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Research Article

First report on the occurrence of invasive black-chin tilapia *Sarotherodon melanotheron* (Ruppell, 1852) in Manila Bay and of Mayan cichlid *Cichlasoma urophthalmus* (Gunther, 1892) in the Philippines

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Abstract

We report the presence of blackchin tilapia, *Sarotherodon melanotheron*, in the coastal waters of Manila Bay and in the river tributary of Hagonoy, Bulacan, suggesting its possible range expansion. The occurrence of the native Central American Mayan cichlid, *Cichlosoma urophthalmus*, is also putatively confirmed for the first time in Hagonoy, Bulacan. While morphological features, morphometric characters, and meristic counts of the current populations match those reported elsewhere for these species, further genetic work is required to confirm their identification and possibly provide information on their source. Negative impacts of the existence of the two species due to competitive interactions have already been anecdotally reported, however, no empirical scientific evidence is available to provide verification. Therefore, studies on the potential ecological impacts should be done, especially because the two species were already reported as notoriously invasive.

Key words: fish invasive species, black-chin tilapia, Mayan cichlid, DNA barcoding, marine invasion

Introduction

In Southeast Asia, the number of non-native fish species introduced and established over the recent decades has increased considerably (De Silva 1989; Pallewatta et al. 2003; Welcomme and Vidthayanon 2003; Tan and Tan 2003). However, documentation of the geographic ranges and biology of non-native Southeast Asian fishes is poorly established in scientific literature and the current knowledge is largely limited to the grey literature or anecdotal observations (Nico et al. 2007; Peh 2010). Despite the growing number of peer-reviewed publications in recent years (e.g. Liang et al. 2005; Chavez et al. 2006; Dudgeon and Smith 2006; Page and Robins 2006; Nico et al. 2007; Kwik et al. 2013), many non-native, introduced species in Southeast Asia still have uncertain geographic distribution and inadequately confirmed identifications (Nico et al. 2007).

Casal et al. (2007) reported that since 1905, already 159 fish species have been introduced into the Philippines. Intoduced non-native fish species that have previously been identified to be invasive and have been known to cause adverse impact include Mozambique tilapia *Oreochromis mossambicus* (Peters, 1852), armored sailfin catfish *Pterygoplichthys* spp., and the recently recorded knife fish *Chitala ornate* (Grey, 1831). The armored sailfin catfish or more popularly knownlocally as "janitor fish" was first introduced in Laguna de Bay during the late 1980s to early 1990s and has been reported to have established in different provinces in the country (Chaves et al. 2006; Hubilla et al. 2007). As early as 2011, the aggressive and highly carnivorous knife fish had been reported to infest Laguna de Bay, Philippines (The Fish Site 2013). Both invasive fishes have caused severe problems due to the decline in local catch of important indigenous fish species from the lake (e.g. milkfish *Chanos* sp. and

