



Identifying the biodiversity of marine jacks (Carangidae) in the freshwater Taal Lake, Philippines using phenotypic features and mitochondrial DNA

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Summary

Jacks, members of the genus *Caranx* (Perciformes: Carangidae), comprise economically-important marine fisheries across the world. In the Philippines, this valued fishery extends from the marine environment into a freshwater volcanic lake, Taal Lake. Two jacks, *Caranx ignobilis* (Forsskål, 1775) and *C. sexfasciatus* (Quoy and Gaimard, 1825), have long been reported from the lake, with recent molecular evidence alluding to genetic divergence between marine and freshwater populations. Here, a combination of phenotypic features and the mitochondrial Cytochrome oxidase *b* gene region were used explicitly to profile the taxonomic relationship between marine and freshwater specimens of *C. ignobilis* and *C. sexfasciatus*. Using molecular data from all known Philippine *Caranx* species, a partial phylogeny of the genus was reconstructed and contrasted to observed colouration, morphological and meristic features. Findings include the first report of *C. papuensis*, in Taal Lake; however, previous reports of *C. sexfasciatus* are not validated. *Caranx ignobilis* was confirmed present, yet several morphological features were unique to lake specimens, including red instead of yellow colouration of the lower jaw, anal fins, and caudal peduncle, and four instead of two canine teeth. Further, *C. ignobilis* sequence distance was 6.3% between marine and lake specimens, whereas *C. papuensis* distance was 0.2%. The combined molecular and phenotypic data suggest that Taal Lake *C. ignobilis* may represent an evolutionarily unique lineage. These data are significant for developing management strategies that have typically overlooked the ecological and evolutionary attributes of the jack fishery.

Introduction

Conservation strategies developed for economically-important species have the dual task of maintaining robust genetic biodiversity of the species, while simultaneously ensuring that the species can continue to be exploited. Jacks, members of the genus *Caranx* (Perciformes: Carangidae), are a highly-valued reef-associated fishery found worldwide. Six jack species occur in the biologically diverse marine habitats of the Philippines, *C. lugubris*, *C. papuensis*, *C. tille*, *C. melampygus*, *C. ignobilis* and *C. sexfasciatus* (Smith-Vaniz, 1999); the latter two have also been reported in the freshwater Taal Lake, Batangas Province (Herre, 1958; Aquilino et al., 2011;

Papa and Mamaril, 2011). Jacks are migratory fish capable of adapting to a wide range of salinities from nearly freshwater to fully marine (Honebrink, 2000). This physiological plasticity enables juveniles to inhabit estuaries, and may allow them to penetrate inland rivers and lakes. Thus, it is believed that jack populations in Taal Lake are sustained by recruits migrating up the Pansipit River from the adjacent Balayan Bay (Fig. 1). However, recent evidence suggests genetic divergence between *C. sexfasciatus* sampled from Taal Lake and from the marine environment (Aquilino et al., 2011) and leaves room for further analysis.

Taal Lake is a caldera lake formed after the collapse of volcanic structures from major eruptions from a few hundred thousand to a few tens of thousands of years ago (Ramos, 2002). Eruptions have continued to shape the hydrology of Taal Lake, including a series of violent eruptions in 1754 that significantly constricted the Pansipit River (Wolfe and Self, 1983; Hargrove, 1991). The 18th century eruptions have been proposed as the origin of the present day configuration and freshwater condition of Taal Lake (Hargrove, 1991; International Lake Environment Committee (ILEC), 1999). Geological events such as these are a candidate mechanism for promoting allopatric speciation, the evolutionary process of lineage divergence due to selection and genetic drift as a result reproductive isolation between geographically separated populations (Futuyma, 2005). Alternatively, if geographically separated populations are isolated in environmentally-distinct habitats, such as marine and fresh water, selective pressures unique to each habitat could favour certain traits and differentiation may occur through ecological speciation (Schluter and Conte, 2009). Over time, marine species isolated in Taal Lake may have diverged from their marine conspecifics and since formed distinct species (Willette et al., 2014), exemplified by the endemic freshwater sardinella *Sardinella tawilis* (Herre, 1927) and endemic Taal sea-snake *Hydrophis semperi* (Garman, 1881).

