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HISTORICAL BIOGEOGRAPHY OF THE LIVEBEARING FISH GENUS *POECILIOPSIS* (POECILIIDAE: CYPRINODONTIFORMES)

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Abstract.—To assess the historical biogeography of freshwater topminnows in the genus *Poeciliopsis*, we examined sequence variation in two mitochondrial genes, cytochrome *b* (1140 bp) and NADH subunit 2 (1047 bp). This widespread fish genus is distributed from Arizona to western Colombia, and nearly half of its 21 named species have distributions that border on the geologically active Trans-Mexican Volcanic Belt (TMVB), a region that defines the uplifted plateau (Mesa Central) of Mexico. We used the parametric bootstrap method to test the hypothesis that a single vicariant event associated with the TMVB was responsible for divergence of taxa found to the north and south of this boundary. Because the single-event hypothesis was rejected as highly unlikely, we hypothesize that at least two geological events were responsible for divergence of these species. The first (8–16 million years ago) separated ancestral populations that were distributed across the present TMVB region. A second event (2.8–6.4 million years ago) was associated with northward dispersal and subsequent vicariance of two independent southern lineages across the TMVB. The geological history of this tectonically and volcanically active region is discussed and systematic implications for the genus are outlined.

Key words.—Bayesian inference, mitochondrial, molecular clocks, parametric bootstrap, phylogeny, phylogeography, vicariance.

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Central America and Mexico comprise a tectonically and volcanically dynamic part of the world that has interested biogeographers for many years (Marshall and Liebherr 2000). This diverse region has served as a corridor for organismic dispersal between the Neartic and Neotropic and represents a transition zone between these provinces (Pielou 1979). This transition zone is presently bisected by a conspicuous physiographic feature known as the Trans-Mexican Volcanic Belt (TMVB; Fig. 1). The TMVB, which defines the southern limit of the massively uplifted Mexican plateau (Mesa Central), has undergone extensive volcanism since the early Miocene (~25 million years ago; Ferrari et al. 1999). Although much attention has focused on the Isthmus of Panama as a barrier to dispersal of marine organisms (Knowlton et al. 1993; Bermingham et al. 1997; Tringali et al. 1999), less attention has focused on the TMVB as a barrier to dispersal of terrestrial and freshwater organisms. Tectonic and volcanic activity has compartmentalized riverine drainages within and bordering the TMVB (Miller and Smith 1986) and provided opportunities for vicariant events in a number of aquatic organisms including salamanders (Lynch et al. 1983; Darda 1994), toads (Mulcahy and Mendelson 2000), and fish (Barbour 1973; Webb 1998).

Two attempts to date vicariant events associated with the TMVB were based on molecular clocks. Webb (1998) relied on fossil data to calibrate a clock for mitochondrial DNA (mtDNA) divergence of cyprinodontiform fish in the family Goodeidae. He identified multiple divergence times across a single geological barrier in the western TMVB for five pairs of species (0.3–9.3 million years ago), which he attributed to separate vicariant events. In contrast, Mulcahy and Mendelson (2000) relied on comparative divergence rates of Asian toads to calibrate a molecular clock for mtDNA divergence among members of the *Bufo valliceps* complex distributed in eastern (i.e., Gulf of Mexico) slope drainages. They concluded that a single Miocene-Pliocene event (4.2–7.6 million years ago) separated populations of these toads north and south of the TMVB.

In this study, we examined roles that the TMVB might have played in diversification of the widespread fish genus *Poeciliopsis* (Cyprinodontiformes: Poeciliidae). These small livebearing topminnows are distributed mostly in Pacific-slope drainages from southern Arizona to western Colombia (Fig. 2), where they comprise a significant part of the native freshwater fish fauna. They inhabit a range of environments from tropical lowland streams to high-altitude lakes, and desert springs and streams. Twenty extant species of *Poeciliopsis* sensu lato are divided into two subgenera, *Aulophallus* and *Poeciliopsis* sensu stricto. The three known *Aulophallus* species, all located in southern Central America away from the TMVB (Fig. 2), are used as an outgroup for the present study, which focuses primarily on the subgenus *Poeciliopsis*. Of the 17 known species of *Poeciliopsis* s.s., only one, *P. infans*, traverses the TMVB. The rest are distributed north (seven species) or south (nine species) of the TMVB; and nine of these species are bounded by the TMVB as northern or southern limit of their respective ranges. Our primary goal was to determine whether divergence of these northern and southern lineages of *Poeciliopsis* s.s. was due to one or more historical events associated with the TMVB. To establish this, we tested the hypothesis that a single vicariant event was responsible for divergence of the northern and southern lineages (i.e., the single-event hypothesis). If this hypothesis is true, then at least one geographical group (i.e., northern or southern) should comprise a monophyletic clade.

Our secondary goal was to conduct the first comprehensive phylogenetic analysis of the genus *Poeciliopsis* s.l. The phylogenetic hypotheses presented here were based on complete

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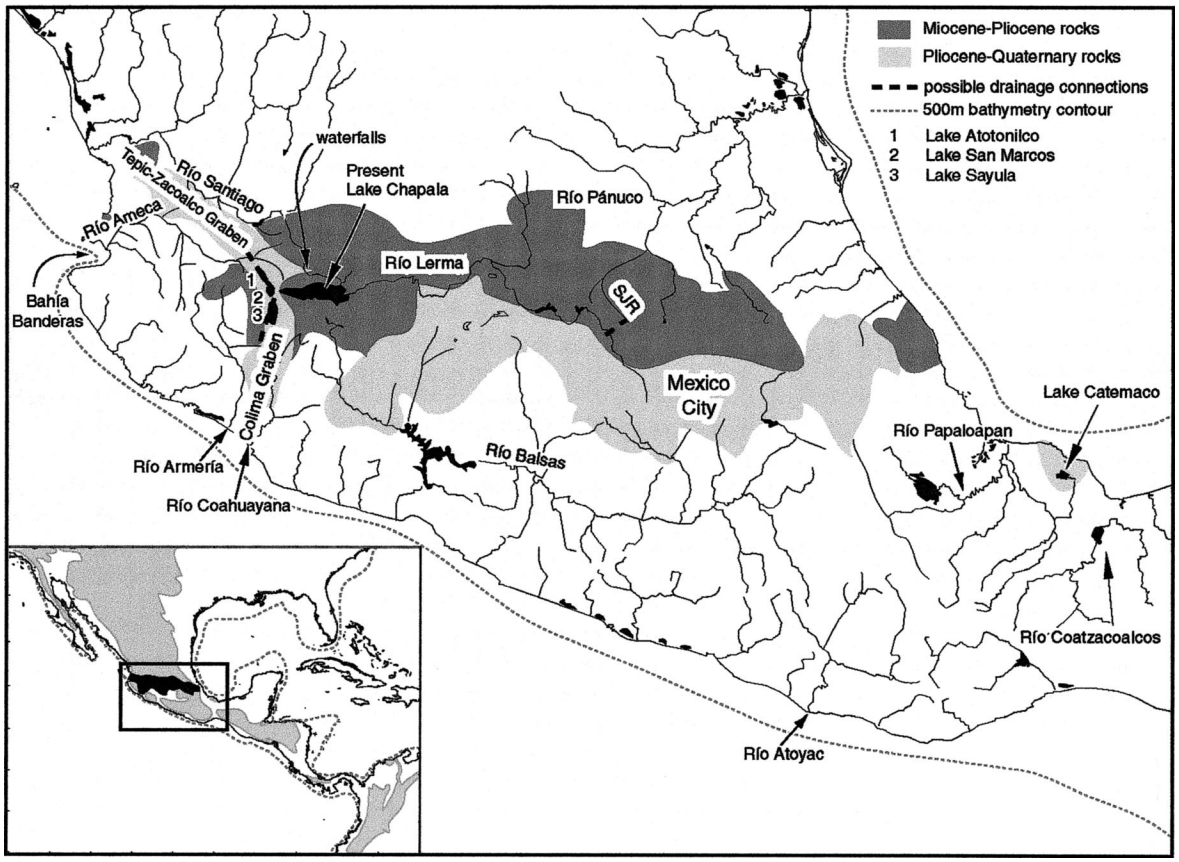

FIG. 1. Geological and hydrographical features of central Mexico: gray-shaded areas are aged volcanic rocks; black-shaded objects are lakes and coastal lagoons (modified from Gómez-Tuena and Carrasco-Núñez 2000). Insert: gray-shaded areas indicate high-altitude regions (> 500 m), and black area indicates the Trans-Mexican Volcanic Belt. Dotted line is a 500-m bathymetric contour.

sequences of two mitochondrial genes, 1140 bp of cytochrome *b* (cyt *b*) and 1047 bp of NADH subunit 2 (ND2). The rapid mutation rate and almost ubiquitous uniparental mode of inheritance of mtDNA make it a powerful marker for phylogenetic and phylogeographic inference (reviewed by Avise 2000). Furthermore, when mtDNA substitution rates are homogeneous across lineages and time, the relative chronology of diversification events may also be estimated from genetic distance data and be compared to known dates of geological events (e.g., Sivasundar et al. 2001). In particular, cyt *b* has been useful in resolving phylogenetic relationships among Actinopterygian fish (reviewed by Lydeard and Roe 1997) and ND2 has been used for solving relationships among cichlids (Kocher et al. 1995), cyprinids (Broughton and Gold 2000), and other Poeciliids (Ptacek and Breden 1998; Breden et al. 1999; Hamilton 2001).

