



# Conservation genetics of the tropical gar (*Atractosteus tropicus*, Lepisosteidae)

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## Abstract

The tropical gar (*Atractosteus tropicus*) is the smallest member of the family Lepisosteidae; yet this species has a large socioeconomic impact in México and Central America where it is traditionally harvested commercially and for subsistence. While natural populations of tropical gar have been dwindling throughout its natural range, it is also an emergent aquaculture species that is produced in local hatcheries and grown out in privately owned ponds. The increased pressure on natural populations of *A. tropicus* and its increasing use in aquaculture production poses potential conflicts for the management and conservation of natural populations. Here, we investigated the population genetic structure of tropical gar populations, including over 200 individuals sampled in México, El Salvador, and Costa Rica. Using 11 microsatellite loci, we identified three genetic clusters with distinct geographic distributions, including a cluster in drainages along the Pacific versant of Central America, a cluster in the Grijalva and Usumacinta River basins that drain into the Gulf of México, and a cluster in the Río San Juan that drains into the Caribbean Sea. Given the degree of divergence observed, these results indicate the potential presence of evolutionary significant units within tropical gar that warrant separate fisheries and conservation management. We also found that tropical gar from an aquaculture facility along the Pacific versant of México were derived from Atlantic versant populations, indicating that individuals have already been translocated across biogeographic boundaries. We discuss how such translocations can negatively impact the natural population structure of tropical gar and provide recommendations for future research and aquaculture practices.

**Keywords** Aquaculture · Conservation · Gar · Microsatellites · Population genetics · Population structure

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## Introduction

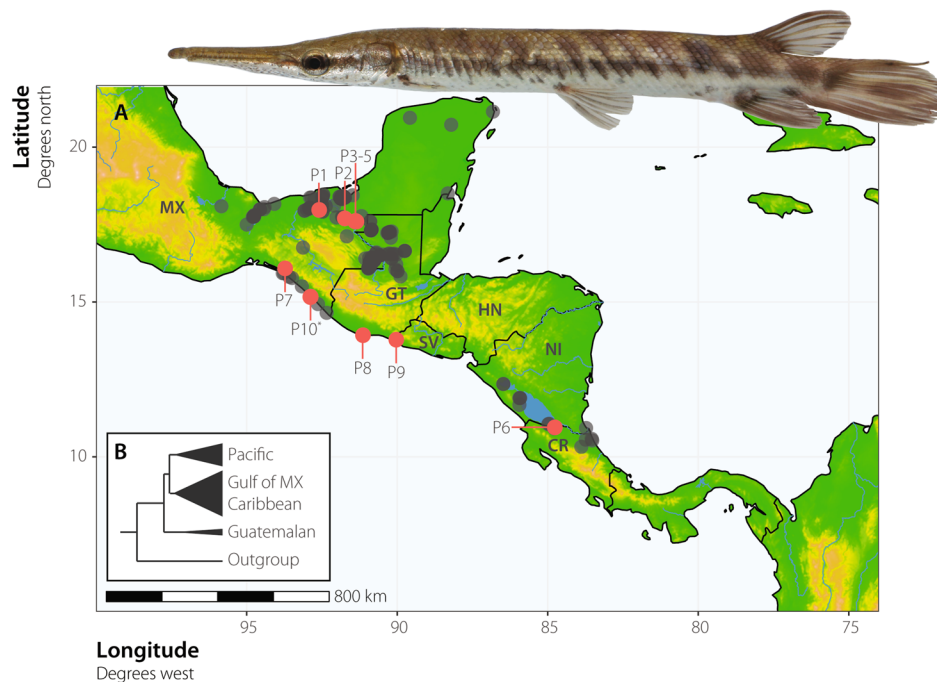
Gars of the family Lepisosteidae are the sole survivors of an ancient group of ray-finned fishes that dates back to the late Jurassic, over 155 million years ago (Brito et al. 2017). There are seven extant species classified into two genera, *Lepisosteus* (4 species) and *Atractosteus* (3 species), which primarily occur in freshwater and coastal habitats of eastern North America, Central America, and Cuba (Berra 2001; Wright et al. 2012; Echelle and Grande 2014). All gars are carnivorous and have cylindrical, elongated bodies covered in ganoid scales. As is typical for sit-and-wait predators, their dorsal and anal fins are set far back on the body near the abbreviated heterocercal caudal fin, and they have long jaws that are densely packed with sharp teeth (Echelle and Grande 2014). In nature, gars are voracious predators that capture invertebrates, fish, and occasionally even small birds and mammals with sideway strikes of their head (Lauder 1980; Porter and Motta 2004; Lemberg et al. 2019).

All gars are large-bodied fish, with longnose gar (*L. osseus*), Florida gar (*L. platyrhincus*), and alligator gar (*A. spatula*) growing more than 2 m in length (García de León et al. 2001; Allan et al. 2005; Mendoza Alfaro et al. 2008; Buckmeier et al. 2016). Accordingly, these species play important ecological roles as the top predators in their habitats (Fry et al. 1999; Akin and Winemiller 2006; Williams and Trexler 2006; Fletcher et al. 2015). However, despite their considerable size, gars are not typically harvested commercially or for subsistence in the United States where most of the species occur. Rather, fishermen and fisheries managers have long targeted gars as nuisance species that were thought to negatively affect more valuable sport fish populations (Scarnecchia 1992). Small commercial fisheries, primarily for *A. spatula*, have only persisted regionally in some southern states (Aguilera et al. 2002), although there is a growing interest in gar as trophy fish that are angled for sport (Smith et al. 2020). Population declines and even local extirpation of gar caused by overfishing (*i.e.*, nuisance animal removal) and habitat loss (Scarnecchia 1992; David et al. 2018), in conjunction with increased demands for trophy quality fish, have led to more widespread efforts to assess and manage gar populations (Glass et al. 2015; Bohn et al. 2017; Smith et al. 2018, 2020).

An important exception with considerable economic importance is the tropical gar, *Atractosteus tropicus* (regionally known as catán, gaspar, machorra, and pejel-agarto). The smallest member of the family (maximum length of ~1.25 m), *A. tropicus* occurs from southern México to Costa Rica (Fig. 1A), where it inhabits backwaters of large rivers, oxbow lakes, lagoons, flood ponds, and

swamps, including some with brackish water (Villa 1982; Bussing 1998; Miller et al. 2005; Sauz-Sánchez et al. 2021). Especially in southern México and Guatemala, tropical gar has historically been a sought-after species of cultural importance (Emery 2017), and it is traditionally consumed because of its nutritional qualities (Manuel et al. 2013; Márquez-Couturier et al. 2015; Barrientos et al. 2018). In 1996 alone, over 530 tons of *A. tropicus* were harvested by fisherman setting gill nets in the Mexican state of Tabasco, making it one of the most important fisheries in the region (Aguilera et al. 2002). However, natural population sizes and catch rates of this species have been declining (Aguilera et al. 2002; Siebe et al. 2005), and some regional populations have even been extirpated (Villa 1982; Mora Jamett et al. 1997; Barrientos-Villalobos and Espinosa de los Monteros 2008). Accordingly, *A. tropicus* is listed as endangered in El Salvador (MARN 2015), but not in other countries. Population loss and decline have been attributed to overexploitation, habitat degradation, and habitat loss (Siebe et al. 2005; Sauz-Sánchez et al. 2021). Although systematic studies assessing the health of natural *A. tropicus* populations are missing and the fishery of this species remains unmanaged, there is also a growing interest in its aquaculture production (Mendoza Alfaro et al. 2008; Márquez-Couturier et al. 2015). Aquaculture of *A. tropicus* is facilitated by its high tolerance to nitrogenous wastes (Aranda-Morales et al. 2021), its ability to breathe atmospheric air and tolerate hypoxia (Burggren et al. 2016; Martínez-Bautista et al. 2022), a thorough understanding of its reproductive biology (Márquez-Couturier et al. 2015), its acceptance of artificial feeds (including *Artemia* nauplii for larvae and juveniles and pellets for adults; Márquez-Couturier et al. 2015; Martínez-Cárdenas et al. 2018), and its excellent food conversion rates and profitability (Palma-Cancino et al. 2019).

