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PHYLOGENETIC ORIGINS AND AGE—BASED PROPORTIONS OF MALACHO (*ELOPS SMITHI*) RELATIVE TO LADYFISH (*ELOPS SAURUS*): SPECIES ON THE MOVE IN THE WESTERN GULF OF MEXICO

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Abstract: Two species of ladyfish occur in the Gulf of Mexico (GOM), *Elops saurus* and *Elops smithi*, that are morphologically indistinguishable except for vertebral counts but can also be identified by mitochondrial DNA haplotypes. Here we expand on previous work, most of which has occurred in Florida, and examine the demography, phylogenetics, geographic distribution, and age—structure of ladyfishes in Texas estuaries. Fishery—independent gill net data demonstrated that ladyfishes increase in abundance from north to south along the Texas coast. The abundance of ladyfishes also increased in Texas waters from 1982–2021, which coincides with recent trends of warmer winters. Genetic data confirmed that both *E. saurus* and *E. smithi* occur in Texas waters; however, *E. smithi* was far less common. Contrary to previous research, we observed higher levels of genetic diversity in *E. saurus* due to larger sample size and thorough sampling of the western portion of its geographic range. Phylogenetic analysis supported the existence of *E. saurus* as a distinct species but indicated that *E. smithi* may be paraphyletic with other species of *Elops*. Otolith analysis showed that the ages of *E. saurus* and *E. smithi* ranged from 0–3 years. The lack of individuals > age—3 suggests that ladyfishes migrate to the offshore GOM at age 3 and do not return to coastal areas. This study enhances knowledge of the biology of ladyfishes in inshore waters of the northwestern GOM. Future management would benefit from expanding this research to the entire geographic range of the genus *Elops*.

Key words: age estimation, genetics, ladyfishes, mitochondrial DNA, phylogenetic analysis

Introduction

Ladyfishes (*Elops* spp., Elopidae, Elopiformes), also known as skipjacks and tenpounders, are coastal fishes found throughout warm temperate, subtropical, and tropical oceans. The recent discovery of genetically distinct cryptic species of *Elops* in the Western Atlantic (McBride et al. 2010) underscores how much is unknown about phylogenetic relationships, phylogeography, and population genetic structure of ladyfishes in the Western Atlantic and elsewhere. The 2 species of *Elops* that inhabit the Western Atlantic, the Ladyfish (*E. saurus*) and Malacho (*E. smithi*), can be distinguished by vertebral and myomere counts (Smith 1989, Smith and Crabtree 2002, McBride and Horodysky 2004, McBride et al. 2010) and mitochondrial DNA (McBride et al. 2010). Hereafter, we use the term "ladyfishes" to refer collectively to 2 or more species of *Elops* and use the scientific name to refer to a specific species of *Elops*. The detection of *E. smithi* seemed to suggest that additional cryptic species of *Elops* may exist (McBride et al. 2010, Levesque 2011); however, there has been no thorough phylogenetic study of *Elops* as of yet. Phylogeographic and population genetic studies are limited to *E. saurus* and *E. smithi* from coastal Florida (McBride et al. 2010) and the Tenpounder (*E. machnata*) from southern India (Ramanadevi and Thangaraj 2013, 2014).

Ladyfishes are a common component of the ichthyofauna of Gulf of Mexico (GOM) estuaries (Hoese and Moore 1998, Levesque 2011). Fisheries—independent resource monitoring by Texas Parks and Wildlife Department (TPWD) showed that ladyfishes are the tenth most commonly caught fish in gill nets (TPWD unpublished); however, substantial knowledge gaps exist in the life history of *E. saurus* and *E. smithi*. Both species inhabit low—salinity areas of rivers and streams as juveniles and transition to higher salinity areas as they mature (McBride et al. 2001, Levesque 2010). Spawning areas of *E. saurus* and *E. smithi* remain a mystery, but it is likely that both species spawn offshore given that *Elops* leptocephali are most often collected in offshore waters (Levesque 2011, Adams et al. 2013) and that Western Atlantic ladyfishes with mature gonads have only been recorded from offshore (Hildebrand 1963). Most studies of age and growth in ladyfishes have relied on laboratory rearing (Gehringer 1959), analysis of scales (Carles 1967), or length frequency distributions (McBride et al. 2001, Levesque 2015). Only a few studies have used otoliths to examine age and growth in ladyfishes (Palko 1984).

Given uncertainties in the genus' population structure, phylogenetics, and the lack of age and growth data, we examined the demography, population genetic structure, and otolith—based age structure of ladyfishes captured in Texas bays. Specifically, we 1) examined the influence of environmental variables on the presence of ladyfishes by analyzing spatial and temporal trends of abundance from 1982–2021 using a long—term fisheries independent dataset maintained by TPWD, 2) used mitochondrial DNA sequencing of field specimens to determine the relative abundance of *E. saurus* and *E. smithi*, 3) assessed the phylogenetic position of Texas ladyfishes relative to other *Elops* species using field—collected specimens as well as online sequence data, and 4) examined otolith increment data to describe the age structure of both ladyfish species along the Texas coast.

Materials and Methods

Demographic analysis

Temporal trends in the abundance of ladyfishes were evalu-

ated using a long—term fisheries—independent monitoring dataset maintained by TPWD as part of its Marine Resource Monitoring Program (Martinez—Andrade 2015). Gill nets have been used to monitor abundance trends of all estuarine—associated finfish species in all of the state's major bays since the 1970s, with the exception of Sabine Lake (sampling began in 1986) and Cedar Lakes (sampling began in 1996). Each bay is divided into 1—min^2 grids aligned with the geographic coordinate system, and grids are sampled with gill nets, bag seines, and trawls using a stratified random design.

Gill nets were deployed in 10 major inshore bays for 10 weeks in spring (April–June) and 10 weeks in fall (September–November) each year throughout the period 1982–2021. Forty—five nets were deployed in each bay across each 10 week season, with 3 exceptions. Twenty nets were deployed in East Matagorda Bay during each season, except spring seasons of 1982–1984 during which 8 nets were deployed and the fall seasons 1982–1983 in which 10 nets were deployed each season. Twenty nets were deployed in Cedar Lakes each season from 1996–1999 and 10 nets each season from 2000–2021. Lastly, no gill nets were set in any Texas bays during the spring season of 2020 due to the COVID—19 pandemic. Each gill net extended 182.9 m from shore and consisted of equally sized panels with 4 different mesh sizes (76, 102, 127, and 152 mm). Upon retrieval of each net, specimens were enumerated and the total length (TL) of each specimen was measured to the nearest millimeter. Latitude, longitude, and water parameters were recorded for each sampling event (temperature [°C], salinity, dissolved oxygen [DO, mg/L], turbidity [ntu], and depth [0.1 m]). Catch—per—unit effort (CPUE) was computed as catch/hr—the number of fish caught divided by the number of hours the net was deployed. Inlet distance was calculated in ArcMap 10.8 (ESRI, Redlands, California) as the distance (km) between the centroid of each sample grid to the nearest Gulf pass. Due to the difficulty of distinguishing species morphologically, demographic data were analyzed with both species combined.

Boosted regression trees (BRTs) were used to examine the influence of latitude, year, bay, inlet distance, temperature, salinity, turbidity, DO, depth, and season on the presence/absence of ladyfishes. The BRT is an ensemble method for fitting statistical models to data that employs 2 algorithms: boosting (a machine learning technique that combines simple models to yield improved performance) and regression trees (models that relate dependent variables to their predictors via recursive binary splits; Elith et al. 2008). One of the main strengths of BRTs is their ability to deal with nonlinear or discontinuous data (Elith et al. 2008), and they have proven useful elucidating patterns in fishery—independent datasets (Froeschke and Froeschke 2011; Montero et al. 2016; Anderson et al. 2017, 2022). Each gill net set between the years 1982–2021 represented a single observation and the overall sample size was 30,198 gill nets (14,894 in spring and 15,304 in fall). The initial model was fit using a tree complexity of 5, learning rate of 0.01, bag fraction of 0.5, and a Bernoulli error distribution. Tenfold cross—validation of training data was used to set the optimal number of trees necessary to minimize deviance and maximize predictive performance to independent test data (n = 15,099). A final simplified model was constructed with the lowest contributing variables excluded. The impact of each variable on the presence/absence of ladyfishes was assessed using partial dependence plots. Partial dependence plots were generated by fitting a generalized additive model (GAM) spline to the plots of explanatory variables against fitted values of catch probability from the BRT. The analysis was performed using R v.3.6.1 (R Development Core Team 2019) and the gbm package (Greenwall et al. 2020) and functions from Elith et al. (2008).

