Shell Variation of Springsnail Populations in the Cuatro Cienegas Basin, Mexico: Preliminary Analysis of Limnocrene Fauna

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ABSTRACT

Geographic variation in shell morphometry is analyzed for three locally endemic springsnail (Gastropoda: Hydrobiidae) species occurring sympatrically in nine limnocrenes of the Cuatro Cienegas Basin, Coahuila, Mexico. Despite some correlation of size-related variables across species, groupings of populations based on multivariate analyses were not very similar among species, nor were they strongly concordant with current drainage configurations in the basin. Groups of populations of *Mexipyrgus churinceanus* Taylor having different patterns of shell sculpture and color banding (and once considered separate species) were not separated similarly on basis of shell size and shape. Inter-population differentiation of these snails was approximately equivalent to that of *Nymphophilus minckleyi* Taylor, whereas *Mexithauma quadripaludium* Taylor was less variable.

INTRODUCTION

One of the more remarkable aquatic faunas of the New World occurs in the small (1,200 km^2), intermontane valley of Cuatro Cienegas, Coahuila, Mexico, which harbors at least 26 locally endemic forms (Cole, 1984; McCoy, 1984; Minckley, 1984; Hershler, 1985). Local aquatic taxa show a great diversity in extent of differentiation relative to adjacent biota, ranging from slightly differentiated populations to highly divergent genera, suggesting both long-term persistence of aquatic habitat and multiple invasions of the valley over a broad time scale (Minckley, 1969). Aquatic organisms are deployed among diverse, spring-fed aquatic habitats that comprise five to seven local drainage systems (Minckley, 1969; LaBounty, 1974), providing what has been termed a "matchless natural laboratory" (Taylor & Minckley 1966:22) for ecological and evolutionary study.

Springsnails (Gastropoda: Hydrobiidae) of the basin are diverse [nine genera (five endemic), 13 species (nine endemic); Hershler, 1985], and occur abundantly in a large number of easily accessible sites, providing an excellent opportunity to study geographic variation of populations, the analysis of which is considered crucial to understanding the speciation process (Gould & Johnston, 1972; Endler, 1977). To date, research on this snail fauna has largely been taxonomic (Taylor, 1966; Hershler, 1985), although geographic variation of one endemic species was partly analyzed (Hershler, 1985; Hershler & Minckley, 1986). Taylor (1966) and Taylor and Minckley (1966) noted apparent diversity in extent of differentiation among local species: *Mexipyrgus* Taylor, an endemic restricted to large (> 25 m^2) springpools (limnocrenes) and stream outflows, is variable enough to have been originally considered as six nominal species (Taylor, 1966; synonymized to monotypy by Hershler, 1985), whereas other snails appear morphologically uniform, at least in the portion of the basin that has been well studied (*i.e.*, all but the southeastern lobe; Hershler, 1985). It was suggested that heightened differentiation of *Mexipyrgus* resulted from marked discontinuity of its habitat:

> . . . it seems that habitat of this genus is more likely to be discontinuous than that of other snails in the area. *Mexipyrgus* lives in soft flocculent ooze or mud in the lagunas, thus not in the shallows where wave action removes the fine particles. Extensive marshy areas with small streams connecting larger water bodies provide no suitable widespread habitat. (Taylor, 1966:188)

Variation within *Mexipyrgus* has largely been discussed in terms of shell sculpture and color banding (Taylor, 1966), characters absent from or poorly developed in other local snails, and there has been no attempt to contrast intraspecific variation among members of the snail fauna using a set of common characters, such as shell morphometric variables. In this paper we provide such a comparison between *Mexipyrgus churinceanus* Taylor and the two other species (both local endemics) common in basin limnocrenes, *Mexithauma quadripaludium* Taylor and *Nymphophilus minckleyi* Taylor (see figure 2). Specifically, we seek to answer the following questions: 1) Do these distantly related snails (Hershler, 1985) show commonality of pattern of geographic variation? 2) Does shell morphometric variation among pop-

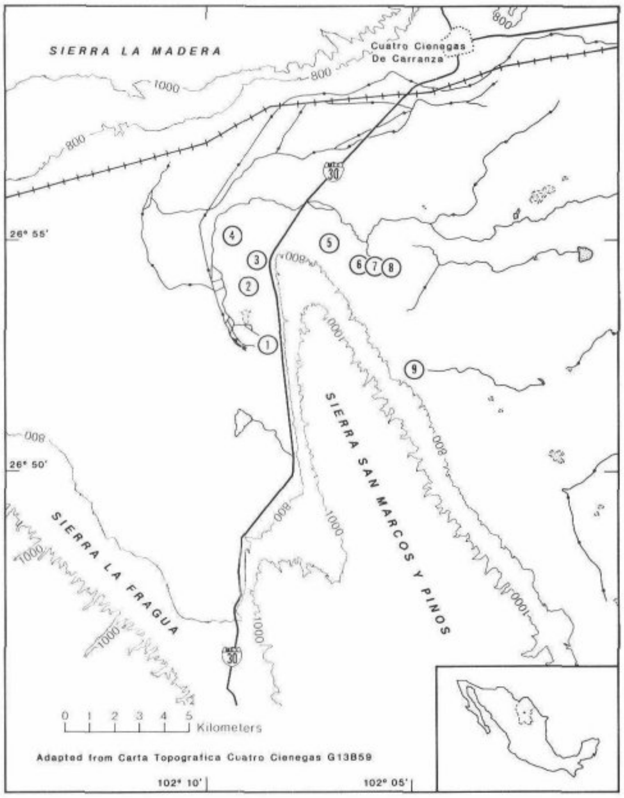


Figure 1. Map of the central portion of the Cuatro Cienegas Basin, showing major drainage features and collecting localities (numbered as in table 1). The inset (lower right) shows locations of the state of Coahuila (dashed line) and Cuatro Cienegas (dot) within Mexico.