The increased pressure on natural populations of *A. tropicus* in conjunction with its increasing use in aquaculture production poses potential conflicts. While increased aquaculture production can relieve fishing pressure on declining natural populations, sharing of brood stocks across aquaculture cooperatives in different geographic regions has the potential to interfere with the natural population genetic structure and patterns of local adaptation (Ward 2006; Östergren et al. 2021). For example, a previous study on mitochondrial DNA (mtDNA) variation in *A. tropicus* has revealed significant differentiation among populations (Barrientos-Villalobos and Espinosa de los Monteros 2008). Specifically, analyses of mtDNA haplotypes indicated a differentiation between populations in river drainages flowing eastward toward the Gulf of México and the Caribbean Sea (from México to Costa Rica) and populations in river drainages flowing westward into the Pacific Ocean (in México



**Fig. 1** **A** Map of the natural distribution (gray dots) and the sampling locations (red dots) of tropical gar used on this study. The number associated with collection localities corresponds to sites in Table 1. Note that the asterisk associated with site 10 indicates samples from an aquaculture population. Country codes correspond to Mexico (MX), Guatemala (GT), El Salvador (SV), Honduras (HN), Nicaragua (NI), and Costa Rica (CR). **B** The cladogram depicts the phylo-

genetic relationship of different clades of tropical gar (relative to an outgroup of the other two *Atractosteus* species) based on mtDNA analyses of Barrientos-Villalobos and Espinosa de los Monteros (2008). Note that branch lengths are not to scale, but the height of each triangle is proportional to the number of mtDNA haplotypes in each clade. The insert is a photograph of a juvenile tropical gar with a standard length of about 125 mm (photo by M. Tobler)

and El Salvador; see cladogram in Fig. 1B). In addition, the identification of divergent and reciprocally monophyletic mtDNA haplotypes from Guatemala has fueled some speculation about the presence of a potentially undescribed species (Barrientos-Villalobos and Espinosa de los Monteros 2008).

In this study, we used nuclear markers (microsatellites) to revisit patterns of population genetic variation in *A. tropicus*. We used samples from nine natural populations in three geographic regions that span the natural distribution of the species—including samples from the Grijalva and Usumacinta River basins that drain into the Gulf of México, the San Juan River basin that drains into the Caribbean Sea, and multiple drainages along the Pacific versant of Central America—to test whether population genetic structure is in concordance with geographic barriers and reflects previous analyses of mtDNA. We predicted significant population genetic differentiation between populations from the Gulf of México/Caribbean slope of Central America and those that occur on the Pacific versant (Barrientos-Villalobos and Espinosa de los Monteros 2008). Since tropical gar are increasingly farmed in different regions of Mexico, we also investigated the provenance of cultured *A. tropicus* from an aquaculture cooperative on the Pacific coast of México to assess the

potential for human-mediated movement of gar across biogeographic boundaries.

## Methods

### Sample collection

Samples of tropical gar were collected between 2009 and 2017 from 10 locations that span much of the range of the species, including 9 natural populations and 1 population associated with an aquaculture facility (see Fig. 1 and Table 1 for an overview). On the Atlantic versant, sampling sites included the Laguna Chilapa in the Río Grijalva drainage, México ( $N=32$  individuals); the Río Chacamax in the Río Usumacinta drainage, México ( $N=20$ ); three proximate sites in the Laguna Canitzán and the main stem of the Río Usumacinta, also in the Río Usumacinta drainage, México ( $N=22+9+16$ ); and one site in the Laguna Caño Negro and nearby rivers, Río San Juan drainage, Costa Rica ( $N=25$ ). On the Pacific versant, sampling sites were in the Río Zanatenco, México (3 individuals); the Canal de Chiquimulilla associated with the Río Los Esclaves, Guatemala ( $N=34$ ); and a site in the Barra de Santiago wetland associated with

**Table 1** Summary information of sites from which tropical gar were sampled, including location, drainage, country, versant, and latitude and longitude

| Site | Location                                             | Drainage                  | Country     | Versant  | Lat/Long        |
|------|------------------------------------------------------|---------------------------|-------------|----------|-----------------|
| P1   | Laguna Chilapa, Centla, Tabasco                      | Río Grijalva              | México      | Atlantic | 17.973, −92.592 |
| P2   | Río Chacamax,<br>La Libertad, Chiapas                | Río Usumacinta            | México      | Atlantic | 17.698, −91.741 |
| P3   | Laguna Canitzán, Tenosique, Tabasco                  | Río Usumacinta            | México      | Atlantic | 17.618, −91.383 |
| P4   | Río Usumacinta, Tabasco, Tenosique, Tabasco          | Río Usumacinta            | México      | Atlantic | 17.601, −91.500 |
| P5   | Río Usumacinta, Boca del Cerro, Tabasco              | Río Usumacinta            | México      | Atlantic | 17.599, −91.354 |
| P6   | Laguna Caño Negro, Alajuela                          | Río San Juan              | Costa Rica  | Atlantic | 10.951, −84.764 |
| P7   | Tonalá, Chiapas                                      | Río Zanatenco             | México      | Pacific  | 16.085, −93.724 |
| P8   | Canal de Chiquimulilla, Aldea Monterrico, Santa Rosa | Río Los Esclavos          | Guatemala   | Pacific  | 13.895, −90.481 |
| P9   | Zanjon del Chino, Ahuachapan                         | Ríos Cara Sucia/San Pedro | El Salvador | Pacific  | 13.754, −90.056 |
| P10  | La Palma, Chiapas                                    | Aquaculture               | México      | Pacific  | 15.173, −92.838 |

All samples were collected from natural populations, except for the population from La Palma (Chiapas, site 10), which represents an aquaculture population

Ríos Cara Sucia and San Pedro of El Salvador ( $N=39$ ). In addition, we obtained 12 samples from an aquaculture cooperative located in La Palma, Chiapas, on the Pacific versant of México. Hence, the total sample size included in this study was 212 individuals.

All individuals for this study were adults (> 60 cm total length) caught with cast nets. A small piece of the caudal or a pelvic fin was removed from each of the individuals with dissection scissors upon capture, and tissues were preserved in 95% ethanol for later processing in the lab. All individuals were immediately released at the original collection site once the tissue sample was secured.

