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Status Assessment of *Clarias* Species in the Philippines: Insights from DNA Barcodes

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ABSTRACT

Catfishes of the genus *Clarias* are important food fishes in aquaculture. In the Philippines, six species are documented but only three, namely *C. batrachus*, *C. macrocephalus,* and *C. gariepinus*, are found in the market today. Of these, *C. macrocephalus* is both native and near threatened. In this study, the cytochrome c oxidase I (COI) gene was amplified for 20 Agusan Marsh, Agusan del Sur specimens provisionally identified as *C. macrocephalus*. These specimens have a different morphology compared to other *C. macrocephalus* specimens previously obtained elsewhere. The COI sequences all matched the Philippine COI sequences of *C. macrocephalus,* thus confirming its identity. Reanalysis of barcode sequences was also conducted to resolve the conflicting claims regarding the status of some *Clarias* species. A total of 179 COI sequences from *Clarias* species present in GenBank were included in the analyses. The average intraspecific and interspecific Kimura-2-Parameter distances were 2.99% and 13.26%, respectively. There was very little sequence diversity observed in the Philippine samples of *C. macrocephalus*. Philippine samples of *C. batrachus* and *C. macrocephalus* formed distinct clades, while

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Philippine *C. gariepinus* specimens clustered with those of other countries, supporting the claim that the former two species are native and the latter was introduced to the country. The status of the other *Clarias* species in the Philippines is also discussed.

Keywords: Catfish, lariidae, COI, DNA barcoding

LAYMAN'S ABSTRACT

Catfishes of the genus *Clarias* are abundant and are important food fishes in the Philippine market. Six *Clarias* species have been reported in the country in the past. Today, however, only *C. batrachus*, *C. gariepinus*, and *C. macrocephalus* are found in the market. The other species have not been seen in recent years. Of these species, *C. macrocephalus* is of primary importance because it is both native to the Philippines and near threatened. As such it is important to identify them from their natural populations. DNA barcoding is a technique that utilizes a single gene to identify an organism to the species level. This was performed in this study by amplifying and sequencing the cytochrome c oxidase subunit I gene (COI) of 20 specimens of *C. macrocephalus* from Agusan Marsh to confirm their identity. These specimens appear different compared to other *C. macrocephalus* specimens from other locations. The COI sequences from Agusan Marsh were highly similar to the sequences of other Philippine *C. macrocephalus,* thus confirming their identity. As for the other *Clarias* species, there are conflicting claims regarding their status. To resolve this, all available barcode sequences of *Clarias* species were analyzed. A total of 179 COI sequences from the GenBank database were included. Philippine samples of *C. batrachus* and *C. macrocephalus* formed distinct groupings, while Philippine *C. gariepinus* specimens grouped with those of other countries. This supports the claim that *C. batrachus* and *C. macrocephalus* are native to the Philippines while *C. gariepinus* is only introduced to the country. The presence of the other *Clarias* species in the Philippines is questionable.

INTRODUCTION

Clarias species are abundant and are important food fishes in the Philippine market. Six *Clarias* species have been reported in the country. Of these, only *C. batrachus*, *C. gariepinus*, and *C. macrocephalus* are commonly found in the market today. The others, *C. nieuhofii, C. meladerma,* and *C. fuscus,* have not been reported in recent years. The status of these *Clarias* species is quite controversial due to conflicting reports from old checklists, recent surveys, as well as current findings.

The status of the Philippine native catfish, *C. macrocephalus*, is of primary interest. While *C. batrachus* and *C. gariepinus* are abundantly used in fish farming, *C. macrocephalus* is listed as near threatened by the International Union for Conservation of Nature (IUCN). Natural populations of *C. macrocephalus* have been declining all over Southeast Asia (Vidthayanon and Allen 2013). In the Philippines, *C. macrocephalus* was previously described by Conlu (1986) as widely distributed. However, the abundance of this species was greatly reduced in the years that followed due to habitat loss, poor water quality, and the presence of larger-sized competitors, *C. batrachus* and *C. gariepinus* (Vidthayanon and Allen 2013).

The presence of *C. macrocephalus* in the Philippines was also reported by Fowler (1941) and Herre (1953). FishBase also indicates that *C. macrocephalus* is native to the Philippines (Froese and Pauly, 2014). 'Native' is defined as naturally occurring in the country as opposed to those that are introduced. Bycontrast, Teugels et al. (1999) claimed that *C. macrocephalus* is a species introduced to the Philippines for aquaculture, falsely citing Conlu (1986), who stated that *C. macrocephalus* is endemic, or is found exclusive, to the Philippines. This, however, is also false since *C. macrocephalus* is also nativeto Cambodia, Laos, Thailand, and Vietnam (Froese and Pauly 2014).

