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Morphometric and genetic characterization of tilapia (Cichlidae: Tilapiini) stocks for effective fisheries management in two mexican reservoirs

Caracterización morfométrica y genética de stocks de tilapias (Cichlidae: Tilapiini) para un efectivo manejo de sus pesquerías en dos presas mexicanas

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RESUMEN

Varias especies de tilapias fueron introducidas a la presa El Infiernillo durante los años sesenta del siglo pasado. Posterior a dichas introducciones, la presa ha sido uno de los sitios importantes de producción nacional para peces. Sin embargo, bajas recientes en las capturas, sugieren que las estrategias de manejo y las prácticas de explotación requieren de ser revisadas. Hasta ahora, dichas prácticas de manejo están limitadas por la falta de métodos para evaluar el status de dichas especies introducidas. En éste trabajo se caracterizan morfométricamente dos géneros y tres especies de dos sitios en la presa El Infiernillo y de un sitio de la presa Zicuirán. Las muestras fueron caracterizadas con 14 *loci* aloenzimáticos y dos marcadores mitocondriales ADN-RFLP, *r16S* y *citocromo b*. Basados en estos marcadores morfológicos y genéticos, se identificó a *Tilapia rendalli* y *Oreochromis aureus* en la presa El Infiernillo y a *Oreochromis mossambicus* para Zicurán. Las especies se separaron en dos géneros en base a diferencias en el número de escamas predorsales y a la morfología del diente faríngeo. Para *Oreochromis*, las especies se separaron con seis variables morfométricas. Los resultados de aloenzimas y PCR-RFLP del mtADN concuerdan con estas diferencias morfológicas. Además, dos poblaciones de *O. aureus* en la presa El Infiernillo parecen morfológica y genéticamente diferentes. Los niveles de diversidad genética (H_e = 0.0567-0.2299 y h = 0.0000-0.4848) son bajos comparados con los reportados para poblaciones manejadas adecuadamente. Dados los niveles de variación de estas poblaciones, sugerimos estrategias de manejo adecuadas.

Palabras clave: Infiernillo, variación genética, tilapias, Zicuirán.

ABSTRACT

Several tilapia species were introduced to the Infiernillo Dam during the 1960's. Following this introduction of tilapia, the dam has been an important fish production site in Mexico. However, recent decreases in tilapia catches suggest that fish management strategies and tilapia exploitation practices need to be revised. To date, management practices are limited by the lack of methods to evaluate the status of these introduced species. Here we morphometrically characterized two genera and three species from two sites in the Infiernillo Dam and a site in the Zicuiran Dam. The samples were characterized for 14 allozyme *loci* and two mitochondrial DNA-RFLP markers, *r16S* and *cytochrome b*. Based on these morphological and genetic markers, we identified *Tilapia rendalli* and *Oreochromis aureus* in the Infiernillo Dam, and *Oreochromis mossambicus* at the Zicuiran reservoir. These species were separated into genera based on differences in the number of predorsal scales and the pharyngeal teeth morphology. For *Oreochromis*, they were further classified into species using six morphometric variables. The allozyme and mtDNA-RFLP results are concordant with

the morphological differences. Furthermore, two populations of *O. aureus* in the Infiernillo Dam appear genetically and morphologically distinct. The levels of genetic variability (H_e = 0.0567-0.2299 and h = 0.0000-0.4848) are low compared to properly managed tilapia populations. Given the levels of variation in these populations, we suggest sustainable genetic management strategies.

Key words: Genetic variation, Infiernillo, Tilapias, Zicuiran.

INTRODUCTION

Tilapias were introduced into Mexico to facilitate the availability of inexpensive, high protein, and low-fat food staples. *Oreochromis aureus* Steindachner 1864, and *Tilapia rendalli* Boulenger 1897, were first introduced into the country in 1964 and 1974, respectively (www.fao.org/fishery/introsp/1866-2598), whereas *O. mossambicus* Peters 1852 was first introduced into the country in 1964 from Auburn University, Alabama (Morales, 1991). Following their introductions, these tilapia species have become widely distributed throughout natural and artificial water reservoirs in tropical and temperate regions of Mexico (Arredondo, 1983). Tilapias (3,385 fry) were first introduced into the Adolfo Lopez Mateos Infiernillo Dam in 1969. In 1987, the tilapias in the dam reached its maximum production of 18,953 metric tons, which made it the main source of tilapia in Latin America (Guzmán Urioestegui, 1994). However, during the late 1990's this tilapia-based fishery faced a drastic decrease in production due to overexploitation (Jiménez-Badillo, 1999) and inbreeding. In contrast, the Zicuiran Dam has been maintained as a lower profile fishery. Records show that in the late 1990's, these two dams were stocked with fry from the same tilapia fry production farm (TFPF). However, Mexican tilapia fisheries have few records to show which among the species and strains were introduced. To enhance the fish stocks and to aid in developing fisheries management legislation, monitoring genetic stock diversity is essential (Bentzen & Thodesen, 2005; Haughton *et al.*, 2006). Genetic monitoring also reveals the past and present genetic variation within the managed fisheries. Because the two dams have similar genetic histories, we studied the fish populations from both dams.