We qualitatively examined trends in fall and spring CPUE data by plotting the mean annual CPUE against year for the Texas coast and for each major bay. Lastly, we computed the mean latitude for catches of ladyfishes, and the relationship between mean latitude and year was evaluated using simple linear regression.

Sample collection for genetic and age—structure analyses

Ladyfishes were collected via bag seines, otter trawls, and gill nets from multiple estuaries in Texas from 2020–2021, during the course of TPWD's Marine Resource Monitoring Program (Martinez—Andrade 2015). Whole fish were placed in plastic bags, stored on ice, and transported back to the field station where the specimens were preserved frozen for genetic and otolith analysis. The body size of specimens was measured as standard length (SL) to the nearest mm.

Genetic analysis

We extracted genomic DNA from each specimen using about 20 mg of fin clip tissue excised with sterile scissors or scalpel. Genomic DNA was isolated from other cellular constituents using Gentra Puregene Tissue Kit (Qiagen, Venlo, Netherlands). We used polymerase chain reaction (PCR) to amplify approximately 700 base pairs (bp) of the cytochrome *b* (cyt*b*) gene which was used to assign species identity. We also amplified and sequenced (circa 655 bp) an additional mitochondrial gene, cytochrome oxidase *c* subunit I (COI). For the COI dataset, we used a subsample (8–12 individuals per estuary) identified as *E. saurus* based on cyt*b* haplotypes. We sequenced all individuals identified as *E. smithi* due to small sample size for this species. PCR was performed separately for each gene.

TABLE 1. *Primers used to amplify and sequence cytochrome* b *(cytb) and cytochrome oxidase subunit I (COI) from specimens of ladyfish (*Elops saurus*) and malacho (*E. smithi*).*

Gene	Primer name	Primer sequence (5′ to 3′)	Source
cyt*b*	Cyb-09H	GTGACTTGAAAAACCACCGTTG	Song et al. (1998)
cyt*b*	Cyb-07L	AATAGGAAGTATCATTCGGGTTTGATG	Taberlet et al. (1992)
COI	FishF1	TCAACCAACCACAAAGACATTGGCAC	Ward et al. (2005)
COI	FishR1	TAGACTTCTGGGTGGCCAAAGAATCA	Ward et al. (2005)

We performed PCR using PuReTaq Ready—To—Go (RTG) PCR beads (GF Healthcare, Piscataway, NJ). PCR reactions were conducted in 25 µL reactions, with each reaction consisting of one RTG bead, 10 pmol of each primer (Table 1), 3.0 µL of genomic DNA, and sufficient double—deionized water to reach the final volume. Cytochrome *b* was amplified using the following protocol: initial denaturation at 94.0°C for 3 min; 25 cycles of denaturation at 94.0°C for 30 s, annealing at 52.0°C for 1 min, and extension at 72.0°C for 1 min; and final extension at 72.0°C for 3 min (McBride et al. 2010). The PCR protocol for COI consisted of initial denaturation at 95.0°C for 2 min; 35 cycles of denaturation at 94.0°C for 40 s, annealing at 54.0°C for 45 s, and extension at 72.0°C for 1 min; and final extension at 72.0°C for 10 min (Ramanadevi and Thangaraj 2013). We used a Techne Prime (Techne, Cambridge, UK) thermal cycler for all PCR amplifications of cytochrome *b* and COI.

PCR products were purified by an enzymatic method (ExoSAP—IT; Thermo Fisher Scientific, Waltham, MA). Primers used for sequencing were identical to those used in PCR. Sequencing reactions for cyt*b* and COI were carried out in 20 µL volumes using the BigDye Termination v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific) using the following protocol: initial denaturation at 96.0°C for 1 min followed by 25 cycles of denaturation at 96.0°C for 10 s, annealing at 50.0°C for 5 s, and extension at 60.0°C for 4 min.

Sequencing reactions were performed using either ABI Veriti (Thermo Fisher Scientific) or TC—512 (Techne) thermal cyclers. Sequencing reactions were precipitated using a solution containing 2.5 µL each of sodium acetate (3 M) and EDTA (100 mM), followed by 100 µL of 100% ethanol. Precipitated sequence extracts were centrifuged at 3,700 RPM for 45 min at 4.0°C. The resulting pellets were washed with 100 µL of 70% ethanol and centrifuged for 30 min at 4.0°C, dried in a vacuum centrifuge for 1 h at 45.0°C, and then rehydrated using Hi—Di formamide (Thermo Fisher Scientific). Rehydrated DNA was loaded on an ABI 3500 Genetic Analyzer (Thermo Fisher Scientific) for separation and detection. We visually inspected electropherograms of each sequence and aligned forward and reverse sequences of each gene and each sample in the computer program Sequencher, version 4.9 (Gene Codes, Ann Arbor, MI).

Cytochrome *b* and COI sequences were aligned using the computer program Clustal X (Larkin et al. 2007). Cytochrome *b* sequences were trimmed to the same length (470 bp) as the haplotypes obtained by McBride et al. (2010), and the COI sequences were trimmed to the length of the shortest sequence in that dataset. We determined the number of haplotypes in the cyt*b* and COI datasets using DnaSP (Rozas et al. 2017). We investigated the evolutionary relationships among the cyt*b* haplotypes identified by McBride et al. (2010, GenBank accession numbers GQ183881–GQ183894) and this study by constructing a median—joining network (Bandelt et al. 1999) using the computer program PopArt, version 1.7 (Leigh and Bryant 2015). The species identity of *Elops* specimens was determined by the position of each haplotype within the cyt*b* haplotype network relative to those haplotypes detected by McBride et al. (2010). We assessed the extent of sequence divergence between *E. saurus* and *E. smithi* cyt*b* haplogroups by computing the proportion of pairwise nucleotide differences (p distance, Nei and Kumar 2000) using the computer program MEGA7 (Kumar et al. 2016). Standard error of sequence divergence was estimated with 1,000 bootstrap replicates. We conducted identical analyses for the COI sequences.

To further assess the evolutionary relationships between *E. saurus* and *E. smithi*, we performed maximum likelihood phylogenetic analyses in MEGA7 using the haplotypes from both datasets and cyt*b* and COI sequences of *Elops* spp. from previous studies available in GenBank. There were more COI sequences of *Elops* spp. available in GenBank relative to cyt*b* sequences. Phylogenetic analysis allowed us to evaluate the genetic relationships of GenBank COI sequences of ladyfishes identified as either *Elops saurus* or *Elops* sp. Kimura's (1980) 2—parameter model with a gamma distribution (K2+Γ) was selected as the most appropriate model of molecular evolution for both genes based on the output from the model selection tool implemented in MEGA7. The reliability of inferred relationships for both phylogenetic analyses was assessed by 1,000 bootstrap replicates (Felsenstein 1985). A single whole mitochondrial genome sequence of the Atlantic Tarpon (*Megalops atlanticus*, GenBank accession number AP004808) was used to root both phylogenies.