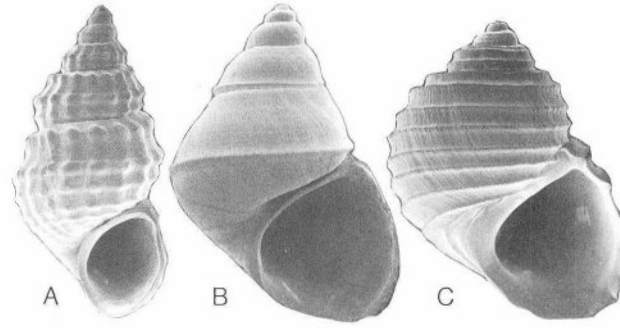


Figure 2. Scanning electron micrographs (printed at same enlargement) of cleaned shells of springsnails from Laguna Tio Candido, Cuatro Cienegas, Mexico: a, *Mexipyrgus churinceanus* (shell height, 6.34 mm); b, *Nymphophilus minckleyi* (6.45 mm); c, *Mexithauma quadripaludium* (6.22 mm).

ulations of *Mexipyrgus churinceanus* exceed that of the two other limnocrene species (above) that occur sympatrically with this species? 3) Are *Mexipyrgus* populations that are well differentiated in terms of shell sculpture and color banding patterns similarly separated by shell size and shape variation?

MATERIALS AND METHODS

Nine limnocrenes were sampled (shown in figure 1 and described in the Appendix), representing the majority of springpools in the study area (all of basin excluding southeastern lobe) where all three forms are common. Only isolated sources (Sites 1–4, 9) or, in cases where springs were connected by stream, upflow pools (Sites 5–8) were considered, in order to reduce possible effects of gene flow from contiguous populations (Hershler & Minckley, 1986). These springs belong to four separate drainage systems of the basin and harbor forms of *Mexipyrgus* referable to four nominal species (see below).

Mexipyrgus churinceanus was collected by sieving soft substrates, while the other two species, associated with hard substrates, were gathered by washing travertine and macrophytes in a bucket. Material was fixed in dilute formalin, and preserved in 70% ethanol.

For each species, about 15 live-collected, fully mature adults, recognizable by their complete and thickened inner shell lips, were randomly selected from collections from each site and dried for morphometric analysis. After whorls were counted (WH), shells were imbedded in clay in standard apertural aspect (figure 2) and shell outlines were drawn using a camera lucida mounted on a WILD M-5 dissecting microscope (12 × or 25 ×). Points on these drawings were digitized and values for the following "standard" shell parameters (1–4) and shell shape descriptors (5–8) (from Raup, 1966, and elsewhere) were generated:

1) Shell height (SH)
2) Shell width (SW)
3) Length of body whorl (LBW)
4) Width of body whorl (WBW)
5) Translation rate (T)
6) Whorl expansion rate (W)
7) Distance of generating curve from coiling axis (D)
8) Aperture shape (SA)

A calculated variable (S), consisting of the addition of SH and SW, was generated to serve as a more realistic measure of size than either shell length or width. Calculation of shape parameters largely followed methods of Kohn and Riggs (1975), with the exception being W, which was measured as the mean of a series of squared ratios of perpendicular distances from coiling axis to sutures (shell in apertural and not apical aspect) at half whorl intervals. The apertural suture was not used, due to frequent loosening of coiling during last half whorl of growth, nor were sutures used from eroded adapical sections of the spire. Digitizing was done using the CONCH software program (Chapman *et al.*, 1988; methodology fully described therein) and a GTCO Micro-Digi Pad 12 × 12 linked to a KAYPRO 2000 microcomputer.

Descriptive statistics for all morphological variables were obtained for each species and locality. The hypotheses of homogeneity of mean differences and variances across localities were tested for each species. An ANOVA model was selected for each variable of each

Table 1. Descriptive statistics for each species at each locality. Data given are mean, standard deviation, and sample size (in parentheses). L = locality; variable abbreviations are given in text.

