

GOVERNOR
Susana Martinez



STATE OF NEW MEXICO
DEPARTMENT OF GAME & FISH

One Wildlife Way
Post Office Box 25112
Santa Fe, NM 87504
Phone: (505) 476-8008
Fax: (505) 476-8123

STATE GAME COMMISSIONERS

JIM McCLINTIC
Chairman
Albuquerque, NM

THOMAS "DICK" SALOPEK
Vice-Chairman
Las Cruces, NM

DR. TOM ARVAS
Commissioner
Albuquerque, NM

SCOTT BIDEGAIN
Commissioner
Tucumcari, NM

ROBERT V. HOFFMAN
Commissioner
Las Cruces, NM

GERALD "JERRY" A. MARACCHINI
Commissioner
Rio Rancho, NM

BILL MONTOYA
Commissioner
Alto, NM

DIRECTOR AND SECRETARY
TO THE COMMISSION
Tod W. Stevenson

Visit our website at www.wildlife.state.nm.us
For information call: (505) 476-8000
To order free publications call: (800) 862-9310

June 29, 2011

Mr. Stephen Robertson, Chief
USFWS - Federal Assistance Division
PO Box 1306
Albuquerque, NM 87103

Attn: Ms. Susan MacMullin, Grant Manager

Dear Mr. Robertson:

Enclosed please find the Department's submission packet of **FINAL** reports for the Section 6 Endangered Species Grant **NM E-56-7 "Macroinvertebrates of Bitter Lake National Wildlife Refuge"** for your review and approval. The packet includes the Final Performance Report and the FINAL SF 425 Federal Financial Report for the period 01/01/2009 through 12/31/2010. The report due date was extended to 6/29/2011. This grant is ready to be closed. We acknowledge that there will be an unspent balance of \$78.75 upon closure.

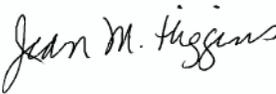
We appreciate your review of these documents and if you have any questions, please feel free to contact me at jean.higgins@state.nm.us or at (505) 476-8012. We appreciate your continued guidance and support.

Sincerely,

Jean M. Higgins,
Federal Assistance Coordinator

FEDERAL FINANCIAL REPORT

(Follow form instructions)

1. Federal Agency and Organizational Element to Which Report is Submitted		2. Federal Grant or Other Identifying Number Assigned by Federal Agency (To report multiple grants, use FFR Attachment)				Page 1	of pages
3. Recipient Organization (Name and complete address including Zip code)							
4a. DUNS Number		4b. EIN		5. Recipient Account Number or Identifying Number (To report multiple grants, use FFR Attachment)		6. Report Type <input type="checkbox"/> Quarterly <input type="checkbox"/> Semi-Annual <input type="checkbox"/> Annual <input type="checkbox"/> Final	7. Basis of Accounting <input type="checkbox"/> Cash <input type="checkbox"/> Accrual
8. Project/Grant Period From: (Month, Day, Year)				To: (Month, Day, Year)		9. Reporting Period End Date (Month, Day, Year)	
10. Transactions						Cumulative	
<i>(Use lines a-c for single or multiple grant reporting)</i>							
Federal Cash (To report multiple grants, also use FFR Attachment):							
a. Cash Receipts							
b. Cash Disbursements							
c. Cash on Hand (line a minus b)							
<i>(Use lines d-o for single grant reporting)</i>							
Federal Expenditures and Unobligated Balance:							
d. Total Federal funds authorized							
e. Federal share of expenditures							
f. Federal share of unliquidated obligations							
g. Total Federal share (sum of lines e and f)							
h. Unobligated balance of Federal funds (line d minus g)							
Recipient Share:							
i. Total recipient share required							
j. Recipient share of expenditures							
k. Remaining recipient share to be provided (line i minus j)							
Program Income:							
l. Total Federal program income earned							
m. Program income expended in accordance with the deduction alternative							
n. Program income expended in accordance with the addition alternative							
o. Unexpended program income (line l minus line m or line n)							
11. Indirect Expense	a. Type	b. Rate	c. Period From	Period To	d. Base	e. Amount Charged	f. Federal Share
				g. Totals:			
12. Remarks: Attach any explanations deemed necessary or information required by Federal sponsoring agency in compliance with governing legislation:							
13. Certification: By signing this report, I certify that it is true, complete, and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent information may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)							
a. Typed or Printed Name and Title of Authorized Certifying Official					c. Telephone (Area code, number and extension)		
					d. Email address		
b. Signature of Authorized Certifying Official					e. Date Report Submitted (Month, Day, Year)		
							
14. Agency use only:							

Standard Form 425
OMB Approval Number: 0348-0061
Expiration Date: 10/31/2011

Paperwork Burden Statement
According to the Paperwork Reduction Act, as amended, no persons are required to respond to a collection of information unless it displays a valid OMB Control Number. The valid OMB control number for this information collection is 0348-0061. Public reporting burden for this collection of information is estimated to average 1.5 hours per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding the burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to the Office of Management and Budget, Paperwork Reduction Project (0348-0060), Washington, DC 20503.

FINAL REPORT

State: New Mexico Project Number: E-56-(4-7)

Project Title: Macroinvertebrates of Bitter Lake National Wildlife Refuge

Study Title: Endangered Species

Contract Period: 3 November 2005 To: 31 December 2010

I. Program Narrative Objectives

1. Process macroinvertebrate voucher material collected from previous field studies that focused on population status surveys (distribution and abundance) of state-listed and federal Candidate hydrobiid spring snails and gammarid amphipods in New Mexico (see Table 1). Particular emphasis will be placed on processing macroinvertebrate collections from Bitter Lake National Wildlife Refuge, Blue Spring, and Willow Spring.
2. Quantify habitat data collected from previous macroinvertebrate studies in New Mexico.
3. Monitor hydrobiid spring snail and gammarid amphipod populations in New Mexico, and conduct exploratory surveys for target species listed in Table 1.
4. Assess threats and provide management recommendations for target species based on data derived from Objectives 1-3 above.

II. Procedures

- A. Continue laboratory processing of field voucher collections for pre- and post-fire studies of BLNWR macroinvertebrates, and monthly samples from previous studies at Blue Spring (*Pyrgulopsis pecosensis*) and Willow Spring (*Pyrgulopsis chupaderae*). Work will be conducted by the Project Leader and consultants.

Lab processing of pre- and post-fire macroinvertebrate collections from BLNWR is ongoing under a professional services contract (11-516-0000-00022) with Miami University (Ohio).

Lab processing of all monthly collections (1997-1999) from Blue Spring and Willow Spring was completed under segment 5, including measurements of shell lengths to construct monthly length frequency histograms for characterizing reproductive periodicity of *P. pecosensis* and *P. chupaderae*. Verification of lowest taxonomic identification for select macroinvertebrates (i.e., copepods, Insecta) is ongoing to facilitate population- and community-level data analyses.

- B. Synthesize results from previous monthly population and habitat studies at BLNWR, Blue Spring, and Willow Spring.

Under the current Section 6 grant (E-66-R), efforts continue to link habitat and population data for *P. chupaderae* (Willow Spring) and *P. pecosensis* (Blue Spring).

- C. Conduct routine population and habitat monitoring of macroinvertebrate species listed in Table 1*.
1. Field monitoring will consist of population abundance estimation, habitat assessment, life history observations, and identification of threats.
 - a. Estimate population densities by benthic samples, dip nets, and/or artificial substrate samples from all occupied habitat types.
 - b. Habitat quantification will include measures of water depth and velocity, substrate type, and hydrophytes.
 - c. Measure physicochemical parameters (water temperature, salinity, specific conductance, total dissolved solids, dissolved oxygen, and pH) at sample sites.

During the grant period, population and habitat monitoring occurred at Bitter Lake National Wildlife Refuge (BLNWR) and Blue Spring. Lack of private land access throughout this grant period prevented monitoring of *P. chupaderae* at Willow Spring.

See Appendix A for a synopsis of monitoring effort at BLNWR and Blue Spring.

- D. Expand exploratory surveys for target species listed in Table 1 with a particular emphasis on documenting the status (distribution and abundance) of *Pyrgulopsis* spp. in the Gila River Basin*.

(* Pending successful landowner-agency liaison relations.)

Appendix B provides information on population surveys (2008, 2009) of *Pyrgulopsis gilae* and *Pyrgulopsis thermalis* in the Gila River basin. This appendix also includes a report by Dr. Robert Hershler (Smithsonian Institution) and Dr. Hsiu-Ping Liu (University of Denver) that is appended as an attachment. The Hershler and Liu (2010) report, conducted under contract with the NMDGF, addresses our current understanding of the taxonomic status of *P. gilae* and *P. thermalis*.

During this grant period, the project biologist co-authored two published papers with Drs. Hershler and Liu:

- Hershler, R., H-P. Liu, and B.K. Lang. 2007. Genetic and morphologic variation of the Pecos assiminea, an endangered mollusk of the Rio Grande region, United States and Mexico (Caenogastropoda: Rissosoidea: Assimineidae). *Hydrobiologia* 579:317-335.

A final revision draft of this paper was provided to the USFWS under Segment E-56-5

(contact the authors above for a PDF copy of the published paper).

- Hershler, R., H-P. Liu, and B.K. Lang. 2010. Transfer of *Cochliopa texana* to *Pyrgulopsis* (Hydrobiidae) and description of a third congener from the lower Pecos River basin. *Journal of Molluscan Studies* 76:245-256.

A final revision draft of this paper is included in Appendix C because this research has not been previously reported to the USFWS (contact the authors above for a PDF copy of the published paper).

- E. Investigate the taxonomic status of the Sangre de Cristo peaclam by reinspection of field voucher material collected from previous surveys.

The project biologist reports no activity under this task, which will require substantial commitment of funding to support morphometric and genetic studies. These tasks are contingent on finding an adequate sample size of living specimens of *Pisidium sanguinichristi* at Middle Fork Lake (type locality; TL), or from another locality which is currently not known to exist because *P. sanguinichristi* is only reported from the TL.

- F. Submit annual reports summarizing activities during the reporting period. These activities will include preliminary analysis of results, identification of threats, and management recommendations.

Reports summarizing annual activities were submitted under all previous grant segments.

- G. Prepare a completion report which summarizes the results of work accomplished under Procedures A-D, including management recommendations for species-specific long-term population/habitat monitoring protocols and assessment of factors posing imminent threats to target species.

For management recommendations, including threat assessment, see Appendix D.

III. Geographic Location

Laboratory: Project headquarters will be located at the New Mexico Department of Game and Fish, Santa Fe, NM.

Field: Population/ habitat monitoring will be conducted in target species' native habitats. Exploratory field investigations will occur within target species' known or presumed historic range.

Prepared by: Brian K. Lang
Brian K. Lang
Project Biologist

Approved by: Dave Holderman
Dave Holderman, Assistant Chief
Conservation Services Division

Approved by: Jean Higgins
Jean Higgins
Federal Aid Coordinator

Approved by: Matt Wunder
Matt Wunder, Chief
Conservation Services Division

4/1/2011

Table 1. State listed and federal Candidate and Species of Concern aquatic mollusks and crustaceans of New Mexico. Species categorized by ecological specialization, geographic region, and conservation status.

Taxa ¹	Species	2011 Status ²			Occurrence ³
		County	State	Federal	
Spring Snails					
Pecos assiminea	<i>Assiminea pecos</i>	Chaves	E	E	BLNWR
Koster's springsnail*	<i>Juturnia kosteri</i>	Chaves	E	E	BLNWR, RCC
Roswell springsnail*	<i>Pyrgulopsis roswellensis</i>	Chaves	E	E	BLNWR, RCC
Pecos springsnail*	<i>Pyrgulopsis pecosensis</i>	Eddy	T	SC	Blue Spring
Chupadera springsnail*	<i>Pyrgulopsis chupaderae</i>	Socorro	E	C	Chupadera Mts.
Gila springsnail *	<i>Pyrgulopsis gila</i>	Grant	T	C	Gila River Basin
New Mexico springsnail *	<i>Pyrgulopsis thermalis</i>	Grant	T	C	Gila River Basin
Aquatic Snails & Bivalves					
wrinkled marshsnail	<i>Stagnicola caperatus</i>	Sandoval	E	-	VCNP, BLNWR
star gyro	<i>Gyraulus crista</i>	Colfax	T	-	Black Lake
lake fingernailclam	<i>Musculium lacustre</i>	Colfax	T	-	Cieneguilla Creek
paper pondshell mussel	<i>Utterbackia imbecillis</i>	San Miguel	E	-	Canadian River
swamp fingernailclam	<i>Musculium partumeium</i>	Union	T	-	Arkansas River Basin
long fingernailclam	<i>Musculium transversum</i>	Union	T	-	Arkansas River Basin
Texas hornshell mussel	<i>Popenaias popeii</i>	Eddy	E	C	Pecos River
Lilljeborg's peaclam	<i>Pisidium lilljeborgi</i>	Santa Fe	T	-	Sangre de Cristo Mts.
Sangre de Cristo peaclam*	<i>Pisidium sanguinichristi</i>	Taos	T	SC	Sangre de Cristo Mts.
Crustaceans					
Noel's amphipod*	<i>Gammarus desperatus</i>	Chaves	E	E	BLNWR

¹ Taxonomic authorities: (a) Turgeon, D. D., A. E. Bogan, E. V. Coan, W. K. Emerson, W. G. Lyons, W. L. Pratt, C. F. E. Roper, A. Scheltema, F. G. Thompson, and J. D. Williams. 1988. Common and scientific names of aquatic invertebrates from the United States and Canada: mollusks. American Fisheries Society Special Publication 16; (b) Hershler, R. and F. C. Thompson. 1987. North American Hydrobiidae (Gastropoda: Rissoacea): Redescription and systematic relationships of *Tryonia* Stimpson, 1865 and *Pyrgulopsis* Call and Pilsbry, 1896. The Nautilus 101(1):25-32.

² 2011 Status: (State) E = Endangered, T = Threatened; (Federal) E = Endangered, C = Candidate, SC = Species of Concern.

³ Acronyms: BLNWR - Bitter Lake National Wildlife Refuge; RCC - Roswell Country Club; VCNP - Valles Caldera National Preserve.

* Species endemic to New Mexico.

Appendix A. Population/habitat monitoring and surveys (2005-2010): Bitter Lake National Wildlife Refuge and Blue Spring.

Population/Habitat Monitoring and Surveys (2005-2010): Bitter Lake National Wildlife Refuge and Blue Spring

During this 5-year grant period, monitoring of state- and federal-listed invertebrates (*Assiminea pecos*, *Juturnia kosteri*, *Pyrgulopsis roswellensis*, *Stagnicola caperata*, *Gammarus desperatus*) occurred annually at Bitter Lake National Wildlife Refuge (BLNWR), Chaves County, and at Blue Spring (*Pyrgulopsis pecosensis*), Eddy County. Additional activities included:

1. Exploratory surveys were conducted in 2008 for all of these listed taxa on BLM lands located immediately adjacent to the eastern limit of BLNWR, specifically McCrea Spring and South Y Canyon Spring;
2. In collaboration with Miami University (Ohio), quarterly monitoring occurred at BLNWR (Bitter Creek and Sago Spring) and Blue Spring from 2007 to 2008;
3. Collection of voucher material (2008-2009) to assess among-population genetic variation of *J. kosteri* and *P. roswellensis*;
4. Distribution surveys were conducted (2009-2010) for all of these listed taxa in Hunter Marsh, BLNWR.

1. Exploratory Surveys (2008) BLM, McCrea Spring and South Y Canyon Spring:

Milford et al. (2001) reported on aquatic vegetation and macroinvertebrate surveys of isolated spring systems situated along the lower Pecos River, Chaves County. In June 2008, NMDGF surveyed McCrea Spring and South Y Canyon Spring (Lang 2009). None of the five listed (state, federal) invertebrates present on BLNWR were found at either site (Figure 1; map provided here from Milford et al. [2001]). These springs are characterized as cold seeps with seasonally variable temperatures and discharge. Aquatic macroinvertebrates present at both sites included: blood worms (Oligochaeta); the pulmonate snail, *Physa acuta* (formerly *virgata*); seed shrimp (Ostracoda); and a variety of insects (Odonata: dragonflies and damselflies; Coleoptera; Diptera: Chironomidae and mosquito larvae). Several sink-hole-related springs described by Milford et al. (2001) were found dry during this survey.

2. Quarterly Monitoring (2007-2008): Macroinvertebrate population monitoring at BLNWR was coordinated with Drs. David Berg and Makiri Sei, Miami University (MU), under a National Science Foundation (NSF) grant titled, “*RUI: Patterns of biodiversity of benthic invertebrates in Chihuahuan Desert springs.*” Sites on the Refuge targeted for this study included Sago Spring and Bitter Creek at “Lost River Pool.”

This NSF project required that inventory be conducted quarterly over a 1-year period and employed quantitative sampling per the “PLOCH method” (Oertli et al. 2005) to assess the longitudinal biodiversity of macroinvertebrates in each aquatic system. Following this method, the number of samples (benthic and sweep) varied exponentially according to the size of the spring source (i.e. “pond habitat”; 4 benthic samples for a 10 m² pond, 8 for 100 m², 16 for 1000 m² and 32 for 10,000 m²). Furthermore, this protocol calls for sampling to assess longitudinal species richness commencing at exponential intervals downstream of the spring source, i.e., 5m,

25m, and 125m. Accordingly, a total of 18 samples (11 benthic, 7 sweep) was collected quarterly for one year (2007-2008) from both BLNWR study sites and Blue Spring.

Preliminary Results: All NSF-monitoring collections from BLNWR are sorted and data analysis is ongoing by MU staff. In general, the abundance of aquatic macroinvertebrates in Bitter Creek at Lost River Pool and Sago Spring appears commensurate to past levels. During this monitoring period, four *Gammarus desperatus* were collected (March 2008) from the spring vent at “Lost River Pool” of Bitter Creek. In April 2010, the project biologist found *G. desperatus* and *Juturnia kosteri* extant in a spring vent located in Dragonfly Spring Run (GPS data [NAD 83, Zone 13S: UTMS = 3704972, 553389; N33°28.974’, W104°25.522’]). These findings confirm the persistence of both species, albeit low level, within the upper reaches of Bitter Creek.

Collections from Blue Spring continue to be processed for data entry and analysis. This task has been supported by a State Wildlife Grant (T-32-P2, Project #13) to Miami University under the Department’s Share with Wildlife Program.

3. Genetic Survey of Hydrobiidae of Bitter Lake National Wildlife Refuge: Previous genetic and ecological research focusing on gammarid amphipods of the Chihuahuan Desert has shown compelling evidence of small-scale, cryptic speciation of *G. desperatus* on BLNWR (Seidel et al. 2009, 2010). Seidel’s studies suggest that management practices should consider isolated populations of *Gammarus* spp. as unique conservation units. Similar, yet unexplored, patterns of genetic diversity could be present within snails of the family Hydrobiidae that co-occur with gammarid amphipods on BLNWR, specifically *J. kosteri* and *P. roswellensis*.

Accordingly, the project biologist collected genetic voucher material (2008, 2009) from isolated populations of hydrobiids on BLNWR (“locality by species” collections shown immediately below). This material is currently being assayed by MU using molecular (mDNA) genetic techniques under the MU NSF grant.

Lake Saint Francis	Bitter Creek at Lost River	Bitter Creek at Flume	Sago Spring	Unit 6 Spring Ditch	Unit 7 Spring Ditch	Unit 7 Marsh	Hunter Marsh
<i>J. kosteri</i>	<i>J. kosteri</i>	<i>J. kosteri</i>	<i>J. kosteri</i>		<i>J. kosteri</i>	<i>J. kosteri</i>	<i>J. kosteri</i>
			<i>P. roswellensis</i>	<i>P. roswellensis</i>		<i>P. roswellensis</i>	

4. Hunter Marsh Surveys (2009-2010): Staff at BLNWR conducted a controlled burn of Hunter Marsh in early March 2009, which facilitated search for surface expressions of spring sources that occur in an otherwise “impenetrable forest” of dense common reed (*Phragmites australis*) and other marsh emergents. The project biologist took advantage of this opportunity to conduct distribution surveys focusing on: *A. pecos*, *J. kosteri*, *P. roswellensis*, *Stagnicola caperata* (Wrinkled marshsnail), *G. desperatus* (Lang 2010).

2009 Qualitative Surveys (presence/absence): Using kitchen sieves, nine sites were surveyed in Hunter Marsh (Figure 2). GIS layers used to create this map included: 2009 National

Agricultural Imagery Program (orthography) and 2009 Bureau of Land Management, New Mexico State Office, Surface Management shape file. All collection sites were documented with GPS (NAD83, Zone 13, UTM) per the project biologist's field catalogue numbering system (Table 1). Voucher material from these sites was retained for ongoing studies comparing among-population genetic similarity/divergence of hydrobiid snails and gammarid amphipods on the Refuge.

2010 Qualitative Surveys (presence/absence): Survey effort in 2010 focused specifically on refining the distribution of *A. pecos* in Hunter Marsh, where a total of 23 sites was sampled using soil sieves and visual search (jeweler's lenses). All sites searched were documented with GPS, but no specimens of *A. pecos* were found, and are thus not mapped in this report.

Results, 2009-2010 Surveys: The April 2009 survey expanded the known range of state and federal listed species in previously unsurveyed areas of Hunter Marsh (Lang 2010). Based on presence of live specimens or empty shells (Table 1), it appears that *J. kosteri* and *G. desperatus* occur in Hunter Marsh where suitable habitat exists, i.e., permanent spring sources. The apparent absence of *P. roswellensis* in Hunter Marsh could be attributed to sampling bias or no historic occurrence in surveyed areas. It was also encouraging to find a new record of *A. pecos* (one empty shell, recent [periostracum intact]; Table 1, BKL09-014) from Hunter Marsh. However, it was discouraging to not find a single specimen in 2010 despite a concerted effort focused specifically on known habitat affinities of this species. Future survey for *A. pecos* is recommended in Hunter Marsh but will require different search methods (e.g., nocturnal) than were employed for the 2010 survey (i.e., diurnal).

Being true to its habitat affinity for ephemeral wetlands, the known range of Wrinkled marshsnail in Hunter Marsh (Lang 2010) has expanded slightly to the west of extant populations previously reported by Lang (2005).

Literature Cited

- Lang, B. K. 2005. Macroinvertebrates of Bitter Lake National Wildlife Refuge. New Mexico Department of Game and Fish, Completion Report, E-56 (1-3), submitted to the Division of Federal Aid, U. S. Fish and Wildlife Service, Region 2, Albuquerque, NM.
- Lang, B. K. 2009. Macroinvertebrates of Bitter Lake National Wildlife Refuge. New Mexico Department of Game and Fish, Performance Report, E-56-6, submitted to the Division of Federal Aid, U. S. Fish and Wildlife Service, Region 2, Albuquerque, NM.
- Lang, B. K. 2010. Macroinvertebrates of Bitter Lake National Wildlife Refuge. New Mexico Department of Game and Fish, Performance Report, E-56-7, submitted to the Division of Federal Aid, U. S. Fish and Wildlife Service, Region 2, Albuquerque, NM.
- Milford, E., E. Muldavin, Y. Chauvin, and M. Reehling. 2001. Spring vegetation and aquatic invertebrate survey 2000. New Mexico Natural Heritage Program, Final Report to the

Bureau of Land Management, Roswell Field Office, under Cooperative Agreement 1422G910A96011 Task Order 13. 24 pp.

New Mexico Department of Game and Fish. 2005. Recovery and conservation plan for four invertebrate species: Noel's amphipod (*Gammarus deseperatus*), Pecos assiminea (*Assimineea pecos*), Koster's springsnail (*Juturnia kosteri*), and Roswell springsnail (*Pyrgulopsis roswellensis*). New Mexico Department of Game and Fish, Conservation Services Division, Santa Fe, New Mexico. 80 pp.

Oertli, B., D. A. Joye, E. Castella, R. Llejuge, A. Lehmann, J.-B. Lachavanne. 2005. PLOCH: a standardized method for sampling and assessing the biodiversity in ponds. *Aquatic Conservation: Marine and Freshwater Ecosystems* 15: 665–679.

Seidel, R. A., B. K. Lang, and D. J. Berg. 2009. Phylogeographic analysis reveals multiple cryptic species of amphipods (Crustacea: Amphipoda) in Chihuahuan Desert spring. *Biological Conservation* 142:2303-2313.

Seidel, R. A., B. K. Lang, and D. J. Berg. 2010. Salinity tolerance as a potential driver of ecological speciation in amphipods (*Gammarus* sp.) from the northern Chihuahuan Desert. *Journal of the North American Benthological Society* 29(3):11161-1169.

Table 1. Aquatic macroinvertebrates collected from Hunter Marsh, Bitter Lake National Wildlife Refuge, April 2009.