Age structure

Sagittal otoliths were extracted from a subset of genetically—identified individuals. Whole otoliths were cleaned of residual tissue with dionized water and a paintbrush, air—dried, embedded in molding trays with a 2:1 mix of epoxy resin and hardener, respectively, and oven—dried at 37°C. Otoliths were transversely sectioned with a saw (Struers Accutom—5™, 4" in blade diameter) at 3,000 rpms where 3—4 serial sections (1 mm thick) were obtained, ensuring that a section contained the otolith core. Otolith sections were mounted to microscope slides with thermoplastic Crystalbond™. Calibrated images of otolith sections were taken with immersion oil and unpolarized transmitted light at 5x magnification using a camera—mounted Nikon Eclipse LV100ND compound microscope and NIS—Elements D imaging software.

Two readers estimated age—at—capture as the number of presumed annuli along the edge of the sulcus from the core area to edge (VanderKooy et al. 2020). Both readers re—aged the otoliths on separate occasions blind to specimen information, and the average percent error (APE) was used as a measure of within—reader precision between the 2 age estimates (Campana 2001) where individuals with APE ≤ 5% (J. Carroll, pers. comm., Florida Fish and Wildlife Conservation Commission, St. Petersburg, FL, USA) were retained for analyses. The final age estimate or consensus age for each individual was determined as the age estimate that both readers independently agreed upon serving as a criterion for among—reader precision (Oele et al. 2015). Further, precision among readers was quantified as the percentage of aged individuals with a consensus age (Oele et al. 2015). A Welch's independent t—test was used

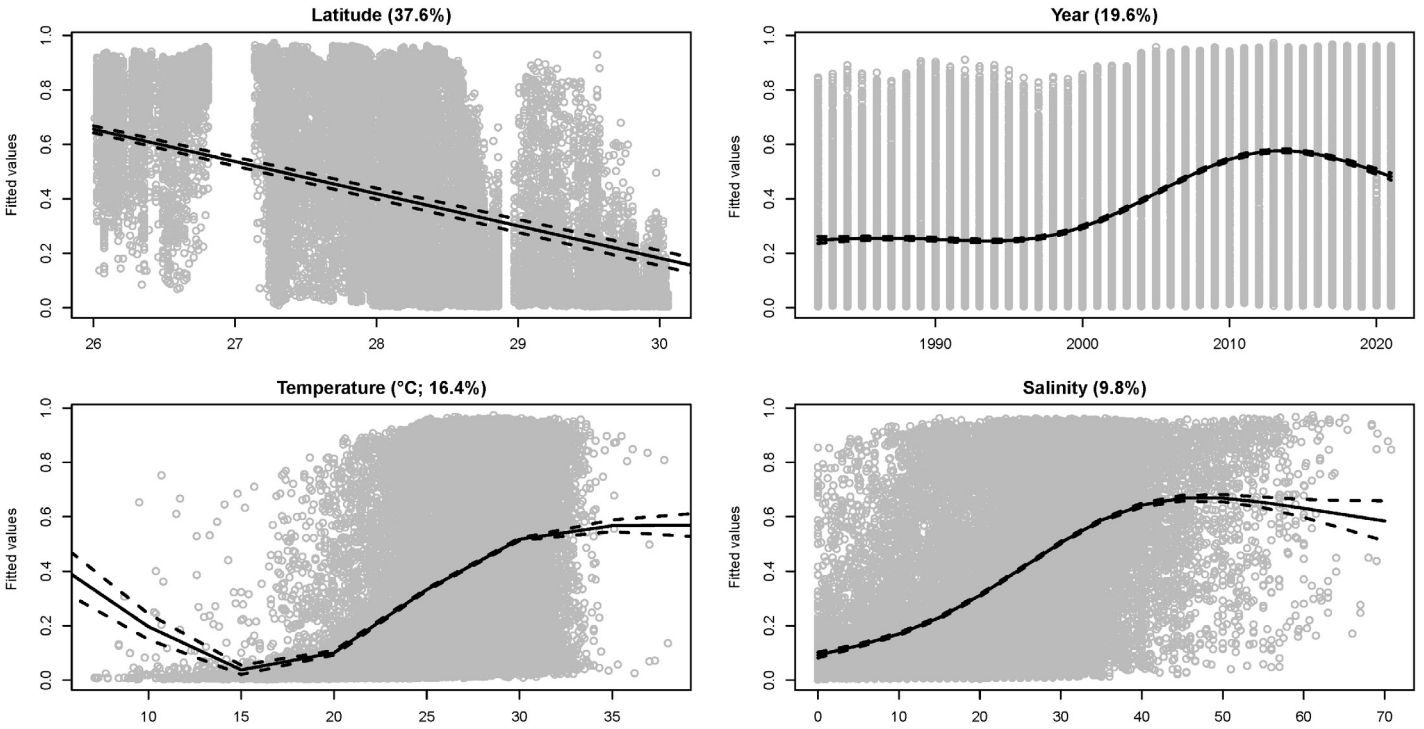

FIGURE 1. *Fitted values of probability of occurrence based on boosted regression tree (BRT) analysis of ladyfishes (*Elops *spp.) in fishery-independent gill nets (1982–2021). Gray circles are fitted values and black splines were fit to the data using generalized additive modeling. Dotted lines represent 95% confidence intervals. The percentage of deviance explained by each independent variable is given in parentheses.*

to test for a significant difference in APE among readers. We used an Evans—Hoenig symmetry test to assess bias within and among reader age estimates (McBride 2015, Nesslage et al. 2022). We used a t—test to test for a significant difference in mean SL among species.

Each *E. saurus* and *E. smithi* individual was assigned to a 25 mm SL class. We created an age—length key with the aged subset of *E. saurus* and *E. smithi* individuals that met the criteria for within— and among—reader precision in age estimates and calculated the proportion of individuals within each SL and age class combination (Ogle 2016). These probabilities were used to randomly assign ages to unaged *E. saurus* and *E. smithi* individuals (Isermann and Knight 2005) within SL classes. All statistical analyses, graphics, calculations, and age assignment were performed in R v.3.6.1 with the following packages: ggplot2 (Wickham 2016), dplyr (Wickham et al. 2020), tidyr (Wickham 2020), and FSA (Ogle et al. 2020).

Results

Demographic analysis

Over the 39—year sampling time series, we observed a total of 51,526 ladyfishes (both species combined). At least one individual was present in 11,520 of 30,198 gill nets deployed (38% catch rate). Boosted regression trees showed that latitude, year, temperature, and salinity explained 83% of the variance of presence/absence of ladyfishes in gill nets. Latitude explained the greatest amount of variance (38%) of presence/absence in gill nets, and the probability of ladyfishes being present in a gill net declined with increasing latitude (Figure 1). Year, temperature, and salinity explained 20%, 16%, and 10%, respectively, of the variance, and presence of ladyfishes was positively correlated with all 3 variables. Other variables (bay, inlet distance, turbid-

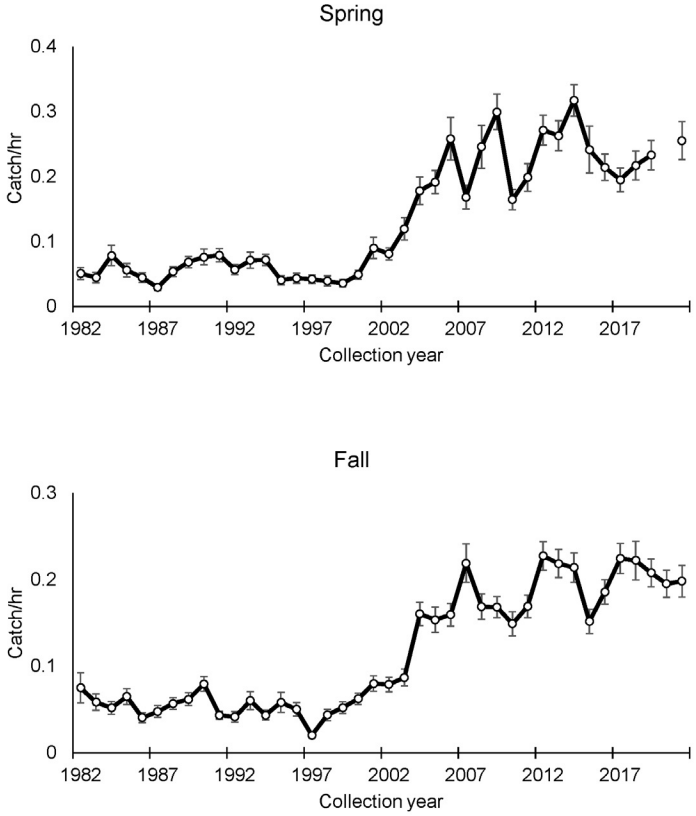

FIGURE 2. *Distribution of mean (± se) seasonal CPUE (catch/hr) of ladyfishes (*Elops *spp.) captured in fishery-independent gill net samples deployed across the Texas coast, 1982–2021.*