	Variable										
	L	SH	SW	S	LBW	WBW	WH	W	D	T	SA
Mexipyrgus churinceanus											
	1	5.14	2.62	7.76	3.37	2.39	6.18	1.90	0.76	6.17	1.32
	(15)	0.39	0.20	0.57	0.21	0.21	0.37	0.16	0.07	0.49	0.06
	2	6.13	3.21	9.34	3.85	3.00	7.03	1.81	0.67	5.87	1.36
	(15)	0.30	0.11	0.35	0.19	0.12	0.57	0.19	0.04	0.53	0.06
	3	4.76	2.48	7.24	2.94	2.32	6.57	1.75	0.68	6.13	1.35
	(15)	0.32	0.21	0.49	0.17	0.19	0.48	0.14	0.05	0.79	0.06
	4	5.02	2.72	7.74	3.44	2.47	6.23	1.97	0.73	5.99	1.35
	(14)	0.23	0.12	0.29	0.16	0.12	0.27	0.16	0.06	0.27	0.07
	5	3.99	2.17	6.16	2.60	2.02	6.28	1.98	0.68	6.19	1.31
	(15)	0.22	0.17	0.38	0.12	0.16	0.38	0.36	0.07	0.64	0.07
	6	7.30	3.98	11.3	4.68	3.55	6.55	1.86	0.61	5.77	1.36
	(14)	0.56	0.33	0.84	0.24	0.36	0.41	0.21	0.04	0.67	0.04
	7	6.48	3.17	9.66	4.13	2.88	7.28	2.05	0.70	6.67	1.36
	(15)	0.30	0.18	0.41	0.24	0.24	0.44	0.40	0.07	0.65	0.06
	8	5.49	2.54	8.03	3.57	2.24	7.03	1.92	0.73	7.13	1.41
	(15)	0.18	0.14	0.25	0.14	0.09	0.61	0.28	0.06	0.87	0.06
	9	7.16	3.62	10.8	4.39	3.45	6.58	1.76	0.70	6.44	1.41
	(15)	0.46	0.22	0.66	0.19	0.26	0.32	0.10	0.07	0.73	0.09
Mexithauma quadripaludium											
	1	7.39	6.44	13.8	6.61	4.72	4.57	2.20	0.62	3.81	1.11
	(15)	0.55	0.41	0.90	0.47	0.30	0.24	0.22	0.05	0.44	0.06
	2	5.82	5.07	10.9	5.09	3.64	4.47	2.26	0.60	3.14	1.13
	(15)	0.41	0.28	0.66	0.42	0.24	0.23	0.32	0.04	0.40	0.06
	3	5.73	4.83	10.6	5.04	3.54	4.63	2.28	0.65	3.50	1.13
	(15)	0.30	0.33	0.59	0.31	0.19	0.25	0.33	0.05	0.60	0.06
	4	6.20	5.45	11.7	5.45	3.99	4.37	2.05	0.62	3.52	1.07
	(13)	0.57	0.36	0.87	0.58	0.25	0.26	0.21	0.06	0.60	0.08
	5	5.77	5.09	10.9	5.14	3.68	4.19	2.36	0.61	3.38	1.12
	(9)	0.61	0.55	1.12	0.52	0.41	0.11	0.41	0.08	0.48	0.05
	6	6.72	6.00	12.7	5.76	4.51	4.44	2.07	0.61	3.34	1.03
	(9)	0.60	0.49	1.07	0.47	0.47	0.41	0.11	0.05	0.48	0.05
	7	5.23	4.50	9.73	4.56	3.27	4.32	2.14	0.63	2.85	1.12
	(15)	0.36	0.22	0.55	0.34	0.20	0.26	0.19	0.04	0.21	0.05
	8	6.07	5.15	11.2	5.18	3.78	4.67	2.20	0.62	3.17	1.10
	(15)	0.48	0.30	0.72	0.42	0.25	0.29	0.25	0.07	0.37	0.06
	9	6.40	5.51	11.9	5.47	4.15	4.63	2.16	0.64	3.51	1.08
	(15)	0.44	0.29	0.67	0.35	0.27	0.30	0.19	0.04	0.56	0.05
Nymphophilus minckleyi											
	1	6.66	5.30	12.0	5.19	4.00	4.80	2.06	0.57	3.24	1.12
	(15)	0.38	0.19	0.53	0.28	0.20	0.34	0.37	0.03	0.29	0.05
	2	5.99	4.56	10.6	4.51	3.76	5.03	1.88	0.59	3.82	1.09
	(15)	0.47	0.31	0.73	0.34	0.22	0.09	0.19	0.05	0.43	0.03
	3	5.12	4.09	9.21	3.96	3.15	4.54	2.01	0.57	3.35	1.10
	(12)	0.43	0.26	0.67	0.31	0.20	0.37	0.26	0.03	0.36	0.04
	4	5.88	4.89	10.8	4.50	3.79	5.39	1.89	0.54	3.32	1.05
	(7)	0.39	0.15	0.42	0.25	0.09	0.35	0.12	0.02	0.33	0.04
	5	5.56	4.34	9.90	4.38	3.48	5.28	2.08	0.60	3.60	1.14
	(15)	0.34	0.23	0.50	0.28	0.19	0.38	0.29	0.03	0.48	0.03
	6	7.17	5.98	13.1	5.29	4.84	5.68	1.86	0.54	3.19	1.13
	(15)	0.30	0.26	0.45	0.24	0.18	0.26	0.16	0.04	0.42	0.05

Table 1. Continued.