The most recent genetic studies on tilapia species focused on reconstructing phylogenetic relationships between species (Pouyaud & Agnëse, 1995; Sodsuk *et al.*, 1995; Feresu-Shonhiwa & Howard, 1998; Klett & Meyer, 2002; Salzburger *et al.*, 2002)

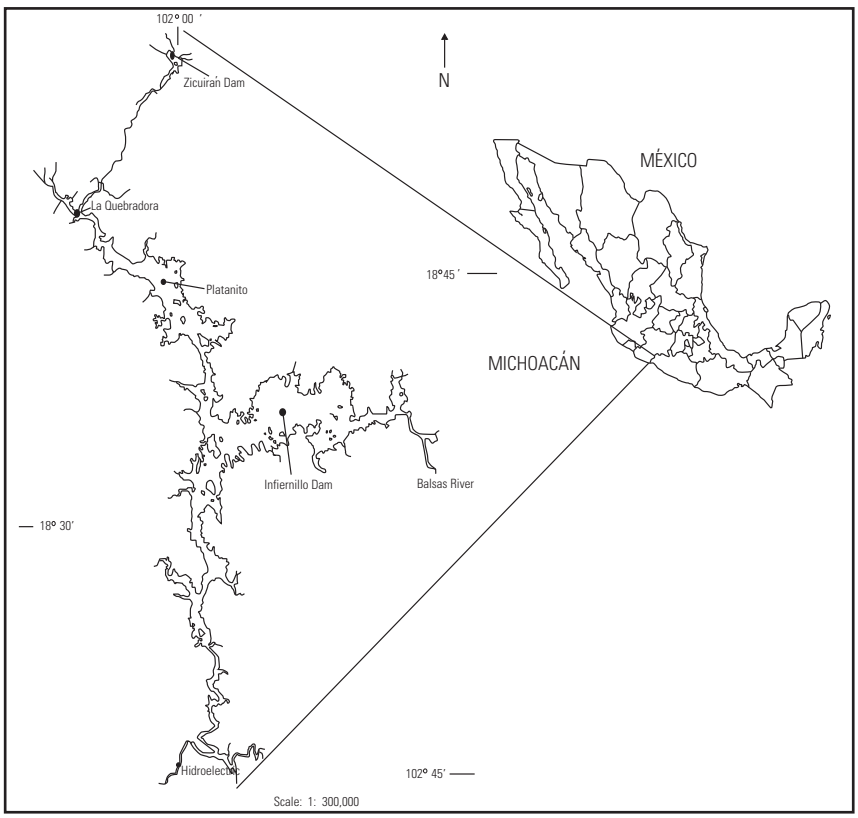

Figure 1. Map of the study area including both the Infiernillo and Zicuirán Dams. The collecting sites of the tilapia samples included in this study are indicated.

while few have focused on tilapia population genetics (Rognon & Guyomard, 1997; Wilson *et al*., 2000; De Silva, 2004). One relevant population genetics study found low genetic variation in farmed and wild tilapia populations, including a sample from Infiernillo, and suggested a monitoring program to improve management practices (Barriga-Sosa *et al*., 2004). To gain a better perspective on the genetic variation in two managed populations, Infiernillo and Zicuirán Dams, we characterized the morphometric and genetic variation of three putative species of tilapia. With these data, we can better understand the history and species composition of these populations. Based on the current genetic variation, we can help develop informed management strategies. We have three objectives for this study: 1) identify which species are present; 2) determine the morphometric and genetic variation within and between populations; and 3) determine the population genetic structure and evaluate if the populations interbreed.

MATERIALS AND METHODS

Study sites. The Infiernillo Dam (120 km in length and 34,600 hectares, surface area) was built in 1963 within the limits of Michoacán and Guerrero states (18º 52' 00" N, 18º 11' 00" N, 101º 03' 00" W and 102º 07' 00" W). The Infiernillo is part of the hydrological region "Cuenca del Río Balsas" and draws 74.64 % of the water in the system (Jiménez-Badillo, 1999). It receives water from the Tepalcatepec, Cupatitzio, Del Marquez, Paso de las Crucitas or Pinzandarán, Balsas, Cutzamala, Tacambaro and Huetamo rivers and from the Churumuco streams (Fig. 1). The Zicuiran Dam (490 hectares, 4.9 km maximum length, and 30 m maximum depth; 18° 56' 15'' N, 101° 55' 30'' W) gets its water from the Zicuiran river, the permanent tributaries El Huamito and El Conguripo and the temporal stream La Manga (Aguilera-Reyes, pers. comm.).

Tilapia sampling. A total of 248 fish were collected between 1999 and 2000 from three collecting sites. From two commercial catch sites at the Infiernillo Dam, 188 organisms were caught (El Platanito (P), N = 79 and La Quebradora (Q), N = 119). The remaining 50 organisms were obtained from culture cages at the Zicuiran Dam (Z, N = 50) (Fig. 1). The three locations are separated by 13-21 km.

Fish were caught with gill nets with a mesh size of 3¼". The fish were immediately frozen in dry ice (-80ºC) and transported to the Laboratorio de Genética y Biología Molecular de la Planta Experimental de Producción Acuícola (PExPA), Universidad Autónoma Metropolitana Unidad Iztapalapa for processing.

Morphological identification of tilapias and variation. Each collection site was considered *a priori* as a discrete group. Fish were first assessed as to whether it belongs to either the genus *Tilapia* or *Oreochromis* following established criteria (Trewavas, 1983) and analysis of the morphology of the pharyngeal teeth (*Oreochromis* - monocuspid and bicuspid and *Tilapia* – tricuspid). To identify fish samples at the species level, we assessed 21 morpho-

