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PRIMARY RESEARCH PAPER

Genetic and morphologic variation of the Pecos assiminea, an endangered mollusk of the Rio Grande region, United States and Mexico (Caenogastropoda: Rissooidea: Assimineidae)

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Abstract *Assiminea pecos* is an endangered species of amphibious gastropod that occupies four widely separated portions of the Rio Grande region in the southwestern United States (Pecos River basin) and northeastern Mexico (Cuatro Cienegas basin). Our statistical and discriminant function analyses of shell variation among the disjunct populations of this species indicate that Mexican specimens differ in their morphometry from those of the United States and can be diagnosed by several characters. We also analyzed variation in the mitochondrial genome by sequencing 658 bp of mitochondrial COI from populations of *A. pecos*, representatives of the other three North American species of *Assiminea*, and several outgroups. Our results indicated substantial divergence of the Mexican population of *A. pecos*, which was consistently depicted as a monophyletic unit nested within or sister to the shallowly structured group comprised of American members of this species. Consistent with our findings, we describe the Mexican population as a new species, which is provisionally placed in the large, worldwide genus *Assiminea* pending further study of the phylogentic relationships of the North American assimineids. Our molecular data suggest that the Rio Grande region assimineids, which are among the few inland members of the otherwise estuarine subfamily Assimineinae, diverged from coastal progenitors in the late Miocene, with subsequent Pleistocene vicariance of Mexican and American species perhaps associated with development of the modern, lower course of the Rio Grande.

Keywords Assiminea · Gastropoda · North America · Systematics · Biogeography · Conservation

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Introduction

The Assimineidae is a diverse family of small, amphibious and terrestrial caenogastropods found in tropical and temperate regions throughout much of the world (Abbott, 1958; Ponder & DeKeyzer, 1998). The North American fauna consists of four amphibious species currently assigned to the large,

cosmopolitan genus *Assiminea*, and referable to the informal *nitida*-complex (*sensu* Abbott, 1958). Two of these species share the brackish coastal habitats typical of other members of the subfamily Assimineinae (Fukuda & Ponder, 2003) and have extremely broad ranges along the Pacific (*A. californica* [Tryon]) and Atlantic-Gulf of Mexico (*A. succinea* [Pfeiffer]) margins (Abbott, 1974). The other two live in spring-riparian settings in Death Valley (*A. infima* Berry) and the Rio Grande region (*A. pecos* Taylor) and are among the few representatives of this subfamily associated with inland aquatic habitats (Fukuda & Ponder, 2003). Both of these ''land-locked'' species are thought to have been derived from coastal progenitors that dispersed inland during the late Tertiary (Berry, 1947; Taylor, 1985) and consequently are of biogeographic interest. They have also become a focus of conservation attention because of threats to their fragile, groundwater-reliant habitats – both have critically imperiled (G1) global heritage rankings (NatureServe, 2006) and *A. pecos* was recently listed as endangered by the USWFS (2005). However, despite the compelling and important features of the North American assimineids, these snails are poorly known taxonomically and additional studies are needed to better clarify their species limits and assess their phylogenetic relationships.

Assiminea pecos, commonly known as the Pecos assiminea (Turgeon et al., 1998), was described from a small number of sites in the upper Pecos River basin near Roswell, New Mexico (including the type locality); lower segment of this watershed near Fort Stockton, Texas; and Cuatro Cienegas basin, Coahuila (Mexico), which drains (in part) to the Rio Grande (Taylor, 1987). Taylor (1987) clearly differentiated *A. pecos* from its North American congeners on the basis of its strongly rounded shell whorls, deep suture, absence of a subsutural thread, and broad umbilicus. However, conspecificity of the widely separated populations assigned to *A. pecos* was not convincingly demonstrated in this study as the only (shell) morphological data provided were from topotypes and all but one of the figured specimens were also from the type locality (Taylor, 1987: Table 1, Fig. 2a–d). There have been no subsequent taxonomic studies of the Pecos assiminea, although several additional populations have been reported from the Roswell and Ft. Stockton areas, and another isolated segment of Pecos River drainage near Balmorhea, Texas (USFWS, 2002, 2005).

As currently envisaged, the Pecos assiminea is composed of four groups of populations separated from each other by ca. 75–760 km (Fig. 1). Given its tight linkage with aquatic habitats, *A. pecos* is likely incapable of dispersing long distances within the highly fragmented drainage of the arid Rio Grande region and consequently its broadly disjunct populations are probably not exchanging genes at present. The fossil record of the Pecos assiminea consists of a few Holocene collections from sites closely proximal to extant colonies (Taylor, 1987) and does not provide insight into the timing of population isolation. Taylor (1985), however, speculated that this snail (which was then undescribed) evolved from Gulf Coastal progenitors that dispersed along a hypothesized brackish paleodrainage in the Rio Grande Valley, with isolation of inland, salt-adapted populations occurring upon inception of the modern, freshwater lower Rio Grande during the Pleistocene. If this hypothesis is correct, Mexican (Cuatro Cienegas) and American (Pecos River basin) populations separated by the putative Rio Grande barrier have long been isolated and may represent divergent conspecific subunits, or separate species.

In this paper we survey morphological and mitochondrial DNA variation of *A. pecos* populations, and assess their evolutionary relationships using molecular evidence. We use our results to re-evaluate the current taxonomic treatment of these populations as a single species and test Taylor's (1985) hypothesis of the biogeographic history of assimineids in the Rio Grande region. Additionally, we discuss the implications of our findings for ongoing efforts to recover the endangered Pecos assiminea (NMDGF, 2004).

Materials and methods

Genetics

Samples from seven populations of *A. pecos* spread across its entire geographic range were

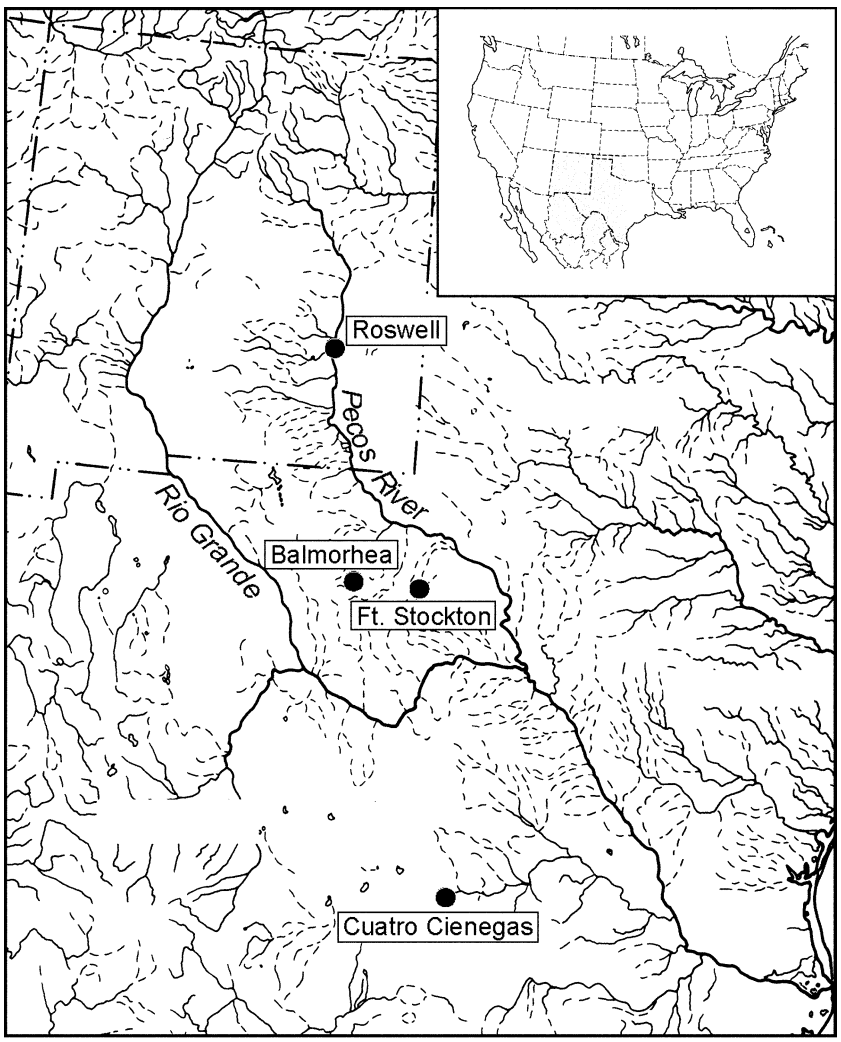

Fig. 1 Map showing the four disjunct areas in the Rio Grande region, United States and Mexico, which comprise the geographic range of *Assiminea pecos*

