Philippine Journal of Science
148 (S1): 251-261, Special Issue on Genomics
ISSN 0031 - 7683
Date Received: 18 Mar 2019

Molecular Phylogeny of Philippine Tigerperches (Perciformes: Terapontidae) Based on Mitochondrial Genes

Reynand Jay C. Canoy[1,2,3], Ian Kendrich C. Fontanilla[1,2], and Jonas P. Quilang[1,2]*

[1]Natural Sciences Research Institute, University of the Philippines
Diliman, Quezon City 1101 Philippines
[2]Institute of Biology, College of Science, University of the Philippines
Diliman, Quezon City 1101 Philippines
[3]Institute of Human Genetics, National Institutes of Health
University of the Philippines Manila, 625 Pedro Gil St., Ermita, Manila 1000 Philippines

The molecular phylogeny of the Philippine tigerperches is first described in this study. Eight species were analyzed: these include one endemic species (*Leiopotherapon plumbeus*); one introduced species (*Bidyanus bidyanus*); and six native species (*Terapon jarbua, Terapon puta, Terapon theraps, Pelates quadrilineatus, Helotes sexlineatus,* and *Mesopristes cancellatus*). Primers were designed to amplify and sequence the 12S rRNA (12S), cytochrome *c* oxidase subunit I (COI), and cytochrome b (CytB) genes. The concatenated 12S, COI, and CytB sequences (3529 bp) were used to construct the phylogeny of the tigerperches using Maximum Parsimony (MP), Neighbor Joining (NJ), Maximum Likelihood (ML), and Bayesian Inference (BI) analyses. All four analyses supported the monophyly of tigerperches. Except for the MP tree, all phylogenetic trees showed that *Terapon jarbua* was the first to have diverged from the rest of the tigerperch species examined. The congeneric *T. jarbua, T. puta* and *T. theraps* did not group together, suggesting their non-monophyly. However, SH test on the unconstrained (actual observation) and constrained (the three congeneric species were forced to group together) NJ trees showed no significant difference ($p = 0.55$). This demonstrated that the monophyly of the genus *Terapon* remains unclear. *Helotes sexlineatus* and *Pelates quadrilineatus* were found to group together based on the three markers, which lends support to assertions in other studies that these taxa are congeneric and should be placed in the same genus *Pelates*. The immediate sister taxa of *B. bidyanus, L. plumbeus, M. cancellatus*, and *Rhynchopelates oxyrhynchus* were not confirmed by the MP, NJ, ML, and BI phylogenetic trees. The inclusion of additional unsampled Philippine species, as well as those from neighboring countries, is recommended to further refine the phylogeny of tigerperches.

Keywords: 12S rRNA, cytochrome b, cytochrome oxidase I, mitochondrial genes, molecular phylogeny, Terapontidae, tigerperches

INTRODUCTION

Tigerperches or grunters (family Terapontidae) are ray-finned fishes (order Perciformes, class Actinopterygii, phylum Chordata) composed of 61 species from 15 genera (Fricke *et al.* 2019) that thrive in marine, coastal, brackish, or freshwater parts of the Indo-West Pacific region (Nelson 2006). They have oblong to oblong-ovate body shape, two spines on the opercle, dorsal fin with 11–14 spines and 8–14 soft rays, anal fin with three spines and 7–15 soft rays, and body length that can reach up to 80 cm (Nelson 2006). They are characterized by having an upper jaw that does not extend beyond the center of orbit (Vari 1999).

*Corresponding Author: jpquilang@up.edu.ph

In the Philippines, historical records show that there are eight tigerperch species (Herre 1953) present, but Froese and Pauly (2019) reported that there are 10 tigerperch species that thrive in lakes, brackish and sea waters. They are locally traded as food fishes and are thus economically and commercially important. In particular, the Philippine endemic *Leiopotherapon plumbeus* – locally known as "ayungin" – is traded around the major lakes of Luzon Island. The native *Mesopristes cancellatus* or "pigek" is highly-priced in Mindanao. It can also be found in Lake Naujan, Lake Mainit, and Kalinwan River. It is an exquisite delicacy and is served at very high prices in restaurants and hotels. A recent study has shown that the "bulidao" fish in Abra River is the same as the "pigek" fish found in Mindanao (Maralit *et al.* 2012). Other tigerperch species such as *Terapon jarbua* and *Pelates quadrilineatus* are commonly caught in coastal areas.

There are few available studies on the tigerperches. Most of them focused on the distribution (Azhagar *et al.* 2009, Rowland 2001); physiology (Chen *et al.* 1998); and morphological and genetic variation (Bostock *et al.* 2006; Quilang *et al.* 2007, 2008) of specific tigerperch species. The taxonomy and monophyly of tigerperches, in particular, were revised by Vari (1978) using morphological characters. Since then, only one phylogenetic study was conducted on the molecular systematics of *Terapon jarbua*, *Terapon theraps*, and *Pelates quadrilineatus* using allozyme analysis and partial 12S sequences (Lee and Tsai 1999).

The advancement of molecular biology has permitted the study of evolutionary relationships at deeper levels. Gene or genomic sequences are now being used to infer phylogenetic relationships (Vandamme 2009). In fishes, mitochondrial genes are routinely used to describe their molecular phylogeny. The complete mitochondrial genome was employed to infer the phylogeny of otophysan fishes (Peng *et al.* 2006) and of the highly diversified percamorph fishes (Yagishita *et al.* 2009). Among the mitochondrial genes that are commonly used in fish phylogeny are the CytB (Akihito *et al.* 2000, Orrell and Carpenter 2004, Sloss *et al.* 2004, Thacker and Hardman 2005, Xiao *et al.* 2005, Doiuchi and Nakabo 2006, Slechtová *et al.* 2006); 12S (Sloss *et al.* 2004, Slechtová *et al.* 2006); 16S rRNA (16S) (Orrell and Carpenter 2004, Doiuchi and Nakabo 2006, Li *et al.* 2008); and COI (Thacker 2003, Thacker and Hardman 2005, Doiuchi and Nakabo 2006) genes. Molecular phylogenetics can be used for species delineation and confirmation of high-value fishes. For instance, Maralit *et al.* (2012) confirmed the species designation of "pigek" fish in Sultan Kudarat and the "bulidao" fish in Abra as *M. cancellatus*, suggesting its widespread distribution and possible utility in stock management.