In recent sampling activities, *C. macrocephalus* specimens from Agusan Marsh, Agusan del Sur were found to be much larger than others. The standard lengths of Agusan Marsh specimens ranged from 22.7–34.8 cm with an average of 29.4 cm, whereas those of Cagayan specimens ranged from 11.2–20.2 cm with an average of 16.3 cm. Conlu (1986) indicated that the size range of *C. macrocephalus*is 20–30cm; however, it was not clear whether the length indicated was the total length or the standard length. The average size was not described as well. The Agusan Marsh and Cagayan specimens were larger and smaller, respectively, than Conlu's estimates. These specimens were still assigned to *C. macrocephalus* due to the characteristic obtuse and rounded shape of their supraoccipital process. Nonetheless, it is still important

to verify whether the large-size and small-size morphotypes are merely variants of the same species or whether they are genetically divergent lineages.

The mitochondrial DNA (mtDNA) cytochrome c oxidase subunit I (COI) gene has been extensively used in the past decade as a DNA barcode for species identification and delineation (Hebert et al. 2004). The COI gene is useful for fast, simple, robust, and precise species identification of fish species (Costa and Carvalho 2007). It has been applied for the detection of cryptic species, possible market mislabeling, and taxa requiring taxonomic re-evaluation (Smith et al. 2008; Ward et al. 2008; Smith et al. 2011).

To date, 179 *Clarias* COI sequences are available in GenBank (Table 1). Some of the sequences have been published in various papers, with some having conflicting claims regarding the status of *Clarias* species in different countries. For example, Wong et al. (2011) observed low sequence identities between Thai specimens of *C. batrachus* and those from GenBank. Although it was not stated, the GenBank sequences were most likely from India since these were the only ones available at that time. In 2012, Bhattacharjee et al. (2012) barcoded catfishes, including *C. batrachus*, from India. They did not include the Thai specimens from the study of

Table 1. List of *Clarias* COI sequences obtained from GenBank

Species	Country	Accession number	Reference
Clarias angolensis	Congo	HM880232	International Barcode of Life, Direct Submission
Clarias batrachus	India	FJ459456-59	Lakra et al. 2011
	India	GQ466399-403	Barman et al. (unpublished)
	India	JN628880,924	Bhattacharjee et al. 2012
	India	KF214293-96; JQ667517-18	Khedkar et al. 2014
	India	JQ699205-208	Aneesha et al. (unpublished)
	India	JX946369	Singh et al. (unpublished)
	India	KF742432	Subedi et al. (unpublished)
	India	KJ720696	Premdass et al. (unpublished)
	Thailand	JF292297-309	Wong et al. 2011
	Philippines	HQ654701	Aquilino et al. 2011
	Philippines	HQ682679-81	Aquino et al. 2011
	Philippines	KC789523-27	Yambot et al. (unpublished)
	Philippines	KF604645-56	Quilang and Yu 2015
	Vietnam	EF609334	Ward and Holmes 2007
Clarias camerunensis	Nigeria	HM882808	Nwani et al. 2011
Clarias dussumieri	India	HM579862	Simi et al. (unpublished)
	India	JQ699209-13	Aneesha et al. (unpublished)
Clarias fuscus	China	JN020071	Wang et al. (unpublished)
	China	KF011504-05	Xiao and Peng (unpublished)

Table 1. List of *Clarias* COI sequences obtained from GenBank (Cont'd.)

Species	Country	Accession number	Reference
Clarias gabonensis	Congo	HM880231,33	International Barcode of Life Direct Submission
	Nigeria	JF510511, HM882815-16,29 32-37	Nwani et al. 2011
Clarias gariepinus	Brazil	GU701825-29	Pereira et al. 2013
	Indonesia	HM345933-34	Muchlisin et al. 2013
	Nigeria	HM882809-14,17, 20-21,23-31	Nwani et al. 2011
	Thailand	JF292310-320	Wong et al. 2011
	Turkey	JQ623925; KC500413-32	Keskin and Atar 2013
	India	JQ699199-203	Aneesha et al. (unpublished)
	India	KF742418	Chaulagain et al. (unpublished)
	India	JX260853	Kalyankar et al. (unpublished)
	Philippines	KF604657-61	Quilang and Yu 2015
	Ethiopia	KF929769	Bentley and Wiley (unpublished)
Clarias jaensis	Nigeria	HM882818-19	Nwani et al. 2011
Clarias macrocephalus	Philippines	KF604662-66	Quilang and Yu 2015
	Thailand	JF292321-37	Wong et al. 2011
Clarias nieuhofii	Malaysia	JF280833-34	Othman et al. (unpublished)
Clarias teijsmanni	Malaysia	JN646093	Sade and Biun 2012

