creek, Owens River basin, Inyo Co., California	AY627956†	DQ251086#
wongi	W1A	Spring near Conway Summit, Mono Lake basin, Mono Co., California	DQ251052#	DQ251078#
wongi	W22A	Spring along Lubken Creek, Owens River basin, Inyo Co., California	DQ251071#	DQ251099#
wongi	W2B	Springs at Little Lake, Rose Valley, Inyo Co., California	DQ251053#	DQ251079#
wongi	W4A	River Spring, Adobe Valley, Mono Co., California	DQ251056#	DQ251080#
wongi	W11A	Jacks Spring, Teels Marsh, Mineral Co., Nevada	DQ251056#	DQ251088#
wongi	W13B	Spring east of McNett Ranch, Fish Lake Valley, Esmeralda Co., Nevada	DQ251063#	DQ251090#
wongi	W15A	Corral Springs, Deep Springs Valley, Inyo Co., California	DQ251064#	DQ251091#
wongi	W5B	Spring in Clark Canyon, Walker River basin, Mono Co., California	DQ251057#	DQ251082#
P. n. sp. 1	–	Spring near north end of Amargosa Gorge, Amargosa River basin, Inyo Co., California	DQ364002	DQ364023
P. n. sp. 2	–	Saratoga Spring, Death Valley, Amargosa River basin, San Bernardino Co., California	DQ364003	DQ364024
P. n. sp. 3	–	Grapevine Springs, Nye County, Amargosa River basin, Nevada	DQ363999	DQ364020
Extralimital drainage				
arizonae	–	Medicine Spring, Bylas, Gila River basin, Graham Co., Arizona	AY627948†	AY628072†
avernalis	–	Muddy Spring, Moapa Valley, Clark Co., Nevada	AF520930§	AY628042†
bacchus	–	Tassi Spring, Grand Wash, Colorado River basin, Mojave Co., Arizona	DQ364005	DQ364026
breviloba	–	Flag Springs (middle), White River Valley, Nye Co., Nevada	AY627928†	AY628050†
californiensis	P30A	Spring tributary to Lytle Creek, Santa Ana River basin, San Bernardino Co., California	DQ364019	DQ364040
californiensis	P31A	Spring tributary to Snow Creek, Santa Ana River basin, Riverside Co., California	AY367481*	AY367540*

(*continued*)

TABLE 1. SAMPLES, CODES, LOCALITIES, AND GENBANK ACCESSION NUMBERS (*continued*)