Field No.	X	Y	Taxa	Specimens	Preservation
09-010	553943	3697484	<i>Stagnicola caperata</i>	4	95% EtOH
	“ “	“ “	<i>Stagnicola caperata</i>	29	empty shells
	“ “	“ “	<i>Physa acuta</i>	21	empty shells
09-011	553756	3697428	<i>Gammarus desperatus</i>	21	95% EtOH
	“ “	“ “	<i>Hyalella sp.</i>	22	95% EtOH
09-012	553736	3697478	<i>Stagnicola caperata</i>	33	95% EtOH
09-013	553550	3697308	<i>Gammarus desperatus</i>	24	95% EtOH
	“ “	“ “	<i>Hyalella sp.</i>	49	95% EtOH
	“ “	“ “	<i>Juturnia kosteri</i>	34	95% EtOH
09-014	553821	3697262	<i>Gammarus desperatus</i>	2	95% EtOH
	“ “	“ “	<i>Hyalella sp.</i>	?	95% EtOH
	“ “	“ “	<i>Assiminea pecos</i>	1	empty shell
	“ “	“ “	<i>Juturnia kosteri</i>	2	95% EtOH
	“ “	“ “	<i>Physa acuta</i>	1	95% EtOH
09-015	553938	3697414	<i>Gammarus desperatus</i>	abundant	no collection
	“ “	“ “	<i>Juturnia kosteri</i>	33	95% EtOH
09-016	554152	3697366	<i>Gammarus desperatus</i>	107	95% EtOH
	“ “	“ “	<i>Juturnia kosteri</i>	9	95% EtOH
09-017	554187	3697461	<i>Gammarus desperatus</i>	12	95% EtOH
09-018	554092	3697230	<i>Gammarus desperatus</i>	6	95% EtOH
	“ “	“ “	<i>Juturnia kosteri</i>	2	empty shells

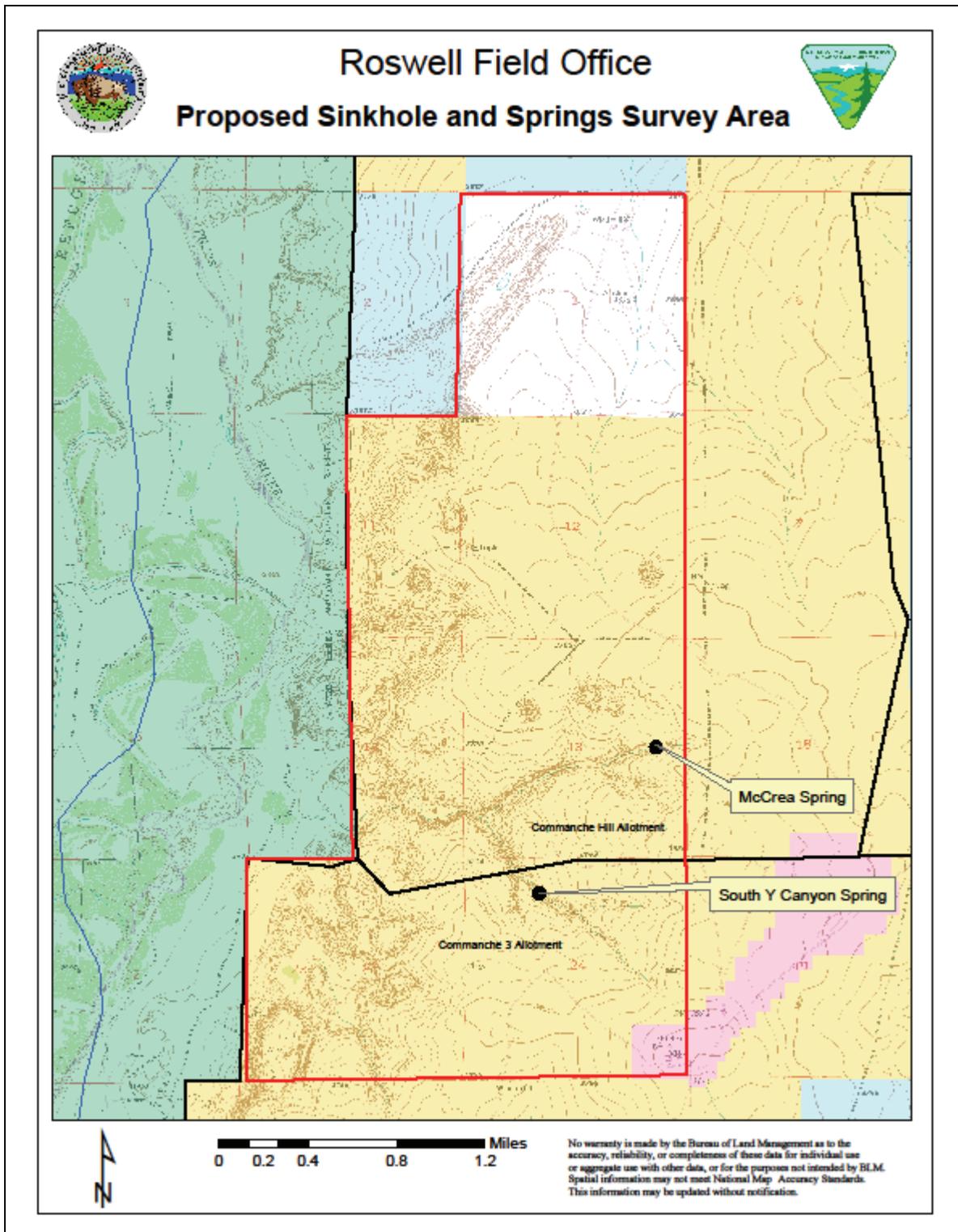


Figure 1. 2008 survey sites on lands administered by the BLM Roswell Field Office (tan) located immediately east of Bitter Lake National Wildlife Refuge (green), Chaves County. Map per Milford et al. (2001).



Figure 2. 2009 survey sites in Hunter Marsh, Bitter Lake National Wildlife Refuge, Chaves County, New Mexico.

Appendix B. 2008-2010 survey for *Pyrgulopsis gilae* and *Pyrgulopsis thermalis* in the Gila River basin, New Mexico.

2008-2010 Survey for *Pyrgulopsis gilae* and *Pyrgulopsis thermalis* in the Gila River Basin, New Mexico.

Under previous grant segments, Lang (2009, 2010) provided information on the distribution of Gila springsnail (*Pyrgulopsis gilae*) and New Mexico springsnail (*Pyrgulopsis thermalis*) based on surveys conducted from 2001 to 2009. Survey results from 2010 along a ca 1.5 miles reach of the West Fork Gila River are also reported here.

Background Information: Distribution

Pyrgulopsis gilae is endemic to numerous thermal springs in the Gila River basin, Catron and Grant counties, New Mexico. This species is known historically from ten populations throughout the basin (Taylor 1983, 1987; Mehlhop 1993):

- Alum Spring, Gila River mainstem, 2.75 miles downstream of the confluence of the East and West forks;
- Jordon Hot Spring, Middle Fork Gila River;
- Two populations on the lower East Fork Gila River (type locality, TL; unnamed spring west [downstream] of TL);
- Fall Spring, upper East Fork Gila River drainage; and
- Five populations from headwater tributaries of the upper East Fork Gila River (i.e., Taylor Creek, three sites; Beaver Creek, two sites).

The endemic *P. thermalis* is known historically from three populations in Grant County (Taylor 1983, 1987; Mehlhop 1993):

- Alum Spring, TL, Gila River mainstem;
- Unnamed spring, lower East Fork Gila River, at TL of *P. gilae*; and
- Unnamed spring, lower East Fork Gila River, west (downstream) of *P. gilae* TL.

These species co-occur at their respective type localities, but apparently partition habitat based on water temperature; *P. thermalis* typically occurs in warmer waters (Taylor 1987, Lang 2002).

Conservation Status

Both species are listed as state threatened (NMDGF 2010) and are considered as candidates for listing (Federal Register 2008) under the Endangered Species Act of 1973. Surveys in 2001, 2002, 2008-2010 indicate that populations and habitats at type localities of both species are stable, including new populations reported by Lang (2009, 2010).

Data Collection and Synthesis

The collection record of *Pyrgulopsis* spp. in the Gila River basin (Taylor 1983, 1987; Mehlhop 1993; Lang 2002, 2009) has been documented by various reporting methods (e.g., geographic descriptors, unsurveyed township-range-section designations, and GPS). Sites surveyed in 2008 and 2009 (Table 1; GPS datum: NAD83, Zone 12S), were mapped (Figure 1) using data

provided by the USFWS (letter to NMDGF dated 18 December 2009). Because most springs surveyed have never been named, the term “Spring Name” in Table 1 most often refers to biologist’s field notes, except for Alum Spring, Fall Spring and Jordan Spring—place names that were obtained from various map sources (e.g., USGS 7.5’ quads, Forest Service maps).

Figure 1 references species occurrences by river reach as “in-set” maps: the upper East Fork Gila River, including Beaver and Taylor creeks (Figure 2); the Middle Fork Gila River (Figure 3); and the lower East Fork Gila River, including Alum Hot Spring located on the Gila River mainstem (Figure 4). For figures 2-4, species occurrences are color coded: yellow = *P. gilae*, blue = *P. thermalis*, red = non-occurrence localities. Surveys in 2009 were also conducted at two sites downstream of Alum Hot Spring: Brock Hot Springs and Turkey Creek Hot Springs.

At most sample sites, water quality parameters (temperature [°C], dissolved oxygen [mg/l, % saturation], specific conductance [μS/cm], salinity [ppt]) were measured using a YSI 85 (Table 1). Due to hydrogeologic setting and site remoteness, spring discharge (depth, velocity) and habitat dimensions (stream width, length; meters) were most often estimated; where habitat conditions required, water temperature was measured using a hand-held thermometer.

Voucher material (genetic, morphologic) was collected (2008, 2009) for taxonomic studies focused on phylogenetic relationships among *Pyrgulopsis* populations of the Gila River basin, New Mexico. Under contract with NMDGF, Dr. Robert Hershler (Smithsonian Institution) and Dr. Hsiu-Ping Lui (Metropolitan State College of Denver) provided a report assessing preliminary results on the genetic and morphologic variation among *Pyrgulopsis* spp. based on these collections. This report is included as “Attachment 1” at end of this appendix.

2008-2010 Survey Results

Since surveys conducted from 2008 to 2010, 43 populations of *P. gilae* and 14 populations of *P. thermalis* have been documented throughout the Gila River basin in New Mexico. While both species occupy similar habitats (isolated spring vents, complexes of spring sources), *P. gilae* occurs more widely distributed throughout the Gila River basin than *P. thermalis* (Figure 2, upper East Fork Gila River; Figure 3, Middle Fork Gila River; and Figure 4, lower East Fork Gila River). The latter species is restricted to similar habitats (isolated springs, spring complexes) of the lower East Fork Gila River and upper Gila River mainstem (Figure 4). Neither species has been found downstream of Alum Spring in the Gila River basin (i.e., Brock Hot Springs and Turkey Creek Hot Springs).

Habitat affinities of *P. gilae* and *P. thermalis*: Spring habitats occupied by both species can be broadly categorized as:

- isolated spring sources (e.g., hill-side seeps situated on broad, alluvial benches; small, stream-side seeps; or “madicolous” habitats—“thin sheet flow of water flowing over rock facies” [sensu Hynes 1970])—that are often situated in shallow entrant canyons and ultimately discharge surface flow to a higher order stream; or
- a complex of spring sources, locally aggregated in shallow entrant canyons, that arise

from groundwater sources (referenced immediately above) that also discharge to a higher order stream.

Across all habitats sampled, salinity ranged narrowly (0.0—0.3 ppt) and dissolved oxygen likewise varied little from 3.1 to 9.1 mg/l (Table 1). While the thermal regime of all spring sources was variable, water temperature of habitat occupied by *P. gilae* ranged from 13.8 to 38.6 °C, whereas *P. thermalis* occurred in more thermal warmer waters (19.5 to 40.4°C). See Table 1 for estimates of spring discharge.

Summary

Field surveys from 2008 to 2010 have increased our knowledge of the distribution of *P. gilae* and *P. thermalis* in the Gila River basin, New Mexico. Both species are more widespread than previously thought. However, recent taxonomic research (Hershler and Liu 2010), indicates that two new species of *Pyrgulopsis* likely occur in the Gila River basin of New Mexico.

Based on genetic analysis of mDNA (COI sequence divergence 3.9-6.1%) and observed shell differences between populations of *P. gilae*, Hershler and Liu (2010) identified three, geographically disparate sub-groups that call for taxonomic recognition:

- Sub-group I: represents the nominal species, *P. gilae*, which is restricted to Alum Spring and springs along the lower reach of the East Fork Gila River;
- Sub-group II: a putative new species, upper reach East Fork Gila River watershed; and
- Sub-group III: a putative new species, Middle Fork Gila River watershed.

With regard to genetic divergence among populations of *P. thermalis*, Hershler and Liu (2010) found that this species is also structured into three geographically, non-overlapping genetic lineages (COI sequence divergence 1.6-3.0%), but they did not observe sufficient morphologic differences among these groups as was observed for the three *P. gilae* sub-groups referenced above. Based on these taxonomic data and close geographic proximity of these genetic lineages, Hershler and Liu (2010) recommended that populations of *P. thermalis* be treated as “conservation units” (*sensu* Moritz 1994) rather than distinct species.

The diversity of habitat types where these species occur has expanded our search image, which calls for more field surveys. Vast areas within the Gila River basin remain unsurveyed:

1. Beaver Creek above Trap Canyon Corral;
2. East Fork Gila River from Black Canyon upstream to Fall Spring;
3. East Fork Gila River from Fall Spring upstream to the confluence of Beaver and Taylor creeks;
4. Middle Fork Gila River upstream of Jordan Canyon; and
5. A re-survey of the West Fork Gila River.

Regarding #5 above, Lang (1998) surveyed ca. 20 miles of the West Fork Gila River from Gila Cliff Dwellings National Monument upstream to Hell’s Hole. While no hydrobiid populations were located, habitat search methods learned from 2008 and 2009 field efforts called

for a re-survey of the West Fork Gila River. To that end, a short reach of the West Fork Gila River (ca. 1.5 miles) from EE Canyon (including search along a ca. 0.75 mile wetted reach of EE Canyon) upstream to an archeological site (NM Lab of Archeology No.: LA148248, primary tracking number; US Forest Service No.: AR-03-06-08-00185, secondary tracking number) was surveyed in 2010 and no hydrobiids were found.

Genetic and morphologic analysis of voucher material collected during this grant period (Hershler and Liu 2010; see Attachment 1 of this appendix) implies that such research should continue to refine potential taxonomic affinities amongst *Pyrgulopsis* spp. of the Gila River basin of New Mexico.

Threats to *P. gilae* and *P. thermalis* detailed in Appendix E (below) are based on current knowledge of the species' biological and ecological requirements, direct and indirect knowledge of environmental factors known to affect hydrobiids and their habitats, and review of the published literature that documents adverse natural and human-induced impacts to aquatic gastropods (Brown et al. 2008).

Literature Cited

- Brown, K. M., B. K. Lang, and K. E. Perez. 2008. The conservation ecology of North American pleurocerid and hydrobiid gastropods. *Journal of the North American Benthological Society*. 27(2):484-495.
- Federal Register. 2008. Endangered and threatened wildlife and plants; Review of species that are candidates or proposed for listing as endangered or threatened; Annual notice of findings on recycled petitions; Annual description of progress on listing actions; proposed rule. 50 CFR Part 17, 73(238):75176-75244.
- Hershler, R. and H-P. Liu. 2010. Genetic variation of threatened springsnails from the Gila River basin, New Mexico. Final report submitted to New Mexico Department of Game and Fish under Contract 10-516-0000-0010. 16 pp.
- Hurt, C. R. 2004. Genetic divergence, population structure and historical demography of rare springsnails (*Pyrgulopsis*) in the lower Colorado River basin. *Molecular Ecology* 13:1173-1187.
- Hynes, H. B. N. 1970. *The Ecology of Running Waters*. University of Toronto Press. 555 pp.
- Lang, B. K. 1998. Status of aquatic mollusks of New Mexico. New Mexico Department of Game and Fish, Performance Report E-20-6 submitted to the Division of Federal Aid, U. S. Fish and Wildlife Service, Albuquerque, NM.
- Lang, B. K. 2002. Status of aquatic mollusks of New Mexico. New Mexico Department of Game and Fish, Completion Report E-20-(5-9) submitted to the Division of Federal Aid, U. S. Fish and Wildlife Service, Albuquerque, NM.

- Lang, B. K. 2009. Macroinvertebrates of Bitter Lake National Wildlife Refuge. New Mexico Department of Game and Fish, Performance Report, E-56-6 , submitted to the Division of Federal Aid, U. S. Fish and Wildlife Service, Region 2, Albuquerque, NM.
- Lang, B. K. 2010. Macroinvertebrates of Bitter Lake National Wildlife Refuge. New Mexico Department of Game and Fish, Performance Report, E-56-7 , submitted to the Division of Federal Aid, U. S. Fish and Wildlife Service, Region 2, Albuquerque, NM.
- Mehlhop, P. 1993. Establishment of a rare mollusc inventory and monitoring program for New Mexico. Year II Progress Report. NMDGF Professional Services Contract No. 80-519-52-Amendment 1.
- Moritz, C. 1994. Defining 'Evolutionarily Significant Units' for conservation. *Trends in Ecology and Evolution* 9: 373-375.
- New Mexico Department of Game and Fish. 2010. Threatened and endangered species of New Mexico: biennial review and recommendations.
- Taylor, D. W. 1983. Endangered species: status investigation of mollusks of New Mexico. Professional Service Contract Nos. 519-69-01 and 519-69-01-A.
- Taylor, D. W. 1987. Fresh-water mollusks from New Mexico and vicinity. New Mexico Bureau of Mines & Mineral Resources Bulletin 116.

Table 1. 2008 and 2009 survey data for *Pyrgulopsis gilae* and *Pyrgulopsis thermalis* of the Gila River basin, New Mexico.

Spring Name	Date	Species	Latitude	Longitude	Tw °C	DO mg/l	DO %	Conductance µS/cm	Salinity ppt	Width cm	Depth cm	Length	Flow
LOWER EAST FORK GILA RIVER													
<i>P. gilae</i> Type Locality Complex													
Site 1 (Pg TL)	19-May-09	gilae	33°11.501	108° 10.450	20.8	6.8	74	315	0.2	45	1.5	~40 m	1 gal/m
Site 1 (Pg TL)	19-May-09	gilae	33°11.501	108° 10.450	20.8	6.8	74	315	0.2	45	1.5	~40 m	1 gal/m
Slick rock (Pg TL)	19-May-09	gilae	33°11.501	108° 10.450	24.5					100	0.5	?	
Slick rock (Pg TL)	19-May-09	thermalis	33°11.501	108° 10.450	24.5								
Slick rock (Pg TL)	19-May-09	gilae	33°11.501	108° 10.450	20.8	6.8	74	315	0.2				
Slick rock (Pg TL)	19-May-09	thermalis	33°11.501	108° 10.450	24.5								
E Slot canyon	19-May-09	gilae	33°11.522	108° 10.447	13.8	9.14	87	61	0	45	2.0	>30 m	
E Slot canyon	18-Aug-09	gilae	33°11.492	108° 10.460	19.8	7.3		434	0.2				1 l/m
Bedrock fissure	19-May-09	thermalis	33°11.508	108° 10.470	27	7	84	46.3	0	15	1.5	20	
Bedrock fissure	19-May-09	thermalis	33°11.508	108° 10.470	27	7	84	46.3	0	15	1.5	20	
floodplain 1	19-May-09	thermalis	33°11.481	108° 10.430	34.5	5.5	75	721	0.3	10	1.5	10	
talus slope	19-May-09	thermalis	33°11.392	108° 10.455	40.2	4.7	74	80	0	20	1.0	10	1 gal/m
talus slope	19-May-09	thermalis	33°11.392	108° 10.455	40.2	4.7	74	80	0	20	1.0	10	1 gal/m
talus slope	18-Aug-09	thermalis	33°11.392	108° 10.455	40.4	4.8	73	482	0.2				
fern	19-May-09	thermalis	33°11.477	108° 10.422	31	ND	ND	ND	ND	300	0.5	20	
rock face	19-May-09	thermalis	33°11.520	108° 10.510	38.6	4.6	70	814	0.3	45	1.5	10	1 gal/m.
head of slough	19-May-09	thermalis	33°11.462	108° 10.426	ND	ND	ND	ND	ND	300	6.0	125	
head of slough	18-Aug-09	thermalis	33°11.462	108° 10.426	33.9	3.1	44	745	0.3				
Spire canyon	19-May-09	gilae	33°11.505	108° 10.503	17.8	7.7	81	532	0.3	10 m	0.5	>20	
Chigger site complex													
warm source	18-Aug-09		33°11.182	108° 10.049	30.6	0.35	4.6	4.5	0	8	2.0	20	< 1 gal/m
warm source 2	18-Aug-09	thermalis	33°11.182	108° 10.049	32.4	4.9		15.1					
warm source 2	18-Aug-09	gilae	33°11.182	108° 10.049	32.4	4.9		15.1	0	8	2.0	20	< 1 gal/m
high warm	18-Aug-09	thermalis	33°11.167	108° 10.097	38	ND	ND	ND	ND				
cool source	18-Aug-09	thermalis	33°11.184	108° 10.044	19.5	ND	ND	ND	ND				
cool source	18-Aug-09	gilae	33°11.184	108° 10.044	19.5	ND	ND	ND	ND				
Unnamed spring	18-Aug-09	none	33°11.185	108° 10.024	40	2.5	ND	780	0.3	20	3.0	15	2 l/s
Eastern canyon	18-Aug-09	gilae	33°11.157	108° 09.989	21.7	6.2	70	26.4	0	15	0.5	20	seep

Table 1. (continued)

Spring Name	Date	Species	Latitude	Longitude	Tw °C	DO mg/l	DO %	Conductance µS/cm	Salinity ppt	Width cm	Depth cm	Length	Flow
LOWER EAST FORK GILA RIVER													
EF Gila seep	19-May-09	glae	33°11.060	108° 09.907	23.3	6	70	230	0.1	30 m	40 m	4000	seep
Werber home source complex													
Werber source	19-May-09	glae	33°11.676	108° 10.826	22.8	8.4	88	602	0.3	15 to 600	1.5	>400	10 g/m
Werber source	19-May-09	thermalis	33°11.676	108° 10.826	22.8	8.4	88	602					
Werber source	19-May-09	thermalis	33°11.676	108° 10.826	ND	ND	ND	ND					
Werber source	19-May-09	glae	33°11.676	108° 10.826	ND	ND	ND	ND					
Werber source	19-May-09	glae	33°11.676	108° 10.826	ND	ND	ND	ND					
Werber source	19-May-09	thermalis	33°11.676	108° 10.826	ND	ND	ND	ND					
canyon mouth	19-May-09	none	33°11.628	108° 10.890									
canyon behind guest house	18-Aug-09	none	33°11.669	108° 10.965									
canyon below guest house	18-Aug-09	none	33°11.727	108° 11.340									
Hot soaking pool	18-Aug-09	none	33°11.446	108° 11.440									
UPPER EAST FORK GILA													
Fall Spring	30-Jul-08	glae	33°17.642	108° 07.812									
Fall Spring	20-May-09	glae	33°17.642	108° 07.812	23.9	7.5	90	280	0.1	45	5.0	400 m	0.15 cfs
Little Fall	30-Jul-09	glae	33°17.707	108° 07.610									
Little Fall	20-May-09	glae	33°17.707	108° 07.610	17.5	6.7	70	54	0	30	1.0	38 m	1 l/m
Little Fall seep	20-May-09	glae	33°17.734	108° 07.607	17.2	6.5		15	0				seep
TAYLOR CREEK													
Below Wall Lake sites													
Below Wall (Big Meadow)	1-Aug-08	glae	33°20.745	108° 05.423									
Below Wall (Big Meadow)	20-May-09	glae	33°20.745	108° 05.423	22	5.3	53	165	0.1				
seep1	20-May-09	glae	33°20.762	108° 05.194	21.9	5.1	60	18.3	0			2	seep
seep 2	20-May-09	glae	33°20.770	108° 05.233	25							6	seep
seep 3	20-May-09	glae	33°20.793	108° 05.235	22							30	
cany. Blw Wall	20-May-09	glae	33°20.911	108° 05.291	20						1.0	15 m	
Blw Wall dam1	20-May-09	glae	33°21.070	108° 05.002	22							15m	seep

Table 1. (continued)

