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# Genetic Divergence in the *Poecilia sphenops* Complex in Middle America

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**Key Word Index** – *Poecilia*; Poeciliidae; Pisces; isozymes; starch gel electrophoresis; allozymes; chemosystematics.

**Abstract** – Based on morphological and allozymic evidence, the *Poecilia sphenops* complex is an array of at least ten biological species ranging from Mexico to Venezuela (systematics are unclear south of Mexico) and not a single polytypic species as some authors have previously suggested. The allozyme data also suggest that the populations of mollies with tricuspid teeth on the Atlantic and Pacific coasts of Mexico now referred to as *P. sphenops (sensu stricto)* may represent at least two biological species. As some of the members of the complex are used as general research animals, experimental biologists should ascertain the specific identity of their stocks.

## Introduction

The shortfin mollies of the *Poecilia sphenops* complex are ubiquitous in fresh and brackish waters of Mexico, Middle America, northern South America and adjacent islands. The morphology of the components of this complex is often superficially similar. This similarity, coupled with the sometimes marked differentiation of some populations, has led to considerable taxonomic confusion, exemplified by two contending views of the systematics of the group. One view, tentatively advanced by Rosen and Bailey [1] and widely held among European biologists (e.g. Zeiske [2]; Parzefall [3–5]) envisions a single, highly variable polytypic species, *P. sphenops,* ranging from the Rio Grande drainage in north-eastern Mexico to coastal Venezuela. The second view (Schultz and Miller [6]; Miller [7, 8]; Bussing [9]) holds that the complex is an assemblage of nine or more biological species with partially overlapping ranges (Table 1). If the second view is correct, the complex would represent one of the major radiations of poeciliid fishes in North and Middle America.

Some of the members of the complex are used widely as experimental animals [2–5, 10–20]. Hence, resolution of this issue is especially important. For example, the cave molly used by Parzefall [15], Peters *et al.* [17] and Zeiske [2] has unicuspid inner teeth. Conclusions about the cave mollies were based on comparisons with surface mollies. However, it is difficult to determine if the surface forms were the unicuspid *P. mexicana* or the tricuspid *P. sphenops.* Erroneous conclusions could be drawn about the behaviour and physiology of cave mollies if *P. sphenops* was used as the surface form instead of *P. mexicana.*

In this paper we present the first substantial non-morphological evidence that many of the components of the complex are genetically distinct, thus arguing against a polytypic interpretation of this complex.

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## Results

Fourteen loci showed no detectable variation in any of the populations surveyed: *Adh* (alchohol dehydrogenase), *Ldh-1* (lactate dehydrogenase-1), *Ldh-3, Prv-1* (parvalbumin-1), *Prv-2, Prv-3, Fum* (fumarate-hydratase), *Gpd* (glyceraldehyde-3-phosphate dehydrogenase), *Idh-1* (isocitrate dehydrogenase-1), *Cpk-2* (creatine kinase-2), *Cpk-1, Ak-1* (adenylate kinase-1), *Aco-1* (aconitate hydratase-1), *Gpi-2* (glucosephosphate isomerase). Fifteen other loci showed electrophoretic variation (Table 2): *Aco-2, Agp* (alpha-glycerol

