

Elimia Comalensis (Gastropoda: Pleuroceridae) from the Edwards Plateau, Texas: Multiple Unrecognized Endemics or Native Exotic?

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***Elimia comalensis* (Gastropoda: Pleuroceridae) from the Edwards Plateau, Texas: Multiple Unrecognized Endemics or Native Exotic?**

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ABSTRACT.—This research explored the distribution, morphological variation, and molecular systematics of *Elimia comalensis* (Gastropoda: Pleuroceridae) using geometric morphometrics ($n = 565$) and mitochondrial DNA sequences ($n = 15$). *Elimia comalensis* was originally documented as endemic to Comal Springs, Comal County, Texas, but recent collections found this species in multiple springs and drainages in Central Texas. Morphometric analyses showed a high amount of morphological overlap with no clear geographic patterning. Phylogenetic analysis of mt COI sequence data indicated that *E. comalensis* represents a single species, with no genetic divergence among isolated populations. We conclude that *E. comalensis* may be an unrecognized native exotic, a species endemic to one area that has been spread by humans and assumed to be part of the natural fauna.

INTRODUCTION

Elimia is the largest and most widely distributed North American genus in Pleuroceridae, a family of gill-breathing, operculate, freshwater snails (Turgeon *et al.*, 1998). *Elimia* species occur across the eastern United States, with known limits of the Edwards Plateau in Texas to the west, the Florida panhandle to the south and southern Canada to the north (Burch and Tottenham, 1980). Of the 156 recognized pleurocerid species *Elimia* accounts for over half (Turgeon *et al.*, 1998). This research explored the distribution, morphological variation and molecular systematics of one *Elimia* species, *E. comalensis* using geometric morphometrics and mitochondrial DNA sequences.

Snails in the genus *Elimia* are important components of freshwater ecosystems in the eastern United States (Newbold *et al.*, 1983; Richardson *et al.*, 1988). In the southeastern U.S., where *Elimia* diversity is the highest (Burch, 1982; Lydeard and Mayden, 1995), *Elimia* may comprise more than 90% of the total macroinvertebrate biomass (Newbold *et al.*, 1983; Richardson *et al.*, 1988). In addition to representing a considerable portion of biomass, *Elimia* may have a beta-richness rivaling most aquatic insect genera in the same region; (Merritt and Cummins, 1995) as well as serving a primary role in structuring communities and nutrient flow. In spite of the important ecological impacts of *Elimia*, the literature pertaining to life history characteristics is surprisingly sparse. Those papers addressing life history features of the genus are almost exclusively limited to examinations of Mobile River basin and Atlantic slope fauna (*e.g.*, Dillon and Davis, 1980; Dillon, 1984a; Huryn *et al.*, 1994). Therefore, large information gaps exist in the literature for the remainder of the *Elimia* species.

This lack of understanding concerning basic biological features of *Elimia* is alarming considering the genus-wide decline in species richness and distribution. Like many

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freshwater faunas globally (Lydeard *et al.*, 2004), pleurocerid snails including *Elimia* are declining due to human impacts including habitat modification, degradation, and destruction. The impacts of this loss have been studied extensively in the Mobile River basin, where pleurocerids historically reached their highest diversity and where the largest number of extinctions has occurred (Neves *et al.*, 1997). Although *Elimia* is highly diverse, this genus is understudied, and the conservation status of most *Elimia* species remains unknown. In addition, basic taxonomic and life history data remain scarce, though recent studies have started illuminating the evolutionary relationships within and among these species (*e.g.*, Lydeard *et al.*, 1997, 1998; Dillon and Frankis, 2004). The gathering of natural history and evolutionary information is a necessary scientific foundation for any plans to conserve and preserve this fauna.

The Balcones *Elimia*, *Elimia comalensis*, exemplifies the small body of knowledge typical of most species in the genus. *Elimia comalensis* was described by Pilsbry (1890) from Comal Creek, New Braunfels, Texas as the smooth form of *Goniobasis* (= *Elimia*) *pleuristriata*. Pilsbry and Ferris (1906) provided a more complete description of the species and illustrated its highly variable shell morphology, including figures of the sculptured form previously considered *G. pleuristriata*. They described the distribution of the species as the system of short rivers intercalated between the Colorado and Nueces river basins comprising the Guadalupe and San Antonio Rivers and their tributaries along the edge of the Edwards Plateau. Recent museum collections confirm this distribution, but expand the range to include springs in the Colorado, Brazos and Nueces basins, and large spring populations in Del Rio at the southwestern corner of the plateau in the Rio Grande drainage (Fig. 1). *Elimia comalensis* occurs in springs and spring-run streams associated with the Edwards Plateau. The current distribution of *E. comalensis* spans from Salado Creek, Bell County, TX to the westernmost limit of the Edwards Plateau in Del Rio, Val Verde Co, TX on the Mexico-Texas boarder. All are fed by the Edwards Aquifer with the exception of Anson Springs in Schleicher County, which are part of the middle Colorado-Concho River drainage. Like many freshwater organisms in Texas, *E. comalensis* may be experiencing declines due to water extraction (Contreras-Balderas and Lozano-Vilano, 1994), habitat modification, and introduced species (Britton, 1991; Howells, 2001). *Elimia comalensis* is under additional pressure because it occurs in areas used for municipal water supplies and recreation (McCarl *et al.*, 1993; McKinney and Watkins, 1993; McKinney and Sharp, 1995).

