



Phylogenetic relationships of assimineid gastropods of the Death Valley–lower Colorado River region: relicts of a late Neogene marine incursion?

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ABSTRACT

Aim A small fauna of amphibious snails (genus *Assiminea* Fleming, 1828) living in association with highly mineralized springs in the Death Valley–lower Colorado River region (DVLRCR) is thought to be a relict of the Bouse Embayment, a putative late Miocene–early Pliocene transgression of the ancestral Gulf of California along the lower Colorado River valley. We analysed the phylogenetic relationships of this fauna using mtDNA sequence data (1171 bp) to determine whether, as would be consistent with this hypothesis, it forms a substantially divergent unit sister to marine coastal congeners.

Location South-western Great Basin and lower Colorado River region, USA.

Methods Two genes [mitochondrial cytochrome *c* oxidase subunit I (COI) and the mitochondrial 16S ribosomal RNA gene] were sequenced for 10 populations of DVLRCR assimineas (*Assiminea infima* Berry, 1947; *Assiminea* sp.). We also sequenced an undescribed population from a spring in the Colorado River delta; western North American Pacific Coastal *Assiminea californica* (Tryon, 1865); the three other congeners that live on the continent; and three Old World assimineids (outgroups). Phylogenies based on the combined data set were obtained using Bayesian methods, and divergence times were estimated using a COI molecular clock for related gastropods.

Results Composite haplotypes of the DVLRCR assimineas, together with that observed in the Colorado River delta population, formed a weakly supported clade that was sister to a clade composed of populations of North American Pacific and Atlantic coastal species. The genetic distance between members of these two clades was $3.46 \pm 0.47\%$ for COI and $1.69 \pm 0.38\%$ for 16S. The former clade was composed of five subunits that differed from each other by 1.29–2.84% (COI) and 0.52–1.98% (16S) sequence divergence.

Main conclusions Application of the COI clock suggests that progenitors of the DVLRCR fauna diverged from coastal ancestors 2.13–1.89 Ma (late Pliocene), several million years after the Bouse Embayment would have been terminated by the establishment of the lower (freshwater) Colorado River. This finding, together with shallow genetic structuring of several DVLRCR lineages that are widely distributed across the topographically complex regional landscape, suggests that the *Assiminea* fauna of this inland area was more likely to have been founded by coastal colonists transported on water birds than through a direct connection with the sea.

Keywords

Amphibious snails, Assimineidae, Bouse Embayment hypothesis, Colorado River, Death Valley, Gastropoda, historical biogeography, marine incursion, mtDNA, North America.

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INTRODUCTION

The biogeographical history of the lower Colorado River region is a controversial subject that has important implications for resolving the early development of this master stream, which is one of the enduring mysteries of western North American geology (Young & Spamer, 2001). The presence of marine biota (e.g. barnacles, fishes, foraminifers, molluscs) in the late Miocene–early Pliocene Bouse Formation, which outcrops along the lower Colorado River valley, has been interpreted as evidence that a marine or brackish arm of the ancestral Gulf of California (Bouse Embayment) penetrated the region as far north as (what is now) Parker, Arizona, during that time period (Metzger, 1968; Smith, 1970; Winterer, 1975; Todd, 1976; Busing, 1990) (Fig. 1). This hypothesis forms an important component of the model of lower Colorado River evolution advanced by Lucchitta (1972, 1979), which contends that this reach of river was formed by headward erosion from sea level in response to regional uplift (Bouse Formation deposits currently lie as much as 550 m a.s.l.; Lucchitta, 1979). More recently, compelling geological evidence has been used to infer that the Bouse Formation was deposited near its current elevations in lacustrine environments (Spencer & Patchett, 1997; Poulson & John, 2003), and the lower Colorado River instead formed as a result of successive spilling of a series of lakes along its valley (Meek & Douglass, 2001; Spencer & Pearthree, 2001; cf. Blackwelder, 1934). Proponents of this model argue that the small number of marine species recorded from the Bouse Formation could

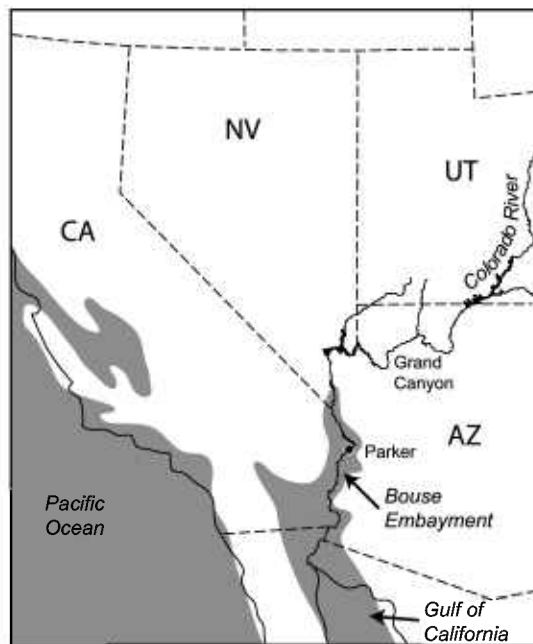


Figure 1 Map of the south-western USA and north-western Mexico showing the Colorado River and areas of late Neogene marine sedimentation, including the putative Bouse Embayment (modified from Christiansen, 1992: plate 7H).