TABLE 1. DISTRIBUTION OF MORPHOLOGICAL FORMS IN *POECILIA* SUBGENUS *MOLLIENESIA*

| Taxa | Range | Collection sites* | Comments |
|---|---|---|---|
| *P. butleri* Jordan 1889 | Pacific coast, Río Fuerté, Mexico. S. to W. Guatemala | M66-18 near Tepic, Nayarit | Unicuspid teeth; M66-18 is N. of *P. sphenops* range |
| *P. catemaconis* Miller 1975 | Laguna Catemaco, Veracruz, Mexico | Not available | Tricuspid teeth; endemic to Laguna Catemaco, only *Poecilia* in the lake |
| *P. chica* Miller 1975 | Río Purificación, and adjacent streams, Jalisco, Mexico | M66-14 Río Apamila, Jalisco | Tricuspid teeth; endemic to R. Purificación and vicinity |
| *P. "gracilis"*† (cf. Regan 1913) | Lago de Petén, Guatemala | M71-29 Arroyo Ixiu, Trib. Lago de Petén | Unicuspid teeth; taxonomic status unclear at this time, perhaps a lacustrine derivative of *P. mexicana* (Fig. 2E and 2F); analysis based on offspring of one female |
| *P. latipunctata* Meek 1904 | Río Tamesí. Tamaulipas, Mexico | Not available | Endemic to R. Tamesí, unicuspid teeth |
| *P. mexicana* Steindachner 1863 | Río San Juan, Mexico, at least to Lago Izabal, Guatemala | M68-29 *P. mexicana* Veracruz, Mexico | Unicuspid teeth |
| | | •M77-39 *P. m. limantouri* Monterrey, Mexico | |
| | | M77-38 *P. mexicana* Corozal, Belize | |
| | | •M77-41 *P. mexicana* Siquirres, Costa Rica | Originally identified as *P. gilli* |
| | | •M77-42 *P. mexicana* Pensharst, Costa Rica | Originally identified as *P. gilli* |
| *P. orri* Fowler 1943 | Costal Yucatan Peninsula (Quintana Roo) S. and E. | M72-1 Belize River mouth, Belize | Unicuspid teeth; M72-1 is sympatric with *P. mexicana* |
| *P. sphenops* Valenciennes 1846 | Just N. of Veracruz to Río Coatzacoalcos, Mexico | M67-2 35 km S. of Veracruz L68-7 Laguna Isleta, W. of Minatitlan, Veracruz | Tricuspid teeth (Fig. 2B); M67-2 Atlantic slope form is sympatric with *P. mexicana* |
| | Pacific slope of Mexico and W. Guatemala | M76-20 Río Marques-Río Balsas near Nuevo Italia Michoacán | Tricuspid teeth; Pacific slope or R. Balsas form, sympatric with *P. butleri* |
| *P. sulphuraria* Alvarez 1948 | W. of Teapa, Tabasco, Mexico | Not available | Unicuspid teeth; inhabits sulphur springs, bright yellow fins |
| *P. vandepolli* van Lidth de Juede 1887 | Coastal Venezuela and the Netherlands Antilles | •M76-48 Curaçao | Unicuspid teeth (Fig. 2C and 2D) |

* • Indicates field samples; no circle indicates samples from laboratory stocks; the first figure given for each collection station is the year of collection.

† The taxonomic status of *Poecilia gracilis* Regan (proposed as a replacement name for *P. petenensis* Gunther) is not clear, the name is preoccupied by Poecilia gracilis Valenciennes, a synonym of *Cniesterodon decemmaculatus* (see [1], p. 77). Regan's fish is therefore referred to as Poecilia "gracilis".

phosphate dehydrogenase), *Cpk-3, Es-5* (esterase-5), *Got-1* (glutamate oxaloacetate transferase-1), *Got-2, Mdh-1* (malate dehydrogenase-1), *Mdh-2, Mdh-3, Gpi-1, Sod* (superoxide dismutase), *6Pgd* (6-phosphoglucose dehydrogenase), *Pgm-1* (phosphoglucomutase-1), *Cpk-1, Xdh* (xanthine dehydrogenase), and *Ldh-2.* Hemoglobins were not surveyed extensively, but there appeared to be three non-polymorphic hemoglobin loci in the few species examined. The resolution of *Idh-2, Es-4, Got-3, Ada* (adenosine deaminase) and *Ald* (aldolase) was poor and these loci were not included in the analysis. In all 29 loci were used in the analyses.

Although a small sample size was used, we feel that eight fish adequately represent any laboratory

