ARTICLE

Species and endemicity status of the therapontid "Pigek", *Mesopristes cancellatus* (Cuvier, 1829) in the Philippines

Benedict A. Maralit[1], Minerva Fatimae H. Ventolero[1], Rosario Segundina P. Gaerlan[2], Macmod D. Mamalangkap[3], Mudjekeewis D. Santos[1,*]

[1] Genetic Fingerprinting Laboratory, Marine Fisheries Research Division, National Fisheries Research and Development Institute, Quezon City, Philippines
[2] Bureau of Fisheries and Aquatic Resources – Region I, Pagdalagan Norte, San Fernando, La Union
[3] Department of Agriculture and Fisheries – ARMM, ORG Compound, Cotabato City

Aside from "Ludong" or President's fish (probably *Cestraeus plicatilis*), "Pigek" is also known in the Philippines as a delectable, highly priced and rare freshwater fish. Early reports indicate that "Pigek" is of the species *Mesopristes cancellatus* and popularly believed to only be found in Rio Grande de Mindanao, Tamontaka River, and Pulangi River in Cotabato. However, there have also been reports that "Bulidao", a fish caught in Abra River, is of the same species as "Pigek" because of their resemblance. Here, we confirmed the identities of "Pigek" and "Bulidao" collected from Rio Grande de Mindanao and Abra River respectively, using morpho-meristics and genetics. Morphological comparison using principal component analysis and three genetic markers: cytochrome oxidase subunit 1 (COI), Control Region, and large subunit ribosomal DNA (16S), revealed that "Bulidao" and "Pigek" are indeed *M. cancellatus* species and are one and the same. Results also suggest that *M. cancellatus* is widely distributed throughout the Philippines contrary to what was previously thought. This bodes well for its management and conservation and calls for further studies to determine whether this fish species originate from or constitute a single stock.

*Corresponding author
Email Address: mudjiesantos@yahoo.com
Submitted: January 23, 2011
Revised: November 28, 2011
Accepted: December 1, 2011
Published: February 9, 2012
Editor-in-charge: Gisela P. Padilla-Concepcion
Reviewers:
Rachel R. Gotanco
Benjamin Vallejo Jr.
Josefina D. Pante

KEYWORDS

Mesopristes cancellatus, control region, CO1, 16S, Pigek, Bulidao

INTRODUCTION

Mesopristes cancellatus is a freshwater fish known as tapiroid grunter under class Actinopterygii, order Perciformes, and family Terapontidae. Its frequent synonym is *Terapon cancellatus* (Vari 2001). The fish is generally distributed from Sumatra eastward through the rest of Indonesia, New Guinea, and Vanuatu to the Solomon Islands; in the north to the Philippines and Taiwan Province of China (Vari 2001). In the Philippines, *M. cancellatus* is locally called as "Pigek." Next to "Ludong" or President's fish (popularly known as *Cestraeus*

plicatilis), the "Pigek" is famous for its delectable taste, towering price, alarming conservation status and confusing taxonomic identity.

The current status of the fish is quite alarming as local fishermen and Regional Bureau of Fisheries and Aquatic Resources (BFAR) data revealed that the average catch over the years has greatly decreased. Actually, "Pigek" is now in danger of becoming extinct because of uncontrolled and unregulated harvesting (Fresco 2002). Presently, efforts are underway to conserve and protect the remaining population because rearing techniques and culture of the fish are difficult to develop.

According to anecdotal reports "Pigek" samples which are found to be Tapiroid terapon of the lone species *M. cancellatus* believed to be found only in the waters of the Southern Philippines, specifically in Rio Grande de Mindanao, Tamontaka River, and Pulangi River in Cotabato (Macabalang 1984). Recently, there have been claims that "Bulidao" being caught downstream of Abra river, located in the Northern part of the Philippines, is the same species as "Pigek" because of their resemblance.

Species identification is almost always efficiently solved by the use of a standardized molecular approach such as DNA barcoding in conjunction with a comprehensive morphological analysis (Hebert et al. 2004; Ward et al. 2005; Hubert et al. 2008; Meyer and Paulay 2005; Barrett and Hebert 2005; Hajibabaei et al. 2006). Actually, barcoding particularly oversees the problem caused by intraspecific phenotypic variation which overlaps that of sister taxa in nature leading to incorrect identifications if based on phenotype only (Pfenninger et al. 2006). Since the inception of DNA barcoding, cryptic variation and often spectacular levels of taxonomic diversity have been detected (Hebert et al. 2004). The cytochrome c oxidase subunit 1 mitochondrial region (COI) is the most popular DNA barcode