Unlike the endemic freshwater *S. tawilis* and *H. semperi*, *Caranx ignobilis* (Forsskål, 1775) and *C. sexfasciatus* (Quoy and Gaimard, 1825) are found beyond Taal Lake in reefal habitats across the Philippines and throughout the Indo-Pacific Region (Smith-Vaniz, 1999). *Caranx ignobilis* and *C. sexfasciatus* juveniles occur in estuaries and are tolerant to a wide range of salinities (Honebrink, 2000); an adaptation that enables the juveniles to penetrate inland waters and

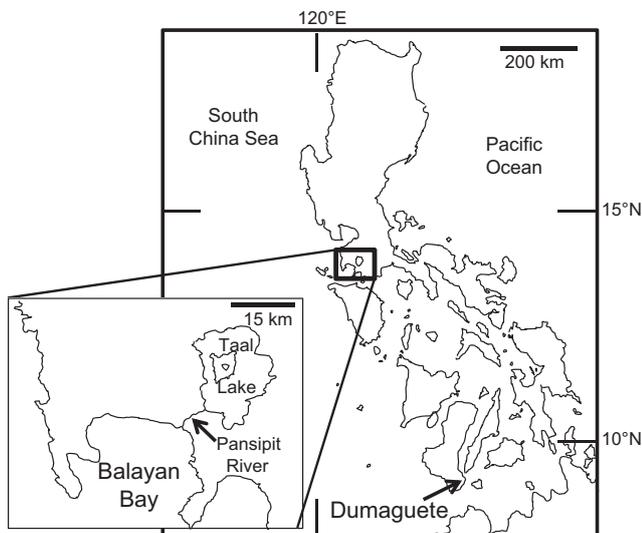


Fig. 1. Sampling site map. Sampling sites, Taal Lake and Dumaguete fish market, Negros Oriental, Philippines. Insert map = Pansipit River, connecting freshwater Taal Lake to adjacent marine Balayan Bay

opens additional habitats for colonization. This ability to establish marine and freshwater cohorts fits within the ecotype concept (Song et al., 1998), and thus the jack populations in Taal Lake are considered freshwater ecotypes of two marine species.

Both *C. ignobilis* and *C. sexfasciatus* were confirmed present in Taal Lake through DNA barcoding of the Cytochrome oxidase I (COI) gene region (Aquilino et al., 2011) and to date, the only molecular study on Taal Lake jacks. *Caranx ignobilis* samples formed a single, highly supported clade with *C. ignobilis* sequences available from the GenBank public database. *Caranx sexfasciatus* samples also formed a single clade with GenBank *C. sexfasciatus* sequences, but intraspecific difference within the clade was high, 3.05% (Aquilino et al., 2011). The high degree of differentiation was attributed to the geographic origin of the samples, the GenBank sequences originating from specimens from Australian waters. Comparison of Taal Lake specimens to marine *Caranx* specimens from Philippine waters was beyond the scope of the Aquilino et al. (2011) study.

Given the importance of the jack fishery in Taal Lake, the inclusion of marine specimens as well as a robust morphological and meristic assessment and a second mtDNA gene region such as the Cytochrome *b* (Cyt *b*) gene region, would provide clarity to the phylogenetic relationship between Taal Lake and marine jack ecotypes. The Cyt *b* gene region is among the most useful genes for testing phylogenetic relationships due to the variable rate of evolution among codon positions (Song et al., 1998; Farias et al., 2001). This molecular marker has shown great utility in inferring molecular phylogenies of marine and freshwater fish taxa, including Carangidae, Clupeidae, and Cichlidae (Farias et al., 2001; Jerome et al., 2003; Reed et al., 2003), and for species-level identification (Lin et al., 2005). Further, it can provide the diagnosable clustering of individuals in relation to their

parental pattern of ancestry, thus describing species per the Phylogenetic Species Concept (Eldredge and Cracraft, 1980).