Spring Name	Date	Species	Latitude	Longitude	Tw °C	DO mg/l	DO %	Conductance µS/cm	Salinity ppt	Width cm	Depth cm	Length	Flow
TAYLOR CREEK													
Below Wall Lake sites													
Blw Wall dam2	20-May-09	glae	33°21.064	108° 04.979	23								seep
Blw Wall dam3	20-May-09	glae	33°21.044	108° 04.853	19.5					20m		20m	seep
Above Wall Lake sites													
Edge Spring	1-Aug-08	glae	33°21.488	108° 04.036	ND	ND	ND	ND	ND				
Edge Spring	20-May-09	glae	33°21.488	108° 04.036	ND	ND	ND	ND	ND				
200M DwnStream Whitewater/Whitetail cny conf	1-Aug-08	glae	33°21.675	108° 03.457	ND	ND	ND	ND	ND				
BEAVER CREEK													
Beaver 1	21-May-09	glae	33°20.433	108° 06.583	22.1	4.75		300	0.2	75	15.0	170	1 cfs
Beaver 1	21-May-09	glae	33°20.433	108° 06.583	22.1	4.75		300	0.2	75	15.0	170	1 cfs
Beaver 2	21-May-09	glae	33°20.480	108° 06.698	24							100 m	
Beaver 3	21-May-09	glae	33°20.478	108° 06.808	24.7	4.9	60	324	0.2	45	13.0	170 m	.75 cfs
Beav mdw1	21-May-09	glae	33°21.516	108° 06.935	22.2	5.7	65	60	0				1 l/m
Beav mdw2 (lower reach)	21-May-09	glae	33°21.556	108° 06.942	19.8	4.1	45	22.4	0				
Beav mdw2 (@ source)	21-May-09	glae	33°21.556	108° 06.942	19.8	4.1	45	22.4	0				
Beaver 4	21-May-09	glae	33°20.413	108° 06.574									
Beaver 5 to	21-May-09	glae	33°20.343	108° 06.450									
Beaver 11	21-May-09	glae	33°20.306	108° 06.367	23.1	5.8		311	0.2	30	1.0		0.1 cfs
end of survey EF Gila downstream	21-May-09		33°19.599	108° 06.308									
end of survey upstream	21-May-09		33°20.566	108° 05.741									
GILA RIVER													
Alum Spring	28-Jul-08	thermalis	33°09.707	108° 12.484	38.6	5.12	96.2	792					
MIDDLE FORK GILA													
Jordan complex													
Jordan (in dispersed cmpgd)	29-Jul-08	glae	33°17.335	108° 16.097									
Jordan (in dispersed cmpgd)	29-Jul-08	glae	33°17.525	108° 16.149									
Downstream extent	1-Oct-09	glae	33°17.479	108° 16.100	31.3								seep
Collection site (field tag reads: Upper Jordan)	1-Oct-09	glae	33°17.543	108° 16.174	30.5					60	1-3	50 m	~0.1 cfs

Table 1 (continued)

Spring Name	Date	Species	Latitude	Longitude	Tw °C	DO mg/l	DO %	Conductance µS/cm	Salinity ppt	Width cm	Depth cm	Length	Flow
MIDDLE FORK GILA													
Upstream extent	1-Oct-09	gilaie	33°17.560	108° 16.193									
"Lower Jordan" per MIM field tag	1-Oct-09	gilaie	33°17.452	108° 16.084									
Summer's Canyon	29-Sep-09	none	33°15.620	108° 13.974									
Mud turtle complex													
site 1 (turtles)	29-Sep-09	gilaie	33°16.444	108° 14.861	25.9							20 m	<0.25 cfs
site 2 (upper canyon)	30-Sep-09	gilaie	33°16.522	108° 14.961	22							5 m	~ 2 l/min
site 3 (maidenhair fern)	30-Sep-09	gilaie	33°16.509	108° 14.939	28.5							30 m	~ 0.1 cfs
site 4 (meadow)	30-Sep-09	gilaie	33°16.485	108° 15.027	19							50 m	
site 5 (@ 5th xing)	30-Sep-09	gilaie	33°16.461	108° 15.079	30.9					100	1-6	30 m	

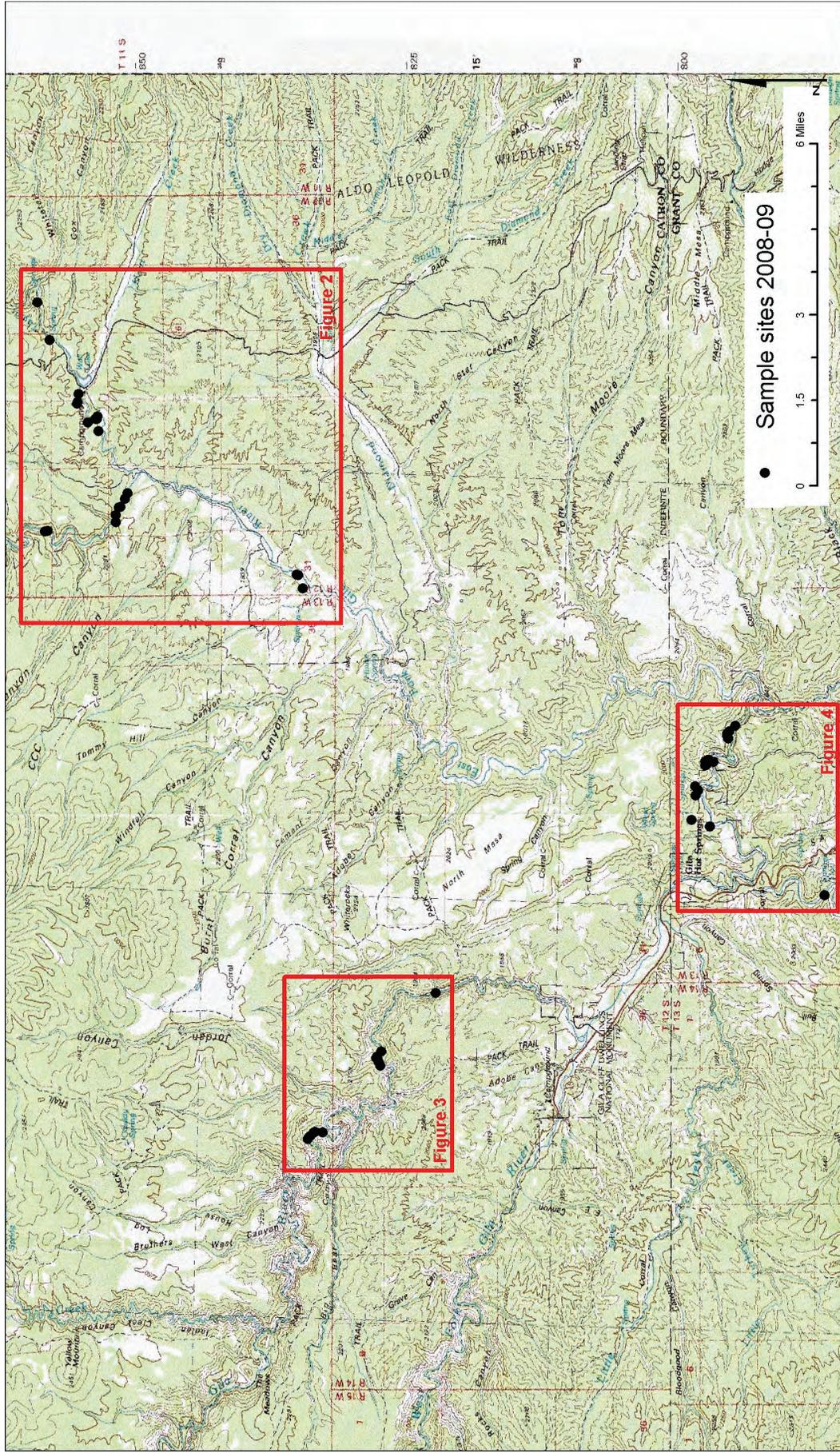


Figure 1. 2008 and 2009 survey sites for *Pyrgulopsis* spp. of the Gila River Basin, New Mexico.

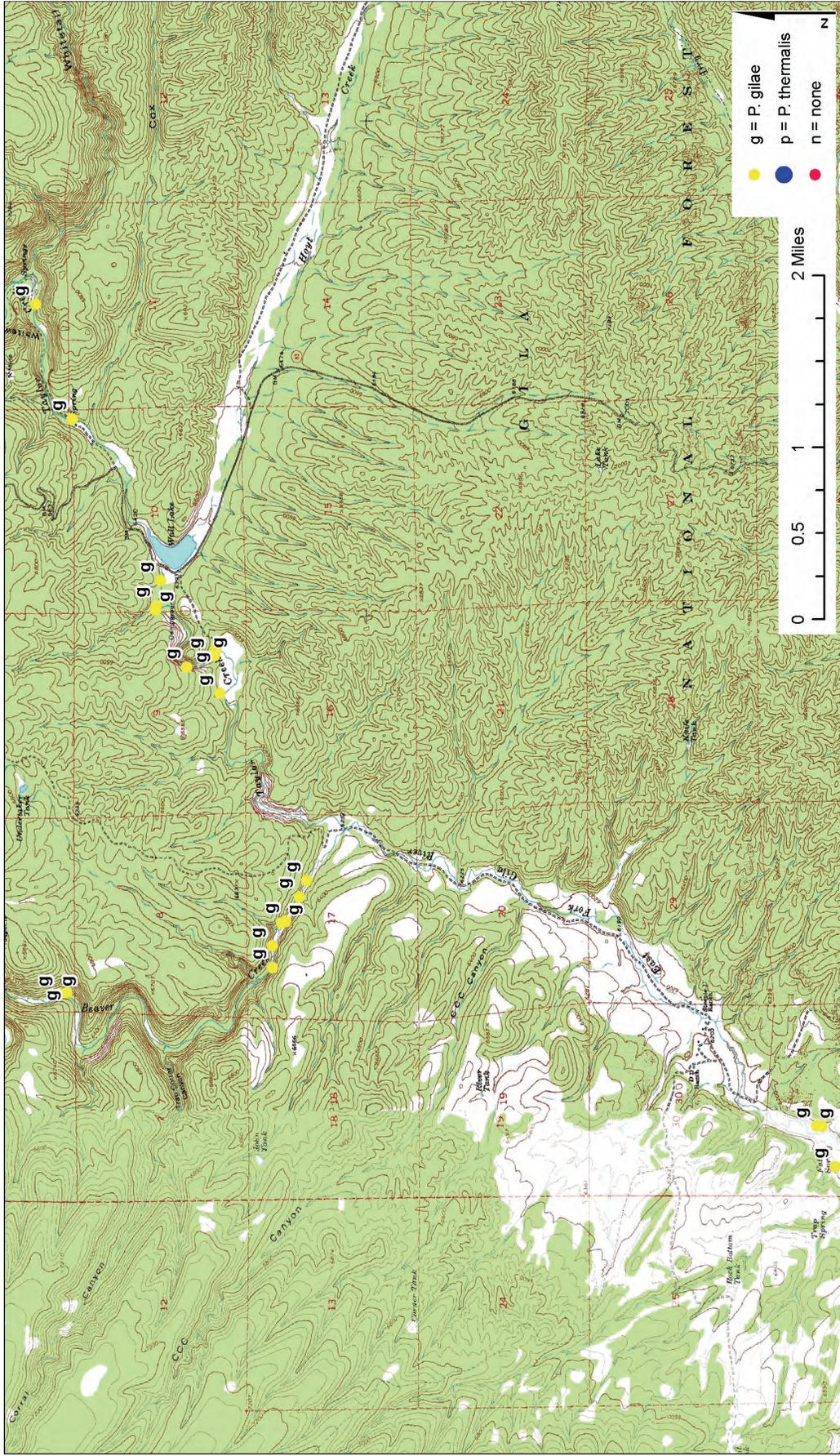


Figure 2. Upper East Fork Gila River *Pyrgulopsis* species occurrences based on 2008 and 2009 surveys of the Gila River Basin, NM.

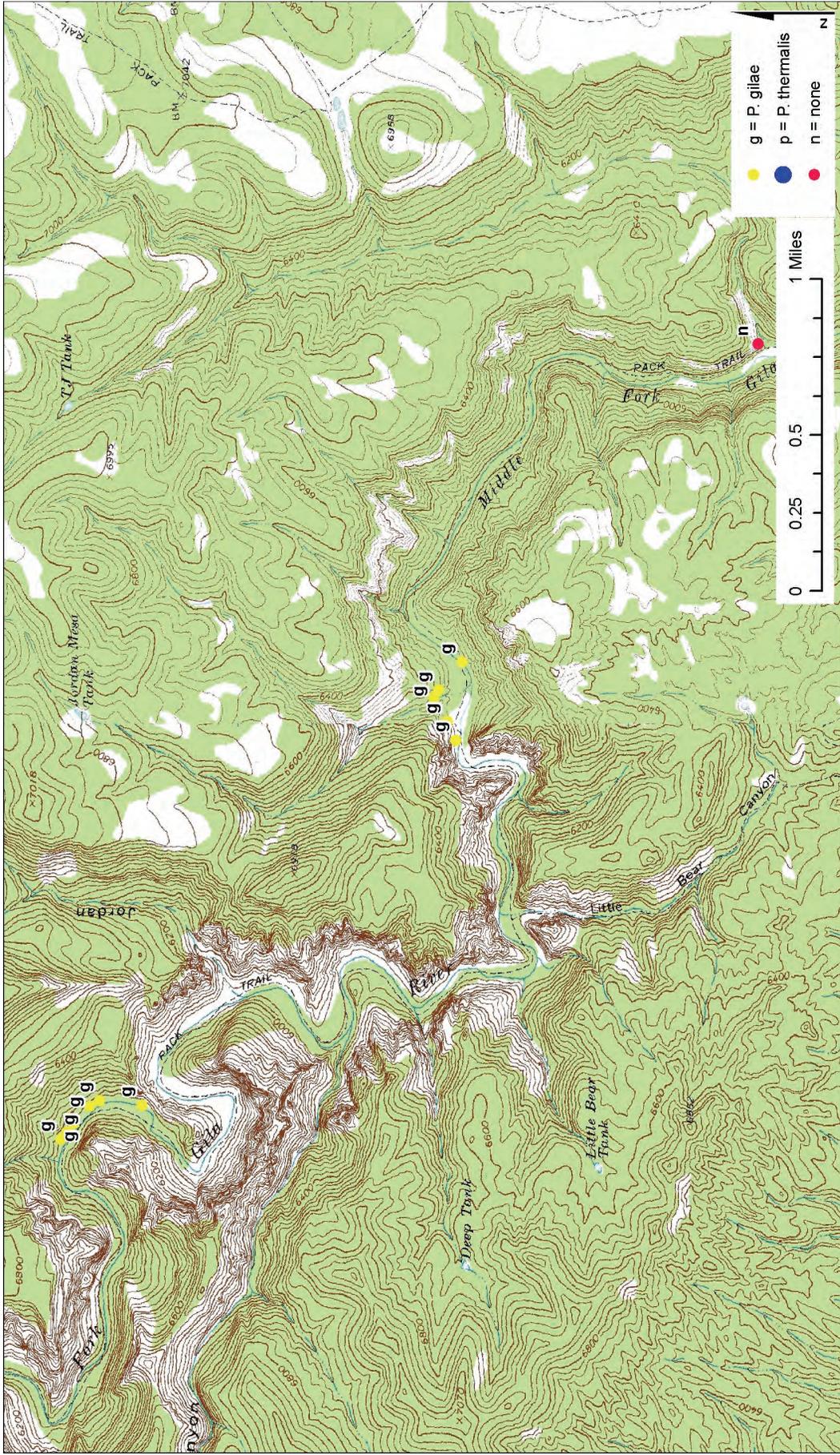


Figure 3. Middle Fork Gila River *Pyrgulopsis* species occurrences based on 2008 and 2009 surveys of the Gila River Basin, NM.

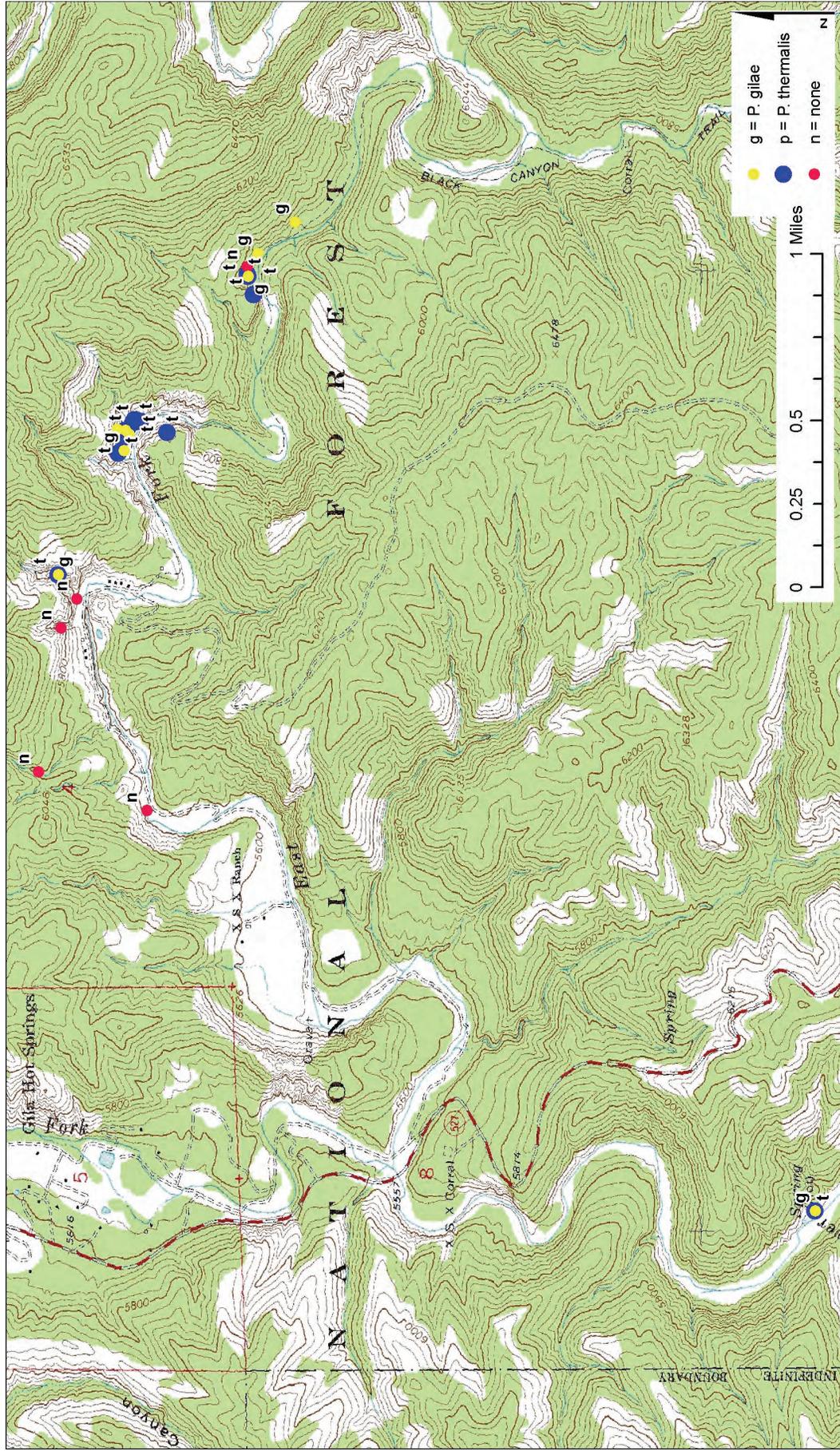


Figure 4. Lower East Fork Gila River *Pyrgulopsis* species occurrences based on 2008 and 2009 surveys of the Gila River Basin, NM.

ATTACHMENT 1

Genetic variation of threatened springsnails from the Gila River basin, New Mexico

Report to the State of New Mexico Department of Game & Fish

13 December 2010

¹Robert Hershler and ²Hsiu-Ping Liu

¹Department of Invertebrate Zoology
Smithsonian Institution, P. O. Box 37012, NHB W-305, MRC 163
Washington, D.C. 20013-7012
Phone 202-633-1747; fax 202-633-0182; email hershler@si.edu

²Department of Biology
Metropolitan State College of Denver
Denver, CO 80217

INTRODUCTION

The purpose of this project was to assess genetic variation among populations of two New Mexico state listed springsnail species, *Pyrgulopsis gilae* and *P. thermalis*, which are distributed among geographically isolated spring habitats in the upper Gila River basin (Taylor 1987, NMDFG 2008). This work was specifically recommended in the state's most recent review of its threatened and endangered species (NMDGF 2008) as an aid to developing management strategies for these two threatened snails. Here we assess genetic variation and structuring within these two species based on analyses of partial sequences of the cytochrome *c* oxidase subunit (COI) mitochondrial gene.

MATERIALS AND METHODS

Specimens

Samples were collected by RH and Brian Lang from multiple sites (*P. gilae*, 12 sites; *P. thermalis*, 5 sites) across the entire geographic ranges of these two species (Fig. 1). Specimens were preserved in 90% ethanol. Locality details and other pertinent information for these samples are in Table 1. Partial COI sequences were obtained from two-ten specimens from each sample. Previously published sequences of these two species (Hurt 2004, Liu & Hershler 2005) and representative sequences from nine other regional congeners were also included in our phylogenetic analyses. Trees were rooted with sequences of *Floridobia floridana* following Hershler *et al.* (2003).

Methods

Genomic DNA was extracted from entire snails using a CTAB protocol (Bucklin 1992). Partial sequences of COI (658 bp) were amplified and sequenced with primers LCO1490 and HCOI2198 (COI) following the protocols of Liu *et al.* (2003). Sequences were determined for both strands and then edited and aligned using Sequencher™ version 4.8.

Sequence divergences (uncorrected p distance) within and between phylogenetic lineages were calculated using MEGA4 (Tamura *et al.* 2007); standard errors were estimated by 1000 bootstrap replications with pairwise deletion of missing data. MrModeltest 2.3 (Nylander 2004) was used to obtain an appropriate substitution model (using the Akaike Information Criterion) and parameter values for the Bayesian analyses. Bayesian inference was performed

using MrBayes 3.12 (Ronquist & Huelsenbeck 2003); separate analyses were conducted for *P. gilae* and *P. thermalis*. In the initial analysis the burnin was set at 10% (10,000 generations) of the chain length (100,000 generations). Three runs were conducted in MrBayes using the HKY + G + I (for *P. gilae*) and HKY + G (for *P. thermalis*) models selected by MrModeltest and the default random tree option to determine when the log likelihood scores reached a stable value (by plotting the log likelihood scores of sample points against generation time). The log likelihood scores started at around -6100 for *P. gilae* and -4600 for *P. thermalis* and quickly converged upon stable values of about -2800 and -2300, respectively. 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 topology with the first 5,000 trees, equal to 50,000 generations, removed to ensure that the chain sampled a stationary portion.

RESULTS

Pyrgulopsis gilae

Eighty-three (83) specimens of *P. gilae* were sequenced for COI (Table 2). The alignment of COI sequences yielded 658 bp, of which 175 sites were variable (26.6%) and 129 were parsimony informative (19.6%). Overall nucleotide composition was biased towards thymine (T) (36.4%) and adenine (A) (25.2%), followed by cytosine (C) (20.2%) and guanine (G) (18.2%) as typically observed in gastropod mitochondrial genes (e.g., Hershler *et al.* 2003). Minor variation involving a few base pair positions was detected in most of the samples; five samples each contained only a single haplotype (G5, G7, G10, G11, G13).

In the Bayesian analysis, specimens of *P. gilae* formed a moderately supported clade that was sister to a clade consisting of *P. deserta* and an undescribed congener from the Rio Mimbres drainage (Fig. 2). *Pyrgulopsis gilae* was structured into three geographically disjunct sub-groups in this tree: sub-group I contained COI sequences from “Alum Spring” (along the mainstem Gila River) and springs along the lower reach of the East Fork Gila River; II was composed of sequences from the upper reach of the East Fork Gila watershed; and III was composed of sequences from the Middle Fork Gila watershed (Fig. 2). These three sub-groups differed from each other by 3.9-6.1% COI sequences divergence while variation within each of these units was minor (Table 3).

Pyrgulopsis thermalis

Twenty-one (21) specimens of *P. thermalis* were sequenced for COI (Table 4). The alignment of COI sequences yielded 658 bp, of which 159 sites were variable (24.2%) and 98 were parsimony informative (14.9%). Overall nucleotide composition was biased towards thymine (T) (39.6%) and adenine (A) (24.7%), followed by cytosine (C) (17.0%) and guanine (G) (18.8%) as typically observed in gastropod mitochondrial genes (e.g., Hershler *et al.* 2003). Minor variation involving a few base pair positions was detected in most of the samples; one sample (T5) was composed of a single haplotype (Table 4).

In the Bayesian analysis, specimens of *P. thermalis* formed a well supported clade that was sister to *P. trivialis* (Fig. 3). *Pyrgulopsis thermalis* was structured into three geographically proximal yet non-overlapping sub-groups in this tree: sub-group I contained COI sequences from “Alum Spring” along the mainstem Gila River; II was composed of sequences from several springs along a lower reach of the East Fork Gila River; and III was composed of sequences from a spring further downflow in the East Fork Gila watershed (Fig. 3). These three sub-groups differed from each other by 1.6-3.0% COI sequence divergence while variation within each of these units was minor (Table 5).

DISCUSSION

Our results confirm a previous finding (Hurt 2004) that both *P. gilae* and *P. thermalis* are structured into multiple genetic lineages. The three sub-groups of *P. gilae* delineated by our analyses are widely separated geographically and their COI sequence divergence (3.9-6.1%) falls well into the range observed for other species of *Pyrgulopsis* (1.1-13.1% for COI; Liu & Hershler 2005). Based on this information and the obvious differences between the shells of these groups of populations (Hershler unpublished), we consider the three subunits of *P. gilae* to be distinct species. In spite of its extremely narrow geographic range, *P. thermalis* is also structured into geographically non-overlapping genetic lineages whose sequence divergence (1.6-3.0%) falls into the range observed for other congeners. However, in this case we did not observe obvious morphologic differences among these groups of populations. Based on this evidence and the close geographic proximity of these lineages we suggest that they be treated as conservation units (*sensu* Moritz 1994) rather than distinct species.