Wong et al. (2011), but they cited the *C. batrachus* accession of Aquilino et al. (2011) from Taal Lake, Philippines as a possible misidentification. Quilang and Yu (2015) addressed this issue by including Philippine *C. batrachus* sequences from Laguna de Bay (Aquino et al. 2011) and from their own specimens obtainedfrom Cagayan and three lakes from Camarines Sur. They observed that all *C. batrachus* specimens clustered within their country of origin. Philippine specimens are closer to those from Thailand with an average distance of 2.6%, whereas the average distance of Philippine and Thai specimens with those from India were 11.3% and 12%, respectively. These results were taken as supporting proof to the status of *C. batrachus* as native to the Philippines (Herre 1924; Herre 1926) even though it was widely believed to have been introduced later (Vallejo 1985; Juliano et al. 1989; ASAP 1996; Table 2).

In subsequent years, more COI sequences of *Clarias* became available in Genbank, enabling the representation of various countries (Table 1). As such, reanalyzing the barcodes would provide more information that could possibly resolve the conflicting claims. In this study, the COI gene was utilized to ascertain the identity of the Agusan Marsh specimens. This was also used to assess the status of other *Clarias* species in the Philippines.

Table 2. Status of *Clarias* species in countries where the COI sequences were obtained

Species	Country	Status	Reference
Clarias batrachus	Philippines	Native	Herre 1924
	Thailand	Native	Welcomme and Vidthayanon 2003
	India	Misidentification	Ng and Kottelat 2008
	Vietnam	Misidentification	Kottelat 1998
Clarias gariepinus	Philippines	Introduced	Juliano et al. 1989
	India	Introduced	Shaji et al. 2000
	Thailand	Introduced	Vidthayanon 2003
	Brazil	Introduced	Vitule et al. 2006
	Indonesia	Introduced	Cambray 2005
	Turkey	Native	Solak et al. 2001
	Nigeria	Native	Olaosebikan and Raji 1998.
	Ethiopia	Native	Rocha 2008
Clarias macrocephalus	Philippines	Native	Conlu 1986
	Thailand	Native	Vidthayanon et al. 1997
Clarias fuscus	Philippines	Native	Herre 1953
	Philippines	Misidentification	Ng 1999
	China	Native	Sudarto et al. 2004

MATERIALS AND METHODS

A total of 20 specimens of *C. macrocephalus* were collected from Agusan Marsh, Agusan del Sur (8.21 N; 125.96 E) from a local contact. These were brought to the Institute of Biology, Molecular Population Genetics Laboratory for processing and identification. Specimens were initially identified based on the morphology of the supraoccipital process (Conlu 1986; Teugels et al. 1999). The weight and length measurements were obtained. Each specimen was photographed on the dorsal and left side views using a Nikon D90 SLR camera. A piece of white muscle tissue was excised from the right body side of each specimen. The tissue was placed in a 2-mL microfuge tube containing absolute ethanol and stored in the freezer until further use.

Approximately 20 mg of the muscle tissue from each specimen was used for DNA extraction using PromegaWizard® Genomic DNA purification kit (Madison, WI) following the manufacturer's protocol. The following primers from Ward et al. (2005) were used for the amplification of approximately 655 bp of the mitochondrial cytochrome c oxidase I (COI) gene:

FISHF1: 5'-TCAACCAACCACAAAGACATTGGCAC-3'

FISHR2: 5'-ACTTCAGGGTGACCGAAGAATCAGAA-3'

PCR amplification was performedin 50-µL volumes. The PCR mix consisted of 1.0 µL of dNTP (0.05 µM), 2.5 µL of each primer (0.1 µM), 5.0 µL of 1X PCR buffer, 0.5 µL of (1.25 U) Taq polymerase (Roche Taq dNTPack), 34.5 µL of ultrapure water, and 4.0 mL of DNA template. The PCR conditions were as follows (Ward et al. 2005): initiation for 2 min at 95°C, followed by 35 cycles of denaturation for 0.5 min at 94°C, primer annealing for 0.5 min at 54°C, and primer extension for 1 min at 72°C. A final extension step at 72°C for 10 min completed the reaction. The PCR products were visualized on 1% agarose gels with ethidium bromide. Bands with approximate size of 650 bp were excised from the gel. The excised gels were then purified with QIAquick® Gel Extraction Kit (QIAGEN, Valencia, CA) following the manufacturer's protocol. The purified DNA products were sent to 1st BASE in Selangor, Malaysia for bidirectional sequencing.

The consensus sequence of each specimen was assembled using the Staden Package v4.10 (Staden et al. 2000). These were aligned and analyzed using MEGA version 6 software (Tamura et al. 2013). The 20 *C. macrocephalus* COI sequence were submitted to GenBank and Barcode of Life Data Systems (BOLD).