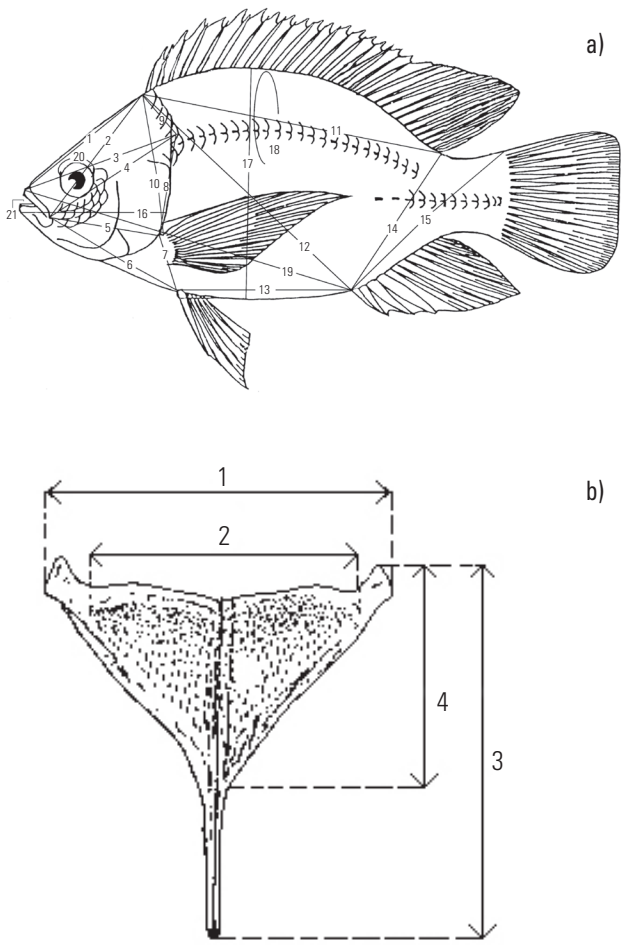

Figure 2 a-b. a) Twenty-one morphometric measurements recorded for each sample (Barriga *et al.,* 2004). b) TPB variables: 1) total width; 2) width of the toothed area; 3) total length; and 4) length of the toothed area (modified from Vreven *et al.,* 1998).

metric variables (*M*) (Vreven *et al*., 1998; Barriga-Sosa *et al*., 2004). All *M* characters were measured to the nearest 0.1 mm using digital calipers (Fig. 2a). The five meristic variables (*m*) recorded for each sample were: a) number of scales of the inferior lateral series and b) number of predorsal scales, c) number of gill rakers (first gill raker), d) number of spines and e) rays of dorsal fin (Barriga-Sosa *et al*., 2004). Once the species are identified, the following morphological variables of the toothed pharyngeal bone (TPB) were assessed to confirm the identity and record morphological variation: a) total width (*ttw*), b) width of the toothed area (*wta*), c) total length (*ttl*), and d) length of the toothed area (*lta*) (Fig. 2b) (Arredondo-Figueroa & Tejeda-Salinas, 1989). An American Optical Stereo Scope microscope model 569 (3.0x) was used to measure the morphological variables of the TPB and the meristic characters.

The *M* (continuous) and *m* (discrete) data were analyzed separately. To evaluate if the data have equal variances, a Bartlett test was done prior to further analyses. The parameters

Table 1. Nomenclature and buffers used for the different enzymes assayed.

Enzyme	E.C number	*Locus* Abbreviation	Tissue
Aspartate amino transferase	2.6.1.1	Aat-1 Aat-2	h h
Esterase	3.1.1.1	Est-1 Est-2	l l
Phosphoglucomutase	5.4.2.2	Pgm	l
Glucose-6-phosphate isomerase	5.3.1.9	Gpi-1 Gpi-2 Gpi-3	h h h
Glycerol-6-phosphate dehydrogenase	1.1.1.49	G6pdh	l
Isocitrate dehydrogenase	1.1.1.42	Idh	m
L-lactate dehydrogenase	1.1.1.27	Ldh	m
Malate dehydrogenase	1.1.1.37	sMdh-1 sMdh-2	h h
Xantin dehydrogenase	1.1.1.204	Xdh	m

Buffers: CAAPM = Citric acid-morpholine pH = 7.0; TG = Tris-glycine pH 8.5. Tissue extracts with constant activity: liver (l), muscle (m) and heart (h).

of the allometric ratios between total length (considered as the independent variable) and the remaining 20 morphometric measures as dependent variables were calculated as follows: $Y_{ij} = aiTlj^{bi}$, where *Tlj* is the total length of organism *j* ; *Yij* is variable *i* of organism *j;* and *ai* and *bi* are the parameters of the allometric ratio between *Tl* and variable *i.* To eliminate differences related to size, morphometric data were transformed according to three different criteria (Barriga-Sosa *et al.*, 2004): i) proportion, representing each character relative to length; ii) logarithmic transformation of each character and iii) normalization of individuals of each group (Lombarte & Lleonart, 1993). Original data (*Mo*) were also analyzed. To account for unequal variances, the TPB data were logarithmically transformed. To compare the TPB data between species, a Tukey test on the transformed data was performed. To reduce the number of variables, the *M* & *m* data were submitted to multivariate analyses using two descriptive and exploratory methods: a) principal component (PC) and reciprocal averaging (RA) and b) discriminate analysis (DA). Statistical and multivariate analyses were carried out as follows: the RA analysis was done by using Co603c (Lleonart, 1991), and the PC and DA analyses were done in STATISTICA version 6.0 (1997).

Allozyme Analysis. Allozyme markers and allele designations were taken from Barriga-Sosa *et al.*, (2002; 2004). The staining protocol was based on Barriga-Sosa *et al.*, (2002). A summary of electrophoretic buffers, enzyme names and numbers, abbreviations, and putative resolved *loci* in liver, muscle and heart tissues in Cellulose Acetate Gel Electrophoresis (CAGE) are presented in Table 1.

For each species and sample, allele frequencies per locus, proportion of polymorphic *loci* (P_{95} criteria), mean number of alleles per locus, and expected (H_e) heterozygosity were calculated. Deviations from the expected Hardy-Weinberg equilibrium (HWE) were tested using the Monte Carlo method (10,000 permutations) in GENEPOP 3.1 (Raymond & Rousset, 1995). To test the significance of allele frequency differences between species and samples, Fisher's combined probability test (Sokal & Rohlf, 1995) was used in TFPGA 1.3 (Miller, 1998). *F* indices (Weir & Cockerham, 1984) were computed with GENEPOP 3.1. When sample size allowed, Weir & Cockerham *F*-statistics (1984) were estimated with F-STAT 2.9.3.2 (Goudet, 2001). Because natural hybridization can occur between congeners of the Tilapinii family (Trewavas, 1983), and because of the uncertainty of species distribution within these water reservoirs, the following parameters were calculated for each subpopulation or sample: *f* or the correlation of genes for individuals in the same subpopulations; and θ_S, the correlation of genes for individuals in different subpopulations. Variances of these parameters were estimated by jack-knifing across *loci*. The value of θ_S and its confidence intervals (95% and 99%) were also computed. *F*-statistics were estimated for significance with 2,000 allele permutations within subpopulations (*f*) and between subpopulations (*F* and θ_S) over all *loci*. Pairwise tests of population differentiation were calculated and Bonferroni corrections were used (Goudet, 2001).