These findings are preliminary in that our samples were analyzed for only a single gene and have not been accompanied by detailed morphologic studies. We anticipate completing the sequencing of a second gene within the next two months; the morphologic component of the project will be completed after larger samples have been obtained and analyzed using standard methods. Upon completion of this work, a taxonomic revision of *P. gilae*, which will include description of two new species, will be prepared and published.

ACKNOWLEDGMENTS

We thank Brian Lang for providing specimens and Justin Davies for generating sequence data that were analyzed in this study.

LITERATURE CITED

- Bucklin, A. 1992. Use of formalin-preserved samples for molecular analysis. *Newsletter of Crustacean Molecular Techniques* **2**: 3.
- Hershler, R., Liu, H.-P. & Thompson, F.G. 2003. Phylogenetic relationships of North American nymphophiline gastropods based on mitochondrial DNA sequences. *Zoologica Scripta* **32**: 357-366.
- Hurt, C.R. 2004. Genetic divergence, population structure and historical demography of rare springsnails (*Pyrgulopsis*) in the lower Colorado River basin. *Molecular Ecology* **13**: 1173-1187.
- Liu, H.-P. & Hershler, R. 2005. Molecular systematics and radiation of western North American nymphophiline gastropods. *Molecular Phylogenetics and Evolution* **34**: 284-298.
- Liu, H.-P., Hershler, R. & Clift, K. 2003. Mitochondrial DNA sequences reveal extensive cryptic diversity within a western American springsnail. *Molecular Ecology* **12**: 2771-2782.
- Moritz, C. 1994. Defining 'Evolutionarily Significant Units' for conservation. *Trends in Ecology and Evolution* **9**: 373-375.
- NMDGF (New Mexico Department of Game and Fish). 2008. Threatened and endangered species of New Mexico. 2008 biennial review. Available from http://www.wildlife.state.nm.us/conservation/threatened_endangered_species/documents/2008BiennialReview.pdf (accessed 8 December 2009).

- Nylander, J.A.A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Ronquist, F. & Huelsenbeck, J.P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**: 1596–1599.
- Taylor, D.W. 1987. Fresh-water molluscs from New Mexico and vicinity. *New Mexico Bureau of Mines & Mineral Resources Bulletin*, **116** 1-50.

Table 1. Samples used for molecular analysis, with sample codes (referred to in the text and figures) and locality details.

Species	Code	Locality (all in New Mexico)	Latitude	Longitude
<i>P. gilae</i>	G1	Grant Co., East Fork Gila River, unnamed spring ca. 1.53 km north and 2.9 km east of State Rte. 527 bridge crossing	33.1917	-108.1742
<i>P. gilae</i>	G2	Grant Co., East Fork Gila River, unnamed spring ca. 1.29 km north and 0.56 km west of confluence with Black Canyon	33.1864	-108.1675
<i>P. gilae</i>	G3	Grant Co., East Fork Gila River, unnamed spring 1.53 km north and 2.38 km east of State Rte. 527 bridge crossing	33.1946	-108.1804
<i>P. gilae</i>	G4	Catron Co., Taylor Creek, ca. 0.32 km south and 0.93 km west of Wall Lake dam (below Wall Lake)	33.3457	-108.0904
<i>P. gilae</i>	G5	Catron Co., East Fork Gila River, hillside spring seep 1.61 km north, 0.97 km east of Burnt Corral Canyon	33.2951	-108.1268
<i>P. gilae</i>	G6	Catron Co., Beaver Creek ca. 0.29 km and 0.40 km west of confluence with Taylor Creek	33.3405	-108.1097
<i>P. gilae</i>	G7	Catron Co., Taylor Creek, Whitewater Canyon, 50 m west of Whitetail Canyon, along south stream bank of Taylor Creek	33.3613	-108.0576
<i>P. gilae</i>	G8	Grant Co., Middle Fork Gila River, ca. 0.97 km north and 0.64 km west of Jordan Canyon	33.2848	-108.2667
<i>P. gilae</i>	G9	Catron Co., East Fork Gila River, Fall Spring 1.61 km north, 0.56 mile east of Burnt Corral Canyon	33.2940	-108.1302
<i>P. gilae</i>	*G10	Catron Co., East Fork Gila River, Fall Spring 1.61 km north, 0.56 km east of Burnt Corral Canyon	33.2940	-108.1302
<i>P. gilae</i>	G11	Grant Co., Middle Fork Gila River, ca. 0.48 km north and 0.48 km west of Jordan Canyon	33.2909	-108.2681
<i>P. gilae</i>	G13	Catron Co., Taylor Creek, ca. 0.80 km north and 1.13 km east of Wall Lake Dam (above Wall Lake)	33.3581	-108.0673
<i>P. gilae</i>	G14	Grant Co., Gila River, "Alum Hot Spring" ca. 1.93 km south and 0.16 km west of State Route 527 bridge crossing	33.1618	-108.2081
<i>P. thermalis</i>	T1	Grant Co., Gila River, "Alum Hot Spring" ca. 1.93 mile south and 0.16 km west of State Route 527 bridge crossing	33.1618	-108.2081
<i>P. thermalis</i>	T2	Grant Co., East Fork Gila River, unnamed spring ca. 1.29 km north and 0.56 km west of confluence with Black Canyon	33.1864	-108.1675
<i>P. thermalis</i>	T3	Grant Co., East Fork Gila River, unnamed spring ca. 1.53 km north and 2.98 km east of State Rte. 527 bridge crossing	33.1899	-108.1742
<i>P. thermalis</i>	T4	Grant Co., East Fork Gila River, unnamed spring 1.53 km north and 2.38 km east of State Rte. 527 bridge crossing	33.1946	-108.1804
<i>P. thermalis</i>	T5	Grant Co., East Fork Gila River, unnamed spring ca. 1.53 km north and 2.90 km east of State Rte. 527 bridge crossing	33.1917	-108.1742

*Very small (juvenile) specimens initially thought to be distinct from *P. gilae*.

Table 2. COI haplotype variation observed in *P. gilae* samples.

Sample	Haplotype	Base pair position								
G1		67	95	115	316	334	424	502	625	
	G1A	A	C	T	T	T	T	G	T	
	G1B	A	C	T	T	T	T	G	T	
G2	G1C	G	T	C	C	C	C	A	C	
		316								
	G2A	C								
	G2B	C								
	G2C	C								
	G2D	C								
	G2E	T								
	G2F	C								
	G2G	C								
	G2H	C								
	G2I	C								
G3	G2J	C								
		319	355							
	G3A	T	A							
	G3B	T	A							
	G3C	A	A							
	G3F	T	G							
G4	G3G	T	A							
	G3J	T	A							
		43	145	190	295	337	347	375	401	557
	G4B	T	T	C	A	G	T	G	C	C
	G4C	T	T	C	A	G	T	G	C	C
	G4D	T	T	C	A	G	T	G	C	C
	G4E	T	T	C	A	G	T	G	C	C
	G4F	T	C	T	G	A	C	G	T	T
G4G	C	T	C	A	G	T	G	C	C	
G5	G4H	C	T	C	A	A	T	C	C	C
	G5A									
	G5B									
	G5C									
	G5D									
	G5E									
	G5H									
	G5I									
G6		199	281	421	601					
	G6A	T	T	C	T					
	G6B	C	C	T	C					
	G6C	C	C	T	C					
	G6D	C	C	T	C					
	G6F	T	T	C	T					
G6G	C	T	T	C						

Sample	Haplotype	Base pair position			
G7	G6H	C	C	T	C
	G6I	C	C	T	C
	G6J	C	C	T	C
	G7A				
	G7B				
	G7C				
	G7D				
	G7E				
	G7F				
	G7G				
	G7H				
	G7I				
G7J					
G8		196	217	613	
	G8A	G	C	C	
	G8B	G	T	C	
	G8C	G	T	T	
	G8D	A	T	C	
	G8E	G	T	C	
	G8G	G	T	C	
G9		89			
	G9A	A			
	G9B	-			
	G9C	-			
	G9D	-			
	G9E	G			
	G9F	G			
	G9G	G			
	G9H	G			
	G9I	G			
G10	G10A				
	G10B				
	G10C				
	G10D				
G11	G11B				
	G11D				
G13	G13A				
	G13B				
	G13C				
	G13D				
	G13E				
	G13F				
	G13G				

Sample	Haplotype	Base pair position
G14	G13H	271
	G14D	C
	G14E	C
	G14F	T

Table 3. Mean COI sequence divergence among *P. gilae* sub-groups. Sub-group I: G1, G2, G3, G14; Sub-group II: G4, G5, G6, G7, G9 G10, G13; Sub-group III: G8, G11.

	Group I	Group II	Group III
Group I	0.006+/-0.002		
Group II	0.061+/-0.009	0.008+/-0.002	
Group III	0.039+/-0.008	0.045+/-0.008	0.002+/-0.001

Table 4. COI haplotype variation observed in *P. thermalis* samples.

Sample	Haplotype	Base pair position				
T1		206	217	318	331	412
	T1H	C	T	C	A	C
	T1I	T	T	C	G	C
	T1J	T	C	T	A	T
	T1K	T	T	T	A	T
T2	T1L	T	T	C	A	C
		166	190	451	502	514
	T2H	A	T	G	A	C
	T2J	A	T	A	G	T
	T2L	G	C	A	A	T
T3	T2M	G	C	A	A	T
		55				
	T3H	C				
	T3I	C				
	T3J	C				
	T3K	C				
T4	T3L	T				
	T3M	C				
		286				
T5	T4J	G				
	T4K	A				
T5						
	T5H					
	T5K					
	T5L					
	T5M					

Table 5. Mean COI sequence divergence among *P. thermalis* sub-groups. Sub-group I: T1; Sub-group II T2, T3, T5; Sub-group III: T4.

	Group I	Group II	Group III
Group I	0.005+/-0.001		
Group II	0.016+/-0.004	0.004+/-0.001	
Group III	0.030+/-0.006	0.023+/-0.005	0.005+/-0.002

Figure 1. Map showing the location of collection localities for *P. gilae* and *P. thermalis*. Sample codes are from Table 1.

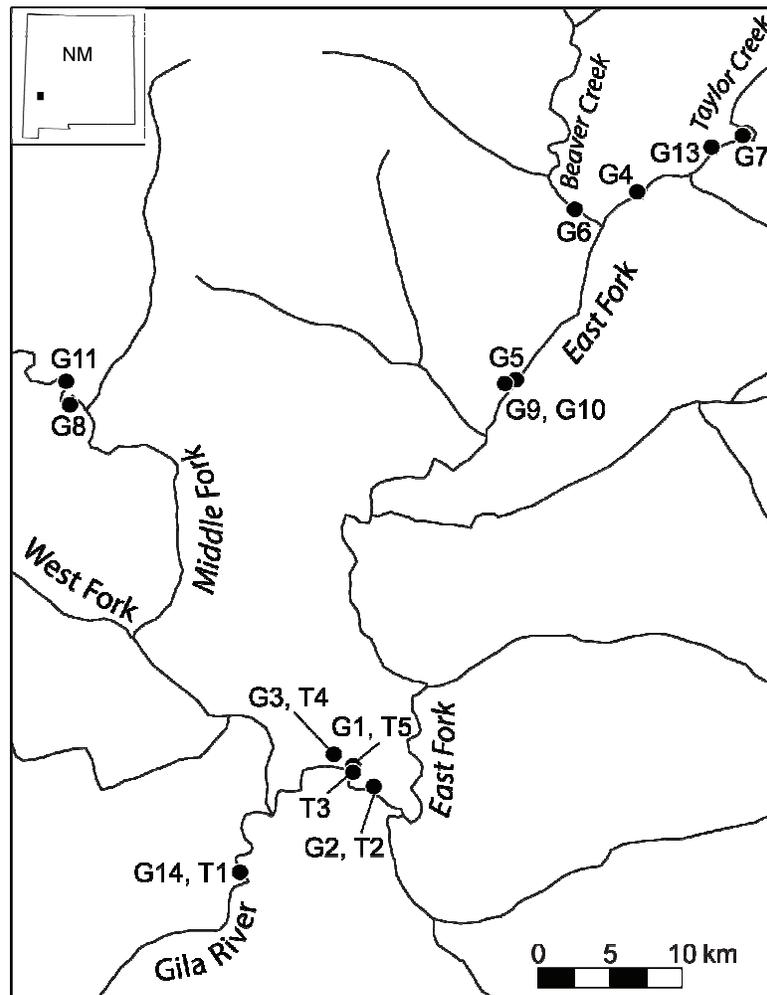


Figure 2. Bayesian tree for *P. gilae* based on mtCOI sequences. GenBank accession numbers and sample codes (from Hurt 2004, Liu & Hershler 2005) are provided for previously published sequences. Posterior probabilities of nodes are provided when $\geq 90\%$. Insert map shows the geographic locations of the three *P. gilae* sub-groups (shaded grey).

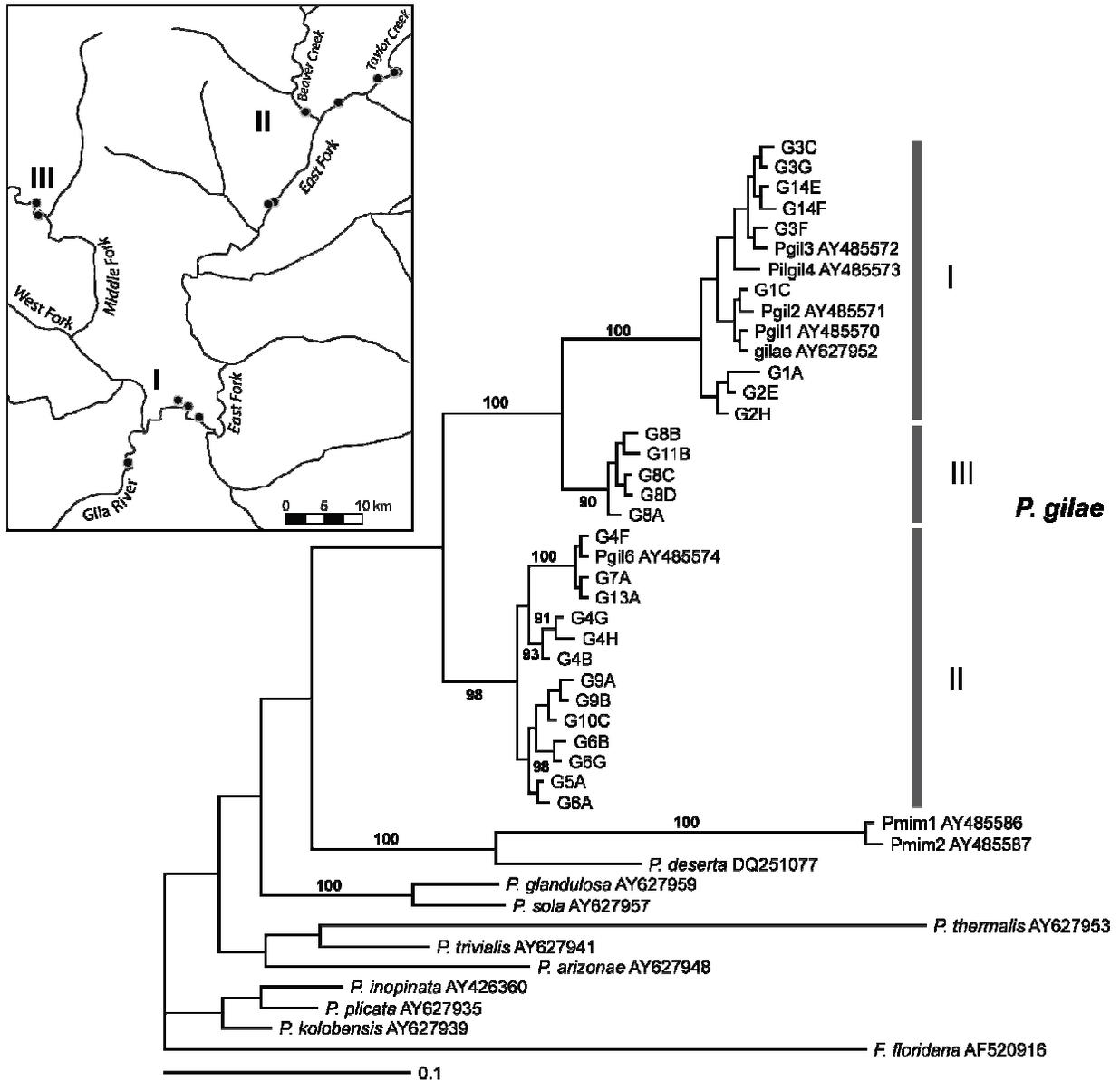
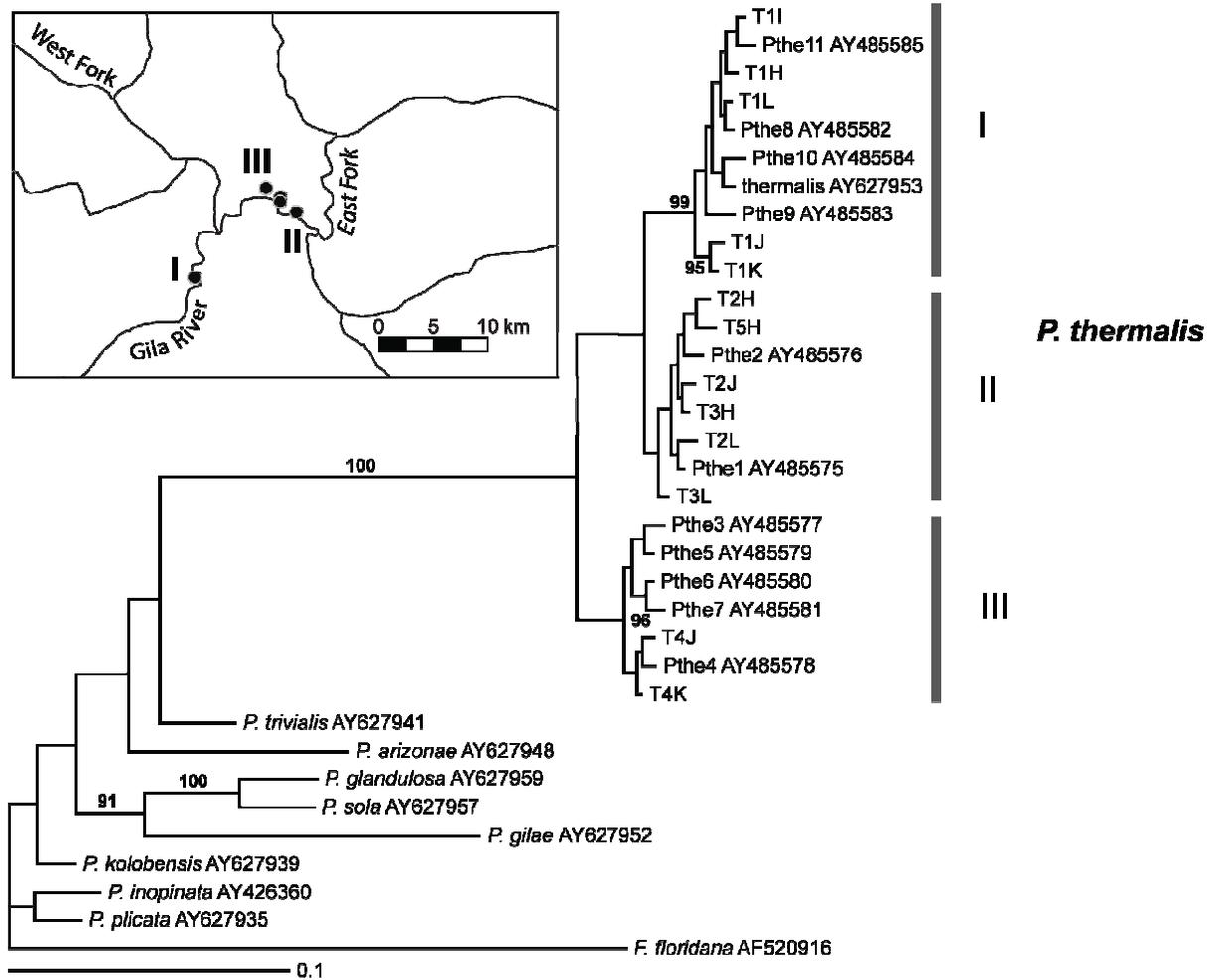


Figure 3. Bayesian tree for *P. thermalis* based on mtCOI sequences. GenBank accession numbers (and specimen codes from Hurt 2004) are provided for previously published sequences. Posterior probabilities of nodes are provided when $\geq 90\%$. Insert map shows the geographic locations of the three *P. thermalis* sub-groups (shaded grey).



Appendix C. Transfer of the Phantom Cave snail, *Cochliopa texana*, to *Pyrgulopsis* (Hydrobiidae) and description of a third congener from the lower Pecos River basin.

TRANSFER OF THE PHANTOM CAVE SNAIL, *COCHLIOPA TEXANA*, TO *PYRGULOPSIS*
(HYDROBIIDAE) AND DESCRIPTION OF A THIRD CONGENER FROM THE LOWER
PECOS RIVER BASIN

ROBERT HERSHLER¹, HSIU-PING LIU² AND BRIAN K. LANG³

¹*Department of Invertebrate Zoology, Smithsonian Institution, PO Box 37012, NHB W-305, MRC 163, Washington, DC 20013-7012, USA;* ²*Department of Biology, Metropolitan State College of Denver, Denver, CO 80217, USA;* ³*New Mexico Department of Game and Fish, One Wildlife Way, Santa Fe, New Mexico 87507, USA*

RUNNING HEAD: TRANSFER OF *COCHLIOPA TEXANA*

Correspondence: R. Hershler; email: hershler@si.edu

ABSTRACT

The Phantom Cave snail (*Cochliopa texana*), a little studied rissooidean gastropod that is locally endemic within the lower Pecos River basin (Texas) and currently a candidate for addition to the Federal list of threatened and endangered species, is redescribed and transferred to the hydrobiid genus *Pyrgulopsis* based on shell and anatomical characters. Specimens from the type locality (Phantom Lake Spring) and San Solomon Spring are larger than those from East Sandia Spring and also differ somewhat in shell shape and shape of the central cusps of the lateral radular teeth. However genetic (mtCOI, NDI) variation within and among these geographically proximal (6-13 km) populations was slight, providing no basis for the recognition of distinct conservation units of this imperiled species. We also describe *P. ignota* new species, which was recently discovered in a different part of the lower Pecos River basin and initially confused with the Phantom Cave snail. These two species differ in shell shape, operculum morphology, and form and glandular ornament of the penis. They are also strongly differentiated genetically from each other and from (thirteen) other regional congeners (pairwise sequence divergence >6.3% for both genes). A Bayesian phylogenetic analysis of the COI and NDI dataset indicated that these two snails are not closely related and that *P. ignota* occupies a basal position relative to other regional congeners.

INTRODUCTION

Although the systematics of North American cochliopid gastropods has been extensively studied and revised for more than four decades (e.g., Taylor, 1966; Thompson, 1968; Taylor, 1987; Hershler & Thompson, 1992; Hershler, 2001; Liu, Hershler & Thompson, 2001; Hershler, Liu & Stockwell, 2002), various problems have yet to be resolved. One of these concerns the identity of the Phantom Cave snail, *Cochliopa texana* Pilsbry, 1935, a tiny, little studied species that is locally distributed in the lower Pecos River basin (west Texas) and a candidate for addition to the Federal list of threatened and endangered wildlife (USFWS, 2001). Pilsbry's (1935) brief description of this species was bereft of anatomical details and he presumably assigned it to *Cochliopa* based on shell shape. Morrison (1946) later restricted *Cochliopa* to three species from Panama following Stimpson's (1865) original diagnosis (also see Thompson & Hershler, 1991). He allocated most of the other snails which had been previously assigned to this genus to *Cochliopina*, but did not treat *C. texana*. Taylor (1966:179) commented that the Phantom Cave snail "is not referable to *Cochliopa* in the strict sense according to Morrison (1946)," but was unable to make a definitive generic assignment for this species, which he classified in the Cochliopinae (*sensu* Taylor, 1966) based on its multispiral operculum with sub-central nucleus. The Phantom Cave snail was not treated in a subsequent review of the cochliopid genera (Hershler & Thompson, 1992) and in recent works is either tentatively retained in *Cochliopa* (e.g., Turgeon *et al.*, 1998) or listed as genus *incertae sedis* (e.g., Burch, 1989).

We recently studied the anatomy of the Phantom Cave snail and determined that it belongs to the North American hydrobiid genus *Pyrgulopsis*. Here we provide a new description and taxonomic assignment for this species and also describe a new congener that was recently discovered in the lower Pecos River basin ca. 150 km from Balmorhea and originally confused with the former. We also use mitochondrial DNA sequence data to further describe variation within these two species and examine their phylogenetic relationships.

MATERIAL AND METHODS

Anatomical study was based on specimens that were relaxed with menthol crystals and fixed in dilute formalin. Snails used for mtDNA sequencing were preserved in 90% ethanol in the field. UTM x-y coordinates (NAD83 datum) are provided when available for a given sample. Types for the new species and other voucher material from this study were deposited in the National Museum of Natural History (USNM) collection. Types and other material of the Phantom Cave snail in the collections of the Academy of Natural Sciences of Philadelphia (ANSP) and University of Minnesota Bell Museum of Natural History (BMNH) were also examined during the course of this study.