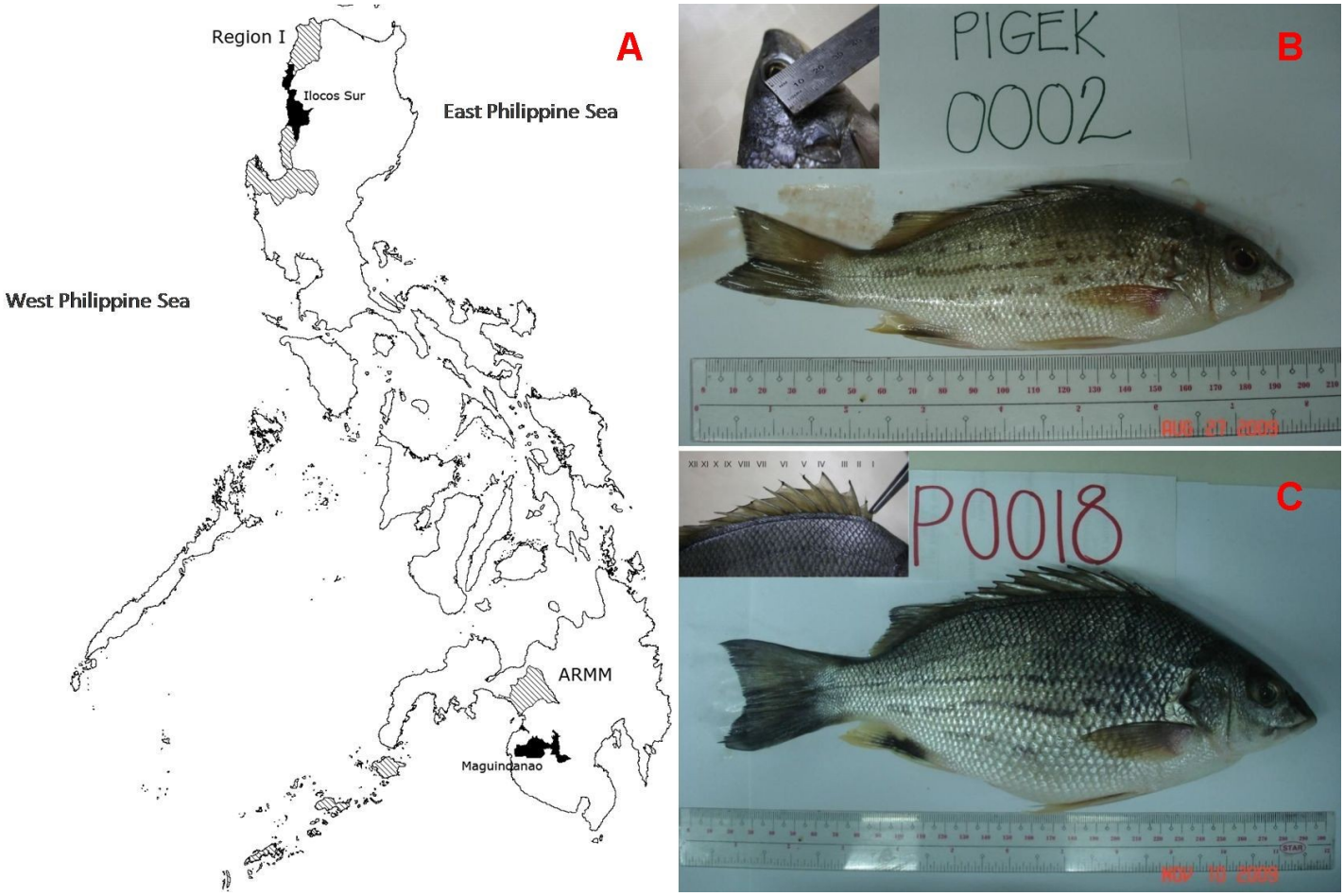

Figure 1. A. Sampling sites on the Philippine Map. One located in Brgy. Tamurong, Puro, Caoayan, Ilocos Sur (Region 1) and another in Rio Grande de Mindanao in Sultan Kudarat, Maguindanao (ARMM). **B.** "Bulidao" captured from the mouth of Abra River. (Inset: Determination of eye diameter for morphometric data). **C.** "Pigek" caught from the river bank of Rio Grande de Mindanao. (Inset: Determination of dorsal fin soft rays for meristic data)

for animals because COI possesses a great range of phylogenetic signal and evolution to allow the discrimination of not only closely allied species, but also phylogeographic groups within a single species (Hebert et al. 2003; Cox & Hebert 2001; Wares & Cunningham 2001). Numerous studies have established the usefulness of COI barcoding in several large groups of animals, such as birds (Hebert et al. 2004), fish (Ward et al. 2005; Hubert et al. 2008), cowries (Meyer and Paulay 2005), spiders (Barrett and Hebert 2005), and Lepidoptera (Hajibabaei et al. 2006). In cases where the COI marker is not sufficiently informative or suitable for a certain taxon (Moritz and Cicero 2004; Will et al. 2005), identified conserved sequences of putative functional importance in the control region of the mitochondrial DNA have provided valuable information on phylogeny, phylogeography and population genetics (Brown et al.1986; Saccone et al.1987; Sbisa` et al.1997; Matson and Baker 2001; Larizza et al. 2002; Reyes et al. 2003; Ketmaier and Bernardini 2005; Iyengar et al. 2006). Other phylogenetic work has also focused on mitochondrial genes encoding ribosomal (12S, 16S) DNA, although broad taxonomic analyses is constrained by the prevalence of insertions and deletions (indels) that greatly complicate sequence alignments (Doyle & Gaut 2000). In the past two decades, the mitochondrial 16S rRNA gene has not only been widely used to explore the phylogenetic relationships of lizards at varying taxonomic levels (Guo et al. 2011) but also in fish (Pondella et al. 2003), sea cucumbers (Byrne et al. 2010) and other organisms.

In this study, we confirmed, using morphological, meristic and genetic analyses, that a) "Pigek" found in Rio Grande de Mindanao and "Bulidao" found in Abra River are *M. cancellatus*.

MATERIALS AND METHODS

Sample collections

A total of 16 samples were gathered from the sampling sites (Figure 1a). Eight "Bulidao" (Figure 1b) were collected from the mouth of Abra River at Brgy. Tamurong, Puro, Caoayan, Ilocos Sur while eight "Pigek" (Figure 1c) were obtained from the river bank of Rio Grande de Mindanao in Sultan Kudarat, Maguindanao. Whole fish samples where chilled on ice until reaching the laboratory for sorting, initial identification, morphological examination, tissue sampling and archival storage. Tissue extraction involved cutting a small piece of muscle tissue from the left dorsal anterior side of the fish.

Morphometric, Meristic and Principal Component Analyses

Documentation involved taking photos of whole fish samples beside a metric ruler. Morphometric characteristics like standard length (SL), head length (HL), length of the dorsal fin with spines (DSL), length of the dorsal fin with soft rays (SRL), eye diameter (ED), length of the anal fin (AFL), pelvic fin (PvFL), pectoral fin (PcFL), caudal fin (CFL) etc. were measured using a 12 inch ruler. Meristics, on the other hand, was determined by counting the spines (NDFS) and soft rays (NDFSR) on the dorsal fin, rays on the anal fin (NAFSR), pelvic fin (NPvFSR), and pectoral fin (NPcFSR), scales along (NLS), above (NRSLL) and below the lateral line, number of cheek scales (NCS) etc. These morphometric and meristic characters (Table 2) were recorded and log transformed before imported to PC-ORD software for Principal Component Analysis. The correlation option was selected in generating the cross products