MATERIALS AND METHODS

Specimens

We examined new DNA sequences from seven outgroup and 40 ingroup OTUs (Table 1). Two outgroup sequences were obtained from the literature. The taxa included all named species within the genus *Poeciliopsis* with the exception of *P. balsas*. The genus *Poeciliopsis* is assigned to the tribe Heterandriini, supertribe Poeciliini, subfamily Poeciliinae (Parenti 1981); thus, we used members of its tribe and other tribes in its subfamily as outgroups. Collection localities for the ingroup samples of *Poeciliopsis* are illustrated in Figure 2. For widespread species, we examined multiple localities when samples were available. Because we were particularly interested in reconstructing the historical hydrography of TMVB basins, we sampled more extensively *P. infans*, the only species that traverses this boundary.

Laboratory Protocols

We followed the manufacturer's protocol for the DNeasy Kit (Qiagen, Inc., Valencia, CA) to extract total DNA from frozen or alcohol-preserved skeletal muscle or fin. Cyt *b* was amplified with the LA and HA primers (Schmidt et al. 1998). ND2 was amplified with a combination of ND2B-L (Broughton and Gold 2000) and ASN (Kocher et al. 1995) or ND2E-H (Broughton and Gold 2000) primers. Polymerase chain reaction (PCR) amplification used *Taq* polymerase (Promega, Madison, WI) with an initial denaturation of 2 min at 95°C; 35 cycles of 1 min at 95°C, 1 min at 57°C (cyt *b*) or 53°C (ND2), and 2 min at 72°C; and a final extension of 10 min at 72°C. PCR products were gel extracted and cleaned with Qiagen Gel Extraction Kit. Both strands of each PCR product were sequenced on ABI 373 or 377 automated sequencers (Perkin-Elmer/ABI, Foster City, CA). The original PCR

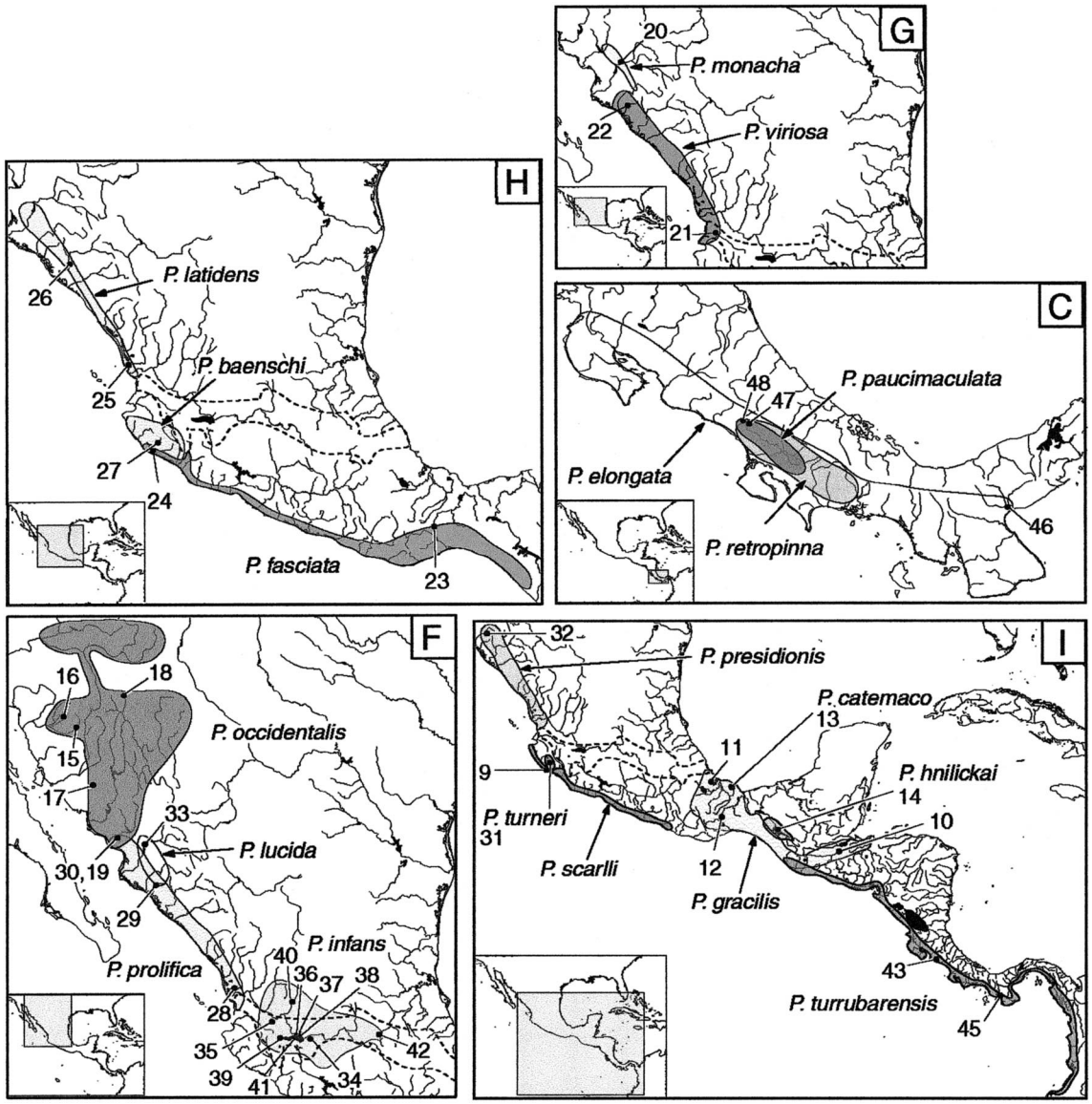

FIG. 2. Species distribution by groups; each group corresponds to a clade in Figure 3. Sampling localities are indicated by numbers and correspond to identification numbers in Table 1. Dotted line corresponds to boundaries of the Trans-Mexican Volcanic Belt.

primers were used for ND2 and four additional internal primers were used for cyt *b* (LA-276:5′-TGCATYTAYCTTCACATC-GG-3′, HA-357:5′-CGAAGGCGGTTATTACRAG-3′, LA-805:5′-AGCCCGAATGATATTTTCTCTTCG-3′, and HA-933:5′-CGGAAGGTRAGGCTTCGTTG-3′; Sanjur 1998).

Sequences were proofread with ABI AutoAssembler software and aligned by eye, based on the inferred amino acids. We used the entire coding regions of cyt *b* and ND2 with the following exception. The coding region of cyt *b* in *P. latidens*, *P. fasciata*, and *P. baenschi* was 21 bp (7 aa) longer than for the other taxa. Because this longer fragment only affected these closely related taxa, we excluded it from phylogenetic analyses, but its addition is illustrated in the tree (Fig. 3). By contrast, the coding region of ND2 was 3 bp (1 aa) shorter in *P. infans*, *P. occidentalis*, *P. prolifica*, and *P. lucida*. This noncoding fragment was included in the phylogenetic analyses, and its deletion is also illustrated in the tree.

Phylogenetic Analyses

All phylogenetic analyses were performed with PAUP*4.0b8 (Swofford 1998), unless otherwise noted. We performed maximum-parsimony (MP), minimum-evolution (ME), neighbor-joining (NJ), and maximum-likelihood (ML) analyses. The MP, ME, and ML analyses were performed by heuristic searches with 50 stepwise random additions and TBR branch swapping. Bootstrap values were based on 1000 replicates for MP, ME and NJ analyses. To minimize computation time, ML bootstrap searches (100 replicates) included only a subset of the OTUs. Reported bootstrap support values were calculated from a 50% majority-rule consensus tree.

TABLE 1. Locality information and GenBank accession numbers for examined OTUs. Identification numbers correspond to collection site numbers in Figure 2.