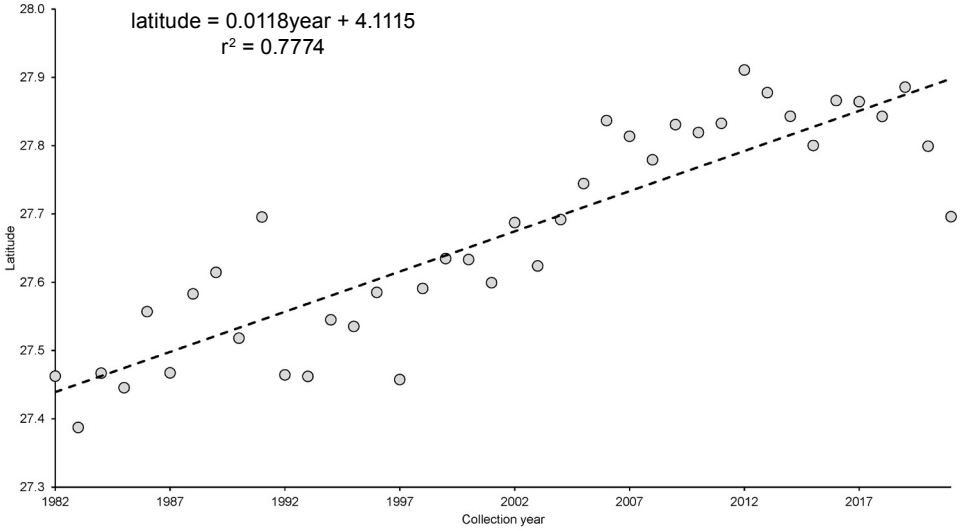

FIGURE 3. *Latitude of the ladyfishes (Elops spp.) catches (both species combined) in fishery-independent gill nets deployed on the Texas coast, 1982–2021. Gray-filled circles represent point means of each year, the dashed line is a trend line (linear regression) fit to the data, and regression parameters and fit are reported in the upper left corner.*

ity, DO, depth, and season) examined in the initial model accounted for < 10% of the variance. Mean CPUE of ladyfishes began an increasing trend in the spring and fall on the Texas coast after 2003 (Figure 2). Similar trends in spring and fall CPUE were observed in each major estuary except for Sabine Lake (Supplemental Figures S1 and S2). Year was positively correlated with mean latitude of ladyfish catches (r^2 = 0.78, p < 0.0001), with mean latitude increasing by 0.01 degrees per year (Figure 3).

Genetic analysis

We obtained cyt*b* sequences for 354 individuals, among which we detected 48 haplotypes. The cyt*b* haplotypes resolved into two haplogroups separated by 4–5 inferred missing haplotypes (Figure 4A). Most of the cyt*b* sequences (n = 322, 91%) were identical to haplotype A or 32 new haplotypes closely related to it, and thus were identified as *Elops saurus*. Fifteen haplotypes were detected among the remaining 32 sequences. These were either identical to one of the *E. smithi* haplotypes (D, E, G, H, I, J, or N, Figure 4A) or closely related to them, and these individuals were identified as *E. smithi*. Haplotypes B, C, F, K, L, and M were not observed in our dataset. The most common (n ≥ 100) haplotypes were the *E. saurus* haplotypes A and Hap15, whereas all of the other haplotypes were less common (n = 1–22). Despite its rarity, *E. smithi* was detected in every bay (1–6 individuals per bay, Figure 5). The uncorrected p distance (± se) between the *E. saurus* and *E. smithi* haplogroups was 1.9% ± 0.5%.

We obtained 128 sequences of COI (541 bp), which represented 7 *E. saurus* and 10 *E. smithi* haplotypes (Figure 4B). The COI haplotype network resembled the one based on cyt*b*. However, the 2 species were separated by 11 inferred missing haplotypes in the COI network versus 4–5 inferred missing haplotypes in the cyt*b* haplotype network. There did not appear to be any geographic pattern to the distribution of haplotypes of either species among Texas estuaries. Sequence divergence based on COI was also larger among the 2 species (p distance = 2.9% ± 0.6% se). Haplotype sequences of cyt*b* and COI were deposited in GenBank as accession numbers OM161024—OM161063 and OM128141—OM128157, respectively.

Phylogenetic analysis of cyt*b* sequences revealed that *E. saurus* haplotypes were part of a monophyletic, albeit weakly supported, clade (bootstrap = 54%, Figure 6). The *E. smithi* haplotypes clustered with sequences of the Hawaiian Ladyfish (*E. hawaiensis*) and a cyt*b* sequence from a whole mitochondrial genome labeled as *E. saurus* (accession number AP004807) as part of a poorly supported clade (bootstrap < 50%). In contrast, maximum likelihood analysis of COI sequences yielded a strongly supported clade (bootstrap = 92%) consisting of *E. saurus* haplotypes and several GenBank sequences identified as either *E. saurus* (n = 7) or *Elops* sp. (n = 2) that were collected from the Atlantic and Gulf Coasts of the United States or Mexico (Figure 7). The *E. smithi* COI haplotypes were recovered as part of a large clade with moderate bootstrap support (77%) that also included *E. hawaiensis* and *E. machanata* and sequences of ladyfishes originally identified as either *E. smithi* (n = 1), *E. saurus* (n = 8), or *Elops* sp. (n = 6) collected from coastal waters of Mexico, Belize, and Brazil (Figure 7). Although bootstrap support was weak overall, phylogenetic analyses of both datasets suggests that 1) the Pacific ladyfish (*E. affinis*) is the most basal lineage within *Elops*, and 2) *E. saurus* is a sister lineage to one formed by *E. hawaiensis*, *E. machnata*, and *E. smithi*.

Age structure

Individuals that were field—sampled for genetics and otolith analysis had an SL at capture that ranged from 91–540 mm with a mean (± sd) of 313 ± 135 mm for *E. saurus* (n = 321), and from 41–531 mm with a mean of 304 ± 148 mm for *E. smithi* (n = 32). A Welch's independent t—test showed no significant differences in mean SL between species (t = 0.303, df = 36.385, p > 0.05). We performed microscopic examination of otolith annuli in 65% of *E. saurus* (n = 210) and 97% of *E. smithi* (n = 31). Of those specimens, 92% of *E. saurus* (n = 193) and 94% of *E. smithi* (n = 29) individuals had age estimates with APE ≤ 5% within readers. A Welch's independent t—test showed