### DNA extraction and microsatellite amplification

The total genomic DNA was extracted from ethanol-preserved fin clips with the DNeasy Blood & Tissue Kit (Qiagen, Inc., Valencia, CA) following the manufacturer's protocol. Extracted DNA was used for polymerase chain reaction (PCR) amplification of 11 tri-nucleotide microsatellites using primers *Asp007* (annealing temperature,  $T_a=56^\circ\text{C}$ ), *Asp035* ( $T_a=56^\circ\text{C}$ ), *Asp040* ( $T_a=56^\circ\text{C}$ ), *Asp053* ( $T_a=53^\circ\text{C}$ ), *Asp054* ( $T_a=56^\circ\text{C}$ ), *Asp057* ( $T_a=56^\circ\text{C}$ ), *Asp066* ( $T_a=56^\circ\text{C}$ ), *Asp072* ( $T_a=56^\circ\text{C}$ ), *Asp122* ( $T_a=60^\circ\text{C}$ ), *Asp159* ( $T_a=58^\circ\text{C}$ ), and *Asp168* ( $T_a=56^\circ\text{C}$ ) (Moyer et al. 2009; Bohn et al. 2013). Microsatellites of all loci were amplified using PCR mix A from Moyer et al. (2009): 50 mM KCl, 10 mM Tris–HCl at pH 8.3, 0.01% gelatin, 2.0 mM  $\text{MgCl}_2$ , 200  $\mu\text{M}$  dNTPs, 0.1875 U Taq polymerase, 0.3  $\mu\text{M}$  of both the forward and reverse primer, 20–100 ng of template DNA, and RNase-free water to a final volume of 12.5  $\mu\text{L}$ . PCR conditions consisted of an initial denaturing at  $94^\circ\text{C}$  for 2 min, and then 35 cycles of  $94^\circ\text{C}$  for 30 s,  $56$ – $60^\circ\text{C}$  (depending on the primer specific annealing temperature) for 1 min, and  $72^\circ\text{C}$  for 1 min. At

the end, a 10-min final elongation step concluded the PCR. The PCR products were separated using capillary electrophoresis on an ABI 377 automated DNA sequencer (Applied Biosystems, Inc.). Genotypes were determined using Genotyper 2.1 software (Applied Biosystems), and Micro-checker v. 2.2.3 (Van Oosterhout et al. 2004) was used to assess the microsatellite data for null alleles and scoring errors.

### Data analysis

Data analysis was conducted in R v. 4.1.1 (R Core Team 2021), unless otherwise noted. We summarized missing data using the `info_table()` function from the `POPPR` package (Kamvar et al. 2014). We then tested for departures from Hardy–Weinberg equilibria using the `hw.test()` function from `PEGAS` (Paradis 2010);  $P$ -values were derived analytically from a  $\chi^2$  statistic and from a Monte Carlo test with 1000 permutations. We also tested for departures from linkage equilibrium using the `pair.ia()` function from `POPPR` (Kamvar et al. 2014). This function calculates a standardized index of association,  $\bar{r}_d$ , which is less biased to variation in sample sizes compared to the classical index of association,  $I_A$  (Agapow and Burt 2001).  $P$ -values for deviations from linkage equilibrium were based on 1000 permutations. In addition, we calculated a series of descriptive statistics, including allelic richness using the `allelic.richness()` function from the `HIERFSTAT` package (Goudet 2005), the number of alleles per locus and the number of polymorphic loci within each population using the `summary()` function in `POPPR` (Kamvar et al. 2014), the observed and expected heterozygosity using the `basic.stats()` function in `HIERFSTAT` (Goudet 2005), and the inbreeding coefficient,  $F_{IS}$ , using the `basic.stats()` function in `HIERFSTAT` (Goudet 2005). We also quantified the number of private alleles in each population with the `private_alleles()` function from `POPPR` (Kamvar et al. 2014).

We used two complementary methods to describe and visualize clusters of genetically related populations and individuals. First, we calculated chord distances (Cavalli-Sforza and Edwards 1967) using the `genet.dist()` function from the `HIERFSTAT` package (Goudet 2005) to generate a neighbor-joining tree using the `nj()` function from the `APE` package (Paradis and Schliep 2019). Second, we conducted a principal component analysis (PCA) using the `dudi.pca()` function from the `ADE4` package (Dray and Dufour 2007).

To identify population structure empirically without a priori designation of defined populations, we used the program `STRUCTURE` (v. 2.3.4; Pritchard et al. 2000), and `STRUCTURE` outputs were processed with the `POPHELPER` package in R (Francis 2017). Specifically, we identified the optimal number of genetically distinct clusters ( $K$ ) using the Evanno method (Evanno et al. 2005), as implemented in the `evannoMethodStructure()` function, and visualized individual cluster assignment probabilities using the `plotQ()` function.

Finally, differentiation between all population pairs was evaluated using  $F_{ST}$  (Weir & Cockerham 1984), as implemented in the `pairwise.WCfst()` function from `HIERFSTAT` (Goudet 2005). We tested for isolation by distance among all natural populations (*i.e.*, excluding the aquaculture population, P10) using a Mantel test with  $F_{ST}$  as the dependent variable and geographic distance (in kilometers) as the independent variable. The Mantel test was conducted with 1000 permutations of the `mantel()` function from the `ECODIST` package (Goslee and Urban 2007).

Note that we conducted all analyses with and without the aquaculture population (P10, because it is a non-natural population) and the population from Río Zanatenco, Chiapas (P7, because of low sample size). However, there were no qualitative differences in the outcome of analyses with or without these populations. Since both populations are

interesting from a conservation management perspective, we only present the full analyses using all 10 populations.

## Results

A total of 212 individual fish were successfully genotyped for 11 microsatellite loci. The missing data rate across all loci and populations was less than 2.3%, and no single population or locus had a missing data rate over 7.7% (Table S1). All loci were polymorphic, and the number of alleles ranged from 3 (*Asp072*) to 18 (*Asp066*; Table S2). Global analyses including all populations revealed significant deviation from both Hardy–Weinberg (Table S3) and linkage equilibria (Figure S1), which is expected from spatially structured populations with non-random mating. However, population-specific analyses found no consistent evidence for deviations from Hardy–Weinberg expectations for specific loci or populations (Table S3), or for deviations from linkage equilibrium (Table S4).

Compared across natural populations, allelic richness varied from 1.493 in the population from El Salvador to 1.971 in the population from Laguna Canitzán in the Río Usumacinta basin (Table 2). Across the same populations, observed heterozygosity varied from 0.209–0.411 and expected heterozygosity from 0.244–0.444 (Table 2). The number of polymorphic loci ranged from 5 to 11 (Table 2). The population from Costa Rica exhibited the highest number of private alleles (10), exceeding the range for all other populations (0–4). Finally, the population from the aquaculture facility exhibited the highest values for allelic richness (2.099) and observed and expected heterozygosity (0.459 and 0.490), exceeding the metrics of all natural populations.