Here, specimens of marine and freshwater *C. ignobilis* and *C. sexfasciatus* were compared to resolve the phylogenetic relationship between populations in the two distinct habitats. Specifically, we used the Cyt *b* gene region to reconstruct a phylogeny inclusive of Philippine marine and freshwater specimens of jacks, with particular focus on the putative highly differentiated *C. sexfasciatus* population in Taal Lake (Aquilino et al., 2011). Morphological and meristic features were included for additional comparison. Our results provide molecular genetic, morphological and meristic data that clarify the *Caranx* species composition of Taal Lake; evaluate the basis for distinct lake and marine ecotypes; and highlight factors that have substantial implications for the management and conservation of the Taal Lake jack fishery.

Materials and methods

Sampling, identification, and tissue preparation

Three specimens of freshwater *C. ignobilis* were obtained at a fish market in Talisay, Batangas, along the shoreline of Taal Lake (14°5'N, 121°1'E; Fig. 1). Marine specimens of *C. sexfasciatus*, *C. tille*, and *C. ignobilis* were collected at Dumaguete fish market, Negros Oriental (9°18'N, 123°18'E). Sampling environment, marine or freshwater, was confirmed with the vendor for all specimens. All samples were brought to Silliman University marine laboratory for morphological observation, meristic measurement, photo-documentation, and tissue sample collection. Fish identifications were based on the taxonomic keys (Myers, 1991; Smith-Vaniz, 1999). Tissue samples were taken from the right dorsal muscles of the fish specimens, preserved in 95% ethanol and stored frozen for subsequent molecular analysis. Taal Lake tissue samples of *Caranx* specimens used in Aquilino et al. (2011) were provided by Dr. Jonas Quilang of the Biology Department-University of the Philippines, and were included in this analysis.

DNA isolation, amplification and sequencing

DNA extraction was carried out following the Chelex[®] protocol (Walsh et al., 1991). Isolated DNA was stored frozen overnight prior to DNA amplification. The mitochondrial Cyt *b* gene region was amplified using the light strand primer (5' – AACTGCAGCCCCTCAGAATGATATTTGTCCTCA – 3') and heavy strand primer (5' – GTGACTTGAAAAACACCGTTG – 3') (Lecomte et al., 2004). PCR reactions were set up using 25 μ l reaction volume containing 14.5 μ l ddH₂O, 2.5 μ l PCR 10 \times buffer, 2.5 μ l dNTPs (8 μ M), 2 μ l MgCl₂ (25 μ M), 1.25 μ l of each primer (10 μ M), 0.25 μ l Taq polymerase (5u μ l⁻¹), and 1 μ l of extracted DNA template. Thermal cycle protocol included an initial denaturing step at 94°C for 7 min, followed by 25 cycles with a denaturing step at 95°C for 30 s, annealing at 45°C for 30 s, extension at 72°C for 45 s, and final extension at 72°C for 7 min. The successful PCR product was assessed by gel electrophoresis, followed by purification and sequencing at the University of California Berkeley DNA sequencing facility.

Sequence data analysis

Sequences were trimmed of oligonucleotide primers, assembled and edited in Geneious Pro 5.45 (Biomatters, Auckland), and all sequences submitted to the public domain database GenBank [Accession numbers pending]. Sequences were imported into MEGA 5 (Tamura et al., 2011) and aligned with available carangid sequence data from the Euteleost Tree of Life (EToL) project database (M. Sanciangco, unpublished data) and a *C melampygyus* sequence [Accession #AP004445] from GenBank (Benson et al., 2006), and explored for molecular evolutionary and phylogenetic patterns. Neighbor-joining (NJ), maximum likelihood (ML) and maximum parsimony (MP) methods were used in reconstructing the phylogenetic trees applying the Tamura–Nei substitution model (Tamura and Nei, 1993). The Tamura–Nei (TN93) with Gamma distribution (+G) model best fit the sequence data per the subroutine function in MEGA v5. Statistical confidence of the tree topology was based on 1000 non-parametric bootstrap replicates (Felsenstein, 1985). Between-group mean sequence distances were calculated in MEGA v5 (Tamura et al., 2011).