Literature pertaining to *Elimia comalensis* is limited to identification manuals (*e.g.*, Cheatum and Fullington, 1971; Burch and Tottenham, 1980) species lists (Strecker, 1935; Turgeon *et al.*, 1998), larval trematode investigations (Lindholm and Huffman, 1979; Tolley-Jordan and Owen, 2005) and government and agency reports (*e.g.*, Britton, 1991). Only one publication has directly addressed *E. comalensis*, demonstrating that individuals in pool and stream regions of Comal Springs exhibit different size distributions (Cheatum and Mouzon, 1934). In terms of conservation status, *E. comalensis* is ranked H (possibly extirpated) on the state, national, and global heritage rank scales (NatureServe, 2005). The species appears restricted to the Edwards Plateau, the largest portion of the Balconian biotic province (Blair, 1951) and an area of high endemism containing many threatened taxa (Diamond *et al.*, 1997). A survey of plant and animal taxa with global ranks of G1 through G3 highlighted the Balconian region as an area of importance for rare species (Diamond *et al.*, 1997), and Neck (1986) documented the region's unique vegetative and vertebrate faunas.

In an effort to increase our knowledge of *Elimia comalensis* and of *Elimia* in general, we explored the distribution, morphological variation and molecular systematics of the species using museum collections, geometric morphometrics and mitochondrial DNA sequences.

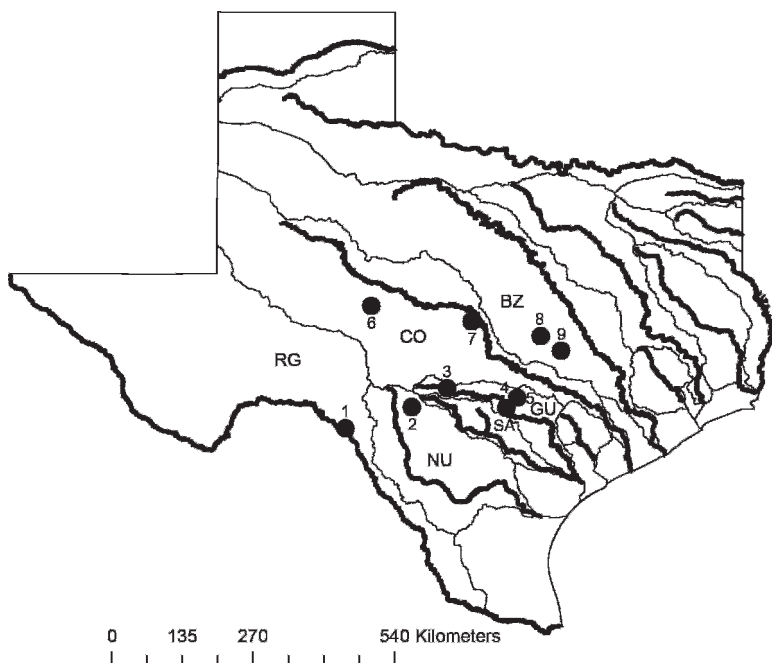


FIG. 1.—Map of Texas showing river drainages and localities where *Elimia comalensis* has been found. Drainages: BZ, Brazos; CO, Colorado; GU, Guadalupe; NU, Nueces; RG, Rio Grande; SA, San Antonio. Localities: C, Comal; D, Del Rio; F, Falls Creek; G, Guadalupe; L, Leakey; M, San Marcos; S, Salado, A, Anson Springs

Based on the current understanding of speciation and distribution in pleurocerids, two hypotheses were formulated to explain what is currently known regarding *E. comalensis*. The first hypothesis stated that *E. comalensis* is comprised of multiple unrecognized species, each occupying springs in single drainage basins. The drainage specificity of pleurocerids and spring endemic gastropods lead us to believe this was the most likely scenario. Minton and Lydeard (2003) showed that species in the pleurocerid genus *Lithasia* grouped by river drainage using gene sequence data, and Thompson (2000) showed that *Elimia* follow similar patterns. Spring-snails in the family Hydrobiidae are also well known for being endemic in springs in the U.S. and Australia (Thompson, 2000; Liu *et al.*, 2003; Perez *et al.*, 2005). If *E. comalensis* follows similar patterns, we predicted that morphological and molecular data would divide *E. comalensis* into reciprocally monophyletic, exclusive groups showing geographic structuring by spring and drainage system.