ID	OTUs examined	Collection sites or reference	GenBank accession no.	
			cyt *b*	ND2
	Tribe Poeciliini			
1	*Poecilia butleri*	Río Acaponeta, Nayarit, Mexico	AF412124	AF412167
2	*Xiphophorus nigrensis*[1]	Breden et al. (1999)		AF031386
	Tribe Gambusiini			
3	*Gambusia* sp.	Río Mayo, Guamuchil, Sonora, Mexico (not native)	AF412123	AF412166
	Tribe Heterandriini			
4	*Heterandria formosa*[1]	Breden et al. (1999)		AF084973
5	*Heterandria formosa*[2]	Everglades drainage, Dade County, Florida	AF412125	
6	*Phallichthys tico*	Río Agua Caliente, near town Fortuna, Costa Rica	AF412127	AF12168
7	*Priaphichthys festae*	San Vicente Thermal Springs, Guayas Province, Ecuador	AF412126	AF412170
8	*Neoheterandria umbratilis*	Río Santa Rosa, Costa Rica	AF412165	AF412169
	Genus *Poeciliopsis* Subgenus *Poeciliopsis*			
9	*Poeciliopsis scarlli*	Río Marabasco, above Cihuatlán, Jalisco, Mexico	AF412159	AF412198
10	*P. gracilis*[2]	Río Jones, Río Motagua, Guatemala	AF412162	
11	*P. gracilis*	Tierra Blanca, Veracruz, Mexico	AF412155	AF412195
12	*P. gracilis*	Río Coatzacoalcos, Oaxaca, Mexico	AF412154	AF412200
13	*P. catemaco*	Laguna Catemaco, Veracruz, Mexico	AF412161	AF412201
14	*P. hnilickai*	Río Girasol, Chiapas, Mexico	AF412156	AF412202
15	*P. occidentalis occidentalis*[2]	Río Magdalena, Sonora, Mexico	AF412140	
16	*P. occidentalis occidentalis*	Río Altar, Sonora, Mexico	AF412141	AF412185
17	*P. occidentalis sonoriensis*	Río Matape, Sonora, Mexico	AF412143	AF412187
18	*P. occidentalis sonoriensis*	Upper Río Yaqui, Black Draw, Arizona, USA	AF412142	AF412186
19	*P. occidentalis sonoriensis*[2]	Río Mayo, Guamuchil, Sonora, Mexico	AF412144	
20	*P. monacha*	Río Fuerte, Sonora, Mexico	AF412131	AF412173
21	*P. viriosa*	San Pedro Lagunillas, Nayarit, Mexico	AF412132	AF412174
22	*P. viriosa*	Río Mocorito, Sinaloa, Mexico	AF412133	AF412175
23	*P. fasciata*	Río Coaztacoalcos, Oaxaca, Mexico	AF412149	AF412193
24	*P. fasciata*	Stream near Cihuatlán, Jalisco, Mexico	AF412150	AF412199
25	*P. latidens*	Río San Pedro Mezquital, Nayarit, Mexico	AF412151	AF412194
26	*P. latidens*[2]	Río Culiacán, Sinaloa, Mexico	AF412152	
27	*P. baenschi*	Río Purificación, Jalisco, Mexico	AF412148	AF412191
28	*P. prolifica*	Río San Pedro Mezquital, Nayarit, Mexico	AF412146	AF412189
29	*P. prolifica*	Río Mocorito, Sinaloa, Mexico	AF412145	AF412188
30	*P. prolifica*	Río Mayo, Guamuchil, Sonora, Mexico	AF412147	AF412190
31	*P. turneri*	Río Purificación, Jalisco, Mexico	AF412158	AF412197
32	*P. presidionis*	Río Mocorito, Sinaloa, Mexico	AF412157	AF412196
33	*P. lucida*	Río Fuerte, Sonora, Mexico	AF412139	AF412184
34	*P. infans*	Río Lerma, Michoacán, Mexico	AF412134	AF412176
35	*P. infans*	Río Ameca, Jalisco, Mexico	AF412135	AF412177
36	*P. infans*[1]	Río Grande de Santiago, Lake Cajititlán, Jalisco, Mexico		AF412178
37	*P. infans*[1]	Río Grande de Santiago, Juanacatlán, Jalisco, Mexico		AF412179
38	*P. infans*	Río Grande de Santiago, Atotonilquillo, Jalisco, Mexico	AF412137	AF412180
39	*P. infans*[1]	Lake Atotonilco, Jalisco, Mexico		AF412181
40	*P. infans*[1]	Río Verde, San Juan de los Lagos, Jalisco, Mexico		AF412182
41	*P. infans*[2]	Lake Chapala, Jalisco, Mexico	AF412136	
42	*P. infans*	Río Pánuco, San Juan del Río, Querétaro, Mexico	AF412138	AF412183
43	*P. turrubarensis*[2]	Stream near Rincón, Puntarenas, Costa Rica	AF412160	
44	*P. turrubarensis*	Río Abrojo, Costa Rica	AF412163	AF412203
45	*P. turrubarensis*	Río San Pedro, at road to Sona, Panama	AF412164	AF412204
	Subgenus *Aulophallus*			
46	*P. elongata*	Agua Dulce, Cocle, Panama	AF412129	AF412172
47	*P. retropinna*[2]	Río Savegre, Puntarenas, Costa Rica	AF412130	
48	*P. paucimaculata*	Río General, near San Isidro, San José, Costa Rica	AF412128	AF412171

[1] ND2 sequence only.
[2] Cytochrome *b* sequence only.

Initial phylogenetic analyses were performed on all OTUs and rooted with members of the Gambusiini and Poeciliini (Table 1). To examine the potential effects of homoplasy, we progressively excluded more distant outgroups based on this initial tree topology: (1) OTUs outside the Heterandriini; (2) OTUs outside the genus *Poeciliopsis*; and (3) OTUs outside the subgenus *Poeciliopsis*.

Selecting an appropriate weighting scheme in MP analyses was not straightforward, as some substitution types appeared to be saturated at particular codon positions (e.g., ND2 third-

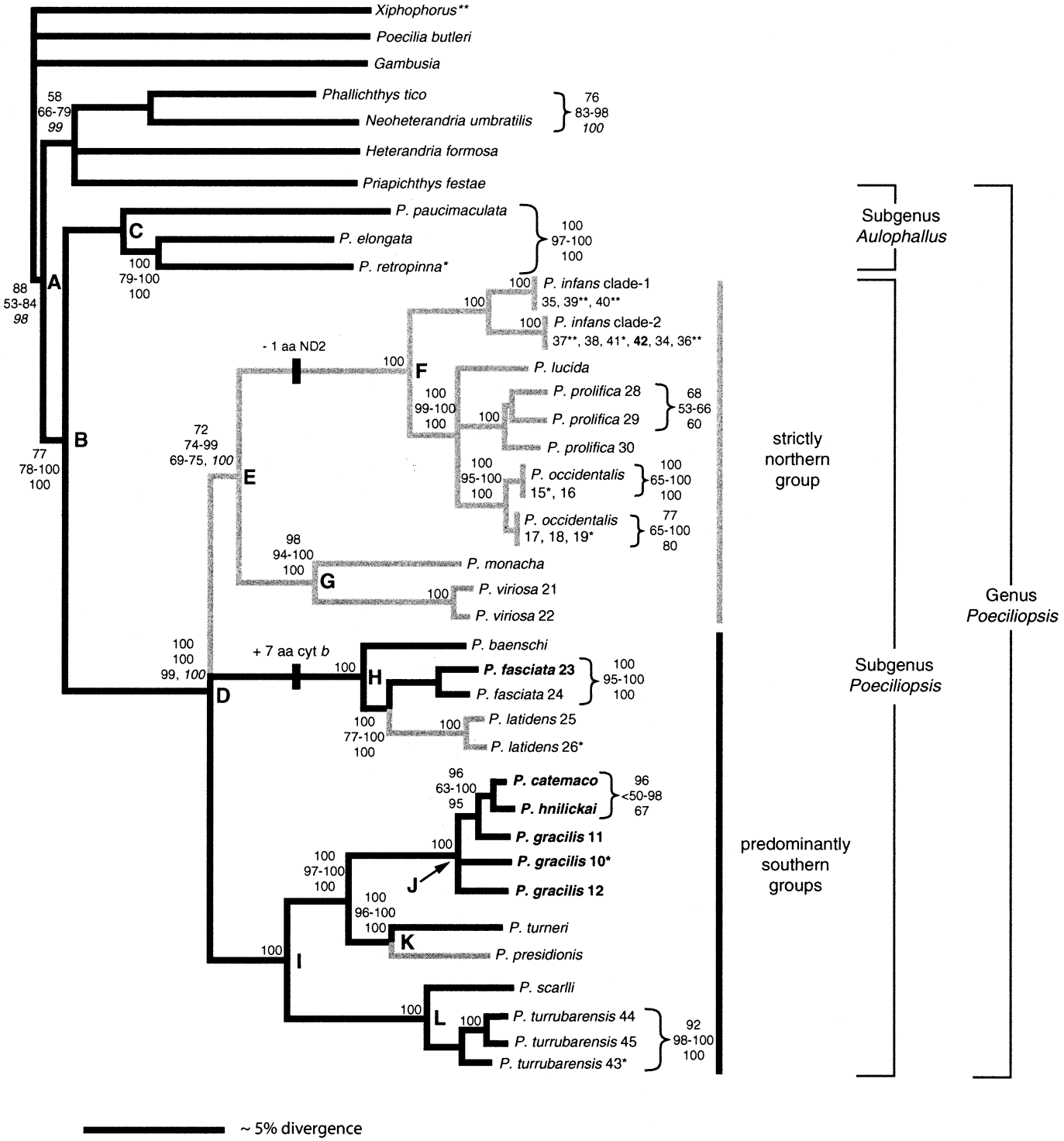

FIG. 3. A strict consensus of the most parsimonious trees based on combined data from cytochrome *b* and ND2. All characters and substitution types received equal weights. Capital letters next to nodes indicate major clades. Numbers in OTU labels distinguish different samples of the same species (identification number, Table 1). Numbers adjacent to nodes indicate support value or ranges for parsimony, distance, and likelihood analyses (from top to bottom, respectively). Parsimony values correspond to bootstrap support with all characters and substitutions weighted equally. Distance values correspond to bootstrap support from neighbor-joining and minimum-evolution analyses, based on the three different substitution models described in the Materials and Methods. Nonitalicized maximum-likelihood values correspond to maximum-likelihood bootstrap support, whereas italicized numbers correspond to Bayesian support. If bootstrap values for a particular node equaled 100% in all analyses, only one value is shown. For the subgenus *Poeciliopsis*, black branches correspond to samples south of the Trans-Mexican Volcanic Belt, gray branches indicate samples on or north of the Trans-Mexican Volcanic Belt, bold OTU labels correspond to Gulf of Mexico slope drainages, and all others correspond to Pacific slope drainages. Rather than showing the relationships among all haplotypes within *P. infans* and *P. occidentalis*, we pooled all closely related sequences