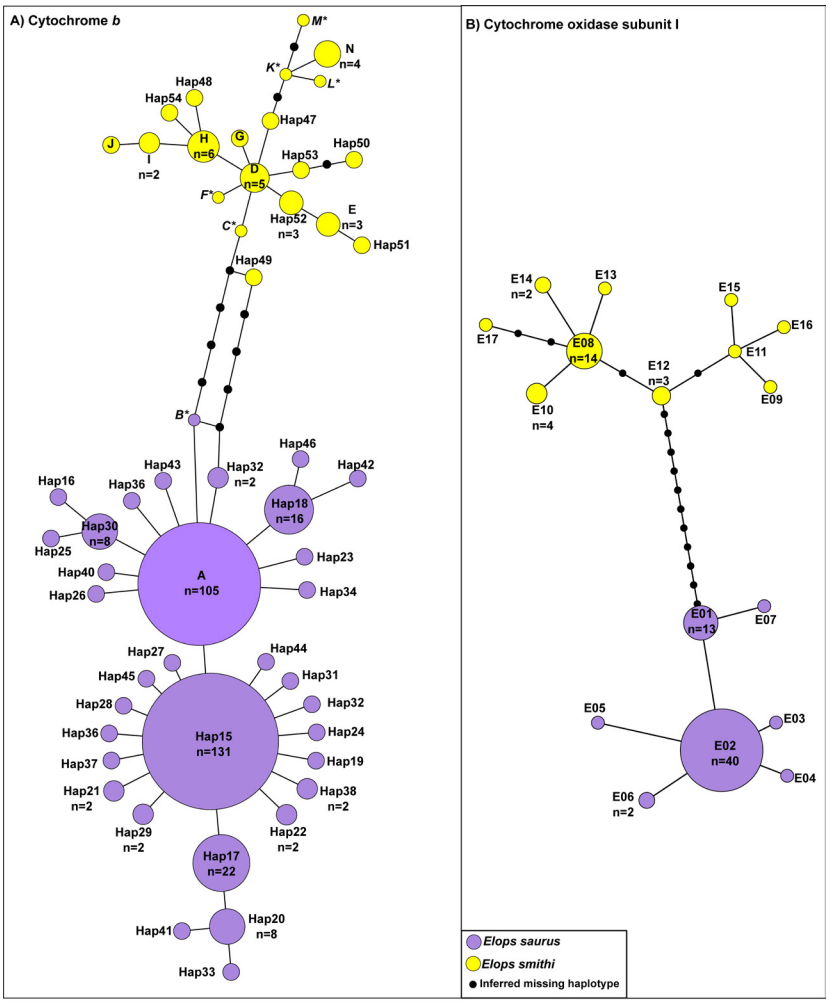

FIGURE 4. *Haplotype networks of* Elops saurus *and* E. smithi. *A. Cytochrome* b *(cytb). B. Cytochrome oxidase c subunit I (COI). Each colored circle represents a unique haplotype, and boldface letters (A, Hap40, E01, etc.) designates individual haplotypes, with the number of individuals (if n > 1) possessing that haplotype in parentheses below the letter. Each line connecting a circle represents a single base substitution and solid black circles represent inferred missing haplotypes. Italicized letters followed by an asterisk (*) represent cytb haplotypes observed by McBride et al. (2010) that were not detected along the Texas coast.*

no significant difference in APE among readers (t = –1.536, df = 401.5, p > 0.05). Most of the individuals of *E. saurus* (90%, n = 174) and *E. smithi* (83%, n = 24) with an APE ≤ 5% also had a consensus age estimate, indicating high precision and agreement in age estimates among readers. An Evans–Hoenig test showed no systematic bias in age estimates within reader 1 (more experienced; X^2 = 0.5, df = 1, p > 1; Table 2A). However, the same test showed systematic bias in age estimates within reader 2 (X^2 = 10.3, df = 2, p < 0.01; Table 2B) and among readers for read 1 (X^2 = 6.5, df = 2, p < 0.05, Table 2C) and read 2 (X^2 = 17.9, df = 2, p < 0.001; Table 2D). This highlights the importance of using precision criteria as quality assurance and control measures to decrease uncertainty in age estimates.

Using the probability–based age–length key, we assigned ages to 112 unaged individuals (111 *E. saurus* and 1 *E. smithi*). As a result, 285 *E. saurus* and 25 *E. smithi* individuals had an estimated age at capture ranging from 0–3 years. Many *E. saurus* (n = 149, 52%) and most *E. smithi* (n = 18, 72%) individuals were young–of–the–year (age 0), and age 2 was the second most frequently captured age class for both species (Table 3). Qualitatively, a similar range of age classes was observed in both species. Growth in both species appeared to be fastest between ages 0 and 1 and slowed considerably between ages 1 and 3 (Supplemental Figure S3). Although growth appeared to be approaching asymptotic size in age 3 individuals, we could not reliably assess growth with standard growth models due to a lack of individuals > age 3 in our sample.

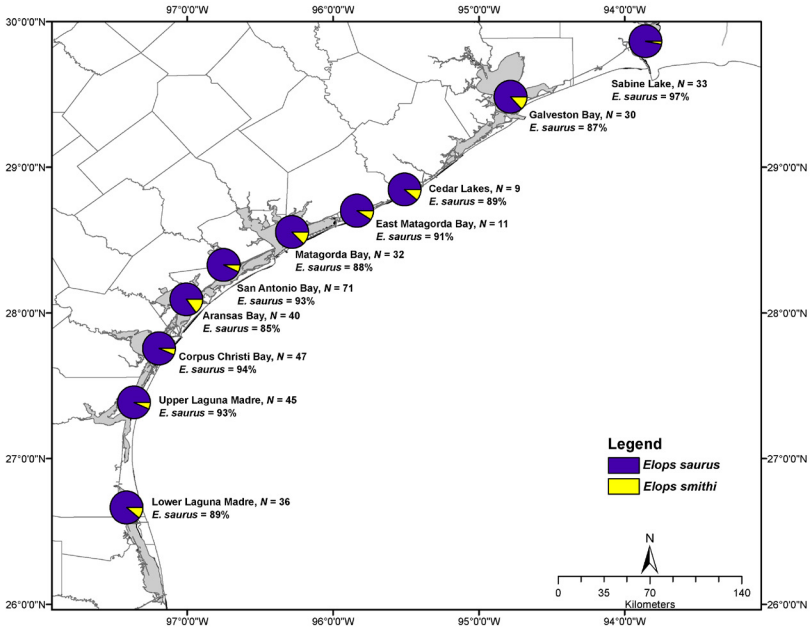

FIGURE 5. *Map showing the geographic distribution of* Elops saurus *and* E. smithi *on the Texas coast, based on specimens sequenced for mitochondrial cytochrome* b. *Pie charts represent the proportions of* E. saurus *and* E. smithi *in samples taken from each major Texas estuary in 2020 and 2021.*

Discussion

Demographic analysis

The abundance of ladyfishes increased from north to south along the Texas coast, which corresponds to the southward cline of increasing temperature and salinity. The increase in mean temperature is driven by a natural climatic cline, whereas the higher salinity of southern bays is due to decreased rainfall and lower freshwater inflows (Tolan 2007). Abundance was generally highest in Corpus Christi Bay and the Lower Laguna Madre, which

are two of the warmest and most saline estuaries on the Texas coast. Greater abundance of ladyfishes in southern Texas bays is similar to coastal abundance trends of other marine species with tropical affinities, such as Gray Snapper (*Lutjanus griseus*; Tolan and Fisher 2008, Anderson et al. 2022), Common Snook (*Centropomus undecimalis*) and Large—Scale Fat Snook (*C. mexicanus*; Anderson et al. 2019).

Ladyfishes also exhibited a sharp increase in overall abundance from 1982–2021 as well as becoming more common on the upper coast of Texas, which may be partly driven by climatic fluctuations. Tolan and Fisher (2008) argued that the increase in Gray Snapper abundance on the Texas coast was due to warmer winters, which allowed more larvae and juveniles to survive each year. Similarly, Hare and Able (2007) proposed that warmer water temperatures in the winter had allowed Atlantic Croaker (*Micropogonias undulatus*) to expand northward along the eastern coast of the United States. Milder winters are also partly responsible for the greater abundance of tropical species in Texas bays, resulting in increasing biodiversity of fish communities during the past 33 years (Pawluk et al. 2021). This trend has been observed in co—distributed subtropical and tropical estuarine and marine taxa (Tolan and Fisher 2008, Armitage et al. 2015, Anderson et al. 2019, 2022, Purtlebaugh et al. 2020), and reflects ongoing tropicalization of the northern GOM and other temperate regions caused by increasingly warmer climate and milder winters (Sagarin et al. 1999, Fodrie et al. 2010, Horta e Costa et al. 2014, Heck et al. 2015, Fujiwara et al. 2019).