PCR-RFLP analysis. Total DNA was extracted from frozen muscle following the DNeasy® (QIAGEN, Valencia, Ca) protocol with the modifications of Barriga-Sosa *et al.*, (2005). Total DNA was quantified with gel electrophoresis, using the standard 123 bp DNA ladder (1 mg/mL, Sigma BioSciences, St. Louis, Mo) as reference marker, visualized under UV light, and photo documented with a Polaroid DS-34 Direct Screen Instant Camera (Polaroid, Co., Cambridge, Ma).

Two mitochondrial DNA or mtDNA genes namely, *r16S* and cytochrome b (*cytb*) were amplified according to conditions described in Barriga-Sosa *et al.*, (2005). The cycling conditions for both genes were established by the results obtained after a series of gradient PCRs at annealing temperatures between 36 and 42ºC. Both genes amplified the best with the following parameters: an initial cycle (94ºC for 1:30 min), annealing (36ºC for 0:30 min), and extension (72ºC for 1:30 min), followed by 30 cycles at 94ºC for 45 sec, 36ºC for 30 sec, and a final extension at 72ºC for 10 min.

The PCR amplicons were purified utilizing the QIAquick® columns (QIAGEN, Valencia, Ca) prior to the restriction digests with *Alu*I, *Hae*III, and *Rsa*I (Boehringer Mannheim, Germany).

Table 2. Species examined and collection site, total sample size per species (N), mean size, size intervals, and collection dates.

Specie s and collection site	N	Mean size (TL mm)	Size intervals (TL mm) ± SD	Date of collection
Oreochromis aureus. Infiernillo Dam, Platanito (AP)	70	182.4	149-231±3.8	February, 1999
O. aureus. Infiernillo Dam, La Quebradora (AQ)	62	218.4	119.5-268±2.3	February, 2000
Tilapia rendalli. Infiernillo Dam, La Platanito (TP)	9	126.1	60-199±15.9	February, 1999
T. rendalli. Infiernillo Dam, La Quebradora (TQ)	57	153.6	122-190±2.0	February, 2000
O. mossambicus. Zicuiran Dam (MZ)	50	180.4	160-221±1.9	February, 2000
	N_T = 248			

N_T = Total sample size

These enzymes were selected based on the presence/absence of restriction sites in partial sequences of both genes (*O. mossambicus* mitochondrial *cytb*, GenBank X81565 and *r16S* genes, GenBank AY597335).

Restriction digests were carried out according to the manufacturer specifications in a final volume of 25 *mL* with 20 ng/*mL* of purified PCR product. Restriction fragments were sized on 2.0% agarose gels with 20 bp (500 ng, BIO-RAD, Hercules, Ca) and 100 bp molecular rulers (250 ng, BIO-RAD, Hercules, Ca) as standard markers. Gels were run for 1.75 to 2 hrs, depending on the sizes of the expected products.

A restriction site presence/absence matrix was created with the GENERATE program in the REAP package (McElroy *et al*., 1992). Haplotype frequencies were calculated in Arlequin 2.000 software (Schneider *et al*., 2000). Haplotype and nucleotide diversity indices (Nei, 1987), and divergence between species (Nei & Tajima, 1981) were computed with D and DA programs inside the REAP package.

RESULTS

Fish identity based on morphological characters and variation. The sample size, mean TL (mm), size intervals (Min-Max), collecting site and date for each species are presented in Table 2. Two genera are identified based on pharyngeal teeth characteristics: *Oreochromis*, with monocuspid and bicuspid teeth, and *Tilapia*, with tricuspid teeth. *Oreochromis aureus* (A) and *T. rendalli* (T) are both identified from two sites at the Infiernillo Dam [Platanito (AP and TP) and Quebradora (AQ and TQ)]. *O. mossambicus* is identified only from Zicuirán (MZ). The morphological data of the TPB confirmed the identity of the samples to the genus and species levels. Significant differences were observed between genera and species in all variables ($p< 0.05$), except *wta* between *O. aureus* vs. *O. mossambicus* and *ttl* between *T. rendalli* vs. *O. mossambicus* (Table 3).

For the meristic data (*m*) RA explains the 97.81% of the variance (Table 4). These data support the separation of fish in the two genera, *Oreochromis* and *Tilapia*. Similarly, DA also resolves the two genera. The single *m* variable accounting for the highest percentage of the variance is the number of predorsal scales (0.83 %).

The *M* data resolved variation within species, but differ in whether the intraspecific variation is from shape or size. With the raw data (*Mo*) and the data transformed proportionally (i) or logarithmically (ii), the first two components of the PC and RA analyses show changes that relates to size. However, the PC and RA analyses with normalized data (iii) show a correlation matrix with different sign and magnitude, a pattern that suggests variation in shape (data available on request) (Corti *et al*., 1988; Cuadras, 1991). To reduce the number of variables, we use the PC because it suggests a clear separation between samples and explains the highest percentage of the variance (Table 4).

The three species are clearly differentiated through these analyses. Overall, six morphometric variables contribute to the species distinctions (with the highest variance percentages): the distance from the end of the mouth opening to the most anterior dorsal fin (variable 2, 0.95); the distance from the end of the mouth opening to the most superior origin of the opercule (3, 0.96); the distance of the origin of the dorsal fin to the origin of anal fin (11, 0.93); the distance of the origin of pelvic fin to the origin of anal fin (12, 0.93); the distance of the origin of the anal fin to the end of dorsal fin (14, 0.94); and the distance of the origin of the anal fin to the upper end of the caudal fin (15, 0.94) (Table 5).