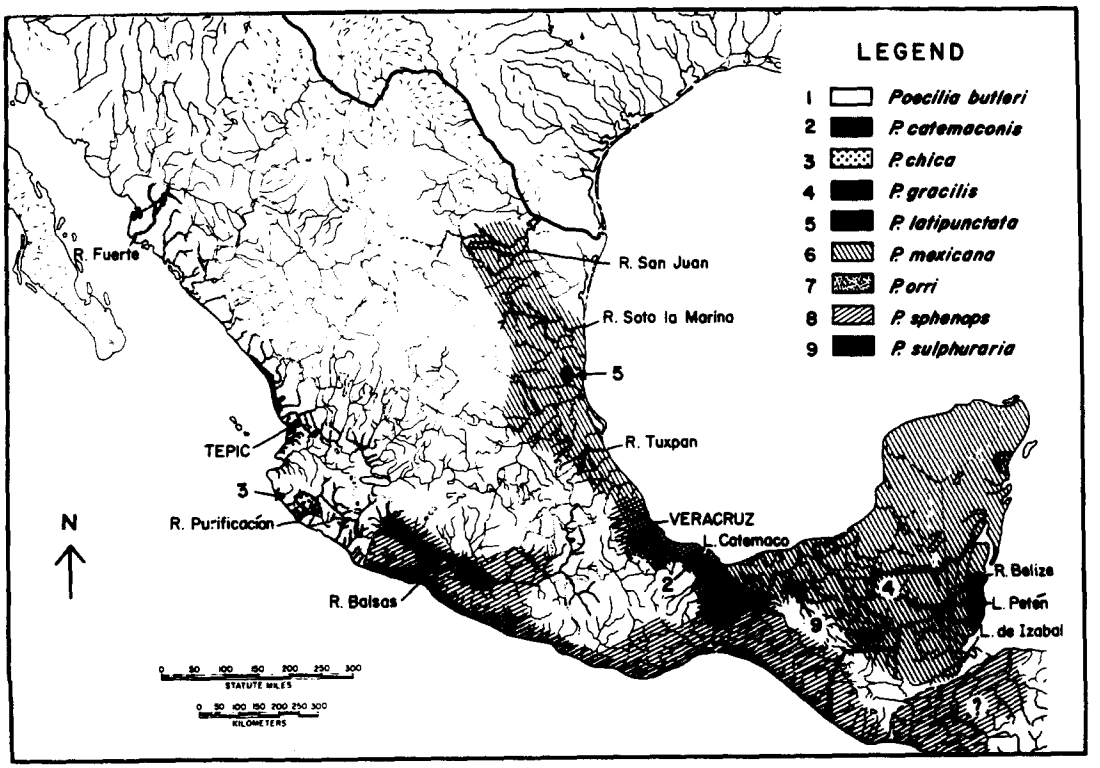


FIG. 1. DISTRIBUTIONS OF *POECILIA* IN MEXICO AND NORTHERN CENTRAL AMERICA.

stock. Although only 16 genomes represented a species, 29 loci were used. Nei [21] has shown that a large number of loci and a few individuals give a smaller variance between populations than a large sample size and a few loci. The laboratory stocks most probably adequately represent the field populations from which they came because: (a) the major alleles of laboratory stocks of the three most widespread species, *P. mexicana, P. sphenops,* (Atlantic) and *P. butleri*, are the same as those found at many locations in the wild [22]; (b) the alleles in the laboratory are still segregating (i.e. there are polymorphisms); (c) field studies of the most widespread species confirm intraspecific Nei similarity values of 0.94–0.99 [22]. *P. chica, P. vandepolli, P. orri* and *P. "gracilis"* occur over a much smaller geographical area (Fig. 1) and the allozymic variation in these fish is not expected to vary as much as in widespread species. The interspecific values (0.65–0.83) fall greatly below the intraspecific values, with *P. mexicana* vs *P. "gracilis"* being the only exception if one considers the Balsas *P. sphenops* to be specifically distinct. Based on this evidence, we conclude that *P. mexicana, P. sphenops, P. butleri, P. chica, P. orri* and *P. vandepolli* represent valid taxa at the specific level. The status of *P. "gracilis", P. gilli* and the Balsas form of *P. sphenops* remain in question as do several species which were not examined in this study (*P. catamaconis* Miller, 1975; *P. latipunctata*, both of which occur in restricted ranges on the Atlantic coast of Mexico). *P. latipinna* is the only sailfin molly that has been examined electrophoretically [23]. *P. velifera* Regan and *P. petenensis* Gunther, the other two sailfins, have yet to be examined. Nei similarity values (*I*) between *P. mexicana* laboratory stocks collected in 1968 and 1979 field collections from the same geographic area (Veracruz, Mexico) were $0.971 \pm 0.00$. Average *I* values between the