Genetic analysis

The results of our genetic analysis based on cyt*b* were broadly similar in pattern to McBride et al. (2010) but also harbored notable differences. We observed more haplotypes in *E. saurus* than McBride et al. (2010), which was likely due to our larger sample size (n = 356 versus n = 56) and sampling in the western portion of the species' geographic range. McBride et al. (2010) suggested that the decreasing population size and geographic distribution from Pleistocene glacial cycles resulted in low genetic diversity of *E. saurus*, which was partially supported by the star—shaped haplotype networks (i.e., star phylogenies) among *E. saurus* cyt*b* and COI haplotypes. Star phylogenies are often indicative of species that have un-

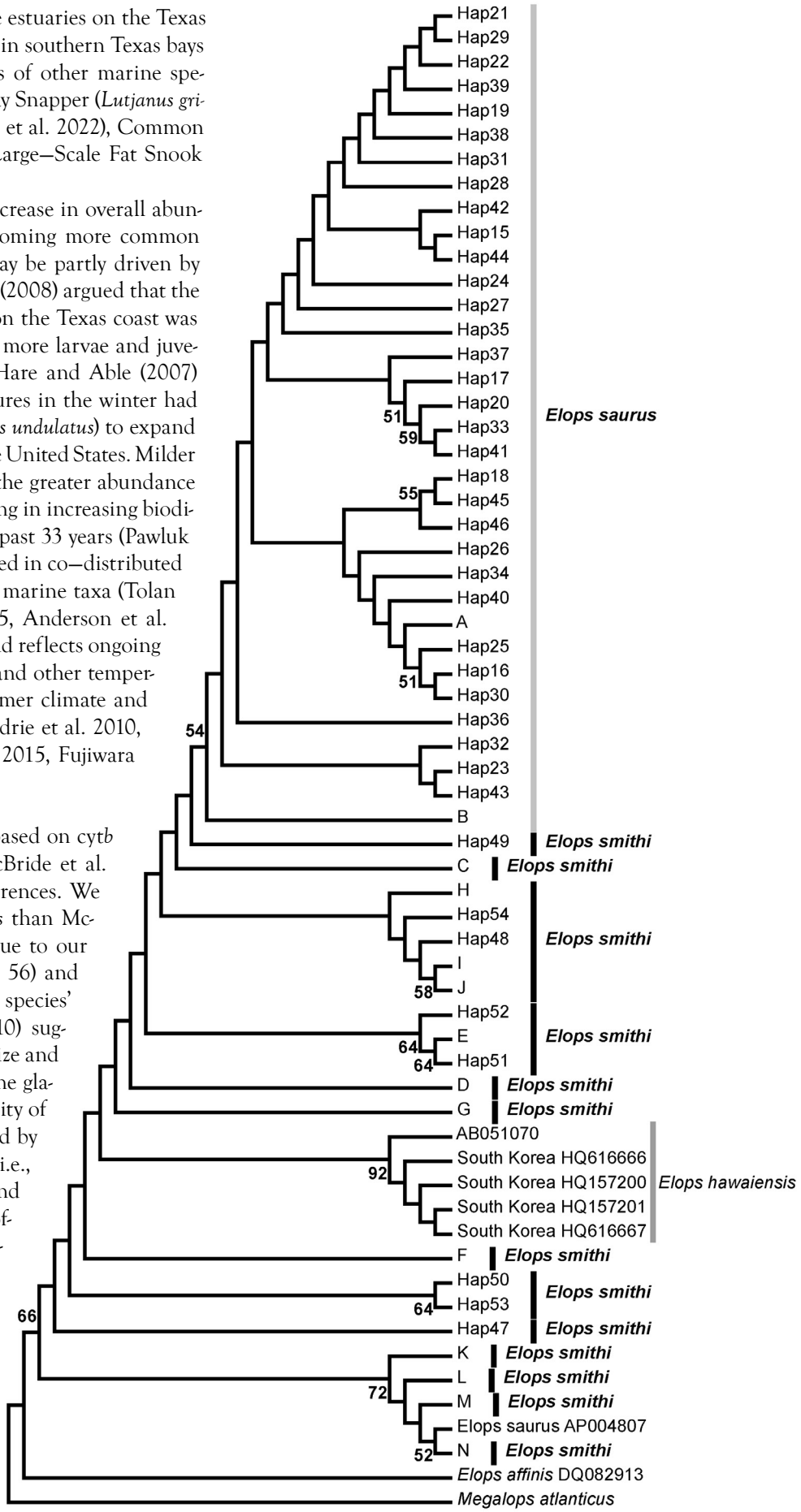

FIGURE 6. *Maximum likelihood phylogenetic tree of* Elops *taxa inferred from mitochondrial cytochrome* b *sequences. Values on branches are bootstrap values (if ≥ 50%). Sequences obtained from GenBank are indicated by accessions numbers and locality information was included in labels if known.*

