SHORT COMMUNICATION

First report of the Non-native Midas Cichlid, *Amphilophus citrinellus* (Gunther, 1864), in Laguna de Bay, Philippines

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──── A B S T R A C T ────

In recent years, increasing global trade, travel, and transport had rapidly increased the rate of introduction and diversity of non-native fish species. Once established, some introduced fish species can become aggressive and dangerously invasive. Here, we provide the first report of the occurrence of a non-native Midas cichlid *Amphilophus citrinellus* in Laguna de Bay using morphological analysis and genetic marker, specifically the mitochondrial gene cytochrome c oxidase subunit 1 (CO1). The results provide important information on the presence of another invasive species in Laguna de Bay that needs to be addressed since this species can competitively exclude, predate, and displace native species.

Non-native species may cause changes in the ecosystems to which they are introduced (Jeschke et al. 2012). These changes can be manifold and potentially damaging to ecosystems and biodiversity. In some cases, these changes are dramatic and may result in the extinction of native species or radical changes in ecosystem functioning (Kulhanek et al. 2011; Simberloff and Gibbons 2014). In recent years, the rate of introduction of alien species in many ecosystems has increased because of increasing global trade, travel, and transport (Joshi 2006). Next to habitat destruction, the introduction of invasive species is the second major cause of loss of biodiversity (IUCN 1999).

Laguna de Bay is the Philippines' largest inland water with 900 km² surface area and the third largest in Southeast Asia (Israel 2007). The lake has numerous economic uses to the surrounding population, one of which is aquaculture. Studies have been conducted on the development and growth of aquaculture in Laguna de Bay. Delmendo and Gedney (1976) reported that the natural food supply of the lake could sustain the rearing of milkfish in the lake. Nicholas and Librero (1977) indicated that fish pen operations in the lake were extremely profitable while Garcia and Medina (1987) pointed out that fish cage culture like fish pen culture was also a highly profitable operation in the lake. The study was supported by Basiao (1989), who indicated that fish cage culture of tilapia in particular, even without supplemental feeding, can be conducted successfully in the lake. The analysis of Israel (2007) showed that fish pen and fish cage culture in Laguna de Bay has significant economic and social contributions not only to the country but also to the bay municipalities.

After more than 40 years, the aquaculture industry in the lake rapidly developed, mainly using species not native to the lake. The lake is now populated with non-native species, including species that are considered invasive and nuisance (Cuvin-Aralar 2014). These non-native species were intentionally introduced for aquaculture, while some were acciden-

tal introductions emanating from the ornamental fish industry. The Asiatic catfish (*Clarias batrachus*) has been recorded to have ecologically displaced the native catfish (*Clarias microcephalus*) in Laguna de Bay (Juliano et al. 1989) and other water bodies in Luzon where it has been introduced. The *Pterydoplicthys dysjunctivus*, locally known as janitor fish, is a nuisance fish that has caused economic losses to fisherfolk in Laguna de Bay by reducing their fish catch with gillnets and fish corals (Chavez et al. 2006). The "clown featherback" (*Chitala ornata*), a native species in Thailand, is believed to have escaped into the lake after a flooding event caused by Typhoon Ondoy in 2009 (Guerrero 2014). As early as 2011, the destructive and highly carnivorous knife fish had been reported to infest Laguna de Bay. The "black-chinned tilapia" *Sarotherodon melanotheron*, a native species in Africa, was reported to be competing for food and space of cultured stocks in the Laguna de Bay (Aquino et al. 2011) and in the brackish fishponds of Bulacan (Ordoñez et al. 2015; Chavez 2013; Cervantes 2013).

Midas cichlid, *Amphilophus citrinellus*, a freshwater and benthopelagic fish, has been reported to have a maximum length of 24.4 cm. According to Conkel (1993), the species coloration is mostly bright orange to orange-red in adults; mature males are larger, with longer fins, and with a distinct hump on their heads. They are mostly found in lakes, omnivorous, eating mostly snails and small fishes; also feeds on insect larvae, worms, and other bottom-dwelling organisms (Yamamoto and Tagawa 2000). This cichlid is a native of Central America, particularly in Nicaragua and Costa Rica. Introduced by the aquarium trade industry, the species escaped into the natural waters (Nico et al. 2007).

Identification of fish species through morphology-based methods has been proven to be extremely difficult even for trained taxonomists when the key characters for identification to the species level are lacking. Hence, DNA based methods, particularly DNA barcoding, have been used in identifying various cichlid species in the Philippines (Ordonez et al. 2015 and 2016). In this study, a non-native cichlid fish species collected in Laguna de Bay was identified using DNA barcoding.

The sample was collected from fishers in Kinagatan, Talim Island, Binangonan, Rizal in February 2019, who caught the fish by hook and line near the island shore by Bureau of Fisheries and Aquatic Resources – National Inland Fisheries Technology Center (BFAR-NIFTC). The whole fish sample was sent to the National Fisheries Research and Develop-

ment Institute – Genetic Fingerprinting Laboratory (NFRDI-GFL) on February 6, 2019, for DNA-based identification (see Fig. 1).