collected between 2000–2004, preserved in 90% ethanol, and used for sequencing of mitochondrial DNA. Two to seven animals were analyzed for each population, totaling 30 specimens. We also analyzed small samples (three-five specimens) of each of the other three North American species of *Assiminea*. Inasmuch as the phylogenetic relationships of the North American assimineids have never been studied, we used as outgroups two members of the family from geographically distant Taiwan—*Paludinella taiwanensis* Habe, *Pseudomphala latericea* (H. & A. Adams)—and rooted our trees with the latter species. Locality and other data for all samples are provided in Table 1.

Genomic DNA was isolated from individual snails using a CTAB protocol (Bucklin, 1992). Six hundred fifty-eight base pairs (bp) of mitochondrial cytochrome c oxidase subunit I were amplified and sequenced with primers COIL1490 and COIH2198 (Folmer et al., 1994) following protocols of Liu et al. (2003). Sequences were determined for both strands and then edited and aligned using Sequencher™ version 3.1.1. New sequences were deposited in GenBank (Accession numbers DQ533841–533866).

Table 1 Samples used for sequencing of COI

Species	Code	Area	Locality and voucher (if available)	Haplotypes	GenBank Accession #	Sample size
Assiminea pecos	A9, A16	Roswell	Impoundment #7, northwest corner, Bitter Lake NWR, Chaves Co., NM; USNM 1007246, USNM 1069820	VI	DQ533848	5
–	A10	Roswell	Sago Springs, Bitter Lake NWR, Chaves Co., NM; USNM 1011495	VI	DQ533847	3
–	A5	Ft. Stockton	Monsanto Spring, Diamond Y Draw, Pecos Co., TX	II, III	DQ533842 DQ533843	2
–	A6, A17	Ft. Stockton	Diamond Y Spring, ca. 200–250 m downflow from source, Diamond Y Draw, Pecos Co., TX; USNM 1069823	VI, VII, VIII, IX	DQ533849 DQ533850 DQ533851 DQ533852	7
–	A8, A31	Ft. Stockton	"Johns Hole," ca. 0.8 km west of TX Hwy 18, Diamond Y Draw, Pecos Co., TX; USNM 1007143, USNM 1085823	IV, V, VI	DQ533844 DQ533845 DQ533846	7
–	A18	Balmorhea	East Sandia Spring, Reeves Co., TX; USNM 1069829	VI, IX	DQ533853 DQ533854	3
–	A3	Cuatro Cienegas	Spring–marsh complex just west of Hwy 30, ca. 2.5 km north of Poza de la Becerra, Coahuila, Mexico, USNM 1085799	I	DQ533841	3
Assiminea californica	–	–	Point San Pablo Yacht Harbor, San Pablo Bay, Contra Costa Co., CA; USNM 1011440	–	DQ533855 DQ533856 DQ533857 DQ533858	5
Assiminea succinea	–	–	Nueces Bay, ca. 1.6 km northwest of Indian Point, west side TX Hwy 181, San Patricio Co., TX; USNM 1011486	–	DQ533859 DQ533860 DQ533861 DQ533862	5
Assiminea infima	–	–	Badwater, Death Valley, Inyo Co., CA; USNM 1068665	–	DQ533863	3
Pseudomphala latericea	–	–	Hao Mei Li Natural Ecological Preservation Area, Bu Dai, Chiayi Co., Taiwan; USNM 1087367	–	DQ533864 DQ533865	2
Paludinella taiwanensis	–	–	Gutter along railway, Chi-Chi, Nantou Co., Taiwan; USNM 1087368, USNM 1087369, USNM 1087370	–	DQ533866	5

Base composition differences were evaluated using the Chi-square test. Phylogenetic relationships were inferred using maximum parsimony (MP), neighbor-joining distance (NJ), maximum likelihood (ML), and Bayesian inference methods. MP, NJ, and ML analyses were performed using PAUP*4.0b10 software (Swofford, 2002) and Bayesian analyses were conducted using MrBayes 3.04 (Huelsenbeck & Ronquist, 2001). MP analyses employed equal weighting, using the heuristic search option with 100 random additions. Modeltest 3.7 (Posada & Crandall, 1998) was used to determine which evolutionary model best fits the data under the Akaike Information Criterion, which was then used to construct ML and Bayesian trees. The NJ tree was generated using genetic distances that were also based on this model. For the Bayesian analyses, 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). Metropolis-coupled Markov chain Monte Carlo simulations were then run 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 on a stationary portion. Node support was evaluated by 10,000 bootstrap pseudo-replicates except for the ML analysis, in which support values were based on 100 replications.

Sequence divergences (uncorrected p distance) within and between phylogenetic lineages were calculated using MEGA3 (Kumar et al., 2004); standard errors were estimated by 1000 bootstrap replications with pairwise deletion of missing data.

Morphology

Standard shell parameters were compared for single samples from each of the four disjunct parts of the Pecos assiminea's geographic range. Sample sizes were not sufficiently large to enable assessment of sexual dimorphism. Instead, 20–30 adult specimens with fully formed inner shell lips were selected from amongst the largest specimens of each sample. The total number of shell whorls was counted (WH) for each specimen; and the height and width of the entire shell (SH, SW), body whorl (HBW, WBW), and aperture (AH, AW) were measured from camera lucida outline drawings using a digitizing pad linked to a personal computer (see Hershler, 1989). In addition, three ratios that estimate aspects of shell shape were generated from the raw data (SW/SH, HBW/SH, AH/SH). Descriptive statistics were generated for each sample; and sample heterogeneity was examined through analysis of variance (ANOVA), with post-hoc testing of differences among means using the Bonferroni correction for multiple comparisons. Discriminant analysis was used to evaluate the extent to which these samples could be differentiated on the basis of the multivariate dataset (excluding ratios). This technique has been successfully used to differentiate closely similar species of other assimineid genera (Fukuda & Ponder, 2003, 2005). Classification matrices based on canonical scores were generated to assess accuracy of assignment of individual specimens to their samples, and the first and second scores were presented as a bivariate plot to enable visual assessment of sample differentiation. A second set of discriminant analyses was conducted using a dataset in which the mensural parameters were log transformed (base 10). All analyses were performed using Systat for Windows 11.00.01 (SSI, 2004).

Shells were further studied and photographed using scanning electron microscopy (SEM). Variation in other aspects of snail morphology (e.g., radula, soft part anatomy) was also examined using standard methods (Hershler, 1998; Hershler et al., 2006a), but was constrained by the quality and quantity of available material.

Results

Genetics

The alignment of the COI dataset yielded 658 bp, of which 179 sites were variable (27.3%) and 175 were parsimony informative (26.6%). Average

base frequencies were 22.6% A, 41.3% T, 15.6% C, and 20.5% G. Based frequencies were homogeneous across all sites (Chi-square = 35.66, df = 147, $P = 1.00$). Mean genetic distances between specimens of *A. pecos* and other North American *Assiminea* species ranged from 10.93–11.71%, while the latter differed from each other by 3.31–5.47%. Distances between the four North American species and other assimineids that were analyzed ranged from 16.63 to 17.72%.