TABLE 2. GENE FREQUENCIES OF SPECIES OF *POECILIA*, SUBGENUS *MOLLIENESIA*

| | *Agp* | | | *Est-5* | | | *Got-1* | | | | | | | *Got-2* | | | | | | | *Mdh-1* | | | *Mdh-2* | | *Mdh-3* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | a | b | c | d | e | f | a | b | c | d | e | f | a | b | c | d | e | f | g | a | b | c | a | b | a | b | c |
| *P. butleri* M66-18 | | 1 | | | | 0.75 | 0.25 | | | | | | | | 1 | | | 1 | | | | | 1 | | | 1 | | 1 | | |
| *P. chica* M66-14 | | 1 | | | | 0.86 | | | 0.14 | 0.70 | | 0.30 | | | | | | | | | 0.64 | 0.36 | 1 | | | | 1 | | 1 | |
| *P. gracilis* M71-29 | 1 | | | | | | 0.86 | | 0.14 | 0.58 | | 0.42 | | | | | | | | | 1 | | | | 1 | 1 | | 1 | | |
| *P. mexicana* M68-29 | | 1 | | | | | 1 | | | 1 | | | | | | | | | | | | 1 | | | 1 | 1 | | 1 | | |
| *P.m. limantouri* *M77-39 | | 1 | | | | | 1 | | | 1 | | | | | | | | | | | | 1 | | | 1 | 1 | | 1 | | |
| *P. mexicana* Belize M77-38 | | 1 | | | | | 0.67 | 0.33 | | 0.28 | | 0.72 | | | | | | | | | 0.10 | | 0.90 | 0.16 | | 0.84 | 1 | | 1 | |
| *P. mexicana* *(*P. gilli?*) M77-41 | | 1 | | | | | 1 | | | 1 | | | | | | | | | | | | 1 | | | 1 | 1 | | 1 | | |
| *(*P. gilli?*) M77-42 | | 1 | | | | | 1 | | | 1 | | | | | | | | | | | | 1 | | | 1 | 1 | | 1 | | |
| *P. orri* M72-1 | | | 1 | | | | 0.83 | | 0.17 | | | 1 | | | | | | | | | 1 | | | 1 | | 1 | | 1 | | |
| *P. sphenopds* Balsas M76-20 | | 1 | | 0.13 | 0.87 | | | | | | | | | 1 | | | | 1 | | | | | | | 1 | | 1 | | 1 |
| Atlantic-1 M67-2 | | 1 | | 0.37 | 0.43 | | | | | | | 1 | | | | 1 | | | | | | | 1 | | 1 | | 1 | | |
| Atlantic-2 L68-7 | | 1 | | | | | | 1 | | | | 1 | | | | | | | 0.83 | 0.17 | | | 1 | | 1 | | 1 | | |
| *P. vandepolli* *M76-48 | | 1 | | | | | 1 | | | | | | 1 | | | | | | | | 1 | | 1 | | 1 | | 1 | | |

TABLE 2 - *CONTINUED*

| | *6Pgd* | | | *Gpi-1* | | | *Sod* | | | | | | *Pgm-1* | | | | *Cpk-3* | | *Xdh1* | | | | | *Ldh-2* | | *Aco-2* | | | *P* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | a | b | c | a | b | c | d | e | f | a | b | c | d | a | b | a | b | c | d | e | i | m | a | b | c | |
| *P. butieri* M66-18 | 0.38 | 0.62 | | | 1 | | | 1 | | | | | 0.67 | 0.33 | | | 1 | | | | | 1 | | | 1 | | | | 0.11 |
| *P. chica* M66-14 | 0.50 | 0.50 | | | 1 | | 1 | | | | | | | | | 1 | 1 | | | 1 | | | | | 1 | | | | 0.14 |
| *P. gracilis* M71-29 | | 1 | | | 1 | | | | | | 1 | | | 1 | | | 1 | | 1 | | | | | | 1 | | | | 0.07 |
| *P. mexicana* M68-29 | | 1 | | | 1 | | | | | 0.07 | 0.93 | | | 1 | | | 1 | | | | | 1 | | | 1 | 0.12 | 0.88 | | 0.07 |
| *P.m. limantouri* *M77-39 | | 1 | | | 1 | | | | | | 1 | | | 1 | | | 1 | | – | | | | | 0.88 | 0.12 | | 1 | | 0.04 |
| *P. mexicana* Belize M77-38 | 0.20 | 0.80 | | | 1 | | | | | | 1 | | | 1 | | | 1 | | – | | | | | | 1 | | 0.95 | 0.05 | 0.21 |
| *P. mexicana* *(*P. gilli?*) M77-41 | 1 | | | | 1 | | | | | | 1 | | | 1 | | | 1 | | – | | | | | | 1 | | 0.12 | 0.88 | 0.04 |
| *(*P. gilli?*) M77-42 | | 1 | | | 1 | | | | | | 1 | | | 1 | | | 1 | | – | | | | | | 1 | | 1 | | 0.00 |
| *P. orri* M72-1 | | 1 | | | 1 | | | | | | | 1 | | 1 | | | 1 | | | 1 | | | | | 1 | | | | 0.03 |
| *P. sphenops* Balsas M76-20 | | | 1 | | | 1 | | 1 | | | | | | | 1 | | | 1 | | 0.37 | 0.63 | | | | 1 | | | | 0.07 |
| Atlantic-1 M67-2 | 0.36 | 0.64 | | 1 | | | | 0.70 | | | 0.30 | | | | 1 | | 1 | | | 0.24 | | 0.76 | | | 1 | | | | 0.14 |
| Atlantic-2 L68-7 | 0.44 | 0.56 | | – | | | | 1 | | | | | | | 1 | | 1 | | | | | 0.44 | 0.56 | | 1 | | | | 0.11 |
| *P. vandepolli* *M76-48 | 1 | | | | | 1 | | 1 | | | | | | | | 1 | 1 | | | | 1 | | | | 1 | | | | 0.00 |

*P* refers to percent of the total number of loci that are polymorphic; M66-18, M66-14, etc. refer to collection sites; asterisks indicate field samples; no asterisk indicates samples from laboratory stocks.