Results

Phenotypic characteristics of Philippine marine and freshwater jacks

Specimens of *C. ignobilis* from Negros Oriental (Fig. 2a) and Taal Lake (Fig. 2b) had similar diagnostic morphological characters, which fit to the species descriptions in Myers (1991) and Smith-Vaniz (1999). They had oblong and compressed bodies with a distinct steep head profile and naked breast. Upper head and body colourations ranged from grey to black. The colour of the dorsal fins and upper lobe of the caudal fins were grey to black, while pectoral fins, anal fins, and lower lobe of the caudal fins were yellow. Taal specimens, however, had distinct pale red colouration at the lower jaw and base of the anal fins and caudal peduncle. Meristics of the specimens also coincided with the measurements found in the references (Table 1) except for the number of canine or conical teeth. *Caranx ignobilis* specimens from Taal Lake had four canines at the lower jaw, while marine specimens only had two. The *C. sexfasciatus* specimens from Negros Oriental (Fig. 2c) had moderately steep and relatively elongated bodies compared to *C. ignobilis*. Colourations of the head and upper body ranged from silvery to grey with shades of dark green, while their lower bodies were silvery-olive to whitish. A small dark spot (smaller than the pupil diameter) above the operculum was visible from each specimen. The upper lobes of the second dorsal fins had white tips. Colour of the fins was mostly grey. The upper limbs of the first gill arch had six gillrakers and the lower limb had 15. Eight spines were observed at the first dorsal fins and one spine with 20 soft fin rays at the second dorsal fins. Anal fins had two detached spines followed by one spine and 16 soft fin rays.

Photographs of *C. sexfasciatus* from Taal Lake used in the Aquilino et al. (2011) were compared to specimens in this study. Photographed of *C. sexfasciatus* had more compressed body with white spot above the operculum lighter scutes,

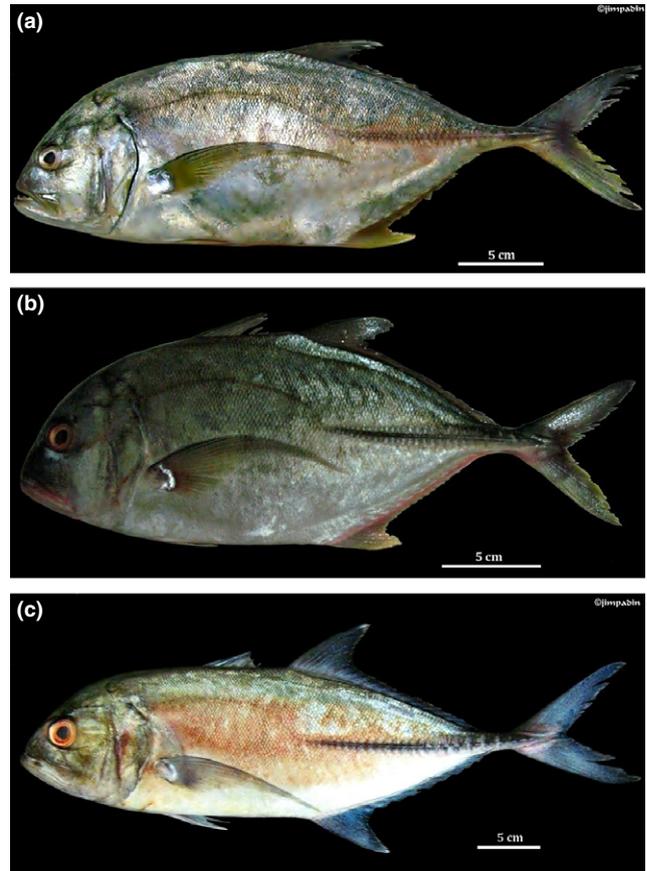


Fig. 2. *Caranx* specimens from marine and freshwater environments, *C. ignobilis* from a marine environment. (a) *C. ignobilis* collected from Dumaguete fish market, Negros Oriental, Philippines (Note dorsal fins and upper lobe of caudal fin grey to black, with yellow pectoral, anal, and lower lobe of the caudal fins); (b) *C. ignobilis* from Taal Lake, Batangas, Philippines (Note distinct pale red colouration at lower jaw and base of anal fin and caudal peduncle); and (c) *C. sexfasciatus* from Dumaguete fish market, Negros Oriental, Philippines

and yellowish anal fin and lower lobe of the caudal fin than observed in marine *C. sexfasciatus* samples. These features are diagnostic of *Caranx papuensis*, rather than *C. sexfasciatus*, which also occur in the Indo-Pacific region and whose juveniles can also be found in estuaries (Honebrink, 2000).