have been transported inland via birds (Spencer & Patchett, 1997). These competing models (well summarized by House *et al.*, 2005) continue to be debated in the recent literature, with much of the discussion focusing on the origin of the Bouse marine biota (see papers cited by Young & Spamer, 2001; Reheis, 2005).

Molecular phylogenetic studies relevant to this subject have focused on terrestrial organisms to date. Most of these investigations delineated late Neogene vicariance of areas separated by the lower Colorado River valley, and attributed this divergence to a dispersal barrier provided by the Bouse Embayment (Lamb *et al.*, 1989; Orange *et al.*, 1999; Riddle *et al.*, 2000; Devitt, 2006; Douglas *et al.*, 2006; Smith & Pellmyr, 2007; Wood *et al.*, 2008; but cf. Avise *et al.*, 1992; Lamb *et al.*, 1992; Mulcahy *et al.*, 2006). However, the existence of this inland arm of the sea was usually accepted as fact in these studies, and consequently there was no consideration of whether the late Neogene barrier identified on the basis of genetic evidence may have instead been a large (Bouse) lake or even the river itself, the inception of which terminated the geologically brief interval of Bouse deposition (≤ 1.5 Myr; House *et al.*, 2008). Here we provide a different perspective of pertinent biogeographical history based on a molecular phylogenetic study of tiny, amphibious snails living in the Death Valley–lower Colorado River (DVLRC) region (> 450 km inland from the head of the Gulf of California), which are thought to be surviving relicts of the Bouse Embayment marine fauna.

These snails belong to the worldwide caenogastropod genus *Assimineia* Fleming, 1828 *sensu lato* (family Assimineidae H. & A. Adams, 1856), which is typically found in estuaries and coastal salt marshes (Abbott, 1958). Fukuda & Ponder (2003) provided morphological evidence that *Assimineia* is a composite group, and suggested that this genus should be restricted to its European type species and a Japanese congener. The generic status of the North American species (and many other species assigned to this genus) has not yet been resolved.

The North American fauna consists of five currently recognized species. *Assimineia succinea* (Pfeiffer, 1840) lives along the Atlantic and Gulf of Mexico coasts (Keen, 1971); *Assimineia californica* (Tryon, 1865) ranges along the Pacific and Gulf of California margins (Abbott, 1974); and three inland species are distributed in the DVLRC (*Assimineia infima* Berry, 1947) and Rio Grande basin (*Assimineia cienegensis* Hershler *et al.*, 2007; *Assimineia pecos* Taylor, 1987), where they typically live in moist riparian habitats along the margins of highly mineralized springs (Hershler, 1987; Taylor, 1987; Sada, 2001). The DVLRC fauna consists of *A. infima*, which inhabits four spring systems in Death Valley (Badwater, Cottonball Marsh, Nevares Springs, Travertine Springs; Hershler, 1987) and taxonomically undescribed populations (closely similar morphologically to *A. infima*; Hershler, 1987) that are distributed in other parts of this valley (Grapevine Springs, Saratoga Spring), the upper portion of the Amargosa River basin (Ash Meadows, Tecopa Hot Springs), Saline Valley (a small hydrographically isolated basin west of Death Valley)

and along the Colorado River (Morrison, 1955; Landye, 1973; Hershler, 1986).

In his original taxonomic description of *A. infima*, Berry (1947: 6) considered this species 'a land-locked *Assimineea* and therefore of marine affiliation.' (A marine coastal origin for the inland *Assimineea* of North America may be inferred by their extremely narrow geographical distribution, and the probable brackish water ancestry of the entire *Assimineid* radiation; Ponder, 1988.) Taylor (1985: 317) suggested that the evolution of this species and undescribed *Assimineea* populations from the DVLCR 'is likely related to the Miocene Bouse embayment', which he conjectured to have possibly extended into Death Valley. The North American *Assimineea* have a scant fossil record that provides little insight into the origin of the DVLCR fauna: the genus has not been recorded from the Bouse Formation, and its only regional fossil occurrence is from early Holocene (10,500 yr BP) deposits in Panamint Valley (Taylor, 1986). Available phylogenetic evidence is also of limited utility, as the DVLCR fauna has not been studied beyond inclusion of several topotypes of *A. infima* in a molecular analysis that focused on congeners living in the Rio Grande basin (Hershler *et al.*, 2007).