Nine COI haplotypes (I-IX, Table 2) were resolved in the 30 specimens of *A. pecos* that were analyzed. The most common haplotype (VI) was shared by five Pecos River basin populations spread among all three segments of this drainage that are inhabited by this species. Each of the other seven haplotypes observed in specimens from this basin were restricted to one (II–V, VII, VIII) or two (IX) populations, and differed from the common haplotype and each other by one-three bp. All three Cuatro Cienegas specimens that were analyzed shared the same haplotype (I), which was differentiated from the Pecos River basin haplotypes by 13 fixed base pair differences. The mean genetic distance between specimens from these two geographic areas was 2.30 ± 0.55% (2.13–2.43%), which was much larger than the differences observed within the Pecos River basin (0.11 ± 0.04%). An unpublished 16S dataset that we gathered shows the same pattern, with substantial sequence divergence between snails of these two areas (1.4–1.8%), and little variation within populations (0–0.4%).

Table 2 COI haplotypes resolved in *Assiminea pecos*

		Base pair position																				
Code	Haplotype	22	76	100	103	254	263	271	283	295	316	349	370	382	407	433	463	511	517	562	583	640
A3A	I	A	T	G	C	A	T	T	C	G	A	A	T	G	C	A	C	G	T	T	A	C
A3B	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A3C	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
A5C	II	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	C	G	T
A5D	III	.	C	.	T	.	C	.	.	A	.	G	C	T	T	G	T	A	A	.	G	T
A8A	IV	.	.	A	T	.	C	.	T	A	G	G	C	T	T	G	T	A	A	.	G	T
A8C	V	.	C	.	T	.	C	C	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A31B	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A31C	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A31D	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A31E	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A31F	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A10A	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A10B	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A10E	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A9A	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A9B	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A16C	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A16E	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A16F	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A6C	VII	.	C	.	T	G	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A6D	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A17B	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A17C	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A17D	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A17E	VIII	.	.	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A17F	IX	C	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A18B	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A18E	VI	.	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T
A18F	IX	C	C	.	T	.	C	.	T	A	.	G	C	T	T	G	T	A	A	.	G	T

Code stands for individual specimens. Periods indicate identical nucleotides as the first sequence

Modeltest selected the General Time Reversible model with variable sites assumed to follow a discrete gamma distribution (e.g., GTR + G; Tavare, 1986) with the following parameter values best fitting our data: A = 0.2403, C = 0.1432, G = 0.1871, T = 0.4294; Rmat = {0.1980 16.4996 2.7368 0.0000 8.8725}; shape of gamma distribution = 0.1556. GTR distance was used to generate a NJ tree and a GTR + G model was used for the ML and Bayesian analyses.

All phylogenetic analyses congruently depicted *A. pecos* as a moderate-strongly supported monophyletic unit sister to a clade composed of the other three North American species, with the Taiwanese outgroups positioned basally (Fig. 2, a MP tree). Specimens from Cuatro Cienegas were consistently resolved as a well supported subclade within *A. pecos*. In the MP and NJ trees, Pecos River basin specimens were also resolved as a monophyletic subunit of *A. pecos* (Fig. 2), whereas in the Bayesian and ML topologies they formed a paraphyletic group in which the Cuatro Cienegas subclade was nested. The resulting trees otherwise differed only in the positioning of terminal branches within well supported clades.

Morphology

Descriptive statistics and ANOVA results (based on the raw dataset) are reported in Table 3. Sample heterogeneity was highly significant for all parameters except aperture height (AH). Post-hoc pairwise testing for each parameter indicated no statistically significant differences among the Pecos River basin samples, which are closely similar in all respects (Fig. 3). In contrast, the Cuatro Cienegas sample (Fig. 4) significantly differed ($P < 0.005$) from all three Pecos River

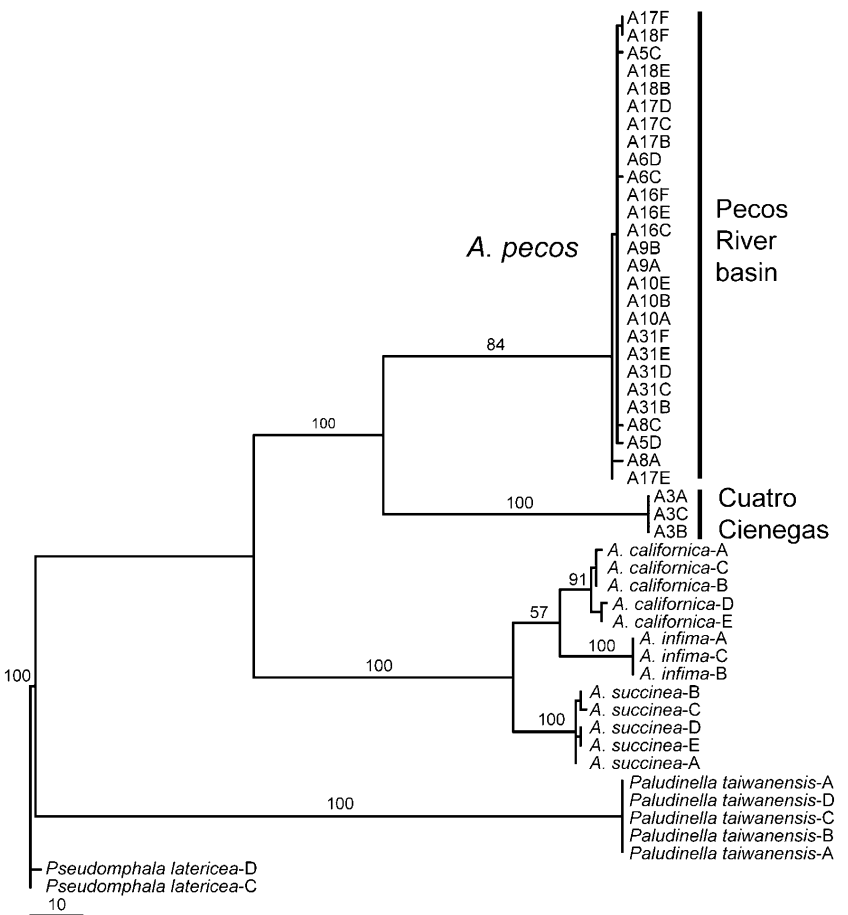

Fig. 2 One of six shortest MP trees (TL = 277, CI = 0.82) depicting phylogenetic relationships of Pecos assiminea populations and division of these into reciprocally monophyletic subunits (Pecos River basin, Cuatro Cienegas). Numbers are bootstrap values for nodal support

Table 3 Variation in shell parameters among *A. pecos* populations

Area					
Parameter	Roswell[a] (25)	Ft. Stockton[b] (20)	Balmorhea[c] (20)	Cuatro Cienegas[d] (30)	*ANOVA
WH	4.67 ± 0.28 4.25–5.25	4.80 ± 0.31 4.35–5.25	4.76 ± 0.25 4.25–5.25	4.43 ± 0.26 4.00–5.00	$^{**}F = 9.38$
SH	2.04 ± 0.14 1.81–2.43	2.09 ± 0.18 1.75–2.35	2.00 ± 0.14 1.71–2.26	1.78 ± 0.11 1.58–2.04	$^{**}F = 24.917$
SW	1.51 ± 0.08 1.37–1.70	1.51 ± 0.09 1.33–1.66	1.46 ± 0.06 1.36–1.58	1.38 ± 0.08 1.27–1.57	$^{**}F = 15.584$
HBW	1.34 ± 0.08 1.22–1.52	1.36 ± 0.08 1.20–1.49	1.32 ± 0.08 1.21–1.49	1.26 ± 0.06 1.17–1.43	$^{**}F = 9.677$
WBW	1.36 ± 0.08 1.19–1.54	1.36 ± 0.09 1.18–1.50	1.31 ± 0.06 1.21–1.45	1.24 ± 0.06 1.13–1.38	$^{**}F = 16.683$
AH	0.82 ± 0.04 0.76–0.95	0.83 ± 0.05 0.74–0.90	0.81 ± 0.04 0.73–0.90	0.80 ± 0.05 0.70–0.95	$F = 1.563$
AW	0.77 ± 0.06 0.67–0.87	0.78 ± 0.04 0.70–0.85	0.75 ± 0.04 0.68–0.83	0.73 ± 0.04 0.65–0.83	$^{**}F = 6.379$
SW/SH	0.74 ± 0.03 0.67–0.81	0.72 ± 0.04 0.65–0.82	0.73 ± 0.04 0.66–0.80	0.78 ± 0.05 0.71–0.87	$^{**}F = 8.84$
AH/SH	0.40 ± 0.02 0.37–0.43	0.40 ± 0.02 0.35–0.43	0.41 ± 0.02 0.37–0.45	0.45 ± 0.03 0.40–0.50	$^{**}F = 30.64$
HBW/SH	0.66 ± 0.02 0.61–0.69	0.66 ± 0.03 0.61–0.72	0.66 ± 0.02 0.62–0.70	0.71 ± 0.03 0.66–0.78	$^{**}F = 30.181$