Molecular comparison of Philippine marine and freshwater jacks

A 424 bp fragment of *Cyt b* was obtained from nine *C. ignobilis*, five *C. sexfasciatus* and one *C. tille* specimens. To reconstruct a more complete *Caranx* phylogeny inclusive of all known *Caranx* species from the region, 11 Carangidae sequences from the EtOL Project (M Sanciangco, unpublished data) were included in the analysis and trimmed manually to 424 bp fragment size for consistency. Sequences of snub nose pompano *Trachinotus blochii* (Lacépède, 1801) were assigned as outgroup. A total of 27 *Cyt b* sequences were aligned in MUSCLE (Edgar, 2004). A 541 nucleotide site alignment was obtained, of which 111 were parsimony-informative and 143

Table 1
Caranx ignobilis measurements

Species	DSR	ASR	FL (cm)	SL (cm)	TL (cm)	DOB (cm)	GR	Scutes
<i>Caranx ignobilis</i> Marine/Dumaguete	VIII-I, 20	II-I, 17	35.5	34.5	40.2	11.5	6-15	31-32
<i>Caranx ignobilis</i> Taal Lake	VIII-I, 20	II-I, 15	28.3	27.3	32.5	10.8	6-15	29-30

Meristic measurements (in cm) from Dumaguete (Negros Oriental) and Taal Lake

DSR-Dorsal spines and rays, ASR-Anal spines and rays, FL-Fork length, SL-Standard Length, TL-Total length, DOB-Depth of body, GR-Gill rakers

were variable sites. The average nucleotide composition for all taxa was as follows: A = 25.6%, T/U = 28.8%, C = 30.5% and G = 15.1%. The estimated transition/transversion (ti : tv) bias ratio was 4.59, with higher rates of transitional substitution for C↔T (55.8%) than A↔G (25.0%).

Sequence distance between congeners ranged from 0.0 to 14.2% (Table 2). The lowest interspecific sequence distance (0.0%) was between *C. melampygyus* and *C. sexfasciatus*, followed by the next lowest (0.2%) between the Taal Lake *C. sexfasciatus* and marine *C. papuensis* specimens. Low sequence distance may suggest mis-identification of specimens. All other interspecific distances were at least 9.1%. Intraspecific sequence distances between marine and freshwater ecotypes were high, comparable to the difference observed between congeners. Taal Lake *C. sexfasciatus* and marine *C. sexfasciatus* differed by 13.2%, whereas the divergence between the marine and Taal Lake *C. ignobilis* populations was 6.3%. Interspecific sequence distance between *Caranx* species and the outgroup *T. blochii* ranged from 22.4 to 25.4% (Table 2).

Phylogenetic reconstructions of Cyt *b* gene data using neighbour-joining (NJ), maximum likelihood (ML), and maximum parsimony (MP) methods generated similar tree topologies (Fig. 3, only ML shown). The six Philippine *Caranx* species formed five clades supported by high bootstrap values. Clades for *C. ignobilis*, *C. tille*, and *C. lugubris* were each monophyletic (Fig. 3). Marine and freshwater *C. ignobi-*

lis sequences generated in this study formed a well-supported monophyletic clade, with the freshwater Taal specimens forming a unique sub-clade from the marine specimen. This partitioning of this sub-clade is consistent with the 6.3% sequence difference estimated between the Taal Lake and marine *C. ignobilis* specimens (Table 2). A clade containing the Taal Lake specimens identified previously as *C. sexfasciatus* by Aquilino et al. (2011) were grouped with marine *C. papuensis* sequences, supporting the suggested misidentification. Marine *C. sexfasciatus* sequences obtained from Dumaguete fish market and the ETOL project formed a separate and highly supported clade, and included the single *C. melampygyus* obtained from GenBank.