Pyrgulopsis species	Specimen code	Locality	COI	NDI
Extralimital drainage (*continued*)				
californiensis	P33A	Spring tributary to Campo Creek, Tijuana River basin, San Diego Co., California	AY627924†	AY628046†
carinifera	–	Muddy Spring, Moapa Valley, Clark Co., Nevada	AY627920†	AY628041†
conica	–	Dripping Spring, Sacramento Wash, Colorado River basin, Mohave Co., Arizona	AY627958†	AY628083†
deaconi	–	Red Spring, Las Vegas Valley, Clark Co., Nevada	AY367483*	AY367542*
deserta	–	Springs tributary to Virgin River, Littlefield, Mohave Co., Arizona	DQ251077#	DQ251106#
diablensis	–	Stream, Del Puerto Canyon, San Joaquin River basin, Stanislaus Co., California	AY627922†	AY628044†
fausta	–	Corn Creek Springs, Las Vegas Valley, Clark Co., Nevada	AY367485*	AY367544*
gibba	P134B	Springs west of Fee Reservoir, Surprise Valley, Modoc Co., California	AY197603**	AY426413††
gibba	P192A	Spring along Hwy 395 below Bridgeport Reservoir, Walker River basin, Mono Co., California	DQ364016	DQ364037
gilae	–	Spring tributary to East Fork Gila River, Grant Co., New Mexico	AY627952†	AY628076†
glandulosa	–	Nelson Place Spring, Verde River basin, Yavapai Co., Arizona	AY627959†	AY628084†
gracilis	–	Emigrant Springs (north), White River Valley, Nye Co., Nevada	DQ364011	DQ364032
greggi	–	Grapevine Creek, Ft. Tejon State Park, San Joaquin River basin, Kern Co., California	AF520943§	AY367546*
hubbsi	–	Crystal Spring, Pahranagat Valley, Lincoln Co., Nevada	AY627918†	AY628039†
kolobensis	P117A	Toquerville Springs, Virgin River basin, Washington Co., Utah	AY627939†	AY628063†
kolobensis	P193B	Spring, southwest of Pinto, Bonneville basin, Washington, Co., Utah	DQ364008	DQ364029
kolobensis	P194A	Spring, Pine Valley, Santa Clara River basin, Washington Co., Utah	DQ364009	DQ364030
kolobensis	P195A	Spring, Kershaw–Ryan State Park, Meadow Valley Wash, Lincoln Co., Nevada	DQ364010	DQ364031
lata	–	Butterfield Springs, White River Valley, Nye Co., Nevada	AY627927†	AY628049†
longiglans	–	Spring, north-northwest of Holbrook Junction, Antelope Valley, Douglas Co., Nevada	DQ364017	DQ364038
longinqua	–	Spring west-southwest of Hunters Spring, Salton Sea basin, Riverside Co., California	DQ364006	DQ364027
marcida	–	Hardy Springs, White River Valley, Nye Co., Nevada	DQ364012	DQ364033
merriami	–	Ash Spring, Pahranagat Valley, Lincoln Co., Nevada	AY627919†	AY628040†
montana	–	Spring, upper Camp Valley, Meadow Valley Wash, Lincoln Co., Nevada	AY627940†	AY628064†
morrisoni	–	Spring, Bubbling Pond Hatchery, Verde River basin, Yavapai Co., Arizona	DQ364007	DQ364028
sathos	–	Flag Springs (north), White River Valley, Nye Co., Nevada	DQ364013	DQ364034
simplex	–	Spring on road east of Irving Power Plant, Verde River basin, Gila Co., Arizona	AY627949†	AY628073†
stearnsiana	–	Stream, Colson Canyon, Sisquoc River basin, Santa Barbara Co., California	AY367490*	AY367553*
taylori	–	Spring tributary to San Luis Obispo Creek below Cuesta Pass, central California coastal drainage, San Luis Obispo Co., California	AY627923†	AY628045†
thermalis	–	Hot Spring, Gila River basin, Grant Co., New Mexico	AY627953†	AY628077†
trivialis	–	Spring at Three Forks, Gila River basin, Apache Co., Arizona	AY627941†	AY628065†
F. floridana	–	Juniper Springs (outflow), St. Johns River basin, Marion Co., Florida	AF520916§	AY628035†

*Sequence reported by Liu et al. (2003).
†Sequence reported by Liu and Hershler (2005).
§Sequence reported by Hershler et al. (2003a).
#Unpublished sequence (Liu and Hershler herein).
**Sequence reported by Hershler et al. (2003b).
††Sequence reported by Hershler and Liu (2004b).

performed with the PAUP*4.0b10 software (Swofford, 2002), and the Bayesian analyses were performed using MrBayes 3.04 (Huelsenbeck and Ronquist, 2001). For distance analysis, an appropriate genetic distance was used to generate a neighbor-joining (NJ) tree (Saitou and Nei, 1987). MP analyses were conducted with equal weighting, using the heuristic search option with tree bisection reconnection branch-swapping and 100 random additions. The appropriate model was applied for the ML analyses. A neighbor-joining tree with appropriate genetic distance was used as the initial topology for branch-swapping. Node support was evaluated by 10,000 bootstrap pseudoreplicates except for the ML analysis, in which support values were based on 100 replications. In the Bayesian approaches, three short runs were first conducted using the default random tree option to determine when the log likelihood sum reached a stable value (by plotting the log-likelihood scores of sample points against generation time). Metropolis-coupled Markov chain Monte Carlo simulations were then run with four chains using the model selected through Modeltest 3.7 for 1,000,000 generations, and Markov chains were sampled at intervals of 10 generations to