**Table 2** Summary statistics for 10 populations of tropical gar sampled for this study

| Site | $N$  | $N_A$ | $R_A$ | $H_O$ | $H_E$ | $F_{IS}$ | $N_{po}$ | $N_{pr}$ |
|------|------|-------|-------|-------|-------|----------|----------|----------|
| P1   | 32   | 4.000 | 1.900 | 0.417 | 0.415 | −0.003   | 10       | 3        |
| P2   | 20   | 3.364 | 1.844 | 0.338 | 0.396 | 0.095    | 11       | 4        |
| P3   | 22   | 4.000 | 1.971 | 0.411 | 0.444 | 0.103    | 10       | 2        |
| P4   | 9    | 2.455 | 1.654 | 0.297 | 0.302 | −0.019   | 6        | 0        |
| P5   | 16   | 3.182 | 1.792 | 0.363 | 0.370 | 0.030    | 10       | 0        |
| P6   | 25   | 3.909 | 1.737 | 0.327 | 0.309 | −0.063   | 7        | 10       |
| P7   | 3    | 2.091 | 1.824 | 0.288 | 0.364 | 0.213    | 5        | 0        |
| P8   | 34   | 2.545 | 1.595 | 0.326 | 0.292 | −0.112   | 7        | 1        |
| P9   | 39   | 2.455 | 1.493 | 0.209 | 0.244 | 0.195    | 7        | 1        |
| P10  | 12   | 3.455 | 2.099 | 0.459 | 0.490 | 0.099    | 10       | 0        |
| Mean | 21.2 | 3.146 | 1.791 | 0.344 | 0.363 | 0.054    | 8.3      | 2.1      |

The table lists the sample size ( $N$ ), the average number of alleles per locus ( $N_A$ ), allelic richness ( $R_A$ ), observed heterozygosity ( $H_O$ ), expected heterozygosity ( $H_E$ ), inbreeding coefficient ( $F_{IS}$ ), the number of polymorphic loci ( $N_{po}$ ), and the number of private alleles ( $N_{pr}$ ). The populations IDs (P1–P10) correspond to Table 1

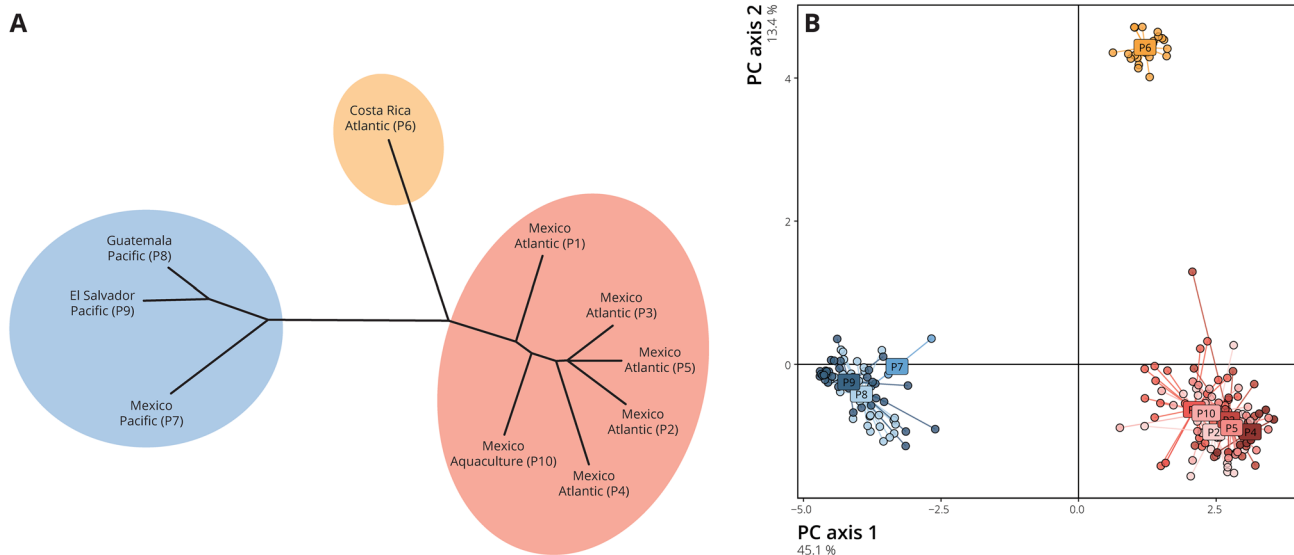
Despite these high levels of genetic diversity, the aquaculture population exhibited no private alleles.

We used a neighbor-joining tree based on population-level allele frequencies and a principal component analysis (PCA) to describe and visualize clusters of genetically related populations and individuals. The neighbor-joining tree (Fig. 2A) indicated a clear division between populations from the Atlantic and Pacific versants of Central America. Within the Atlantic cluster, the population from Costa Rica was divergent from the populations in México. Most notably, the population from the aquaculture facility clustered with the Atlantic populations from México, even though the facility is located on México's Pacific versant. The PCA confirmed the patterns of the neighbor-joining tree and recovered three distinct clusters (Fig. 2B), with a clear separation of the Atlantic and Pacific populations along axis 1 and separation of the Costa Rican and Mexican populations

along axis 2. Again, the aquaculture population clustered with populations from the Atlantic side of México.

Finally, the identification of three distinct population clusters was supported empirically by the STRUCTURE analysis (best supported number of clusters  $K=3$ ; Table 3; Fig. 3). The three clusters included (1) populations from the Atlantic drainages of the Grijalva and Usumacinta in México, (2) the population from the Atlantic versant of Costa Rica, and (3) the populations from the Pacific drainages in México, Guatemala, and El Salvador. The aquaculture population once again clustered with populations from México's Atlantic drainages (Fig. 3).

Pairwise  $F_{ST}$  values between population pairs ranged across two orders of magnitude, from 0.007 between two populations on México's Atlantic versant (P3 and P5) and 0.722 between a Mexican population on the Atlantic versant (P4) and the population from El Salvador (P9; Table 4).

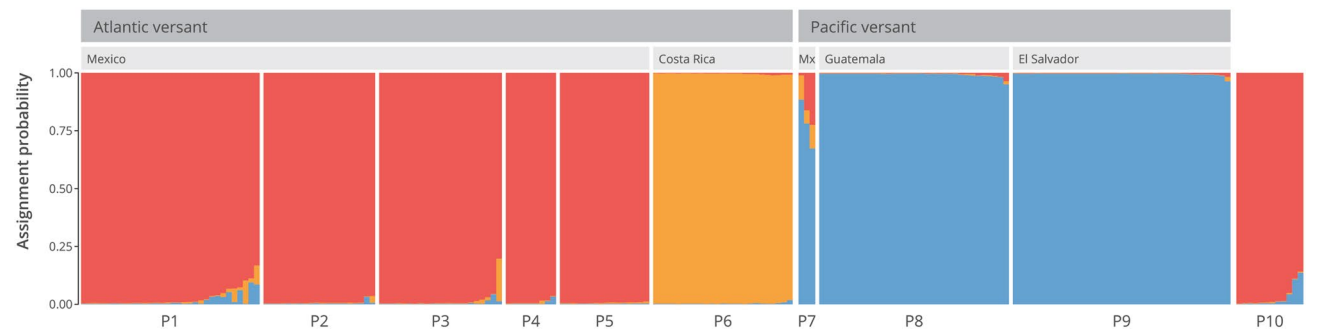


**Fig. 2** **A** Unrooted neighbor-joining tree constructed with chord distances estimated from the allele frequency data of 10 populations of tropical gar. The populations are labeled by country of origin, versant (Atlantic or Pacific), and the identification number as listed in Table 1. Note that three distinct clusters are recovered: populations from the Atlantic versant in México form the cluster highlighted in red, including the aquaculture population from La Palma that is

located on the Pacific versant (site 10); the southernmost population from Costa Rica forms the orange cluster; and the Pacific versant populations from México, Guatemala, and El Salvador form the blue cluster. **B** Results from a principal component analysis (PCA) are consistent with the neighbor-joining tree, uncovering three clear clusters of related individuals (colors correspond to those in panel A). Populations IDs (P1-P10) correspond to Table 1