This study was designed in recognition of the importance of correct identification of species and the value of inferring phylogenetic relationships. In this study, the molecular phylogeny of economically important Philippine tigerperches was determined using three mitochondrial genes: 12S, COI, and CytB. This is the first study to evaluate the phylogeny of eight Philippine terapontid species, including the endemic *L. plumbeus*.

MATERIALS AND METHODS

Specimen Collection

Sampling activities were conducted to collect the tigerperch species in the Philippines. These included the hiring of fishermen to catch fishes and procuring fish samples as they were unloaded in fish landing sites and local fish ports. Coordination with the Philippine Bureau of Fisheries and Aquatic Resources (BFAR) was also done to secure other tigerperch fish samples. Standard morphometric measurements (weight, total length, fork length, standard length, and body depth) and meristic counts (dorsal fin, pectoral fin, ventral fin, anal fin, lateral line scales, number of rows above and below the lateral line, and upper left gill rakers) were taken from each fish specimen. The morphological keys of Vari (1999) in the Food and Agriculture Organization's Species Identification Guide for Fishery Purposes were used to identify the species of each individual. A total of eight species of tigerperches were collected in this study (Table 1, Figure 1).

The muscle tissue of the pectoral region at the right side of each of the specimens was excised and stored in absolute ethanol and kept at –20 °C until DNA extraction. All of the collected specimens were stored in 95% ethanol as voucher specimens at the Institute of Biology, College of Science, University of the Philippines Diliman, Philippines.

DNA Extraction, Primer Design, and PCR Amplification

DNA extraction was performed using the Promega Wizard® SV Genomic DNA Purification System (Madison, WI, USA) with 20 mg/mL proteinase K solution from Roche Applied Science (Germany). Briefly, 20 mg of the muscle tissue was incubated overnight in the digestion solution. Genomic DNA was then extracted following the manufacturer's protocol.

Primers were designed (Table 2) using Primer3Plus (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi; Untergasser *et al.* 2007) to amplify and

Table 1. Summary of tigerperch species included in the study.

Species	Status	No. of individuals collected	Collection site
Bidyanus bidyanus (Mitchell, 1838)	Introduced	2	BFAR, Dagupan City
Helotes sexlineatus (Quoy & Gaimard, 1825)	Native	6	Iloilo Fish Port
Leiopotherapon plumbeus (Kner, 1864)	Endemic	6	Binangonan, Rizal
Mesopristes cancellatus (Cuvier, 1829)	Native	6	Butuan City; Cotabato City
Pelates quadrilineatus (Bloch,1790)	Native	6	Navotas Fish Port
Terapon jarbua (Forsskål, 1775)	Native	6	Navotas Fish Port
Terapon puta Cuvier, 1829	Native	6	Agoo, La Union
Terapon theraps Cuvier, 1829	Native	4	Iloilo City

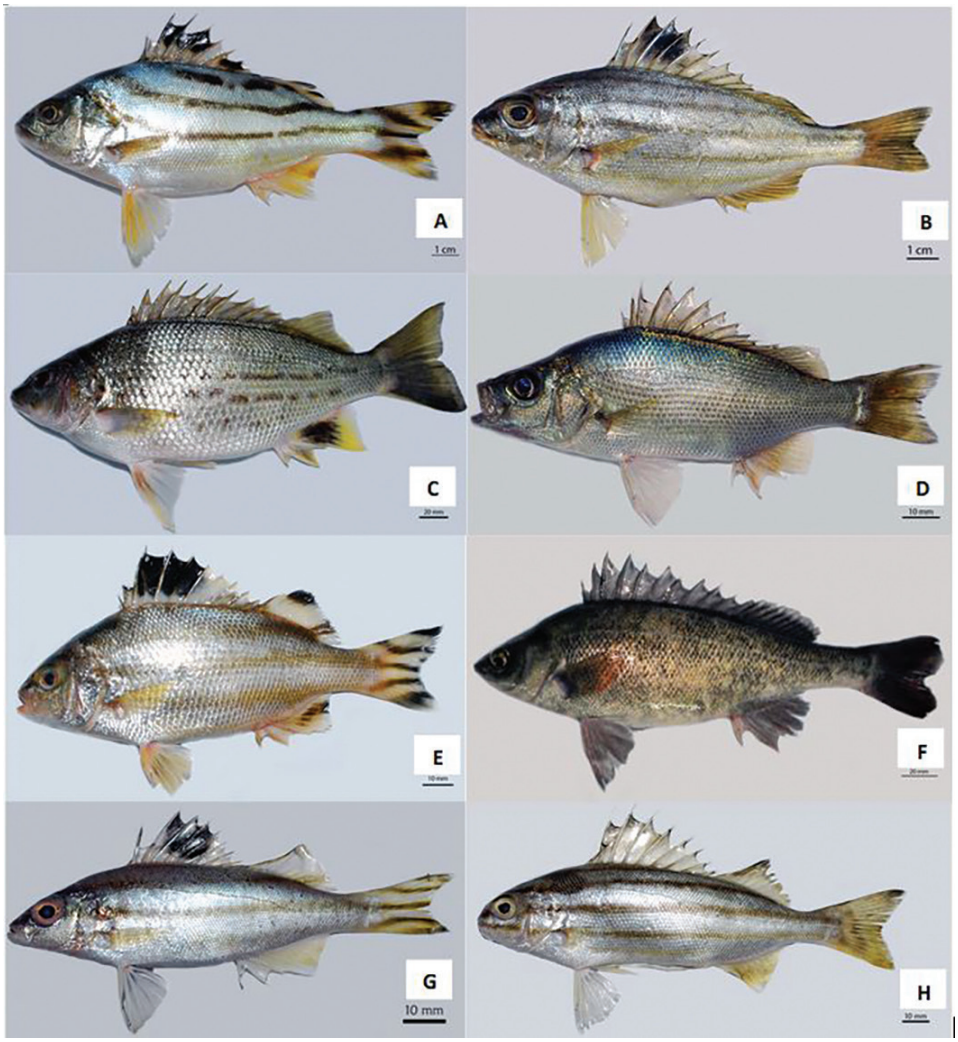

Figure 1. Philippine tigerperch species included in this study: *Terapon jarbua* (A), *Pelates quadrilineatus* (B), *Mesopristes cancellatus* (C), *Leiopotherapon plumbeus* (D), *Terapon theraps* (E), *Bidyanus bidyanus* (F), *Terapon puta* (G), and *Helotes sexlineatus* (H).