A second and competing hypothesis stated that *Elimia comalensis* represents a single widespread species occupying springs in disjunct basins. In this scenario we predicted that *E. comalensis* populations would show none or some degree of molecular geographic structure although with non-exclusive populations and non-diagnostic morphological patterns of variation. A pattern of high genetic variation without formation of exclusive lineages would be similar to that observed in *E. proxima* across drainages in the Appalachians (Dillon, 1984b). The explanation of no genetic diversity is confounded by the need to explain the distribution of populations across unconnected basins and the inability of Pilsbry and Ferris (1906) to find it in San Felipe Springs in Del Rio, Texas in the early twentieth century.

METHODS

A total of 565 individuals were used in the morphological analysis (Table 1). Digital photographs of individual shells were taken with a tripod-mounted, KODAK EasyShare CX6330 Digital Camera. Shells were leveled in clay to make the longitudinal axis of the shell parallel to the table surface to assure accuracy in length measurements, and a ruler was placed in each picture to provide a scale measurement. Input files for tpsDig were generated from the digital picture filenames using tpsUtil 1.10 (Rohlf, 2003). Seven homologous landmarks (Fig. 2) along with two scale landmarks located on the ruler were digitized using tpsDig 1.31 (Rohlf, 2003). Although much debate exists on the topic of selection of landmarks for gastropod shells (*e.g.*, Johnston *et al.*, 1991; Stone, 1998), landmarks were chosen in this study for their repeatability among individuals. The apex of the shell was not used as a landmark as many individuals had heavily eroded apices.

Geometric morphometrics were used to examine shape variation through principal components analysis (PCA) and canonical variance analysis (CVA). PCA is a technique for simplifying descriptions of variation among individuals while CVA simplifies descriptions of differences between groups (Zelditch *et al.*, 2004). In traditional morphometric analysis, PCA generally suffers from the overwhelming influence of size across the newly generated axes. However, in geometric morphometric analysis, all individuals are rescaled to be the same size thus eliminating size as a factor, with the result that shape can be more thoroughly examined. In PCA, no *a priori* assumptions are needed to group individuals, in contrast, CVA determines the set of axes which best discriminates between groups; therefore an *a priori* assumption of group membership is necessary. For CVA analysis, individuals were examined grouped by locality as well as by drainage, since pleurocerids generally tend to be unique by drainage (Chambers, 1978, 1980, 1990; Dillon and Davis, 1980; Stiven and Kreiser, 1995; Thompson, 2000).

All data analyses were performed using the IMP Suite (Sheets, 2003). The landmark coordinates from tpsDig were imported into CoordGen6f. CoordGen was then used to convert landmark coordinates into Procrustes distances using least squares Procrustes superimposition methods. Procrustes superimposition methods are generally favored over other superimposition methods such as Bookstein coordinates or sliding baseline registration because landmarks are not constrained to a baseline that may transfer variance from the baseline to other landmarks (Zelditch *et al.*, 2004) and because generalized Procrustes methods have been shown to produce estimates with the least error and no pattern of bias (Rohlf, 2003). One of the suture points from the second body whorl posteriorly and the anterior end of the aperture were used as baseline coordinates (Fig. 2). Initial comparisons between all population combinations were performed in TwoGroup6c to determine if there were significant shape differences assessed by Goodall's F. The significance level was adjusted using a Bonferroni correction for multiple pairwise comparisons. A PCA was performed with PCAGen6g on the data with *a posteriori* groups assigned by locality. A CVA was also generated using CVAGen6h with groups assigned by locality or drainage.

Live specimens of *Elimia comalensis* for use in molecular studies were collected by hand and stored in 95% ethanol. Voucher specimens are housed at the Museum of Natural History at the University of Louisiana at Monroe. DNA extraction followed a modified CTAB-proteinase K method (Sagahi-Marooof *et al.*, 1984) with chloroform extraction using a small piece of tissue from the distal end of the head of the specimen. Extracted DNA was stored at 4° C for subsequent amplification. Genomic DNA served as a template for PCR amplification of a portion of the mitochondrial cytochrome oxidase *c* subunit I (COI) gene

TABLE 1.—Systematic list of taxa used in the molecular and morphometric study. Specimens are deposited at the Florida Museum of Natural History (FLMNH) and University of Louisiana at Monroe Museum of Natural History (ULM; uncataloged but available from RLM). Each unique locality also has a letter identifier. Asterisks (*) indicate that all museum specimens for that locality were included in the morphometric analyses