**Table 3** STRUCTURE Harvester results for the best-supported number of genetic clusters ( $K$ ) for the populations sampled for this study

| $K$ | Replicates | Mean lnP ( $K$ ) | Stdev lnP ( $\sigma$ ) | ln' ( $K$ ) | l ln''( $K$ ) | $\Delta K$ |
|-----|------------|------------------|------------------------|-------------|---------------|------------|
| 2   | 10         | -4208.11         | 0.2079                 | —           | —             | —          |
| 3   | 10         | -3593.86         | 0.3836                 | 614.25      | 473.88        | 1235.50    |
| 4   | 10         | -3453.49         | 34.0654                | 140.37      | 65.73         | 1.92       |
| 5   | 10         | -3378.85         | 6.9693                 | 74.64       | 35.57         | 5.10       |
| 6   | 10         | -3339.78         | 2.9716                 | 39.07       | 68.10         | 22.91      |
| 7   | 10         | -3368.81         | 11.4307                | 29.03       | 37.20         | 3.25       |
| 8   | 10         | -3360.64         | 17.2228                | 8.17        | —             | —          |



**Fig. 3** Results of STRUCTURE analyses for K=3. Tropical gar populations are structured by versant (Atlantic vs. Pacific) and geography (México vs. Costa Rica). P10 represents an aquaculture population located on the Pacific versant of México. Populations IDs (P1-P10) correspond to Table 1

**Table 4** Pairwise  $F_{ST}$ -values for all population pairs of tropical gar

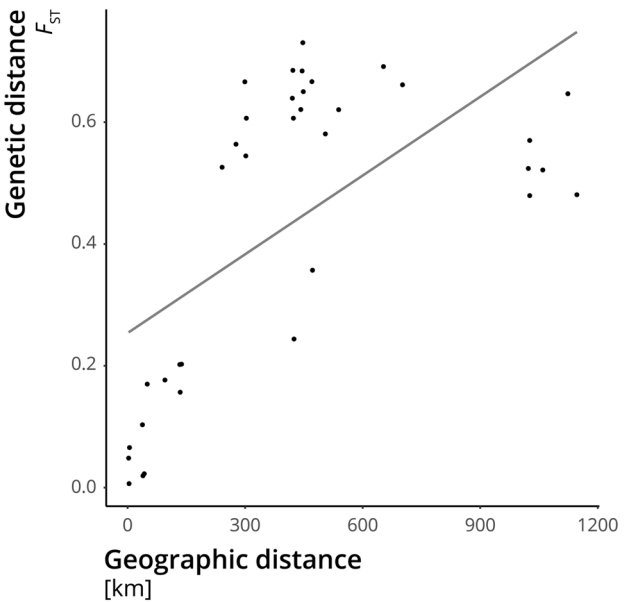
|     | P1    | P2    | P3     | P4    | P5    | P6    | P7    | P8    | P9    |
|-----|-------|-------|--------|-------|-------|-------|-------|-------|-------|
| P2  | 0.180 |       |        |       |       |       |       |       |       |
| P3  | 0.155 | 0.021 |        |       |       |       |       |       |       |
| P4  | 0.200 | 0.103 | 0.047  |       |       |       |       |       |       |
| P5  | 0.208 | 0.023 | *0.007 | 0.066 |       |       |       |       |       |
| P6  | 0.477 | 0.515 | 0.476  | 0.559 | 0.531 |       |       |       |       |
| P7  | 0.521 | 0.561 | 0.537  | 0.651 | 0.600 | 0.633 |       |       |       |
| P8  | 0.579 | 0.610 | 0.604  | 0.678 | 0.644 | 0.662 | 0.252 |       |       |
| P9  | 0.619 | 0.654 | 0.648  | 0.722 | 0.687 | 0.692 | 0.347 | 0.169 |       |
| P10 | 0.084 | 0.072 | 0.043  | 0.122 | 0.087 | 0.468 | 0.493 | 0.587 | 0.632 |

Note that all comparisons were significantly different from zero ( $P < 0.001$ ), except for P3/P5 (marked with an asterisk). The populations IDs (P1-P10) correspond to Table 1 in the main manuscript

With a single exception (P3/P5), all pairwise  $F_{ST}$  values were statistically significant. There was a significant correlation between geographic and genetic distance (Mantel test:  $r = 0.320$ ,  $P = 0.003$ ), indicating isolation by distance (Fig. 4).

Discussion

The tropical gar (*Atractosteus tropicus*) is the smallest species within the family Lepisosteidae, yet this emergent aquaculture species has perhaps the highest socioeconomic impact both in terms of subsistence and commercial fisheries. Our study of the population genetic structure in this species revealed deep divergences among populations in different regions of México and Central America. We identified three genetic clusters that likely represent ancient genetic lineages with distinct geographic distributions. Most prominently, there was a divide between populations inhabiting rivers on the Atlantic versus the Pacific versant, a pattern commonly observed in other freshwater fishes of this region (McCafferty et al. 2012; McMahan et al. 2013; Picq et al.



**Fig. 4** Genetic distance ( $F_{ST}$ ) plotted as a function of geographic distance (km) for all pairwise comparisons of the nine wild-caught populations of tropical gar (Mantel test:  $r = 0.320$ ,  $P = 0.003$ )

2014; Palacios et al. 2016; Ward et al. 2022). In addition, populations from the Atlantic versant were further split into a northern cluster from the Grijalva-Usumacinta River basins in México and a southern cluster from the Río San Juan basin in Costa Rica.

Our findings based on the analysis of nuclear microsatellite markers exhibit commonalities and differences with a prior population genetic study that used analyses of mitochondrial DNA (Barrientos-Villalobos and Espinosa de los Monteros 2008). Both studies recovered a clear split between populations from the Atlantic and the Pacific versants, reflecting extended periods of geographic isolation and a pattern of isolation by distance, which suggests limited gene flow. In contrast, there was no clear pattern of differentiation in mitochondrial haplotypes between Atlantic versant populations in México and Costa Rica. Instead, Barrientos-Villalobos and Espinosa de los Monteros (2008) documented deeply divergent mitochondrial haplotypes from the lower Río Usumacinta and the Petén region of Guatemala that formed a sister clade to all other tropical gar samples, including individuals from the Atlantic and Pacific versants (Fig. 1B), which led to speculation about a putatively undescribed species. However, analyses of nuclear markers in our study did not corroborate the cryptic species interpretation. Although we were not able to obtain samples from Guatemala's Petén region, there was no evidence for a divergent population in sites associated with the Río Usumacinta. Hence, the divergent mitochondrial haplotypes likely represent ancient polymorphisms that have been maintained within populations of tropical gar, rather than distinct evolutionary lineages. Future analyses will nonetheless require a more comprehensive coverage of the species' range to obtain a complete picture of genetically distinct lineages present within the species, including the Ríos Papaloapan and Coatzacoalcos in México, the Petén region in Guatemala, and occurrences around Lago Xolotlán and Lago Cocibolca in Nicaragua.