Table 2. Primers used in this study. Forward and reverse primers were mixed and matched to amplify each gene. Internal primers were used to sequence the middle region of COI and CytB genes.

Gene	Forward primer (5' to 3')	Reverse primers (5' to 3')	Internal primers (5' to 3')	Approx. product size (bp)
12S	LPak12s01 (TAAGATGRGCCCTRRAAAGC)	Pak12s01H (CARGGTRATCCGAWTTGCAC)		1000
	Lpak12s02 (TAAGATGRGCCCTRRAAAGC)	Pak12s02H (CGAWTTGCACGGATGACTTC)		
	Lpak12s03 (GCGTAGCTTAMYYAAAGCAT)	Pak12s031H (GGATGACTTCTCRGTGTAAGGG)		
	Lpak12s04 (CACTGAAGATKTTAAGATGGG)	Pak12s041H (GTGTAAGGGAGATGCTTTTCTA)		
COI	LPakCOI01 (CCACCGCTTAAAAACTCAGC)	PakCOI01H (CCWAYWYAYGGGGGTTCRAY)	InLPakCOI01 (CACGAAGTCTGAACAAAAGTTCAC)	1600
	LPakCOI02 (CACCTCRRRRCTTGGTAAGA)	PakCOI02H (GGTTCRAYTCCYYCCTTTCT)	InPakCOI01H (GTGAACTTTTGTTCAGACTTCGTG)	
	LPakCOI03 (TMTATGGGGCTACAATCCACC)	PakCOI03H (GGTWATGTGRTTGGCTTGAAAC)		
CytB	LpakCytB01 (CCMGGAYTYTAACCAGGAMY)	PakCytB01H (GGCGCTCTGRRRYTGAGCTA)	InLPakCytB01 (GAAACAGGYTCAAAYAACCC)	1200

sequence the 12S, COI, and CytB mitochondrial genes. The primer sequences were based on the mitogenome of *Rhynchopelates oxyrhyncus* from Japan and of two other closely related species: *Enoplosus armatus* (Enoplosidae) and *Girella punctata* (Kyophosidae) (Yagishita *et al.* 2009). Forward and reverse primers were mixed and matched to obtain successful amplification of the three genes.

PCR amplification was done using Roche dNTPack (Germany), having the following components in a 50-µL reaction: 0.2 µM dNTP mix; 0.5 µM forward primer; 0.5 µM reverse primer; 1X PCR buffer (100 mM Tris-Cl, 15 mM $MgCl_2$, 500 mM KCl, pH 8.3); and 1.25 U *Taq* polymerase. For the PCR amplification of 12S, the PCR conditions consisted of an initial step of 95 °C for 2 min; followed by 35–40 cycles of 94 °C for 30 sec, 54 °C for 1 min, and 72 °C for 1 min; final extension of 72 °C for 10 min; and then cool down at 4 °C until gel visualization. For the PCR amplification of COI and CytB, the PCR conditions were as follows: an initial step of 95 °C for 2 min; followed by 35–40 cycles of 94 °C for 1 min 30 sec, 56°C for 1 min 30 sec, and 72°C for 1 min 30 sec; final extension of 72 °C for 10 min; and then cool down at 4 °C until gel visualization.

DNA Sequencing and Sequence Alignment

PCR amplicons were run on 1% agarose gels with 1% ethidium bromide for visualization. Amplicons with the expected sizes (~1000 bp for 12S, ~1600 bp for COI, and ~1200 bp for CytB) were cut from the gel and were then purified using Qiagen Qiaquick® Gel Extraction Kit (Valencia, CA, USA) following the manufacturer's protocol. Purified PCR products were sent to 1stBASE Pte. Ltd. in Selangor Darul Ehsan, Malaysia for bidirectional sequencing using the genetic analyzer from Life Technologies with BigDye v3.1 cycle sequencing kit chemistry. For COI and CytB amplicons, internal sequencing primers (Table 1) were used to sequence the middle regions of COI and CytB genes. The sequences generated by 1stBase were evaluated to obtain the consensus sequences for each sample using Staden Package v4.10 (Staden *et al.* 2000).

At most two individuals were analyzed for each species collected. Outgroup sequences (Table 3) were included based on the study of Yagishita *et al.* (2009) for the subsequent phylogenetic analyses. Sequence alignment for each gene was done using the CLUSTAL W algorithm in BioEdit 7.0.5.3 (Hall 1999). The 12S sequences were further aligned manually according to secondary structural elements established for fishes (Wang and Lee 2002) that are partitioned into stems and loops. Ambiguous alignment at the loop regions was excluded in the analysis. COI and CytB sequences were also checked for premature stop codons.

Site Saturation Test, Homogeneity Test, and DNA Substitution Model Testing

Site saturation was checked computationally for each gene by the Xia test (Xia *et al.* 2003, Xia and Lemey 2009) in DAMBE (http://dambe.bio.uottawa.ca). Homogeneity of phylogenetic information among the three genes was checked by the partition homogeneity test (Farris *et al.* 1994) in PAUP* 4.0b10 (Swofford 2002) at $p < 0.05$ for significant difference. The optimum model of DNA substitution was determined by jModeltest 2.1.4 (Guindon

and Gascuel 2003, Darriba *et al.* 2012) among the 1,624 models of DNA substitution. The Akaike Information Criterion (AIC) (Akaike 1973), Bayesian Information Criterion (BIC) (Schwarz 1978), and Decision Theory Performance-based Selection (Minin *et al.* 2003) were used as the model selection strategies.

Construction of Phylogenetic Trees and Testing the Tree Hypothesis

MP analysis (Fitch 1977) was performed in PAUP* 4.0b10 (Swofford 2002). This analysis involved the heuristic search of the most parsimonious trees of random, stepwise additions and tree bisection reconnection of branch swapping with 10,000 pseudoreplications for bootstrap resampling.