obtain 100,000 sample points. The sampled trees with branch lengths were used to generate a 50% majority rule consensus tree, where the first 5000 trees, equal to 50,000 generations, were removed to ensure that the chain sampled a stationary portion. Sequence divergences (uncorrected *p* distance) within and between phylogenetic lineages were calculated using MEGA3 (Kumar et al., 2004), and standard errors were estimated by 1000 bootstrap replications with pair-wise deletion of missing data. A molecular clock hypothesis for the COI data set was tested using the likelihood ratio test (Felsenstein, 1981) based on the ML topology under the best model selected with and without the constraint of a molecular clock. Tajima relative rate tests of clocklike behavior (Tajima, 1993) were also performed for each phylogenetic lineage using MEGA 3.0.

RESULTS

Sequence Data

The alignment of COI sequences yielded 658 bp, of which 243 sites were variable (36.9%) and 196 were parsimony informative (29.8%). Average base frequencies for COI were 24.99% A, 37.12% T, 19.38% C, and 18.53% G. Base frequencies were homogeneous across all sites ($\chi^2 = 56.48$, df = 237, $P = 1.0$). We analyzed 530 bp of NDI, of which 251 sites were variable (47.4%) and 204 were parsimony informative (38.5%). Average base frequencies for this gene were 28.17% A, 36.86% T, 18.87% C, and 16.11% G. Base frequencies were homogeneous across all sites ($\chi^2 = 71.02$, df = 237, $P = 1.0$). New sequences were deposited in GenBank (accession numbers DQ363999–DQ364040).

Modeltest selected the General Time Reversible (GTR) model (Tavare, 1986); some sites were assumed to be invariant, and variable sites were assumed to follow a discrete gamma distribution (e.g., GTR + I + G), as the best fit for the data set using the Akaike information criterion. The optimized parameters were base frequencies of A = 0.3116, C = 0.1646, G = 0.1323, T = 0.3915; Rmat = {2.1762 36.9774 0.4286 2.1233 26.4015}; shape of gamma distribution = 1.0332; and proportion of invariant sites = 0.5264.

Phylogenetic Reconstruction

All phylogenetic analyses yielded trees with short, weakly supported basal branches (Fig. 3, a neighbor-joining tree). Similar short, weakly supported basal branches were obtained in a recent analysis of a large set of congeners sampled across the entire range of *Pyrgulopsis* and interpreted as evidence of a rapid pulse of diversification coincident with late Tertiary tectonic upheaval of the western landscape (Liu and Hershler, 2005).

The Death Valley system fauna was depicted in all analyses as a polyphyletic assemblage (Fig. 3). Regional species formed eight consistently resolved lineages (A, C–I), of which six (A, C, E, F, G, H) received strong bootstrap support and a high Bayesian posterior probability. The other two lineages (D, I) each consisted of a single population. A ninth lineage (B) was resolved in the NJ tree (Fig. 3), but it was depicted as paraphyletic in the other analyses. Five lineages are restricted to the Amargosa River basin (Fig. 3): Two of these consist of single populations (D, I); one is composed of a flock of six species endemic to the Ash Meadows spring oasis (F); and two are composed of populations that are more widely distributed in this basin (B, G). A sixth lineage (A) is composed of two species endemic to the northern portion of Owens Valley. Each one of the remaining three lineages, which consist of one (C, H) or more (E) species, is distributed within the Death Valley system as well as one or more extralimital basins. One of these lineages (E) was subdivided into three geographically disjunct, moderate to strongly supported subclades (E1–E3, Fig. 3).