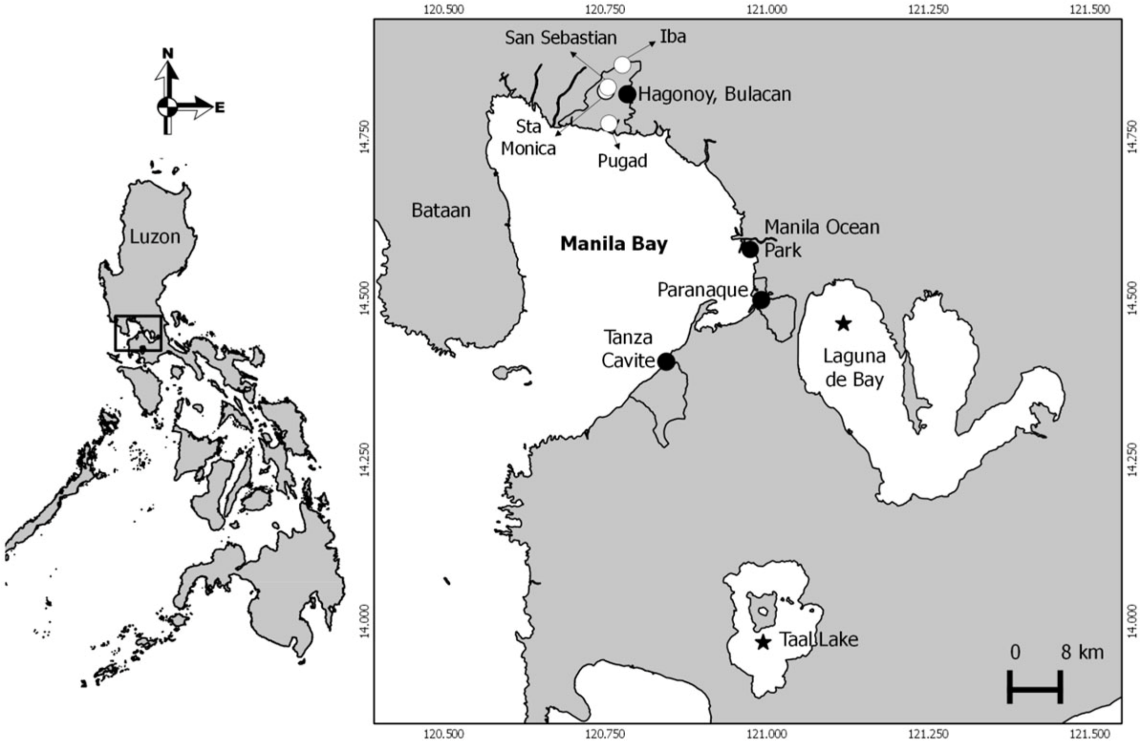

Figure 1. Map of Philippines and southern Luzon with locations where *S. melanotheron* marked in black filled circles and *C. urophthalmus* marked in white circles. *S. melanotheron* has been also reported to occur in Taal Lake, Batangas (Aquilino et al. 2011) and in Laguna de Bay (Aquino et al. 2011) marked by stars.

shrimp; Sotelo 2013) by preying upon or replacing the commonly fishes harvested here (Chavez et al. 2006; Official Gazette 2013). Most of these introduced alien species entered the country through the ornamental fish trade and aquaculture industry (Casal et al. 2007).

Other invasive fish species recently reported from the coastal waters of Manila Bay and fish ponds in Hagonoy, Bulacan include the anecdotally identified Blackchin tilapia (*Sarotherodon melanotheron*) and "flowerhorn" of unknown taxonomic identity. *S. melanotheron* has been reported to occur in the two open water lakes Taal Lake in Batangas (Aquilino et al. 2011) and Laguna de Bay (Aquino et al. 2011), while "flowerhorn" have not yet been reported from natural inland water bodies in the Phillipines. In this paper, we employed established, morphological approaches to investigate the taxonomic identity of the two anecdotally identified tilapia fish species that appeared to be introduced and could pose a serious threat to livelihoods and native ecosystems in Manila Bay and adjacent waters.

Materials and methods

S. melanotheron specimens from Manila Bay (MBAY) were collected from the landing sites in Tanza, Cavite (14°21′07.08″N; 120°50′18027″E) and Fisherman's Wharf in Paranaque City (14°30′ 03.91″N; 120°57′50.89″E) on October 31, 2013 while specimens from San Sebastian, Hagonoy, Bulacan (HAG; 14°49′59.96″N; 120°43′59.87″E) were collected on October 25, 2013 from the local public market (Figure 1). Each sample was examined for distinguishing morphological features for *S. melanotheron*, according to FishBase (Froese and Pauly 2012) and Trewavas et al. (1983). Seven meristic and twenty four morphometric characters were also measured (Adepo-Gourene et al. 1994; El-Zaeem et al. 2012) (Appendices 1 and 2). Morphometric measurements were taken three times with a standard ruler and the mean of the three was calculated and used in the analysis. All characters were measured in millimetres (mm). The characters were converted to ratios of the standard length (SL) or head length (HL). We selected some of these characters and compared with the measurement reported by Omoniyi and Agbon (2008). Also, we investigated if there were physical differences between specimens from marine (MBAY) and freshwater habitats (HAG). To confirm that *S. melanotheron* thrive in the waters of Manila Bay, we captured samples inthe vicinity (~55 meters) of the coast adjacent to Manila Ocean Park (MOP), Pasay City (approx. 14°34′46.3686″N, 120°58′21.183″E) using fishnets. No measurements were taken for these samples.