TABLE 3. GENETIC DISTANCES (*D*) AMONG SPECIES OF THE *POECILIA SPHENOPS* COMPLEX ARE GIVEN ABOVE THE DIAGONAL, AND GENETIC IDENTITIES (*I*) BELOW THE DIAGONAL

| | butler M66-18 | chica M66-14 | gracil M71-29 | mexica M68-29 | mexica M77-39 | mexica M77-38 | mexica M77-41 | mexica M77-42 | orri M72-1 | spheno M76-20 | spheno M67-2 | spheno L68-7 | vandep M76-48 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *P. butleri* M66-18 | | 0.28 | 0.34 | 0.03 | 0.32 | 0.25 | 0.28 | 0.28 | 0.30 | 0.52 | 0.26 | 0.29 | 0.33 |
| *P. chica* M66-14 | 0.76 | | 0.31 | 0.25 | 0.31 | 0.25 | 0.27 | 0.27 | 0.29 | 0.37 | 0.30 | 0.31 | 0.19 |
| *P. gracilis* M71-29 | 0.71 | 0.73 | | 0.12 | 0.13 | 0.10 | 0.10 | 0.10 | 0.19 | 0.60 | 0.34 | 0.37 | 0.31 |
| *P. mexicana* M68-29 | 0.74 | 0.78 | 0.88 | | 0.03 | 0.03 | 0.04 | 0.04 | 0.24 | 0.51 | 0.30 | 0.34 | 0.29 |
| *P. mexicana* *M77-39 | 0.73 | 0.73 | 0.87 | 0.97 | | 0.05 | 0.06 | 0.08 | 0.26 | 0.58 | 0.32 | 0.36 | 0.35 |
| *P. mexicana* M77-38 | 0.78 | 0.78 | 0.90 | 0.97 | 0.95 | | 0.06 | 0.07 | 0.20 | 0.50 | 0.25 | 0.27 | 0.25 |
| *P. mexicana* *M77-41 | 0.75 | 0.76 | 0.90 | 0.97 | 0.94 | 0.94 | | 0.08 | 0.23 | 0.53 | 0.24 | 0.32 | 0.32 |
| *P. mexicana* *M77-42 | 0.75 | 0.75 | 0.90 | 0.93 | 0.92 | 0.93 | 0.93 | | 0.25 | 0.53 | 0.29 | 0.32 | 0.32 |
| *P. orri* M72-1 | 0.74 | 0.75 | 0.83 | 0.79 | 0.77 | 0.82 | 0.80 | 0.80 | | 0.58 | 0.25 | 0.26 | 0.23 |
| *P. sphenops* M76-20 Balsas | 0.59 | 0.69 | 0.55 | 0.60 | 0.56 | 0.60 | 0.59 | 0.59 | 0.56 | | 0.44 | 0.47 | 0.34 |
| *P. sphenops* M67-2 Atlantic | 0.77 | 0.74 | 0.71 | 0.74 | 0.72 | 0.78 | 0.79 | 0.75 | 0.78 | 0.64 | | 0.08 | 0.25 |
| *P. sphenops* L68-7 Atlantic | 0.75 | 0.73 | 0.69 | 0.71 | 0.70 | 0.76 | 0.72 | 0.72 | 0.77 | 0.62 | 0.93 | | 0.25 |
| *P. vandepolli* *M76-48 | 0.72 | 0.83 | 0.73 | 0.70 | 0.70 | 0.78 | 0.73 | 0.73 | 0.75 | 0.71 | 0.78 | 0.78 | |

M66-18, M66-14, etc. refer to collecting sites; asterisks indicate field samples; no asterisk indicates samples from laboratory stocks.