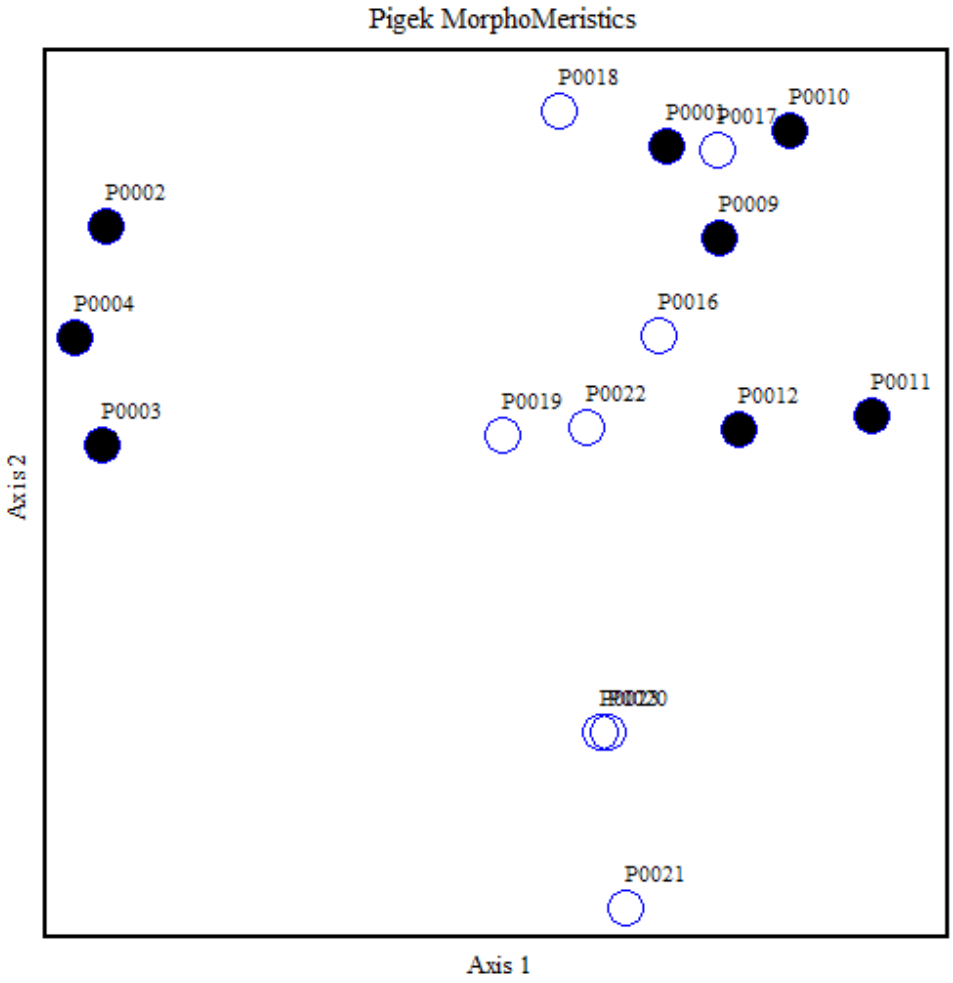

Figure 2. Scatter plot of 16 "Pigek" samples using 19 morphometric and meristic data for Principal Component Analysis. Abra samples in darkened circles while Rio Grande samples in light circles. No significant loadings on Axis 1 while Axis 2 was primarily based on the "caudal fin length and number of cheek scales"

matrix until the ordination graph was produced. Variables without variance were discarded from the analysis.

Genetic Analysis

DNA was extracted according to Santos et al. (2010) with minor modifications. Ethanol preserved tissues were rinsed with de-ionized H_2O. After which approximately 150 mg were sliced off with an uncontaminated disposable razor. The tissues were then minced and placed in a properly marked 1.5 mL Eppendorf tubes containing Cetyl Trimethyl Ammonium Bromide (CTAB) Extraction buffer (600 uL 2% CTAB pH 8.5, 30 uL of 1% Proteinase K) and incubated overnight in a water bath at 55 °C with occasional shaking. After incubation, 600 uL of chloroform: isoamyl (3:1) solution was added to each of the sample, shaken by hand for about 3 min. and then centrifuged for 5 min. at 8,000 rpm. The upper aqueous supernatant was then transferred in newly marked 1.5 mL tubes, avoiding inclusion of the organic phase. The described steps of clean-up after incubation were done twice. DNA precipitation was then carried out by mixing 50 uL of 3M Sodium Acetate (NaOAc) and 900 uL 95 % ethanol to the tubes containing the supernate. These were then hand shaken for 3 min. and placed overnight in a –20 °C freezer. After precipitation, the tubes are spun in a microcentrifuge at 13,000 rpm for 30 min and then the aqueous phase was carefully pipetted out leaving the DNA pellet at the bottom of the tube. The pellet was then rinsed by adding 500 uL 70% ethanol and spun for 13,000 rpm for 3 min. before removal of ethanol. The previously described rinsing step was done twice. The DNA pellet was air dried for 10 minutes and rehydrated in 300 uL of 1X TE buffer (1 mM EDTA, 10 mM Tris-HCl, pH 8.0). Resulting stock DNA extracts were stored in cryo vials at -20 °C.

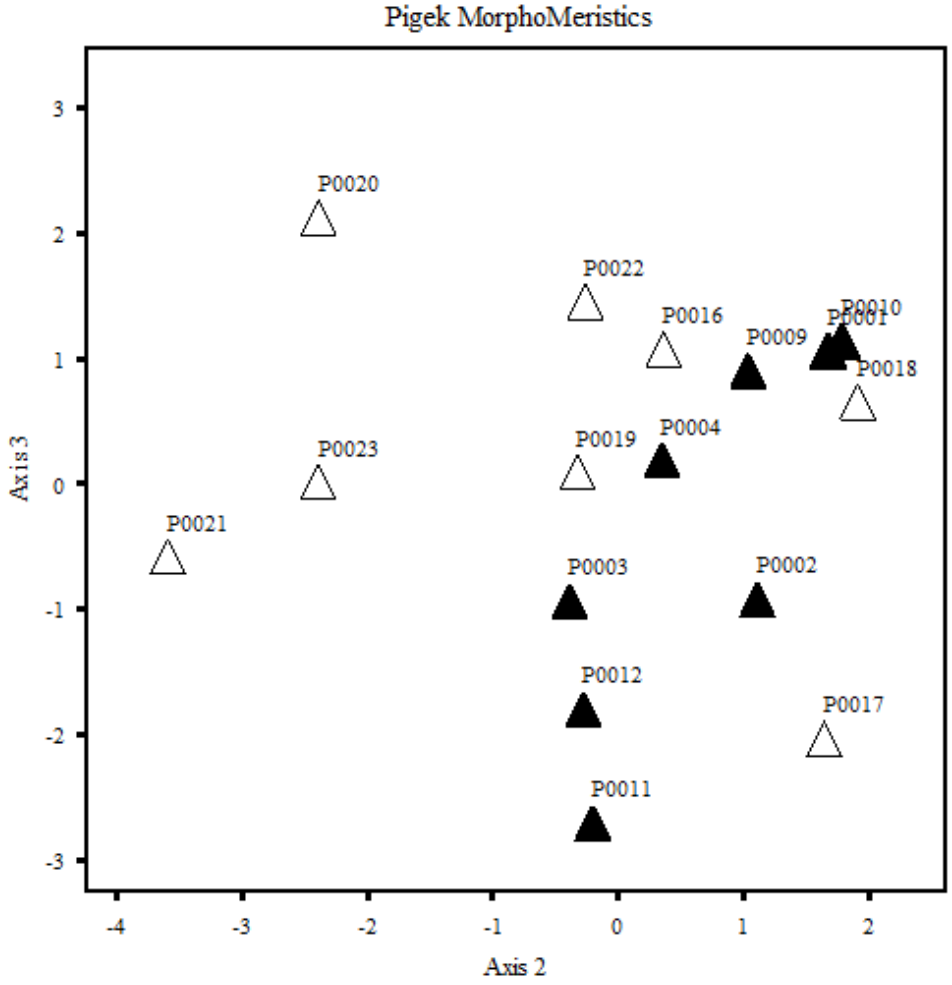

Figure 3. Scatter plot of 16 "Pigek" samples using 19 morphometric and meristic data for Principal Component Analysis. Abra samples in darkened triangles while Rio Grande samples in light triangles. Axis 2 was primarily based on the "caudal fin length and number of cheek scales" while axis 3 was based on variations of "the number of soft rays on the dorsal fin and anal fin."