	Variable									
L	SH	SW	S	LBW	WBW	WH	W	D	T	SA
7	5.69	4.53	10.2	4.13	3.74	5.22	1.76	0.58	3.45	1.07
(11)	0.29	0.27	0.54	0.19	0.26	0.14	0.04	0.05	0.24	0.05
8	7.03	5.55	12.6	5.32	4.35	5.23	1.88	0.57	3.34	1.18
(10)	0.25	0.36	0.55	0.28	0.27	0.18	0.29	0.04	0.35	0.07
9	7.48	5.85	13.3	5.61	4.61	5.47	1.83	0.59	3.21	1.17
(15)	0.46	0.30	0.68	0.35	0.30	0.27	0.13	0.06	0.32	0.06

species unless very significant heterogeneity of variance existed, in which case the generalized Welch test was used to consider mean differences. Pearson correlations were computed across species pairs for population means of each variable. Principal component analysis (PCA) was applied separately to each species data matrix to assess and compare groupings of specimens without *a priori* assumptions. Because the units of measurement were distinct and non-comparable, the analyses were performed on correlation matrices. Discriminant analysis (DA) was used to determine assignment of specimens to the locality groupings on basis of shell size and shape. This analysis was computed in a stepwise manner in order to identify measurements contributing to significance of discrimination. Selection criterion was maximization of Mahalanobis D-squared between closest pairs of localities. The *a posteriori* procedure of classification analysis was performed to determine possible error of specimen assignment to locality. When SL and SW were replaced by their sum (S), slightly better locality separation resulted in the multivariate analyses and these results are reported.

Computations were performed using SYSTAT (Wilkinson, 1986) on an IBM-XT, and SPSSX Ver. 2 on an IBM 4381 VM/CMS system at the Smithsonian Institution.

RESULTS

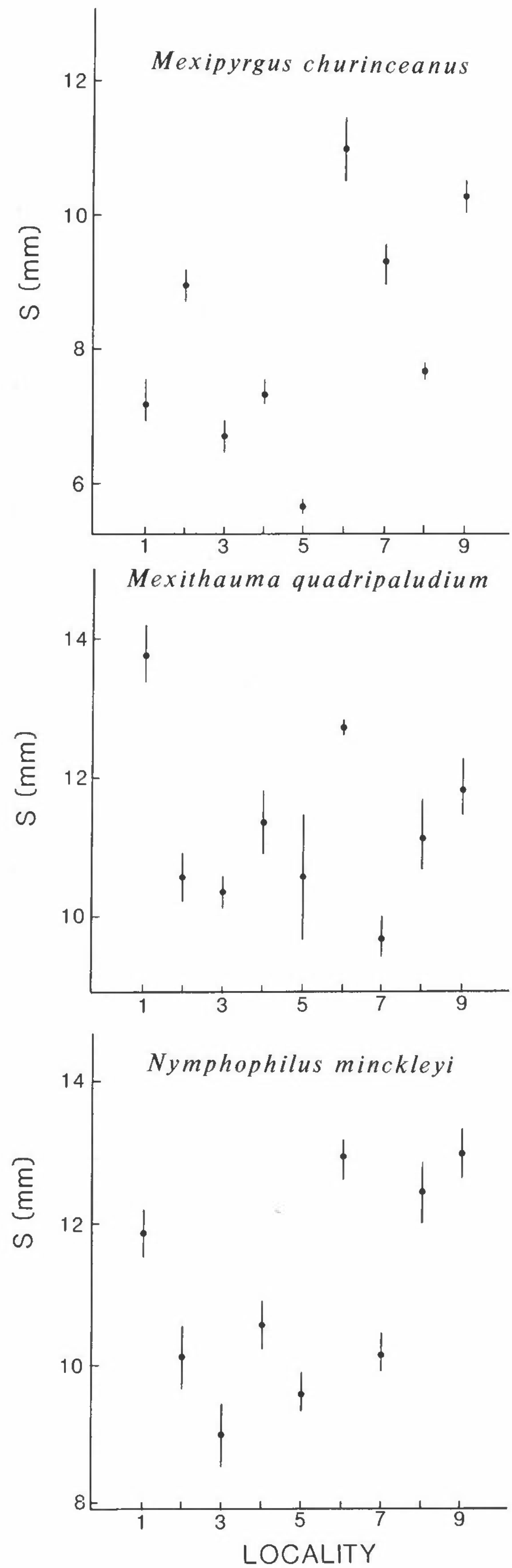
Descriptive summary statistics for each species by locality are in table 1. Results of locality tests for both mean differences and variance are in table 2. Heterogeneity of variance was more pronounced for *Mexipyrgus churinceanus* (significant for six variables at $P < 0.01$ level) than for the other species. Inter-locality variation was marked for each species, and in all but three cases (LBW, *Mexipyrgus churinceanus*; W, D, *Mexithauma quadripaludium*) the hypothesis of mean equality of variables across localities was rejected at 0.05 level. As an example, inter-locality variation in the size-indicator variable, S (= SH + SW), is shown in figure 3, with significant differences ($P < 0.05$) indicated by non-overlapped confidence intervals. Size range for each species is considerable: significant differences (*i.e.*, absence of overlap in figure 3) are especially numerous among populations of *Mexipyrgus churinceanus*, with overlap more prevalent among *N. minckleyi* populations and particularly pronounced for those of *Mexithauma quadripaludium*.