The "flowerhorn" specimens were caught in four different locations: in Hagonoy, Bulacan: Santa Monica (approx. 14°50′41.9244″ N; 120° 43′9.303″E) on August 25, 2013, and Iba (approx. 14°51′57.0816″N; 120°45′52.11″E), San Sebastian (approx. 14°49′50.502″N; 120°44′30.2202″E), and Pugad (approx. 14°46′27.0798″N; 120°44′38.223″E) on June 17, 2014 (Figure 1). The fish ponds in which the specimens were captured in these areas are connected to the river tributaries that provide local water supply. Meristic counts and morphometric characters were measured for all samples of "flowerhorn". Additional distinguishing characters were also examined in accordance with the methods of Nico et al. (2007) and these were then compared with data reported by Nico et al. (2007).

Interviews with local fisherfolk were also conducted to gather information including the approximate date when *S. melanotheron* and "flowerhorn" were first observed, their possible mode of introduction and observed effects on the fish catch.

Four samples of *S. melanotheron* (MOP01, MBAY07, HAG02, HAG06) and three "flowerhorn" fish (FH01, FH02, FH06) were randomly selected and subjected to DNA barcoding. Muscle tissue samples (approximately 1 gram) were taken from the dorsal portion of each fish, stored in 96% ethyl alcohol and kept frozen at -20°C until use. DNA extraction was performed using a modified cetyltrimethylammonium bromide (CTAB) extraction method (Doyle and Doyle 1987) as described by Santos et al. (2010). Amplification of the ~650 bp barcode region of COI was done using C_FishF1t1/C_FishR1t1 primer cocktails described by Ivanova et al. (2007) with slight modifications. PCR reactions were carried out in a final volume of 25–26 μL. The reaction mixture comprised 7.5μL ddH2O, 10 μL BSA (Bovine Serum Albumin), 2.5 μL 10x PCR Buffer with 1.5 mM $MgCl_2$, 0.25 $MgCl_2$ (25 mM), 0.125 μL dNTPs (10 mM), 0.25μL 0.01mM of each primer, 0.125 μL KAPA Taq DNA Polymerase and 2–3 μL of DNA template. The PCR reaction profile included an initial step of 2 min at 95°C and 35 cycles of 30 sec at 94°C, 40 sec at 54°C, and 1 min at 72°C, with a final extension at 72°C for 10 min. PCR products were visualized on 1% agarose gel. Unclean PCR amplicons were sent to Macrogen in Korea for purification and forward and reverse sequencing using 3730/3730xl DNA Analyzer. Sequence electropherograms from two strands were assembled and manually corrected using MEGA 5 (Tamura et al. 2011). All sequences were subjected to online BLASTn searches at the National Centre for Biotechnology Information (Altschul et al. 1990) and BOLD-IDS at the Barcode of Life Datasystems (Ratnasingham et al. 2007).

Results and discussion

Identification of many species from the family Cichlidae has been challenging not only because of its megadiversity (>1,300 species) and unresolved taxonomy problems but also due to the widespread introduction of many species, morphological and color variation within species, marked overlap in color patterns and morphological characters among different taxa, and the proliferation of natural and artificial cichlid hybrids (Lever 1996; Fuller et al. 1999; Kullander 2003; Miller et al. 2005). Therefore, the positive identification of introduced cichlids requires caution (Nico et al. 2007). In this paper, we identified two non-native and invasive cichlid species occurring in the Philippines based on strong morphological evidence.

Blackchin tilapia (Sarotherodon melanotheron)

A total of 47 samples were collected from two areas (Figure 1): (1) Hagonoy, Bulacan (N=16) and Manila Bay (N=31) adjacent to Paranaque City and Tanza, Cavite. The taxonomic determination of the blackchin tilapia specimens in this study was inferred from the description of FishBase (Froese and Pauly 2012), Trewavas et al. (1983) and other online resources (e.g. Nico and Neilson 2013; Beras 2005). Frequency distribution of key characters is summarized in Table 1. The identification of the MBAYand HAG samples matched to *S. melanotheron* based on the presence of the black pigmentation mostly on the underside of the head, the cleithrum and the apices of caudal and soft dorsal fins (Figure 2). In the Philippines, the black blotches located on the lower part of the head were the basis of farmers for referring to this species with the local name "tilapiang Gloria".

Table 1. Frequency distribution of the distinguishing characters of *S. melanotheron* from Manila Bay (MBAY) and Hagonoy, Bulacan (HAG) based from FishBase (Froese and Pauly 2012) and Trewevas (1983).