Three primers pairs (Table 1) were used for amplification of the genetic markers. A 25 uL reaction mixture was prepared containing water, 1x PCR Buffer, 0.2 mM dNTP's, 0.5 uM primers (for control region and 16S) or 0.8 uM primers (for CO1), 2 mM MgCl, 1 unit Taq polymerase and 1 ul of DNA template. They were run on a thermal cycler with the following PCR cycling parameters: Initial denaturation at 94 °C for 10 min; 36 cycles of 94 °C for 30 s, 50 °C for 45 s, 72 °C for 45 s; and a final extension of 72 °C for 10 min (for 16S and Control Region amplification) or 94 °C initial denaturation for 1 min followed by 5 cycles of 94 °C for 1 min, 45 °C for 1 min and 30 secs, 72 °C for 1 min and 30 secs; another 35 cycles of 94 °C 1 min, 50 °C for 1 min and 30 secs, 72 °C for 1 min; and a final extension of 72 °C for 5 minutes (for CO1 amplification). After the reaction, amplicons were electrophoresed through a 1 % agarose gel stained with Ethidium Bromide and submerged in TAE buffer. Bi-directional capillary sequencing using Big Dye Terminator method followed.

DNA sequences were edited and aligned using alignment explorer packaged in MEGA version 4.0 (Tamura et al. 2007). A comprehensive phylogenetic analysis of the genus *Mesopristes* is beyond the scope of this study. We do, however, present the phylogenetic positions of our *M. cancellatus* samples relative to each other.

In conjunction with the morpho-meristic analysis, a cladogram for each marker was inferred. *Rhynchopelates oxyrhynchus* marker sequences from GenBank were used as outgroup sequences for the genetic analyses. The bootstrap consensus tree inferred from 500 replicates and 64238 seeds was taken to represent the evolutionary history of the taxa analyzed and branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) is shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura-Nei method (Tamura and Nei 1993). All phylogenetic analyses were done in MEGA version 4.0 (Tamura et al. 2007).

RESULTS AND DISCUSSION

Gross Morphology and Meristics

Eight "Pigek" and "Bulidao" samples each were analyzed. In accordance with the descriptions made by Vari (2001), they closely resembled characteristics in published descriptions of *Mesopristes cancellatus*. The observed parameters in all of the samples fall well within the range of the different diagnostic characters of *M. cancellatus*. The dorsal part of the body was mainly gray to grayish brown and silvery white on the ventral side when fresh. Likewise, 5 dark oblique vertical bars were seen dorsally above the uppermost stripe of the 3 horizontal stripes which were found longitudinally. These coloration or markings, however, were mostly prominent on small individuals and less prominent fading into the background on larger individuals. Length and weight of all the samples ranged from 192mm to 310mm and 80g to 515g respectively. Head length (HL) ranged from 46mm to 80mm while eye diameter (ED) was from 12mm to 20mm. Likewise, all of the fish samples had 12 spines (NDFS) and either 10 or 11 soft rays (NDFSR) on the dorsal fin. Three prominent spines and 8 or 9 soft rays (AFSR) could also be observed on the anal fin. Five soft rays were found on the pelvic fin (NPvFSR) while 12 or 13 on the pectoral fin (NPcSR). Number of scales on the lateral line (NLS) ranged from 49 to 58, cheek scales (NCS) from 4 to 7 and number of rows of scales above the lateral line is either 8 or 9. The fish also had a very distinguishable basal blotch on the anal fin. It could also be noted that the post temporal bone is expanded, serrate and not covered with skin and scales. The opercular spine does not extend beyond the margin of the opercle lobe with a similar serrate preopercle. Average measurements for "Pigek" and "Bulidao" samples were determined and listed in Table 2.

Morphological Identity

For Principal Component Analysis, "Pigek" and "Bulidao" samples were analyzed using 19 characters (Table 2). All values obtained from metrics and meristics were log transformed to minimize the effect of extremely high and low values; and more or less equalize the distance units between measurements and frequencies used in the dataset. It is for the same reason for the use of correlation option in generating the cross products matrix.

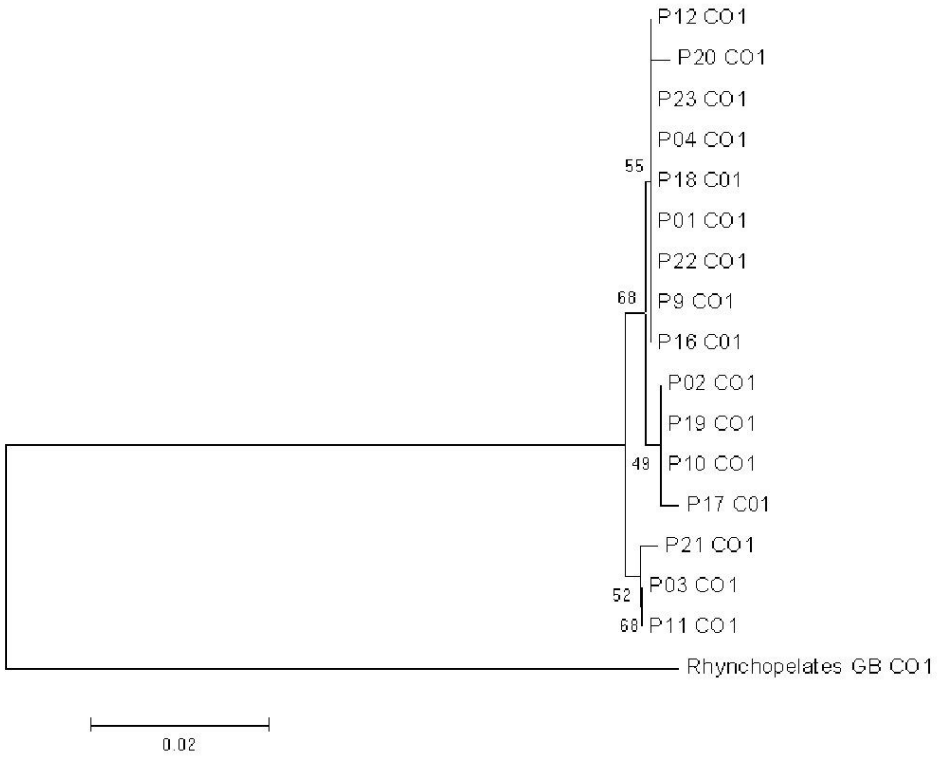

Figure 4. Figure 3. Evolutionary relationships of 17 taxa inferred using the Neighbor-Joining method (Saitou and Nei 1987). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura-Nei method (Tamura and Nei 1993) and are in the units of the number of base substitutions per site. Gaps and missing data in 467 positions were eliminated from the dataset (Complete deletion option). Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007).