The whole sample was examined for morphological features. Morphometric measurements (in cm) were taken with a standard ruler. Muscle tissues of about 150 mg were obtained from the fish sample, then preserved in 95% ethanol and stored at -20 °C.

DNA extractions were carried out in triplicate coded as 19R1FC 1, 19R1FC 2, and 19R1FC 3. DNA was extracted using 10% Chelex based on the protocol of Walsh et al. (1991). The quality and quantity of the DNA extracts were measured using NanoPhotometer (IMPLEN – Guill – Bern Corporation). The DNA template concentrations ranged from 130-140 ng/ μL.

Figure 1. Sample (labelled 19R1FC) submitted by BFAR-NIFTC for morphological and molecular identification. Photo by John T. dela Peña.

A region of cytochrome c oxidase I (~600 bp) gene was amplified using the following primers: VF2_t1 (5'TGTAAAACGACGGCCAGTCAACCAAC-CACAAAGACATTGGCAC3'), FishF2_t1 (5'TGTA AAACGACGGCCAGTCGACTAATCATAAA-GATATCGGCAC3'), FishR2_t1 (5'CAGGAAACA GCTATGACACTTCAGGGTGACCGAAGAAT-CAGAA3'), and Fr1d_t1 (5'CAGGAAACAGCT AT-GACACCTCAGGGTGTCCGAARAAYCARAA3') (Ward et al. 2005; Ivanova et al. 2007). The 25 - μl PCR reactions consisted of water, 2.5 μL 10x PCR buffer with 1.5 mM MgCl^2, 2.5 μL MgCl^2 (25mM), 2.5 μL dNTP's (2mM), 2 μL of each primer (10μM), 0.2 unit Taq polymerase and 2 μl of template. The PCR cocktails were subjected to the following conditions: initial step of 94°C for 5 min, 35 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 1 min, and a final exten-

sion of 72°C for 5 min. Product amplicons were electrophoresed in 1% agarose gel stained with ethidium bromide and submerged in 1x TAE buffer. Standard sequencing and DNA purification were outsourced to Macrogen Inc., Korea.

Resulting bi-directional sequence electropherograms were visualized, aligned, and manually edited using Geneious Pro 6.1 software (Biomatters Ltd., Aukland, New Zealand). Voucher CO1 sequences were obtained using BLAST (blast.ncbi.nlm.nih.gov) and BOLD (www.boldsystems.org). MEGA 7.0 (Tamura et al. 2013) was used for aligning DNA sequences together with the reference sequences. Species identification was inferred using Neighbor-Joining (NJ) tree based on the Kimura 2-parameter (K2P) model with 500 bootstrap replications (Tamura et al. 2013). Mean genetic distance was computed between the sample and the reference sequence using K2P model (Kimura 1980).

Midas cichlid, *Amphilophus citrinellus*

The sample submitted weighted 200 g, a total length of 17.4 cm, a standard length of 13.5 cm, a body depth of 4.5 cm, had 26 dorsal fin rays, and 13 anal fin rays. *Amphilophus citrinellus* is a member of the cichlid species assemblage (*Amphilophus* spp.),

a group of closely related, morphologically similar species thought to comprise recent adaptive radiation (Barluenga and Meyer 2010). It resembles a cichlid species because it exhibits a wide variety of striking color morphs that range from oranges, yellows, whites, and combinations of these colors. The taxonomic determination of Midas cichlid was inferred from the description of FishBase (Froese and Pauly 2014) and Nico and Neilson (2013).