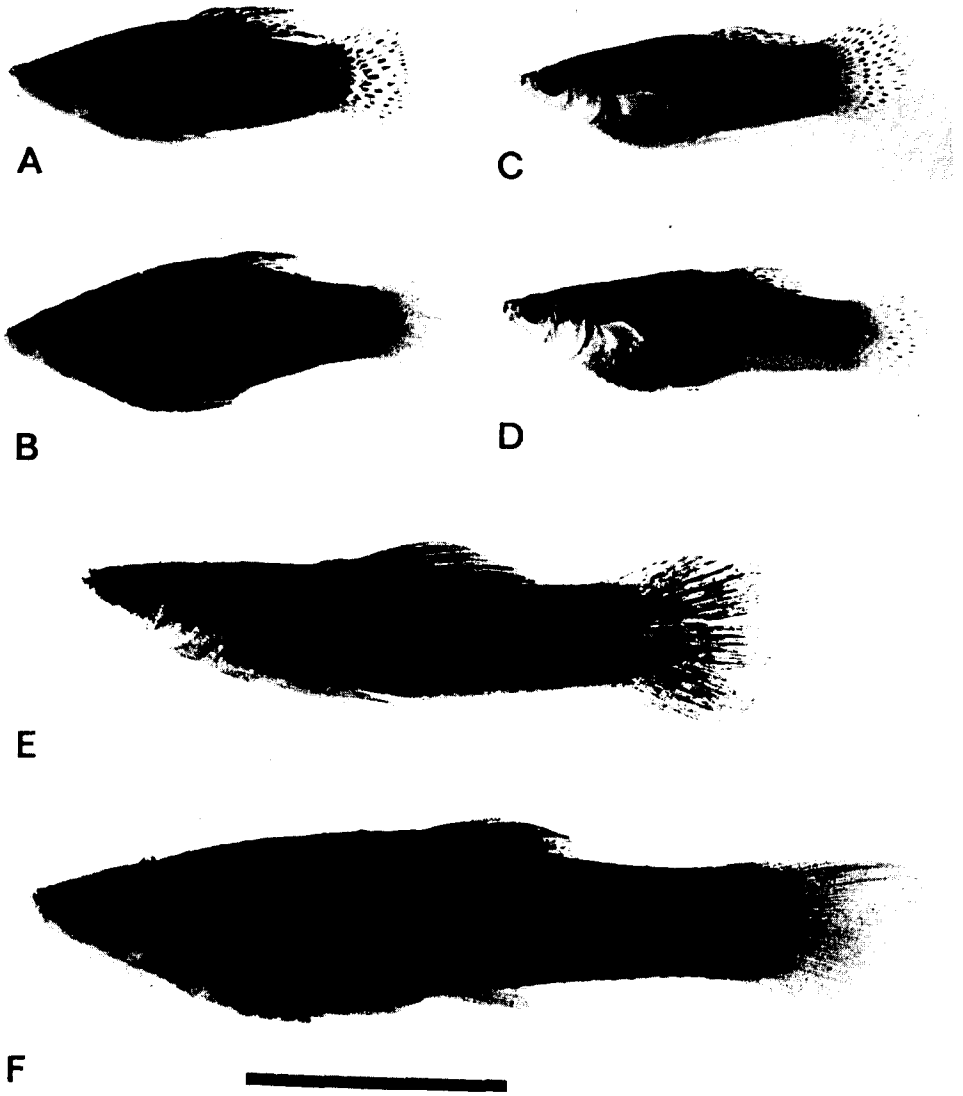


FIG. 2. REPRESENTATIVES OF THE *POECILIA SPHENOPS* COMPLEX ARE FIGURED HERE. Recent photographs of other species in the complex have been figured in ref. [6] (*P. mexicana* and *P. sphenops*); ref. [18] (*P. mexicana*); ref. [8] (*P. catamaconis* and *P. chica*). (A) *Poecilia orri* from San Roque Creek, Belize UMMZ 202029, male 36.7 mm, (B) female, 44.6 mm; (C) *P. vandepolli* from Pescadera Bay beach, Curaçao UMMZ 19927, 2, male 36.5 mm, (D) female 42.2 mm; (E) *P. "gracilis"* from Laguna de Petén, Guatemala UMMZ 143710, male 58.0 mm, (F) female, 83.6 mm. Scale bar = 30 mm.

Veracruz *P. sphenops* laboratory stocks (collected in 1967 and 1968) and the 1979 Veracruz field collections were $0.943 \pm 0.02$. Between drainage *I* values range from 0.96 to 0.98 in *P. mexicana* and 0.95 to 0.98 in *P. sphenops* (22). The average *I* value between 24 field populations (500 individuals) of *P. mexicana* and 22 field populations (365 individuals) of *P. sphenops* was $0.721 \pm 0.01$. The average *I* value between one laboratory population of *P. mexicana* and two of *P. sphenops* was $0.725 \pm 0.02$.

The morphologically most similar mollies (*P. mexicana, P. sphenops* and *P. butleri*) are genetically quite distinct (Tables 2 and 3). Although there were very few polymorphisms within a presumptive species, the interspecific differences were great. The *P. sphenops* sample from the Balsas drainage on the Pacific coast of Mexico had unique alleles at 10 of the 29 loci examined. *P. vandepolli* (Fig. 2C and 2D) from Curaçao had no polymorphic loci although several alleles showed fixed differences from the other species. *P. mexicana mexicana* and *P. mexicana limantouri* share all alleles. There are frequency differences, however, at *Aco-1* and *Ldh-2*.