position transitions). Although commonly used, the practice of down-weighting transitions rarely improves phylogenetic estimates and may, in some cases, be counter-productive (Broughton et al. 2000). Thus, we weighted all codon positions and types of substitution equally. However, to assess the robustness of our data to different assumptions, we also applied different transition:transversion weighting schemes (i.e., 1:2, 1:4, 2:1, and 4:1) to all positions simultaneously, as well as to ND2 third, and cyt *b* third, separately. Nevertheless, only well-supported (> 50% bootstrap support) discrepancies among equally weighted and differentially weighted analyses are reported. Pairwise distances used for NJ and ME analyses were based on three different DNA substitution models: Kimura two-parameter (K2P, Kimura 1980), general time reversible (GTR, Rodríguez et al. 1990), and the ML distance model selected with the likelihood-ratio test (LRT) described below.

To fit the best model for the ML and ML-based distance analyses, we used the hierarchical LRT implemented by Modeltest 3.06 program (Posada and Crandall 1998), following the criteria of Huelsenbeck and Rannala (1997) and Huelsenbeck and Crandall (1997). Starting with a NJ tree based on Jukes-Cantor distances (Jukes and Cantor 1969), we calculated likelihood scores for 56 different substitution models. Then, likelihood scores were compared hierarchically with a LRT. A mixed chi-square distribution (i.e., 50:50 mixture of X^2_{k-1} and X^2_k) was used to construct a LRT for comparisons involving invariable sites and/or rate heterogeneity among sites (following Goldman and Whelan 2000; Ota et al. 2000).

Bayesian Inference

Nonparametric bootstrapping can produce unreliable results (Zharkikh and Li 1992; Hillis and Bull 1993) and unclear interpretations (e.g., Felsenstein and Kishino 1993). Thus, to obtain an additional measure of support for nodes in the tree, we also used the Bayesian method of phylogenetic inference (Rannala and Yang 1996) as implemented in the program MrBayes 2.0 (Huelsenbeck 2000). We assumed the GTR model and a discrete approximation (four categories) of the gamma distribution shape parameter for among-site rate variation. We ran 300,000 generations of four simultaneous Markov chain Monte Carlo chains and sampled trees and branch lengths every 10 generations. Because the log-likelihood sum (of the four chains) reached stability after approximately 30,000 generations, we excluded the first 3000 trees and used the remaining 27,000 to compute a 50% majority-rule consensus tree. Following Huelsenbeck (2000), the percent of times a clade occurred among the sampled trees was interpreted as the probability of that clade existing.

Parametric Bootstrap

We used a parametric bootstrapping method (Efron 1985; Felsenstein 1988) to test the single-event hypothesis and assess whether our data provided enough resolution for nodes that received low nonparametric bootstrap support. A parametric bootstrap test of monophyly involves obtaining score estimates for the trees recovered first with and then without the constraint of monophyly and then comparing the difference in scores with the distribution of differences generated by simulation. Although a likelihood approach is recommended for obtaining score estimates (Huelsenbeck et al. 1996), due to the prohibitive computation time for such a large number of OTUs, we had to adopt MP as a computational alternative (Hillis et al. 1996; see also Ruedi et al. 1998; Emerson et al. 2000). DNA sequences were simulated with the Seq-Gen program (ver. 1.2.4, Rambaut and Grassly 1997). For a given null hypothesis (e.g., all OTUs north of TMVB are monophyletic), we first constructed a constrained NJ tree under the K2P model. Then we used the LRT method as described above to select the substitution model and estimate ML branch lengths. One hundred datasets were simulated for each constrained topology, and MP-heuristic searches (with 50 stepwise random additions) were conducted with and without a specified constraint. The distribution of tree-length differences between constrained and unconstrained simulated trees was compared with the observed tree-length differences. If the probability of obtaining the empirical tree-length difference was less than 5%, we rejected the null hypothesis of monophyly.

Molecular Clocks

Molecular clocks can be calibrated from well-dated biogeographic scenarios (e.g., the rise of the Isthmus of Panama) or for fossil data (reviewed by Caccone et al. 1997) or substitution rates are borrowed from related taxa that evolve at the same rate as the taxa under study or for which rate differences can be adjusted (e.g., Mulcahy and Mendelson 2000). Because fossil data are not available for *Poeciliopsis*, we were unable to calibrate an independent molecular clock for the genes examined in this study. However, we could assume a crude molecular clock for cyt *b* K2P divergences, ranging between 1% and 2% per million years based on K2P divergence rates estimated for mitochondrial genes in other teleost fishes (Bermingham et al. 1997), including other cyprinodontiforms (García et al. 2000). Additionally, we restricted our estimates to cyt *b* because the molecular clock hypothesis was rejected for ND2. Clocklike behavior of gene trees was evaluated with a LRT on the likelihood scores of trees generated with and without molecular clock constraints. Degrees of freedom were calculated as (number of taxa) − 2, following Huelsenbeck and Rannala (1997).

RESULTS

Altogether, we examined 82 sequences (42 cyt *b* and 40 ND2; GenBank accession numbers in Table 1) from 48 fish

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into one of the two most divergent haplotype clades in each of these species. Scale (bottom left) corresponds approximately to 5% divergences based on K2P distances and both genes combined. Note: Molecular clock calibrations referred to in text assumed K2P distances based on cytochrome *b* only. One asterisk denotes a relationship based on cytochrome *b* sequences only. Two asterisks denotes a relationship based on ND2 sequences only.

specimens. Apparently, because of variation in the priming sites or poor template quality of some specimens, we could not obtain both sequences from 11 OTUs. We observed no evidence for heteroplasmy in the sequences of either mitochondrial gene. Two observations suggest that the sequences examined herein are not nuclear-integrated copies of these mitochondrial genes (e.g., Zhang and Hewitt 1996). First, we found no premature stop codons disrupting the reading frame in any of the samples. Second, nucleotide content in the light strand for both genes was strongly biased against guanine in all samples: cyt *b* (average: 24.7% A, 31.6% C, 14.5% G, and 29.2% T); and ND2 (average: 26.6% A, 33.9% C, 11. 7% G, and 28.4% T). Anti-G bias is characteristic of mitochondrial, but not the nuclear, genes in other fishes (Meyer 1993; Kocher et al. 1995; Ptacek and Breden 1998).

We evaluated nucleotide saturation by plotting transitions and transversions against the uncorrected distances separately for each codon position (results not shown). Substitutions at cyt *b* first and second positions and ND2 second positions did not appear to be saturated. Cyt *b* third and ND2 first positions appeared to be at an early stage of transitions saturation around 20% and 35% divergence (K2P per gene, based on all codon positions), respectively. ND2 third-position transitions seemed to be saturated at distances greater than 20%, whereas transversions appeared to be at an early stage of saturation at distances greater than 40%.

Phylogenetic Analyses

Altogether, 973 of the approximately 2187 nucleotide sites were parsimony-informative (577 ND2 and 396 cyt *b*). Maximum divergence (K2P corrected) among the ingroup OTUs was 24% for cyt *b* and 42% for ND2, whereas the maximum divergence including outgroup OTUs was 25% for cyt *b* and 47% for ND2. The substitution models selected according to the hierarchical LRTs contained multiple parameters, including a different rate for almost every type of substitution, unequal base frequencies, as well as a specific proportion of invariable sites and a specific gamma shape parameter (discrete approximation; four categories) for rate differences among sites.

In general, tree topologies obtained under all our different assumptions (i.e., genes, phylogenetic methods, substitution models, and taxon sampling and character weighting schemes) were similar, and most of the discrepancies were not supported by bootstrap values above 50%. Separate examination of each gene provided less phylogenetic resolution than analyses of the combined dataset, so we report results from the combined analyses (Fig. 3) and discuss inconsistencies between the two genes. Combining the two genes was appropriate because a partition of homogeneity test (Farris et al. 1994) failed to detect heterogeneity between them.

All phylogenetic analyses of the combined dataset supported monophyly of the tribe Heterandriini (Fig. 3, node A) with nonparametric bootstrap support of 53–88% and Bayesian support of 98%. Within the Heterandriini, all analyses supported monophyly of the genus *Poeciliopsis* (node B) with bootstrap values of 77–100% and Bayesian support of 100%. As anticipated from traditional taxonomy (Rosen and Bailey 1963), this genus comprised two well-supported and reciprocally monophyletic clades (nodes C and D) corresponding to the subgenera *Aulophallus* and *Poeciliopsis*. Within *Aulophallus*, *P. paucimaculata* appeared as sister-taxon to a group containing *P. elongata* and *P. retropinna* (cyt *b* sequences alone; 79–100% bootstrap support).