Species	Locality	n (DNA)	n (morphometrics)	Accession No.
<i>Elimia</i>				
<i>E. comalensis</i>	Salado Creek at Interstate 35, Salado, Bell Co., TX (S)	3	42	ULM
	Comal Creek, New Braunfels, Comal Co., TX (C)	3		ULM
	Comal Creek, New Braunfels, Comal Co., TX	-	926*	FLMNH 74061
	Comal Creek, New Braunfels, Comal Co., TX	-		FLMNH 74062
	Comal Creek, New Braunfels, Comal Co., TX	-		FLMNH 74063
	Comal Creek, New Braunfels, Comal Co., TX	-		FLMNH 74064
	Comal Creek, New Braunfels, Comal Co., TX	-		FLMNH 74065
	Comal Springs Park, New Braunfels, Comal Co., TX	-		FLMNH 258418
	Comal Creek, New Braunfels, Comal Co., TX	-		FLMNH 283580
	San Marcos River, San Marcos, Hays Co., TX (M)	3		ULM
	San Marcos River Park, San Marcos, Hays Co., TX	-	33*	FLMNH 20002
	San Marcos River, San Marcos, Hays Co., TX	-		FLMNH 74058
	San Marcos River, San Marcos, Hays Co., TX	-		FLMNH 74059
	San Marcos Spring, San Marcos, Hays Co., TX	-		FLMNH 232139
	San Marcos River, San Marcos, Hays Co., TX	-		FLMNH 267951
	South Fork Guadalupe River, Ingram, Kerr Co., TX (G)	-	28	FLMNH 283553
	Leakey Springs Creek, Leakey, Real Co., TX (L)	-	66	FLMNH 283557
	Leakey Springs Creek, Leakey, Real Co., TX	3		ULM
	Falls Creek, San Saba, San Saba Co., TX (F)	-	10	FLMNH 74060
	San Felipe Springs, Del Rio, Val Verde Co., TX (D)	-	160*	FLMNH 232134
	San Felipe Springs, Del Rio, Val Verde Co., TX	-		FLMNH 232135
	San Felipe Springs, Del Rio, Val Verde Co., TX	-		FLMNH 283962
	San Felipe Springs, Del Rio, Val Verde Co., TX	3		ULM
	Anson Springs, Schleicher Co., TX (A)	-	-	ULM
	San Gabriel, Milam Co., TX (B) collection only	-		

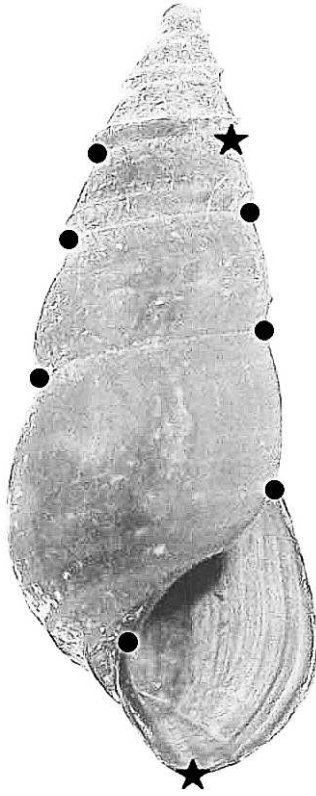


FIG. 2.—Landmarks used in morphological analyses. Landmarks indicated by stars were used as the baseline

using the methods of Minton and Lydeard (2003). The COI PCR primers used for this study were GASCOIH and GASCOIL (Minton and Lydeard, 2003) which amplify an approximately 1 kb gene fragment. The amplification regime included an initial denaturation (92° C for 120 s), 30 cycles of denaturation (92° C for 40 s), annealing of the primers (40° C for 40 s), and primer extension (72° C for 90 s), and a final extension (72° C for 120 s). After the first five cycles, the annealing temperature was raised to 50° C. COI amplicons were purified using a Qiagen Qiaquick PCR purification kit and cycle sequenced using ABI BigDye 3.1 chemistry. Sequences were analyzed on an ABI 3100 Genetic Analyzer.

Sequences were assembled in BioEdit 5.0.9 (Hall, 1999) and aligned by eye to existing COI sequences from Minton and Lydeard (2003; Appendix B) along with other *Elimia* sequences generated for the project. Outgroup taxa are available on Genbank: *E. hydei*, F435775; *E. laqueata*, DQ464059; *E. cf. obovata*, AF435759, AF435760; *E. proxima*, DQ868389; *E. virginica*, DQ464058; *Io fluviatilis*, AF435776, AF 435777, AF435778; *Leptoxis crassa anthonyi*, AF435772, AF435773, AF435774; *L. praerosa*, AF435779, AF435780, AF435781, AF435782; *Lithasia armigera*, AF435739, AF435740, AF435741, AF435742, AF435743; *Li. duttoniana*, AF435744, AF435745; *Li. geniculata geniculata*, AF435755; *Li. g. fuliginosa*, AF435747, AF435748, AF435749, AF435750, AF435751, AF435752, AF435753, AF435754; *Li. g. pinguis*, AF435763, AF435764; *Li. jayana*, AF435756; *Li. lima*, AF435757, AF435758; *Li. salebrosa*