Discussion

The present study includes the first report of *C. papuensis* in Taal Lake, Philippines, and confirms the presence of *C. ignobilis* in the lake. Although *C. sexfasciatus* has been reported multiple times in Taal Lake (Herre, 1958; Aquilino et al., 2011), data here do not corroborate these reports. Re-evaluation of putative *C. sexfasciatus* specimens examined in Aquilino et al. (2011) indicates these were misidentified. Instead, diagnostic colouration patterns and original Cyt *b* phylogenetic reconstructions identify them as *C. papuensis*, a species also found in the marine habitats of the Philippines and wider Indo-Pacific region (Smith-Vaniz, 1999). Herein, the

Table 2
Estimates of evolutionary divergence over sequence pairs between groups show as per cent distance

	A	B	C	D	E	F	G	H	I	J	K
A <i>C. lugubris</i> (EToL)	█	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.04	0.02
B <i>C. papuensis</i> (EToL)	13.0	█	0.02	0.03	0.02	0.02	0.02	0.00	0.03	0.04	0.02
C <i>C. sexfasciatus</i> (EToL)	11.6	13.3	█	0.02	0.02	0.02	0.00	0.02	0.02	0.04	0.00
D <i>C. tille</i> (EToL)	13.0	14.4	12.2	█	0.02	0.02	0.02	0.03	0.00	0.04	0.02
E <i>C. ignobilis</i> (DGT)	12.6	12.2	12.8	10.3	█	0.01	0.02	0.02	0.02	0.03	0.02
F <i>C. ignobilis</i> (Taal Lake)	9.6	9.1	12.8	10.7	6.3	█	0.02	0.02	0.02	0.03	0.02
G <i>C. sexfasciatus</i> (DGT)	11.5	13.2	0.1	12.0	12.6	12.7	█	0.02	0.02	0.04	0.00
H <i>C. sexfasciatus</i> (Taal Lake)	12.9	0.2	13.2	14.2	12.3	9.2	13.0	█	0.03	0.03	0.02
I <i>C. tille</i> (DGT)	12.6	14.0	11.8	0.3	9.9	10.4	11.7	13.8	█	0.04	0.02
J <i>T. blochii</i> (EToL)	25.4	22.9	23.5	24.8	22.8	22.4	23.4	22.8	24.4	█	0.04
K <i>C. melampygyus</i> (GenBank)	11.5	13.2	0.1	12.0	12.6	12.7	0.0	13.0	11.7	23.4	█

Between-group mean distances below the diagonal

Standard Error estimates above the diagonal

In parentheses: origins of sequence data; DGT and Taal Lake indicate this study, EToL is from the EToL project and GenBank from the NCBI GenBank public database

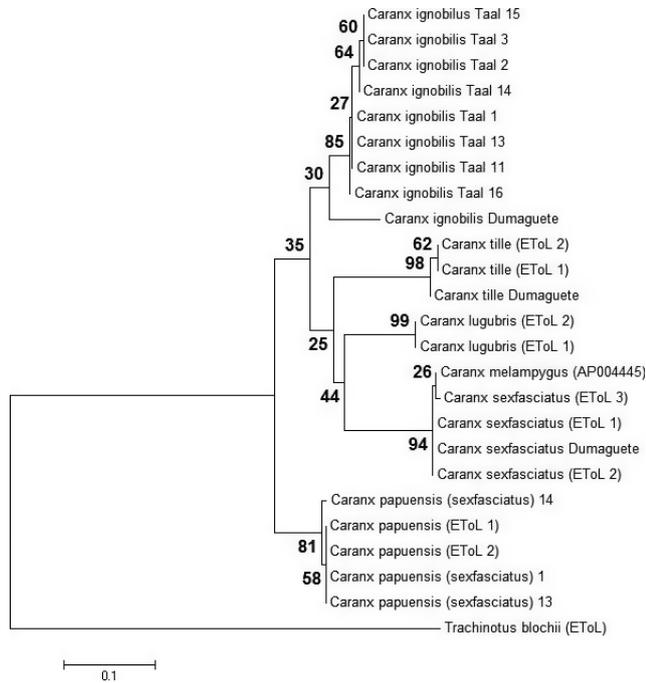


Fig. 3. *Caranx* Cyt *b* phylogeny. Maximum Likelihood tree illustrating reconstructed Cyt *b* phylogeny of Philippine *Caranx* species from Taal Lake and marine environment. Reconstruction based on 1000 bootstrap replicates (illustrated by node support number). In parentheses: source of sequence data not obtained in this study (either EToL project or GenBank). Note three *C. papuensis* labels followed by '(sexfasciatus)', signifying samples previously believed to be *C. sexfasciatus* in Aquilino et al., 2011

putative *C. sexfasciatus* specimens from Aquilino et al. (2011) and used in this study will be referred to accurately as *C. papuensis*. Thus, this study confirms the present of two *Caranx* species in Taal Lake, *C. papuensis* (this study) and *C. ignobilis* (Herre, 1958; Aquilino et al., 2011; Papa and Mamaril, 2011), but not *C. sexfasciatus* (Herre, 1958; Aquilino et al., 2011).