In this paper we assess the molecular phylogenetic relationships of the DVLCR *Assimineea*, representatives of the other four North American congeners, and outgroups consisting of three other members of the family *Assimineidae*. Our main goal is to evaluate whether the DVLCR snails form a divergent unit that is sister to North American coastal congeners consistent with their hypothesized derivation from Bouse Embayment progenitors. A relatively deep split between the DVLCR fauna and its coastal sister group is expected under this scenario, as vicariance presumably would have occurred 5.3–4.8 Ma when the (freshwater) lower Colorado River became established (Dorsey *et al.*, 2007; House *et al.*, 2008) and the Bouse Embayment receded. However, we acknowledge that a finding of a divergent DVLCR *Assimineea* clade consistent with this hypothesis does not preclude the possibility that this cladogenic event was instead the product of long-distance transport of coastal snails on birds, as per the ongoing controversy regarding the origin of the Bouse marine biota. Given that it may not be possible to distinguish between ancient vicariance and ancient dispersal in such a case, we also assess whether genetic signatures of geologically recent spread across regional topographic barriers suggestive of avian phoresy (*vide* Liu *et al.*, 2003; Liu & Hershler, 2007) are evident elsewhere in our data set, in order to gauge its importance in shaping the biogeographical history of the DVLCR *Assimineea*.

MATERIALS AND METHODS

Each of the 10 areas in the DVLCR where *Assimineea* lives was sampled during 2004–05. Specimens were preserved in 90% ethanol for sequencing mitochondrial DNA. Five to eight snails from each of these samples were sequenced for mitochondrial cytochrome *c* oxidase subunit I (COI). The

mitochondrial 16S ribosomal RNA gene (16S) showed less variation than COI in a preliminary analysis, thus only two to three specimens from each population were sequenced for this marker. Our analysis also included representatives of the North American coastal congeners (*A. californica*, *A. succinea*) and the inland *Assimineea* fauna of the Rio Grande region (*A. cienegensis*, *A. pecos*). Unfortunately, our sampling of the Pacific Coastal species (*A. californica*) did not include a collection from the Gulf of California as we were unable to find living specimens at several historic sites along the northern shoreline of this water body (this species may be extinct in this region owing to recent commercial development; Carol Skoglund, personal communication to R.H., April 2007). However, during our search in the Colorado River delta we sampled an undescribed population (shells of which closely resemble those of the DVLCR *Assimineea*) in El Doctor spring, and included these specimens in our analysis. We used two Asian *Assimineid*s, *Solenomphala taiwanensis* (Habe, 1942) and *Pseudomphala latericea* (H. & A. Adams, 1864), as outgroups and rooted our trees with the type species of the family, *Assimineea grayana* Fleming, 1828. Vouchers were deposited in the collection of the National Museum of Natural History, Smithsonian Institution (USNM). Locality and other data for all samples are provided in Table 1.

Genomic DNA was isolated from individual snails preserved in 70% ethanol using a cetyl trimethyl ammonium bromide (CTAB) protocol (Bucklin, 1992). Partial sequences of COI were amplified via polymerase chain reaction (PCR) using the primers LCO1490 and HCO2198 (Folmer *et al.*, 1994) for all samples except *A. succinea* from New York. Several forward and reverse primers were designed specifically to amplify the COI sequence for specimens from this locality based on other *Assimineea* sequences, and LCO1490 and *Assimineea*R3 (5'-GCA-GGATCAAAGAACGCAGT-3') worked the best of these. A segment of the 16S gene was amplified using primers 16Sar-L and 16Sbr-H (Palumbi *et al.*, 1991). Amplifications were conducted in 25- μ L total volume containing 5 μ L Invitrogen (Carlsbad, CA, USA) optimizer buffer F (10 mM MgCl₂ pH 9.0) for COI or buffer D (17.5 mM MgCl₂ pH 8.5) for 16S, 2.5 μ L dNTPs (2.5 mM each), 1.25 μ L of each primer (10 μ M), 1 unit *Taq* polymerase, 1 μ L template (*c.* 100 ng double-stranded DNA) and 13.8 μ L sterile water. The PCR temperature profile began with a preheating step at 94°C for 2 min. The following 35 cycles began with a denaturing step at 94°C for 1 min, an annealing step at 45°C for COI or 55°C for 16S for 1 min, and an extension step at 72°C for 2 min. The final extension step was extended for another 7 min. The amplified PCR product was sequenced using a dye terminator cycle sequencing reaction (Beckman Coulter CEQ 8000; Fullerton, CA, USA) as described by Liu *et al.* (2003). Sequences were determined for both strands and then edited and aligned using SEQUENCHER ver. 4.7.