No other rotation was used.

The first 5 components displayed eigenvalues greater than 1. However, only the first three components, which accounted for approximately 71.87% of the total meaningful variance (Supplementary Table A), were retained for the analysis. For interpretation, an item was said to load on a given component if the absolute value of its factor loading was .40 or greater for that component, and was less than .40 for others. Using these criteria, no items were found to load on the first component, thus, indicating the homogeneity of the analyzed characteristics among the samples on this axis. No specific character defined the relationship of any of the samples on axis or component 1 (Supplementary Table B). This is a preliminary indication that no specific morpho-meristic pattern in axis I may be observed in the samples in relation to the sampling sites. Meanwhile, two items loaded on the second component, thus, any correlation on this axis would primarily be based on the "caudal fin length and number of cheek scales." On the third component, however, any correlation, based on 9.29% meaningful variance, between the characters on this axis would be mainly on variations of "the number of soft rays on the dorsal fin and anal fin" (Supplementary Table B). When the resulting ordination was graphed on scatter plots using components one vs two and two vs three, no clustering related to the geographic origin of the samples could be observed (Figure 2 and 3). In the context of species differentiation, it can be inferred that the samples do not differ exclusively from one another based on the principal component analysis of the examined morphometric and meristic characters.

Genetic Identities

Using BLASTn, DNA sequences of the samples using the three markers are approximately 87% identical with the blotched terapon, *Rhynchopelates oxyrhynchus* (accession: AP011064.1). The value of the identity does not, however, suggest that the samples are indeed *Rhynchopelates*. Instead, this indicates that the among the terapontid sequences in GenBank, the nearest genetic sister group of the samples is Rynchopelates. Examination of the samples and comparison with published descriptions reveal that they are different from *Rhynchopelates* and are most likely *Mesopristes*. The average genetic distance over all sequence pairs using Kimura 2-parameter model is 0.020 for CO1, .033 for the control region and 0.004 for 16S, indicating homogeneity in the sequences. This is further supported when pairwise sequence alignment is done and high identity scores of 96 to 99% within datasets are observed. Based on these observations of the DNA sequence characteristics presented by the markers, there is a high probability, therefore, that the samples are identical to each other and no difference can be seen in relation to geographic origin. The Genbank voucher sequence of *Rhynchopelates oxyrhynchus* was used as outgroup to determine the interrelatedness the samples and construct a neighbor joining tree for each of the markers. Primarily, cladogram construction to describe phylogenetic relationships in the study was based on mtDNA CO1 (Folmer et al. 1994) because there is a preliminary assumption that it possesses a great range of phylogenetic signal and evolution to allow the discrimination of not only closely allied species, but also

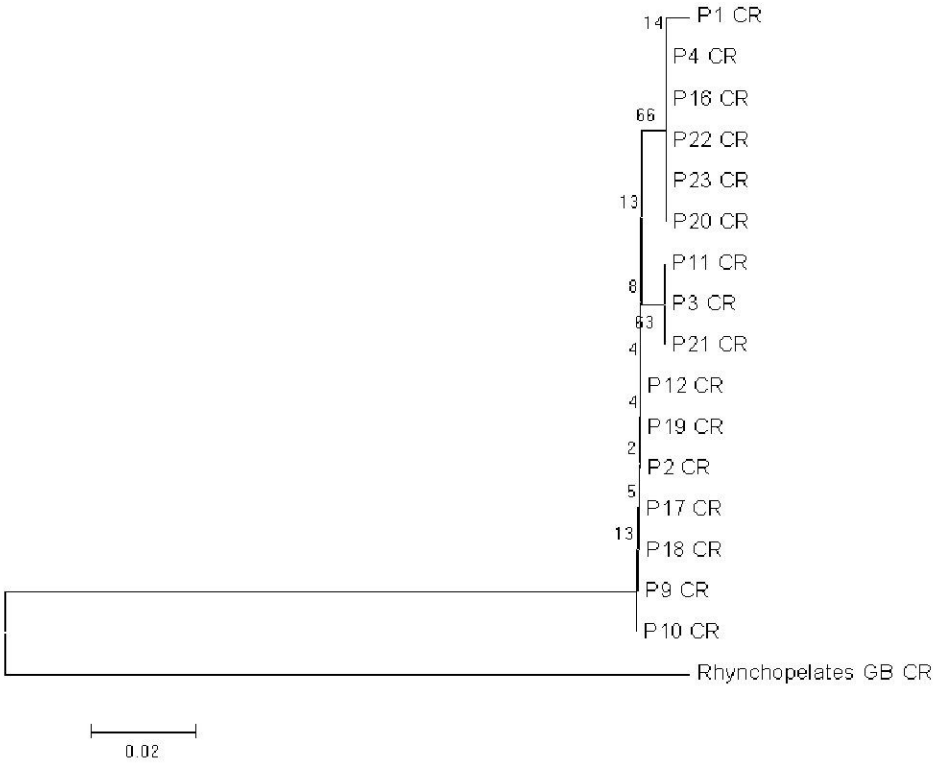

Figure 5. Evolutionary relationships of 17 taxa based on CR marker and inferred using the Neighbor-Joining method (Saitou and Nei 1987). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura-Nei method (Tamura and Nei 1993) and are in the units of the number of base substitutions per site. Gaps and missing data in 224 positions were eliminated from the dataset (Complete deletion option). Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007).

phylogeographic groups within a single species (Hebert et al. 2003; Cox & Hebert 2001; Wares & Cunningham 2001). Although, as shown, no geographic based grouping or a highly supported genetic pattern was observed (Figure 4). Proven to be highly conserved for this particular species with only 4 parsimony informative sites and 6 unique haplotypes, mtDNA CO1 strongly suggests conspecificity in all the samples.