The identified optimum model of DNA substitution was used in the NJ (Saitou and Nei 1987), ML (Felsenstein 1981), and BI analyses. NJ analysis was performed in PAUP* 4.0b10 (Swofford 2002) at 10,000 pseudoreplicates for bootstrap resampling. On the other hand, ML analysis was performed in PhyML 3.0 (Guindon and Gascuel 2003) at 1,000 pseudoreplicates for bootstrap resampling. In the ML analysis, Nearest Neighbor Interchange was used to estimate tree topologies with BioNJ as the starting tree. BI analysis was performed using MrBayes v3.0b4 (Huelsenbeck and Ronquist 2001, Ronquist *et al.* 2005). The characteristics of the optimum model of DNA substitution was used to set the model in which the trees were based (number of kinds of substitution, gamma distribution, and proportion of invariant sites). Starting trees were randomly chosen. For the BI analysis, Monte Carlo Markov chains were run for 10,000,000 generations – trees being sampled for every 100 generations for a total of 100,000 trees. The number of samples discarded was 99,001 (burn-in).

The Shimodaira and Hasegawa (1999) (SH) test implemented in PAUP* 4.0b10 (Swofford 2002) was performed to compare the unconstrained NJ tree (actual

Table 3. GenBank sequences used in this study.

Species	Family	GenBank accession number	Source
Bidyanus bidyanus	Terapontidae	KF999833, KF999834, KF999849, KF999850, KF999867, KF999868	This study
Helotes sexlineatus	Terapontidae	KF999837, KF999838, KF999853, KF999854, KF999870, KF999871	This study
Leiopotherapon plumbeus	Terapontidae	KF999829, KF999830, KF999845, KF999846, KF999861, KF999862	This study
Mesopristes cancellatus	Terapontidae	KF999827, KF999828, KF999843, KF999844, KF999859, KF999860	This study
Pelates quadrilineatus	Terapontidae	KF999825, KF999826, KF999841, KF999842, KF999857, KF999858	This study
Terapon jarbua	Terapontidae	KF999823, KF999824, KF999839, KF999840, KF999855, KF999856	This study
Terapon puta	Terapontidae	KF999835, KF999836, KF999851, KF999852, KF999869	This study
Terapon theraps	Terapontidae	KF999831, KF999832, KF999847, KF999848, KF999863, KF999864, KF999865, KF999866	This study
Rhynchopelates oxyrhynchus	Terapontidae	AP011064	Yagishita *et al.* (2009)
*Histiopterus typus**	Pentacerotidae	AY491978	Yagishita *et al.* (2009)
*Enoplosus armatus**	Enoplosidae	AP006008	Yagishita *et al.* (2009)
*Girella punctata**	Kyphosidae	AP011060	Yagishita *et al.* (2009)
*Kuhlia mugil**	Kuhliidae	AP011065	Yagishita *et al.* (2009)
*Oplegnathus fasciatus**	Oplegnathidae	AP006010	Yagishita *et al.* (2009)

*Outgroup

observation) of *T. jarbua*, *T. puta*, and *T. theraps* with the corresponding constrained tree (the three species were forced to cluster together). Constrained and unconstrained NJ trees were compared at $p < 0.05$ for significant difference. SH test assesses a set of selected trees having a null hypothesis that all trees are equally good explanation of the data (Schmidt 2009). When there is evidence to reject the null hypothesis, some or all trees are not equally good explanation of the data (Schmidt 2009).

The obtained 12S, COI, and CytB gene sequences were submitted to GenBank and were assigned accession numbers that are given in Table 3.

RESULTS AND DISCUSSION

Only a little saturation was evident in the aligned sequences of 12S (891 bp), COI (1557 bp), and CytB (1081 bp) genes based on the Xia test (Xia *et al.* 2003, Xia and Lemey 2009) where – for each set of aligned sequences – the observed index of site saturation (*Iss*) was significantly lower from the critical index of site saturation (*Iss.c*) for both symmetrical and asymmetrical trees (Table 4).

Table 4. Results of Xia test showing the index of site saturation of each gene used for the phylogenetic analyses.

Gene	*P*-value	*Iss*	*Iss.c* (symmetrical tree)	*Iss.c* (assymetrical tree)
12S	0.0000	0.1256	0.7571	0.5753
COI	0.0000	0.2005	0.7876	0.6230
CytB	0.0000	0.2203	0.7683	0.5922

Table 5 shows the base frequencies and sequence variation of 12S, COI, and CytB aligned tigerperch sequences used in this study. Among the 12S sequences, A was observed to be the dominant base (30%), which was even more pronounced in the loop region (33%). The dominance of A in the 12S sequences, especially in the loop region, was also seen in percid fishes (Sloss *et al.* 2004). In COI sequences, both C and T accounted for 58% of the bases. For the individual codon positions, the dominant bases were G (32%) for the 1st, T (41%) for the 2nd, and C (36%) for the 3rd. In the CytB sequences, C was dominant (33%) overall and in the 1st (27%) and 3rd (45%) codon positions while T (41%) was dominant in the 2nd. The GC composition (48%) in COI sequences was similar to Australian teleost fishes (47%) (Ward *et al.* 2005) and the same with sinipercid fishes (48%) (Chen *et al.* 2010). On the other hand, the GC composition in CytB sequences (48%) was similar to percid fishes (47%) (Sloss *et al.* 2004) but slightly higher compared to sinipercid fishes (46%).

The loop region of 12S sequences had more variable (25% *vs.* 14%) and more parsimony informative (41% *vs.* 38%) sites compared to the stem region. This is expected as the stem region should be conserved to maintain the overall secondary structure of 12S (Wang and Lee 2002). This was particularly observed in 23 gobioid fishes where rates of nucleotide substitution were lower in stems than in loops, producing less phylogenetic information (Wang and Lee 2002). In COI and CytB sequences, the 3rd codon position was observed to be the most variable (81% and 86%, respectively) and contained the most number of parsimony informative sites (72% and 71%, respectively). This is also expected as the degeneracy of the genetic code allows changes at the third codon position while retaining the original amino acid.