Likelihood ratio tests rejected clocklike behavior of sequences for the COI data set ($P < 0.001$). However, Tajima's relative rate test did not reject clocklike behavior of COI sequences within Death Valley system lineages, indicating that the application of a molecular clock is appropriate for these data. We derived a molecular clock rate based on the divergence of a species (*P. perturbata*) that is endemic to a volcanic tableland in northern Owens Valley composed of the Bishop Tuff, which was erupted from the Long Valley caldera 0.7589 Ma (Sarna-Wojcicki et al., 2000). Assuming that *P. perturbata* evolved subsequent to the formation of this tableland, then the 1.23% COI divergence of this species relative to its sister, *P. owensensis* (Liu et al., 2003), implies a clock rate of 1.62% per million years. This substitution rate is similar to the 1.83% ± 0.21% calibration for this gene based on divergence of European hydrobiid snails (Wilke, 2003). Using our calibration, we estimated minimum and maximum ages of Death Valley system springsnail lineages based on mean COI divergence within lineages, and between lineages and their sister taxa, respectively (Table 2).

DISCUSSION

The application of a molecular clock is laden with difficulties (Arbogast et al., 2002; Heads, 2005; Ho and Larson, 2006). A perfect molecular clock is obtainable only in the unrealistic situation in which sequence divergence is a linear function of time, rate of change is equal across all nucleotide positions, and there are no errors in phylogenetic reconstruction and rate calibration (Hillis et al., 1996). Even if all these conditions are satisfied, confidence limits can still be substantial due to stochastic variation (Hillis et al., 1996). Given these problems, caution should be used in estimating absolute divergence times based on molecular data. Nonetheless, this method does provide a means of roughly calculating divergence times that can be especially useful for studies of taxa such as *Pyrgulopsis,* which have a phylogenetically uninformative fossil record. Based on our local COI molecular clock, the six lineages (A, B, D, F, G, I) that are endemic to the Death Valley system appear to have initially diverged from their closest relatives ca. 1.9–3.6 Ma (Table 2). This finding is congruent with the middle to late Pliocene inception of topographic closure of the Death Valley system in its present configuration (e.g., Reheis et al., 2002; Cox et al., 2003), which presumably