Phylogenetic relationships were inferred using Bayesian inference in MrBayes ver. 3.12 (Ronquist & Huelsenbeck, 2003). In order to provide a readable tree, only one sequence per haplotype per population was used in the phylogenetic analyses. Base compositional differences were first evaluated

Table 1 Samples used for genetic analysis with codes, locality details and GenBank accession numbers.

Species	Code	Locality	COI	16S
<i>Assiminea infima</i> Berry, 1947	A23B	Badwater, Death Valley, Inyo County, CA	<i>n</i> = 7 DQ533863*	<i>n</i> = 3 EF667328
–	A21C	Travertine Springs, second spring from east, Death Valley, Inyo County, CA (USNM 1068663)	<i>n</i> = 7 EF667300	<i>n</i> = 3 EF667326
–	A22C	Nevaras Springs, main spring at source, Death Valley, Inyo County, CA (USNM 1068664)	<i>n</i> = 5 EF667301	<i>n</i> = 2 EF667327
–	A24A, A24AA	Cottonball Marsh, Death Valley, Inyo County, CA (USNM 1068666)	<i>n</i> = 7 EF667302 EF667303	<i>n</i> = 3 EF667329
<i>Assiminea</i> sp.	A28C	Blue Point Spring, Colorado River basin, Clark County, NV (USNM 1071941)	<i>n</i> = 6 EF667308	<i>n</i> = 3 EF667333
<i>Assiminea</i> sp.	A20A	Outflow from Kings Pool, Ash Meadows, Nye County, NV (USNM 1068662)	<i>n</i> = 7 EF667299	<i>n</i> = 3 EF667325
<i>Assiminea</i> sp.	A26AA	Northern-most of Tecopa Hot Springs, Inyo County, CA (USNM 1068668)	<i>n</i> = 8 EF667305 EF667306	<i>n</i> = 2 EF667331
<i>Assiminea</i> sp.	A27	Saratoga Spring, Death Valley, San Bernardino County, CA (USNM 1068661)	<i>n</i> = 7 EF667307	<i>n</i> = 3 EF667332
<i>Assiminea</i> sp.	A30AA	Grapevine Springs, Death Valley, Inyo County, NV (USNM 1074314)	<i>n</i> = 7 EF667309	<i>n</i> = 2 EF667334
<i>Assiminea</i> sp.	A25B	Salt marsh (Saline Marsh), Saline Valley, Inyo County, CA (USNM 1068667)	<i>n</i> = 5 EF667304	<i>n</i> = 3 EF667330
<i>Assiminea</i> sp.	A15A	Spring-fed laguna at El Doctor, Colorado River delta, Sonora, Mexico (USNM 1072100)	<i>n</i> = 4 EF667296	<i>n</i> = 2 EF667316
<i>Assiminea californica</i> (Tryon, 1865)	A1A, A1B, A1D, A1E	Point San Pablo Bay Yacht Harbor, Contra Costa County, CA	<i>n</i> = 5 DQ533855* DQ533856* DQ533857* DQ533858*	<i>n</i> = 4 EF667312 EF667313 EF667314 EF667315
–	A29A	Tidal canal, Venice, Los Angeles County, CA (USNM 1070685)	<i>n</i> = 4 EF667297	<i>n</i> = 2 EF667317
<i>Assiminea cienegensis</i> Hershler <i>et al.</i> , 2007	A3A	Spring-marsh complex c. 2.5 km north of Poza de la Becerra, Cuatro Cienegas basin, Coahuila, Mexico	<i>n</i> = 3 DQ533841*	<i>n</i> = 1 EF667321
<i>Assiminea grayana</i> Fleming, 1828	–	Salt marsh, Medway Estuary, Kent, UK (USNM 1096073)	<i>n</i> = 4 EF667310	<i>n</i> = 1 EF667338
<i>Assiminea pecos</i> Taylor, 1987	A16C	Impoundment #7, Bitter Lakes National Wildlife Refuge, Pecos River basin, Chaves County, NM	<i>n</i> = 5 DQ533848*	<i>n</i> = 1 EF667322
–	A17D	Diamond Y Spring, Diamond Y Draw, Pecos River basin, Pecos County, TX	<i>n</i> = 7 DQ533849* DQ533850* DQ533851* DQ533852*	<i>n</i> = 1 EF667323
–	A18B	East Sandia Spring, Pecos River basin, Reeves County, TX	<i>n</i> = 3 DQ533853* DQ533854*	<i>n</i> = 1 EF667324
<i>Assiminea succinea</i> (Pfeiffer, 1840)	A32B	Salt marsh, Jamaica Bay Wildlife Refuge, New York, NY (USNM 1083440)	<i>n</i> = 8 EF667298	<i>n</i> = 4 EF667320
–	A2A, A2B	Nueces Bay, c. 1.6 km northwest of Indian Point, San Patricio County, TX	<i>n</i> = 5 DQ533859* DQ533860* DQ533861* DQ533862*	<i>n</i> = 2 EF667318 EF667319
<i>Solenomphala taiwanensis</i> (Habe, 1942)	–	Gutter along railway, ChiChi, Nantou County, Taiwan	<i>n</i> = 5 DQ533866*	<i>n</i> = 3 EF667335