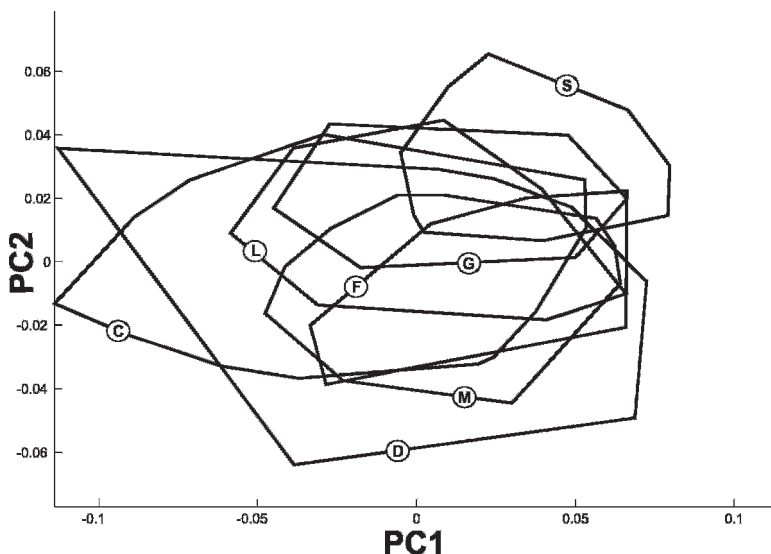


FIG. 3.—Polygon plot of PC2 versus PC1. Localities: C, Comal; D, Del Rio; F, Falls Creek; G, Guadalupe; L, Leakey; M, San Marcos; S, Salado

salebrosa, AF435765; *Li. s. florentiana*, AF435746; *Li. verrucosa*, AF435766, AF435767, AF435768, AF435769, AF435770, AF435771; *Pleurocera canaliculatum*, AF435783; *P. prasina-tum*, AF435784; *Pleurocera* sp., EF116551; *P. walkeri*, AF435785. Specific locality information for nonfocal taxa is contained in each Genbank record and in Minton and Lydeard (2003). Minton and Lydeard (2003) showed no base composition bias or saturation using this fragment in a previous, more inclusive analysis of pleurocerid species, so no modifications were made in the analyses. The aligned data matrix was analyzed under maximum parsimony in NONA 2.0 (Goloboff, 1998) with the following settings: keep 1000 total trees, run 100 replicates of TBR branch swapping, hold 10 trees per replicate, followed by one additional round of TBR branch swapping on all shortest trees (h1000;h/10;mult*100;max*). To test the internal stability of the data, jackknife support (1000 replicates, 37% deletion; Farris *et al.*, 1996) and Bremer decay (Bremer, 1994) values were generated in T.N.T. (Goloboff *et al.*, 2001).

RESULTS

Pair-wise comparisons of all populations assessed by Goodall's F test showed that snails from each locality had significantly different ($p < 0.01$) shapes. In the PCA, the first axis (PC1) explained 47% of the variation and the second (PC2) explained 14% of the total variation. There was no clear separation of populations within the PCA, as all populations overlapped with at least one other (Fig. 3). CVA of each population yielded five distinct axes ($p < 0.05$) where all means were significantly different from each other, although populations overlapped in all scatterplots (Fig. 4). When grouped by drainage, a jackknifed classification matrix based on CVA correctly placed individuals in their corresponding groups 75% of the time for the Guadalupe drainage, 86% for the Nueces, 75% for the Rio Grande, 86% for the Colorado and 86% for the Brazos.

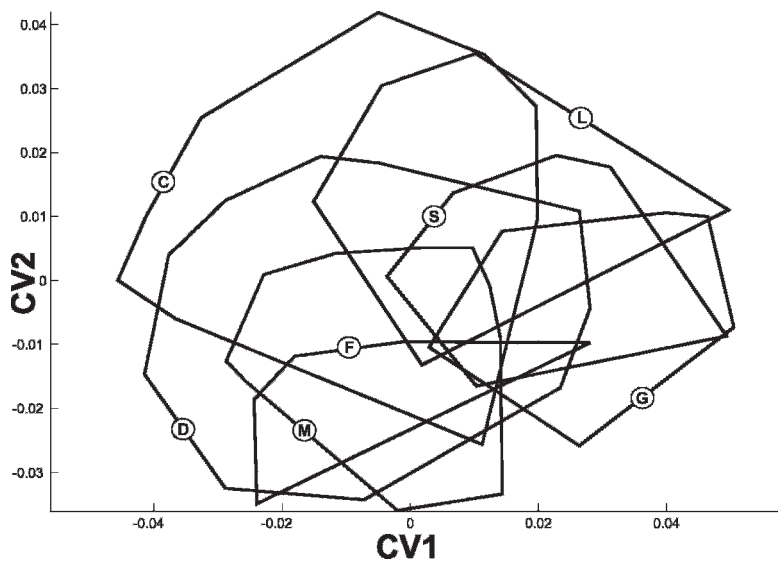


FIG. 4.—Polygon plot of CV2 versus CV1. Localities: C, Comal; D, Del Rio; F, Falls Creek; G, Guadalupe; L, Leakey; M, San Marcos; S, Salado