Table 5. Base frequencies and sequence variation of each gene used for the phylogenetic analyses. The corresponding percentages with respect to the number of aligned sites (for the number of variable sites) and with respect to the number of variable sites (for the number of parsimony informative sites) are enclosed in parentheses.

Gene		% composition				No. of aligned sites	No. of variable sites	No. of parsimony informative sites
		A	C	G	T			
12S		30	27	23	20	891	175 (20%)	70 (40%)
	stem	26	27	26	20	446	64 (14%)	24 (38%)
	loop	33	26	21	20	445	111 (25%)	46 (41%)
COI		24	29	19	29	1557	481 (31%)	322 (67%)
	1st codon	24	23	32	21	519	47 (9%)	16 (34%)
	2nd codon	18	26	15	41	519	13 (3%)	1 (8%)
	3rd codon	29	36	10	25	519	421 (81%)	305 (72%)
CytB		24	33	15	28	1081	358 (33%)	236 (66%)
	1st codon	25	27	25	23	361	43 (12%)	16 (37%)
	2nd codon	20	26	13	41	360	6 (2%)	0 (0%)
	3rd codon	29	45	6	20	360	309 (86%)	220 (71%)

The phylogenetic information among the three sets of sequences was found to be homogenous ($p > 0.05$) as revealed by the partition homogeneity test (Farris *et al.* 1994). The three sets of sequences were then concatenated (3529 bp) for further phylogenetic analysis.

Three models of selection strategies were employed (Posada 2009) to select the optimum model of DNA substitution. There are cases when AIC would choose a different model compared to BIC, but the latter tends to choose the more accurate and reliable model of DNA substitution (Acquah 2010, Evans and Sullivan 2011). For this study, all three selection strategies found TIM2+Γ+I as the optimum model of DNA substitution.

Four methods of phylogenetic tree construction (MP, NJ, ML, and BI) were used in this study. All four methods supported the monophyly of tigerperches with very high bootstrap support (100% for ML, NJ, and MP) and posterior probability (1.0 for BI). Correspondingly, this provided support that the characteristic features of the family – having post-temporal bone covered with skin and scales, not expanded posteriorly, and its posterior margin not serrate (Vari 1999) – are derived for Terapontidae. However, the trees differed from the cladogram constructed by Vari (1978) using comparative morphology to depict the generic relationships of terapontid fishes. Vari (1978) used 33 synapomorphic morphological characters such as extrinsic swim bladder muscle, transversely divided swim bladder, third pharyngobranchial process, urohyal form, and other morphological characters that either describe the anatomy or counts the number of formation of parts. The species *Leiopotherapon*, *Terapon*, and *Pelates* were among the tigerperch species that Vari (1978) studied. The cladogram in Vari (1978) showed that *Leipotherapon* was the first to have diverged and *Terapon* and *Pelates* only came later. In contrast, this study showed *T. jarbua* to have diverged first from the rest of the tigerperch species.

Figure 2 shows the ML tree that incorporates the ML, NJ, and MP bootstraps – as well as the BI posterior probabilities. The other tigerperch species included in this study, *Bidyanus bidyanus* and *Rhynchopelates*