The sample sequences matched with GenBank sequences JN024800, JN024798, and HQ654655 of Amphilophus citrinellus using BLAST analysis. These sequences formed a distinct monophyletic clade with a bootstrap value of 100% in the NJ tree (Fig, 2). To further support the inferred species identity of the sample, the genetic distances were also computed and are shown in Table 1. Computation of mean genetic distance between the fish sample and voucher sequences occurring at 0.000-0.002. The threshold value for species delineation among the included species was set a 3.0%-3.5% (Ward et al. 2009). These values of nucleotide differences fall below the threshold value and are very low strengthen positive identification of the sample as Amphilophus citrinellus. The CO1 sequences of the sample were submitted to BOLD with the following accession number: 19R1FC_MN796349.
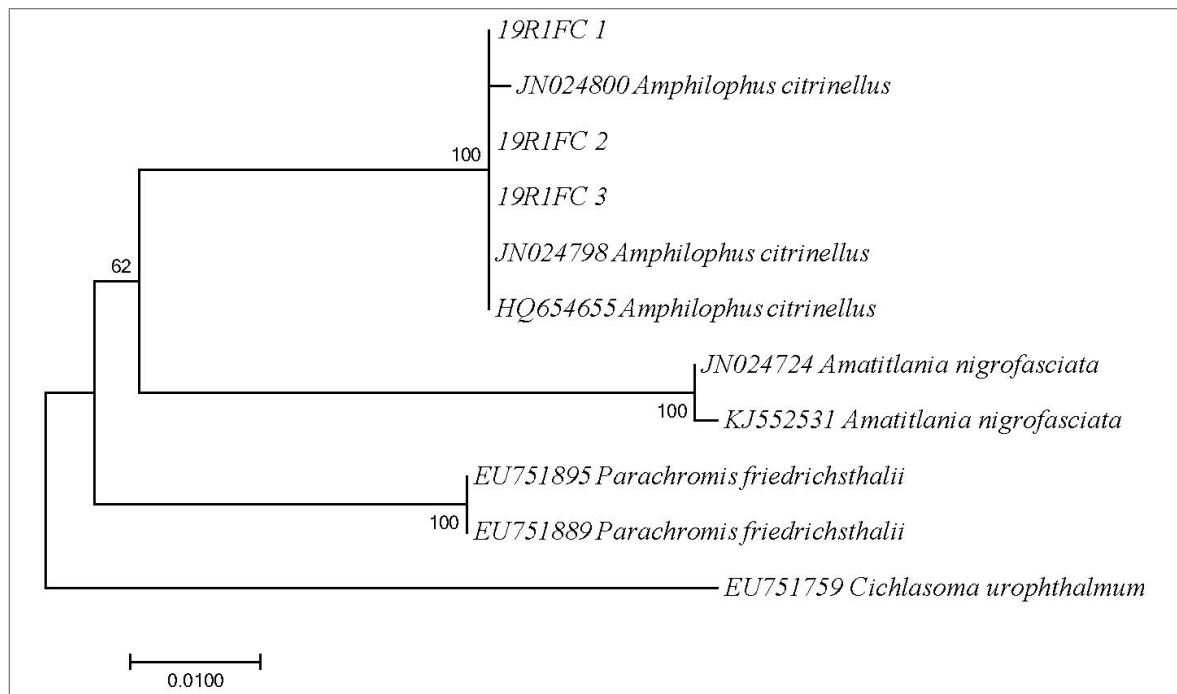

Figure 2. Neighbor-Joining tree generated using Kimura 2-parameter model of mtDNA COI sequences from the sample with the voucher sequences. GENBANK accession numbers are shown next to the species name. [Outgroup: Cichlasoma urophthalmum (Ordonez et al. 2015)]. The analysis involved 11 nucleotide sequences.

Table 1. Computed genetic distances between the sample submitted and voucher sequences from GenBank using Kimura 2-parameter model. The number of base substitutions per site from between sequences is shown. Standard error estimate(s) are shown above the diagonal and were obtained by a bootstrap procedure (500 replicates). The analysis involved 11 nucleotide sequences.

19R1FC_1		0.000	0.000	0.000	0.002	0.000	0.010	0.010	0.011	0.011	0.012
19R1FC_2	0.000		0.000	0.000	0.002	0.000	0.010	0.010	0.011	0.011	0.012
19R1FC_3	0.000	0.000		0.000	0.002	0.000	0.010	0.010	0.011	0.011	0.012
JN024798_*Amphilophus_citrinellus*	0.000	0.000	0.000		0.002	0.000	0.010	0.010	0.011	0.011	0.012
JN024800_*Amphilophus_citrinellus*	0.002	0.002	0.002	0.002		0.002	0.010	0.010	0.011	0.011	0.012
HQ654655_*Amphilophus_citrinellus*	0.000	0.000	0.000	0.000	0.002		0.010	0.010	0.011	0.011	0.012
EU751895_*Parachromis_friedrichsthalii*	0.058	0.058	0.058	0.058	0.060	0.058		0.000	0.012	0.012	0.012
EU751889_*Parachromis_friedrichsthalii*	0.058	0.058	0.058	0.058	0.060	0.058	0.000		0.012	0.012	0.012
JN024724_*Amatitlania_nigrofasciata*	0.069	0.069	0.069	0.069	0.071	0.069	0.077	0.077		0.002	0.013
KJ552531_*Amatitlania_nigrofasciata*	0.071	0.071	0.071	0.071	0.073	0.071	0.079	0.079	0.002		0.014
EU751759_*Cichlasoma_urophthalmum*	0.086	0.086	0.086	0.086	0.088	0.086	0.084	0.084	0.099	0.101	

Ordoñez et al. (2015) reported the presence of blackchin tilapia, *Sarotherodon melanotheron*, in the coastal waters of Manila Bay and the occurrence of Mayan cichlid, *Cichlosoma urophthalmus*, in Hagonoy, Bulacan for its range expansion and for the negative impacts of its existence due to the competitive interactions. The current infestation of Midas cichlid in Laguna de Bay is considered to be similar to the introduction of the Mayan cichlid and the blackchin tilapia. There is no evidence yet regarding their negative impact on both environmental and economic aspects.

This is the first record of Midas cichlid in Laguna de Bay. This species has been reported in Taal Lake in 2011 (Aquilino et al. 2011). When and how this species was introduced in the Philippines is unknown. We suspect that the species was introduced through ornamental fish trade, intentionally farmed in fishponds, and escaped during flooding events.

In this study, we identified *Amphilophus citrinellus* through DNA barcoding of a specimen collected from the island shore of Laguna de Bay. It is a native cichlid of Central America. This study provided vital information that could be used in the development of management actions for its control. Thorough monitoring should be carried out to determine the current distribution and the future spread of the species in the country.

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