Sample sizes are in parentheses. Values are mean ± standard deviation, and range

[a] USNM 1086379, Sago Springs

[b] USNM 1007143, ''John's Hole''

[c] USNM 1086193, East Sandia Spring

[d] USNM 1086441, spring–marsh complex north of Poza de la Becerra

* Df for all parameters were 3, 91

** Highly significant ($P \leq 0.01$)

basin samples for all but two parameters (AH, AW), and for AW significantly differed ($P < 0.016$) from two (Roswell, Ft. Stockton) samples.

The discriminant function analysis revealed significant differences among samples (Wilk's lambda = 0.258, $F = 7.034$, df = 21, $P < 0.0001$). Most of the variation (90.4%) was explained by the first canonical function, on which SH, SW, and WBW had the highest loadings. A plot of the scores for the first two functions (Fig. 5) showed extensive overlap of the Pecos River basin samples, and substantial differentiation of Cuatro Cienegas shells. The classification matrix correctly distinguished 90% of the Cuatro Cienegas specimens, with considerably lower values (50–64%) for the Pecos River basin samples. Only four of 27 misclassified specimens from the Pecos River basin were attributed to the Cuatro Cienegas sample. The log transformed dataset yielded closely similar results to the above in all respects, suggesting that distinctiveness of the Cuatro Cienegas specimens cannot be solely attributed to their smaller shells. This is also evidenced by the ratio data (Table 3), which indicated that the Cuatro Cienegas shells are broader and have larger apertures and body whorls (relative to shell height) than specimens of the Pecos River basin. Examination of available material suggests that the Cuatro Cienegas shells (Fig. 4) can be further differentiated from those of the Pecos River basin (Fig. 3) by their flatter whorls, narrower whorl shoulders, narrower umbilicus, and thinner lip.

Discussion

Our main goal was to describe and analyze variation within the Pecos assiminea and assess the evolutionary and taxonomic status of its

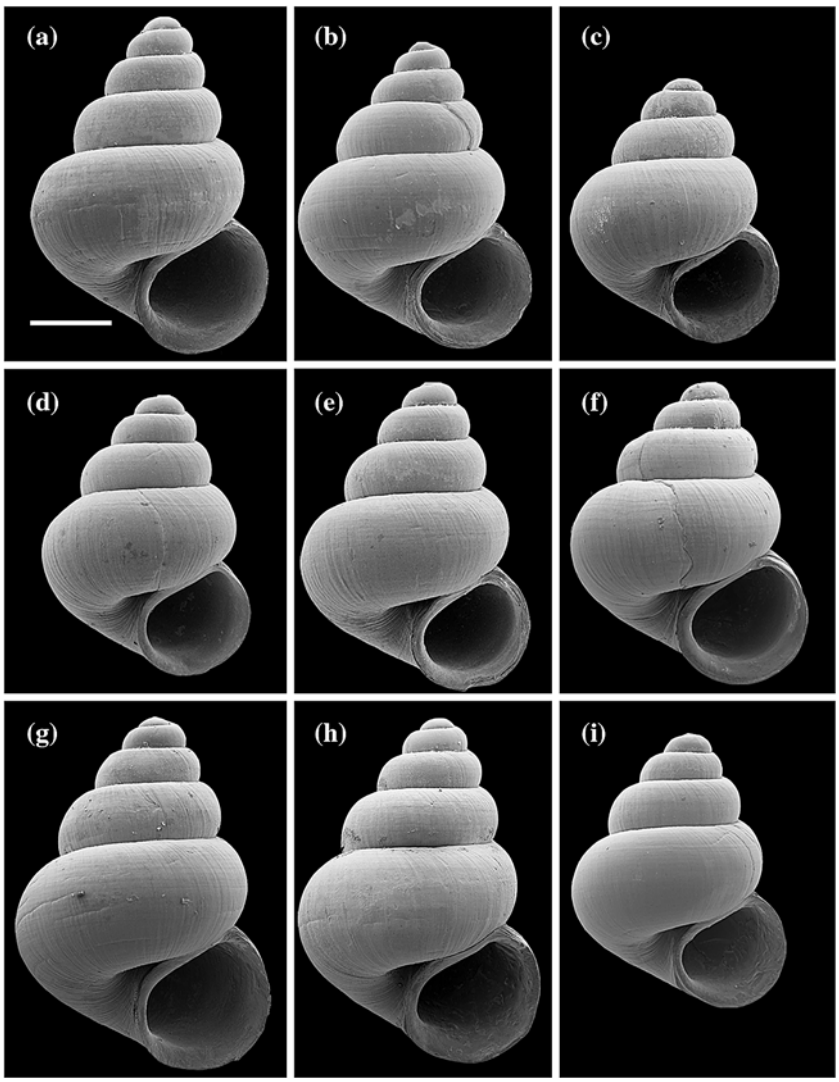

Fig. 3 (**a**–**i**) Pecos assiminea from the Pecos River basin. (**a**–**c**) USNM 1004623, Sago Springs, terminus of spring run, Bitter Lake National Wildlife Refuge, Chaves Co., NM; (**d**–**e**) USNM 1014738, East Sandia Spring, Reeves Co., TX; (**f**), USNM 1086193, East Sandia Spring, Reeves Co., TX; (**g**–**i**), USNM 1007143, ''Johns Hole,'' Diamond Y Draw, Pecos Co., TX. Scale bar = 500 μm

disjunct groups of populations. We have shown that *A. pecos* populations distributed among the type locality area and two other hydrographically separate portions of the Pecos River watershed are closely similar morphologically, and have limited genetic diversity and extensive sharing of haplotypes. These results confirm the conspecificity of these populations and suggest that they could be managed as a single conservation unit. However, given that these three groups of populations are distributed among habitat patches separated by broad expanses of desert, it is likely that they are reproductively isolated from each other. If so, it would be desirable to preserve populations in each of these areas in order to maximize the long term genetic potential and viability of the Pecos assiminea. On the other hand, it is possible, although less likely in our view, that these demes are linked by contemporary gene flow (e.g., facilitated by passive trans-