Variation in the number of cusps on the radular teeth was assessed using the method of Hershler *et al.* (2007). Other methods of morphological study and descriptive terminology are those used in recent taxonomic investigations of *Pyrgulopsis* (Hershler, 1998; Hershler *et al.*, 2003). Shell data were analysed using Systat for Windows 11.00.01 (SSI, 2004).

The molecular phylogenetic analysis included the two species treated herein, *P. davisii*, which is the only other congener found in the lower Pecos River basin, and 12 other regional members of the genus. Two species of *Floridobia* were used as outgroups based on the close relationship between this eastern North American genus and *Pyrgulopsis* (Liu & Hershler, 2005); the type species of the former, *F. floridana*, was used as the root. Prior to our final analysis we

performed a comprehensive (unpublished) survey of mtDNA variation within *Pyrgulopsis* to confirm that close relatives of the Phantom Cave snail and the new species had not been omitted.

Genomic DNA was extracted from entire snails using a CTAB protocol (Bucklin, 1992). A 658 bp segment of cytochrome c oxidase subunit I (COI) corresponding to “Folmer’s fragment” (Folmer *et al.*, 1994) and a 530 bp segment of NADH dehydrogenase subunit I (NDI) (Liu, Hershler & Clift, 2003) were amplified and sequenced with primers LCO1490 and HCOI2198, and ND43F and RND592F respectively following protocols of Liu *et al.* (2003). Sequences were determined for both strands and then edited and aligned using Sequencher™ version 4.8. *Pyrgulopsis chupaderae*, *P. metcalfi*, *P. roswellensis* and the two congeners treated herein were newly sequenced for this study; sequences for the other species were from our previously published investigations (Hershler, Liu & Thompson, 2003; Liu & Hershler, 2005). We sequenced eight specimens from each sample of the two species treated herein to assess variation. Sample information and GenBank accession numbers for the sequenced specimens utilized in this study are in Table 1. The 26 new sequences reported herein were deposited in GenBank under accession numbers GQ904200-GQ904225 (Table 1).

Sequence divergences (uncorrected p distance) were calculated using MEGA4 (Tamura *et al.*, 2007). Phylogenetic relationships were inferred using Bayesian inference in MrBayes 3.12 (Ronquist & Huelsenbeck, 2003). MrModeltest (Nylander, 2004) selected the General Time Reversible model (GTR + G), which best fit the data under the Akaike Information Criterion. In the initial Bayesian analysis the burn-in was set at 10% (10,000 generations) of the chain length (100,000 generations). Three runs were conducted in MrBayes using the General Time Reversible model (GTR + 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 -10,000 and quickly converged upon a stable value of about -5,670 after 8,000 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.

SYSTEMATIC DESCRIPTIONS

Family Hydrobiidae Troschel, 1857

Subfamily Nymphophilinae Taylor, 1966

Genus *Pyrgulopsis* Call & Pilsbry, 1886

Type species, *Pyrgula nevadensis* Stearns, 1883, by original designation.

Diagnosis: Liu & Hershler, 2005: 296.

***Pyrgulopsis texana* (Pilsbry, 1935) new combination**

(Figs 1-3)

Cochliopa texana Pilsbry, 1935: 91-92, fig. 5a-b [type locality Phantom Lake near Toyahvale, Reeves Co., Texas].—Baker, 1964: 177 (lectotype selection).—Dundee & Dundee, 1969: 205-210, figs. 1, 2, 3a (notes on morphology).—Williams *et al.*, 1985: 26.—Taylor, 1987: 40-41 (new record from San Solomon Spring, notes on ecology).—Turgeon *et al.*, 1998: 73,

217.—Besse, 2002: x (new record from East Sandia Spring).
“*Cochliopa*” *texana* Pilsbry. Taylor, 1966: 179.—Taylor, 1975: 188-189 (summary of literature citations).—Burch, 1980: 130, fig. 317 (illustration from Pilsbry, 1935).—Burch, 1989: 130, fig. 317.

Types: Figured lectotype, ANSP 163887; paralectotypes (from same lot), ANSP 420561.

Referred material: TEXAS. *Jeff Davis County*: USNM 421498, 4.5 miles (7.2 km) southwest of Toyahvale, 9/1934.—USNM 873212, USNM 894372, ca. 160 feet (49 m) inside entrance to Phantom Cave, 8/6/1979, 6/25/1968.—USNM 894371, ca. 50 feet (15 m) inside entrance to Phantom Cave, 6/25/1968.—BMNH uncat., Phantom Cave, 2 miles (3.2 km) west of Toyahvale, 8/5/1967.—USNM 1068972, USNM 1121838, Phantom Lake Spring, at mouth of cave, 6/15/2000, 12/2/2008.—BMNH uncat., Phantom Lake Spring, Kingston Ranch, 6/25/1968.—BMNH uncat., Phantom Lake Spring outflow, Kingston Ranch house, 4/10/1965, 6/25/1968.—USNM 884954, canal outflow just below Phantom Lake Spring. 7/3/1995. USNM 874850, USNM 874851, USNM 883951, USNM 883953, canal outflow ca. 50 feet (15 m) below Phantom Cave, 7/14/1973, 7/14/1973, 4/3/1995, 4/3/1995. BMNH uncat., Phantom Spring outflow, first lateral north, 6/25/1968.—BMNH uncat., Phantom Lake Spring outflow, second lateral south, 6/25/1968. *Reeves County*: BMNH uncat., Phantom Lake Spring outflow, Joe Kingston Ranch, 11/5/1981—BMNH uncat., spring-fed creek, Toyahvale (ex UMMZ 60851).—USNM 874849, canal outflow 0.57 mile (0.92 km) below Phantom Cave, 11/18/1971.—USNM 894373, canal outflow 1.45 mile (2.33 km) below Phantom Cave, 6/25/1968.—BMNH uncat., San Solomon Spring, Balmorhea State Park, 11/5/1981.—USNM 1116250, USNM 1123756, San Solomon Spring, main canal from pool, N 3424124, E 615904, Zone 13, 6/4/2008, 3/29/2009.—USNM 1116591, East Sandia Spring, ca. 30 feet (9 m) downflow from source, N 3429301, E 621404, Zone 13., 6/5/2008—USNM 1003865, East Sandia Spring, 6/15/2000.

Diagnosis: A small species of *Pyrgulopsis* having a depressed valvatiform-trochiform, perforate shell with highly convex whorls. Penis simple (lacking lobes and glands); having short, conical, well demarcated filament that is densely pigmented (white-yellow) internally.

Description: Shell depressed valvatiform or trochiform (Fig. 1A-C), apex often eroded; height about 1.2-2.0 mm; whorls 3.0-4.0. Periostracum tan or dark brown. Protoconch near planispiral, about 1.2 whorls, diameter about 290 μ m (Fig. 1D); initial 0.75-1.0 whorl strongly wrinkled (Fig. 1E), remaining portion smooth. Teleoconch whorls highly convex, rarely shouldered; sculpture of collabral growth lines. Aperture ovate, slightly angled above. Inner lip usually adnate, rarely slightly disjunct, usually slightly thickened internally, rarely thick; columellar shelf usually absent, rarely narrow; outer lip usually thin, rarely slightly thickened, strongly prosocline, often sinuate near base. Umbilicus well developed, perforate. Shell measurements (mean \pm standard deviation in parentheses): height, 1.46-1.97 mm (1.67 \pm 0.15); width, 1.50-1.89 mm (1.70 \pm 0.10); body whorl height, 1.31-1.70 mm (1.48 \pm 0.12); body whorl width, 1.20-1.53 mm (1.34 \pm 0.09); aperture height, 0.85-1.15 mm (0.98 \pm 0.06); aperture width, 0.83-1.04 mm (0.93 \pm 0.05); shell width/height, 0.90-1.14 (1.02 \pm 0.06); body whorl height/shell height, 0.84-0.92 (0.89 \pm 0.02); aperture height/shell height, 0.52-0.67 (0.59 \pm 0.04); number of whorls, 3.25-4.0

(3.54 ± 0.22) (paratypes, ANSP 420561, $n=30$); height, 1.02-1.36 mm (1.16 ± 0.07); width, 1.09-1.32 mm (1.19 ± 0.06); body whorl height, 0.89-1.08 mm (0.98 ± 0.05); body whorl width, 0.88-1.04 mm (0.96 ± 0.04); aperture height, 0.60-0.73 mm (0.64 ± 0.03); aperture width, 0.57-0.66 mm (0.61 ± 0.03); shell width/height, 0.88-1.11 (1.03 ± 0.06); body whorl height/shell height, 0.76-0.88 (0.84 ± 0.02); aperture height/shell height, 0.49-0.60 (0.56 ± 0.03); number of whorls, 3.25-3.75 (3.46 ± 0.16) (USNM 1003865, $n=30$).

Operculum thin, light amber, broadly ovate, multispiral with sub-central nucleus (Fig. 1F, G); last 0.5-1.0 whorl strongly frilled on outer side, last 0.25 whorl sometimes frilled on inner side (Fig. 1H); attachment scar border variably thickened on inner side (Fig. 1H, I). Radula taenioglossate (Fig. 2A), with about 62 well-formed rows of teeth. Central teeth about 40 μ m wide, cutting edge weakly to strongly concave (Fig. 2B, C); lateral cusps 3-6; central cusp narrow, pointed, often parallel-sided proximally; basal cusp 1, very small; basal tongue U-shaped, slightly shorter than lateral margins. Lateral tooth face slightly taller than wide; central cusp hoe-like (Fig. 2D) or narrow-pointed (Fig. 2E); lateral cusps 0-3 (inner), 1-4 (outer); outer wing broad, sometimes flexed, about 170% length of cutting edge; basal tongue well developed. Inner marginal teeth having 18-29 cusps (Fig. 2F); fourth to sixth cusp from outer edge enlarged. Outer marginal teeth having 33-47 cusps (Fig. 2G); inner edge sometimes having an elongate, weakly delineated wing. Cephalic tentacles dark brown dorsally except for pale patches surrounding eyes, ventral surfaces pale. Snout dark brown, distal lips pale. Foot dark brown, sole pale or pigmented with scattered brown granules. Pallial roof, visceral coil dark brown or black dorsally. Ctenidium well developed, positioned a little in front of pericardium; ctenidial filaments about 20, broadly triangular, lateral surfaces having prominent ridges. Osphradium narrow, positioned posterior to middle of ctenidium. Hypobranchial gland small, positioned between anterior portion of kidney and pallial genital duct. Prostate gland rather large, bean-shaped, with about 40% of length in pallial roof. Anterior vas deferens opening from ventral edge of prostate gland a little in front of pallial wall, section of duct on columellar muscle usually having a prominent bend, rarely straight. Penis medium-sized, base rectangular, inner edge smooth (Figs. 2H, 3A); filament short, strongly tapered, demarcated from base by slight constriction (Fig. 2H, I), nearly horizontal. Penis lacking a lobe and superficial glands. Penial duct very narrow, near outer edge, straight or having a few weak undulations. Filament densely pigmented internally (white-yellow). Female glandular oviduct and associated structures shown in Figure 3B-D. Coiled oviduct a simple, circular loop. Bursa copulatrix medium-sized, narrowly ovate, horizontal, largely overlapped by albumen gland. Bursal duct short, narrow or medium width, slightly broader distally, opening from distal edge, junction with common duct a little behind posterior wall of pallial cavity. Seminal receptacle very small, finger-shaped, positioned along ventral edge of bursa. Albumen gland having short pallial section. Capsule gland composed of two distinct tissue sections. Genital aperture a terminal pore.

Distribution and habitat: *Pyrgulopsis texana* is distributed in three springs in the vicinity of Balmorhea (Fig. 4). This species is concentrated near the sources of these springs and typically found on hard substrates where it is often extremely abundant (Dundee & Dundee, 1969; Taylor, 1987; personal observations, RH, BKL).

Remarks: The Phantom Cave snail is excluded from the Cochliopidae by the absence of the

female sperm tube (confirmed in section) that is diagnostic of this family (Hershler & Thompson 1992). (Note that this group was recently elevated to separate family status by Wilke *et al.*, 2001.) We transfer this species to the hydrobiid genus *Pyrgulopsis* based on the combination of its wrinkled protoconch microsculpture, simple penis, superficial position of the bursa copulatrix and its duct on the albumen gland, single seminal receptacle opening to the distal arm of the coiled oviduct, and junction of the bursal duct and common duct of the oviduct and seminal receptacle behind the posterior wall of the pallial cavity (Liu & Hershler, 2005).

Pyrgulopsis texana is readily distinguished from geographically proximate *P. davisi* and *P. ignota* (described below) by its more depressed shell shape and simple penis. It is also differentiated from these congeners by its more convex shell whorls, larger umbilicus, sub-central operculum nucleus, strongly frilled operculum whorls and mtDNA sequences (COI, 11.6%, 9.7%; NDI, 13.4%, 11.7%, respectively). *Pyrgulopsis texana* is differentiated from other congeners included in this study by 9.1-12.8% (COI) and 11.6-17.4% sequence divergence (NDI). The simple penis of *P. texana* is shared with many congeners (Hershler & Sada, 2002). In the Bayesian analysis (Fig. 5) *P. texana* was positioned as a terminal clade sister to one of these species, *P. bernardina*, albeit without strong support.

Shells from East Sandia Spring (Fig. 1C) are smaller and have a smaller (relative to shell height) aperture and taller than those from the type locality (Fig. 1A) (t-test, separate variance, $P < 0.001$, $df = 40.5-57.4$; from data summarized above) and San Solomon Spring (Fig. 1B) (the latter could not be statistically analyzed as few specimens in this population have an uneroded shell apex.) The East Sandia Spring population also differed from the other two in that the central cusps of the lateral radular tooth were often narrow (Fig. 2E) and pointed rather than hoe-shaped (Fig. 2D). We did not observe any anatomical differences between these populations. Mitochondrial DNA sequence variation within *P. texana* was minimal (Fig. 5, Table 2), both within (0-0.2%, COI; 0-0.6%, NDI) and among (0.1-0.2%, COI; 0.2-0.5%, NDI) populations. Seven of the eight sequenced specimens from the East Sandia Spring population had haplotypes that were shared with one (NDI, San Solomon Spring) or both (COI) of the other two populations. This finding does not support recognition of distinct conservation units of *P. texana*, which is not surprising given that the springs inhabited by this snail are separated from each other by only 6-13 km and may have been integrated (through Toyah Creek) prior to recent decreases and re-routing of surface flows (USFWS, 2007).

In an unpublished doctoral dissertation, Fullington (1978:40) provided a synonymy for the Phantom Cave snail which mistakenly indicated this species had been transferred to *Cochliopina* by Taylor (1966).

Radular count data were from USNM 883953, USNM 1003865 and BMNH uncat. (San Solomon Spring).

***Pyrgulopsis ignota* new species**

(Figs 6-8)

Types: Holotype, USNM 1127357, Caroline Springs, just below the first pond downflow from spring-fed lake, Terrell County, Texas, N 3373903, E 230992, Zone 14, coll. RH and J. Jerry Landye, 3/30/2009. Paratypes (from same lot), USNM *****.

Etymology: Based on the Greek *ignotus* in reference to the unusual penial morphology of this

snail. We propose that “Caroline Springs pyrg” be used as the vernacular name.

Referred material: TEXAS. *Terrell County:* USNM 1120337 USNM 1123945, USNM 116592, Caroline Springs, outflow from first pond below spring-fed lake, ca. 200 m below pump house, N 231017, E 3373900, Zone 14, 9/17/2008, 3/11/2009, 6/9/2008.

Diagnosis: A small species of *Pyrgulopsis* having a trochoid shell with weakly to moderately convex whorls. Penis having a large lobe and short filament; penial ornament consisting of a large pad-like gland on the dorsal surface of the lobe.

Description: Shell trochoidal, often appearing decollate owing to erosion of apex (Fig. 6A-C); height about 1.3-1.5 mm; whorls about 3.5. Periostracum tan or orange. Protoconch near planispiral, about 1.3 whorls, diameter about 300 μ m (Fig. 6D), initial portion weakly wrinkled and sometimes having a few short spirals (Fig. 6E). Teleoconch whorls weakly to moderately convex, last 0.25 whorl sometimes strongly shouldered; sculpture of collabral growth lines, later whorls having numerous weak spiral striae (crossed by growth lines) (Fig. 6C). Aperture ovate, strongly angled above. Inner lip usually adnate, rarely slightly disjunct, thickened internally; columellar shelf absent; outer lip usually thin, rarely slightly thickened, prosocline, weakly sinuate. Umbilicus chink-like or absent. Shell measurements (mean \pm standard deviation in parentheses): height, 1.41 mm; width, 1.23 mm; body whorl height, 1.23 mm; body whorl width, 1.02 mm; aperture height, 0.82 mm; aperture width, 0.71 mm; shell width/height, 0.87; body whorl height/shell height, 0.88; aperture height/shell height, 0.58; number of whorls, 3.75 (holotype, USNM 1123757); height, 1.37-1.63 mm (1.48 \pm 0.08); width, 1.15-1.35 mm (1.26 \pm 0.07); body whorl height, 1.19-1.43 mm (1.28 \pm 0.08); body whorl width, 0.96-1.12 mm (1.03 \pm 0.06); aperture height, 0.76-0.92 mm (0.84 \pm 0.05); aperture width, 0.69-0.78 mm (0.74 \pm 0.04); shell width/height, 0.82-0.88 (0.85 \pm 0.02); body whorl height/shell height, 0.84-0.89 (0.86 \pm 0.02); aperture height/shell height, 0.55-0.59 (0.56 \pm 0.01); number of whorls, 3.50-3.75 (3.66 \pm 0.13) (paratypes, USNM *****, $n=8$).

Operculum somewhat thickened, amber, multispiral with eccentric nucleus (Fig. 6F, G); last 0.25 whorl sometimes frilled on outer side (Fig. 6G), weak rim sometimes present along outer edge (Fig. 6H); attachment scar border variably thickened, sometimes prominently so almost all around. Radula taenioglossate (Fig. 7A), with about 52 well-formed rows of teeth. Central teeth about 18 μ m wide, cutting edge highly convex (Fig. 7B); lateral cusps 5-8; central cusp narrow, pointed, parallel-sided proximally, sometimes distally bifurcate; basal cusp 1, small; basal tongue V-shaped, about as long as lateral margins. Lateral tooth face rectangular, angled; central cusp pointed, parallel-sided proximally (Fig. 7C); lateral cusps 3-4 (inner), 5-8 (outer); outer wing narrow, flexed, about 240% length of cutting edge; basal tongue well developed. Inner marginal teeth having 27-32 cusps, fourth cusp from outer edge enlarged (Fig. 7D). Outer marginal teeth having 29-36 cusps; inner edge with short wing near mid-length (Fig. 7E). Cephalic tentacles light grey or dark brown except for pale distal tips and sometimes pale patches surrounding eyes. Snout similarly pigmented, distal lips pale. Foot dark brown along anterior and posterior edges, light grey centrally, sole pigmented with scattered grey granules. Pallial roof, visceral coil dark brown or black dorsally. Ctenidium positioned a little in front of pericardium; ctenidial filaments about 13, narrowly triangular, lateral surfaces smooth. Osphradium narrow, positioned

well posterior to middle of ctenidium. Hypobranchial gland large, almost completely overlapping pallial genital duct and rectum. Prostate gland very small, pea-shaped, with about 33% of length in pallial roof. Anterior vas deferens opening from ventral edge of prostate gland a little in front of pallial wall, section of duct on columellar muscle having a slight bend. Penis large, base rectangular, folded along inner edge and basal portion of outer edge; filament short, narrow, tapering, slightly oblique; lobe large, rectangular (sometimes distally rounded), horizontal or slightly oblique (Fig. 8A). Dorsal side of lobe having large slightly raised, pad-like structure (Fig. 7F) containing numerous ovate, glandular units (Fig. 8A). Ventral surface of penis with distinct swelling centrally, but without gland (Fig. 8B). Penial duct narrow, near outer edge, almost straight. Penial filament pigmented with black granules proximally, mostly along inner edge. Female glandular oviduct and associated structures shown in Figure 8C-E. Coiled oviduct circular or posterior-oblique; proximal section sometimes pigmented with a few black granules. Bursa copulatrix small, elongate-ovate, horizontal, largely overlapped by albumen gland. Bursal duct as long or slightly longer than bursa, narrow, opening from distal edge, shallowly embedded in albumen gland distally, junction with common duct a little in front of posterior wall of pallial cavity. Seminal receptacle small, sac-like, positioned along antero-dorsal edge of bursa. Albumen gland almost entirely visceral. Capsule gland composed of three distinct tissue sections. Genital aperture a terminal slit.

Distribution and habitat: *Pyrgulopsis ignota* is endemic to Caroline Springs (also known as T5 Springs), which is located about 24 km (15 miles) south-southeast of Sheffield (Fig. 4). The outflow of this large spring (which discharges through several vents) courses through a large lake and several ponds (all man-made) before entering Independence Creek. *Pyrgulopsis ignota* was found (abundantly) on cobble in the outflow of the first pond below the lake (Fig. 9A, B). Caroline Springs is currently being managed by the Nature Conservancy, which acquired the property on which it is situated (Oasis Ranch) in 2000 (Karges, 2003).

Remarks: This species was initially confused (in the field) with *P. texana* when first discovered owing to their generally similar shells. Upon closer inspection *P. ignota* was readily differentiated from its congener by the features listed in the “Remarks” section above as well as by its more strongly angled shell aperture. The occurrence of a large glandular pad on the dorsal surface of the penis is unique to *P. ignota* within the genus (Hershler & Sada, 2002). The junction of the bursal duct and common duct of the oviduct and seminal receptacle in front of the pallial wall (Fig. 8C) is also unique within *Pyrgulopsis*, but is more posteriorly positioned than in the eastern North American genus *Marstonia* (Hershler, 1994, fig. 5c).

The sequence divergence between *P. ignota* and other congeners included in this study ranged from 6.3-10.3% for COI and 8.3-13.5% for NDI. Variation among the eight specimens analyzed for this species was minimal (0.4±0.2% for both genes). In the Bayesian tree *P. ignota* occupied a well supported basal position.

Radular count data were from USNM *****.

ACKNOWLEDGEMENTS

Collecting permits were provided by the Texas Parks and Wildlife Department (Phantom Lake and San Solomon Springs) and Nature Conservancy (East Sandia and Caroline Springs).

Yolanda Villacampa measured shells and prepared the scanning electron micrographs. Karolyn Darrow prepared the anatomical drawings. Jessica Marn assisted with DNA extraction and amplification. This study was funded (in part) by an award (to RH) from the Upper Colorado Regional Office, Bureau of Reclamation (Interagency Acquisition No. 08-AA-40-2782).