Table 1 (Continued).

Species	Code	Locality	COI	16S
<i>Pseudomphala latericea</i> (H. & A. Adams, 1864)	–	Hao Mei Li Natural Ecological Preservation Area, Bu Dai, Chiayi County, Taiwan	<i>n</i> = 2 DQ533864* DQ533865*	<i>n</i> = 2 EF667336 EF667337

Voucher information is provided for samples that were newly sequenced in this study. Single examples of each haplotype observed in a sample were deposited in Genbank. *n*, number of specimens.

*Hershler *et al.* (2007).

using the χ^2 -test in PAUP* ver. 4.0b10 (Swofford, 2002). Incongruence length differences (ILD) were also calculated in PAUP* 4.0b10 using the partition-homogeneity test (Farris *et al.*, 1994; ILD), to assess whether the two mitochondrial data sets differed in their phylogenetic signals. We conducted 500 replicates of the ILD test using only parsimony-informative sites. MrModeltest (Nylander, 2004) was then used to determine which evolutionary model best fits the data under the Akaike information criterion. In the initial Bayesian analysis, we set burn-in (the initial set of generations preceding convergence on stable likelihood values) at 10% (10,000 generations) of the length of the chain (100,000 generations). Three runs were conducted in MrBayes using the general time reversible model (GTR + I + G) selected by MrModeltest and 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 ln-likelihoods started around -7700 and quickly converged on a stable value of *c.* -4300 after 10,000 generations. For the final run, Metropolis-coupled Markov chain Monte Carlo simulations were performed with four chains 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 with the first 5000 trees (equal to 50,000 generations) removed, to ensure that the chain sampled a stationary portion.

Sequence divergences (uncorrected *p* distance) within and between phylogenetic lineages with all sequences were calculated using MEGA3 (Kumar *et al.*, 2004); standard errors were estimated by 1000 bootstrap replications with pairwise 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 maximum likelihood topology under the Jukes-Cantor model selected with and without the constraint of a molecular clock. In the absence of a COI molecular clock for the family Assimineidae, we used calibrations derived for other members of the superfamily Rissosoidea (*Hydrobia*, 1.83% Myr⁻¹, Wilke, 2003; *Pyrgulopsis*, 1.62% Myr⁻¹, Liu & Hershler, 2007).

RESULTS

A total of 658 base pairs (bp) of COI were analysed. Overall nucleotide composition was biased towards thymine (T)

(41.2%) and adenine (A) (21.9%), followed by guanine (G) (21.6%) and cytosine (C) (15.3%). Base frequencies were homogeneous among species ($\chi^2 = 32.92$, d.f. = 111, *P* = 1.00). The total length of 16S varied from 502 bp in *A. pecos* and *A. cienegensis* to 512 bp in *Solenomphala taiwanensis*. The total aligned data matrix of 16S sequences was 513 bp. Average base frequencies for this gene were 34.6% A, 32.5% T, 19.3% G and 13.6% C. There was no significant base frequency bias among species ($\chi^2 = 6.21$, d.f. = 78, *P* = 1.00). New sequences were deposited in GenBank (accession numbers EF667296–EF667310 and EF667312–EF667338). Likelihood ratio tests could not reject clock-like behaviour of the COI sequences ($\chi^2 = 36.07$, d.f. = 25, *P* = 0.07).

The ILD tests did not reveal significant incongruence between the COI and 16S sequences (*P* = 0.98) and thus we used the combined data set of 1171 bp for our phylogenetic analysis. In the Bayesian tree, (composite) haplotypes of the DVLCR assimineas, together with that observed in the El Doctor specimens, formed a little supported (posterior probability = 41%) monophyletic group (DVLCR clade) that was sister to another poorly supported (67%) clade composed of *A. californica* and the Texas population of *A. succinea* (Fig. 2). The well-supported (96%) clade containing these two units was sister to the New York haplotype of *A. succinea*, and this more inclusive group was most closely related to haplotypes observed in species of the Rio Grande basin (*A. cienegensis*, *A. pecos*). Within the DVLCR clade, the Colorado River basin (Blue Point Spring) haplotype was basally positioned, with remaining haplotypes forming a well-supported (98%) subclade structured into four well-supported (99–100% posterior probability) lineages. One of these lineages consists of specimens from two (Death Valley) populations of *A. infima* while the other three are composed of specimens from multiple drainage basins (Saline Marsh + Saratoga Spring + El Doctor lineage) or widely separated portions of a single basin (Grapevine Springs + Tecopa Hot Springs; Kings Pool + Travertine Springs + Nevares Springs) (Fig. 3).