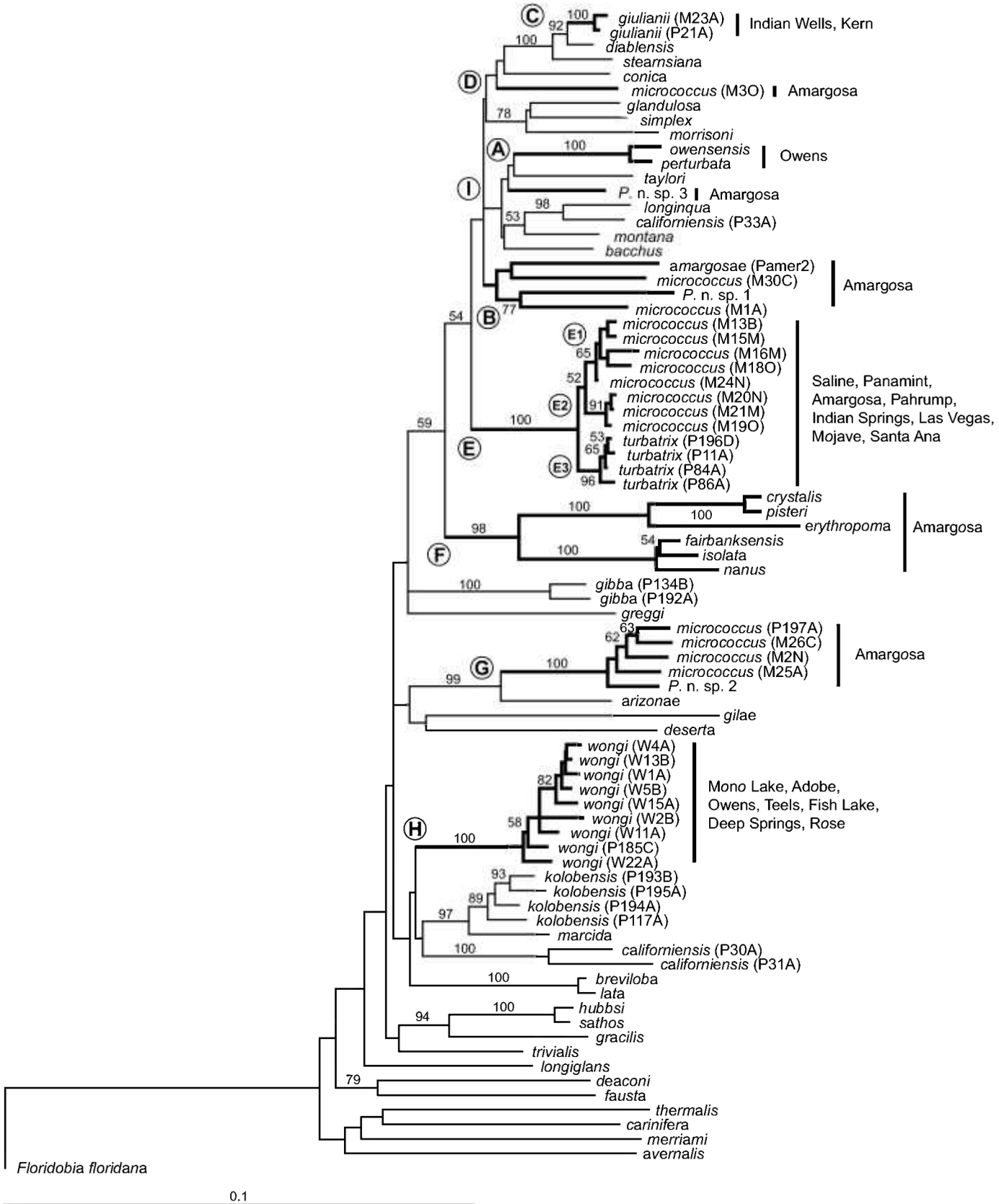

Figure 3. Neighbor-joining (NJ) tree inferred from combined data set based on General Time Reversible (GTR) distance. Lineages containing Death Valley system species have thickened branches and are labeled A–I. The geographic distributions of these lineages are indicated to the right of the vertical lines. Subunits of widely ranging lineage E are labeled E1–E3. Numbers indicate bootstrap support (when >50%).

TABLE 2. COI SEQUENCE DIVERGENCE (UNCORRECTED *P* DISTANCE) AND ESTIMATED AGES OF DEATH VALLEY SYSTEM LINEAGES

Lineage	Sequence divergence (%)		Age of lineage divergence (m.y.)	Sister taxa used
	Within lineage	Between lineage and sister(s)		
Endemic to Death Valley system				
A	1.23 ± 0.41	4.47 ± 0.82	2.76 ± 0.51 (maximum)	*P. taylori*
B	5.89 ± 0.69	—*	3.64 ± 0.43 (minimum)	—
D	–§	3.01 ± 0.56	1.86 ± 0.35 (maximum)	*P. conica, P. diablensis, P. giulianii, P. stearnsiana*
F	4.85 ± 0.59	—*	2.99 ± 0.36 (minimum)	—
G	1.91 ± 0.38	4.67 ± 0.70	2.88 ± 0.43 (maximum)	*P. arizonae*
I	–§	4.18 ± 0.66	2.58 ± 0.41 (maximum)	*P. owensensis, P. perturbata, P. taylori*
Nonendemic				
C	0.00	1.12 ± 0.40	0.69 ± 0.25 (maximum)	*P. diablensis*
E	1.13 ± 0.27	—*	0.69 ± 0.17 (minimum)	—
H	0.85 ± 0.20	—*	0.52 ± 0.12 (minimum)	—

Note: Age of lineage divergence is based on molecular clock rate of 1.62% per m.y. Mean values are followed by standard deviation.
*Sister not well delineated.
§Lineage composed of single sequence.

established a minimum date for divergence of regional lineages. The only one of these lineages to have a strongly supported sister relationship is "G," from the Amargosa River basin, which was depicted as being closest to a species living along the upper Gila River (*P. arizonae*), a major tributary to the lower Colorado River (Fig. 3). This relationship also implies an old divergence event, as any prior hydrographic connection between these two areas surely preceded establishment of the present course of the lower Colorado River after 6 Ma (Howard and Bohannon, 2004; Lucchitta and Jeanne, 2004), and it is paralleled by molecular phylogenetic evidence of middle Pliocene vicariance between pupfishes of the Death Valley system and the lower Colorado River region (Echelle et al., 2005).