Phylogenetic reconstruction of the Cyt *b* gene region revealed two distinct patterns for the marine and Taal Lake *Caranx* species. *Caranx papuensis* sampled from the marine and freshwater lake environments were indistinguishable (Table 2, Fig. 3) and constitute two ecotypes of a single species living in two habitats (Turesson, 1922). This result is consistent with reports of juvenile and adult *C. papuensis*, as well as numerous other jack species, originating from marine environments and inhabiting fresh and brackish waters (Hubbs, 1961; Winemiller and Leslie, 1992; McBride, 1995; Smith-Vaniz, 1999; Boseto and Jenkins, 2006; Froese and Pauly, 2011). Although further investigation is recommended, the lack of any morphological or genetic variation between marine and freshwater *C. papuensis* may suggest a single population maintained by recruits to Taal Lake from the adjacent marine environment via the Pansipit River (Fig. 1).

In contrast, the Taal Lake *C. ignobilis* and marine *C. ignobilis* specimens exhibited discrete meristic, colouration, and genetic differences. Unique features of the Taal Lake *C. igno-*

bilis include four lower jaw canines instead of the diagnostic two of marine *C. ignobilis* (Table 1), pale red instead of yellow colouration along the lower jaw and at the base of anal fin and caudal peduncle (Fig. 2a, b), and a 6.3% sequence difference between the marine and freshwater populations (Table 2). The 6.3% Cyt *b* sequence distance between the two clades is much higher than any other intraspecific distance observed here, and is more typical of interspecific sequence distance observed in fish in general (Johns and Avise, 1998). The high Cyt *b* sequence distance described here is consistent with the high sequence distance in the COI gene region between marine and Taal Lake *C. ignobilis* specimens (Aquilino et al., 2011). Further, our phylogeny (Fig. 3) inclusive of all known *Caranx* species of the Philippines and broader Indo-West Pacific Region (Smith-Vaniz, 1999) rules out other potential species with which the Taal Lake *C. ignobilis* might be misidentified. Rather, the distinct clustering of Taal Lake *C. ignobilis* individuals is consistent with the species delimitation criteria of the Phylogenetic Species Concept (Eldredge and Cracraft, 1980). Further, the substantiating phenotypic differentiation of the Taal Lake *C. ignobilis* population from the marine *C. ignobilis* fit within several other accepted species concepts (Hausdorf, 2011). However, the taxonomic distinction between freshwater and marine populations of species can be highly contentious and is often left unresolved (Taylor, 1999). Thus, until additional data can be obtained, the current accepted Latin binomial *Caranx ignobilis* for both the marine and Taal Lake populations should be maintained. Still, evidence presented here does indicate that the Taal Lake *C. ignobilis* is genetically-distinct and morphologically-divergent from marine *C. ignobilis*, and may warrant recognition as an Evolutionarily Significant Unit, a population possessing a combination of natural history, morphological, and/or genetic traits concordantly distinct from other species populations (Ryder, 1986; Moritz, 1994).

Jacks are a highly important fishery across Southeast Asia, in the Philippines and in Taal Lake. A key first step in managing a valued fishery is to profile the stock composition and biodiversity accurately (Begg et al., 1999). This is also critical for species conservation objectives, including local strategies for unique habitats such as Taal Lake (Escudero, 2012). Data presented here identify two jack species in Taal Lake and indicate that they possess dissimilar patterns between their marine and freshwater populations. This dissimilarity may be the result of a distinct evolutionary lineage of the lake *C. ignobilis* population. Not only do these data advance understanding of the aquatic biodiversity of both Taal Lake and the Philippine Islands, but they also provides a scientific framework for developing conservation and management strategies for this economically-important, multi-species fishery.

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