Eleven haplotypes were observed in the DVLCR samples for the COI gene, while 10 haplotypes were observed for 16S. All of these haplotypes were restricted to single populations, except one for each gene, which was shared by the Badwater and Cottonball Marsh populations. COI and 16S sequence divergence of the DVLCR *Assiminea* lineages and closely related coastal populations are summarized in Tables 2 and 3, respectively. The mean genetic distance between specimens of

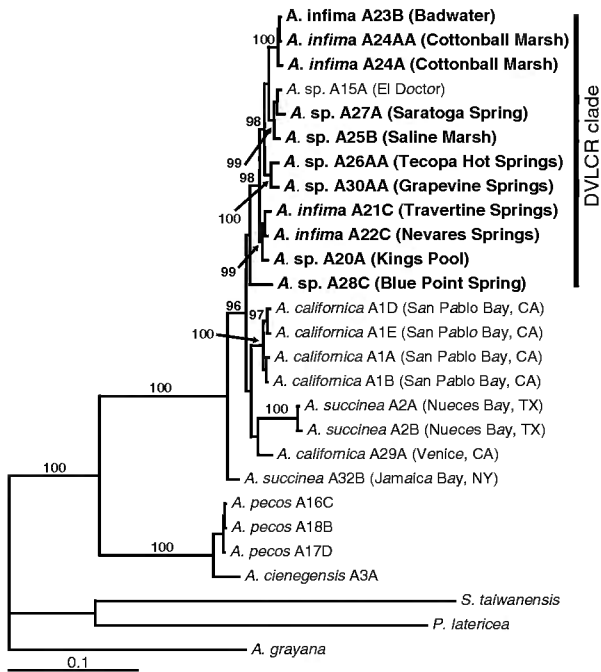


Figure 2 Bayesian topology based on the combined COI + 16S data set depicting the phylogenetic relationships of DVLCR haplotypes (larger, bold font). Posterior probabilities for branch nodes are shown when $\geq 95\%$.

the DVLCR clade and its coastal sister group was $3.46 \pm 0.47\%$ (2.28–5.43%) for COI and $1.69 \pm 0.38\%$ (0.99–2.18%) for 16S. The five subunits of the DVLCR clade differed from each other

by 1.29–2.84% (COI) and 0.52–1.98% (16S) sequence divergence.

DISCUSSION

Our phylogenetic analysis of COI and 16S haplotypes suggests that the DVLCR snails are most closely related to coastal populations, and were derived independently of the other inland North American assimineid fauna that lives in the Rio Grande basin (*A. cienegensis*, *A. pecos*). Whereas the Rio Grande clade is thought to have originated in the late Miocene (Hershler *et al.*, 2007), the DVLCR fauna appears to be the product of a more recent evolutionary event: our COI sequence data suggest that the split between its progenitors and their coastal relatives occurred 2.13–1.89 Ma (late Pliocene), with subsequent divergence of major lineages within this clade taking place 1.75–0.7 Ma (early to mid-Pleistocene). (These may be considered maximum estimates as we have not attempted to correct for ancestral haplotype polymorphism; *vide* Edwards & Beerli, 2000.)

These results suggest that divergence of the DVLCR *Assimineina* clade occurred more than 2 Myr after the Bouse Embayment of the Gulf of California receded (with the inception of the lower Colorado River) and thus may not have been related to this putative marine incursion. The geographical structuring of genetic diversity provides additional evidence that this inland snail fauna did not originate through a direct connection with the sea. The close similarity of haplotypes from El Doctor and two DVLCR springs (Saline Marsh and Saratoga Springs, 0.61 and 0.76% for COI; 0.20 and