For percentage of polymorphic loci (*P* values, see Table 2), 14 of the 29 loci examined vary interspecifically, but within a species, only 0–5 loci were polymorphic. *P. mexicana* from Belize had the largest number of polymorphisms with 6 of 29 loci polymorphic. The M77-42 stock of *P. mexicana* from Costa Rica and the *P. vandepolli* stock (the only island population analysed) showed no polymorphisms and no unique alleles.

*Sod, 6Pgd, Mdh-1, Mdh-2* and *Mdh-3* each show unique allelic patterns for many of the species (Table 2). There are marked interspecific differences in the Nei identity and distance values [24], but few differences among populations of the same species (Table 3), except for *P. sphenops*, which may represent more than one species.

Distributional (Atlantic vs Pacific drainages) or morphological criteria (unicuspid vs tricuspid inner teeth) did not cluster the samples in groups with greater than average similarity (see Table 4, and Fig. 3).

TABLE 4. MEAN VALUES OF NEI IDENTITY VALUES

| | X | SD | SE | N |
|---|---|---|---|---|
| Interspecific all species | 0.725 | 0.080 | 0.015 | 28 |
| Tricuspid species | 0.630 | 0.090 | 0.050 | 3 |
| Unicuspid species | 0.758 | 0.056 | 0.019 | 9 |
| Atlantic coast species | 0.790 | 0.067 | 0.027 | 6 |
| Pacific coast species | 0.675 | 0.085 | 0.050 | 3 |
| Average of each species with all other species | | | | |
| *P. butleri* | 0.719 | 0.060 | 0.023 | 7 |
| *P. chica* | 0.750 | 0.042 | 0.016 | 7 |
| *P. gracilis* | 0.734 | 0.107 | 0.041 | 7 |
| *P. mexicana* | 0.751 | 0.090 | 0.034 | 7 |
| *P. orri* | 0.743 | 0.076 | 0.029 | 7 |
| *P. sphenops* (Balsas) | 0.617 | 0.053 | 0.020 | 7 |
| *P. sphenops* (Atl.) | 0.733 | 0.053 | 0.020 | 7 |
| *P. vandepolli* intraspecific | 0.750 | 0.042 | 0.016 | 7 |
| *P. mexicana* | 0.948 | 0.018 | 0.006 | 10 |
| *P. sphenops* | 0.730 | 0.173 | 0.100 | 3 |
| *P. sphenops* no Balsas pop. | 0.930 | | | 1 |

Mean (X), Standard Deviation (SD), Standard Error of the Mean (SE), Number of Comparisons (N).

## Discussion

The widespread mollies of the *Poecilia sphenops* species complex have posed a taxonomic problem for the past century. Morphologically, all presumptive species strongly resemble one another (Fig. 2). Allozymic information apparently can serve as a tool for interpreting the evolutionary relationships in the *P. sphenops* complex, or at least for distinguishing the species. The results of an allozyme analysis of ten populations of seven presumptive species showed that each presumptive species was defined by relatively non-variable "diagnostic" alleles (Tables 2 and 3). Although laboratory animals do not provide information on the geographical variation of a species, comparison of our stocks with field-caught samples showed that the laboratory stocks provided good approximations to the species as a whole. Such evidence is concordant with the findings of Nei [21] and Gorman and Renzi [25], who showed that if geographical variation is small within a species then allozymic data from small samples can validly represent species for interspecific comparisons. Our allozymic data support the concept of a multispecific complex [8] rather than a single, morphologically variable species. Allozyme data also support the specific status of *P. orri* and *P. vandepolli* (Fig. 1), both of which have been either unrecognized or treated as subspecies of *Poecilia sphenops* [1].