The subgenus *Poeciliopsis*, the principal focus of this study, could be subdivided into four well-supported clades defined by nodes F, G, H, and I (Fig. 3). Members of clade F share a deletion of one amino acid at the 3′ end of ND2, and members of clade H share an addition of seven amino acids at the 3′ end of cyt *b*. Although not similarly defined by synapomorphic deletions or insertions, members of clades G and I also were well supported (bootstrap values of 94–100%). Nevertheless, the relationships among *Poeciliopsis* s.s. lineages varied somewhat among the different phylogenetic methods and genes.

Node E (Fig. 3) defined a clade of *Poeciliopsis* species found on or north of the TMVB. Node E was recovered in all analyses except for two of MP weighting schemes. Bootstrap support for this strictly northern group ranged between 72% and 99% and Bayesian support was 100%. Other clades in the subgenus *Poeciliopsis* (defined by nodes H and I) occur south of the TMVB, with the exception of two species (*P. presidionis* and *P. latidens*). Recognizing these exceptions, we subsequently refer to these clades as the ‘‘predominantly southern’’ groups.

Evolutionary relationships between the strictly northern and predominantly southern clades were complex. All distance-based analyses supported reciprocal monophyly of the two groups—((H,I)E)—at 64–97% bootstrap support. MP analyses also recovered this relationship, but bootstrap support was low ($< 65\%$). ML did not support this relationship; instead, it joined H with E—(I(H,E)). Although bootstrap support for (I(H,E)) was poor ($< 50\%$), Bayesian support was good 83%. To test whether the ML topology, (I(H,E)), was better than the alternative topologies, (E(H,I)) and (H(I,E)), we employed the Shimodaira-Hasegawa test (Shimodaira and Hasegawa 1999), with RELL optimization and 1000 bootstrap replicates, assuming the same model and parameters as the original ML analyses. Failing to reject the null hypothesis, we lacked the power to discriminate among these three alternative topologies. To further assess whether we had the power to reject particular hypothesized relationships among these clades, we used the parametric bootstrap method to test seven alternative hypotheses, where combined letters correspond to hypothesized monophyletic relationships: FH, FI, GH, GI, FGH, FHI, and GHI. We could not test the relationships HI, FG, and FGI because they were among the best trees in the equally weighted MP analysis based on all OTUs and both genes. However, we could reject the hypothesized monophyly for FH, GH, and FHI ($P < 0.01$). Because of the low power to resolve relationships among nodes E, H, and I, we illustrated their relationship as a trichotomy (Fig. 3).

We found a single discrepancy between phylogenetic analyses of individual genes. Within node F, *P. lucida*, *P. occidentalis*, and *P. prolifica* formed a monophyletic group. However, hierarchical relationships among these OTUs were not fully resolved because each gene favored a different hypothesis. Most of the cyt *b* analyses favored the relationship ((*lu-*

cida, *occidentalis*) *prolifica*) with 74–90% bootstrap support and decay index of 1 (Donoghue et al. 1992), whereas ND2 analyses favored the relationship (*lucida* (*occidentalis*, *prolifica*)) with bootstrap values of 62–84% and decay index of 2. Similarly, different assumptions (phylogenetic methods and weighting schemes) also affected the outcome. For each gene separately, we tested whether the best tree obtained with one gene was significantly better than the best tree obtained with the other gene, using the Shimodaira-Hasegawa test with RELL optimization and 1000 bootstrap replicates and assuming the same model and parameters as the original ML analyses. Neither of these tests was significant, indicating that our data lacked enough power to solve this relationship, and thus, we portray it as a trichotomy (Fig. 3).

Testing the Single Vicariant Event Hypothesis

We conducted two parametric bootstrap analyses to test the hypothesis that divergence of northern versus southern species of the subgenus *Poeciliopsis* was caused by a single vicariant event associated with the TMVB. The first test constrained all OTUs found on or north of the TMVB to monophyly, and the second test constrained all OTUs found south of the TMVB to monophyly. Both tests rejected the monophyly hypothesis ($P < 0.01$). Species living north of the TMVB include all members of the strictly northern clade plus two highly divergent species, *P. latidens* and *P. presidionis*, from the predominantly southern clades. Three separate cladistic events were associated with species-level divergence across the TMVB.

Apparently, these three cladistic events occurred during different geological periods. Because sequence divergence in ND2 did not occur in a clocklike fashion, our estimates of divergence times were based solely on cyt *b*. K2P divergence between members of the strictly northern (node E) versus predominantly southern clades (nodes H and I) was 16 ± 1.4%. Assuming a rate of 1–2% sequence divergence (K2P) per million years, we estimated that this event took place between 8 million and 16 million years ago. Divergence of the lineages leading to *P. latidens* and *P. presidionis* occurred more recently; each of the northern species differed from its southern cognate, *P. fasciata* and *P turneri*, by 5.6% and 6.4%, respectively. We estimate that isolation of the two lineages occurred at 2.8–5.6 million and 3.2–6.4 million years ago, respectively.

Discussion

Phylogenetic analyses of mitochondrial gene sequences did not support the hypothesis that a single vicariant event associated with the TMVB was responsible for the division of the fish subgenus *Poeciliopsis* into discrete northern and southern clades. Instead, divergence among lineages of this subgenus occurred during at least two distinct time periods. Although we do not advocate that our molecular clock datings are exact, we use them to place the cladistic events in a reasonable geological context and hypothesize geological and climatic events that could have caused them. Based on divergence in cyt *b* sequences, we hypothesize that the first event occurred between 8 million and 16 million years ago, separating members into one strictly northern versus two predominantly southern clades. Prior to this period, ancestors of the strictly northern clade must have been distributed across the region presently defined by the TMVB. Because *Poeciliopsis* are absent from the Gulf of Mexico drainages north of the TMVB, these ancestors were probably restricted to Pacific slope drainages. This western region has undergone intense volcanism since the Miocene (~25 million years ago; Ferrari et al. 1999). It is easy to imagine that uplift of the western TMVB (now ~2000 m above sea level) fragmented low-elevation river basins and disrupted connections between populations lying to the north and south.

More recent events (2.8–6.4 million years ago) were associated with northward dispersal of the ancestors of *P. latidens* and *P. presidionis*. The overlap of estimated divergence dates and geographical distributions of these two groups suggests that they occurred roughly at the same time and probably as a result of the same events. Northward dispersal is likely because all relatives of the two species live south of the TMVB. We initially considered three possible dispersal routes following the first event: (1) marine dispersal; (2) coastal stream-capture via widened river deltas during Pleistocene low sea levels; and (3) headwater stream-capture through inland connections. Although *Poeciliopsis* are primarily freshwater fish, some, including *P. presidionis* and *P. latidens*, tolerate elevated salinities in coastal lagoons (Rosen and Bailey 1963). However, no *Poeciliopsis* have been captured in the open ocean. Deltaic stream-capture also is unlikely in this region because the continental shelf west and southwest of the TMVB is very narrow, particularly around Bahía Banderas (Fig. 1). Low sea levels in this region would not have exposed a substantial shelf for the formation of deltaic connections. Mountains to the southeast of Bahía Banderas are 76–99 million years old, predating the expected origin of the subgenus *Poeciliopsis*. If the continental shelf in this region had a similar topography throughout the Tertiary (2–65 million years ago), we hypothesize that the ancestors of *P. latidens* and *P. presidionis* dispersed northward via the Colima graben, an inland gap through the TMVB.

Although volcanism in the Colima graben, which presently contains one of the largest active volcanoes in Mexico (Volcán Colima), began 10.1 million years ago, widespread volcanism began 4.6 million years ago (Wallace et al. 1992). Ancestors of *P. latidens* and *P. presidionis* were probably distributed in coastal drainages south of this region, such as the present Ríos Armería and Coahuayana (Fig. 1). To the immediate north, faulting of the Colima graben during the late Pliocene (~2–3.5 million years ago; Luhr and Carmichael 1981), created a series of lakes and possibly a river that could have connected the Armería or Coahuayana to Lake Sayula (lake 3; Fig. 1), in the northern Colima graben. Lacustrine deposits in parts of the Colima graben (Luhr and Carmichael 1981) support this scenario. Through this connection, ancestors of *P. latidens* and *P. presidionis* may have dispersed north into Lake Sayula. Subsequent volcanism during the late Pliocene or early Pleistocene (~1.5–3.5 million years ago) probably closed this southern connection. By this time, Lake Sayula was part of the large Jalisco Paleolake that also encompassed Lake Chapala and the present-day endorheic (i.e., land-locked) lakes Zacoalco, Atotonilco, and San Marcos (Rosas-Elguera and Urrutia-Fucugachi 1998). These

lakes probably drained to the northwest through the Tepic-Zacoalco graben before they attained their present levels. Through these basins, ancestors of *P. presidionis* and *P. latidens* could have dispersed from Lake Sayula to Pacific-slope drainages north of the TMVB. We suspect that the dispersal and isolation of the ancestors of *P. latidens* and *P. presidionis* occurred during the late Pliocene (~2–3.5 million years ago), which is roughly encompassed by our estimated divergence times of 2.8–6.4 million years ago.