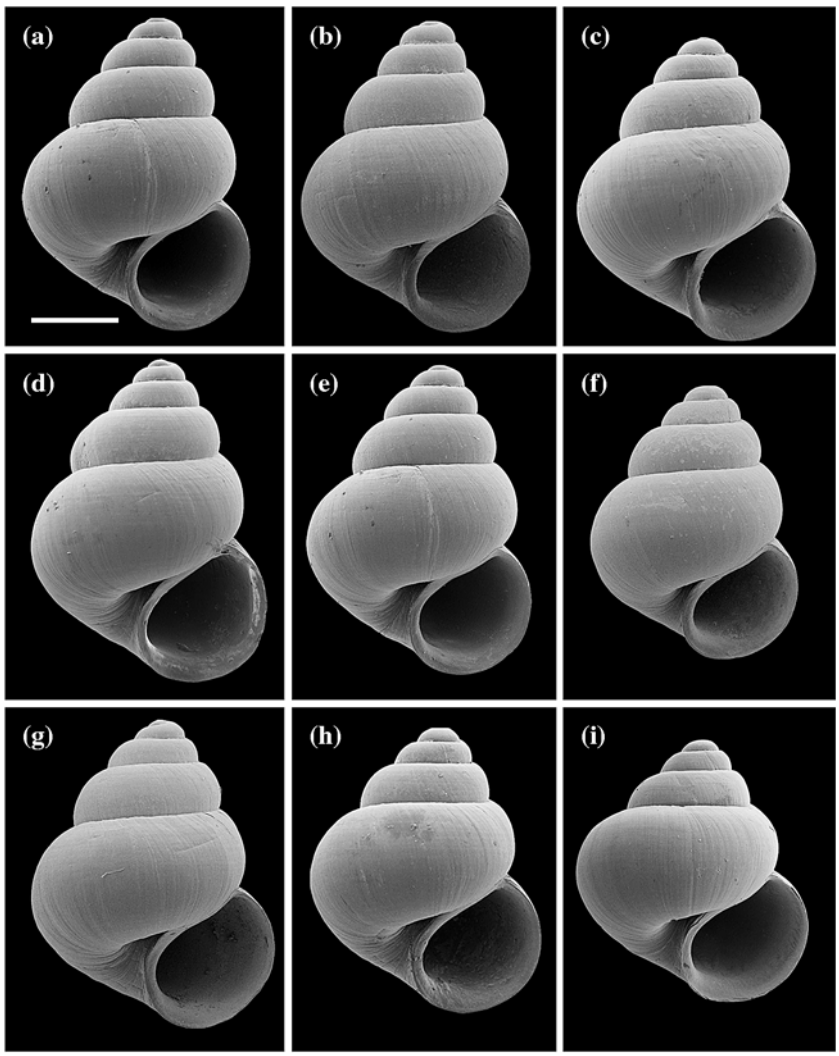

Fig. 4 **(a–i)** Pecos assiminea from the Cuatro Cienegas basin. **(a–i)** USNM 1086441, spring-marsh complex just west of Hwy 30, ca. 2.5 km north of Poza la Becerra, Coahuila, Mexico. Scale bar = 500 μm

port on waterfowl or through periodic flooding), in which case treatment as a single, widespread population is appropriate. Additional studies using more rapidly evolving genetic markers should be undertaken to evaluate these alternatives and help guide plans to conserve and manage the endangered Pecos assiminea.

Our findings also indicated that the Pecos assiminea population in the Cuatro Cienegas basin is morphologically and genetically divergent relative to Pecos River basin snails and can be diagnosed using either of these criteria. The COI sequence divergence between specimens of this population and those of the Pecos River basin (2.30 ± 0.55%) is within the range observed among congeners of other North American gastropods belonging to the superfamily Rissooidea (e.g., *Tryonia*, 1.3–14.8%, Hershler et al., 1999; *Pyrgulopsis*, 1.1–13.1%, Liu & Hershler, 2005; *Taylorconcha*, 1.31%, Hershler et al., 2006b). Although a molecular clock is not available for the Assimineidae, application of the COI calibration of 1.83 ± 0.21% per million years (my) derived for other members of the superfamily Rissooidea (Wilke, 2003) suggests that the broadly disjunct Mexican and American popula-

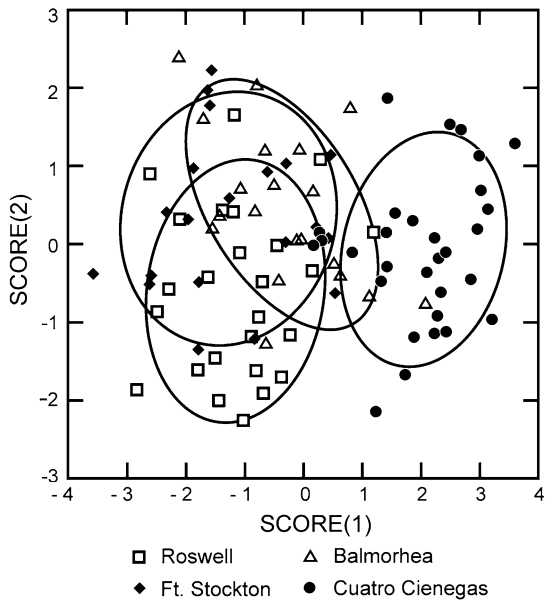

Fig. 5 Plot of the first two discriminant function scores based on seven shell parameters from four populations of the Pecos assiminea, showing divergence of Cuatro Cienegas specimens. Confidence ellipses have $P = 0.6827$

tions of *A. pecos* have been separated for 1.13 ± 0.27 my to 1.42 ± 0.34 my, and thus have had independent evolutionary histories since at least the mid-Pleistocene. Note that the application of molecular clock is laden with difficulties and is constrained in this study by the application of a non-local clock based on a single calibration point (Wilke, 2003). Given these problems, the divergence times estimated above should be considered tentative.

Our analysis of mitochondrial DNA sequences indicates that divergence of these two groups has progressed (at least) to the point where they do not share haplotypes and Cuatro Cienegas specimens form a monophyletic unit nested within the Pecos River basin group (''simple paraphyly,'' Omland et al., 2006). Although reciprocal monophyly is commonly considered a necessary criterion for defining species limits (Sites & Marshall, 2004), others have argued that recently evolved species may have non-monophyletic patterns owing to incomplete lineage sorting (Baker et al., 2003; Omland et al., 2006) and other correlates of their youthful evolutionary status (Funk & Omland, 2003; Kondo et al., 2004). Following this reasoning, we contend that our evidence of substantial divergence and long isolation of the broadly allopatric Mexican and American populations assigned to *A. pecos* amply justifies treating these as distinct congeners, even though they may not have yet achieved the evolutionary stage of reciprocal monophyly. Accordingly we describe the former as a new species (*A. cienegensis*) below.

The large molecular sequence divergence (10.93–11.71%) between assimineids of the Rio Grande region (*A. cienegensis*, *A. pecos*) and their North American congeners suggests a need to reassess a prior model for the biogeographic history of these snails. As discussed above, Taylor (1985) speculated that these inland snails became isolated from Gulf Coastal progenitors as a result of the inception of the modern freshwater lower Rio Grande, which is currently thought to have occurred 1.0–0.75 Ma when the river breached a former impoundment in the El Paso region (Mack, 1997; Cole et al., 2001). Our sequence divergence data suggest that the clade composed of Rio Grande assimineids instead evolved ca. 6.39–5.97 Ma (late Miocene), well prior to the assembly of the modern, integrated Rio Grande. Additional evidence of the antiquity of this clade is provided by our finding that it evolved prior to the split between Atlantic-Gulf Coastal (*A. succinea*) and Pacific (*A. californica*, *A. infima*) congeners (Fig. 2), which can be attributed to the Neogene rise of the Isthmus of Panama. This finding is also consistent with fossil evidence that assimineid snails have lived along the Gulf Coast since the late Oligocene or early Miocene (e.g., *Assiminea aldra* Dall, Tampa Member, Arcadia Formation; age *fide* Brewster-Wingard et al., 1997).

We speculate that the Rio Grande assimineid clade may have originated as a result of stranding of ancestral estuarine snails following one of the series of marine transgressions that occurred along the Gulf Coast during the Neogene (Hosman, 1996). Alternatively, it is possible that inland habitats were colonized in association with the long history of fluvial sedimentation to the northwest Gulf of Mexico that preceded formation of the modern Rio Grande (Galloway et al., 2000; Galloway, 2005). Although our data indicate

that inception of the lower Rio Grande in the mid-Pleistocene well postdated the divergence of this inland clade in conflict with Taylor's (1985) biogeographic hypothesis, this major hydrographic event nonetheless may have contributed to the subsequent 1.25 ± 0.30 Ma vicariance of Mexican (*A. cienegensis*) and American (*A. pecos*) elements of this fauna.

Taxonomy

Family Assimineidae H. & A. Adams
Subfamily Assimineinae

Genus *Assiminea* Fleming, 1828
(type species, *A. grayana* Fleming)
Assiminea cienegensis, n. sp.
Figs. 4, 6–10

Assiminea sp.—Taylor, 1966: 208 (shells from northernmost pool of Pozos del la Becerra, 14 km southwest of Cuatro Cienegas).