On the other hand, it is widely accepted that mitochondrial DNA (mtDNA) control region evolves faster than protein encoding genes, (with few exceptions) and thus, are frequently utilized for population genetic and phylogenetic studies of fishes (Tang et al. 2006). It is in this context that the control region was also used in the analysis to confirm the previous results. In agreement, the analysis also produced an NJ Tree showing no distinct genetic pattern (Figure 5). This can be accounted to the very low number of parsimony informative characters (2) and having only 4 unique haplotypes in the dataset, as also found in the CO1 marker. Although a slower rate of substitution in CR was previously found in salmonid fishes (Bernatchez and Danzmann 1993; Shedlock et al. 1992), this may not be the case as the K2P genetic distance of the marker in this study is comparably higher than that of the CO1. This supports the previous result that the samples generally belong to one species for having a popularly believed variable CR marker (Lee et al. 1995) to be highly conserved among the samples.

Further phylogenetic analysis proceeded with 16S as marker because the mitochondrial gene for 16S ribosomal RNA was also proven to be as useful as the CO1 and CR markers in phylogenetic studies, providing information on the systematics of terrestrial and marine vertebrates (Allard et al. 1992; Milinkovitch et al. 1993). These genes have also been successfully used in reconstructing phylogenies for marine fishes, such as Centropomidae (snooks) and Epinephilnae (groupers) (Craig et al. 2001; Tringali et al. 1999). Actually, it is believed to be powerful for phylogenetic questions since its secondary structures of loop and stem regions allows both conservative and relatively variable regions of the mitochondrial genome to be analyzed with a potential to resolve both deep and shallow clades (Pondella II 2003). With agreement to previous results, however, the NJ tree showed no geographic based grouping or any particular genetic pattern (Figure 6). This 16S marker (Meyer et al. 1994) also proved to be conserved among the samples, with only one parsimony informative character and three unique haplotypes; thus, resulting again to low bootstrap support. With these three markers, the overall result leads to the conclusion that samples are very much genetically identical to each other. It may be noted that the outgroup, which is presumed to be the nearest sister group as it has the highest identity match among all available sequences in GenBank through BLAST, is seen as genetically distant from the group indicating that there may still be a missing *stem taxon* in its evolution. This is, however, beyond the scope of this study but nevertheless warrants a more comprehensive phylogenetic study of the genus and the family Terapontidae.

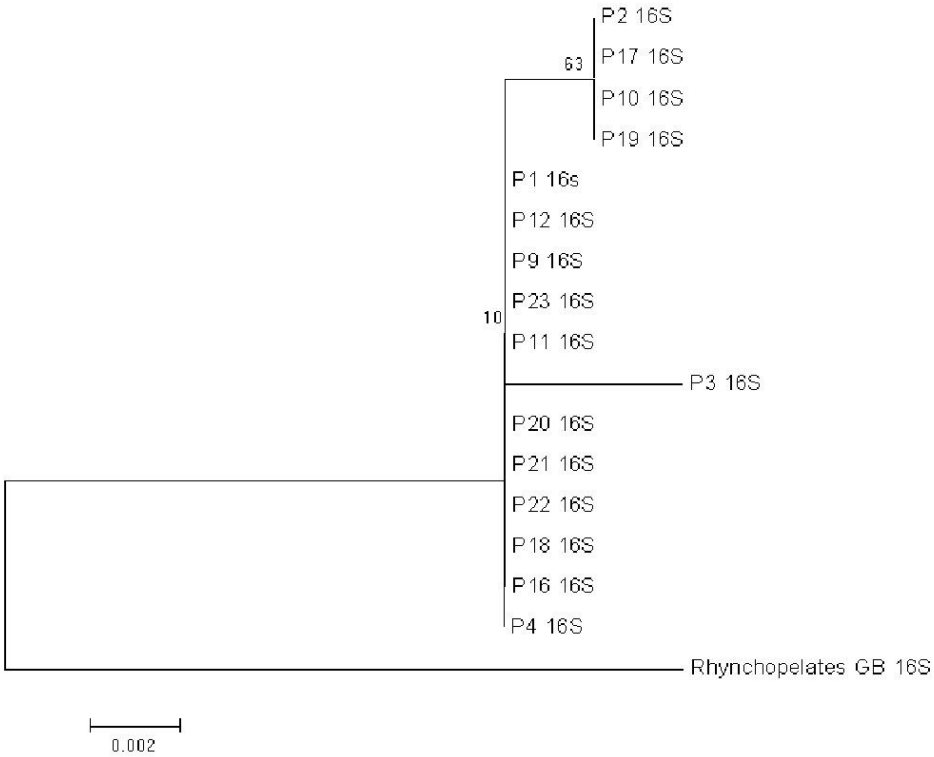

Figure 6. Evolutionary relationships of 17 taxa based on 16S marker and inferred using the Neighbor-Joining method (Saitou and Nei 1987). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed (Felsenstein 1985). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura-Nei method (Tamura and Nei 1993) and are in the units of the number of base substitutions per site. Gaps and missing data in 506 positions were eliminated from the dataset (Complete deletion option). Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007).

Name	Sequence (5' to 3')	Source	Target size
16Sar	CGC CTG TTT ATC AAA AAC AT	Meyer et al., 1994	~630 bp
16Sbr	CCG GTC TGA ACT CAG ATC ACG T	Meyer et al., 1994	
CRA	TTC CAC CTC TAA CTC CCA AAG CTA G	Lee et al., 1995	~450 bp
CRE	CCT GAA GTA GGA ACC AGA TG	Lee et al., 1995	
LCO1490	GGT CAA CAA ATC ATA AGA TAT TGG	Folmer et al., 1994	~710 bp
HCO2198	TAA ACT TCA GGT GAC CAA AAA ATC A	Folmer et al., 1994	

Table 1. (Left) Primers for PCR Amplification of markers used in phylogenetic analyses.

Table 2. (Bottom) Nineteen Recorded morphometric and meristic characters of 16 Pigek and Bulidao samples used in principal component analysis.