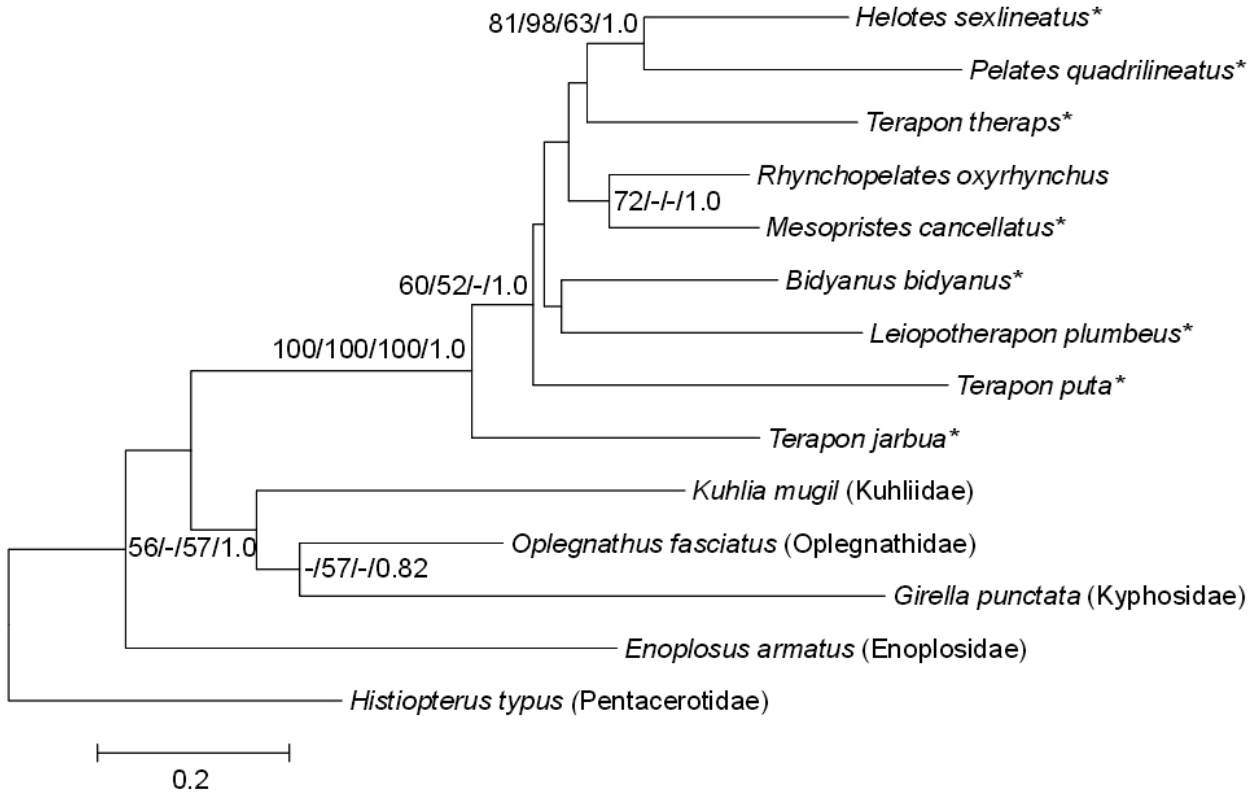

Figure 2. ML phylogenetic tree of the Philippine tigerperches based on the concatenated sequences (3529 bp) of 12S, COI, and CytB. The optimum model of DNA substitution was TIM2+Γ+I, and Nearest Neighbor Interchange was used to estimate tree topologies with BioNJ as the starting tree. The Philippine tigerperch species collected in this study are marked with an asterisk (*). The Japanese native tigerperch *Rhynchopelates oxyrhynchus* was included in the analysis along with the other closely related species as outgroups: *Histiopterus typus* (Pentacerotidae), *Enoplosus armatus* (Enoplosidae), *Girella punctate* (Kyphosidae), *Kuhlia mugil* (Kuhliidae), and *Oplegnathus fasciatus* (Oplegnathidae). The bootstrap support values are indicated in the branches along with the bootstrap support values of NJ and MP analyses and posterior probabilities of BI analysis, respectively. Only bootstrap support values greater than 50 and posterior probabilities greater than 0.80 are shown. Scale bar indicates two nucleotide changes for every 10 nucleotides.

oxyrhynchus, were observed to be placed within the clade of Philippine tigerperch species collected. *B. bidyanus* is an introduced tigerperch species from Australia and is currently being cultured for potential commercial purposes by the BFAR in Dagupan City, Pangasinan, Luzon, Philippines. On the other hand, *R. oxyrynchus* is a native tigerperch species in Japan (Froese and Pauly 2014). Therefore, no geographical segregation among tigerperch species is apparent in this study. The placement of *T. jarbua* with respect to the rest of the species supported the findings of Davis *et al.* (2012) that freshwater terapontid fishes arose from euryhaline terapontid fishes.

The congeneric *T. jarbua*, *T. puta*, and *T. theraps* did not group together. Their placements in the phylogenetic trees were supported by considerable to high bootstrap values and posterior probabilities. The relative positions of the three *Terapon* species were also similar to the phylogenetic construction of Davis *et al*. (2012) describing the evolutionary relationships of Australian terapontid fishes using combined mitochondrial and nuclear sequence data (5952 bp), habitat association, and dietary ecology. This suggests that the characteristic features of *Terapon* species [*e.g.*, having lower opercular spine greatly developed, extending beyond margin of the opercular lobe; lobes of caudal fin with distinct oblique stripes; and spinous dorsal fin with large black blotch on middle rays (Vari 1999)] could possibly be homoplasious. However, results of the SH test (Shimodaira and Hasegawa 1999) on the unconstrained (actual observation) and constrained (*T. jarbua*, *T. puta*, and *T. theraps* were forced to cluster together) NJ trees showed no significant difference (p = 0.55). This demonstrates that there is no definitive support for the non-monophyly of the genus *Terapon*. Lee and Tsai (1999) proposed the transfer of *T. theraps* to a new genus *Pseudoterapon* because *T. jarbua* and *T. theraps* had very low Nei's genetic similarity and had very wide genetic distance using allozyme analysis. Both species did not cluster together in the UPGMA, NJ, and Wagner trees constructed from the isozyme data and 12S sequences.

Helotes sexlineatus and *P. quadrilineatus* were observed to group together with high bootstrap support (81% for ML, 98% for NJ, and 63% for MP) and posterior probability (1.0 for BI). The two species used to be congeneric, but *H. sexlineatus* was recently reclassified as the sole species of the genus *Helotes*. There is confusion on the taxonomic classification of *H. sexlineatus*. Froese and Pauly (2014) noted that a new replacement name would be needed if this particular species is placed again in the genus *Pelates*. This study shows the close relationship between the two genera. Moreover, the K2P distance of the two species was computed to be 13.4%, which is well within the K2P distance range of congeneric species (0–20.63%) observed by Ward *et al.* (2005). This can be further supported if other species of *Pelates* can be sampled and subjected to the same analyses as what was done in this study. The results may show the monophyly of the genus *Pelates* or it may show that *H. sexlineatus* is well within the clade of *Pelates*, which would give further support for the return of *H. sexlineatus* to the genus *Pelates*. If *H. sexlineatus* is returned to the genus *Pelates*, then the characteristic features of *Pelates* species [*e.g.*, having 66–87 lateral-line scales; distinct patch of black pigmentation on anterior portion of spinous dorsal fin; body with distinct, dark, longitudinal stripes; and lobes of caudal fin without prominent oblique transverse black pigmentation (Vari 1999)] are supported.

The exact placements of *B. bidyanus*, *L. plumbeus*, *M. cancellatus*, and *R. oxyrhyncus* within the tigerperch clade were not corroborated among MP, NJ, MI, and BI phylogenetic trees. The molecular markers used in this study (12S, COI, CytB) seemed to be insufficient to identify their respective immediate sister taxa; alternatively, perhaps the Philippine tigerperch species that were not collected in this study (*Mesopristes argenteus* and *Mesopristes iravi*) could be their closest relative. This could be the reason why the described relationships of the collected tigerperch species could not be supported by each of the phylogenetic analyses performed. This is also the case for *L. plumbeus*, which is endemic to the Philippines. This study only focused on the Philippine tigerperch species, so its immediate closest relative may not be in the Philippines but elsewhere. It would be interesting to sample the remaining Philippine tigerperch species and other *Leiopotherapon* species in Australia and examine their evolutionary relationship. This can also show the monophyly of the genus *Leiopotherapon*. Australia is an older land mass compared to the Philippines, so it would provide more insight into their phylogeny. Moreover, many species of this family are confined in Australia and nearby regions, so the species from Australia may be the first in the phylogeny line of this family. Therefore, the inclusion of other tigerperch species from neighboring countries may be able to provide more information and resolution to the phylogeny of tigerperches.