Assiminea pecos.—Taylor, 1987: 8–9 [in part, Mexico: Cuatro Cienegas basin, Coahuila].

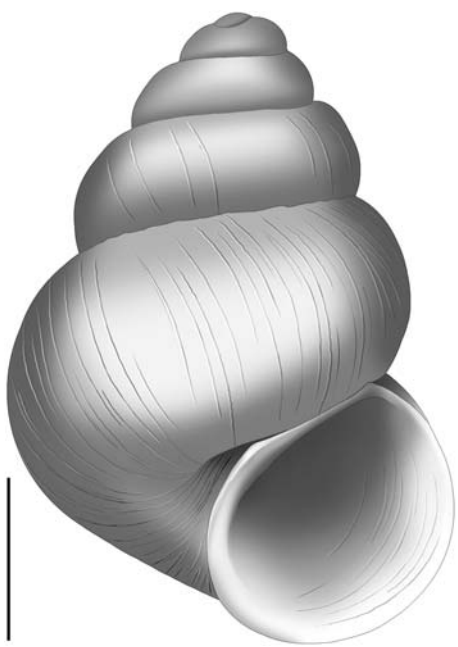

Fig. 6 *Assiminea cienegensis*, holotype, USNM 10171402, southern portion of spring-marsh complex just west of Hwy 30, ca. 2.5 km north of Poza de la Becerra, Cuatro Cienegas basin, Coahuila, Mexico. Scale bar = 1.0 mm

Material examined

Holotype: USNM 1071402, southern portion of spring-marsh complex just west of Hwy 30, ca. 2.5 km north of Poza de la Becerra, Cuatro Cienegas basin, Coahuila, Mexico, ca. 26°53′ N, 102°8′ W. Collected by R. Hershler, June 1, 1981. Paratypes: USNM 1086641, from same collection as above. Other material: USNM 1085799, ibid., 26°53′39.4″ N; 102°8′24.3″ W. Collected by R. Hershler & J. J. Landye, August 2–3, 2005.

Diagnosis

Assiminea cienegensis is distinguished from other North American assimineids by its smaller, broader shell. The shells of *A. cienegensis* also differ from those of its closely similar sister species (*A. pecos*) in having a lower spire, less convex whorls, narrower whorl shoulders, broader umbilicus, and thinner lip. *Assiminea cienegenesis* can be further distinguished from *A. pecos* by the weaker pigmentation of the head–foot; presence of dark pigment along the right edge of the osphradium; much larger coiled section of the rectum in the pallial roof; and smaller, weakly differentiated glandular oviduct.

Description

Shell (Figs. 4, 6) very small (SH usually < 2.0 mm), broadly conical to ovate-conic, thin, translucent. Protoconch (Fig. 7a, b) of 1.6 smooth, slightly convex whorls. Teleoconch of 2.5–3.5 weak or moderately convex, narrowly shouldered whorls; suture medium impressed; sculptured with growth lines, and occasional, weak spiral threads on later whorls. Shell color clear-white; periostracum amber. Aperture wide, pyriform; outer lip thin, slightly prosocline, weakly sinuate; peristome complete, very thin, parietal lip curved, broadly adnate; columellar lip thick, curved, slightly reflected, partly covering umbilical region. Umbilicus open.

Operculum (Fig. 7c) pyriform, inner edge weakly sinuate; paucispiral, horny, thin, transparent, slightly concave. Muscle scar elongate, extending along most of inner surface (Fig. 7d).

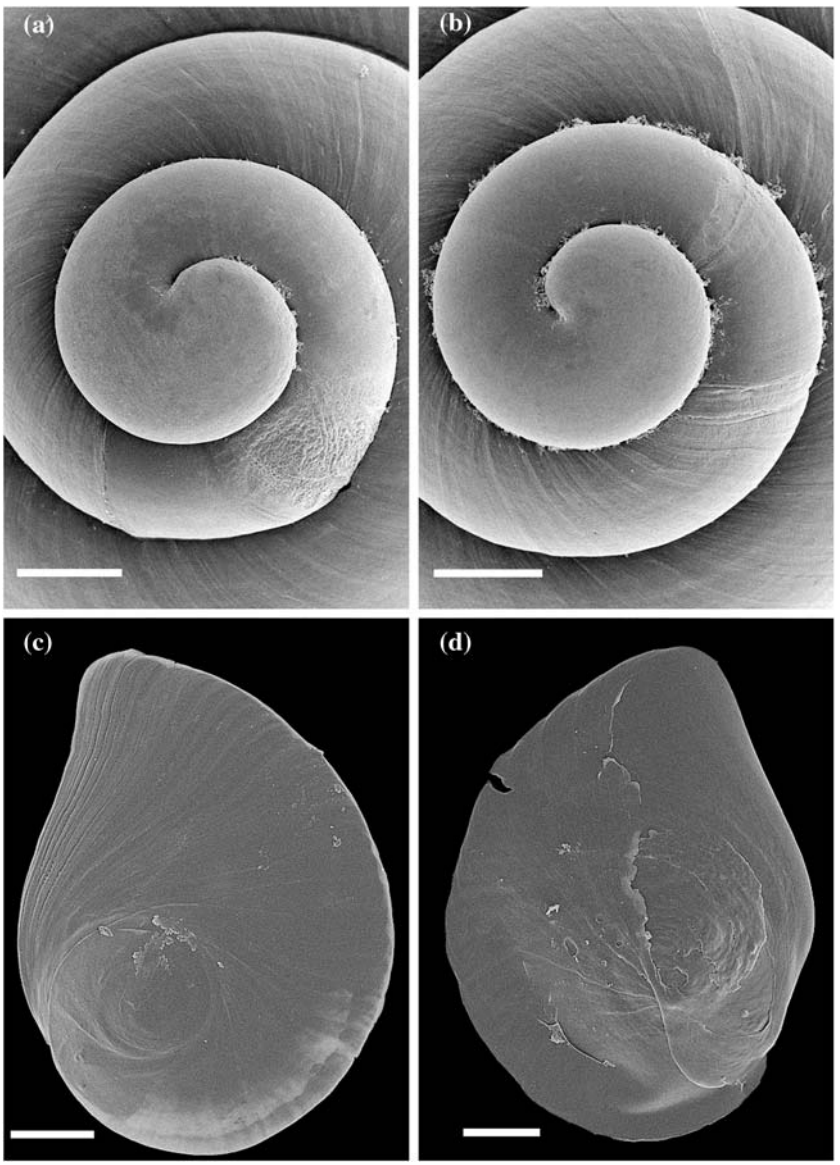

Fig. 7 (**a–d**) Shells and opercula of *A. cienegensis*, USNM 1086441. (**a**, **b**) Shell apex, showing smooth protoconch. (**c**) Outer side of operculum. (**d**) Inner side of operculum. Scale bars = 100 μm

Head–foot usually pale except for black eyespots; occasional specimens having scattered black granules on snout, neck, and bases of eye stalks (Fig. 8a). Eye stalks short. Small, ciliated swelling usually present just behind right eyestalk (Figs. 8a, 9a–c). Snout moderately long, bilobed. Foot large, anterior edge horizontal or slightly rounded, distal end tapering. Anterior mucus gland of two small units that open to middle of transverse slit. Posterior mucus gland absent. Omniphoric grooves (Fig. 9c) well developed.

Pallial cavity large. Pallial roof pale. Kidney opening having fleshy lips; kidney with little or no pallial component. Gill consisting of two tiny, finger-like filaments on posterior section of efferent vein near left edge of pallial cavity (Fig. 8b). Osphradium small, narrow, right edge darkly pigmented (Fig. 8b). Hypobranchial gland not seen in dissection.