A total of 15 specimens, three from each of five populations, were sequenced. Additional individuals and those from the remaining populations collected failed to amplify. Four haplotypes were found across all populations, and no haplotype was unique to any single population (Table 2). The only differences among haplotypes were third codon position changes at two sites. Maximum parsimony analysis yielded 30 most parsimonious trees (969 steps: CI = 0.46; Fig. 5). All specimens of *Elimia comalensis* formed an unresolved monophyletic group in the strict consensus tree, sister to the single Mobile Basin representative of the genus included in the analysis, *E. hydei*. Jackknife and Bremer values

TABLE 2.—Geographic distribution and differences between the four COI haplotypes in *Elimia comalensis*. Nucleotide positions are based on the aligned data matrix of 890 positions. Genbank numbers for haplotypes: A = DQ868390, B = DQ868391, C = DQ927300, D = DQ927301

	Haplotype			
	A	B	C	D
Comal	X		X	X
Del Rio	X	X		X
Leakey	X	X		X
Salado	X	X	X	
San Marcos	X	X	X	

Haplotype	Pos. 790	Pos. 855
A	C	T
B	T	T
C	C	G
D	T	G

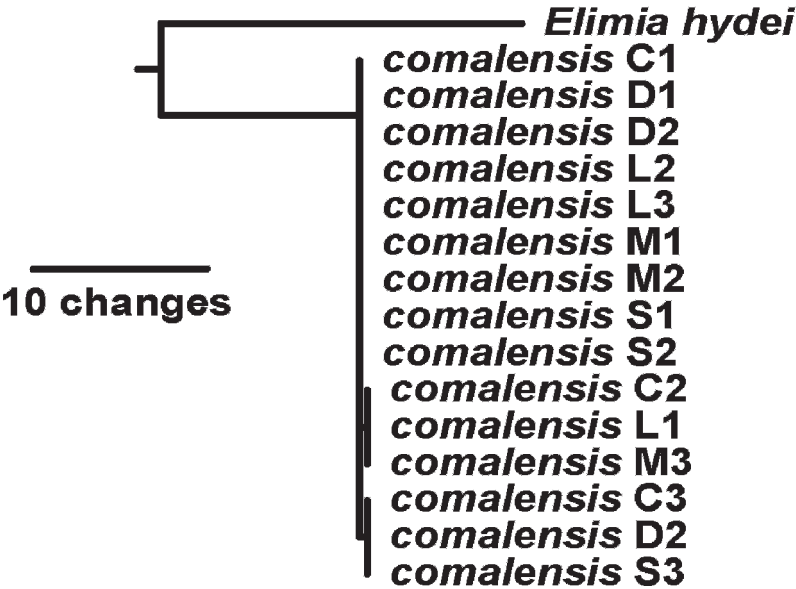
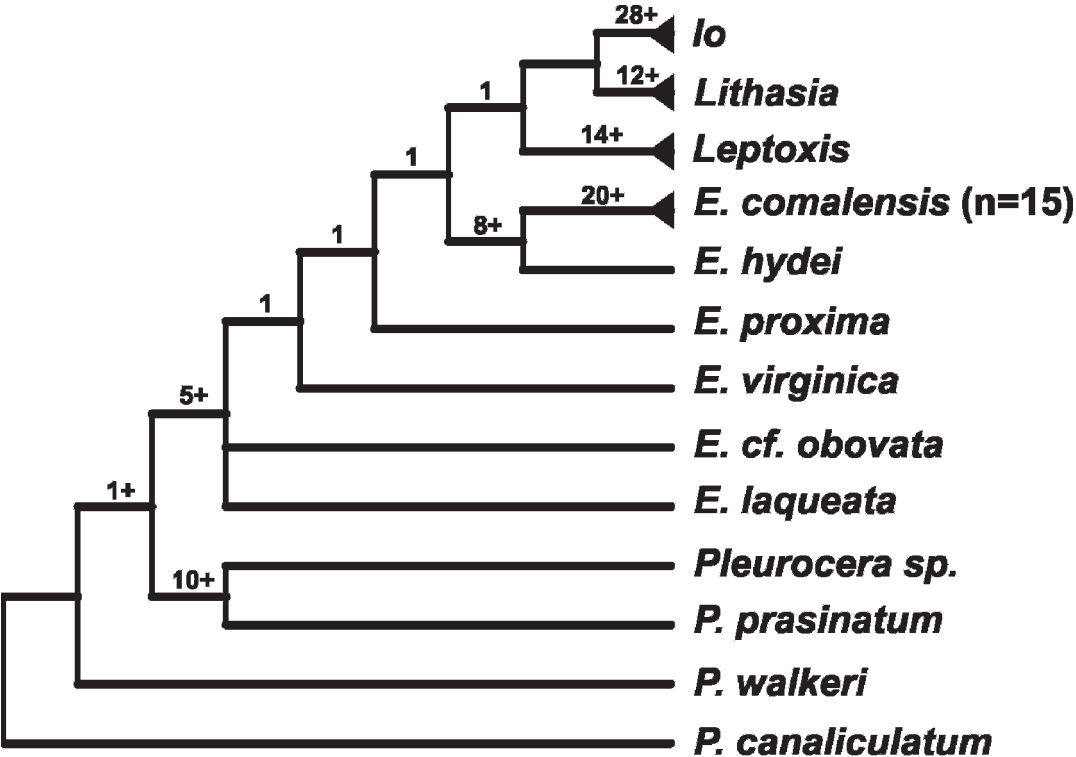
supported the monophyly of the *E. comalensis* specimens and the sister group relationship to *E. hydei* representing the Mobile Basin taxa.