Our finding that the single lineage endemic to Owens Valley (A) is not closely related to Amargosa River lineages suggests that the occasional late Quaternary overflow of Lake Panamint (which formed the terminus of the pluvial Owens River drainage) into Death Valley (Smith, 1976) did not result in genetic exchange between Amargosa and Owens River springsnail faunas. Our results indicate that the clade composed of the Owens Valley lineage (A) and a western California species (*P. taylori*) diverged from an Amargosa River lineage (I) as early as 2.8 Ma (Table 2), which well preceded this brief interval of basin integration.

In contrast with the deep divergence of lineages endemic to the Owens or Amargosa River basins, three widely ranging lineages (C, E, H) are shallowly structured among regional and extralimital basins (Fig. 3); the estimated age of separation of their populations ranges from 0.5 to 0.7 Ma (Table 2). Lineage H (*Pyrgulopsis wongi*) is widely distributed in the Owens River drainage and also ranges among basins to the north, south, and east of this large watershed (Fig. 4). The estimated 0.5 Ma divergence of this lineage suggests that its distribution among a series of (currently isolated) basins that formed the upper portion of the late Quaternary Owens River drainage (Mono Lake basin, Adobe Valley, Owens Valley, Rose Valley) may have resulted partly from dispersal within this pluvial system. However, the inclusion within this shallowly structured lineage of other populations that live in basins which either have been isolated from the Death Valley system since their late Neogene inception (Deep Springs Valley, Fish Lake Valley, Teels Marsh) or which had a pre-Quaternary connection with the region (Walker Lake basin; Reheis et al., 2002), is enigmatic with respect to hydrographic history. To the south, lineage C (*P. giulianii*) is distributed in Indian Wells Valley, which formed another segment of the pluvial Owens River drainage, and along the Kern River, which drains to San Joaquin Valley (Fig. 4). Populations sampled from these two basins shared the same COI haplotype (their NDI sequences differed by four bp, 0.75%), yet there is no evidence of geologically recent integration of these two areas, which are separated by the southern Sierra Nevada. The third widely ranging lineage (E) is composed of populations of *P. micrococcus* and *P. turbatrix* structured into three subunits that are distributed in the northwest (E1), southwest (E2), and northeast (E3) parts of the Death Valley system (Fig. 5). The shallow divergence of these subclades, for which the estimated time of separation is 0.7 Ma (Table 2), suggests that the distribution of lineage E among portions of the three (now separated) drainages that comprised the late Quaternary Death Valley system could have resulted from widespread dispersal within this pluvial watershed (but see Liu et al., 2003).[1] However, each of these subunits also contains populations living in other basins that were not

[1]Note that we were unable to locate the single previously documented springsnail population (*P. micrococcus*) from the Mojave River basin (Hershler and Pratt, 1990). We did, however, sample a population from Lucerne Valley (M19O), which is thought to have drained to the Mojave River during the Quaternary (Cox et al., 2003).