Mouth opens between muscular lips; buccal mass occupying most of snout. Radula sac short. Radula taenioglossate (Fig. 10a), consisting of about 40 well developed tooth rows. Central teeth (Fig. 10b) longer than wide; median cusp long, pointed, flanked by 1–2 shorter cusps; basal cusps 2–3, pointed, obliquely arranged; lateral sides

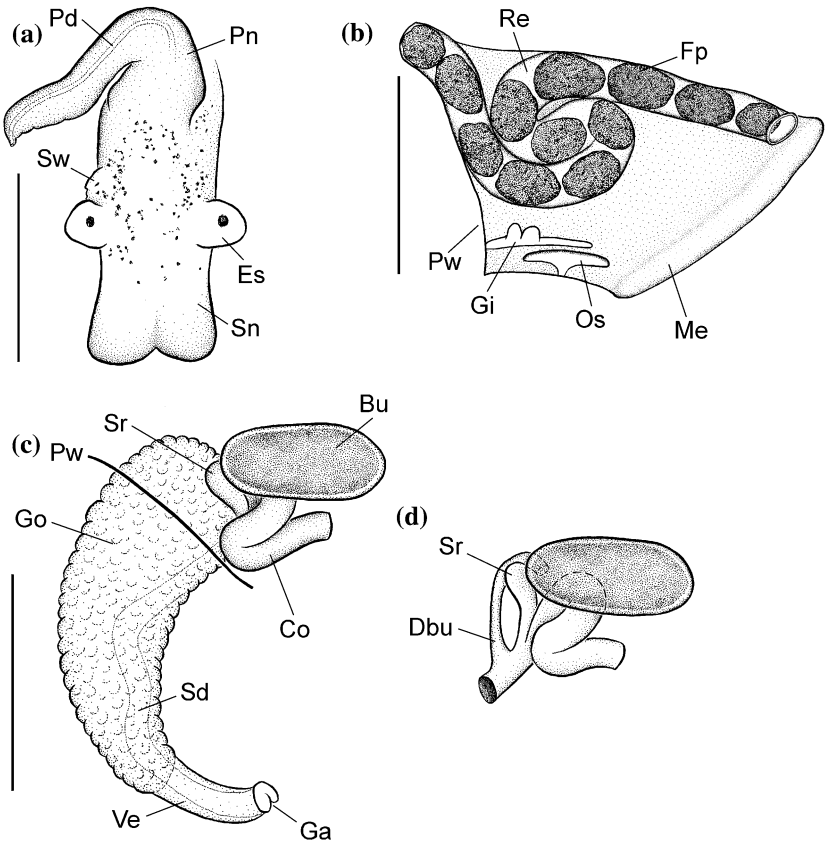

Fig. 8 (**a–d**) Anatomy of *A. cienegensis*, USNM 1085799. (**a**) Head and penis. Scale bar = 1.0 mm. (**b**) Contents of pallial cavity. Scale bar = 250 μm. (**c**) Female glandular oviduct and associated structures. Scale bar = 250 μm. (**d**) Bursa copulatrix and associated structures. Scale as in (**c**). Bu = bursa copulatrix, Co = coiled oviduct, Dbu = bursal duct, Es = eye stalk, Fp = fecal pellet, Ga = genital aperture, Gi = gill, Go = glandular oviduct, Me = mantle edge, Os = osphradium, Pd = penial duct, Pn = penis, Pw = posterior wall of pallial cavity, Re = rectum, Sd = sperm duct, Sn = snout, Sr = seminal receptacle, Sw = swelling behind right eye stalk, Ve = vestibule

nearly vertical; base with large, V-shaped basal tongue. Lateral teeth (Fig. 10c, d) larger than central teeth, taller than wide, with long basal process; median cusp long, pointed, flanked on both sides by 2–3 small cusps; lateral flange very thin, narrow, attached to base of tooth, often difficult to discern in material prepared for scanning electron microscopy (Fig. 10d). Inner marginal teeth (Fig. 10e) with curved sides; cutting edge with 5–8 large, pointed cusps; base rounded. Outer marginal teeth (Fig. 10f) with 13–18 small, pointed cusps; edges of teeth nearly parallel sided, outer edge very thin.

Testis small (ca. 0.5 whorl), pale, consisting of 5–6 simple lobes. Seminal vesicle composed of thick, tightly clustered coils; originating from near middle of testis. Posterior vas deferens opening to ventral edge of prostate gland just behind pallial wall. Prostate gland large, bean-shaped, with about 50% of length in pallial roof. Anterior vas deferens originates from anterior end of prostate gland, straight, narrow. Penis (Figs. 8a, 9d) large, narrow-elongate, coiled clockwise or nearly straight, attached to near middle of head well behind eye stalks. Distal end of penis terminates in small papilla. Penial duct nearly straight, centrally positioned, narrow.

Ovary a simple, yellow sac. Female glandular oviduct and associated structures shown in Fig 8C, D. Oviduct runs to posterior wall of pallial cavity and makes a single posterior, or posterior-oblique loop (Co). Seminal receptacle

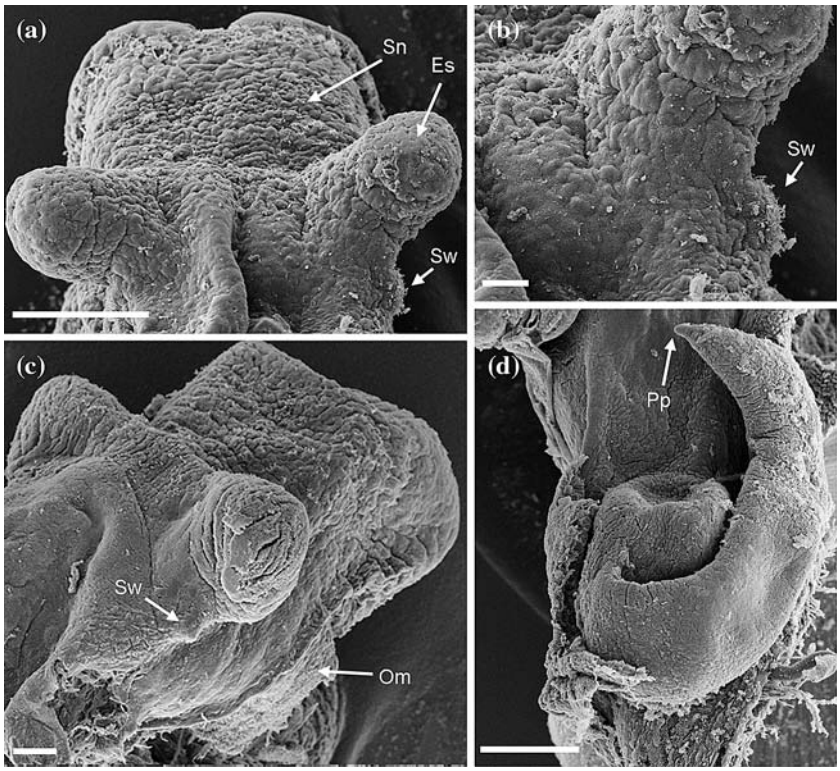

Fig. 9 (**a–d)** Scanning electron micrographs of head and penis of *A. cienegensis*, USNM 1085799. (**a**) Head, showing small, ciliated swelling behind right eye stalk. Scale bar = 100 μm. (**b**) Close-up of swelling. Scale bar = 20 μm. (**c**) Oblique view of head–foot, showing swelling and omniphoric groove. Scale bar = 10 μm. (**d**) Penis. Scale bar = 100 μm. Es = eye stalk, Om = omniphoric groove, Pp = penial papilla, Sn = snout, Sw = swelling behind eye stalk

(Sr) small, pouch-like, with pink sheen, opens to oviduct slightly anterior to coiled portion, anterior to bursa copulatrix, overlapping posterior edge of glandular oviduct, duct about as long as pouch. Bursa copulatrix (Bu) large, ovate, largely or entirely posterior to glandular oviduct, containing dark brown material. Bursal duct (Dbu) long, narrow, strongly curved, originating from right side near anterior edge, sometimes expanded distally. Glandular oviduct short, without obvious division into albumen and capsule glands, distal portion forming nonglandular vestibule (Ve). Sperm duct undulates within glandular oviduct. Genital aperture (Ga) at anterior end of vestibule, forming a muscular papilla.