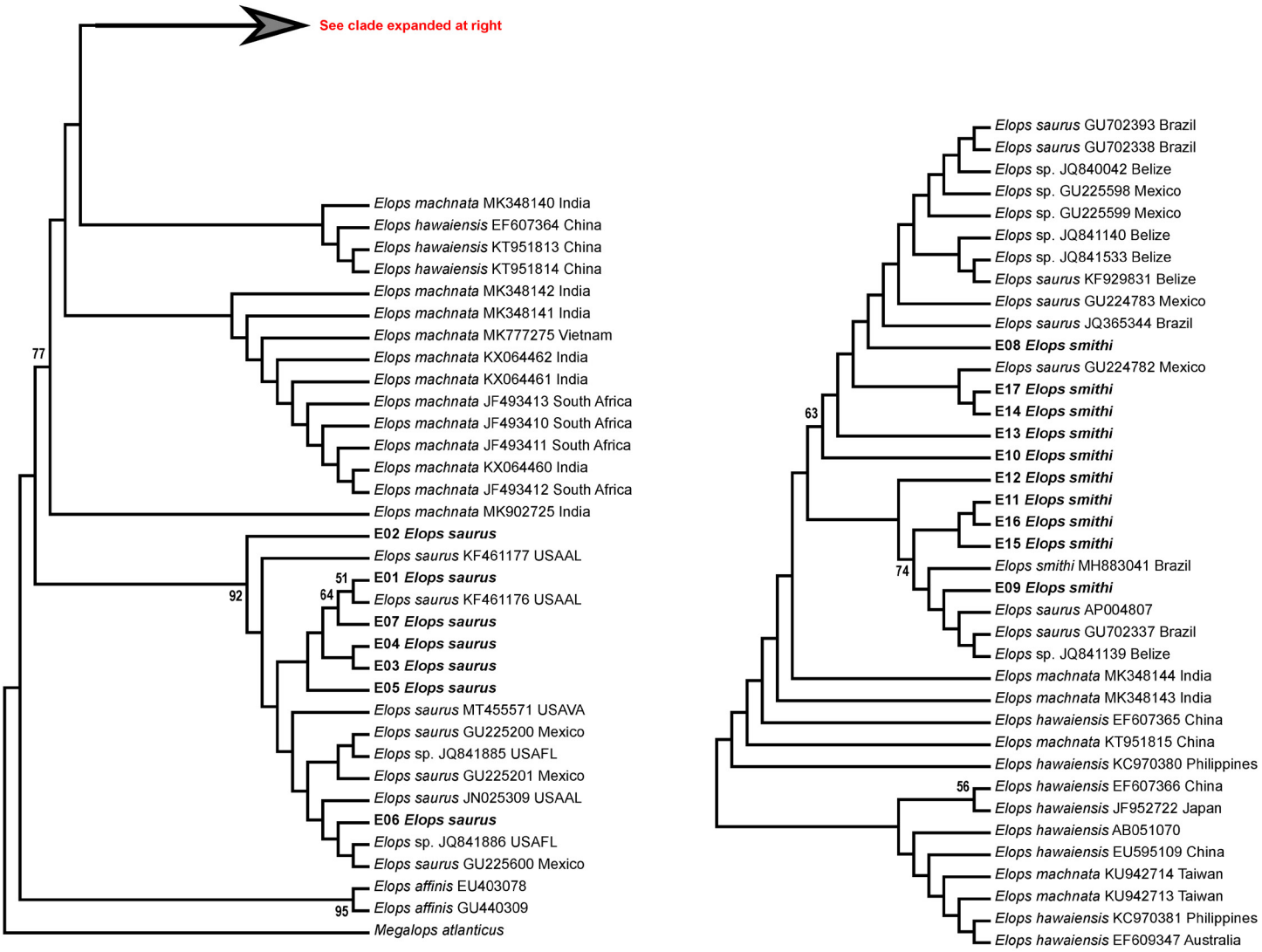

FIGURE 7. *Maximum likelihood phylogenetic tree of* Elops *taxa inferred from 470 bp of mitochondrial COI sequences. Values on branches are bootstrap values (if ≥ 50%). Sequences obtained from GenBank are indicated by accessions numbers and locality information was included in labels if known.*

dergone rapid demographic expansion after a previous decline (Slatkin and Hudson 1991). If ladyfishes in the GOM underwent a demographic decline in the past, the most likely causes were the climatic and sea level fluctuations of the Pleistocene. The global mean temperature was 6°C cooler than today during the Last Glacial Maximum of the Pleistocene (26,500–19,000, Tierney et al. 2020) and mean sea surface temperatures in the GOM were 1°–2°C cooler in winter and 1°C cooler in summer (Brunner 1982). It is likely that much of the GOM and western Atlantic was too cold for many warm—temperate or tropical species. Pleistocene sea level fluctuations appear to be linked to population bottlenecks of many marine taxa (Ludt and Rocha 2015). Sea level of the GOM was 120 m lower than today at the peak of the Last Glacial Maximum (Donoghue 2011), which may have reduced important nursery habitats for estuarine—dependent marine organisms. The overall warmer climate and rising sea levels of the Holocene (11,650 years ago–present) would have likely promoted the demographic and range expansion of ladyfishes. Post—Pleistocene expansions have been inferred for several co—distributed species (Pruett et al. 2005, Mobley et al. 2010, Drum and Kreiser 2012, Escatel—Luna et al. 2015, Williford et al. 2021).

The results of our genetic analysis support earlier morphological studies suggesting that *E. smithi* is rare in the western GOM (McBride and Horodysky 2004, McBride et al. 2010). The relative rarity of *E. smithi* in our samples compared to those from Florida is probably due to the fact that Texas is farther from the Caribbean Sea where spawning likely occurs (McBride and Horodoysky 2004, McBride et al. 2010). Despite its rarity, we detected additional *E. smithi* cyt*b* haplotypes in the western GOM. The absence of previously reported cyt*b* haplotypes for *E. smithi* (C, F, L, and M) and *E. saurus* (B) was probably due to the overall rarity of these haplotypes. Among the 56 Florida ladyfishes used for genetic analysis, McBride et al. (2010) detected these haplotypes in ≤ 2 individuals.

Comparison of our results to those of McBride et al. (2010) suggests that genetic diversity of *E. saurus* is lower in Florida than in Texas. Apparent lower genetic diversity of Florida *E.*

TABLE 2. *Reader agreement on otolith-based age-frequency age estimates of ladyfishes (*Elops saurus *and* E. smithi*) from the Texas coast. A. Within reader 1. B. Within reader 2. C. Read 1 age estimates between readers. D. Read 2 age estimates between readers.*

A Age read 2 (yr)	Age read 1 (yr)				
0	0	1	2	3	4
1	150	2	-	-	-
2	-	37	1	-	-
3	-	3	43	1	-
4	-	-	-	3	1
	-	-	-	-	

C Reader 2 age (yr)	Reader 1 age (yr)				
0	0	1	2	3	4
1	149	6	2	-	-
2	-	21	2	1	-
3	1	14	33	-	1
4	-	-	-	3	-
	-	-	-	-	-

B Age read 2 (yr)	Age read 1 (yr)			
0	0	1	2	3
1	151	-	-	-
2	4	21	1	-
3	2	3	44	-
	-	-	4	11

D Reader 2 age (yr)	Reader 1 age (yr)			
0	0	1	2	3
1	148	2	1	-
2	3	21	2	-
3	1	15	32	1
	-	-	12	3

saurus may be an artifact of smaller sample size used by McBride et al (2010). Another possible cause is that the eastern GOM and the western Atlantic represent areas that have been more recently colonized by *E. saurus*. Genetic diversity of a species usually decreases from the center of the range towards the margins (Austerlitz et al. 1997, Eckert et al. 2008); under this hypothesis, post—Pleistocene expansion of ladyfishes may have occurred in the western GOM first, followed by the eastern GOM and western Atlantic. Finally, the lack of additional cyt*b* haplotypes in Florida may be due to population structure that coincides with an east—west suture zone in the GOM, which has been observed in several co—distributed marine species (Portnoy and Gold 2012 and references therein, Viricel and Rosel 2014, Seyoum et al. 2017, 2018, Portnoy et al. 2021).

The interpretation of the results from our phylogenetic analyses is difficult due to the lack of foundational studies of the *Elops* genus. Previous phylogenetic studies of elopomorph fishes that used more than one species of *Elops* are limited to Obermiller and Pfeiler (2003) and Ramanadevi and Thangaraj (2013). Obermiller and Pfeiler (2003) examined the relationships between 4 species of *Elops*, including one referred to as *Elops* sp. (*Elops smithi* was referred to as *Elops* sp. prior to being officially named in 2010.) Obermiller and Pfeiler (2003) recovered a clade of Atlantic species, *E. saurus* and *E. smithi*, and a sister clade composed of 2 Pacific species, *E. hawaiensis* and *E. affinis*. In contrast, our results suggest that *E. affinis* and *E. saurus* are more closely related, and *E. smithi* is more closely related to *E. hawaiensis*.

Ramanadevi and Thangaraj (2013) conducted phylogenetic analysis on several mitochondrial genes of ladyfishes, which were largely congruent with our results. Analysis of a small set of cyt*b* sequences by Ramanadevi and Thangaraj (2013) revealed that *E. smithi* and *E. hawaiensis* were more closely related to one another than either was to *E. affinis*. Ramanadevi and Thangaraj's (2013) evaluation of a dataset of *Elops* COI sequences yielded results similar to ours. They recovered 2 clades of ladyfishes originally identified as *E. saurus*, with one of the clades more closely related to *E. affinis* and a second clade more closely related to *E. machnata* and *E. hawaiensis*. Analyses based on COI suggested that *E. smithi* is more closely related to Pacific species of *Elops* than to either of the New World species, *E. saurus* and *E. affinis*. The commonality between our results and Ramanadevi and Thangaraj (2013), in contrast to those obtained by Obermiller and Pfeiler (2003), may be due the genetic markers used (cyt*b* and COI versus 12S and 16S ribosomal RNA) and different numerical species representation (multiple individuals of several species versus single individuals).