One of the key findings of our study was that the aquaculture population (located on the Pacific versant of México) was genetically derived from populations on the Atlantic versant, which belong to a different genetic cluster than more proximate populations in rivers that flow westward. This affinity to Atlantic versant populations was confirmed through the neighbor-joining tree, principal component analysis, and the analysis of population structure, suggesting that brood stock for use in aquaculture has been transported across biogeographic boundaries. Follow-up inquiries with the operators of the facility have confirmed that the brood stock at the aquaculture cooperative in La Palma was originally derived from fish produced in a facility from Tabasco (Arias-Rodriguez, personal communication). Transfer of fish across biogeographic boundaries is potentially problematic if we consider the aquacultural practices of the region.

Aquaculture cooperatives breed tropical gar in their facilities and then sell juveniles to landowners that stock them in ponds for rearing to adult size and eventual harvesting. However, individual fish can escape into natural systems, especially during flooding periods that are common in this region and trigger spawning in *A. tropicus*. This scenario has been documented in other species, especially cichlids of the genus *Oreochromis*, which are commonly used in aquaculture in the same region and have become invasive nuisance species as a consequence (Esselman et al. 2013). Interestingly, mixing of natural stocks may have occurred already. Two of three individuals genotyped from a natural population on México's Pacific versant (P7) exhibit a comparatively high putative ancestry from the Atlantic clusters. Whether this finding is a consequence of human activity remains to be tested, especially using higher sample sizes from surrounding populations. At least in theory, the observed pattern may also be a consequence natural gene flow mediated through stream capture in the intersecting river systems of the upper Río Grijalva (although tropical gar does not typically occur in those habitats), or it may reflect ancestral genetic variation that is shared and has been maintained across geographic regions.

The sharing of brood stock among hatcheries could significantly impact the population genetic structure of tropical gar in the future, as new facilities producing gar are continuously appearing. Currently, there are at least six known facilities producing tropical gar in the Mexican state of Tabasco, two in Campeche, one in Veracruz (all associated with water bodies connected to the Gulf of Mexico), and two in Chiapas (both associated with Pacific drainages; Arias-Rodriguez, personal communication). One facility for which production numbers are available (at the Universidad Juárez Autónoma de Tabasco) sells about 200,000 individuals annually. The current practice is that brood stocks are recruited from laboratory-reared individuals and widely shared across facilities, because acclimating wild-caught fish to hatchery conditions is difficult and time consuming. Wild-caught tropical gar have to be fed with live fish, and transitioning them to artificial feeds is not always successful. In addition, wild-caught fish will only spawn in the rainy season even in captivity, which limits productivity. Using breeders recruited from laboratory-born individuals circumnavigates these problems as they readily accept artificial feeds and can be spawned year-round.

Moving forward, potential ecological and evolutionary differences among genetic clusters should be considered in the management of tropical gar populations and aquaculture practices. Aquaculture can be regulated at the local, regional, and international levels to avoid trafficking and mixing of brood stock across biogeographic boundaries and maintain the genetic structure documented in our study. Such regulations would help to prevent

introgression among deeply divergent genetic clusters that could represent evolutionary significant units with different fisheries and conservation management needs. However, for such regulations to be effective, there are several pressing questions that will need to be addressed through future research. From an aquaculture perspective, developing methods for the more effective recruitment of wild-caught individuals as breeders in captive populations should have high priority. Establishment of captive breeders from local populations will reduce the need of sharing brood stock across regions, and it will open opportunities to investigate how different stocks perform in captive care, with concomitant insights about potential differences between native and non-native bloodlines that could impact natural populations. Reliance on local brood stock would also facilitate the frequent turnover of breeding individuals to prevent hatchery adaptation, as documented in other freshwater fishes (Frankham 2008; Christie et al. 2012, 2014). From a fisheries and conservation management perspective, it will be critical to investigate whether the different genetic clusters documented here also differ in aspects of their phenotypes, especially in terms of physiological, behavioral, and life history traits that could mediate local adaptation. These data will be critical to identify potential evolutionary significant units and designate conservation priorities. Future work will also need to investigate the extent and nature of potential introgression using genomic tools to better understand the risks associated with aquacultural releases. In the meantime, tropical gar farmers can be informed that introgression from non-local populations has the potential to reduce survival and reproductive rates, with concomitant negative effects for natural populations (Araki et al. 2008). Proactive fisheries and aquaculture management will hopefully help to counteract recent trends of population decline and local extirpation of tropical gar (Villa 1982; Mora Jamett et al. 1997; Aguilera et al. 2002; Siebe et al. 2005), such that future generations might continue to enjoy traditional pejelagarto dishes served in the region.

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work. MPM, LAR, and MT analyzed the data. MT made the figures and wrote the manuscript. All authors reviewed the manuscript.

## Declarations

**Competing interests** The authors declare no competing interests.

## References

- Agapow P-M, Burt A (2001) Indices of multilocus linkage disequilibrium. *Mol Ecol Notes* 1:101–102. <https://doi.org/10.1046/j.1471-8278.2000.00014.x>
- Aguilera C, Mendoza R, Rodríguez G, Márquez G (2002) Morphological description of alligator gar and tropical gar larvae, with an emphasis on growth indicators. *Trans Am Fish Soc* 131:899–909. [https://doi.org/10.1577/1548-8659\(2002\)131%3c0899:MDOAGA%3e2.0.CO;2](https://doi.org/10.1577/1548-8659(2002)131%3c0899:MDOAGA%3e2.0.CO;2)
- Akin S, Winemiller KO (2006) Seasonal variation in food web composition and structure in a temperate tidal estuary. *Estuaries Coasts* 29:552–567. <https://doi.org/10.1007/BF02784282>
- Allan JD, Abell R, Hogan Z et al (2005) Overfishing of inland waters. *Bioscience* 55:1041–1051. [https://doi.org/10.1641/0006-3568\(2005\)055\[1041:OOIW\]2.0.CO;2](https://doi.org/10.1641/0006-3568(2005)055[1041:OOIW]2.0.CO;2)
- Araki H, Berejikian BA, Ford MJ, Blouin MS (2008) Fitness of hatchery-reared salmonids in the wild. *Evol Appl* 1:342–355. <https://doi.org/10.1111/j.1752-4571.2008.00026.x>
- Aranda-Morales SA, Peña-Marín ES, Jiménez-Martínez LD et al (2021) Expression of ion transport proteins and routine metabolism in juveniles of tropical gar (*Atractosteus tropicus*) exposed to ammonia. *Comp Biochem Physiol C Toxicol Pharmacol* 250:109166. <https://doi.org/10.1016/j.cbpc.2021.109166>
- Barrientos C, Quintana Y, Elías DJ, Rodiles-Hernández R (2018) Peces nativos y pesca artesanal en la cuenca Usumacinta, Guatemala. *Revista Mexicana de Biodiversidad* 89:S118–S130. <https://doi.org/10.22201/ib.20078706e.2018.4.2180>
- Barrientos-Villalobos J, Espinosa de los Monteros A (2008) Genetic variation and recent population history of the tropical gar *Atractosteus tropicus* Gill (Pisces: Lepisosteidae). *J Fish Biol* 73:1919–1936. <https://doi.org/10.1111/j.1095-8649.2008.01993.x>
- Berra TM (2001) Freshwater fish distribution. University of Chicago Press, Chicago
- Bohn S, Barraza E, McMahan C et al (2013) Cross amplification of microsatellite loci developed for *Atractosteus spatula* in *Atractosteus tropicus*. *Rev Mex Biodivers* 84:1349–1351. <https://doi.org/10.7550/rmb.32705>
- Bohn S, Kreiser BR, Daugherty DJ, Bodine KA (2017) Natural hybridization of lepisosteids: implications for managing the alligator gar. *N Am J Fish Manage* 37:405–413. <https://doi.org/10.1080/02755947.2016.1265030>
- Brito PM, Alvarado-Ortega J, Meunier FJ (2017) Earliest known lepisosteoid extends the range of anatomically modern gars to the Late Jurassic. *Sci Rep* 7:17830. <https://doi.org/10.1038/s41598-017-17984-w>
- Buckmeier DL, Smith NG, Schlechte JW et al (2016) Characteristics and conservation of a trophy alligator gar population in the middle Trinity River, Texas. *J Southeast Assoc Fish Wildl Agencies* 3:33–38
- Burggren WW, Bautista GM, Coop SC et al (2016) Developmental cardiorespiratory physiology of the air-breathing tropical gar, *Atractosteus tropicus*. *Am J Physiol Regul Integr Comp Physiol* 311:R689–R701. <https://doi.org/10.1152/ajpregu.00022.2016>