Characters		MBAY	HAG (N=16)
		(N=31)	1
Gold coloration on operculum	Present	27	15
	Absent/not distinct	4	15
Pigmentation on lower head	Present	26	1
	Absent	5	14
Pigmentation on tips of caudal fin and dorsal fin ray	Very distinct	7	1
	Less distinct	10	1
	Absent	14	15
White lower lip	Distinct	25	1
	Not distinct	6	12
Caudal peduncle length-depth ratio	0.6 – 0.9	30	4

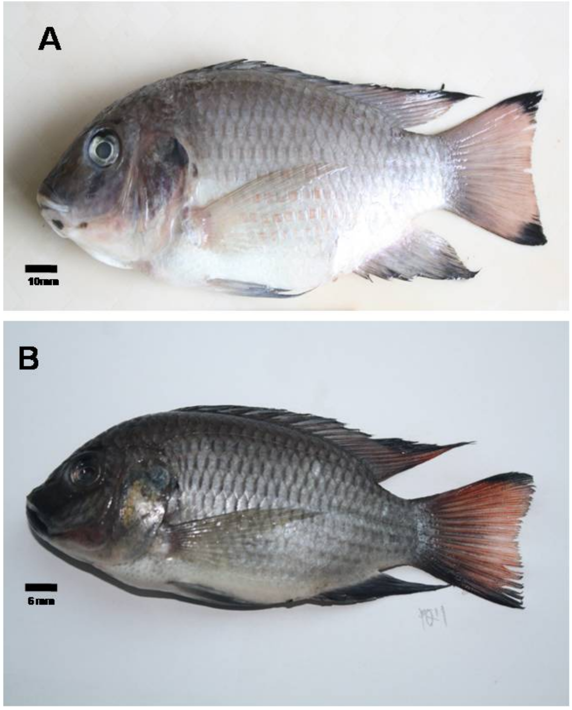

Figure 2. *S. melanotheron* from (A) Hagonoy, Bulacan (SL = 159 mm) and (B) Manila Bay (SL = 89 mm). Photo: B.J. Catacutan.

Some variations on the melanic areas were also observed, i.e. there were samples with black blotches around the lower mouth but less distinct or no pigmentation on the dorsal fin and caudal fin, characters evident in some specimens caught from MBAY. A distinct golden coloration of the operculum was observed in almost all specimens from MBAY but was rare in HAG samples. The white color of the lower lip was present in all specimens exceptone from HAG while it was to less distinct in some fish from MBAY. In general, most of the samples from the two areas were of a darker color on the dorsal area, mostly metallic grey, which becomes lighter towards the ventral side. A metallic yellow to orange streaks were also evident on the body of most specimens from both sampling locations. Our samples were very similar to the *S. melanotheron* specimens collected by Aquilino et al. (2011) from Taal Lake, Batangas based on pictures available at the BOLD database (http://www.boldsystems.org). The summary of selected meristic counts and morphometric characters for the two species investigated in this study are presented in Appendix 1 and 2, respectively. The comparison of our result on meristic counts and morphometric measurements with the data of Omoniyi and Agbon (2008) shows a minimal variation (Table 2). The only noticeable deviation is that, in our study, the caudal peduncle depth was generally longer than the caudal peduncle length, the opposite case can be inferred from the result of their study. But according to the key to species of '*Sarotherodon* of West Africa' by Teugels and Thys van den Audenaerde (2003), one character of *S. melanotheron* that distinguishes the species from other *Sarotherodon* species is the length of caudal peduncle should be 0.6–0.9 times the depth. The caudal peduncle length-depth ratio of almost all specimens in this study was within the range described for *S. melanotheron* (Table 1).

There were observed variations in the phenotypic characters of some of the samples which could possibly be brought about by hybridization to a related species such as *Oreochromis* spp. (Figure 4). However, this would require further investigation.

S. melanotheron is a demersal fish usually found in fresh to brackish waters (Campbell 1987). It is a tropical West African native occurring from Senegal to Zaire and southern Cameroon (Trewevas 1983; Robins et al. 1991). This species has been introduced in different countries including Europe and Asia (Pullin and Lowe-McConnell 1982). It can tolerate salinities of 0 to 40 ppt (Dial and

Table 2. Comparison between selected measurements of locally caught *S. melanotheron* against measurements by Omoniyi and Agbon (2008).