For each of the three species, only three principal components were significant and yielded meaningful information. Eigenstructures show that the first component in each analysis is dominated by size and size-correlated variables (table 3), and explains 39–46% of the total variation. For *Mexipyrgus churinceanus*, two shape variables (T, SA) dominate the second component which explains almost 20% additional variability, and a sixth variable (W) is the sole measure of importance on the third axis, explaining 14%. For the other two species, size (PC1) explains 10% more variation than for *Mexipyrgus churinceanus*, whereas shape (PC2 and 3) explains only slightly less. Weights for shape parameters are spread over both the second and third axes, making interpretations of these more difficult.

Figure 4, consisting of plots of the first three PC's for each species with locality means (centroids) indicated, allows comparison of relative locations of populations in PC space among species. Spread of centroids is largely along PC1, as expected. Each plot has one or two tight clusters of a few centroids, with cluster segregation more

Table 2. Results of Analysis of Variance, or Welch's test; and Bartlett's tests for homogeneity of variance for the nine localities (**: $P < 0.01$; *: $0.01 < P \leq 0.05$; ns: $P > 0.05$).

	Variable									
	SH	SW	S	LBW	WBW	WH	W	D	T	SA
Mexipyrgus churinceanus										
Mean	**	**	**	ns	**	**	**	**	**	**
Variance	ns	*	**	*	*	ns	**	ns	*	ns
Mexithauma quadripaludium										
Mean	**	**	**	**	**	**	ns	ns	**	**
Variance	ns	ns	ns	ns	ns	ns	*	ns	*	ns
Nymphophilus minckleyi										
Mean	**	**	**	**	**	**	**	**	**	**
Variance	ns	ns	ns	ns	ns	*	**	*	ns	ns



obvious for *Mexipyrgus churinceanus* and *N. minckleyi* than for *Mexithauma quadripaludium*.

Results of discriminant analyses on locality are in table 4. Size (S) is the most heavily weighted variable on DF1 for *Mexithauma quadripaludium* and *N. minckleyi*, with LBW weighting negatively; while LBW and S are approximately equally and positively weighted on this function for *Mexipyrgus churinceanus*. As with the principal components analysis, the first discriminant axis explained almost 10% more variation for *Mexipyrgus churinceanus* than for the other two species. Note that the shape parameters W and D were not correlated with any of the functions for any of the species.

Entry of the first variable (S) alone for *Mexipyrgus churinceanus* yielded significant ($P < 0.01$) separation of mean values for all but a single locality pair (1, 4). While entry of four additional variables significantly separated this final pair ($P < 0.05$), the significance level decreased to 0.082 after addition of all remaining variables. Entry of the first variable for *Mexithauma quadripaludium* (WBW) and *N. minckleyi* (S) resulted in significant separation of all but eight and five pairs, respectively; further addition of remaining variables left four and one pairs still unseparated.

Classification error rates for individual specimens indicated considerable overlap of populations, and varied across localities as follows: *Mexipyrgus churinceanus*, 53–100% correct classification [87% (overall)]; *Mexithauma quadripaludium*. 40–87% [62% (overall)]; *N. minckleyi*, 60–100% [84% (overall)]. *Mexithauma quadripaludium* was the poorest classified overall, with less than 60% classification in five of nine localities. Only three discriminant functions were significant for this species ($P < 0.05$), compared to six for the other two. Additional analyses using only shape parameters yielded considerably poorer classification [ranging from 26–39% (overall) for each species], and confirmed the low discriminating power of these variables (see Hershler & Sada, 1987).

Differentiation among drainage systems was also examined. The study area encompasses four local drainages (as recognized by LaBounty, 1974, with localities allocated to these as follows (following notes in Appendix): Becerra System (Locality 1); El Garabatal (2–4); Rio Mesquites System (5–8); and Tio Candido System (9). Clustering of populations in PC-space (figure 4) does not closely follow partitioning of localities into drainages, as indicated by considerable spread of centroids representing localities from El Garabatal (2–4), and from the interconnected springs at Los Remojos (6–8).

For further analysis, localities were re-grouped into drainage systems. A discriminant analysis on each species

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Figure 3. Plots of S (SH + SW) *vs.* locality for each species. Filled circles represent medians; and bars denote simultaneous confidence intervals around the median, constructed so that if parentheses do not overlap, population medians are different at 95% confidence level.

Table 3. Results of Principal Components Analyses on each species. Only factor coefficients having weights > 0.25 are listed. I = *Mexipyrgus churinceanus*; II = *Mexithauma quadripaludium*; III = *Nymphophilus minckleyi*; Eig. = eigenvalue; % V. = % of variance explained.