Location	Code	Morphometrics											Meristics					
		W	TL	FL	SL	HL	ED	DSL	SRL	PcFL	PvFL	AFL	CFL	NDFS	NDFSR	NAFSR	NPvFSR	NPcFSR	NLS	NCS	NRSLL
Cagayan River	R0001	365	287	275	220	71	15	85	40	55	55	40	70	12	10	8	5	12	58	5	8
	R0002	80	192	182	145	47	14	56	30	31	35	30	45	12	10	8	5	13	58	6	8
	R0003	90	189	178	141	46	13	57	30	31	36	30	50	12	10	9	5	13	55	5	8
	R0004	90	193	182	144	46	12	54	28	30	39	28	48	12	10	8	5	13	56	5	8
	R0009	270	310	290	228	70	15	90	35	55	55	42	70	12	10	8	5	12	54	6	9
	R0010	350	305	297	240	80	15	101	53	55	55	40	75	12	10	8	5	12	55	6	8
	R0011	340	310	300	250	79	20	90	47	55	60	47	55	12	11	9	5	12	54	7	9
	R0012	515	305	295	247	75	15	87	45	53	57	45	63	12	11	9	5	13	58	6	8
	Average	263	261	250	202	64	15	78	39	46	49	38	60	12	10	8	5	13	56	6	8
Rio Grande	R0016	360	286	272	225	75	15	80	45	52	55	45	68	12	10	8	5	13	56	5	8
	R0017	230	275	260	210	65	15	89	39	50	55	60	65	12	11	8	4	12	54	6	8
	R0018	185	285	250	195	70	15	80	33	50	50	35	65	12	10	8	5	12	54	6	8
	R0019	380	250	250	205	55	15	75	39	49	47	48	47	12	10	8	5	13	52	6	8
	R0020	355	275	271	235	78	13	76	39	51	51	48	53	12	10	8	5	13	51	4	9
	R0021	445	285	278	232	66	13	78	43	48	55	46	47	12	11	9	5	13	53	4	9
	R0022	325	269	252	205	66	14	75	44	47	52	45	55	12	10	8	5	12	49	5	8
	R0023	400	272	271	222	63	15	80	45	48	52	50	45	12	10	9	5	13	49	5	8
	Average	335	272	263	216	67	14	79	41	49	52	47	56	12	10	8	5	13	52	5	8

CONCLUSION

The study now suggests that "Pigek" is not only found in Mindanao but also in Abra River in Luzon. Since published reports from a lot of sources suggest that *M. cancellatus* is widely distributed in the Philippines and the Asia Pacific Region, there is high possibility that the fish species can be found in other freshwater bodies in the country as reported (Vari 2001). This bodes well for the management of this threatened species in the Southern Philippines as new sources and habitat are found in other parts of the country. It would then be interesting to note in the future whether the Abra and Rio Grande samples constitute / originated from a single stock and determine their true evolutionary history and phylogeography. Voucer sequences of "Pigek" (JN704357 and JN704358) and Bulidao (JN704355 and JN704356) are now available in BOLD.

ACKNOWLEDGEMENT

The authors wish to thank Ms. Ysabelle Laureen M. Mejia from Pamantasan ng Lungsod ng Maynila for initial sorting, gross examination and taking of pictures of the samples. Likewise, our deepest thanks to Demian Willette and Ms. Abigail Garcia for their important insights in the study.

CONFLICT OF INTEREST

None.

CONTRIBUTION OF INDIVIDUAL AUTHORS

Samples were collected by Rosario Segundina Gaerlan and Macmod Mamalangkap. Framing of the hypotheses and experimental design, laboratory work, data analysis, interpretation, & manuscript preparation were contributed by Minerva Ventolero, Benedict Maralit

and Mudjekeewis Santos.

REFERENCES

Allard MW, Miyamoto MM, Jareche L, Kraus F, Tennant, MR. DNA systematics and evolution of the artiodactyls family Bovidae. Proc. Natl. Acad. Sci. USA 1992; 89: 3972–3976.

Barrett RDH, Hebert PDN. Identifying spiders through DNA barcodes. Can. J. Zool. 2005; 83: 481–491.

Bernatchez L, Danzmann RG. Congruence in control-region sequence and restriction-site variation in mitochondrial DNA of brook charr (Salvelinus fontinalis Mitchill). Mol. Biol. Evol. 1993; 10: 1002–1014.

Brown GG, Gadaleta G, Pepe G, Saccone C, Sbisa E. Structural conservation and variation in the D-loop-containing region of vertebrate mitochondrial DNA. J. Mol.Biol. 1986; 192: 503–511.

Byrne M, Rowe F, Uthicke S. Molecular taxonomy, phylogeny and evolution in the family Stichopodidae (Aspidochirotida: Holothuroidea) based on COI and 16S mitochondrial DNA. Molecular Phylogenetics and Evolution. 2010; 56: 1068–1081.

Craig MT, Pondella II, DJ, Franck JPC, Hafner JC. On the status of the serranid fish genus Epinephelus: evidence for paraphyly based on 16s rDNA sequences. Mol. Phylogenet. Syst. 2001; 19: 121–130.

Cox AJ, Hebert PDN. Colonization, extinction and phylogeographic patterning in a freshwater crustacean. Mol. Ecol. 2001; 10: 371–386.

Doyle JJ, Gaut BS. Evolution of genes and taxa: a primer. Plant Mol. Biol. 2000; 42: 1–6.

Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 1985; 39: 783-791.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates, Mol. Mar. Biol. Biotechnol 1994; 3: 294–299.

Fresco MCO. Are you familiar with pigek? BAR Chronicle 2002; 3(12) Last accessed: Sept. 21, 2011 (http://www.bar.gov.ph/barchronicle/2002/jun02_16-30_pigek.asp).

Guo X, Dai X, Chen D, Papenfuss T, Ananjeva N, Melnikov D, Wang Y. Molecular Phylogenetics and Evolution. 2011; 61(2): 400-412.

Hajibabaei M, et al. DNA barcodes distinguish species of tropical Lepidoptera. Proc. Natl. Acad. Sci. U. S. A. 2006; 103: 968–971.

Hebert PDN, et al. Biological identifications through DNA barcodes. Proc. R. Soc. Lond. B. Biol. Sci. 2003; 270: 313–321.

Hebert PDN, et al. Identification of birds through DNA barcodes. PLoS Biol. 2004; 2: e312.

Hubert N, Hanner R, Holm E, Mandrak NE, Taylor E, et al. Identifying Canadian Freshwater Fishes through DNA Barcodes. PLoS ONE 2008; 3(6): e2490. doi:10.1371/journal.pone.0002490.

Iyengar A, Diniz FM, Gilbert T, Woodfine T, Knowles J, Maclean N. Structure and evolution of the mitochondrial control region in oryx. Mol. Phylogenet. Evol. 2006; 40: 305–314.

Ketmaier V, Bernardini C.Structure of the mitochondrial control region of the Eurasian otter (*Lutra lutra*; Carnivora, Mustelidae): patterns of genetic heterogeneity and implications for conservation of the species in Italy. J. Hered. 2005; 96: 318–328.

Larizza A, Pesole G, Reyes A, Sbisa E, Saccone C. Lineage specificity of the evolutionary dynamics of the mtDNA D-Loop region in rodents. J.Mol.Evol. 2002; 54: 145–155.