CONCLUSION

This is the first study to describe the molecular phylogeny of the Philippine tigerperches, which includes eight species based on 3529 nucleotides from three mitochondrial DNA markers. This study supported the monophyly of tigerperches as seen in the MP, NJ, ML, and BI phylogenetic trees. The non-monophyly of the genus *Terapon* does not have definitive support in this study as the SH test showed no significant difference

in the unconstrained and constrained NJ trees. The close relationship between the previously congeneric *H. sexlineatus* and *P. quadrilineatus* was supported in this study, which suggests that the two species should be placed in the same genus as shown in the phylogenetic trees constructed. On the other hand, the immediate sister taxa *B. bidyanus*, *L. plumbeus*, *M. cancellatus*, and *R. oxyrhyncus* were not corroborated by the phylogenetic trees constructed. Addition of tigerperch species from the Philippines and other neighboring countries not included in this study is recommended to refine the molecular phylogeny of the family.

ACKNOWLEDGMENTS

The authors would like to thank the following: the Natural Sciences Research Institute of the University of the Philippines (UP) Diliman for funding this research (Project BIO-11-1-03); Institute of Biology of UP Diliman for providing the facilities and laboratory logistic support; Institute of Human Genetics of UP Manila for providing support for the analysis; Navotas Fish Port officials for giving access to their fish port; and the BFAR in Butuan City, Dagupan City, and Iloilo City for providing fish samples.

CONFLICTS OF INTEREST

There are no conflicts of interest in this study.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

RJC Canoy conducted the field sampling activities and experiments, performed the data analysis and prepared the manuscript. JP Quilang conceptualized the study, conducted field sampling activities, supervised the experiments, and assisted in the data analysis and manuscript preparation. IKC Fontanilla assisted in the data analysis and manuscript preparation.

REFERENCES

ACQUAH HDG. 2010. Comparison of Akaike information criterion (AIC) and Bayesian information criterion (BIC) in selection of an asymmetric price relationship. J Dev Agric Econ 2(1): 1–6.

AKAIKE H. 1973. Information theory and an extension of the maximum likelihood principle. In: Second International Symposium on Information Theory. Petrov BN, Csaki F eds. Budapest (Hungary): Akademiai Kiado. p. 267–281.

AKIHITO, IWATA A, KOBAYASHI T, IKEO K, IMANISHI T, ONO H, UMEHARA Y, HAMAMATSU C, SUGIYAMA K, IKEDA Y, SAKAMOTO K, FUMIHITO A, OHNO S, GOJOBORI T. 2000. Evolutionary aspects of gobioid fishes based upon a phylogenetic analysis of mitochondrial cytochrome b genes. Gene 259: 5–15.

AZHAGAR S, ANBALANGAN T, VEERAPAN N. 2009. Distribution and abundance of finfish larvae along the Bay of Bengal (South East Coast of India). Curr Res J Biol Sci 1(1): 14–17.

BOSTOCK BM, ADAMS M, LAURENSON LJB, AUSTIN CM. 2006. The molecular systematics of *Leiopotherapon unicolor* (Günther, 1859): Testing for cryptic speciation in Australia's most widespread freshwater fish. Biol J Linn Soc 87: 537–552.

CHEN SF, HUANG BQ, CHIEN YY. 1998. Histochemical characteristics of sonic muscle fibers in tigerperch, *Terapon jarbua*. Zool Stud 37(1): 56–62.

CHEN D, GUO X, NIE P. 2010. Phylogenetic studies of sinipercid fish (Perciformes: Sinipercidae) based on multiple genes, with first application of an immune-related gene, the virus-induced protein (viperin) gene. Mol Phylogenet Evol 55: 1167–1176.

DARRIBA D, TABOADA GL, DOALLO R, POSADA D. 2012. jModelTest 2: More models, new heuristics and parallel computing. Nat Methods 9(8): 772.

DAVIS AM, UNMACK PJ, PUSEY BJ, JOHNSON JB, PEARSON RG. 2012. Marine-freshwater transitions are associated with the evolution of dietary diversification in terapontid grunters (Teleostei: Terapontidae). J Evol Biol 25: 1163–1179.

DOIUCHI R, NAKABO T. 2006. Molecular phylogeny of the stromateoid fishes (Teleostei: Perciformes) inferred from mitochondrial DNA sequences and compared with morphology-based hypotheses. Mol Phylogenet Evol 39: 111–123.

EVANS J, SULLIVAN J. 2011. Approximating model probabilities in Bayesian information criterion and decision-theoretic approaches to model selection in phylogenetics. Mol Biol Evol 28(1): 343–349.

FARRIS JS, KALLERSJO M, KLUGE AG, BULT C. 1994. Testing significance of incongruence. Cladistics 10: 315–319.

FELSENSTEIN J. 1981. Evolutionary trees from DNA sequences: A maximum likelihood approach. J Mol Evol 58: 131–144

FITCH WM. 1997. On the problem of discovering the most parsimonious tree. The Am Nat 111: 223–257.

FRICKE R, ESCHMEYER WN, VAN DER LAAN R eds. 2019. Eschmeyer's Catalog of Fishes: Genera, Species, References. Retrieved from http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp on 14 Aug 2019.

FROESE R, PAULY D. 2014. FishBase. Retrieved from www.fishbase.org on 01 Jun 2014.

FROESE R, PAULY D. 2019. FishBase. Retrieved from www.fishbase.org on 14 Aug 2019.

GUINDON S, GASCUEL O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52: 696–704.

HALL TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41: 95–98.

HERRE A. 1953. Check list of Philippine fishes. Washington, DC: United States Government Printing Office. 977p.

HUELSENBECK JP, RONQUIST FR. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

LEE SC, TSAI MP. 1999. Molecular systematics of the thornfishes genera *Terapon* and *Pelates* (Perciformes: Teraponidae) with reference to the new genus *Pseudoterapon*. Zool Stud 38(3): 279–286.

LI J, WANG X, KONG X, ZHAO K, HE S, MAYDEN RL. 2008. Variation patterns of the mitochondrial 16S rRNA gene with secondary structure constraints and their application to phylogeny of cyprinine fishes (Teleostei: Cypriniformes). Mol Phylogenet Evol 47: 472–487.

MARALIT B, VENTOLERO MF, GAERLAN RS, MAMALANGKAP M, SANTOS M. 2012. Species and endemicity status of the therapontid "Pigek", *Mesopristes cancellatus* (Cuvier, 1829) in the Philippines. Philipp Sci Lett 5(1): 30–39.