REFERENCES

- BAKER, H.B. 1964. Type land snails in the Academy of Natural Sciences of Philadelphia. Part III. Limnophile and thalassophile Pulmonata. Part IV. Land and fresh-water Prosobranchia. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **116**: 149-193.
- BESSE, H.C. 2002. Introduction. In: *Springs of Texas* Volume 1 (G. Brune), 2nd edition, i-xvi. Texas A&M University Press, College Station.
- BUCKLIN, A. 1992. Use of formalin-preserved samples for molecular analysis. *Newsletter of Crustacean Molecular Techniques*, **2**: 3.
- BURCH, J.B. 1980. North American freshwater snails. Species list, ranges and illustrations. *Walkerana*, **1**: 81-215.
- BURCH, J.B. 1989. *North American freshwater snails*. Malacological Publications, Hamburg, MI.
- DUNDEE, D.S & DUNDEE, H.A. 1969. Notes concerning two Texas molluscs, *Cochliopa texana* Pilsbry and *Lyrodes cheatumi* Pilsbry (Mollusca: Hydrobiidae). *Transactions of the American Microscopical Society*, **88**: 205-210.
- FOLMER, O., BLACK, M., HOEH, W., LUTZ, R. & VRIJENHOEK, R. 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**: 294-299.
- FULLINGTON, R.W. 1978. *The Recent and fossil freshwater gastropod fauna of Texas*. [Unpublished] doctoral dissertation, North Texas State University, Denton.
- HERSHLER, R. 1994. A review of the North American freshwater snail genus *Pyrgulopsis*. *Smithsonian Contributions to Zoology*, **554**: 1-115.
- HERSHLER, R. 1998. A systematic review of the hydrobiid snails (Gastropoda: Rissooidea) of the Great Basin, western United States. Part I. Genus *Pyrgulopsis*. *Veliger*, **41**: 1-132.
- HERSHLER, R. 2001. Systematics of the North and Central American aquatic snail genus *Tryonia* (Rissooidea: Hydrobiidae). *Smithsonian Contributions to Zoology*, **612**: 1-53.
- HERSHLER, R. & SADA, D.W. 2002. Biogeography of Great Basin aquatic snails of the genus *Pyrgulopsis*. In: *Great Basin aquatic systems history* (eds. R. Hershler, D.B. Madsen, D.R. Currey, eds), pp. 255-276. *Smithsonian Contributions to the Earth Sciences*, **33**.
- HERSHLER, R. & THOMPSON, F.G. 1992. A review of the aquatic gastropod subfamily Cochliopinae (Prosobranchia: Hydrobiidae). *Malacological Review Supplement*, **5**: 1-140.
- HERSHLER, R., LIU, H.-P. & STOCKWELL, C.A. 2002. A new genus and species of aquatic gastropods (Rissooidea: Hydrobiidae) from the North American Southwest: phylogenetic relationships and biogeography. *Proceedings of the Biological Society of Washington*, **115**: 171-188.
- HERSHLER, R., LIU, H.-P. & THOMPSON, F.G. 2003. Phylogenetic relationships of North American nymphophiline gastropods based on mitochondrial DNA sequences. *Zoologica Scripta*, **32**: 357-366.
- HERSHLER, R., FREST, T.J., LIU, H.-P. & JOHANNES, E.J. 2003. Rissooidean snails from

- the Pit River basin, California. *Veliger*, **46**: 275-304.
- HERSHLER, R., LIU, H.-P., FREST, T.J. & JOHANNES, E.J. 2007. Extensive diversification of pebblesnails (Lithoglyphidae: *Fluminicola*) in the upper Sacramento River basin, northwestern United States. *Zoological Journal of the Linnean Society*, **149**: 371-422.
- KARGES, J. 2003. Aquatic conservation and The Nature Conservancy in west Texas. In: Aquatic fauna of the northern Chihuahuan Desert. Contributed papers from a special session within the thirty-third annual symposium of the Desert Fishes Council (G.P. Garrett & N.L. Allan, eds), pp. 141-150. *Museum of Texas Tech University Special Publications*, **46**.
- LIU, H.-P. & HERSHLER, R. 2005. Molecular systematics and radiation of western North American nymphophiline gastropods. *Molecular Phylogenetics and Evolution*, **34**: 284-298.
- LIU, H.-P., HERSHLER, R. & CLIFT, K. 2003. Mitochondrial DNA sequences reveal extensive cryptic diversity within a western American springsnail. *Molecular Ecology*, **12**: 2771-2782.
- LIU, H.-P., HERSHLER, R. & THOMPSON, F.G. 2001. Phylogenetic relationships of the Cochliopinae (Rissooidea: Hydrobiidae): an enigmatic group of aquatic gastropods. *Molecular Phylogenetics and Evolution*, **21**: 17-25.
- MORRISON, J.P.E. 1946. The nonmarine mollusks of San José Island, with notes on those of Pedro González Island, Pearl Islands, Panamá. *Smithsonian Miscellaneous Collections*, **106**: 1-49, plates I-III.
- NYLANDER, J.A.A. 2004. MrModeltest v2. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- PILSBRY, H.A. 1935. Western and southwestern Amnicolidae and a new *Humboldtiana*. *Nautilus*, **48**: 91-94.
- RONQUIST, F. & HUELSENBECK, J.P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**: 1572-1574.
- SSI (SYSTAT SOFTWARE, INC.). 2004. *Systat® for Windows®*. Richmond (CA).
- STIMPSON, W. 1865. Diagnoses of newly discovered genera of gastropods, belonging to the sub-fam. Hydrobiinae of the family Rissoidae. *American Journal of Conchology*, **1**: 52-54, plate 8, figure 1.
- TAMURA, K., DUDLEY, J., NEI, M. & KUMAR, S. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*, **24**: 1596-1599.
- TAYLOR, D.W. 1966. A remarkable snail fauna from Coahuila, México. *Veliger*, **9**: 152-228, plates 8-19.
- TAYLOR, D.W. 1975. Index and bibliography of late Cenozoic freshwater Mollusca of western North America. *University of Michigan Museum of Paleontology Papers on Paleontology*, **10**: 1-384.
- TAYLOR, D.W. 1987. Fresh-water molluscs from New Mexico and vicinity. *New Mexico Bureau of Mines & Mineral Resources Bulletin*, **116**: 1-50.
- THOMPSON, F.G. 1968. *The aquatic snails of the family Hydrobiidae from peninsular Florida*. University of Florida Press, Gainesville.
- THOMPSON, F.G. & HERSHLER, R. 1991. New hydrobiid snails (Mollusca: Gastropoda; Prosobranchia: Truncatelloidea) from North America. *Proceedings of the Biological Society of Washington*, **104**: 669-683.

- TURGEON, D.D., QUINN, J.F., BOGAN, A.S., COAN, E.V., HOCHBERG, F.G., LYONS, W.G., MIKKELSEN, P.M., NEVES, R.J., ROPER, C.F.E., ROSENBERG, G., ROTH, B., SCHELTEMA, A., THOMPSON, F.G., VECCHIONE, M. & WILLIAMS, J.D. 1998. *Common and scientific names of aquatic invertebrates from the United States and Canada: Mollusks*. Second edition. American Fisheries Society Special Publication, **26**.
- USFWS (UNITED STATES FISH AND WILDLIFE SERVICE). 2001. 50 CFR Part 17. Endangered and threatened wildlife and plants; review of plant and animal species that are candidates or proposed for listing as endangered or threatened, annual notice of findings on recycled petitions, and annual description of progress on listing actions; proposed rule. *Federal Register*, **66**: 54808-54832.
- USFWS (UNITED STATES FISH AND WILDLIFE SERVICE). 2007. Species assessment form for the Phantom Cave snail. Available from http://ecos.fws.gov/docs/candforms_pdf/r2/G00X_I01.pdf (accessed 15 September 2009).
- WILKE, T., DAVIS, G.M., FALNIOWSKI, A., GIUSTI, F., BODON, M. & SZAROWSKA, M. 2001. Molecular systematic of Hydrobiidae (Mollusca: Gastropoda: Rissooidea): testing monophyly and phylogenetic relationships. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **151**: 1-21.
- WILLIAMS, J.E., BOWMAN, D.B., BROOKS, J.E., ECHELLE, A.A., EDWARDS, R.J., HENDRICKSON, D.A. & LANDYE, J.J. 1985. Endangered aquatic ecosystems in North American deserts with a list of vanishing fishes of the region. *Journal of the Arizona-Nevada Academy of Science*, **20**: 1-62.

FIGURE CAPTIONS

Figure 1. Scanning electron micrographs of shells and opercula of *P. texana*. **A.** Shell, USNM 883954. **B.** Shell, USNM 1123756. **C.** Shell, USNM 1003865. **D.** Shell apex, showing protoconch sculpture, USNM 883954. **E.** Close up of protoconch sculpture, USNM 883954. **F, G.** Opercula, outer side, USNM 883953, BMNH uncat. (San Solomon Spring), respectively. **H, I.** Opercula, inner side, USNM 883953. Scale bars **A** = 0.5 mm; **D, E** = 100 μm ; **F** = 250 μm . **B, C** printed to same scale as **A**; **G-I** printed to same scale as **F**.

Figure 2. Scanning electron micrographs of radula and critical point dried penis of *P. texana*. **A.** Portion of radula ribbon, USNM 883953. **B, C.** Central radula teeth, USNM 883953, USNM 1003865, respectively. **D, E.** Lateral and inner marginal teeth, USNM 883953, USNM 1003865, respectively. **F.** Inner marginal tooth, USNM 883953. **G.** Outer marginal teeth, USNM 883953. **H.** Penis, dorsal surface, USNM 883954. **I.** Close up of distal penis (dorsal surface), USNM 883954. Abbreviations: Pf, penial filament; Lw, lateral wing. Scale bars **A** = 20 μm ; **B-G** = 10 μm ; **H** = 100 μm ; **I** = 20 μm .

Figure 3. Reproductive anatomy of *P. texana*, USNM 883954. **A.** Penis, dorsal surface (pigment in filament uniformly stippled). **B.** Female glandular oviduct and associated structures (viewed from left side). **C.** Bursa copulatrix. **D.** Seminal receptacle. Abbreviations: Ag, albumen gland; Bu, bursa copulatrix; Cd, common duct of seminal receptacle and coiled oviduct; Cg, capsule gland; Cov, coiled oviduct; Dbu, bursal duct; Ga, female genital aperture; Pd, penial duct; Pf, penial filament; Pw, posterior wall of pallial cavity; Sr, seminal receptacle; Vc, ventral channel of capsule gland. Scale bars **A** = 0.5 mm; **B** = 250 μm . **C, D** printed to same scale as **B**.

Figure 4. Shaded relief map of the lower Pecos River basin (USGS hydrologic subregion 1307) showing the distributions of *P. texana*, *P. ignota* and the third congener (*P. davisii*) found in this watershed.

Figure 5. Bayesian tree based on the combined COI and NDI dataset. Posterior probabilities for nodes are provided when $\geq 90\%$. The two species treated in this paper are highlighted with bold-face type. Terminals are labeled as in Table 1.

Figure 6. Scanning electron micrographs of shells and opercula of *P. ignota*. **A.** Holotype, USNM 1123757. **B, C.** Shells, USNM *****. **D.** Shell apex, showing protoconch sculpture, USNM *****. **E.** Close up of protoconch sculpture (arrow indicating spiral elements), USNM *****. **F.** Operculum, outer side, USNM *****. **G, H.** Opercula, inner side, USNM *****. Abbreviation: Rm, rim. Scale bars **A** = 0.5 mm; **D, E** = 100 μm ; **F** = 250 μm . **B, C** printed to same scale as **A**; **G, H** printed to same scale as **F**.

Figure 7. Scanning electron micrographs of radula and critical point dried penis of *P. ignota*, USNM *****. **A.** Portion of radula ribbon. **B.** Central radular teeth. **C.** Lateral radular tooth. **D.** Inner marginal teeth. **E.** Inner and outer marginal teeth. **F.** Distal penis, dorsal surface. Abbreviations: Gp, pad-like gland; Lw, lateral wing. Scale bars **A** = 10 μm ; **B** = 2 μm ; **C** = 12 μm ; **D, E** = 10 μm ; **F** = 100 μm .

Figure 8. Reproductive anatomy of *P. ignota*, USNM *****. **A.** Penis, dorsal surface. **B.** Penis, ventral surface. **C.** Female glandular oviduct and associated structures (viewed from left side). **D.** Bursa copulatrix. **E.** Seminal receptacle. Abbreviations: Ag, albumen gland; Bu, bursa copulatrix; Cd, common duct of seminal receptacle and coiled oviduct; Cg, capsule gland; Cov, coiled oviduct; Dbu, bursal duct; Ds, distal swelling; Ga, female genital aperture; Gp, pad-like gland; Pd, penial duct; Pf, penial filament; Pl, penial lobe; Pw, posterior wall of pallial cavity; Sr, seminal receptacle; Vc, ventral channel of capsule gland. Scale bars **A, B** = 0.5 mm; **C** = 250 μ m. **D, E** printed to same scale as **C**.

Figure 9. Photographs of the type locality area of *P. ignota*. **A.** First pond below the lake fed by Caroline Springs, with the outlet (where snails were found) to the right. **B.** Stream outlet about 5 m downflow from above pond (scale provided by J. Jerry Landye). Photograph taken by RH on 3/29/2009.

Table 1. Locality details and GenBank accession numbers for COI and NDI sequences.

Species (code)	Locality	GenBank accession number	
		COI	NDI
<i>acarinata</i>	La Tecla Vieja, Cuatro Cienegas basin, Coahuila, Mexico	AY627954*	AY628034*
<i>arizonae</i>	Medicine Spring, Bylas, Gila River drainage, Graham Co., AZ	AY627948*	AY628072*
<i>bernardina</i>	Spring, El Chorro, Rio de Bavispe drainage, Sonora, Mexico	AY627951*	AY628075*
<i>chupaderae</i>	Willow Springs, Rio Grande drainage, Socorro Co, NM	GQ904209	GQ904223
<i>davisi</i>	Spring tributary to Limpia Creek, Pecos River drainage, Jeff Davis Co., TX	AY627950*	AY628074*
<i>gilae</i>	Spring tributary to East Fork Gila River, Grant Co., NM	AY627952*	AY628076*
<i>ignota</i> (Ct3, BKL2)	Caroline Spring, Pecos River drainage, Terrell Co., TX	GQ904203-GQ904206	GQ904215-Q904218
<i>manantiali</i>	Santa Tecla canal at Puerto Salado, Rio Salado drainage, Coahuila, Mexico	AY627955*	AY628079*
<i>metcalfi</i>	Naegele Spring, Rio Grande drainage, Presidio Co., TX	GQ904210	GQ904224
<i>minckleyi</i>	East Cold Spring, Cuatro Cienegas basin, Coahuila, Mexico	AY627917*	AY628034*
<i>pecosensis</i>	Blue Spring, Pecos River drainage, Eddy Co., NM	AF520909 [†]	AY628081*
<i>roswellensis</i>	Sago Spring, Pecos River drainage, Chaves Co., NM	GQ904211	GQ904225
<i>texana</i> (Ct4, BKL4)	Phantom Lake Spring, Pecos River drainage, Jeff Davis Co., TX	GQ904207, GQ90428	GQ904219-GQ904222
<i>texana</i> (Ct1, BKL1)	San Solomon Spring, Pecos River drainage, Reeves Co., TX	GQ904200	GQ904212
<i>texana</i> (Ct2, BKL3)	East Sandia Spring, Pecos River drainage, Reeves Co., TX	GQ904201, GQ904202	GQ904213, GQ904214
<i>thermalis</i>	Hot Spring, Gila River drainage, Grant Co., NM	AY627953*	AY628077*
<i>trivialis</i>	Spring, Three Forks, Gila River drainage, Grant Co., NM	AY627941*	AY628065*

Species (code)	Locality	GenBank accession number	
		COI	NDI
<i>F. floridana</i>	Juniper Springs, St. Johns River drainage, Marion CO., FL	AF520916 [†]	AY628035*
<i>F. winkleyi</i>	Salt marsh, Scarborough, Saco River drainage, Cumberland Co., ME	AY520917 [†]	AY628036*

*Liu & Hershler (2005); [†]Liu *et al.* (2003)

Table 2. COI and NDI variation within *P. texana*.

Specimen	Base pair position									
	COI		NDI							
	302	343	009	032	035	038	102	233	371	479
Phantom Lake Spring										
Ct4A	G	A	C	T	C	A	G	G	T	A
Ct4B	A	—	T	—	—	G	A	A	—	—
Ct4C	G	—	C	—	—	—	—	G	—	—
Ct4D	—	—	—	—	—	—	—	—	—	—
BKL4A	—	—	—	—	—	—	—	—	—	—
BKL4B	—	—	—	—	—	—	—	—	—	—
BKL4C	—	—	—	—	—	A	G	—	—	—
BKL4D	—	—	—	—	T	G	A	—	—	G
San Solomon Spring										
Ct1A	G	A	C	T	C	G	A	G	C	A
Ct1B	—	—	—	—	—	—	—	—	—	—
Ct1C	—	—	—	—	—	—	—	—	—	—
Ct1D	—	—	—	—	—	—	—	—	—	—
BKL1A	—	—	—	—	—	—	—	—	—	—
BKL1B	—	—	—	—	—	—	—	—	—	—
BKL1C	—	—	—	—	—	—	—	—	—	—
BKL1D	—	—	—	—	—	—	—	—	—	—
East Sandia Spring										
Ct2A	G	A	C	T	C	G	A	G	C	A
Ct2B	—	—	—	—	—	—	—	—	—	—
Ct2C	—	—	—	—	—	—	—	—	—	—
Ct2D	—	—	—	—	—	—	—	—	—	—
BKL3A	—	G	—	C	—	—	—	—	T	—
BKL3B	—	A	—	T	—	—	—	—	C	—
BKL3C	—	—	—	—	—	—	—	—	—	—
BKL3D	—	—	—	—	—	—	—	—	—	—