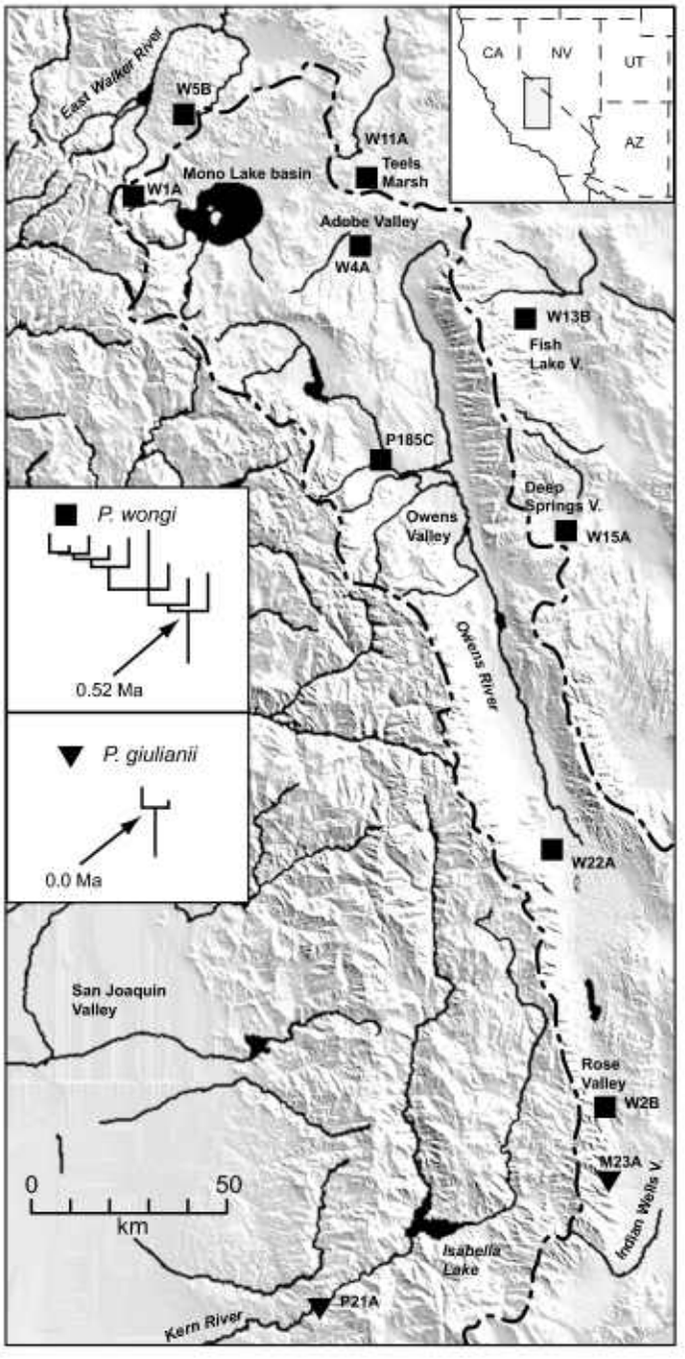

Figure 4. Geographic distribution of mtDNA sequences of lineages H (*P. wongi*) and C (*P. giulianii*). Portions of the tree shown in Figure 3 are inserted, with estimated (minimum) age of pertinent nodes indicated. Hydrographic boundary of the Death Valley system is indicated with a dashed line.