One surprising result of our study was the number of divergent alleles detected in the *P. sphenops* population from the Balsas drainage. The Nei identity values between the Balsas

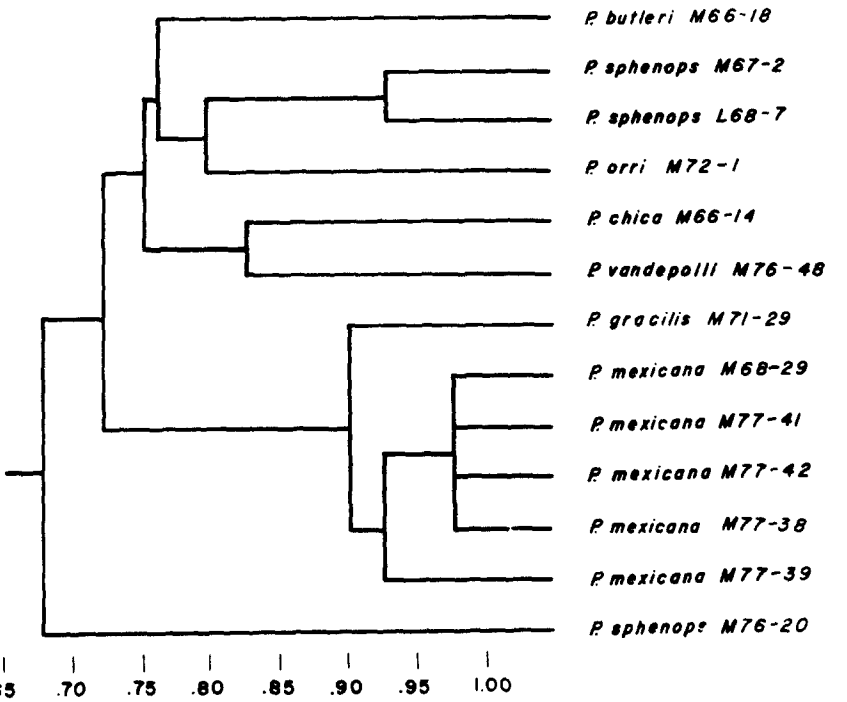


FIG. 3. THIS CLUSTER MAP IS PRESENTED AS A VISUAL REPRESENTATION OF TABLE 3 BASED ON NEI IDENTITY VALUES. No evolutionary relationships are implied. This figure was constructed using SAS hierarchical clustering technique [43].

population and the other populations were unusually low, falling between 0.50 and 0.65 (Tables 3 and 4). Several authors [6, 27] have previously suggested that the Río Balsas populations are morphologically different from other Mexican *P. sphenops*. Our Balsas population is clearly distinct from the other mollies examined. However, we have not yet examined other populations of *P. sphenops* from the Pacific drainages and at present do not know if the distinctiveness of the Río Balsas sample reflects specific level divergence or extreme geographic variation. In addition, morphological data [6] suggest three tricuspid forms on the Pacific slope of Mexico, instead of merely one form.

South of the Mexican border the systematics of the *P. sphenops* complex are still unclear. Faunas disturbed by tectonic activity and stream capture complicate the picture [26]. Allozymic data may eventually clarify this issue.

The allozymic distinctiveness of the members of the *P. sphenops* complex and their interfertility in the laboratory suggest that the complex will be a useful genetic system for mapping large numbers of allozyme loci as has been done with *Xiphophorus* [28].

Much evidence suggests that genomes are essentially mosaic with respect to their patterns of evolutionary change; in many groups there is almost no correlation among morphological, allozymic, chromosomal or other types of genetic differentiation [29–36]. The shortfin mollies provide yet another example of this phenomenon. Their morphological divergence has been, on the whole, minimal and they are interfertile in the laboratory, but the allozyme data indicate that their evolution has involved marked genetic divergence on at least one level of organization.

## Experimental

Allozyme surveys were conducted on ten populations of seven presumptive species representing field-caught specimens and laboratory stocks maintained at The University of Michigan Museum of Zoology (Table 1). Three stocks of *P. sphenops* were used; two from the Atlantic slope and one from the Pacific slope of Mexico. *P. vandepolli* (collection site M76-48, Table 1 contains locality information) from Curaçao, *P.*

*mexicana limantouri* [37], and *P. mexicana* (cf. *P. gilli*, M77-41, M77-42) were field-collected and maintained alive until tissue extracts were prepared; eight fish were scored for each enzyme (Table 2). Enzyme staining solutions and buffers routinely used in Bruce J. Turner's laboratory for allozyme analyses using starch gel electrophoresis were similar to those in general use [38–42]. Field specimens were frozen on dry ice and held at −90° until extracts of tissues could be prepared. Laboratory animals were stored at −90° for later dissection or were frozen at 0° and dissected within 2 h. Extracts for electrophoresis were prepared in advance and stored at −90°; immediately prior to electrophoresis, they were thawed and centrifuged at 15 000–20 000 *g* at 4° for 20–30 min. Alleles were assigned letters according to their mobility, the slowest alleles being *a*; a1, a2, etc. were used to designate alleles between a and b. Loci were assigned numbers according to their mobility; the slowest anodal locus was designated 1, a more anodal locus would then be 2.

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