Morphometric measurements	Awb reservoir[a]		Lagos lagoon[a]		MBAY[b]	HAG[b]
	Range (mm)	Mean (mm) ± S.D.	Range (mm)	Mean (mm) ± S.D.	Mean (mm) ± S.D	Mean (mm) ± S.D.
Standard length	120.0 – 150	134.6 ± 1.46	121.0 – 150.0	134.3 ± 1.17	105.3 ± 17.1	144.5 ± 7.4
Head length	43.0 – 56.0	46.6 ± 0.24	41.0 – 59.0	45.8 ± 0.18	37.5 ± 7.2	50.7 ± 3.3
Head depth	19.0 – 26.0	24.2 ± 0.14	18.0 – 24.0	23.1 ± 1.06	19.7 ± 3.2	24.0 ± 1.8
Body width	18.0 – 24.0	22.8 ± 0.12	16.0 – 23.0	19.3 ± 0.15	16.4 ± 2.9	21.5 ± 1.9
Eye diameter	12.0 – 15.0	13.4 ± 0.13	13.0 – 15.0	13.3 ± 0.11	7.5 ± 1.6	10.3 ± 1.0
Caudal peduncle length	17.0 – 26.0	22.4 ± 0.38	20.0 – 27.0	22.8 ± 0.41	12.1 ± 3.0	21.1 ± 1.8
Caudal peduncle depth	15.0 – 21.0	20.6 ± 0.11	15.0 – 20.0	17.9 ± 0.21	17.4 ± 3.4	23.4 ± 1.6
Meristic characters						
Dorsal fin spine	15 – 17	15.6 ± 0.49	15 – 17	15.5 ± 0.41	15.1 ± 0.3	14.4 ± 3.4
Dorsal fin rays	10 – 12	10.9 ± 0.41	10 – 13	11.0 ± 0.63	11.1 ± 0.8	10.5 ± 0.5
Pectoral fin rays	12 – 13	12.1 ± 0.22	12 – 14	12.2 ± 0.39	12.6 ± 0.8	12.8 ± 0.8
Pelvic fin rays	6	6	6	6	5	5.6 ± 0.4

[a]sites investigated in Omoniyi and Agbon's (2008) study
[b]sites investigated in this study

Wainright 1983; Campbell 1987) and can travel freely between fresh and saltwater environments (Trewevas 1983). Because of its wide salinity tolerance, it has been reported to occur in pure seawater. For example, *S. melanotheron* can be found in coastal and lagoon waters, as well as the freshwater reservoirs in Hawaii (Wu and Yang 2012). *S. melanotheron* has been reported to compete with the native species in freshwater springs in Florida, causing malnourishment and disease in largemouth bass *Micropterus salmoides* (Lacépède, 1802) and bluegill *Lepomis macrochirus* (Rafinesque, 1819) (Courtenay et al. 1974). Similarly, negative impacts were also reported in HAG where local farmers attribute the decrease in harvest of more valuable fishes to appearance of blackchin tilapia in the municipality. Local famers claimed that the fish preys on the larvae or juveniles of farmed species seeded in the area including milkfish *Chanos chanos* (Forsskål, 1775), tilapia (*Oreochromis* spp), and possibly giant tiger shrimp *Penaeus monodon* (Fabricius, 1798) and/or white-leg shrimp *Litopenaeus vannamei* (Boone, 1931). This could be due to the carnivorous nature of blackchin tilapia fry, which feed on smaller fish larvae, eggs, insects, and newly hatched fry of other reared species (Campbell 1987). *S. melanotheron* was also reported to plague the fish ponds in the nearby province of Bataan, causing decrease in fish catch (e.g. tilapia) of fish-farmers operating in the affected area. These observed declines in catch are also probably due to competition of food resources by the *S. melanotheron*, since already established populations of the fish have been associated with a reduction of aquatic vegetation due to overgrazing (Courtenay et al. 1974). *S. melanotheron* has been harvested and sold in the market at a relatively cheaper price than *Oreochromis* spp. This may be because the species has not been well accepted by consumers due to its small size and unappealing taste. According to anecdotal reports, *S. melanotheron* has occurred in Bulacan and Bataan since 2011. Therefore, it is very likely that this species has already established populations in these areas.