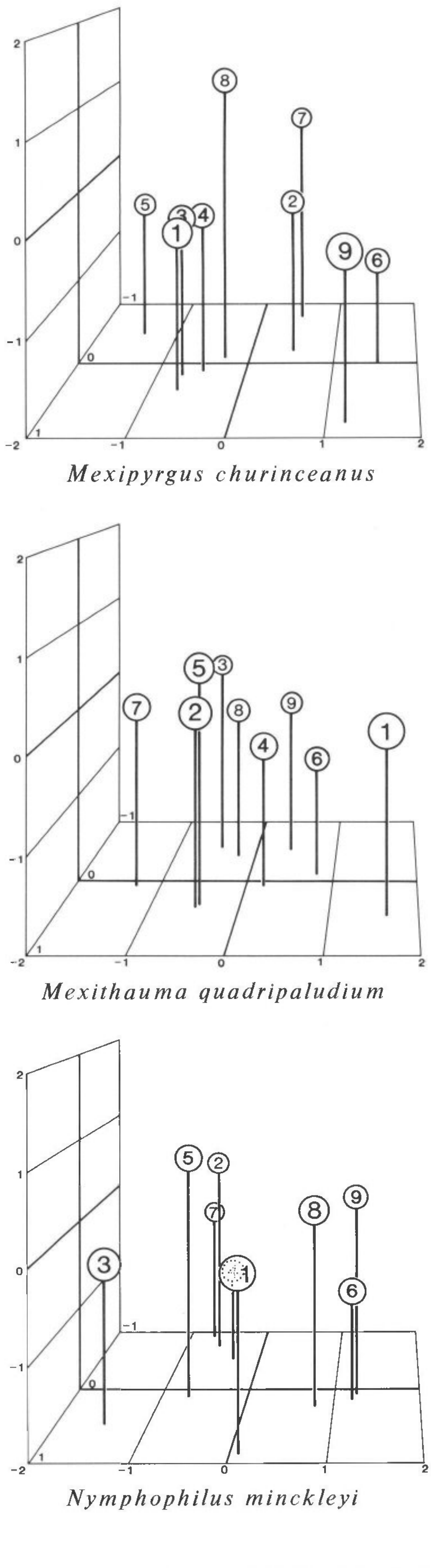
	I			II			III		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
S	0.30			0.30			0.28		
LBW	0.30			0.30			0.27		
WBW	0.29			0.30			0.27		
T		0.55						0.44	−0.45
SA		0.44			0.43	0.59		0.36	0.31
W			−0.77		0.55				0.69
D					0.43	−0.64		0.60	
WH					−0.32	−0.37			−0.32
Eig.	3.22	1.51	1.10	3.19	1.22	1.00	3.49	1.35	1.11
% V.	39.2	19.8	13.8	45.5	17.4	14.4	45.6	16.9	13.9

using all variables was able to separate the four drainage groups only for *Mexipyrgus churinceanus*; only two functions were significant for the other species. Results of these analyses are in table 5. Note the low weights of S on DF1 for *Mexithauma quadripaludium* and *N. minckleyi* compared to these for *Mexipyrgus churinceanus*, and the relatively small amount (*ca.* 40%) of variation explained by the first function for all three species. Classification error rates for individual specimens were similar for the three species: *Mexipyrgus churinceanus*, 61–93% [68% (overall)]; *Mexithauma quadripaludium*, 46–93% [60% (overall)]; *N. minckleyi*, 57–100% [77% (overall)], with highest classification for Drainage 1 (87–100%) and lowest for Drainage 3 (46–61%).

There is similarity of pattern of size variation for the three species: populations having large-sized shells are concentrated in the southeastern portion of the study area (figure 3, Localities 6–9). For standard shell measurements, population means were significantly correlated for *Mexipyrgus churinceanus* and *Nymphophilus minckleyi* ($r > 0.61$, $P < 0.05$ for S, SL, SW, WBW) and *Mexithauma quadripaludium* with *Nymphophilus minckleyi* ($r > 0.60$, $P < 0.05$ for S, SL, SW, WBW, LBW). There were no significant correlations between *Mexipyrgus churinceanus* and *Mexithauma quadripaludium*. Population means for whorl number and shape parameters were not correlated across species with one exception (*Mexithauma quadripaludium* with *Nymphophilus minckleyi*, $r = 0.64$, $P < 0.05$ for W).

Table 4. Results of Discriminant Analyses on locality for each species. Standardized function coefficients (> 0.25) and pooled within group correlation coefficients (> 0.25) of each function with each original variable are listed. I = *Mexipyrgus churinceanus*; II = *Mexithauma quadripaludium*; III = *Nymphophilus minckleyi*; Eig. = eigenvalue; % V. = % of variance explained; C.C. = canonical correlation.

	I			II			III		
	DF1	DF2	DF3	DF1	DF2	DF3	DF1	DF2	DF3
				Standardized coefficients					
S	0.64		0.89	0.94	−0.66		1.51		−1.61
LBW	0.69	0.90	−0.95	−0.56	1.88	2.02	−1.09	−1.43	1.63
WBW	−0.35	−1.13		0.56	−0.94	−2.15	0.35	0.91	
D									
T			0.39			0.65		0.30	
SA							0.34		
WH		0.49	0.61			0.79		0.48	0.77
Eig.	13.3	2.19	0.61	3.13	0.56	0.45	8.95	2.61	0.56
% V.	79.2	13.0	3.6	70.9	12.7	10.1	69.3	20.2	4.3
C.C.	0.96	0.83	0.62	0.87	0.60	0.56	0.95	0.85	0.60
				Correlation coefficients					
S	0.89	−0.38		0.88	0.31		0.84	−0.36	
LBW	0.94			0.74	0.54		0.61	−0.53	0.38
WBW	0.64	−0.65		0.93			0.83		
T		0.34	0.31						
SA					0.46				0.34
WH			0.72			0.77	0.30	0.38	0.78



Despite these correlations, concordance of pattern among plots of PC scores for the three species is not impressive, although there is similarity in order along size-related PC1 (figure 4). Note that the tightest clustered centroids for *Mexipyrgus churinceanus* (Localities 1, 3, 4) are widely separated for the other two species. Similarly, divergent "outlying" centroids for given species (*Mexithauma quadripaludium*, 1; *N. minckleyi*, 3) are not so differentiated in the other species.