Figure 1

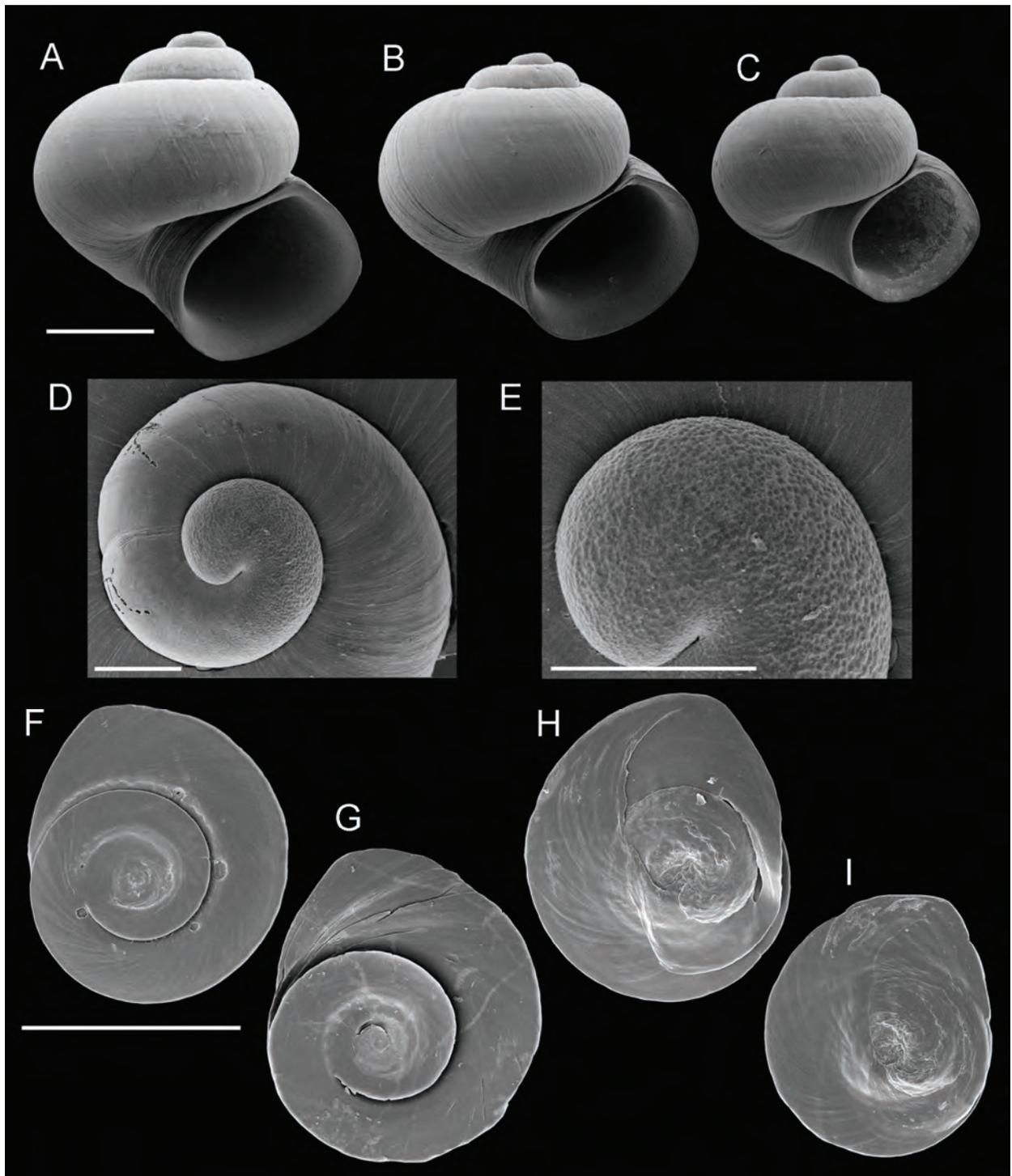


Figure 2

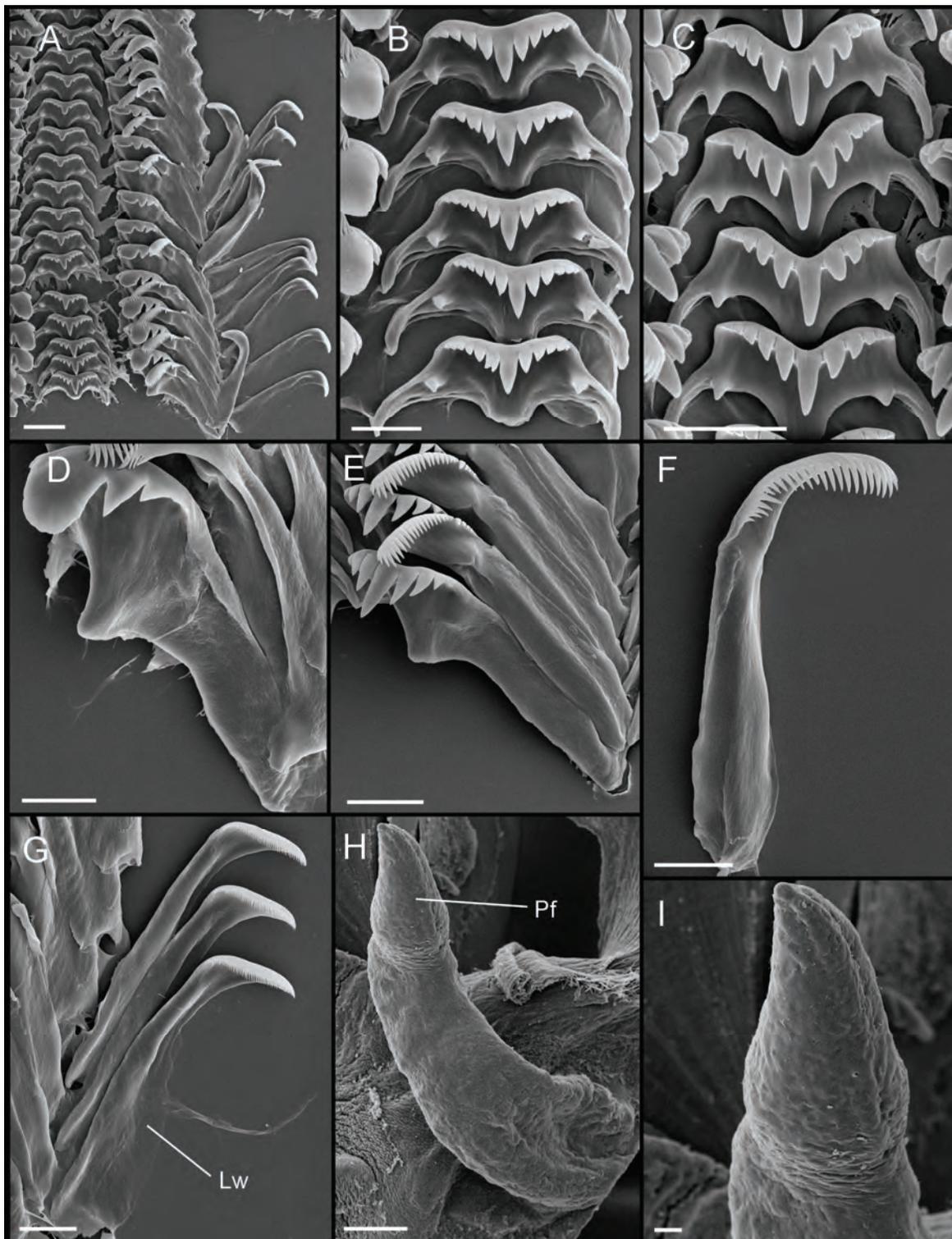


Figure 3

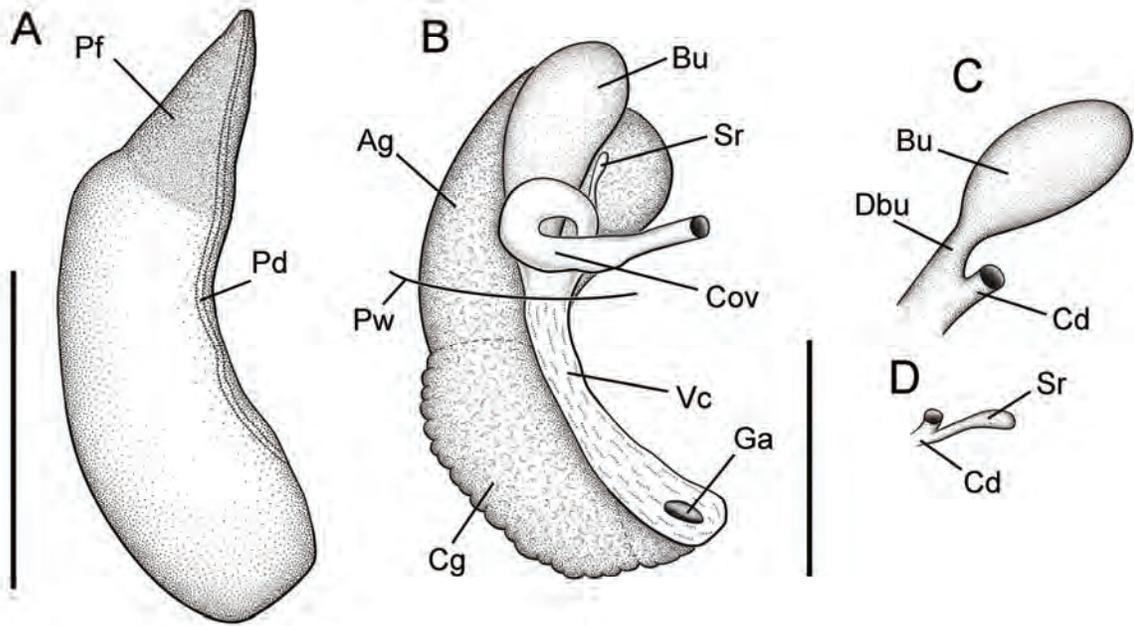


Figure 4

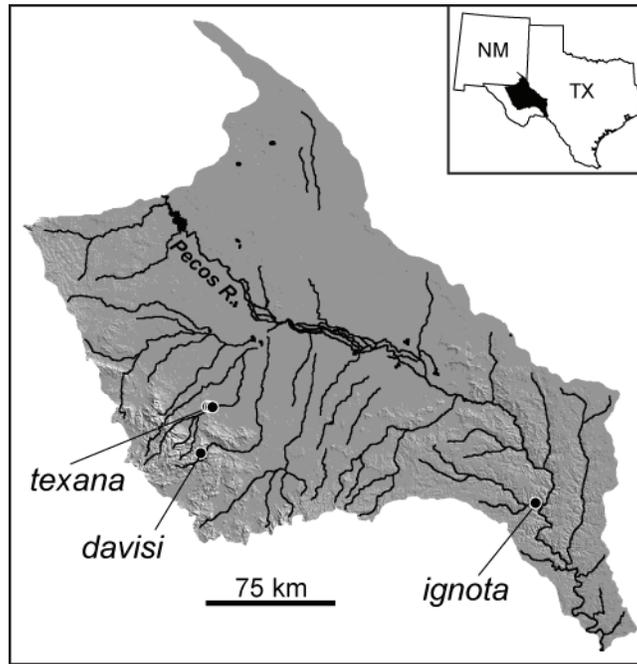


Figure 5

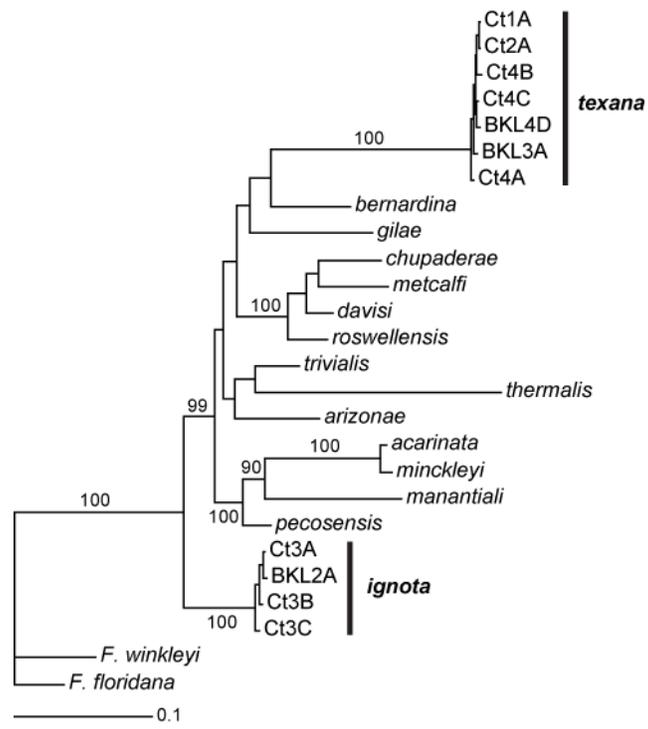


Figure 6

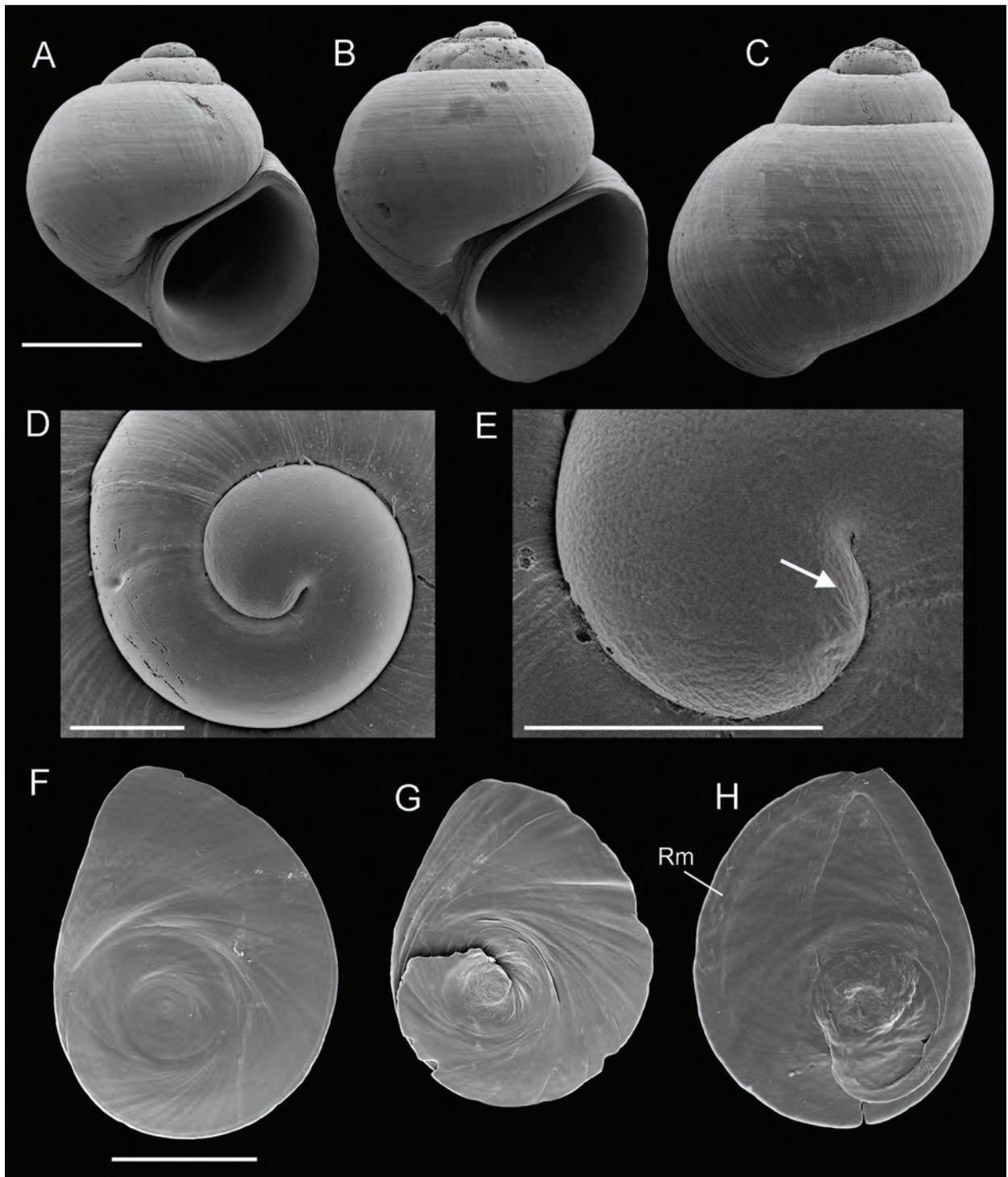


Figure 7

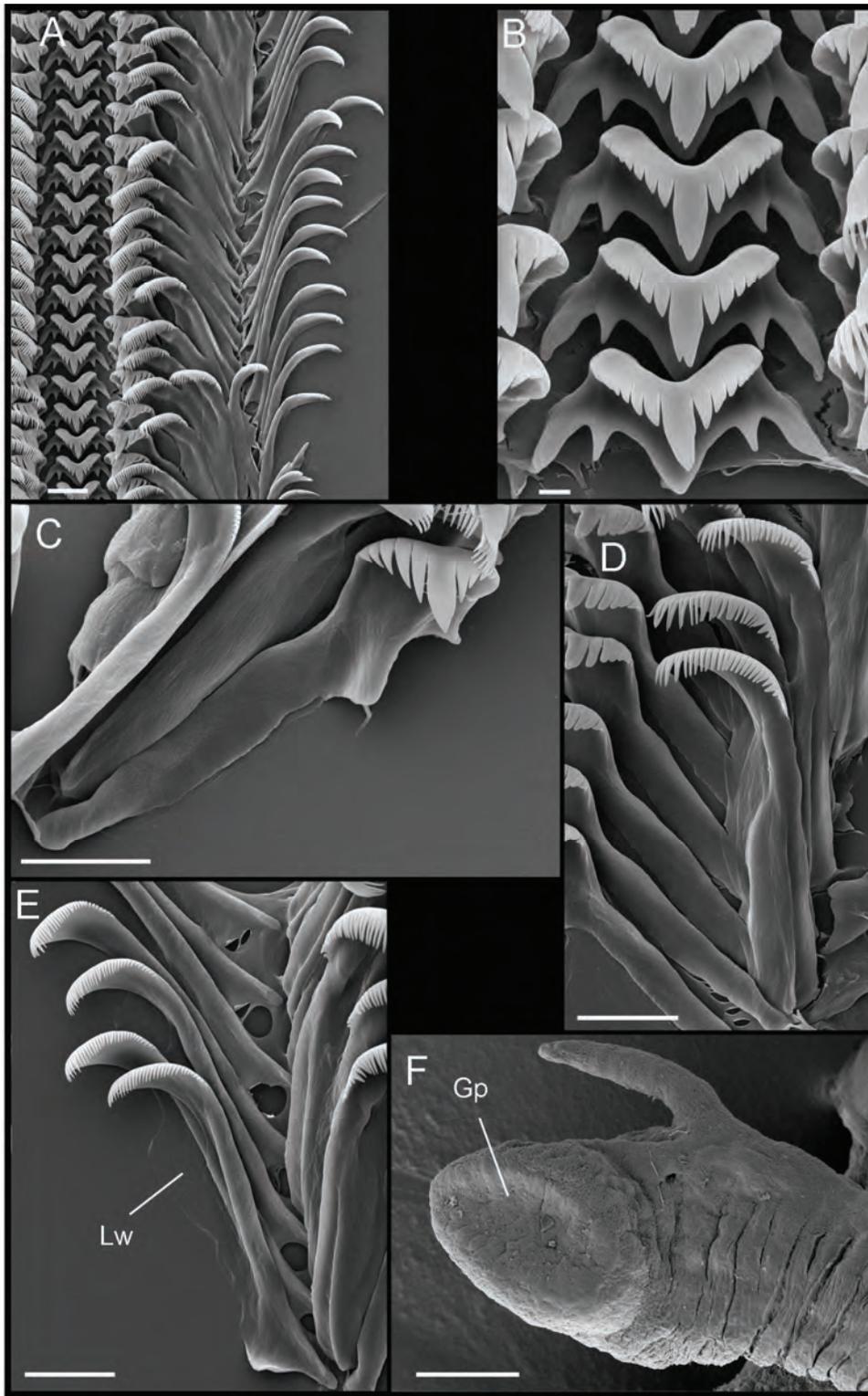


Figure 8

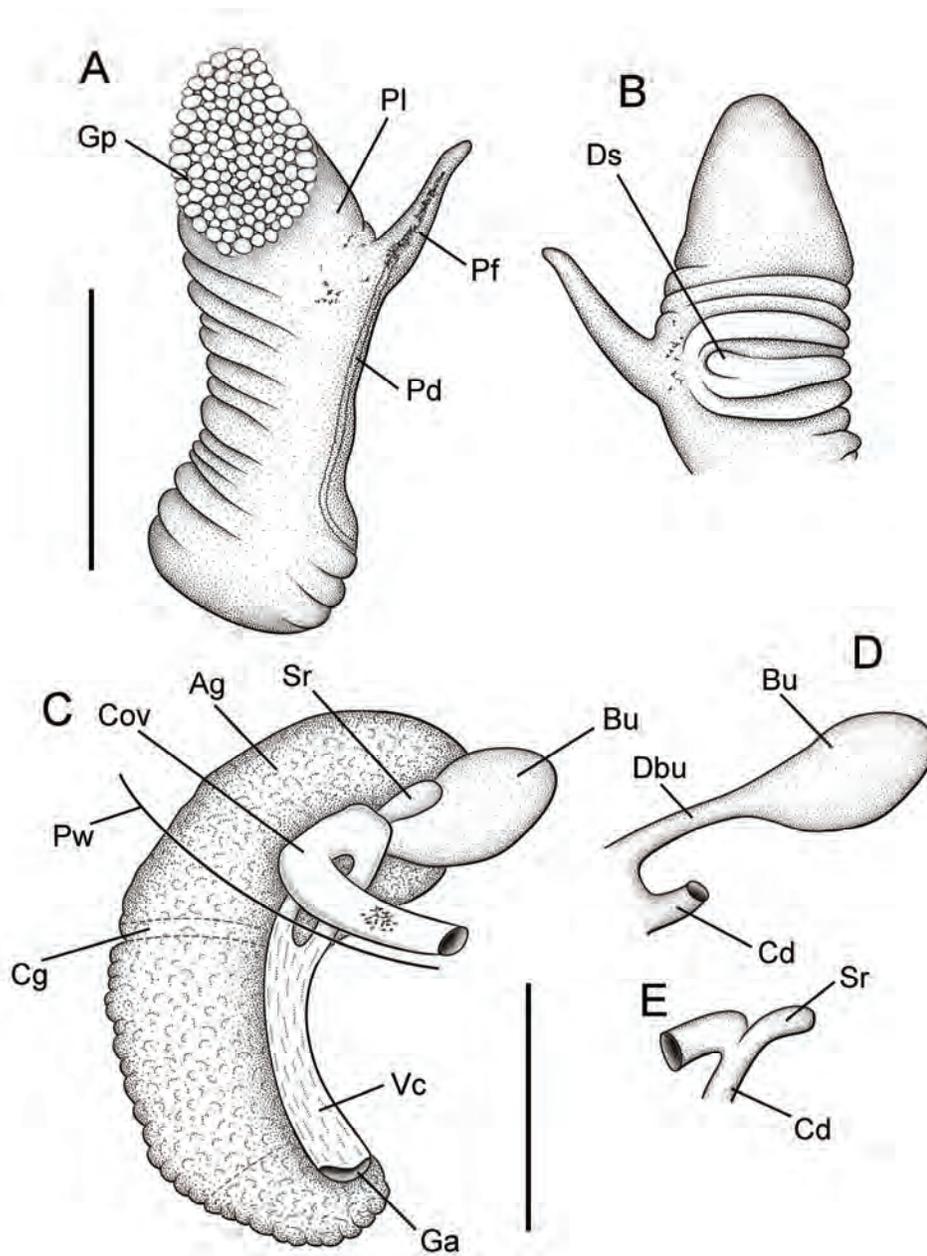


Figure 9



Appendix D. Management recommendations for aquatic macroinvertebrate species of New Mexico with state and federal conservation status.

Management Recommendations for Aquatic Macroinvertebrate Species of New Mexico with State and Federal Conservation Status

Management recommendations described below pertain to aquatic macroinvertebrates of New Mexico (Table 1, p. 5) with state or federal conservation status (endangered, threatened, species of concern). All taxa are recognized as Species of Greatest Conservation Need (SGCN) under the Comprehensive Wildlife Conservation Strategy for New Mexico (NMDGF 2006).

Invertebrates of Bitter Lake National Wildlife Refuge

Status: Recovery and conservation planning for four macroinvertebrates of BLNWR (Pecos assimineia, *Assimineia pecos*; Koster's springsnail, *Juturnia kosteri*; and Roswell springsnail, *Pyrgulopsis roswellensis*; Noel's amphipod, *Gammarus desperatus*) is provided by NMDGF (2005). This state plan identifies three outcomes ("Objective Parameters") to promote the objectives of conservation (maintaining extant populations *in situ*), habitat restoration for reintroduction management, and protection by establishing refuge populations to prevent extinction.

Statutory mandates of the New Mexico Wildlife Conservation Act amendments of 1995 stipulate that these activities use existing resources and funding, to the extent possible, to implement the plan. The NMDGF has no jurisdiction over habitats currently occupied by these species, or historic range where reintroduction might be considered. Accordingly, successful implementation of this plan will require collaboration with state, federal, and local government entities, and private landowners. Currently, the NMDGF is working with the U. S. Fish and Wildlife Service (Region 2, Ecological Services State Office of New Mexico and Texas), Bureau of Land Management, New Mexico Interstate Stream Commission, Texas Parks and Wildlife Department, and private landowners (e.g., The Nature Conservancy).

Threats: While populations of these four invertebrates are stable under current refuge management plans (Research Management Consultants, Inc. 1998), off-refuge land use practices within areas of the Roswell Artesian Basin pose threats to their long-term viability on BLNWR (NMDGF 2005, Federal Register 2005).

Regional ground water pumping for agriculture, municipal water supplies, and oil and gas industry operations continue in the Basin (BLM 1994, USFWS 1997). Any increases in ground water extraction similar to aquifer drawdown levels that occurred in the Basin from the 1950's to 1970's could lead to habitat impacts on BLWNR. Oil and gas development is ongoing within areas of the Basin that Balleau Groundwater, Inc. (1996, 1999) identified as primary ground water source-zones for surface waters at BLNWR. Such extractive processes and industry operations can result in aquifer drawdown, alter aquitard hydraulics, and contaminate ground and surface waters in New Mexico (Hennighausen 1969; Jercinovic 1982, 1984; Longmire 1983;

Quarles 1983; Boyer 1986; Richard 1988a, 1988b; Rail 1989; Richard and Boehm 1989a, 1989b; Balleau Groundwater, Inc. 1996; Martinez et al. 1998).

Aquatic mollusks are acutely sensitive to ground and surface water contaminants (Havlik and Marking 1987, Eisler 1987, Green and Trett 1989, Augspurger 2003). There is increased risk from degradation of ground and surface water quality posed by sewage contamination (i.e., municipal waste, septic discharge) from urban encroachment in aquifer recharge-discharge areas along the western bounds of BLNWR (Federal Register 2005). Illicit dumping of domestic contaminants (e.g., pesticides, herbicides, waste oil, etc.) in sinkholes is known to contaminate ground water resources in karst areas of the United States (White et al. 1995, Zokaites 1997) and in New Mexico (Bitner and Graves 1992, McQuillan et al. 1989).

Natural stochastic events, such as fire or drought, could adversely impact invertebrate populations at BLNWR. Although Lang (2001a) demonstrated short-term fire effects on the physicochemical conditions in Bitter Creek following the March 2000 Sandhill Fire, the long-term impact of these effects, whether beneficial or adverse, on the aquatic biota and riparian corridor remain undetermined (Lang 2002, 2005). Prolonged drought may affect hydrologic conditions on BLNWR by reducing discharge through refuge surface waters while concomitantly affecting aquatic physicochemical conditions and concentrating potential contaminants. Aquatic invasive species represent a threat to native wildlife and habitats at BLNWR (NMDGF 2008).

Aquatic ecosystems and macroinvertebrates are likely to show strong responses to global climate change (Xenopoulos et al. 2005, Burgmer et al. 2007).

Management Recommendations: Six strategies were identified in the recovery plan (Section 3.5) to address issues related to conservation of these species. Numerous activities stipulated under the Action Plan (Section 3.6) are already in progress that encompass these strategies. These activities include:

1. Monitor habitats and populations of these four invertebrates on BLNWR.
2. Continue research on species-specific autecology (population demography, habitat affinities, life history) and gammarid amphipod genetics.
3. Explore wetland restoration of North Spring, Roswell Country Club, with the intent to reintroduce these four species at this site. Specific recommendations here would be to consider first lowering of the lake level to induce flow of North Spring, while installing a fish barrier to exclude non-native fishes from the stream channel. An alternative approach, which is considered more costly and physically intrusive, would be to rehabilitate North Spring by constructing a stream channel and fish barrier to create a flowing system. Either option, or other alternative designs, will require consensus among stakeholders, especially the Roswell Country Club. With particular regard to gammarid amphipods, any repatriation effort should strive to reintroduce *Gammarus desperatus* sensu stricto (Cole 1981) to North Spring, as there is further evidence of cryptic species

of gammarids on BLWNR (Gervasio et al. 2004, Seidel and Berg 2008, Seidel et al. 2009).

4. Collaborate with personnel of BLWNR to expand the range of these four macroinvertebrates by introducing them into a recently created stream course along the western limit of refuge units (impoundments) 3, 5-7, and 15. Habitat suitability assessment is recommended prior to any introduction. Similar comments per #3 above (last sentence) pertain to introductions, which may require intra-specific genetic studies among isolated populations of these invertebrates to elect the most appropriate founding populations from all candidate populations on the refuge.
5. Continue exploratory surveys for these species on public and private land in Chaves County.
6. Efforts to establish captive populations of these species in refugia (Recovery and Conservation Plan Strategy 6) will require substantial logistical support that well-exceeds past and current levels of funding. Such activities require dedicated space and full-time staff to maintain aquaculture facilities and captive populations, while also carrying out research studies to assess the efficacy of controlled propagation (see Federal Register 2000, Lang 2001b, Lang et al. 2006, Shuster et al. 2005).

Under the state Share with Wildlife Program, the NMDGF is contracting with Dr. David Rogowski, Department of Natural Resources Management, Texas Tech University, Lubbock, for development of captive rearing methods and basic life history research for *J. kosteri* and *P. roswellensis*. This project is funded under State Wildlife Grant T-32-P2, Project No. 30, "Investigations into propagation of Koster's and Roswell springsnails."

7. It is recommended that any reintroduction of these species occur only within known historic or current range, as identified in the state recovery plan (NMDGF 2005).

Pyrgulopsis chupaderae

Status: *Pyrgulopsis chupaderae* is known from two isolated springs along the southwest flank of the Chupadera Mountains, Socorro County, New Mexico. Monthly monitoring of the *P. chupaderae* in the native habitat spanned the period May 1997 to July 1998. Routine population and habitat monitoring ceased in September 1999 because a change in land ownership has since precluded access.

Threats: Imminent threats include local/regional ground water depletion, diversion or impoundment of spring flow, loss of riparian vegetation, and overgrazing of the watershed during extended drought (Taylor 1983; NMDGF 1988; Lang 2001a, Lang 2002). These threats could be exacerbated by subdivision development on range lands surrounding Willow Spring. Introduction of aquatic invasive species can result in elimination of spring snail populations by

predation or habitat degradation (e.g., non-native crayfish; Fernandez and Rosen 1996, NMDGF 2008) or by direct competition for food sources (i.e., New Zealand mudsnail; <http://www.esg.montana.edu/aim/mollusca/nzms/nzmsbib.html>). Aquatic ecosystems and macroinvertebrates are likely to show strong responses to global climate change (Xenopoulos et al. 2005, Burgmer et al. 2007).

Management Recommendations:

1. Renegotiate site access to monitor populations of *P. chupaderae* in its native habitat.
2. Synthesize results from previous population and habitat studies.
3. Recommend development of a habitat management plan in cooperation with the land owner (e.g., “candidate conservation agreement”; Federal Register 1997) that perpetuates the existence of this species in its native habitat. This conservation plan should consider balancing water stewardship wisely to meet the needs of historic land use practices, which, heretofore, have allowed for persistence of native habitat critical to the survival of *P. chupaderae*.
4. Establish a refuge population of *P. chupaderae*.

Pyrgulopsis gilae

Status: The “metapopulation” of *P. gilae* consists of 43 widely disjunct populations in the Gila River basin, Catron and Grant Counties, New Mexico (Taylor 1987, Mehlhop 1993, Lang 2002; Hurt 2004; see Appendix B [this report]).

Threats: During previous grant segments, the population at the type locality (East Fork Gila River; Taylor 1987) was found stable (Lang 2009). Lang (2002) reported that recreational bathing at Alum Spring (Gila River) may adversely impact this species within and downstream of an existing bathing pool. Any efforts to increase discharge from the perched spring source that discharges flow to this pool could have adverse impacts to *P. gilae*, as this species is most abundant within the spring brook of Alum Spring nearest the springhead. Benefits potentially afforded *P. gilae* by signage to eliminate bathing in Alum Spring must be weighed against potential detriment (e.g., vandalism, purposeful water contamination, etc.) precipitated by misinterpretations of such a posting as limiting recreational use of this site.

Natural stochastic events (drought, forest fire, sedimentation, flooding), wetland habitat degradation from recreational bathing, and poor watershed management (e.g., over-grazing, forest over-harvesting) represent primary threats to *P. gilae* populations on federal and private lands (Taylor 1983, 1987; NMDGF 1988; Mehlhop 1993). Fire suppression chemicals could have potentially deleterious effects on *P. gilae* populations (McDonald and Hamilton 1995). Introduction of non-native crayfish and the New Zealand mudsnail can adversely impact spring

snails and aquatic habitats (Fernandez and Rosen 1996, NMDGF 2008, <http://www.esg.montana.edu/aim/mollusca/nzms/nzmsbib.html>). Long-term persistence of this species is contingent upon protection of spring sources, their outflows, and the riparian corridor immediately adjacent to these habitats. Aquatic ecosystems and macroinvertebrates are likely to show strong responses to global climate change (Xenopoulos et al. 2005, Burgmer et al. 2007).

Management Recommendations:

1. Continue surveys to document the status of all known populations of *P. gilae* in the Gila River basin. Expand surveys to unexplored reaches within the basin (see Appendix B, this report).
2. Recommend allocation of federal funding to assess genetic and morphologic divergence of *P. gilae* within and among geographically isolated populations throughout the Gila River basin. The taxonomic affinities of such geographically isolated populations are poorly understood, seldom studied, and should be adequately explored prior to development of appropriate management options (Weins 1996). Genetic divergence between disjunct populations may warrant taxonomic re-evaluation of the species, which in turn could confer specific management recommendations particular to genetically and morphologically distinct populations (management units) relative to current ownership and land-use practices.