Recently, *S. melanotheron* was also reported in Manila Bay and although not included in the morphology and meristics analyses, samples were also identified and collected in Manila Bay near Manila City, specifically Manila Ocean Park (Figure 3). Manila Bay is a major area for fishery and aquaculture while inland aquaculture is one of the major sources of livelihood in Hagonoy, Bulacan. Introduction of invasive species therefore poses a serious threat to the livelihoods and ecosystems in these areas and has already occurred in Laguna de Bay with the infestations by janitor and knife fishes. We suspect that the *S. melanotheron* population in MBAY might have come from nearby fishponds and later might have escaped into the coastal waters through flooding events. Intentional introduction by fish-farmers to enhance fishery production in the area is also a possible pathway of introduction. However, the method of introduction into the country still remains unknown.

Figure 3. *S. melanotheron* caught in Manila Bay within the vicinity of Manila Ocean Park. Photo: M.D. Santos.

Table 3. Comparison between selected measurements of *C. urophthalmus* caught in the Philippines against measurements by Nico et al. (2007) using non-native (Thailand and Florida) and native *C. urophthalmus* (Guatemala and Mexico).

		Thailand (N = 37)	Florida (N = 22)	Guatemala and Mexico (N = 49)	Philippines (N = 30)
Number of lateral anal-fin spines	5	3	5	2	6
	6	34	16	38	19
	7		1	9	5
	Mean ± SD	5.9 ± 0.28	5.8 ± 0.5	6.1 ± 0.46	6.0 ± 0.61
Number of dorsal-fin spines	15	2	1	2	9
	16	30	21	24	13
	17	5		22	8
	18			1	
	Mean± SD	16.1 ± 0.43	16.0 ± 0.21	16.4 ± 0.61	16.0 ± 0.76
Number of lateral bars	7	43	20	47	27
	8	1	2	2	3
	Mean± SD	7.0 ± 0.15	7.1 ± 0.29	7.0 ± 0.2	7.1 ± 0.31
Midline blotch on 4th lateral bar	Present (distinct)	7	4	10	15
	Present (faint)	34	9	11	11
	Absent	4	9	28	4

Table 4. Identification summary based on consensus barcoded sequence of selected samples using BLASTN search from GenBank and BOLD Identification System (BOLD-IDS).

Sample name	Genbank Accession ID	(GenBank) BLASTn		BOLD-IDS	
		Species match	% similarity	Species match	% similarity
MBAY07	KM212015	*S. melanotheron*	99–100%	*S. melanotheron*	100%
HAG02	KM212018	*S. melanotheron*	99–100%	*S. melanotheron*	100%
HAG06	KM212014	*S. melanotheron*	99–100%	*S. melanotheron*	100%
MOP*	KM212016	*S. melanotheron*	99–100%	*S. melanotheron*	100%
FH06**	KM212019	*C. urophthalmus*	99%	*C. urophthalmus*	99.51–100%
FH02**	KM212020	*C. urophthalmus*	99%	*C. urophthalmus*	99.22–99.69%
FH01**	KM212017	*C. urophthalmus*	99–100%	*C. urophthalmus*	99.48–100%

**S. melanotheron* from Manila Ocean Park
***C. urophthalmus* samples

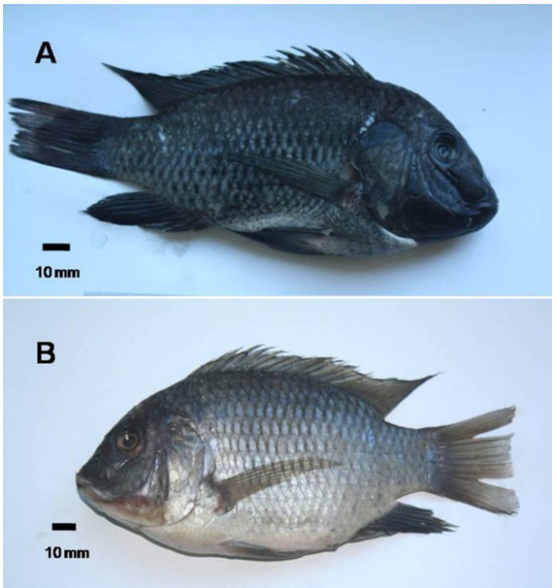

Figure 4. *S. melanotheron* specimens that show different features from the rest of the samples collected for this study. In A (SL = 151 mm), body coloration is different, lower lip is not white, but it has a distinct gold coloration on operculum (MBAY sample). Right side of the fish was taken due to the damaged portion on its left side. In B (SL = 145.5 mm), no pigmentation can be observed on lower head, caudal, and dorsal fin ray (HAG sample). Photo: J.F.F. Ordoñez.