As mentioned above, the populations of *Mexipyrgus* considered in this study are referable to four nominal species (*fide* Taylor, 1966) on basis of shell sculpture and color banding: *Mexipyrgus churinceanus* (Localities 1, 2, 4); *Mexipyrgus mojarralis* (5); *Mexipyrgus lugoi* (6–8); and *Mexipyrgus carranzae* (9) (the form present at Locality 3 is distinctive and not clearly referable to any nominal species). These nominal species are poorly segregated on the PC axes: extent of separation of *mojarralis* (5) and *carranzae* (9) from other centroids, for instance, is exceeded by that seen among three populations referable to *churinceanus* in Los Remojos spring complex (6–8).

DISCUSSION

For all three species, most inter-population variation involved shell size and size-correlated variables. Commonality of geographic variation patterns was indicated by significant correlation of population means for some of these variables across two of three species pairs. The extent of this commonality was not, however, impressive when groupings of populations based on multivariate analyses were examined.

A strong correlation between geographic variation patterns and current drainage configurations was not apparent for any of the species, suggesting that in this example development of intraspecific diversity of shell morphometry may be related to ecological as well as historical factors (see Chernoff, 1982, for general discussion of this subject), although we acknowledge the possibility that the poor correlation with current drainage configuration may be obscured by historically complex basin hydrography (Minckley, 1969; Hershler & Minckley, 1985). Springpools concentrated around the northern tip of Sierra de San Marcos (where the study area is located) are highly uniform in water quality (Minckley & Cole, 1968): single measurements taken by us during the study indicated, for instance, that temperature and conductivity ranged among the nine springpools from 25.5–34.5 °C (seven localities differing by < 4 °C) and 1,825–3,500 micromhos/cm (seven localities differing by < 430 micromhos/cm), respectively. Pools do differ con-

←

Figure 4. Three-dimensional plots of PC centroids for the three species (X axis, PC1; Y, PC2; Z, PC3), standardized and viewed in perspective. Sizes of balls indicating centroids are scaled to heighten perspective.

Table 5. Results of four-group Discriminant Analyses on drainage system for each species. Standardized function coefficients (> 0.25) and pooled within group correlation coefficients (> 0.25) of each function with each original variable are listed. I = *Mexipyrgus churinceanus*; II = *Mexithauma quadripaludium*; III = *Nymphophilus minckleyi*; Eig. = eigenvalue; % V. = % of variance explained; C.C. = canonical correlation.

	I			II			III		
	DF1	DF2	DF3	DF1	DF2	DF3	DF1	DF2	DF3
					Standardized coefficients				
S	2.28	0.72	2.39	−0.49	2.11	0.84	0.87	1.76	−2.78
LBW	−2.53	0.71		1.43	−3.12	−0.46	1.41	−1.49	1.35
WBW	1.05	−0.98	−1.87		1.23	−0.91	−1.50		1.71
W									0.61
D	0.52	−0.46	0.66		0.57				−0.67
T		0.55				0.87			
SA	0.38				0.42			0.67	
WH	−0.36		−0.63			0.51		0.53	
Eig.	3.21	1.51	1.10	3.24	1.26	1.03	3.49	1.35	1.11
% V.	40.2	18.8	13.8	40.4	15.8	12.9	43.6	16.9	13.9
C.C.	0.642	0.499	0.431	0.710	0.426	0.262	0.744	0.717	0.217
					Correlation coefficients				
S	0.58	0.55		0.89	0.32		0.49	0.63	
LBW	0.48	0.58		0.96			0.61	0.57	
WBW	0.65	0.36		0.80	0.50		0.83		
W									0.61
D		−0.36	0.59						−0.50
T		0.47		0.40		0.68			−0.33
SA	0.31				−0.32			0.46	
WH		0.44	−0.47		0.31	0.48		0.68	

siderably in other potentially important parameters such as size, substrate composition, and abundance of molluscivorous cichlid fishes and the relationship between these features and shell geographic variation merits further study.

Groupings of populations of *Mexipyrgus churinceanus* on basis of shell size and shape were not strongly concordant with allocation of these to nominal species defined by shell color banding pattern differences. Furthermore, both univariate and multivariate analyses showed that these populations were no more differentiated in terms of shell size and shape than were those of seemingly monomorphic *Nymphophilus minckleyi*. These results suggest that evolution within *Mexipyrgus* has been mosaic, with development of striking diversity in shell color banding patterns and a few other features (including aspects of shell sculpture, and penial lobation pattern) coupled with unremarkable divergence in shell morphology.