Lee WJ, Conroy J, Howell WH, Kocher TD. Structure and evolution of teleost mitochondrial control regions, J. Mol. Evol 1995; 41**:** 54–66.

Macabalang SD, Gani AA, Kamad IC. Biological investigation and survey of pigek. BFAR XII Res. J. 1984: 17.

Matson CW, Baker RJ.DNA sequence variation in the mitochondrial control region of red-backedvoles (Clethrionomys). Mol.Biol.Evol. 2001; 18: 1494–1501.

Milinkovitch MC, Orti G, Meyer A. Revised phylogeny of whates suggested by mitochondrial ribosomal DNA sequences. Nature 1993; 361: 346–348.

Meyer A, Morrisey JM, Schartl M. Recurrent origin of a sexually selected trait in Xiphophorus fishes inferred from a molecular phylogeny, Nature 1994; 368: 539–542.

Meyer CP, Paulay G. DNA barcoding: error rates based on comprehensive sampling. PLoS Biol. 2005; 3: e422.

Moritz C, Cicero C. DNA barcoding: promise and pitfalls. PLoS Biol. 2004; 2: e354.

Pondella II D, Craig M, Franck J. The phylogeny of Paralabrax (Perciformes: Serranidae) and allied taxa inferred from partial 16S and 12S mitochondrial ribosomal DNA sequences. Molecular Phylogenetics and Evolution. 2003; 29: 176–184.

Reyes A, Nevo E,Saccone C. DNA sequence variation in the mitochondrial control region of subterranean mole rats, *Spalax ehrenbergi* superspecies, in Israel. Mol. Biol.Evol. 2003; 20: 622–632.

Saccone C, Attimonelli M, Sbisa E. Structural elements highly preserved during the evolution of the D-loop-containing region in vertebrate mitochondrial DNA. J. Mol.Evol. 1987; 26: 205–211.

Santos M, Lopez G, and Barut N. A Pilot Study on the Genetic Variation of Eastern Little Tuna (Euthynnus affinis) in Southeast Asia. Philippine Journal of Science. 2010; 139 (1): 43-50.

Shedlock AM, Parker JD, Crispin DA, Pietsch TW, Burmer GC. Evolution of the salmonid mitochondrial control region. Mol. Phylogenet. Evol. 1992; 1: 179–192.

Sbisa` E,Tanzariello F, Reyes A, Pesole G, Saccone C. Mammalian mitochondrial D-loop region structural analysis: identification of new conserved sequences and their functional and evolutionary implications. Gene 1997; 205: 125–140.

Tang Q, Liu H, Mayden R, Xiong B. Comparison of evolutionary rates in the mitochondrial DNA cytochrome b gene and control region and their implications for phylogeny of the Cobitoidea (Teleostei: Cypriniformes). Molecular Phylogenetics and Evolution. 2006; 39: 347–357.

Tamura K, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution 2007; 24: 1596-1599.

Tamura K & Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution.1993; 10:512-526.

Tringali MD, Bert TM, Seyoum S, Bermingham E, Bartolacci D. Molecular phylogenetics and ecological diversification of the transisthmian fish genus Centropomus (Perciformes: Centropomidae). Mol. Phylogenet. Evol. 1999; 13: 193–207.

Vari RP. *Mesopristes cancellatus*. In: Carpenter, K.E.; Niem, V.H., ed(s). FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific. Volume 5. Bony fishes part 3 (Menidae to Pomacentridae). Rome: FAO, 2001: pp. 3310.

Ward RD, et al. DNA barcoding Australia's fish species. Phil. Trans. R. Soc. Lond. B Biol. Sci. 2005; 360: 1847–1857.

Wares JP, Cunningham CW. Phylogeography and historical ecology of the North Atlantic intertidal. Evolution 2001; 12: 2455–2469.

Will KW, Mishler BD, Wheeler QD. The perils of DNA barcoding and the need for integrative taxonomy. Syst. Biol. 2005; 54: 844– 851.

Supplementary Material

Table 3. Extracted Variances from Different Axes using PCA of morpho-meristic data set

VARIANCE EXTRACTED, FIRST 10 AXES

AXIS	Eigenvalue	% of Variance	Cum.% of Var.	Broken-stick Eigenvalue
1	9.415	49.553	49.553	3.548
2	2.477	13.035	62.588	2.548
3	1.764	9.285	71.873	2.048
4	1.387	7.297	79.171	1.714
5	1.025	5.394	84.565	1.464
6	1.000	5.263	89.828	1.264
7	0.887	4.668	94.496	1.098
8	0.432	2.273	96.769	0.955
9	0.190	0.999	97.767	0.830
10	0.146	0.767	98.535	0.719

Table 4. First Six Eigenvectors and the Corresponding factor loadings of each morphomeristic character

FIRST 6 EIGENVECTORS

	Eigenvector					
Characte	1	2	3	4	5	6
TL	0.3210	-0.0261	0.0701	0.0591	-0.0590	0.0000
FL	0.3194	-0.0689	0.0686	0.0631	-0.0272	0.0000
SL	0.3148	-0.1261	0.0618	0.0554	-0.0438	0.0000
HL	0.3041	-0.0004	0.1453	0.1116	-0.1251	0.0000
ED	0.2165	0.1679	-0.3109	0.2237	0.4053	-0.0001
NDFS	0.0000	0.0000	0.0000	0.0000	0.0002	1.0000
DSL	0.3164	0.0891	0.0245	-0.0107	-0.0018	0.0000
NDFSR	0.1418	-0.1421	-0.5762	-0.1047	-0.3167	0.0000
SRL	0.2736	-0.1282	0.0219	0.0487	0.1254	0.0000
AFL	0.2568	-0.1810	-0.0721	-0.3978	0.1004	0.0000
NAFSR	0.0272	-0.3736	-0.4516	0.2785	0.0325	0.0000
NPvFSR	-0.0558	-0.1715	0.2959	0.7059	0.1714	0.0000
PvFL	0.3184	-0.0447	0.0235	-0.0185	-0.0760	0.0000
NPcFSR	-0.1723	-0.3846	-0.0428	0.0922	-0.1595	0.0000
PcFL	0.3158	0.0130	0.1241	0.0117	0.0095	0.0000
CFL	0.1960	0.4075	0.1709	0.0516	-0.3195	0.0000
NLS	-0.0637	0.3441	-0.2244	0.3416	-0.5826	0.0001
NCS	0.0683	0.4238	-0.3761	0.1554	0.3883	-0.0001
NRSLL	0.1168	-0.3001	-0.0140	0.1639	-0.1838	0.0000