Mayan cichlid (Cichlasoma urophthalmus)

A total of thirty samples of putative "flowerhorn" were collected in four different barangays (Figure 1): Santa Monica (N=16), Iba (N=10), San Sebastian (N=2), and Pugad (N=2). All samples were subjected to morphological examination. Figure 5 shows representative samples taken from these barangays. All individuals exhibit almost uniform color (yellow to olive-brown), patterns and body shapes. However, some samples with unusual morphology have also been recorded (e.g. Figure 6). According to the suggestion of Nico et al. (2007), there are several more important traits useful in distinguishing *C. urophthalmus*: 1) seven (rarely 8) prominent dark bars on body (the first an oblique along nape that crosses near the lateral line origin and the seventh or posterior-most bar positioned on the caudal peduncle); 2) conspicuous, dark blotch centred above the caudal fin base and often outlined by a light halo (this blotch may be nearly round, oval square, or vertically elongate, and is noticeably darker than the black body bands); 3) caudal fin rounded; 4) anal-fin spines 5–7 (usually 6); 5) dorsal-fin spines 14–18 (usually 16); and 6) well developed canine, unicuspid teeth in both jaws. A comparison was made between the populations (native and non-native) examined in their study and the Philippine population used in this study using the data on the frequency distribution of selected characters employed by Nico et al. (2007) (Table 3). No striking deviation was found between the Philippine population and the reference populations. Another key character, the presence of another dark blotch on the center of the 4^{th} vertical bar (Miller et al. 2005), could be observed in some samples though most appeared faint. The morphometric measurements and the meristic counts are listed in Appendix 1 and 2, respectively. However, distinguishing characters including lateral lines and ocellus are not always convenient in identifying *C. urophthalmus* because similar features can also be observed in many Middle American cichlids that are now found in Southeast Asia such as *Cichlasoma festae* Buolenger (1899) in Singapore (Tan and Tan 2003) and *Archocentrus octofasciatus* (Regan, 1903) in Thailand (Welcomme and Vidthayanon 2003). In addition, a few "flowerhorn" varieties artificially created from hybridizing cichlid species also resemble *C. urophthalmus* such as hybrids of *C. trimaculatum* (Gunther 1867) (Nico et al. 2007). This may be the reason the fish is referred to as "flowerhorn". Similar to *S. melanotheron*, there were a few *C. urophthalmus* samples that showed variation in morphology (Figure 6).

This is the first record of Mayan cichlid in the Philippines. The name "flowerhorn" was assigned by the local farmers because of the pattern on its body that was believed to resemble that of the true ornamental "flowerhorn" fish (*Cichlasoma* spp. hybrids). When and how this species was introduced in the Philippines is unknown. We suspect that the fish was introduced through aquarium fish trade, deliberately farmed in fishponds and escaped during flooding events. At present, Mayan cichlid are sold as food fish in local market, often mixed with tilapia, milkfish and shrimp. *C. urophthalmus* is native to the New World tropics and introduced populations of this are regarded as highly invasive (Nico et al. 2007). A possible reason for its introduction is most likely for ornamental purposes since the locals identify it as "flowerhorn", an ornamental aquarium fish. Both *S. melanotheron* and *C. urophthalmus* arebelieved to have caused the decline in catch of other important food-fish.