- Bussing WA (1998) Peces de las Aguas Continentales de Costa Rica—freshwater fishes of Costa Rica, 2nd edn. Editorial de la Universidad de Costa Rica, San José
- Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. *Evolution* 21:550–570. <https://doi.org/10.1111/j.1558-5646.1967.tb03411.x>
- Christie MR, Ford MJ, Blouin MS (2014) On the reproductive success of early-generation hatchery fish in the wild. *Evol Appl* 7:883–896. <https://doi.org/10.1111/eva.12183>
- Christie MR, Marine ML, French RA, Blouin MS (2012) Genetic adaptation to captivity can occur in a single generation. *Proc Natl Acad Sci USA* 109:238–242. <https://doi.org/10.1073/pnas.1111073109>
- David SR, King SM, Stein JA (2018) Introduction to a special section: angling for dinosaurs—status and future study of the ecology, conservation, and management of ancient fishes. *Trans Am Fish Soc* 147:623–625. <https://doi.org/10.1002/tafs.10072>
- Dray S, Dufour AB (2007) The ade4 package: implementing the duality diagram for ecologists. *J Stat Softw* 22:1–20. <https://doi.org/10.18637/jss.v022.i04>
- Echelle AA, Grande L (2014) Lepisosteidae: gars. In: Warren Burr MLBM (ed) *Freshwater fishes of North America*, vol 1. John Hopkins University Press, Baltimore, pp 243–278
- Emery KF (2017) Zooarchaeology of the Maya. In: Albarella U, Rizzetto M, Russ H et al (eds) *The Oxford handbook of zooarchaeology*. Oxford University Press, Oxford
- Esselman PC, Schmitter-Soto JJ, Allan JD (2013) Spatiotemporal dynamics of the spread of African tilapias (*Pisces: Oreochromis* spp.) into rivers of northeastern Mesoamerica. *Biol Invasions* 15:1471–1491. <https://doi.org/10.1007/s10530-012-0384-9>
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* 14:2611–2620. <https://doi.org/10.1111/j.1365-294X.2005.02553.x>
- Fletcher DE, Lindell AH, Stillings GK et al (2015) Trophic variation in coastal plain stream predatory fishes. *Southeast Nat* 14:373–396. <https://doi.org/10.2307/26454496>
- Francis RM (2017) pophelper: an R package and web app to analyse and visualize population structure. *Mol Ecol Resour* 17:27–32. <https://doi.org/10.1111/1755-0998.12509>
- Frankham R (2008) Genetic adaptation to captivity in species conservation programs. *Mol Ecol* 17:325–333. <https://doi.org/10.1111/j.1365-294X.2007.03399.x>
- Fry B, Mumford PL, Tam F et al (1999) Trophic position and individual feeding histories of fish from Lake Okeechobee, Florida. *Can J Fish Aquat Sci* 56:590–600. <https://doi.org/10.1139/f98-204>
- García de León FJ, González-García L, Herrera-Castillo JM et al (2001) Ecology of the alligator gar, *Atractosteus spatula*, in the Vicente Guerrero Reservoir, Tamaulipas, México. *Southwest Nat* 46:151–157. <https://doi.org/10.2307/3672523>
- Glass WR, Walter RP, Heath DD et al (2015) Genetic structure and diversity of spotted gar (*Lepisosteus oculatus*) at its northern range edge: implications for conservation. *Conserv Genet* 16:889–899. <https://doi.org/10.1007/s10592-015-0708-2>
- Goslee SC, Urban DL (2007) The ecodist package for dissimilarity-based analysis of ecological data. *J Stat Softw* 22:1–19
- Goudet J (2005) Hierfstat, a package for R to compute and test hierarchical F-statistics. *Mol Ecol Notes* 5:184–186. <https://doi.org/10.1111/j.1471-8286.2004.00828.x>
- Kamvar ZN, Tabima JF, Grünwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ* 2:e281. <https://doi.org/10.7717/peerj.281>
- Lauder GV Jr (1980) Evolution of the feeding mechanism in primitive actinopterygian fishes: a functional anatomical analysis of *Polypterus*, *Lepisosteus*, and *Amia*. *J Morphol* 163:283–317. <https://doi.org/10.1002/jmor.1051630305>
- Lemberg JB, Shubin NH, Westneat MW (2019) Feeding kinematics and morphology of the alligator gar (*Atractosteus spatula*, Lacépède, 1803). *J Morphol* 280:1548–1570. <https://doi.org/10.1002/jmor.21048>
- Manuel M-C, Wendi A-F, Emilio I-D (2013) Common pool resources dilemmas in tropical inland small-scale fisheries. *Ocean Coast Manag* 82:119–126. <https://doi.org/10.1016/j.ocecoaman.2013.06.004>
- MARN (2015) Listado oficial de especies de vida silvestre amenazadas o en peligro de extinción. Ministerio de Medio Ambiente y Recursos Naturales, San Salvador, El Salvador
- Márquez-Couturier G, Vázquez-Navarrete CJ et al (2015) State of the art of biology and breeding of tropical gar (*Atractosteus tropicus*). *Agroproductividad* 8:44–51
- Martínez-Bautista G, Martínez-Burguete T, Peña-Marín ES et al (2022) Hypoxia- and hyperoxia-related gene expression dynamics during developmental critical windows of the tropical gar, *Atractosteus tropicus*. *Comp Biochem Physiol A Mol Integr Physiol* 263:111093. <https://doi.org/10.1016/j.cbpa.2021.111093>
- Martínez-Cárdenas L, Parra-Parra VG, Ramos-Resendiz S et al (2018) Effect of feeding frequency on growth and survival in juvenile gar, *Atractosteus tropicus* Gill, 1863, in culture conditions. *Lat Am J Aquat Res* 46:1034–1040. <https://doi.org/10.3856/vol4-issue-fullt-ext-16>
- McCafferty SS, Martin A, Bermingham E (2012) Phylogeographic diversity of the lower Central American cichlid *Andinoacara coeruleopunctatus* (Cichlidae). *Int J Evol Biol* 2012:780169. <https://doi.org/10.1155/2012/780169>
- McMahan CD, Davis MP, Domínguez-Domínguez O et al (2013) From the mountains to the sea: phylogeography and cryptic diversity within the mountain mullet, *Agonostomus monticola* (Teleostei: Mugilidae). *J Biogeogr* 40:894–904. <https://doi.org/10.1111/jbi.12036>
- Mendoza Alfaro R, González CA, Ferrara AM (2008) Gar biology and culture: status and prospects. *Aquac Res* 39:748–763. <https://doi.org/10.1111/j.1365-2109.2008.01927.x>
- Miller RR, Minckley WL, Norris SM (2005) *Freshwater fishes of Mexico*. University of Chicago Press, Chicago
- Mora Jamett M, Cabrera Peña J, Galeano G (1997) Reproducción y alimentación del gaspar *Atractosteus tropicus* (Pisces: Lepisosteidae) en el Refugio Nacional de Vida Silvestre Caño Negro, Costa Rica. *Rev Biol Trop* 45:861–866
- Moyer GR, Sloss BL, Kreiser BR, Feldheim KA (2009) Isolation and characterization of microsatellite loci for alligator gar (*Atractosteus spatula*) and their variability in two other species (*Lepisosteus oculatus* and *L. osseus*) of Lepisosteidae. *Mol Ecol Resour* 9:963–966. <https://doi.org/10.1111/j.1755-0998.2009.02519.x>
- Östergren J, Palm S, Gilbey J et al (2021) A century of genetic homogenization in Baltic salmon—evidence from archival DNA. *Proc Biol Sci* 288:20203147. <https://doi.org/10.1098/rspb.2020.3147>
- Palacios M, Voelker G, Arias Rodríguez L et al (2016) Phylogenetic analyses of the subgenus *Mollienesia* (*Poecilia*, Poeciliidae, Teleostei) reveal taxonomic inconsistencies, cryptic biodiversity, and spatio-temporal aspects of diversification in Middle America. *Mol Phylogenet Evol* 103:230–244. <https://doi.org/10.1016/j.ympev.2016.07.025>
- Palma-Cancino DJ, Martínez-García R, Álvarez-González CA et al (2019) Bioeconomic profitability analysis of tropical gar (*Atractosteus tropicus*) grow-out using two commercial feeds. *Lat Am J Aquat Res* 47:433–439. <https://doi.org/10.3856/vol47-issue3-fullt-ext-5>
- Paradis E (2010) pegas: an R package for population genetics with an integrated-modular approach. *Bioinformatics* 26:419–420. <https://doi.org/10.1093/bioinformatics/btp696>