Table 3. Results of the Tukey test (Multiple Comparisons), showing significant differences ($p< 0.05$) between samples (1, 2 & 3) for the four variables recorded for the Toothed pharyngeal bone.

Dependent variable	Sample (I)	Sample (J)	Mean Difference (I-J)	SE	*p*
ttw	1	2	-0.2481*	0.03030	0.000
		3	-0.1162*	0.03042	0.001
	2	1	0.2481*	0.03030	0.000
		3	0.1319*	0.02106	0.000
	3	1	0.1162*	0.03042	0.001
		2	-0.1319*	0.02106	0.000
wta	1	2	-0.2414*	0.02463	0.000
		3	-0.1367*	0.02473	0.000
	2	1	0.2414*	0.02463	0.000
		3	0.1046*	0.01712	0.000
	3	1	0.1367*	0.02473	0.000
		2	-0.1046*	0.01712	0.000
ttl	1	2	-0.2065*	0.03526	0.000
		3	-0.1491*	0.03540	0.000
	2	1	0.2065*	0.03526	0.000
		3	0.0574	0.02450	0.053
	3	1	0.1491*	0.03540	0.000
		2	-0.0574	0.02450	0.053
lta	1	2	-0.1799*	0.03906	0.000
		3	0.0096	0.03921	0.968
	2	1	0.1799*	0.03906	0.000
		3	0.1895*	0.02714	0.000
	3	1	-0.0096	0.03921	0.968
		2	-0.1895*	0.02714	0.000

1 = *O. aureus*; 2 = *T. rendalli*, 3 = *O. mossambicus*; *ttw* = teeth total width; ***wta*** = width of the toothed area; *ttl* = total length; *lta* = length of the toothed area; SE= standar error.

The first two discriminant functions resolved from the morphometric variables (*M* iii) show 96.1 and 3.9 relative percentage of classification and a canonical correlation of 0.90 (data not shown). In the plot of this analysis, AQ appears in the right section of the plot, whereas AP appears close to MZ in the lower middle and TQ and TP are on the left side (Fig. 3). With this analysis, AQ, TP and MZ are 100% correctly classified, TQ is 93.5% correctly classified and AP is 82.8% correctly classified (Table 6).

Allele Frequencies and Allozyme Variation. Only one locus was detected for Idh, G6pdh, Pgm and Xdh, while Aat, Mdh and Est have two *loci* and Gpi three *loci*. Eight *loci* are polymorphic (Table 7), while the remaining 5 *loci* (locus 1 for Idh, Mdh, Xdh and *loci* 1 and 2 for Aat) are monomorphic in all samples. With the P_{95} criteria, TP, MZ and AQ have the highest number of polymorphic *loci* (50%), followed by TQ (37.5%), while AP (25%) had the fewest polymorphic *loci*. A similar pattern is observed for H_e (ranges from 0.0567 to 0.2299). Significant differences in the allele frequencies of seven polymorphic *loci* are found in most pairwise comparisons (Table 7). Pairwise comparisons of TP–TQ and TP–MZ allele frequencies are not significantly different. Therefore, all *T. rendalli* samples are regarded as a single sample (population TI) for further analyses. Most samples showed departure from HWE at one or more *loci* (Table 7). An overall deficiency of heterozygotes is observed in all populations (average jack-knifed *F*, 0.427, *S.E.* 0.105). Significant levels of intra-specific differentiation are found in *O. aureus* (AQ and AP, F_{ST} 0.4109, $p< 0.5$).

Variation in *r16S* and *cytb*. The *r16S* and *cytb* amplicons from 31 fish (AQ = 12, TI = 11, and MZ = 8) are 620 bp and 550 bp, respectively. Enzymes *Alu* I, *Hae* III and *Rsa* I all cut the amplified *r16S* and *cytb* products, generating 12 restriction fragments (Table 8), corresponding to five *r16S*/*cytb* haplotypes (Table 9). None of the five *r16S*/*cytb*

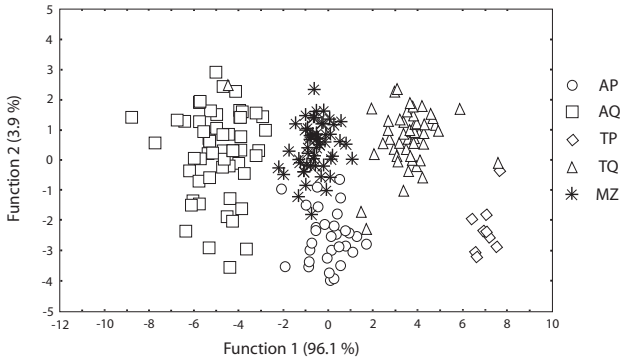

Figure 3. Plot of first and second discriminant functions of the discriminant analysis (DA) using 21 morphometric normalized characters (iii) and five samples: AP = *Oreochromis aureus* from Platanito, AQ = *Oreochromis aureus* from La Quebradora, TP = *Tilapia rendalli* from Platanito, TQ = *Tilapia rendalli* from La Quebradora and MZ = *Oreochromis mossambicus* from Zicuiran.

haplotypes is shared between populations. Haplotypes AAACAA and AAABAA are unique to AQ. TI is monomorphic with all individuals showing the unique haplotype AAAAAA. In MZ, all individuals have haplotypes AABDBB or AAADBB. Haplotype diversity (*h*) ranges from 0.0000 ± 0.0000 in TI to 0.4848 ± 0.1059 in AQ, and nucleotide diversity (π) ranges from 0.0000 in TI and MZ to 0.0187 in AQ (Table 10).

DISCUSSION

Despite problems in one of its major reservoirs, tilapia production continues in Mexico. This production has generated social and economic benefits to local rural areas, and results to an estimated national income of 70 million USD. The Infiernillo Dam is one of the most important tilapia producers at the national level. Although the production in the Zicuiran Dam is not as important, it satisfies the demand of local populations.