MININ V, ABDO Z, JOYCE P, SULLIVAN J. 2003. Performance-based selection of likelihood models for phylogeny estimation. Syst Biol 52: 674–83.

NELSON JS. 2006. Fishes of the World, 4th ed. New Jersey: John Wiley & Sons, Inc. 601p.

ORRELL TM, CARPENTER KE. 2004. A phylogeny of the fish family Sparidae (porgies) inferred from mitochondrial sequence data. Mol Phylogenet Evol 32: 425–434.

PENG Z, WANG J, HE S. 2006. The complete mitochondrial genome of the helmet catfish *Cranoglanis bouderius* (Siluriformes: Cranoglanididae) and the phylogeny of otophysan fishes. Gene 376: 290–297.

POSADA D. 2009. Selections of models of DNA evolution with jModelTest. In: Methods in Molecular Biology. New York: Humana Press. p. 93–112.

QUILANG JP, BASIAO ZU, PAGULAYAN RC, RODEROS RR, BARRIOS EB. 2007. Meristic and morphometric variation in the silver perch, *Leiopotherapon plumbeus* (Kner, 1864), from three lakes in the Philippines. J Appl Ichthyol 23: 561–567.

QUILANG JP, BASIAO ZU, PAGULAYAN RC, RODEROS RR, CAO EP. 2008. Low isozyme variation in native and transplanted populations of the endemic Philippine silver perch, *Leiopotherapon plumbeus* (Kner, 1864), from three lakes in the Philippines. Philipp Agric Sci 91: 99–103.

RONQUIST F, HUELSENBECK JP, VAN DER MARK P. 2005. MrBayes 3.1 Manual. Retrieved from http://mrbayes.csit.fsu.edu/manual.php on 01 Jan 2013.

ROWLAND SJ. 2001. Record of the banded grunter *Amniataba percoides* (Teraponidae) from the Clarence River, New South Wales. Aust Zool 31(4): 603–607.

SAITOU N, NEI M. 1987. The Neighbor-joining method for estimating and testing minimum evolution trees. Mol Biol Evol 4: 406–425.

SCHMIDT HA. 2009. Testing Tree Topologies. In: The Phylogenetic Handbook: A Practical Approach to Phylogenetic Analysis and Hypothesis Testing, 2nd ed. Cambridge, UK: Cambridge University Press. p. 381–404.

SCHWARZ G. 1978. Estimating the dimension of a model. Ann Stat 6: 461–464.

SHIMODAIRA H, HASEGAWA M. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. Mol Biol Evol 16: 1114–1116.

SLECHTOVÁ V, BOHLEN J, FREYHOF J, RÁB P. 2006. Molecular phylogeny of the Southeast Asian freshwater fish family Botiidae (Teleostei: Cobitoidea) and the origin of polyploidy in their evolution Mol Phylogenet Evol 39: 529–541.

SLOSS BL, BILLINGTON N, BURR BM. 2004. A molecular phylogeny of the Percidae (Teleostei, Perciformes) based on mitochondrial DNA sequence. Mol Phylogenet Evol 32: 545–562.

STADEN R, BEAL KF, BOONFIELD JK. 2000. The Staden Package, 1998. Methods Mol Biol 132: 115–130.

SWOFFORD DL. 2002. PAUP* 4.0b10. Sunderland, MA: Sinauer Associates.

THACKER CE. 2003. Molecular phylogeny of the gobioid fishes (Teleostei: Perciformes: Gobioidei). Mol Phylogenet Evol 26: 354–368.

THACKER CE, HARDMAN MA. 2005. Molecular phylogeny of basal gobioid fishes: Rhyacichthyidae, Odontobutidae, Xenisthmidae, Eleotridae (Teleostei: Perciformes: Gobioidei). Mol Phylogenet Evol 37: 858–871.

UNTERGASSER A, NIJVEEN H, RAO X, BISSELING T, GEURTS R, LEUNISSEN JAM. 2007. Primer3Plus, an enhanced web interface to Primer3. Nucleic Acids Res 35: w71–w74.

VANDAMME AM. 2009. Basic concepts of molecular evolution. In: The Phylogenetic Handbook: A practical Approach to Phylogenetic Analysis and Hypothesis Testing, 2nd ed. Cambridge, UK: Cambridge University Press. p.1–29.

VARI RP. 1978. The terapon perches (Percoidei, Teraponidae): A cladistics analysis and taxonomic revision. Bull Am Mus Nat Hist 159(5): 175–340.

VARI RP. 1999. Terapontidae. In: FAO Species Identification Guide for Fishery Purposes. The Living Marine Resources of the Western Central Pacific Vol. 5. Rome: FAO. p. 3305–3316.

WANG HY, LEE SC. 2002. Secondary structure of mitochondrial 12S rRNA among fish and its phylogenetic applications. Mol Biol Evol 19(2): 138–148.

WARD RD, ZEMLAK TS, INNES BH, LAST PR, HEBERT PDN. 2005. DNA barcoding Australia's fish species. P Roy Soc B-Biol Sci 360: 1847–1857.

XIA X, XIE Z, SALEMI, M, CHEN L, WANG Y. 2003. An index of substitution saturation and its application. Mol Phylogenet Evol 26: 1–7.

XIA X, LEMEY P. 2009. Assessing substitution saturation with DAMBE. In: The Phylogenetic Handbook: A Practical Approach to DNA and Protein Phylogeny Testing, 2nd ed. Cambridge, UK: Cambridge University Press. p. 615–630.

XIAO H, CHEN SY, LIU ZM, ZHANG RD, LI WX, ZAN RG. 2005. Molecular phylogeny of *Sinocyclocheilus* (Cypriniformes: Cyprinidae) inferred from mitochondrial DNA sequences. Mol Phylogenet Evol 36: 67–77.

YAGISHITA N, MIYA M, YAMANOUE Y, SHIRAI SM, NAKAYAMA K, SUZUKI, N, SATOH, TP, MABUCHI K, NISHIDA M, NAKABO T. 2009. Mitogenomic evaluation of the unique facial nerve pattern as a phylogenetic marker within the perciform fishes (Teleostei: Percomorpha). Mol Phylogenet Evol 53: 258–266.