Native fish populations are known to decrease when *C. urophthalmus* population increases probably because of competition pressures for food and space (Trexler et al. 2000) or alternatively through

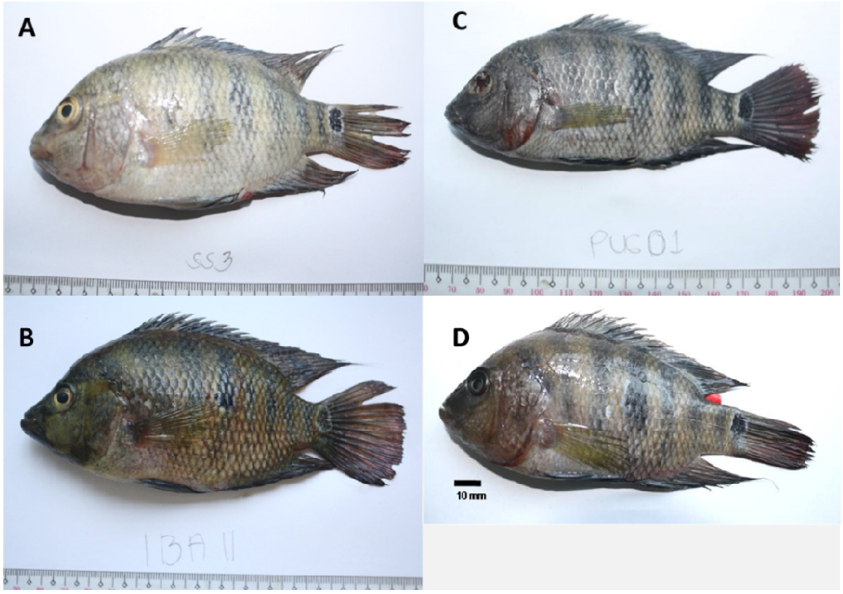

Figure 5. Specimens identified as *C. urophthalmus* collected from different barangays in Hagonoy, Bulacan: (A) San Sebastian (SL = 120 mm); (B) Iba (SL = 123 mm); (C) Pugad (SL = 105 mm); (D) Santa Monica (SL = 105 mm). Photo: J.F.F. Ordoñez

Figure 6. One of the few of the samples of *C. urophthalmus* that have different color pattern and body shape (SL = 142.5 mm). Most specimens taken from Iba have similar appearances to the fish in this photo. Photo: J.F.F. Ordoñez.

predation effects (Ferriter et al. 2006; Porter-Whitaker et al. 2012). The distribution and establishment of *C. urophthalmus* in the Philippines requires further assessment.

Species identification using DNA barcoding

Rapid identification of plants and animal at the species level can be performed using DNA barcoding (Hebert et al. 2003; Hollingsworth et al. 2009). It involves the use of short DNA sequences from a standard and agreed-upon position in the genome that can be compared to a universal barcode library for identification purposes (Aquino et al. 2011). *Cytochrome c oxidase subunit I* (COI) gene has been designated as the standard region for barcoding in animals (Hebert et al. 2003; Hajibabaei et al. 2007). This method is a very useful tool for fast and accurate identification of species and has been applied in numerous fisheries-related studies including food authentication (Maralit et al. 2011; Wallace et al. 2012), illegal trade of wildlife such as protected and endangered species (Teletchea et al. 2005; Asis et al. 2014) and larval fish identification (Hubert et al. 2010; Agmata et al. 2013; Asis et al. 2013; Thomas et al. 2013). Partial COI sequences of several *S. melanotheron* and *C. urophthalmus* specimens were generated. All putative blackchin tilapia and "flowerhorn" were genetically matched to *S. melanotheron* and *C. urophthalmus*, respectively. All samples had 99–100% similarity in both databases (Table 4). Thus, the result from the sequence search using BLASTn and BOLD-IDS support our morphological identification, proving the utility of DNA barcoding in rapid and accurate species identification.

Conclusion

Positive species identification of an invasive organism is one of the vital steps in order to develop management actions for its control. In this study, we identified *S. melanotheron* in the coastal waters of Manila Bay and in fishponds of

Hagonoy, Bulacan. We also reported the first record of *C. urophthalmus*, a native cichlid of Central America, in Philippine waters. However, due to the well-recognised variability in the Cichlidae, further genetic analysis is required to confirm their identification. Although there is no evidence yet regarding their negative impact on both ecological and economic aspects, thorough monitoring should be carried out to determine the distribution and future spread of both species in the Philippines.

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Supplementary material

The following supplementary material is available for this article:

Appendix 1. Frequency distribution selected meristic counts of *C. urophthalmus* and *S. melanotheron* caught in Manila Bay (MBAY) and Hagonoy, Bulacan (HAG).

Appendix 2. Frequency distribution selected morphometric characters of *C. urophthalmus* and *S. melanotheron* caught in Manila Bay (MBAY) and Hagonoy, Bulacan (HAG).

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