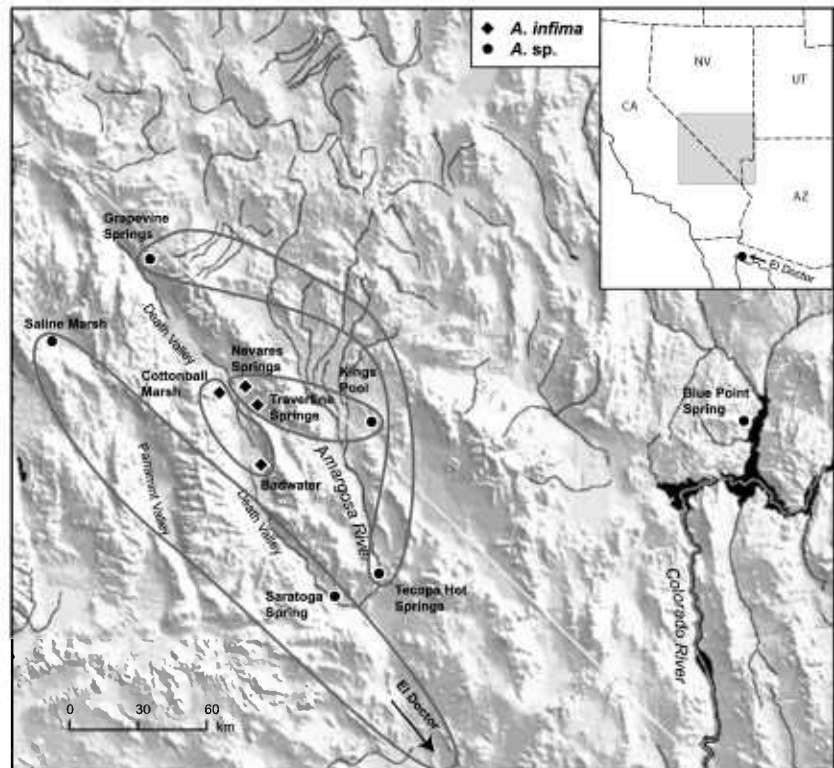


Figure 3 Shaded relief map showing geographical distribution of DVLCR assimineina lineages (ellipses). The Saline Marsh + Saratoga Spring lineage also includes the El Doctor population (insert).

Table 2 COI sequence divergence (mean p distance \pm SD) within and among DVLCR *Assiminea* lineages and North American coastal congeners.

Code	DVLCR <i>Assiminea</i> lineages					<i>Assiminea californica</i>		<i>Assiminea succinea</i>	
	A23,24	A15,25,27	A26,30	A20,21,22	A28	A29	A1	A2	A32
A23,24	0.08 \pm 0.06								
A15,25,27	1.29 \pm 0.38	0.49 \pm 0.18							
A26,30	1.62 \pm 0.47	1.58 \pm 0.40	0.43 \pm 0.18						
A20,21,22	1.94 \pm 0.51	1.71 \pm 0.41	1.62 \pm 0.41	0.45 \pm 0.17					
A28	2.48 \pm 0.58	2.63 \pm 0.55	2.84 \pm 0.59	2.15 \pm 0.52	0.00 \pm 0.00				
A29	3.23 \pm 0.65	2.88 \pm 0.60	3.27 \pm 0.63	2.65 \pm 0.55	3.50 \pm 0.65	0.00 \pm 0.00			
A1	3.13 \pm 0.66	2.65 \pm 0.56	2.40 \pm 0.55	2.28 \pm 0.52	2.59 \pm 0.57	2.30 \pm 0.52	0.43 \pm 0.18		
A2	5.47 \pm 0.89	5.08 \pm 0.84	5.27 \pm 0.83	5.43 \pm 0.86	5.17 \pm 0.85	4.26 \pm 0.75	4.10 \pm 0.77	0.24 \pm 0.14	
A32	3.16 \pm 0.69	3.25 \pm 0.67	2.85 \pm 0.62	3.01 \pm 0.64	3.33 \pm 0.67	3.16 \pm 0.69	2.96 \pm 0.65	4.33 \pm 0.82	0.00 \pm 0.00

Table 3 16S sequence divergence (mean p distance \pm SD) within and among DVLCR *Assiminea* lineages and North American coastal congeners.

Code	DVLCR <i>Assiminea</i> lineages					<i>Assiminea californica</i>		<i>Assiminea succinea</i>	
	A23,24	A15,25,27	A26,30	A20,21,22	A28	A29	A1	A2	A32
A23,24	0.00 \pm 0.00								
A15,25,27	0.67 \pm 0.32	0.32 \pm 0.17							
A26,30	0.79 \pm 0.33	1.06 \pm 0.38	0.53 \pm 0.26						
A20,21,22	0.52 \pm 0.29	0.70 \pm 0.31	0.72 \pm 0.29	0.19 \pm 0.13					
A28	1.19 \pm 0.44	1.86 \pm 0.54	1.98 \pm 0.55	1.61 \pm 0.51	0.00 \pm 0.00				
A29	0.99 \pm 0.41	1.66 \pm 0.51	1.58 \pm 0.49	1.11 \pm 0.42	0.99 \pm 0.43	0.00 \pm 0.00			
A1	1.44 \pm 0.51	2.10 \pm 0.61	2.03 \pm 0.59	1.46 \pm 0.52	1.44 \pm 0.51	1.24 \pm 0.47	0.10 \pm 0.10		
A2	1.39 \pm 0.49	2.05 \pm 0.59	1.63 \pm 0.51	1.51 \pm 0.49	1.39 \pm 0.49	1.29 \pm 0.46	1.34 \pm 0.46	0.20 \pm 0.20	
A32	1.58 \pm 0.54	2.10 \pm 0.60	2.18 \pm 0.60	1.71 \pm 0.55	1.98 \pm 0.60	0.99 \pm 0.44	1.83 \pm 0.57	1.88 \pm 0.58	0.00 \pm 0.00