It is intriguing that these snails and *N. minckleyi*, which differ greatly in microhabitat and presumed potential for gene flow between populations at spring sources, have similar levels of intraspecific shell morphometric divergence, whereas *Mexithauma quadripaludium*, which broadly overlaps in niche with the latter (Hershler, 1984), is less variable. The possibility that these patterns reflect differing times of origin of lineages within the basin is not currently testable due to absence of fossil evidence.

ACKNOWLEDGEMENTS

Fieldwork was supported by Smithsonian Research Opportunities Fund #1233F650. Assistance in the field was provided by Dr. W. L. Minckley, Dr. P. Marsh, A. Riggs, and C. Riggs. The assistance of staff of the Scanning Electron Microscopy Laboratory at NMNH in photographing shells is gratefully acknowledged. Ms. Molly Kelly Ryan, staff illustrator of the Department of Invertebrate Zoology (NMNH), generously prepared figures on very short notice. Paul Greenhall (NMNH-IZ) performed the laborious task of gathering shell morphometric data. Drs. M. G. Harasewych, R. Dillon, and an anonymous reviewer provided useful criticism of the manuscript.

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APPENDIX

Springpools sampled are numbered as in figure 1. Locality names are either those of Minckley (1969) or reflect local usage. Locality data represent air distances from Cuatro Cienegas. Dates of collection are given in parentheses. Catalog numbers (USNM) for voucher material (dry shell plus alcohol specimens) from each locality are given in following order: *Nymphophilus minckleyi* Taylor, *Mexipyrgus churinceanus* Taylor, and *Mexithauma quadripaludium* Taylor.

1. Poso de la Becerra [(south pool) 3-IX-86], 13.7 km W-SW of Cuatro Cienegas. A once enormous spring area (over a kilometer long) significantly reduced in size by canal development in early 1960's (see Taylor, 1966:162; Minckley 1969: figs. 15, 16). Currently consisting of two large springpools (each *ca.* 50 × 125 m^2) connected by short section of stream. Spring orifices few and large, occurring in deepest (to 7 m) portion of pools. Water lily stands extensive. Spring and outflow constituting a major drainage of basin now feeding canals. USNM 857909, 857918, 857927.

Sites 2–4 represent three springs in the area known as El Garabatal, located north of Poso de la Becerra and east of Becerra's outflow. El Garabatal drains to the north and west, and may be considered either a separate, small drainage or a subset of the Rio Mesquites system, the largest drainage in the basin.

2. Lagunas de Juan Santos (5-IX-86), 12.8 km W-SW of Cuatro Cienegas. Largest of El Garabatal springs consisting of series of relatively shallow (depths to 2.0 m), inter-connected lagunas fed by springs emerging along pool margins. Pool area somewhat larger than that of Poso de la Becerra. Extensive marshes bordering near-entirety of spring's perimeter. Water lily stands extensive. Stream outflow extending several hundred meters before terminating in shallow marshy area. USNM 857912, 857921, 857930.

3. Unnamed small spring, known to biologists working in area as North Spring (3-IX-86), 12.0 km W-SW of Cuatro Cienegas. Small springpool *ca.* 12 × 18 m, about a meter deep, with *ca.* 20% coverage by water lily. Outflow entering second pool (not a spring); discharge from latter extending 50 m before disappearing into hole. USNM 857910, 857919, 857928.

4. Unnamed large spring (5-IX-86), 11.8 km W-SW of Cuatro Cienegas. Roughly circular springpool, *ca.* 70 m across; depths not exceeding 3.5 m. Water lily dense in center of pool. Outflow feeding small marsh. USNM 857916, 857925, 857934.

5. "West" Laguna in El Mojarral (4-IV-86), 9.0 km SW of Cuatro Cienegas. Moderate-sized spring (26 × 59 m; Arnold, 1972:12), with depths to 4.5 m. Orifices few in number, cavernous. Water lily stands few, relatively thin. Water exiting spring via both a shallow, surficial stream and single, tubular, subsurface vent. Stream outflow entering "East" Laguna, which in turn drains into Rio Mesquites. USNM 857908, 857917, 857926.

Sites 6–8 are inter-connected springpools known locally as Los Remojos, 9.0 km S-SW of Cuatro Cienegas. Site 6 drains into a large pool receiving discharge from a second pool fed by outflows from Sites 7 and 8. System draining into Rio Mesquites.

6. Northernmost of Los Remojos springs (5-IX-86). Pool *ca.* 18 × 32 m. Depths generally > 1.5 m; water lily common. USNM 857913, 857922, 857931.

7. Intermediate Los Remojos spring (5-IX-86). Pool *ca.* 28 × 47 m, shallow (< 1.0 m). Water lily uncommon. USNM 857914, 857923, 857932.

8. Southernmost of Los Remojos springs (5-IX-86). Pool moderately large (22 × 42 m), with depths increasing in southern end of pool to 4.0 m. Water lily common. USNM 857915, 857924, 857933.

9. Laguna Tio Candido (5-IX-86), 12.5 km S-SW of Cuatro Cienegas. Large spring (*ca.* 45 × 100 m) extensively vegetated by water lily and other macrophytes. Depths generally from 2.0–4.0 m. Outflow extending eastward, comprising part of a major system positioned south of Rio Mesquites drainage. USNM 857911, 857920, 857929.