Poeciliopsis infans is the only species in this genus that lives almost entirely in the region defined by the TMVB (Fig. 2F). Mitochondrial haplotypes in this species define two clades that may also have been separated by events in the Jalisco Paleolake region. Clade 1 includes haplotypes from Río Ameca, Río Verde (lower Santiago drainage), and Lake Atotonilco (lake 1; Fig. 1). Clade 2 includes haplotypes from the Río Lerma, Lake Chapala, upper Santiago drainage, and Río Pánuco (Fig. 1). Presently, upper (clade 2) and lower (clade 1) Santiago are separated by a series of high cascades and waterfalls to the north of Lake Chapala (Fig. 1). Similarly, headwaters of the Río Ameca and Lake Atotonilco (clade 1) are presently separated from the west end of Lake Chapala (clade 2) by less than 100 m of elevation, but were historically connected (Palmer 1926; Smith et al. 1975). These connections could have been disrupted by Pleistocene volcanism that also isolated other basins in the Jalisco Paleolake region (Rosas-Elguera and Urrutia-Fucugachi 1998) and/or by a drop in the level of Lake Chapala, associated with a drier climate or penetration of a new outlet to the Río Santiago at Chapala's northeastern corner (Miller and Smith 1986). The historical Ameca-Chapala connection is supported by the distributions of cognate species-pairs in the fish taxa *Notropis* (Chernoff and Miller 1986), *Chirostoma* (Barbour 1973), *Ictalurus* (Miller and Smith 1986), and Goodeinae (Webb 1998). However, the five goodeine species-pairs exhibit a wide range of sequence divergence (0.3–9.3%), suggesting several cycles of connectivity and isolation (Webb 1998). Assuming a divergence rate of 1–2% per million years for cyt *b*, we estimated that *P. infans* clades 1 and 2 (~ 4% sequence divergence) diverged 2–4 million years ago, a period that may overlap with the presumed Pleistocene isolation of the Jalisco Paleolake basins.

Within clade 1, the close relationship between Río Verde (lower Santiago) and Río Ameca samples (~ 0.2% based on ND2) suggests that these populations have been connected until very recently. Recent connections of Ameca and Santiago tributaries could have been possible through stream capture (e.g., headwaters of both drainages are only a few kilometers apart in the vicinity of one of the volcanoes in this region, Volcán Ceboruco). Recent volcanism in the Tepic-Zacoalco graben (Nelson and Carmichael 1984) could have disrupted such connections.

At the eastern end of its range, we sampled a small population of *P. infans* at San Juan del Río (site 42; Fig. 2F, and SJR; Fig. 1) in a headwater tributary of the Río Pánuco, a Gulf of Mexico drainage. It closely resembled *P. infans* clade 2 haplotypes (0.1–0.28% sequence divergence) found in the Río Lerma. It is hypothesized that the San Juan del Río tributary drained westward to the Lerma basin before it was captured by the eastward draining Río Pánuco (Tamayo and West 1964). It is worth noting that the Pánuco is the only Gulf of Mexico drainage containing five goodeine species that are otherwise restricted to Mesa Central drainages (Webb 1998). Although levels of divergence vary greatly among the Pánuco and Mesa Central populations, one species, *Goodea atripinnis*, exhibits a sequence divergence (0.21%) comparable to that of *P. infans* lineages from these drainages.

The Isthmus of Tehuantepec is another region that may be an important avenue for dispersal across Mexico. Nearly traversed by the Río Coatzacoalcos, this low-altitude, narrow isthmus is the only region in Mexico where multiple groups of aquatic and riparian animals appear to have spread between Gulf of Mexico and Pacific drainages (Mulcahy and Mendelson 2000; Savage and Wake 2001). Closely related lineages of *P. fasciata* are spread across this region. Pacific slope *P. fasciata* (24) were 2.5% divergent from Gulf of Mexico slope *P. fasciata* in the upper Coatzacoalcos (23). Detailed phylogeographic studies of fish and other aquatic organisms may shed light on the historical hydrography of this dynamic region.

Another named species in the *P. gracilis* complex, *P. catemaco*, is endemic to Lake Catemaco (Fig. 1), a crater lake isolated from the Río Papaloapan by a series of waterfalls, some of which are as high as 60 m. Los Tuxtlas volcanic mass, which contains this crater, formed during the Quaternary (0–2 million years ago; West 1964), but unfortunately, precise dates are not known. Based on cyt *b*, we hypothesize that *P. catemaco* has been isolated from other members of the *P. gracilis* complex for 0.75–1.5 million years. *Poeciliopsis gracilis* lineages from localities just below Lake Catemaco were not available for this study, so we cannot draw more precise inferences regarding an event that isolated *P. catemaco*. However, molecular studies of cognate populations from six fish genera also found in Lake Catemaco, including three poeciliids (*Poecilia*, *Xiphophorus*, and *Heterandria*) may allow dating of the event that separated these crater-lake populations.

Absence of *Poeciliopsis* from Gulf of Mexico slope drainages north of the TMVB (excepting *P. infans* in upper Pánuco) is curious, because other poeciliid genera (*Poecilia*, *Xiphophorus*, *Heterandria*, and *Gambusia*) cross this boundary. *Belonesox* is the only other poeciliid genus with a similar northern limit along the Gulf of Mexico slope. Volcanism associated with the TMVB in eastern Mexico region began 12–17 million years ago (Ferrari et al. 1999), and a volcanic event estimated at 5 million years ago is believed to have isolated populations of toads north and south of this area (Mulcahy and Mendelson 2000). *Poeciliopsis* and *Belonesox* may have arrived from the south in this area following this event. The continental shelf is wide in this region and northward dispersal through coastal stream-capture may have been possible during Pleistocene low sea levels, but apparently it did not occur.

Coastal stream-capture may have facilitated dispersal of *Poeciliopsis* north and south of the TMVB on the Pacific slope of Mexico. Four lowland species, *P. prolifica*, *P. viriosa*, *P. presidionis*, and *P. latidens*, have overlapping distributions north of the TMVB (Fig. 2F–I). The continental shelf is relatively wide in this region, and the formation of interconnecting river deltas during Pleistocene low sea levels is plau-

TABLE 2. Traditional and recommended classification of species in the genus *Poeciliopsis.*

Traditional classification	Recommended based on nodes in Fig. 3
Subgenus *Aulophallus* (Hubbs 1926)	Subgenus *Aulophallus* (node C)
P. elongata	*P. elongata*
P. retropinna	*P. retropinna*
P. paucimaculata	*P. paucimaculata*
Subgenus *Poeciliopsis* (Regan 1913)	Subgenus *Poeciliopsis* (node B)
latidens-fasciata complex (Meyer et al., 1986):	*latidens-fasciata* complex (node H)
P. latidens	*P. latidens*
P. fasciata	*P. fasciata*
P. baenschi	*P. baenschi*
gracilis complex (Meyer et al., 1986)	*gracilis* complex (node J)
P. gracilis	*P. gracilis*
P. catemaco	*P. catemaco*
P. hnilickai	*P. hnilickai*
P. lutzi [1]	
occidentalis complex (Meyer et al., 1986)	*Leptorhaphis* [2] complex (node F)
P. occidentalis	*P. occidentalis*
P. lucida	*P. lucida*
P. infans	*P. infans*
P. monacha	*P. prolifica*
P. viriosa	
	monacha-viriosa complex (node G)
	P. viriosa
	P. monacha
turrubarensis complex (Meyer et al., 1986)	*turrubarensis* complex (node L)
P. maldonadoi (fossil)	*P. maldonadoi* (fossil)
P. turrubarensis	*P. turrubarensis*
P. scarlli	*P. scarlli*
P. presidionis	
Not placed in any complex	*presidionis-turneri* complex (node K)
P. balsas	*P. presidionis*
P. prolifica	*P. turneri*
P. turneri [3]	

[1] Rosen and Bailey (1963) consider *P. lutzi* a synonym of *P. gracilis.* Meyer et al. (1986) list it as a valid species, but provide no supporting evidence. We follow Rosen and Bailey (1963).
[2] Miller (1960) grouped *P. occidentalis, P. lucida,* and *P. infans* in the Leptorhaphis species group.
[3] Miller (1975) suggests that *P. turneri* is most closely related to *P. gracilis.*

sible. Similarly, the widespread distributions of *P. fasciata*, *P. scarlli*, and *P. turrubarensis* in Pacific-slope drainages south of the TMVB suggests they might have dispersed during low sea levels, although the continental shelf is narrow in places. Both coastal and headwater stream-capture may have affected the dispersal of the widespread endangered species *P. occidentalis* (Vrijenhoek et al. 1985; Vrijenhoek 1998). Sometimes, downstream populations in adjacent rivers are more similar to each other than each is to upstream populations in its own river, a possible earmark of recent coastal connections. In other cases, interdigitating headwaters of rivers have closely related populations of these fish. Also, as previously discussed, *P. infans* provides a good example of dispersal by headwater capture through the Jalisco Paleolake region. To distinguish coastal from headwater stream-capture, detailed population genetic studies of individual species are needed to assess coancestries between upstream and downstream populations within and between river systems.