Re-stocking Mexican lakes and reservoirs is a common practice. For example, 200,000 tilapia fry were re-stocked in the Infiernillo Dam between 1998 and 2000, and at the Zicuiran Dam, 635,000 tilapia fry were re-stocked from 1999-2002 (Gobierno del Estado de Michoacán, oficio HOO/CPATZ/334). In both cases, the re-stocked tilapias had no species identity or genetic information and were only recognized as "tilapias". Likewise, there are no reliable records to assist in adequate fisheries management. Here, we identify the tilapias from these reservoirs and report the level of their genetic diversity. These data can be used for improving the management practices of tilapia in the biggest reservoir of Mexico that will optimize productivity.

The tilapia genera and species were easily identified by tooth characteristics as previously reported (Liem, 1974; Trewavas, 1983; Arredondo-Figueroa & Tejeda-Salinas, 1989), and the number of predorsal scales (PDS). Among the six morphological variables that aid in the discrimination of species and populations, two variables are related to feeding habits and four to swimming capacity and maintenance of the animals in the water column. These differences could relate to known differences in tilapia species' habitat preferences. *Oreochromis mossambicus* avoids strong currents and prefers turbid planktonic waters. Although *O. mossambicus* can coexist with *Tilapia rendalli*, only

Table 4. Cumulated percentage of the variance in three components for multivariate analyses (MA), principal component (PC) and reciprocal averaging (RA)

Characters	Data	MA	Component I	Component II	Component III
		PC	74.94	80.47	84.1
	Original (o)	RA	28.97	45.82	60.43
		PC	36.79	51.9	59.09
	Proportions (i)	RA	26.9	43.89	54.84
Morphometric		PC	77.49	82.43	85.62
	Log (ii)	RA	27.49	47.75	63.49
		PC	69.8	75.93	80.12
	Normalization (iii)	RA	29.88	46.98	61.34
		PC	38.85	59.89	77.55
Meristic	*Original* (o)	RA	61.42	89.36	97.81

Table 5. Factorial coefficients I, II and III of the PC analysis for 21 mor phometric normalized variables (iii).

Morphological variable	Factorial Coefficient		
	I	II	III
Distance from the anterior end of the upper mandible to the origin of the dorsal fin (1)	0.8458	-0.1356	0.1128
Distance from the end of the mouth opening to the most anterior dorsal fin (2)	0.9565	-0.1099	0.0584
Distance from the anterior end of the upper mandible to the most superior origin of the opercule (3)	0.9323	-0.1727	0.0462
Distance from the anterior end of the mouth opening to the most superior origin of the opercule (4)	0.9195	-0.1795	0.0773
Distance from the end of the mouth opening to the origin of the pectoral fin (5)	0.8965	-0.2702	0.0797
Distance from the end of the mouth opening to the first ray of the pelvic fin (6)	0.861	-0.1771	0.0711
Distance from the origin of the pectoral fin to the most the first spine of the pelvic fin (7)	0.3871	0.8218	-0.0967
Distance from the origin of the pectoral fin to the (8)	0.7487	-0.188	-0.2652
Distance from the upper origin of the opercule to the first ray of the dorsal fin (9)	0.7298	0.2156	-0.0546
Distance from the origin of the pectoral fin to the to the first ray of the dorsal fin (10)	0.8627	0.3047	-0.2086
Distance from the origin of the dorsal fin to the origin of anal fin (11)	0.9621	0.0504	0.0324
Distance of the origin of pelvic fin to the origin of anal fin (12)	0.9347	0.1175	0.031
Distance from the origin of the first ray of the pelvic fin to the most anterior end of the first ray of the anal fin (13)	0.8155	0.1936	-0.0177
Distance of the origin of the anal fin to the end of dorsal fin (14)	0.9402	0.0446	-0.0434
Distance of the origin of the anal fin to the upper end of the caudal fin (15)	0.9414	-0.0165	0.0181
Head width (16)	0.7336	-0.1849	-0.4566
Body depth (17)	0.8666	0.2919	0.0046
Body width (18)	0.5399	0.1526	0.6538
Distance from the origin of the upper mandible to the origin of the anal ray (19)	0.9132	0.0129	0.0758
Orbit diameter (20)	0.6096	-0.1574	0.1844
Length of the inferior mandible (21)	0.8714	-0.1321	-0.1928

O. mossambicus was found in the Zicuirán Dam. *Oreochromis aureus* also inhabits phyto- and zooplanktonic waters (Trewavas, 1983). Interestingly, both *O. aureus* & *T. rendalli* have two differentiated forms in different sites in the Infiernillo Dam. Such morphometric variation has been previously observed for tilapia species and strains, both wild and cultured (Eknath *et al.*, 1991; Vreven *et al.*, 1998; Barriga-Sosa *et al.*, 2004; Narváez *et al.*, 2005). In most cases, the environment has a direct influence in shape differentiation. For example, Barriga-Sosa *et al.*, (2004) reported al least two forms of *O. aureus* in Mexico (from Infiernillo and from the TFPF "Los Amates" in the state of Veracruz). However, this report is the first to document intra-lacustrine or intra-reservoir variation in tilapias.

The *O. aureus* stock from the Infiernillo Dam is derived from 3, 685 tilapia fry introduced in 1966 (original brooders from Auburn University, Alabama, USA) (Guzmán Urioestegui, 1994) and from 200,000 "tilapia" fry re-stocked in the dam between 1998 and 2000, most probably from the TFPF "Los Amates", Veracruz (Gobierno del Estado de Michoacán, oficio HOO/CPATZ/334). These initial and re-stocking events could have introduced the intra-reservoir morphological differences observed in *O. aureus*

Table 6. Discriminate classification of tilapias based on 21 morphometric normalized variables (iii).