DISCUSSION

Based on distributional, morphological and molecular data, our data support the hypothesis that *Elimia comalensis* is a single species with a puzzling distribution. This distribution is restricted to Central Texas spring-run streams in six distinct drainage basins: the Brazos, Colorado, Guadalupe, Nueces, San Antonio and Rio Grande. All are found associated with the Balconian Physiographic province, except for the Rio Grande which forms a separate hydrological unit. The flora and fauna of the Balconian region tend to be endemic; therefore it was plausible to expect that *E. comalensis* in the Balconian drainage basins may represent a different species than those occurring outside of it. *Elimia comalensis* populations in San Felipe Springs (Del Rio) are even more interesting because of the possibility they were not present in the early twentieth century. Pilsbry and Ferris (1906:165) "...saw no sign of *Goniobasis* in the tributaries of the Rio Grande, where our collecting was extensive enough to have found it if it existed." Based on these observations, our initial expectation was that *E. comalensis* would be comprised of multiple unrecognized species each occupying springs in single drainage basins.

Morphometric analyses showed a high amount of overlap with no clear geographic patterning. Although the PCA and CVA show a great deal of overlap over all localities, however this overlap is not complete; when viewed in pairs, some drainage populations show a moderate amount of morphological separation. Separation of the Brazos and Rio Grande populations is particularly interesting because these populations are at the extreme eastern and western ends of the range for *Elimia comalensis*. Comal and Del Rio populations exhibited a much greater amount of variation than other populations, though this is possibly an artifact of unequal sample sizes. Del Rio and Comal Springs populations are better represented in museum holdings, and sample sizes from those populations are larger than the other populations. The ability of the morphometrics to distinguish drainages seems to be at odds with the lack of genetic variability. It is possible that the morphometric analysis is confounded by the unequal samples sizes or small sample sizes of some population samples. However, phenotypic plasticity in shell form is common in freshwater mollusks (Ortmann, 1920), and especially in pleurocerids (Rosewater, 1960; Dillon, 1984a; Stein and Stansbery, 1984), so differences between populations were not unexpected and may reflect slight differences in shape due to unique local environments. There are some drainage level differences in shape of shells, but this shape difference is typically not pronounced, not found in all individuals of each population, and not confined to discrete landmarks. This evidence, combined with the lack of fixed genetic differences, indicates that these do not merit taxonomic subdivision.

Phylogenetic analysis of COI data indicated that *Elimia comalensis* represents a single species, with no genetic divergence among widely separated populations. This outcome is unusual given that *E. comalensis* is a spring endemic and has a discontinuous distribution. This leaves the species' puzzling distribution unexplained. Even if *E. comalensis* formed a single species, we would expect to find some evidence of geographic structuring in these isolated spring populations. A lack of genetic variation has several potential explanations: balancing selection acting on COI haplotypes; vicariance due to historical connections among drainages; natural, recent gene flow in sufficient quantity to effectively have panmictic populations over all of central Texas; the genetic marker used is not sufficiently variable to assess divergence; or recent human-mediated introductions. A selective sweep of



an advantageous COI haplotype across Texas seems unlikely and would assume high levels of gene flow in the past. Vicariance has been convincingly demonstrated in other arid-land spring snail taxa (Hershler and Sada, 2002; Perez *et al.*, 2005), with current distributions reflecting fragmentation of large Pleistocene lakes; however the history of the Edwards Plateau seems to preclude a vicariance explanation. In the Cretaceous the Edwards Plateau region was covered by an oceanic environment. In the early Cenozoic (the Tertiary) the sea retreated, establishing the current drainage systems (draining toward the Southeast). This seems to effectively rule out vicariance as a primary process in the observed haplotype distributions across these springs. Natural gene flow across unconnected freshwater systems seems highly unlikely, given that freshwater snails are poor dispersers, and many spring snails are endemic to only a few hydrologically related springs. The genetic marker used, a portion of COI, has shown sufficient variability to separate different populations of pleurocerids across genera (Minton and Lydeard, 2003; Sides, 2005). Therefore, human-mediated introductions remains as the most plausible and parsimonious explanation for the observed patterns.