Pyrgulopsis pecosensis

Status: Taylor (1987) reported two populations of the endemic *P. pecosensis* from perennial tributaries of the Black River, Eddy County, New Mexico: Blue Spring (type locality) and Castle Spring. Extirpation of the Castle Spring population is attributed to adverse land-use practices in the watershed (Landye 1981, NMDGF 1988, Mehlhop 1992). The habitat and population of *P. pecosensis* in Blue Spring was monitored monthly at two localities from July 1997 to September 1998. Thereafter, Blue Spring has been monitored annually or biannually during all grant segments from 1999 to 2010. *P. pecosensis* of Blue Spring appears stable under current grazing pressure and irrigation withdrawals.

Threats: Taylor (1983) identified ground water depletion as the primary threat to extant populations of *P. pecosensis*. Extirpation of the Castle Spring population was attributed to a number of factors including flood scour, ground water depletion, and possible contamination from an upstream livestock tank (Landye 1981, NMDGF 1988, Mehlhop 1992). Regional ground water withdrawals for agriculture and oil and gas industry operations (exploration, storage, transfer and refining) are ongoing in the Black River valley and adjacent aquifers in Eddy County (BLM 1997). Such extractive processes and industry operations are known to deplete aquifers and to contaminate ground and surface waters in New Mexico (Hennighausen 1969; Jercinovic 1982, 1984; Longmire 1983; Quarles 1983; Boyer 1986; Richard 1988a, 1988b; Rail 1989; Richard and Boehm 1989a, 1989b; Balleau Groundwater, Inc. 1996; Martinez et al.

1998).

Aquatic mollusks are acutely sensitive to ground and surface water contaminants (Havlik and Marking 1987, Eisler 1987, Green and Trett 1989, Augspurger et al. 2003). Richard (1988a, 1988b) and Richard and Boehm (1989a, 1989b) documented ground water contamination of domestic and agricultural/range wells in the upper Black River valley (i.e., Washington Ranch, Ballard Wells) by petroleum-derived hydrocarbons and sulfides. Richard and Boehm (1989b) reported “severe” sulfide contamination of Blue Spring, a regionally significant artesian spring that is a primary hydrologic source for the Black River (Hendrickson and Jones 1952). These authors indicated that gas contamination originating up-gradient was likely transported about 20 miles down-gradient to Blue Spring. Such long distance transport of ground water is common in karst, evaporite rock (White et al. 1995, Martinez et al. 1998). This raises long-term concerns for surface water quality of the Blue Spring wetland complex and the Black River, especially considering the current proliferation of petroleum industry operations in the Black River valley.

Accordingly there is increasing demand for developing ground-water wells to meet future needs for oil and gas exploration and production. This demand for ground water mining under prolonged drought could reduce flow through the Blue Spring system, resulting in habitat loss while also increasing salinity and potentially concentrating contaminants. Introduction of non-native crayfish and the invasive New Zealand mudsnail can adversely impact spring snails and aquatic habitats (Fernandez and Rosen 1996, NMDGF 2008, <http://www.esg.montana.edu/aim/mollusca/nzms/nzmsbib.html>).

Aquatic ecosystems and macroinvertebrates are likely to show strong responses to global climate change (Xenopoulos et al. 2005, Burgmer et al. 2007).

Management Recommendations:

1. Recommend exploring options for a conservation agreement (e.g., Federal Register 1997) with the private landowner of Blue Spring that provides a mechanism for species and habitat conservation compatible with past and present land-use practices. Such an agreement should also consider collaboration with other state agencies (e.g., Oil Conservation Division, Office of State Engineer, Interstate Stream Commission, NM Environment Department) to insure conformance with statutes and regulations for development of petroleum and ground-water wells while also complying with compact agreements for interstate conveyance of surface waters to the State of Texas.
2. Financial support under Section 6 will facilitate the project biologist’s efforts for continued population monitoring and to process voucher collections, compile a database, analyze data, and synthesize reports.
3. Continue routine monitoring of *P. pecosensis* in Blue Spring.

Pyrgulopsis thermalis

Status: The “metapopulation” of *P. thermalis* consists of 14 disjunct populations in the lower East Fork Gila River and the upper mainstem Gila River, Grant County, New Mexico (Taylor 1987, Mehlhop 1993, Lang 2002; Hurt 2004). See Appendix B (this report) for more information.

Threats: All known populations were found stable during the period 2001 to 2010 (Lang 2009, Lang 2010). However, recreational bathing at Alum Spring (type locality; Taylor 1987) may have adverse impacts to this species (Lang 2002). Providing that physical habitat disturbances associated with this type of use are limited to stone pools frequented by bathers, this species appears tolerant of current levels of visitation. However, any efforts to increase flow from the perched spring source that discharges water to these pools could have adverse impacts to *P. thermalis*, as this species is restricted to the spring brook of Alum Spring nearest the springhead. Benefits potentially afforded this species by signage to alert bathers of this fact must be weighed against potential detriment precipitated by misinterpretations of such a posting as limiting recreational use of this site.

Natural stochastic events (drought, forest fire, sedimentation, flooding), wetland habitat degradation from recreational bathing, and poor watershed management (e.g., over-grazing, forest over-harvesting) represent primary threats to *P. thermalis* populations on federal and private lands (Taylor 1983, 1987; NMDGF 1988; Mehlhop 1993). Fire suppression chemicals could have potentially deleterious effects on *P. thermalis* populations (McDonald and Hamilton 1995). Introduction of non-native crayfish and the New Zealand mudsnail can adversely impact spring snails and aquatic habitats (Fernandez and Rosen 1996, NMDGF 2008, <http://www.esg.montana.edu/aim/mollusca/nzms/nzmsbib.html>). Long-term persistence of this species is contingent upon protection of spring sources, their outflows, and the riparian corridor immediately adjacent to these habitats. Aquatic ecosystems and macroinvertebrates are likely to show strong responses to global climate change (Xenopoulos et al. 2005, Burgmer et al. 2007).

Management Recommendations:

- (1) Continue population monitoring to document the status of all known populations of *P. thermalis* in the Gila River basin. Expand survey area to malacologically unexplored reaches within the basin (see Appendix B, this report).
- (2) Recommend allocation of federal funding to assess genetic and morphologic divergence of *P. thermalis* within and among geographically isolated populations throughout the Gila River basin. The taxonomic affinities of such geographically isolated populations are poorly understood, seldom studied, and should be adequately explored prior to development of appropriate management options (Weins 1996). Genetic divergence between disjunct populations may warrant taxonomic re-evaluation of the species, which in turn could confer specific management recommendations particular to genetically and morphologically distinct populations (management units) relative to current ownership

and land-use practices.

Pisidium sanguinichristi

Status: Taylor (1987) described *P. sanguinichristi* as a narrowly restricted peaclam endemic to Middle Fork Lake, Questa Ranger District, Carson National Forest. In 1995, the NMDGF commenced annual population monitoring of *P. sanguinichristi* in response to a multi-agency conservation effort initiated by the U. S. Forest Service (1996). *Pisidium sanguinichristi* has not been collected in New Mexico since Taylor's species description. However, two collections in September 1999 yielded four specimens of *Pisidium* sp. from Middle Fork Lake that conchologically resembled the putative *Pisidium sanguinichristi*. This stimulated reinspection of voucher material from Middle Fork Lake collected during previous grant segments (1995-1999) to obtain sufficient sample sizes for genetic and morphologic studies. Lack of adequate funding and staffing has precluded further study.

Threats: Whereas the remoteness and ownership of Middle Fork Lake (Carson National Forest) afford some measure of protection, the site experiences intense periods of seasonal recreational use (USFS 1996). Threats include natural stochastic events (fire, drought), shoreline destabilization (erosion and sedimentation due to foot and vehicular traffic), contamination from chemicals used in forest fire suppressants, and placer mining runoff (Taylor 1983, NMDGF 1988, McDonald and Hamilton 1995, USFS 1996). Aquatic invasive species pose a threat to all native aquatic biota and ecosystems in New Mexico (NMDGF 2008). Aquatic ecosystems and macroinvertebrates are likely to show strong responses to global climate change (Xenopoulos et al. 2005, Burgmer et al. 2007).

Management Recommendations:

1. Continue sphaeriid inventory in high-elevation wetland habitats throughout the Sangre de Cristo Mountains. Expand this effort to include the Jemez Mountains.
2. Conduct morphometric study of *P. sanguinichristi* and *P. milium* shells. While conchology may help resolve outstanding taxonomic questions (NMDGF 1996), significant ecophenotypic variation in shell morphology and hinge dentition of sphaeriid clams manifested by local environmental influences (Herrington 1962, Mackie 2007) could render such an effort futile. Moreover, genetic study is contingent upon securing an adequate sample size of the putative *P. sanguinichristi*.

Stagnicola caperata

Status: Southern populations of this widely distributed marshsnail are disjunct in New Mexico, Texas, and higher elevations of the western states (Bequaert and Miller 1973; Taylor 1983, 1985). In New Mexico, Taylor (1983) first reported populations of *S. caperata* from Valles

Caldera National Preserve (VCNP) and in Hunter Marsh, Bitter Lake National Wildlife Refuge. While the former population is extant, extirpation of the latter population (between 1983-1985) was attributed to extensive wetland habitat loss, alteration, and sewage contamination (Taylor 1985). However, *S. caperata* was recently (2003-2004) documented from live specimens in: Hunter Marsh; grassland pools on the Valle Grande, VCNP; and high-elevation vernal pools in the Costilla River drainage, Vermejo Park Ranch, Taos County (Lang 2005). Additional records of Wrinkled marshsnail, occurring immediately west of Hunter Marsh on City of Roswell property, were reported by Lang (2010).

Threats: Water contamination from sewage effluent and habitat modification by removal of wetland vegetation or wetland drying represent primary threats (Taylor 1983, NMDGF 1988). Aquatic ecosystems and macroinvertebrates are likely to show strong responses to global climate change (Xenopoulos et al. 2005, Burgmer et al. 2007).

Management Recommendations:

1. The Department should continue statewide surveys of low- and high-elevation, ephemeral wetland habitats.
2. There is considerable habitat diversity and morphologic variation in shell characters between low-elevation populations (BLNWR; Leon Creek, Diamond Y Preserve, Pecos County, TX) and high-elevation populations (VCNP, Vermejo Park; B. Lang, *pers. obs.*). Taxonomic study is recommended to compare low- and high-elevation populations using genetic (mtDNA) and morphologic characters to assess the validity of this observation.

Literature Cited

- Augspurger, T., A. E. Keller, M. C. Black, W. G. Cope, and F. J. Dwyer. 2003. Water quality guidance for protection of freshwater mussels (Unionidae) from ammonia exposure. *Environmental Toxicology and Chemistry* 22(11):2569-2575.
- Balleau Groundwater, Inc. 1996. Interrelation of groundwater and surface water at Bitter Lake National Wildlife Refuge. A report prepared for the U.S. Department of Justice. 16 pp. (with addenda).
- Balleau Groundwater, Inc. 1997. Annotated abstract of water-rights administration Bitter Lake National Wildlife Refuge. A report prepared for the U.S. Department of Justice. 22 pp. (with addenda).
- Balleau Groundwater, Inc. 1999. Source-water protection zones for Bitter Lake National Wildlife Refuge. A report prepared for the U.S. Department of Justice. 42 pp.
- Bequaert, J. C. and W. B. Miller. 1973. The mollusks of the arid southwest: with an Arizona check list. University of Arizona Press, Tucson, AZ. 271 pp.
- Bitner, M. J. And T. Graves. 1992. Effect of lot sizes on potential ground-water contaminants from conventional septic-tank systems: numerical modeling. Policy Coordinating Committee. 47 pp.
- Boyer, D. G. 1986. Differences in produced water contaminants from oil and gas operations in New Mexico—implications for regulatory action. Pp. 291-316, *In Proceedings of the Conference on Southwestern Ground Water Issues* (National Water Well Association, Publisher).
- Bureau of Land Management. 1994. Draft resource management plan/environmental impact statement for the Roswell Resource Area, Roswell, New Mexico, and draft resource management plan amendment/environmental impact statement for the Carlsbad Resource Area, Carlsbad, New Mexico. Bureau of Land Management, Roswell, NM.
- Bureau of Land Management. 1997. Carlsbad Approved Resource Management Plan, Amendment and Record of Decision, Carlsbad Resource Area, Roswell District, New Mexico. BLM-NM-PT-98-004-1610.
- Burgmer, T., H. Hillebrand, and M. Pfenninger. 2007. Effects of climate-driven temperature changes on the diversity of freshwater macroinvertebrates. *Oecologia* 151:03-103.
- Cole, G. A. 1981. *Gammarus desperatus*, a new species from New Mexico (Crustacea: Amphipoda). *Hydrobiologia* 76:27-32.

- Eisler, R. 1987. Polycyclic aromatic hydrocarbon hazards to fish, wildlife, and invertebrates: a syntopic review. U.S. Fish and Wildlife Service. Contaminant Hazard Reviews Report No. 11, Biological Report 85 (1.11).
- Federal Register. 1996. Endangered and threatened wildlife and plants; Review of plant and animal taxa that are candidates for listing as endangered or threatened species. 50 CFR Part 17. 61(40):7596-7613.
- Federal Register. 1997. Announcement of draft policy for candidate conservation agreements. 50 CFR Part 17. 62(113):32183-32188.
- Federal Register. 2000. Policy of controlled propagation of species listed under the Endangered Species Act. 50 CFR Part 17. 65(183):56916-56922.
- Federal Register. 2005. Endangered and Threatened Wildlife and Plants; Listing Roswell springsnail, Koster's tryonia, Pecos Assiminea, and Noel's Amphipod as Endangered with Critical Habitat; Final Rule. 50 CFR Part 17, 70(152):46303-46333.
- Fernandez, P.J., and P.C. Rosen. 1996b. Effects of an introduced crayfish (*Orconectes virilis*) on the stream habitat of the Chiricahua leopard frog (*Rana chiricahuensis*) at Three Forks, White Mountains, Arizona. *In*: Effects of the introduced crayfish *Orconectes virilis* on native aquatic herpetofauna in Arizona. Final Report, IIPAM Project No. I94054. Heritage Program, Arizona Game and Fish Department, Phoenix, AZ.
- Green, J. and M. W. Trett. 1989. The fate and effects of oil in freshwater. Elsevier Science Publishing Co., Inc., New York.
- Gervasio, V., D. J. Berg, B. K. Lang, N. L. Allan, and S. I. Guttman. 2004. Genetic diversity in the *Gammarus pecos* species complex: implications for conservation and regional biogeography in the Chihuahuan Desert. *Limnology and Oceanography*. 49(2):520-531.
- Havlik, M. E. and L. L. Marking. 1987. Effects of contaminants on naiad mollusks (Unionidae): a review. U. S. Fish and Wildlife Service, Resource Publication 164.
- Hendrickson, G. E., and R. S. Jones. 1952. Geology and ground-water resources of Eddy County, New Mexico. U.S. Geological Survey, Ground-Water Report 3, 169 pp.
- Hennighausen, F. H. 1969. Meters and their effects in the Roswell Artesian Basin in Chaves and Eddy counties, New Mexico. Pp. 29-33, *In* 14th Annual New Mexico Water Conference (Water Resources Institute).
- Herrington, H. B. 1962. A revision of the Sphaeriidae of North America (Mollusca: Pelecypoda). University of Michigan Museum of Zoology. Miscellaneous Publications No. 18.

- Hurt, C. R. 2004. Genetic divergence, population structure and historical demography of rare springsnails (*Pyrgulopsis*) in the lower Colorado River basin. *Molecular Ecology* 13:1173-1187.
- Jercinovic, D. E. 1982. Assessment of refined petroleum-product contamination problems in surface and ground waters of New Mexico. Water Pollution Control Bureau, New Mexico Environmental Improvement Division, EID/WPC-82/5.
- Jercinovic, D. E. 1984. Petroleum-product contamination of soil and water in New Mexico. New Mexico Environmental Improvement Division, EID/GWH84/2.
- Landye, J. J. 1981. Current status of endangered, threatened, and/or rare mollusks of New Mexico and Arizona. Endangered Species Office, U. S. Fish and Wildlife Service, Final Report 13.
- Lang, B. K. 2001a. Status of aquatic mollusks of New Mexico. New Mexico Department of Game and Fish, Annual Performance Report (E-20-8) submitted to the Office of Federal Aid, U. S. Fish and Wildlife Service, Albuquerque, New Mexico.
- Lang, B. K. 2001b. New Mexico endangered invertebrates: monitoring and management. New Mexico Department of Game and Fish, Completion Report E-37(1-5) submitted to the Office of Federal Aid, U. S. Fish and Wildlife Service, Albuquerque, NM.
- Lang, B. K. 2002. Status of aquatic mollusks of New Mexico. New Mexico Department of Game and Fish Completion Report E-20-(5-9) submitted to the Division of Federal Aid, U. S. Fish and Wildlife Service, Albuquerque, NM.
- Lang, B. K. 2005. Macroinvertebrate of Bitter Lake National Wildlife Refuge. NMDGF Completion Report E-56-(1-3) submitted to the Division of Federal Aid, U. S. Fish and Wildlife Service, Albuquerque, NM.
- Lang, B. K. 2008. Macroinvertebrate of Bitter Lake National Wildlife Refuge. NMDGF Performance Report E- 56-5 submitted to the Division of Federal Aid, U. S. Fish and Wildlife Service, Albuquerque, NM.
- Lang, B. K. 2009. Macroinvertebrates of Bitter Lake National Wildlife Refuge. New Mexico Department of Game and Fish, Performance Report, E-56-6 , submitted to the Division of Federal Aid, U. S. Fish and Wildlife Service, Region 2, Albuquerque, NM.
- Lang, B. K. 2010. Macroinvertebrates of Bitter Lake National Wildlife Refuge. New Mexico Department of Game and Fish, Performance Report, E-56-7 , submitted to the Division of Federal Aid, U. S. Fish and Wildlife Service, Region 2, Albuquerque, NM.

- Lang, B. K., D. A. Kelt, and S. M. Shuster. 2006. The role of controlled propagation on an endangered species: demographic effects of habitat heterogeneity among captive and native populations of the Socorro isopod (Crustacea: Flabellifera). *Biodiversity and Conservation* 15(12):3909-3935.
- Longmire, P. A. 1983. Petroleum-product contamination of ground and surface water: a literature review. Ground Water Section, Water Pollution Control Bureau, New Mexico Environmental Improvement Division. EID/WPC-83/7.
- Mackie, G.L. 2007. *Biology of Freshwater Corbiculid and Sphaeriid Clams of North America*. Ohio Biological Survey, Columbus, OH. 436 pp.
- Martinez, J. D., K. S. Johnson, and J. T. Neal. 1998. Sinkholes in evaporite rocks. *American Scientist* 86:38-51.
- McDonald, S. F. and S. J. Hamilton. 1995. Fire retardant and foam suppressant chemicals may be toxic to aquatic invertebrates and algae. National Biological Service. NBS Information Bulletin 35.
- McQuillan, D. M., M. J. Jasper, and B. H. Swanson. 1989. Ground-water contamination by septic-tank use: a field study in the Albuquerque south Valley - West Mesa Region, Bernalillo County, New Mexico. New Mexico Health and Environment Department, Environmental Improvement Division, Ground Water Bureau.
- Mehlhop, P. 1992. Establishment of a rare mollusc inventory and monitoring program for New Mexico. Progress Report. NMGF Professional Services Contract 80-519-52.
- Mehlhop, P. 1993. Establishment of a rare mollusc inventory and monitoring program for New Mexico. Year II Progress Report. NMGF Professional Services Contract No. 80-519-52-Amendment 1.
- New Mexico Department of Game and Fish. 1988. Handbook of species endangered in New Mexico. Account: A-299.
- New Mexico Department of Game and Fish. 1996. Status of aquatic and terrestrial mollusks of New Mexico. New Mexico Department of Game and Fish, Completion Report E-20(1-4) submitted to the Office of Federal Aid, U. S. Fish and Wildlife Service, Albuquerque, New Mexico.
- New Mexico Department of Game and Fish. 2000. Threatened and endangered species of New Mexico: biennial review and recommendations. 116 pp.
- New Mexico Department of Game and Fish. 2005. Recovery and conservation plan for four invertebrate species: Noel's amphipod (*Gammarus desperatus*), Pecos assiminea

- (*Assiminea pecos*), Koster's springsnail (*Juturnia kosteri*), and Roswell springsnail (*Pyrgulopsis roswellensis*). 80 pp.
- New Mexico Department of Game and Fish. 2006. Comprehensive Wildlife Conservation Strategy for New Mexico. 635 pp.
- New Mexico Department of Game and Fish. 2008. New Mexico aquatic invasive species management plan. 107 pp.
- New Mexico Department of Game and Fish. 2010. Threatened and endangered species of New Mexico: biennial review and recommendations.
- New Mexico Statutes Annotated. 1995. Interstate Stream Commission Water Conservation Program: Pecos River Portion. NMSA Supplement 72-5-28.
- Quarles, J. 1983. Groundwater contamination in the United States. University of Pennsylvania Press, Philadelphia.
- Rail, C. D. 1989. Groundwater contamination: sources, control, and preventative measures. Technomic Publishing Company, Inc. Lancaster, PA.
- Research Management Consultants, Inc. 1998. Final comprehensive conservation plan and Environmental Assessment: Bitter Lake National Wildlife Refuge.
- Richard, M. 1988a. Natural gas contamination at Rattlesnake Springs, Carlsbad Caverns National Park: review of the geohydrology in the vicinity of Rattlesnake Springs and the contamination problem. Report 1. National Park Service Contract RFQ 7029-8-0025.
- Richard, M. 1988b. Natural gas contamination at Rattlesnake Springs, Carlsbad Caverns National Park: report of the first field investigation, August 1988. Report 2. National Park Service Contract RFQ 7029-8-0025.
- Richard, M. and A. Boehm. 1989a. Natural gas contamination at Rattlesnake Springs, Carlsbad Caverns National Park: report of the second field investigation, March, 1988. Report 3. National Park Service Contract RFQ 7029-8-0025.
- Richard, M. and A. Boehm. 1989b. Natural gas contamination at Rattlesnake Springs, Carlsbad Caverns National Park: final summary of the investigation. Report 4. National Park Service Contract RFQ 7029-8-0025.
- Seidel, R. A. and D. J. Berg. 2008. Genetic assessment of the *Gammarus pecos* species complex (Crustacea: Amphipoda) of New Mexico. Final Report to New Mexico Department of Game and Fish under Share with Wildlife Contract No. 04-516.0000-0093.

- Seidel, R. A., B. K. Lang, and D. J. Berg. 2009. Phylogeographic analysis reveals multiple cryptic species of amphipods (Crustacea: Amphipoda) in Chihuahuan Desert springs. *Biological Conservation* 142:2303-2313.
- Shuster, S. M., M. P. Miller, B. K. Lang, N. Zorich, L. Huynh, and P. Keim. 2005. The effects of controlled propagation on an endangered species: genetic differentiation and divergence in body size among native and captive populations of the Socorro isopod (Crustacea: Flabellifera). *Conservation Genetics* 6:355-368.
- Taylor, D. W. 1983. Endangered Species: Status investigation of mollusks of New Mexico. Professional Service Contract Nos. 519-69-01 and 519-69-01-A.
- Taylor, D. W. 1985. Aquatic molluscs from Pecos County, Texas. Unpublished manuscript submitted to the New Mexico Department of Game and Fish.
- Taylor, D. W. 1987. Fresh-water mollusks from New Mexico and vicinity. New Mexico Bureau of Mines & Mineral Resources Bulletin 116.
- United States Department of Justice. 1996. United States of America's statement of claim for reserved surface water and groundwater rights for Bitter Lake National Wildlife Refuge. Roswell Basin Section, RAB 923 and 1793, Denver, CO.
- United States Fish and Wildlife Service. 1997. Biological opinion on the Roswell Resource Area Resource Management Plans. Section 7 Consultation #2-22-96-102.
- United States Forest Service. 1996. Habitat conservation assessment for the Sangre de Cristo peaclam (*Pisidium sanguinichristi*).
- White, B. W., D. C. Culver, J. S. Herman, T. C. Kane, and J. E. Mylroie. 1995. Karst lands: the dissolution of carbonate rock produces unique landscapes and poses significant hydrological and environmental concerns. *American Scientist* 83:450-459.
- Weins, J. A. 1996. Wildlife in patchy environments: metapopulations, mosaics, and management. Pp 53-84, *In* Metapopulations and Wildlife Conservation (Ed., D. R. McCollough). Island Press.
- Xenopoulos, M. A., D. M. Lodge, J. Alcamo, M. Märker, K. Schulze, and D. P Van Vuurens. 2005. Scenarios of freshwater fish extinctions from climate change and water withdrawal. *Global Change Biology* 11:1557-1564.
- Zokaites, C. 1997. Living on karst: a reference guide for landowners in limestone regions. Cave Conservancy of the Virginias. 25 pp.