	Predicted group					
Actual	Classification %	AP	AQ	TP	TQ	MZ
AP	82.8	29	0	0	0	6
AQ	100	0	62	0	0	0
TP	100	0	0	9	0	0
TQ	93.5	2	1	1	72	1
MZ	100	0	0	0	0	60
Total	95.4	31	63	10	72	67

AP & TP = *Oreochromis aureus* and *Tilapia rendalli* from El Platanito, El Infiernillo dam; AQ & TP = *O. aureus* and *Tilapia rendalli* from La Quebradora, El Infiernillo dam; MZ = *O. mossambicus* from Zicuiran dam.

and *T. rendalli*. However, it remains a possibility that environmental factors promote differentiation within the dam.

The allele distribution for the allozymes discriminates between the two tilapia genera. For example, allele *b* of loci Est-1 and Est-2 are unique to samples TI and MZ, respectively, suggesting that these can be used as diagnostic markers to discriminate between *T. rendalli* and *O. mossambicus*. Although each of these two *loci* were found in other tilapinii studies (McAndrew & Majumdar, 1983; Feresu-Shonhiwa & Howard, 1998; Appleyard & Mather, 2002; De Silva, 2004), only McAndrew & Majumdar (1983) report both *loci* and used them to discriminate between con-specifics (*O. andersonii* Castelnau 1861 and *O. mossambicus*).

The levels of genetic variability observed in this study (H_e, Table 7), are within the range of what has been previously been reported for both wild and cultured species and populations. However, they are relatively low compared to those recently reported for properly managed populations. Wild and cultured populations of *O. niloticus* had low levels of H_e (Macaranas *et al*., 1995), and wild *Oreochromis spp*. of Lake Malawi had intermediate levels of H_e (Sodsuk *et al*., 1995). In a comparison of natural and cultured populations of *O. niloticus* and *T. zilli*, both had high levels of variation, suggesting proper management strategies in these fisheries (Rognon *et al*., 1996). In a study about mitochondrial introgression between *O. aureus* and *O. niloticus* in natural and cultured populations, in which allozymes were also utilized, levels of variation ranged from 0.0 for a natural population to 0.71 for cultured organisms (Rognon & Guyomard, 2003). The low H_e observed in AP might result from high fishing rates. This site and Nuevo Centro account for 66.9 % of the Infiernillo dam total tilapia production (2,280 and 1,988 ton/year, respectively). Such high production may directly impact tilapia population sizes. Additionally, eviscerated fish products are dumped at these sites (Jiménez-Badillo, 1999), producing anoxic conditions, and causing the fish to move to other areas of the dam. Furthermore, seasonal droughts or water level fluctuations create bottleneck processes in fish populations that can lead to low H_e and rapid allele frequency change (Rognon & Guyomard, 1997; Sturmbauer *et al*., 2001) or maintain their genetic variation (Chakraborty & Nei, 1977; Sodsuk *et al*., 1995). El Platanito has drought periods between January and May, when the water level drops to 40 m. This event drives fish populations to deeper and more confined areas such as Pinzandarán (60 m), an area close to the dam curtain with pronounced hills (Jiménez-Badillo, 1999). In this region, seasonal pools with peculiar physicochemical conditions attract fishes with surprising morphological features, suggesting high phenotypic plasticity among tilapias (Falk *et al*., 2000). The cycles of flow into the dam allow for a variety of microclimates. These microclimates could contribute to population subdivision and differentiate semi-isolated genetic units (van Open *et al*., 1997). This mechanism of population subdivision might be the case of *O. aureus* at the Infiernillo, as AP and AQ show significant levels of intra-specific genetic differentiation (θ_{ST} = 0.4343, p< 0.5), which is concordant with both differentiation at Gpi-3 and morphological differentiation (as mentioned earlier by AD analysis). The observed morphological and genetic differentiation could be the initial stages of allopatric speciation of *O. aureus* populations, separated by approximately 13 km within the Infiernillo Dam. To determine if the differentiation is intraspecific variation or the initial stages of speciation, further genetic studies with codominant DNA markers, a yearly sampling strategy and more extensive sampling are required.

Departure from HWE is evident in samples AQ and MZ, which have heterozygous deficiencies at three and four *loci*, respectively. Such deficiencies indicate an absence of random mating (F_{IS}). AP and TI each have only one locus deficient for heterozygotes. Heterozygote deficit can result from the further population substructure, the Wahlund effect or inbreeding. For MZ, the deficit may be explained by inbreeding, because the tilapia in the Zicuirán Dam is isolated from the Infiernillo Dam by 19-21 km of seasonal streams. Furthermore, *O. mossambicus* is the only "tilapia" introduced in this dam. In contrast, the heterozygote deficit of *O. aureas* could result from the Wahlund effect with two possible scenarios. First, *O. aureus* comes from two different origins, each of which differs in allele frequencies. Second, significant allele frequency differences are accumulated as the two populations are isolated in the dam. The Fisher's *P* results indicate significant differences between AP and AQ in five out of eight polymorphic *loci* (Gpi 1-3, G6pdh a nd Ldh). Linkage disequilibrium (LD, based on 14000 permutations) was significant overall, and for the first scenario suggesting two distinct origins for AQ. Both characteristics, significant differences in allele frequencies and LD, are classic features of a Wahlund effect, but further studies are required for more conclusive evidence.

Table 7. Allelic frequencies of polymorphic *loci* for the *Oreochromis* and *Tilapia* species studied and F_{IS} for all samples analyzed.