One possible explanation for the widespread distribution of *Elimia comalensis* is that individuals may have been accidentally introduced to new springs. Mosquitofish (*Gambusia* sp.) introductions across Texas have been ongoing since the early 1900's in an effort to combat mosquito populations and many of the *Gambusia* stocks were taken from Comal Springs (R. Howells, Texas Parks and Wildlife, pers. comm.), the type locality for *E. comalensis*. It is possible that reproductively viable *E. comalensis* or their eggs were inadvertently taken from Comal Springs during collection of *Gambusia* and introduced elsewhere. This scenario explains why there was no genetic differentiation of populations as well as why there was little to no discernible pattern of shell variation, as there has not been sufficient time for population differentiation. Additionally, an ecologically similar snail *Melanoides tuberculata* has been spread through human agency recently through many of the same spring systems, lending further circumstantial evidence to the likelihood of recent human spread of *E. comalensis*.

Since *Elimia comalensis* has likely been spread to springs where it did not originally occur, it could be impacting those local ecosystems. At Comal Springs, *E. comalensis* reaches densities up to roughly 250 per square meter (authors' unpublished data), a density considered "high" for many pleurocerids (Houp, 1970; Stewart and Garcia, 2002). Pleurocerids are known to have major impacts on the invertebrate and algal communities. For example, pleurocerids in high densities can reduce productivity and biomass of grazing-intolerant algal species (Hill *et al.*, 1995; Rosemond *et al.*, 2000), and can have similar impacts on invertebrate taxa by outcompeting them for food resources or physically displacing them from the substrate (Hawkins and Furnish, 1987; Hill, 1992). *Elimia comalensis* also serves as a host for trematode species that could potentially infect native species (Lindholm and Huffman 1979; Tolley-Jordan and Owen 2005). In these ways, *E. comalensis* may act as an unrecognized native exotic, a species endemic to one area, in this case Comal Springs, that has been spread across Texas and assumed to be part of the natural fauna. These introductions appear to be recent; introductions into San Felipe Springs in Del

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FIG. 5.—Top: strict consensus of 30 most parsimonious trees. All *Elimia comalensis* formed a single, unresolved clade sister to *E. hydei*. Numbers on branches are Bremer support; those with a (+) indicate jackknife values >63%. Bottom: partial phylogram from one of the 30 trees showing the *E. comalensis* – *E. hydei* relationship. Localities: C, Comal; D, Del Rio; L, Leakey; M, San Marcos; S, Salado

Rio have likely occurred in the last century, and snails were first found in Anson Springs in the last few decades (N. Strenth, Angelo State University, pers. comm.). This is particularly troublesome at San Felipe Springs where three state-listed endangered fish species occur. One species, *Dionda diaboli*, is an obligate algivore and would likely directly compete with *E. comalensis* for food (López-Fernández and Winemiller, 2005). Much more information and effort has been directed toward the invasive alien species *Melanoides tuberculata*, first recorded as being in Texas in 1964 (Murray, 1964) and becoming established in some of the same systems as *E. comalensis* in the following decades (Dundee, 1974; Howells, 1992). *M. tuberculata* is similar to *E. comalensis* ecologically and many of the same threats and impacts documented for *M. tuberculata* may apply to *E. comalensis* where it occurs as an exotic. Both species must be treated as exotic when found outside their natural range and monitored for their impacts on native ecosystems.

Although its range appears to be enhanced by humans, *Elimia comalensis* poses a conservation and management conundrum. It remains a species that is limited in distribution to highly human impacted isolated sites. In portions of those sites the species appears to be non-native, and is certainly having undetermined impacts on the native flora and fauna. *E. comalensis* was originally described from Comal Springs, a diverse and endangered spring system. Comal Springs is unique because it is one of the largest spring systems in the southwestern US and is considered one of the most diverse spring ecosystems in the world; its diversity is largely comprised of endemic species. Comal Springs and nearby San Marcos Springs shelter seven federally endangered species and several additional endangered species (Votteler, 1998) are found in the Edwards aquifer, the source of the springs. Human activities impacting spring-fed and lotic environments favored by *E. comalensis* may put the species at risk. Water flow in Comal Springs is dependent upon water use practices in the Edwards Aquifer, which has been the subject of lengthy, acrimonious and litigious debate. Major human impact factors that affect Comal Springs and have been shown to imperil and extirpate gastropods in other areas of the United States are the following: residential, urban and industrial development (Palmer, 1986); recreational water use (USFWS, 2002); pesticide runoff (USFWS 2000); and human modification of the river systems through damming (USFWS, 1994; Bogan *et al.*, 1995). All of these impact factors are present in the local environment of *E. comalensis*, yet their effects on the species remain unstudied. By studying the life history, systematics, and distribution of *E. comalensis* and other spring-dependent endemic species, we are providing the baseline data necessary to convince stakeholders, managers, and legislators of the importance of managing water resources for habitat preservation.

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