Systematic Implications

Evolutionary relationships among species in the genus *Poeciliopsis* inferred from the present molecular analysis are broadly consistent with previous systematic studies (Table 2). Analyses of individual mitochondrial genes and the combined data clearly support monophyly of the genus. Addi-

tionally, the present molecular data support division of the genus *Poeciliopsis* into two previously recognized subgenera *Aulophallus* and *Poeciliopsis*. However, these molecular data are less useful for assessing higher relationships within the family Poeciliidae because of limited taxon sampling and problems with substitutional saturation. Although the data are consistent with monophyly of the tribe Heterandriini, to properly evaluate this relationship, all members of the tribe should be examined. Moreover, additional outgroups from all tribes in the subfamily Poeciliinae and a lineage outside the subfamily (to root the tree) need to be examined.

Based on present data, the subgenus *Poeciliopsis* can be split into six complexes of closely related sibling species (Table 2). Sequences of *P. balsas* need to be examined to establish its relationship to other *Poeciliopsis*. As previously recognized, the *latidens-fasciata* complex (Fig. 3) was fully supported by present data (Fig. 3). Other recognized species complexes required revision, and new complexes were erected. Inferred evolutionary relationships among named species within the *gracilis* species complex conflict with their present taxonomy. It should be remembered, however, that on very short evolutionary time scales, gene trees are not necessarily equivalent to species trees (Avise 1989). Results with these mitochondrial genes may be due to lineage sorting among species that diverged recently, subsequent reticulation, or both. *Poeciliopsis* species are known to hybridize broadly, although hybrids between some distantly related species generate all-female lineages that clone (Schultz 1977). Nevertheless, as presently composed, the species *P. gracilis* may not be monophyletic. Additional work based on more comprehensive biogeographic sampling and additional nuclear gene markers is warranted to better resolve relationships in this complex.

The present data helped to resolve relationships in two predominantly southern species complexes (Table 2). We supported Meyer et al.'s (1986) distinction of the *turrubarensis* complex regarding affiliation of *P. turrubarensis* and *P. scarlli*, but we recommend that *P. presidionis* be included in a new complex with *P. turneri*. The *presidionis-turneri* complex is clearly affiliated with the *gracilis* complex, not the *turrubarensis* complex (Fig. 3).

The strictly northern group of species (defined by node E; Fig. 3) was previously recognized by Meyer et al. (1986) as the *occidentalis* complex. It contains two divergent complexes: the *monacha-viriosa* complex (node G); and what Miller (1960) defined as the *Leptorhaphis* group. Our molecular data clearly placed *P. prolifica* within the *Leptorhaphis* group (node F), even though hierarchical relationships among *P. lucida*, *P. occidentalis*, and *P. prolifica* could not be solved. Miller (1960) recognized a number of morphological traits that *P. prolifica* shares with the *Leptorhaphis* species, but was uncertain of its affiliation because of its highly divergent life history. Adult *P. prolifica* females are very small (< 25 mm); they produce tiny eggs, have short interbrood intervals (~ 7 days), and exhibit extreme superfetation (gestating females simultaneously contain up to five embryonic stages in their ovaries; Thibault and Schultz 1978).

Higher-level relationships within the subgenus *Poeciliopsis* remain unresolved. For example, relationships among the three major clades defined by nodes E, H, and I are portrayed as a trichotomy (Fig. 3). The branches connecting these three nodes are very short, contrasting sharply with the long branch leading to the subgenus *Aulophallus*. If divergence among the three groups occurred rapidly, as we inferred from the gene tree, subsequent researchers will need to examine very long sequences of slowly evolving genes to confidently resolve hierarchical relationships.

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Literature Cited

Avise, J. C. 1989. Gene trees and organismal histories: a phylogenetic approach to population biology. Evolution 43: 1192–1208.

———. 2000. Phylogeography. Harvard Univ. Press, Cambridge, MA.

Barbour, C. D. 1973. A biogeographical history of *Chirostoma* (Pisces: Atherinidae): a species flock from the Mexican Plateau. Copeia 1973:553–556.

Bermingham, E., S. S. McCafferty, and A. P. Martin. 1997. Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. Pp. 113–128 *in* T. D. Kocher and C. A. Stepien, eds. Molecular systematics of fishes. Academic Press, San Diego, CA.

Breden, F., M. B. Ptacek, M. Rashed, D. Taphorn, and C. A. Figueiredo. 1999. Molecular phylogeny of the live-bearing fish genus *Poecilia* (Cyprinodontiformes: Poeciliidae). Mol. Phylogenet. Evol. 12:95–104.

Broughton, R. E., and J. R. Gold. 2000. Phylogenetic relationships in the North American cyprinid genus *Cyprinella* (Actinopterygii: Cyprinidae) based on sequences of the mitochondrial ND2 and ND4L genes. Copeia 2000:1–10.

Broughton, R. E., S. E. Stanley, and R. T. Durrett. 2000. Quantification of homoplasy for nucleotide transitions and transversions and a reexamination of assumptions in weighted phylogenetic analysis. Syst. Biol. 49:617–627.

Caccone, A., M. C. Milinkovitch, V. Sbordini, and J. R. Powell.