Among ladyfishes in the western Atlantic, *Elops smithi* is the "low—count" species and is characterized by smaller number of myomeres (74–78 total) and vertebrae (73–80, usually 75–78) compared to *E. saurus*, the "high—count" species (79–86 total myomeres and 79–87 vertebrae but usually 81–85; Smith 1989, Smith and Crabtree 2002, McBride and Horodysky 2004, McBride et al. 2010). However, *E. saurus* and *E. smithi* occasionally overlap in vertebral counts (79–80), which could lead to mistakes in identification. It is possible that GenBank COI sequences labeled *E. saurus* in Ramanadevi and Thangaraj's (2013) second clade, which we used in our analysis as well, were misidentified *E. smithi*. Although *E. smithi* was not named until 2010, most of the GenBank COI sequences of *Elops* spp. from the western Atlantic were from specimens collected after Smith (1989) reported the existence of a possible second species in the

TABLE 3. *Count (n) and frequency (%) of genetically-identified* Elops saurus *and* E. smithi *from the Texas coast within age classes 0–3. Age estimates were either otolith-based or assigned using an age-length key.*

Taxa	Age class (yr)	n	%
E. saurus	0	149	52.0%
	1	46	16.0%
	2	86	30.0%
	3	4	1.4%
E. smithi	0	18	72.0%
	1	1	4.0%
	2	5	20.0%
	3	1	4.0%

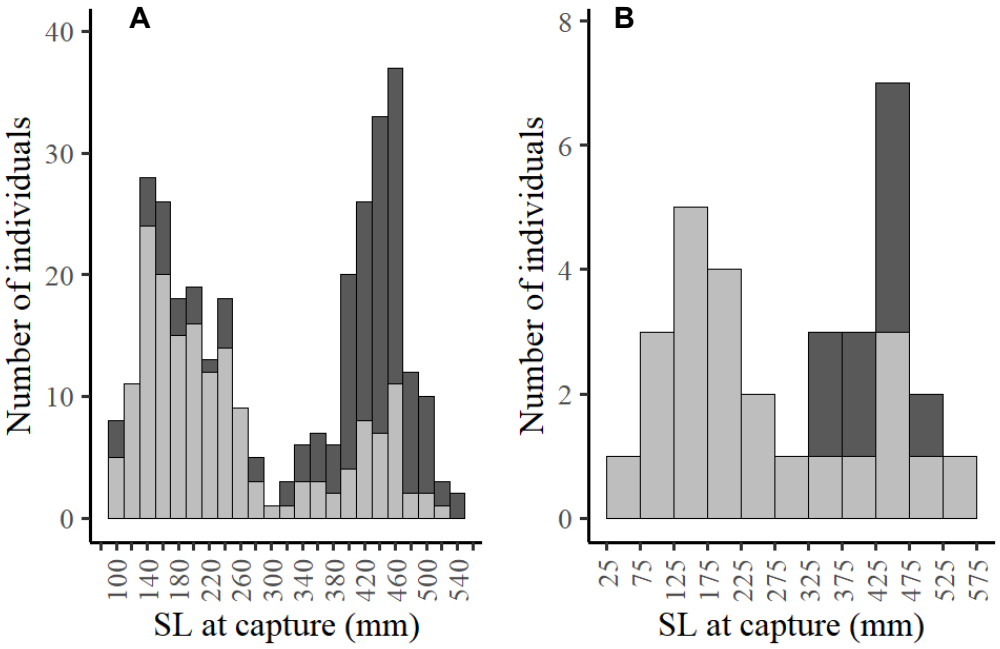

FIGURE 8. *Frequency distribution of standard length (SL) at capture along the Texas coast for 2 species of* Elops. *A.* E. saurus *(n = 321). B.* E. smithi *(n = 32). Lighter-shaded regions: subset of aged* E. saurus *(n = 174) and* E. smithi *(n = 24) that met the criteria for within- and among-reader precision in otolith-based age estimates. Darker-shaded regions: total number of fishes captured. Binwidth (width of the bars in the histogram) is 20 mm for* E. saurus *and 50 mm for* E. smithi.

western Atlantic. GenBank entries and published studies (Valdez–Moreno et al. 2010, April et al. 2011, de Oliveira Ribeiro et al. 2012, Weigt et al. 2012, Guimarães–Costa et al. 2019) associated with the sequences did not specify whether vertebral or myomere counts had been used to identify specimens of ladyfishes.

Mismatches between genetic and morphological identification are often caused by incomplete lineage sorting (Funk and Omland 2003), where closely related species continue to share mitochondrial haplotypes after reproductive isolation has been achieved. Incomplete lineage sorting may partially explain our results and those of Ramanadevi and Thangaraj (2013) and Obermiller and Pfeiler (2003). Incomplete lineage sorting may also account for discordance between morphological and genetic identification, which occurred in 7 (13%) of the specimens examined by McBride et al. (2010). Occasional hybridization between *E. saurus* and *E. smithi* may also be a source of discordance between morphological and genetic identifications. However, incomplete lineage sorting and hybridization does not explain the close relationship of *E. smithi*, *E. machnata*, and *E. hawaiensis*. These 3 species may represent a polymorphic, pantropical species, whereas *E. saurus* and *E. affinis* represent species adapted to subtropical and warm–temperate climates.

The present study and previous work (Obermiller and Pfeiler 2003, Ramanadevi and Thangaraj 2013) were hampered by the reliance on one or 2 mitochondrial genes and limited taxon sampling. Single gene trees are often insufficient for determining species limits and reconstructing evolutionary relationships within a genus due to past interspecific hybridization, gene duplication resulting in nuclear pseudogenes, and incomplete lineage sorting in the mitochondrial genome (Degnan 1993, Funk and Omland 2003, Waters et al. 2010). Inference of evolutionary relationships among ladyfishes has also been hampered by the fact that no phylogenetic study to date has included the West African Ladyfish (*E. lacerta*) and the Senegalese Ladyfish (*E. senegalensis*) and by prior limited geographic sampling of other species. Dense taxon and geographic sampling, especially in areas of sympatry, generally produce more robust phylogenetic trees, increasing the chances of detecting cryptic species, and are more useful for species delimitation (Omland et al. 1999, Avendaño et al 2017, Cicero et al. 2021). We concur with McBride et al. (2010) and Levesque (2011) that the genus *Elops* is a prime candidate for a rigorous phylogenetic analysis and possibly taxonomic revision. A future multilocus phylogenetic analysis of *Elops* would benefit from an extensive geographic sampling of all putative species of ladyfishes, especially in areas of sympatry, and incorporate morphological data into the analysis, providing an ideal basis for a thorough taxonomic review of *Elops*.

Age structure

Our age data indicate that ladyfish found in Texas use estuarine habitats during ages 0–3. The finding of no individuals > age 3 suggested that older individuals are either very rare or rarely found inshore. To attempt to understand the lack of older individuals in our current study, we also examined data from historical angler surveys conducted by TPWD to assess the size distribution of ladyfish landed during inshore versus offshore angling trips; no significant differences were detected. Similarly, length–frequencies of ladyfish found in Florida estuaries indicate that at least 3 age classes are present throughout the year and few individuals are older than 2–3 years (McBride et al. 2001).

The increased representation of age 0 and 2 ladyfish in our study is likely due to gear bias, with smaller age 1 fish falling within the size gap (120–250 mm) between the bag seine and gill net gears employed by TPWD; thus age 1 fish are probably underrepresented in our data set. Support for this hypothesis comes from the bimodal nature of ladyfish sizes in gill nets in the fall but not the spring, which might be simply driven by expected growth into the gill net gear size of age 1 individuals be-

tween the months of June and September and increasing catch efficiency of gill nets with increasing length (Levesque 2013).

The age structure observed in our data suggests that maximum age of ladyfish in Texas's estuarine habitats is likely to be 3 years. This is consistent with previously observed ages of ladyfishes in Florida estuaries, indicating that inshore populations of ladyfishes are primarily composed of immature individuals with maturity likely occurring after emigration to offshore habitats (McBride et al. 2001). Our data circumstantially support the hypothesis of emigration to offshore habitats around age 3, and the older fish noted in previous studies (Hildebrand 1963), Palko 1984) likely occur in offshore habitats in Texas. The biology of larger, mature ladyfishes in offshore habitats in Texas and throughout the range of the species represent an important knowledge gap that could be addressed with further studies.

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