- Paradis E, Schliep K (2019) ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* 35:526–528. <https://doi.org/10.1093/bioinformatics/bty633>
- Picq S, Alda F, Krahe R, Bermingham E (2014) Miocene and Pliocene colonization of the Central American Isthmus by the weakly electric fish *Brachyhypopomus occidentalis* (Hypopomidae, Gymnotiformes). *J Biogeogr* 41:1520–1532. <https://doi.org/10.1111/jbi.12309>
- Porter HT, Motta PJ (2004) A comparison of strike and prey capture kinematics of three species of piscivorous fishes: Florida gar (*Lepisosteus platyrhincus*), redfin needlefish (*Strongylura notata*), and great barracuda (*Sphyræna barracuda*). *Mar Biol* 145:989–1000. <https://doi.org/10.1007/s00227-004-1380-0>
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959. <https://doi.org/10.1093/genetics/155.2.945>
- R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
- Sauz-Sánchez de J, Rodiles-Hernández R, Andrade-Velázquez M, Mendoza-Carranza M (2021) Modelling the potential distribution of two tropical freshwater fish species under climate change scenarios. *Aquat Conserv* 31:2737–2751. <https://doi.org/10.1002/aqc.3663>
- Scarnecchia DL (1992) A reappraisal of gars and bowfins in fishery management. *Fisheries* 17:6–12. [https://doi.org/10.1577/1548-8446\(1992\)017%3c0006:arogab%3e2.0.co;2](https://doi.org/10.1577/1548-8446(1992)017%3c0006:arogab%3e2.0.co;2)
- Siebe C, Cram S, Herre A, Fernández-Bruces N (2005) Distribución de metales pesados en los suelos de la llanura aluvial baja del activo Cinco Presidentes, Tabasco. In: Gold-Bouchot G, Agraz-Hernandez C (eds) Botello AV, Rendón von Osten J. Diagnóstico y Tendencias. Universidad Autónoma de Campeche, Golfo de México Contaminación e Impacto Ambiental, pp 431–450
- Smith NG, Daugherty DJ, Brinkman EL et al (2020) Advances in conservation and management of the alligator gar: a synthesis of current knowledge and introduction to a special section. *N Am J Fish Manage* 40:527–543. <https://doi.org/10.1002/nafm.10369>
- Smith NG, Daugherty DJ, Schlechte JW, Buckmeier DL (2018) Modeling the responses of alligator gar populations to harvest under various length-based regulations: implications for conservation and management. *Trans Am Fish Soc* 147:665–673. <https://doi.org/10.1002/tafs.10040>
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: Software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4:535–538. <https://doi.org/10.1111/j.1471-8286.2004.00684.x>
- Villa J (1982) Peces nicaragüenses de agua dulce. Fondo de Promoción Cultural del Banco de América, Managua, Nicaragua
- Ward RD (2006) The importance of identifying spatial population structure in restocking and stock enhancement programmes. *Fish Res* 80:9–18. <https://doi.org/10.1016/j.fishres.2006.03.009>
- Ward SJ, McMahan CD, Khakurel B et al (2022) Genomic data support the taxonomic validity of Middle American livebearers *Poeciliopsis gracilis* and *Poeciliopsis pleurospilus* (Cyprinodontiformes: Poeciliidae). *PLoS ONE* 17:e0262687. <https://doi.org/10.1371/journal.pone.0262687>
- Williams AJ, Trexler JC (2006) A preliminary analysis of the correlation of food-web characteristics with hydrology and nutrient gradients in the southern Everglades. *Hydrobiologia* 569:493–504. <https://doi.org/10.1007/s10750-006-0151-y>
- Wright JJ, David SR, Near TJ (2012) Gene trees, species trees, and morphology converge on a similar phylogeny of living gars (Actinopterygii: Holostei: Lepisosteidae), an ancient clade of ray-finned fishes. *Mol Phylogenet Evol* 63:848–856. <https://doi.org/10.1016/j.ympev.2012.02.033>

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