1997. Mitochondrial DNA rates and biogeography in European newts (genus *Euproctus*). Syst. Biol. 46:126–144.
Chernoff, B., and R. R. Miller. 1986. Fishes of the *Notropis calientis* complex with a key to the southern shiners of Mexico. Copeia 1986:170–183.
Darda, D. M. 1994. Allozyme variation and morphological evolution among Mexican salamanders of the genus *Chiropterotriton* (Caudata: Plethodontidae). Herpetologica 50:164–187.
Donoghue, M. J., R. G. Olmstead, J. F. Smith, and J. D. Palmer. 1992. Phylogenetic relationships of Dipscales based on *rbc*L sequences. Ann. MO. Bot. Gard. 79:249–265.
Efron, B. 1985. Bootstrap confidence intervals for a class of parametric problems. Biometrika 72:45–58.
Emerson, B. C., P. Oromi, and G. M. Hewitt. 2000. Interpreting colonization of the *Calathus* (Coleoptera: Carabidae) on the Canary Islands and Madeira through the application of the parametric bootstrap. Evolution 54:2081–2090.
Farris, J. S., M. Kallersjo, A. G. Kluge, and C. Bult. 1994. Testing significance of incongruence. Cladistics 10:315–319.
Felsenstein, J. 1988. Phylogenies from molecular sequences: inference and reliability. Annu. Rev. Genet. 22:521–565.
Felsenstein, J., and H. Kishino. 1993. Is there something wrong with the bootstrap of phylogenies? A reply to Hillis and Bull. Syst. Biol. 42:193–200.
Ferrari, L., M. López-Martínez, G. Aguirre-Díaz, and G. Carrasco-Núñez. 1999. Space-time patterns of Cenozoic arc volcanism in central Mexico: from the Sierra Madre Occidental to the Mexican Volcanic Belt. Geology 27:303–306.
García, G., G. Wlasiuk, and E. P. Lessa. 2000. High levels of mitochondrial cytochrome *b* divergence in annual killifishes of the genus *Cynoleibas* (Cyprinodontiformes, Rivulidae). Zool. J. Linn. Soc. 129:93–110.
Goldman, N., and S. Whelan. 2000. Statistical tests of gamma-distributed rate heterogeneity in models of sequence evolution in phylogenetics. Mol. Biol. Evol. 17:975–978.
Gómez-Tuena, A., and G. Carrasco-Núñez. 2000. Cerro Grande Volcano: the evolution of a Miocene stratocone in the early Trans-Mexican Volcanic Belt. Tectonophysics 318:249–280.
Hamilton, A. 2001. Phylogeny of *Limia* (Teleostei: Poeciliidae) based on NADH dehydrogenase subunit 2 sequences. Mol. Phylogenet. Evol. 19:277–289.
Hillis, D. M., and J. J. Bull. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Syst. Biol. 42:182–192.
Hillis, D. M., B. K. Mable, and C. Moritz. 1996. Applications of molecular systematics: the state of the field and a look to the future. Pp. 515–543 *in* D. M. Hillis, C. Moritz, and B. K. Mable, eds. Molecular systematics. Sinauer Associates, Sunderland, MA.
Hubbs, C. L. 1926. Studies of the fishes in the order Cyprinodontes. VI. Misc. Publ. Mus. Zool. Univ. Mich. 13:1–31.
Huelsenbeck, J. P. 2000. MrBayes: Bayesian inference of phylogeny. Distributed by the author, Dept. of Biology, Univ. of Rochester, Rochester, NY.
Huelsenbeck, J. P., and K. A. Crandall. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. Annu. Rev. Ecol. Syst. 28:437–466.
Huelsenbeck, J. P., and B. Rannala. 1997. Phylogenetic methods come of age: testing hypotheses in an evolutionary context. Science 276:227–232.
Huelsenbeck, J. P., D. M. Hillis, and R. Jones. 1996. Parametric bootstrapping in molecular phylogenetics: applications and performance. Pp. 19–45 *in* J. D. Ferraris and S. R. Palumbi, eds. Molecular zoology. Wiley-Liss, New York.
Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules. Pp. 21–132 *in* H. N. Munro, ed. Mammalian protein metabolism. Academic Press, New York.
Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111–120.
Knowlton, N., L. A. Weight, L. A. Solorzano, D. K. Mills, and E. Bermingham. 1993. Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the Isthmus of Panama. Science 260:1629–1632.
Kocher, T. D., J. A. Conroy, K. R. McKaye, J. R. Stauffer, and S. F. Lockwood. 1995. Evolution of NADH dehydrogenase subunit 2 in East African cichlid fish. Mol. Phylogenet. Evol. 4:420–432.
Luhr, J. F., and I. S. E. Carmichael. 1981. The Colima volcanic complex, Mexico. Part II. Late-Quaternary cinder cones. Contrib. Mineral. Petrol. 76:127–147.
Lydeard, C., and K. J. Roe. 1997. The phylogenetic utility of the mitochondrial cytochrome *b* gene for inferring relationships among Actinopterygian fishes. Pp. 285–304 *in* T. D. Kocher and C. A. Stepien, eds. Molecular systematics of fishes. Academic Press, San Diego, CA.
Lynch, J. F., D. B. Wake, and S. Y. Yang. 1983. Genic and morphological differentiation in Mexican *Pseudoeurycea* (Caudata: Plethodontidae), with description of a new species. Copeia 1983: 884–894.
Marshall, C. J., and J. K. Liebherr. 2000. Cladistic biogeography of the Mexican transition zone. Biogeography 27:203–216.
Meyer, A. 1993. Evolution of mitochondrial DNA in fishes. Pp. 3–38 *in* P. W. Hochachka and T. P. Mommsen, eds. Biochemistry and molecular biology of fishes. Elsevier, Amsterdam.
Meyer, M. K., A. C. Radda, R. Riehl, and W. Feichtinger. 1986. *Poeciliopsis baenschi* n. sp., un noveau taxon de Jalisco, Mexique (Teleostei, Poeciliidae). Rev. fr. Aquariol. 12:79–84.
Miller, R. R. 1960. Four new species of viviparous fishes, genus *Poeciliopsis* from northwestern Mexico. Occas. Pap. Mus. Zool. Mich. 433:1–9.
———. 1975. Five new species of Mexican poeciliid fishes of the genera *Poecilia*, *Gambusia*, and *Poeciliopsis*. Occas. Pap. Mus. Zool. Mich. 672:1–36.
Miller, R. R., and M. L. Smith. 1986. Origin and geography of the fishes of central Mexico. Pp. 487–517 *in* C. H. Hocutt and E. O. Wiley, eds. The zoogeography of North American freshwater fishes. John Wiley and Sons, New York.
Mulcahy, D. G., and J. R. Mendelson III. 2000. Phylogeography and speciation of the morphologically variable widespread species *Bufo valliceps*, based on molecular evidence from mtDNA. Mol. Phylogenet. Evol. 17:173–189.
Nelson, S. A., and I. S. E. Carmichael. 1984. Pleistocene to recent alkalic volcanism in the region of Sanganguey volcano, Nayarit, Mexico. Contrib. Mineral. Petrol. 85:321–335.
Ota, R., P. J. Waddell, M. Hasegawa, H. Shimodaira, and H. Kishino. 2000. Appropriate likelihood ratio tests and marginal distributions for evolutionary tree models with constraints on parameters. Mol. Biol. Evol. 17:798–803.
Palmer, R. H. 1926. Tectonic setting of Lago de Chapala. Pan-Am. Geol. 45:125–134.
Parenti, L. R. 1981. A phylogeographic analysis of cyprinodontiform fishes (Teleostei, Atherinomorpha). Bull. Am. Mus. Nat. Hist. 168:335–557.
Pielou, E. C. 1979. Biogeography. John Wiley and Sons, New York.
Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.
Ptacek, M. B., and F. Breden. 1998. Phylogenetic relationships among the mollies (Poeciliidae: *Poecilia*: *Mollienesia* group) based on mitochondrial sequences. J. Fish Biol. 1998:64–81.
Rambaut, A., and N. C. Grassly. 1997. Seq-Gen: an application for the Monte Carlo simulation of DNA sequence evolution along phylogenetic trees. Comput. Appl. Biosci. 13:235–238.
Rannala, B., and Z. Yang. 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. J. Mol. Evol. 43:304–311.
Regan, C. T. 1913. A revision of the cyprinodont fishes of the subfamily Poeciliinae. Proc. Zool. Soc. Lond. 11:977–1018.
Rodríguez, F., J. L. Oliver, A. Marín, and J. R. Medina. 1990. The general stochastic model of nucleotide substitution. J. Theor. Biol. 142:485–501.
Rosas-Elguera, J., and J. Urrutia-Fucugachi. 1998. Tectonic control of volcano-sedimentary sequence of the Chapala graben, western Mexico. Int. Geol. Rev. 40:350–362.
Rosen, D. E., and R. M. Bailey. 1963. The poeciliid fishes (Cy-

prinodontiformes), their structure, zoogeography, and systematics. Bull. Am. Mus. Nat. Hist. 126:1–176.
Ruedi, M., M. Auberson, and V. Savolainen. 1998. Biogeography of Sulawesian shrews: testing for their origin with a parametric bootstrap on molecular data. Mol. Phylogenet. Evol. 9:567–571.
Sanjur, O. I. 1998. Molecular systematics and evolution of fish in the genus *Poeciliopsis* (Cyprinodontiformes: Poeciliidae). Ph.D. diss., Rutgers University, New Brunswick, NJ.
Savage, J. M., and M. H. Wake. 2001. Reevaluation of the status of taxa of Central American caecilians (Amphibia: Gymnophiona), with comments on their origin and evolution. Copeia 2001:52–64.
Schmidt, T. R., J. P. Bielawski, and J. R. Gold. 1998. Molecular phylogenetics and evolution of the cytochrome *b* gene in the cyprinid genus *Lythrurus* (Actinopterygii: Cypriniformes). Copeia 1998:14–22.
Schultz, R. J. 1977. Evolution and ecology of unisexual fishes. Evol. Biol. 10:277–331.
Shimodaira, H., and M. Hasegawa. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol. Biol. Evol. 16:1114–1116.
Sivasundar, A., E. Bermingham, and G. Ortí. 2001. Population structure and biogeography of migratory freshwater fishes (*Prochilodus*: Characiformes) in major South American rivers. Mol. Ecol. 10:407–417.
Smith, M. L., T. M. Cavender, and R. R. Miller. 1975. Climatic and biogeographic significance of a fish fauna from the Late Pliocene-Early Pleistocene of the Lake Chapala Basin, Jalisco, Mexico. Pp. 29–38 *in* Studies on Cenozoic paleontology and stratigraphy in honor of Claude W. Hibbard. Vol. 12. Univ. of Michigan Papers in Paleontology.
Swofford, D. L. 1998. PAUP*: phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Sunderland, MA.
Tamayo, J. L., and R. C. West. 1964. The hydrography of Middle America. Pp. 84–121 *in* R. Wauchope and R. C. West, eds. Handbook of Middle American indians. Univ. of Texas Press, Austin, TX.
Thibault, R. E., and R. J. Schultz. 1978. Reproductive adaptations among viviparous fishes (Cyprinodontiformes: Poeciliidae). Evolution 32:320–333.
Tringali, M. D., T. M. Bert, S. Seyoum, E. Bermingham, and D. Bartolacci. 1999. Molecular phylogenetics and ecological diversification of the transisthmian fish genus *Centropomus* (Perciformes: Centropomidae). Mol. Phylogenet. Evol. 13:193–207.
Vrijenhoek, R. C. 1998. Conservation genetics of freshwater fish. J. Fish Biol. 53(Suppl. A):394–412.
Vrijenhoek, R. C., M. E. Douglas, and G. K. Meffe. 1985. Conservation genetics of endangered fish populations in Arizona. Science 229:400–402.
Wallace, P., I. S. E. Carmichael, K. Righter, and T. A. Becker. 1992. Volcanism and tectonism in western Mexico: a contrast of style and substance. Geology 20:625–628.
Webb, S. A. 1998. A phylogenetic analysis of the Goodeidae (Teleostei: Cyprinodontiformes). Ph.D. diss., University of Michigan, Ann Arbor, MI.
West, R. C. 1964. Surface configuration and associated geology of Middle America. Pp. 33–83 *in* R. Wauchope and R. C. West, eds. Handbook of Middle American indians. Univ. of Texas Press, Austin, TX.
Zhang, D.-X., and G. M. Hewitt. 1996. Nuclear integrations: challenges for mitochondrial DNA markers. Trends Ecol. Evol. 11: 247–251.
Zharkikh, A., and W.-H. Li. 1992. Statistical properties of bootstrap estimation of phylogenetic variability from nucleotide sequences. I. Four taxa with a molecular clock. Mol. Biol. Evol. 9: 1119–1147.

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