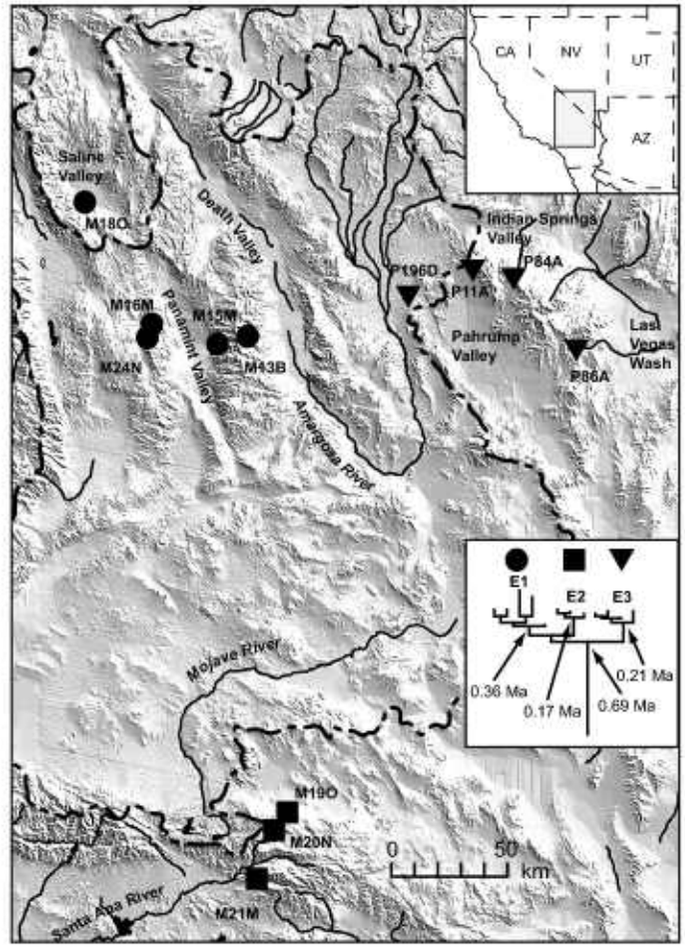

Figure 5. Geographic distribution of mtDNA sequences of lineage E and its subunits (E1–E3). A portion of the tree shown in Figure 3 is inserted, with estimated (minimum) age of pertinent nodes indicated. Hydrographic boundary of the Death Valley system is indicated with a dashed line.

integrated with the Death Valley system during the Quaternary (E1, Saline Valley; E2, Santa Ana River basin; E3, Indian Springs Valley, Pahrump Valley, Las Vegas Wash). The estimated time of population divergence within the subunits of lineage E ranges from 0.2 to 0.4 Ma, suggesting that isolation occurred well subsequent to the hydrographic separation of these basins and the Death Valley system.

The recurring pattern within three Death Valley system lineages of shallowly diverged populations that range among basins that have long been hydrographically separated suggests that, in these places, springsnails have dispersed by means other than spread through continuous aquatic habitat. Two previously proposed mechanisms for such dispersal—passive transport on waterfowl (Liu et al., 2003) and stream capture across drainage divides (Hershler and Sada, 2002)—may have played a role in shaping the biogeography of these widely ranging lineages. For example, colonization of Saline Valley by springsnails (lineage E) likely resulted from avian transport, since the only known population in this deeply downthrown basin lives in a large marsh frequented by birds. On the other hand, the distribution of other populations (also belonging to lineage E) in high-elevation springs on both sides of drainage divides (e.g., Transverse Ranges, E2) is more likely to have resulted from headwater transfers.

Our study shows that the Death Valley system springsnail fauna parallels regional pupfishes and *Tryonia* gastropods in containing endemic lineages that diverged coincident with or prior to the formation of the contemporary regional landscape. These faunas are also similar in having elements confined to the Amargosa River and Owens River basins that are more closely related to (separate) extralimital taxa than to each other. The occurrence of multiple ancient lineages in the Death Valley system is consistent with the previously postulated late Tertiary origin of the modern springsnail fauna and the putatively limited vagility of these animals. However, our findings also show that, contrary to previously proposed models of western North American molluscan biogeography (Taylor, 1966, 1985; Hershler and Pratt, 1990), the evolutionary history of the Death Valley system springsnails has been shaped not only by ancient vicariance, but also by geologically recent (Pleistocene) dispersal. Finally, we provide strong evidence that, again contrary to traditional interpretations by malacologists (Taylor, 1985; Taylor and Bright, 1987), springsnail dispersal has not been restricted to spread within continuous reaches of aquatic habitats. (Note that geologists have also suggested that some interbasinal biotic transfers within this region may have resulted from "nonaquatic" dispersal; e.g., Spencer and Patchett, 1997.) Our finding that springsnail biogeography is not as closely linked with hydrographic history as previously thought suggests that the distributions of these animals should not be used as tools for tracing paleodrainages (*fide* Taylor, 1966, 1985; Hershler and Pratt, 1990) without corroborating evidence that dispersal has predominantly occurred within integrated aquatic systems. Given the difficulties involved in studying dispersal of aquatic invertebrates using direct means (Bilton et al., 2001), it is likely that genetic research will continue to provide the main source of relevant evidence. More generally, we expect that the use of molecular-based methods will lead to a renaissance in the biogeographic study of the remarkable aquatic molluscan fauna of western North America.

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