0.40% for 16S, respectively) and the nesting of the former within a well-supported DVLCR clade (Fig. 2) provide evidence of geologically recent dispersal between the head of the Gulf of California and the DVLCR (presumably a secondary invasion of the coast in this case). The shallow structuring (mean COI and 16S divergence $<$ 0.55%) of each of the three *Assiminea* lineages, which are widely distributed in the DVLCR (Fig. 3), provides additional evidence of recent dispersal across the regional landscape. The most striking example is provided by the limited divergence (0.76% for COI; 0.40% for 16S) observed in the population in Saline Valley, a deeply downthrown basin that has been hydrographically isolated for several million years (Liu *et al.*, 2003), relative to snails living in Saratoga Spring (Amargosa River basin). Similar patterns of geographical structuring of mitochondrial DNA sequence variation were recently documented for DVLCR hydrobiid snails (involving some of the sites that are inhabited by *Assiminea*) and attributed to dispersal on birds migrating along the Pacific Flyway in eastern California (Liu *et al.*, 2003; Liu & Hershler, 2007; see Hershler *et al.*, 2005 for El Doctor spring). This mode of dispersal appears to be even more tenable for *Assiminea*, which receives oxygen directly from the air when out of the water (e.g. *A. californica*, Fowler, 1977), than for gill-breathing hydrobiids.

The evidence described here, together with the absence of Bouse Formation deposits in any of the areas occupied by the DVLCR *assiminea*s, leads us to conclude that this fauna was probably founded by colonists that were transported from marine coastal habitats on birds. This finding does not provide support for the Bouse Embayment hypothesis, and may lend credence to the suggestion that some of the small marine species found in late Neogene fossil deposits of the DVLCR also could have been transported from the coast on water birds. Nonetheless, the presence of other marine biota in the Bouse Formation, including fishes that were unlikely to have been introduced inland by birds (Todd, 1976), continues to be a significant obstacle to accepting the lake-spilling model for the origin of the lower Colorado River (Turak, 2000; Lucchitta *et al.*, 2001), which is otherwise strongly supported by geological evidence (House *et al.*, 2005, 2008; Spencer *et al.*, 2008).

The DVLCR *assiminea*s provide a striking counterpart to ostracodes, which are also thought to have been introduced into regional springs from coastal habitats on birds during the Pleistocene (Forester, 1999; Forester *et al.*, 2005; Jayko *et al.*, 2008). Future studies could explore this interesting parallel further by assessing whether the distribution of these snails is closely correlated with water chemistry, as has been shown for

regional ostracodes (Forester, 1991), and whether the unique inland occurrence (within western North America) of the DVLCR *assimineas* is related to this aspect of ecology.

Our study suggests a need for taxonomic revision, as *A. infima* and the two North American coastal species were found to be non-monophyletic groups that contain substantially divergent populations. Furthermore, the weak support for the DVLCR clade suggests that its origin may be more complex than described here, thus also meriting further investigation.

ACKNOWLEDGEMENTS

We thank the following individuals for collection of specimens and/or assistance in the field: David Franz, James J. Landye, James McLean, Linda Manning, Don W. Sada and Tim Worsfold. We also thank the National Park Service (Death Valley National Park) and the US Fish and Wildlife Service (Ash Meadows National Wildlife Refuge) for providing collecting permits. Tom Quinn (University of Denver) and Sara Oyler-McCance (US Geological Survey) generously shared bench space and equipment in the Rocky Mountain Center for Conservation Genetics and Systematics. Rick Forester, Kyle House, George Jefferson and Storrs Olson provided useful input relevant to some of the ideas expressed in this manuscript. The manuscript also benefited from comments provided by two reviewers (K. House, H. Fukuda). This project was funded, in part, by an award from the Death Valley Natural Association, which was obtained through the support of Linda Greene, Division Chief, Resources Management Division, Death Valley National Park.

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Editor: Robert McDowall