Distribution and habitat

Taylor (1987) suggested that this snail may be widespread in both the western (internal drainage) and eastern (Rio Salado drainage) portions of the Cuatro Cienegas basin based on the occasional occurrence of empty shells in aquatic collections. The only area where we found living specimens of *A. cienegensis* is the immediate vicinity of the type locality, which is in the western lobe of the basin (Fig. 11). Snails were found in small stands of sedges emerging from slightly elevated mounds in areas where the groundwater was very close to the land surface. Collections were made by pulling apart the hard, salt-encrusted exterior of these mats to expose the moist, black-colored bases of vegetation on which the snails live. Specimens were less commonly found on riparian vegetation alongside spring brooks.

Remarks

Assimineids which have small, thin, brownish shells and live in association with aquatic habitats

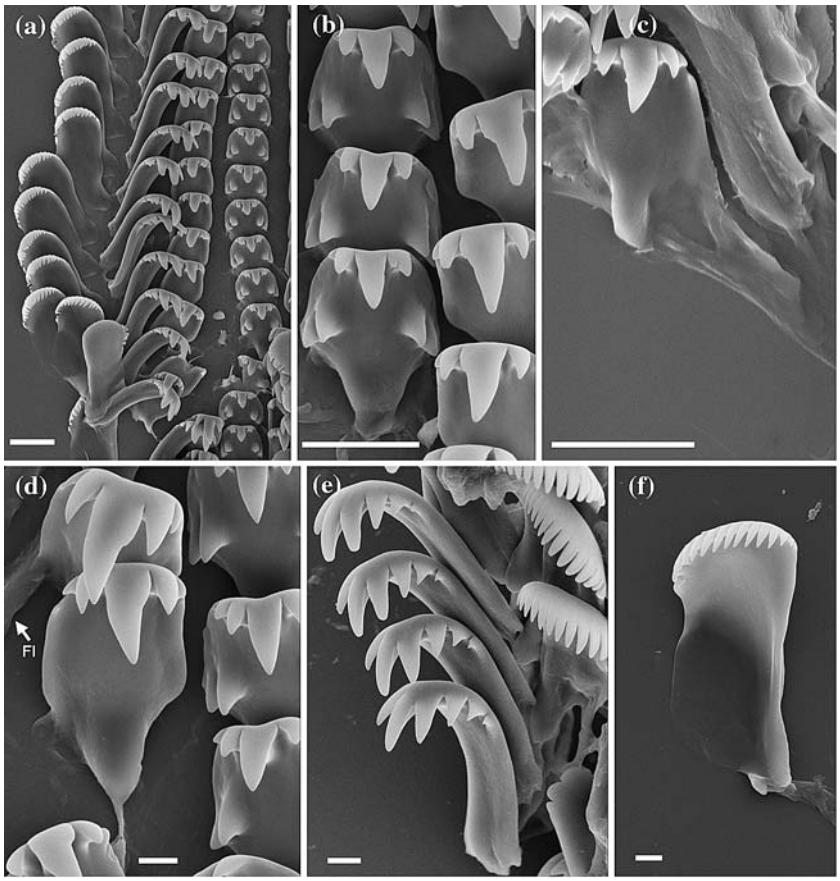

Fig. 10 (**a**–**f**) Scanning electron micrographs of the radula of *A. cienegensis*. (**a**) Portion of radula ribbon, USNM 1086641. Scale bar = 10 μm. (**b**) Central and lateral teeth, USNM 1085799. Scale bar = 2 μm. (**c**) Lateral tooth, USNM 1086441. Scale bar = 10 μm. (**d**) Lateral teeth, USNM 1085799. Scale bar = 2 μm. (**e**) Inner (to left) and outer (right) marginal teeth, USNM 1085799. Scale bar = 10 μm. (**f**) Outer marginal tooth, USNM 1085799. Scale bar = 2 μm. Fl = flange of lateral tooth

have traditionally been placed in the large, worldwide genus *Assiminea* (Abbott, 1958), and the new species described herein is provisionally allocated to the genus on this basis. In a recent review of the generic names of the Assimineidae, Fukuda & Ponder (2003) noted that species attributed to *Assiminea* are morphologically diverse, and suggested that the genus should be restricted to its European type species and other snails that share its various distinctive features. *Assiminea cienegensis* (and the other North American assimineids) does not have any of these characters nor does it well conform morphologically to other genus-group taxa diagnosed by Fukuda & Ponder (2003). Note that *A. cienegensis* differs from *Angustassiminea*, to which Abbott (1974) assigned *A. californica* and *A. succinea* (but see Fukuda & Mitoki, 1996), in lacking spiral sculpture below the suture and in the umbilical region of its shell, and in having a shorter female sperm duct (see Fukuda & Mitoki, 1996; Fukuda & Ponder, 2003, for descriptions of *Angustassiminea*). Although *A. cienegensis* and the North American assimineids ultimately will have to be transferred to another genus, a confident assessment of their generic status must await a more comprehensive study of phylogenetic relationships than has been provided herein.

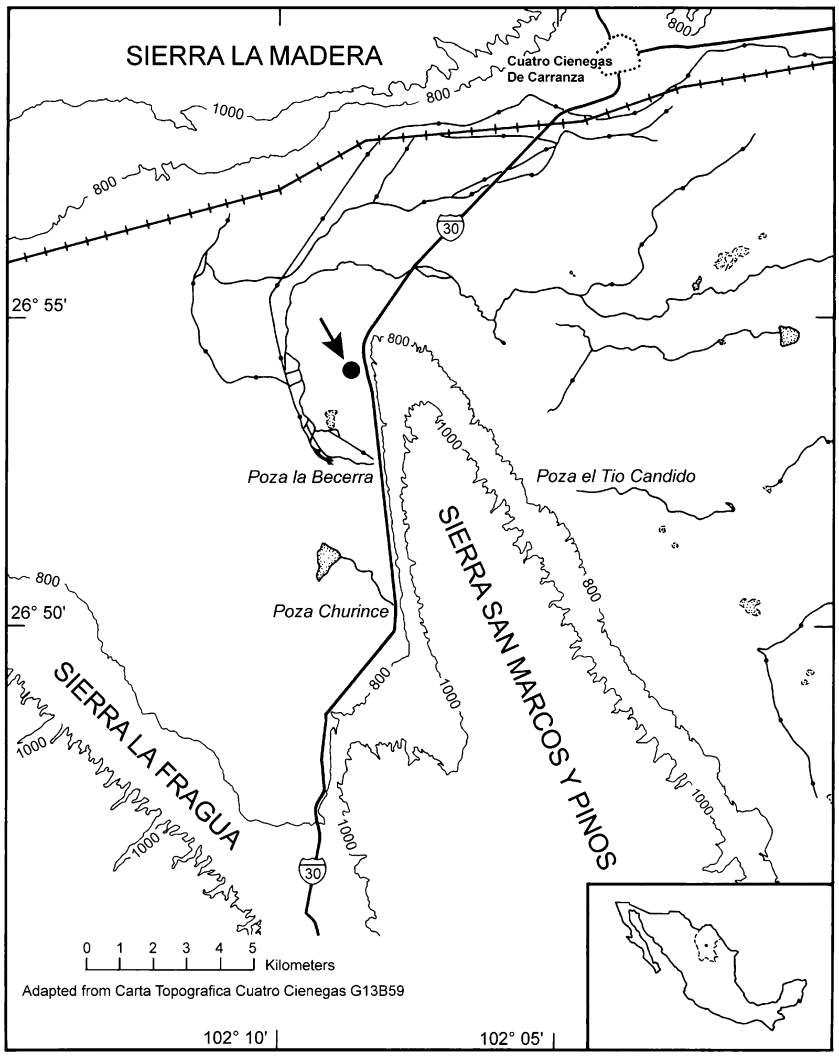

Fig. 11 Map showing location of type locality of *A. cienegensis*. Inset shows location of the Cuatro Cienegas basin in Coahuila state (Mexico)

Etymology

Named for the Cuatro Cienegas basin.

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