Spp/Sample	AP	AQ	TP	TQ	MZ	Fisher's P
Locus						
Est-1						
(N)	35	62	9	57	50	0.0000
A	1.0000	1.0000	0.5000	0.5263	1.0000	
B	0.0000	0.0000	0.5000	0.4737	0.0000	
♦F_{IS}	----	----	-1.000	-0.898	----	
Est-2						
(N)	35	62	9	59	51	0.0000
A	1.0000	1.0000	1.0000	1.0000	0.7843	
B	0.0000	0.0000	0.0000	0.0000	0.2157	
♦F_{IS}	----	----	----	----	+0.314*	
Gpi-1						
(N)	29	57	6	47	54	0.0000
A	0.8793	0.5614	0.5000	0.7979	0.3796	
B	0.0000	0.3509	0.0000	0.1702	0.3704	
C	0.1207	0.0877	0.5000	0.0319	0.2500	
♦F_{IS}	-0.120	+0.563*	-0.250	+0.053*	+0.247*	
Gpi-2						
(N)	20	57	5	47	54	0.0000
A	1.0000	0.6316	1.0000	0.9574	0.9815	
B	0.0000	0.2807	0.0000	0.0213	0.0185	
C	0.0000	0.0877	0.0000	0.0213	0.0000	
♦F_{IS}	----	+0.597*	----	-0.022	-0.010	
Gpi-3						
(N)	20	57	8	49	54	0.0000
A	0.0000	0.8684	1.0000	0.9592	0.9630	
B	0.0000	0.0000	0.0000	0.0204	0.0185	
C	1.0000	0.1316	0.0000	0.0204	0.0185	
♦F_{IS}	----	+0.283*	----	-0.021	+0.493*	
G6pdh						
(N)	26	62	5	53	53	0.0000
A	0.1346	0.0000	0.5000	0.2830	0.6698	
B	0.8654	1.0000	0.5000	0.7170	0.3302	
♦F_{IS}	+0.519*	----	-0.091	-0.106	+0.706*	
Ldh-1						
(N)	20	59	9	60	56	0.0000
A	1.0000	0.8390	0.8889	1.0000	0.8661	
B	0.0000	0.0508	0.0000	0.0000	0.1071	
C	0.0000	0.1102	0.1111	0.0000	0.0268	
♦F_{IS}	----	-0.136	-0.067	----	-0.118	
Pgm						
(N)	35	62	9	60	60	0.1464
A	1.0000	1.0000	1.0000	0.9750	1.0000	
B	0.0000	0.0000	0.0000	0.0250	0.0000	
♦F_{IS}	----	----	----	-0.017	----	
◎$P_{0.95}$	25.00	50.00	50.00	37.50	50.00	
H_e	0.0567	0.1991	0.2299	0.1828	0.2250	

◎ A locus is polymorphic if the frequency of the most common allele does not exceed 95% (± S. E.); N = sample size; H_e = expected heterozygosity and P= Fisher's P among samples. ♦F_{IS} = 0 for a randomly mating population, Positive values indicate a deficiency of heterozygotes. * Locus does not conform to Hardy-Weinberg proportions, *P<0.05, Samples TP and TQ were combined for this analysis (see text).

Table 8. Restriction fragments and their sizes (in base pairs) generated by the digestion with three enzymes in each of the amplified *r16S* (620 bp) and *cyt b* (550 bp) products for the ***Oreochromis*** and ***Tilapia*** species studied. The restriction patterns ranged from one to four morphs (A-D)

Gene	*r16S*				*cyt b*							
Enzyme	*Alu*I	*Hae*III	*Rsa*I		*Alu*I				*Hae*III		*Rsa*I	
Morph	A	A	A	B	A	B	C	D	A	B	A	B
	370	360	620	360	350	370	350	550	380	290	390	390
	250	260		260	150	130	180		170	140	160	90
					50	50	20			120		70

Table 9. Show the mtDNA haplotypes generated with three restriction enzymes (RE) of the amplified r*16S* and ***cyt b*** for the ***Oreochromis*** and *Tilapia* species studied.

	Patterns obtained with RE					
	r16S			*cytb*		
Haplotypes	*Alu*I	*Hae*III	*Rsa*I	*Alu*I	*Hae*III	*Rsa*I
1	A	A	A	C	A	A
2	A	A	A	B	A	A
3	A	A	A	A	A	A
4	A	A	B	D	B	B
5	A	A	A	D	B	B

Based on five unique *r16S/cytb* haplotypes, genetic diversity indices were measured. Similar to the results from the allozyme data, the observed genetic variation based on mtDNA RFLP is low compared to that of other tilapia species, both wild and cultured. Despite the low variation in these markers, they provide sufficient resolution to define species and genera. Low levels of genetic variation were also found in three *O. alcalicus* populations in two lakes (Wilson *et al*., 2000). Despite a low number of haplotypes, high levels of genetic variation were found for *O. niloticus* and a red hybrid tilapia (Romana-Eguia *et al*., 2004).

The tilapias from the Infiernillo Dam are declining due to fishing pressure and drastic environmental cycles. The rainy and dry seasons promote the formation of microclimates that can create bottlenecks that might be playing important roles in micro-evolutionary processes and maintenance of genetic diversity. By implementing three new strategies, the management of tilapia stocks can be greatly improved. First, DNA-based molecular markers can be used as a non-invasive method to identify and characterize tilapias for re-stocking. Second, periodic assessments of the standing genetic diversity of populations can be used to evaluate the health of a population to define management strategies. Third, maintaining genetic heterogeneity in reproductive stocks can allow for constant and predictable tilapia production that satisfies the national demand. Federal management agencies would benefit from a national genetic database for stock management containing molecular marker information on the re-stocked tilapias. These genetic markers can improve assessment and characterization of diversity in stocks, which increases the chances that re-stocked tilapia fisheries are being managed effectively for long-term survival.

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Table 10. MtDNA haplotypes, number of haplotypes in each sample, haplotype (*h*) and nucleotide diversity indices (π) (Nei, 1987; Nei and Tajima 1981) for the *Oreochromis* (AQ = *O. aureus*, La Quebradora; MZ = *O. mossambicus*, Zicuiran) and *Tilapia* (*T. rendalli*, La Quebradora) species studied. N= number of organisms analyzed

	Oreochromis		*Tilapia*
	AQ	MZ	TQ
	N = 12	N = 8	N = 11
Haplotype			
1	8	0	0
2	4	0	0
3	0	0	11
4	0	6	0
5	0	2	0
No. haplotypes/sample	2	2	1
h	0.4848 ± 0.1059	0.4286 ± 0.1687	0.0000 ± 0.0000
π	0.0187	0.0000	0.0000

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