Additional sequences were downloaded from GenBank (Table 1). All *Clarias* COI sequences from BOLD have corresponding GenBank accessions. As such, only GenBank accessions were noted. Pairwise genetic distances within species and between species were calculated using the Kimura 2-Parameter (K2P) model of nucleotide substitution (Kimura 1980). A neighbor-joining (NJ) tree was constructed at 1000 bootstrap replicates (Saitou and Nei 1987) using the K2P model.

RESULTS

Identity of C. macrocephalus specimens

Out of the 20 *C. macrocephalus* specimens from Agusan Marsh, two distinct haplotypes were observed: one haplotype, represented by 19 sequences, matched *C. macrocephalus* from Cagayan (accession numbers KF604663-65) with 100% identity based on BLASTn results; and the other haplotype matched the same sequences above but with 99.7% identity.

K2P distances within and between species

A total of 199 COI sequences representing 11 species were analyzed. The average within- and between-species K2P distances were 2.99% and 13.26%, respectively (Table 3). Average within-species K2P distance greater than 2% was observed for

C. batrachus (6.98%) and *C. fuscus* (5.60%; Table 4). Some intraspecific K2P distances were very high, if only *a priori* species assignments were considered, such as those for *C. batrachus* and *C. fuscus* sequences. To facilitate subsequent analyses, highly diverging conspecific sequences were given *a posteriori* assignments based on their phylogeny (Figure 1). For *C. batrachus*, the sequences were grouped according to country, namely Philippines, Thailand, and India. Indian *C. batrachus* sequences still exhibited very high within-group distances and were further subdivided into the following: India-1, which forms a distinct clade; India-2, which clustered with *C. macrocephalus* sequences; and India-3, which clustered more closely with Philippine and Thai sequences of *C. batrachus*. For *C. fuscus*, the three available sequences were subdivided into the following: *C. fuscus* -1, which corresponds to an accession from Yunnan province; and *C. fuscus*- 2, which represents two accessions from the Guangxi region. Sequences of *C. macrocephalus* were also subdivided into Philippine and Thai samples for comparison. The subgroups described above were treated as separate species for calculations of average within- and between-species genetic distances (Tables 4 and 5). The average K2P distances within subgroups were lower compared to the whole.

Species delineation

Of the 11 species, three, namely *C. angolensis, C. camerunensis,* and *C. teijsmanni*, were represented by only one sequence. The average K2P distances between these three species and others range from 5.2% to 16.1% (Table 5). Five others, namely *C. dussumieri, C. gabonensis, C. gariepinus, C. jaensis,* and *C. nieuhofii*, form distinct clades with average K2P distances within species ranging from 0.00% to 0.98% (Table 4) and a minimum average distance of 5.2% with a non-conspecific (Table 5). The clades representing these five species all had 99% bootstrap support (Figure 1). The remaining three species, namely *C. batrachus, C. macrocephalus,* and *C. fuscus*, did not form distinct clades and were not completely delineated. Six *C. batrachus* sequences from India (India-2) and one *C. batrachus* sequence from Vietnam clustered with *C. macrocephalus* (Figure 1). One *C. fuscus* sequence (China-1) grouped with Philippine sequences of *C. batrachus*, while the other two (China-2) formed a distinct clade (Figure 1).

Table 3. Summary of percent K2P genetic distances within and between species

	K2P Genetic Distance (%)				
	N	**Minimum**	**Average**	**Maximum**	**Standard error**
Within Species	5063	0.00	2.99	14.24	0.06
Between species	14638	0.00	13.26	17.14	0.02

Table 4. Average intraspecific percent K2P genetic distances

Species	K2P Genetic Distance (%)
Clarias angolensis (N=1)	-
Clarias batrachus – ALL (N=59)	6.98
Clarias batrachus – Philippines (N=21)	0.09
Clarias batrachus – Thailand (N=13)	0.12
Clarias batrachus – India ALL (N=24)	6.98
Clarias batrachus – India 1 (N=17)	0.99
Clarias batrachus – India 2 (N=6)	0.58
Clarias batrachus – India 3 (N=1)	-
Clarias batrachus – Vietnam (N=1)	-
Clarias camerunensis (N=1)	-
Clarias dussumieri (N=6)	0.31
Clarias fuscus – ALL (N=3)	5.60
Clarias fuscus – 1 (N=1)	-
Clarias fuscus – 2 (N=2)	0.31
Clarias gabonensis (N=12)	0.64
Clarias gariepinus (N=70)	0.98
Clarias jaensis (N=2)	0.00
Clarias macrocephalus – ALL (N=42)	0.50
Clarias macrocephalus – Philippines (N=25)	0.04
Clarias macrocephalus – Thailand (N=17)	0.60
Clarias nieuhofii (N=2)	0.00
Clarias teijsmanni (N=1)	-

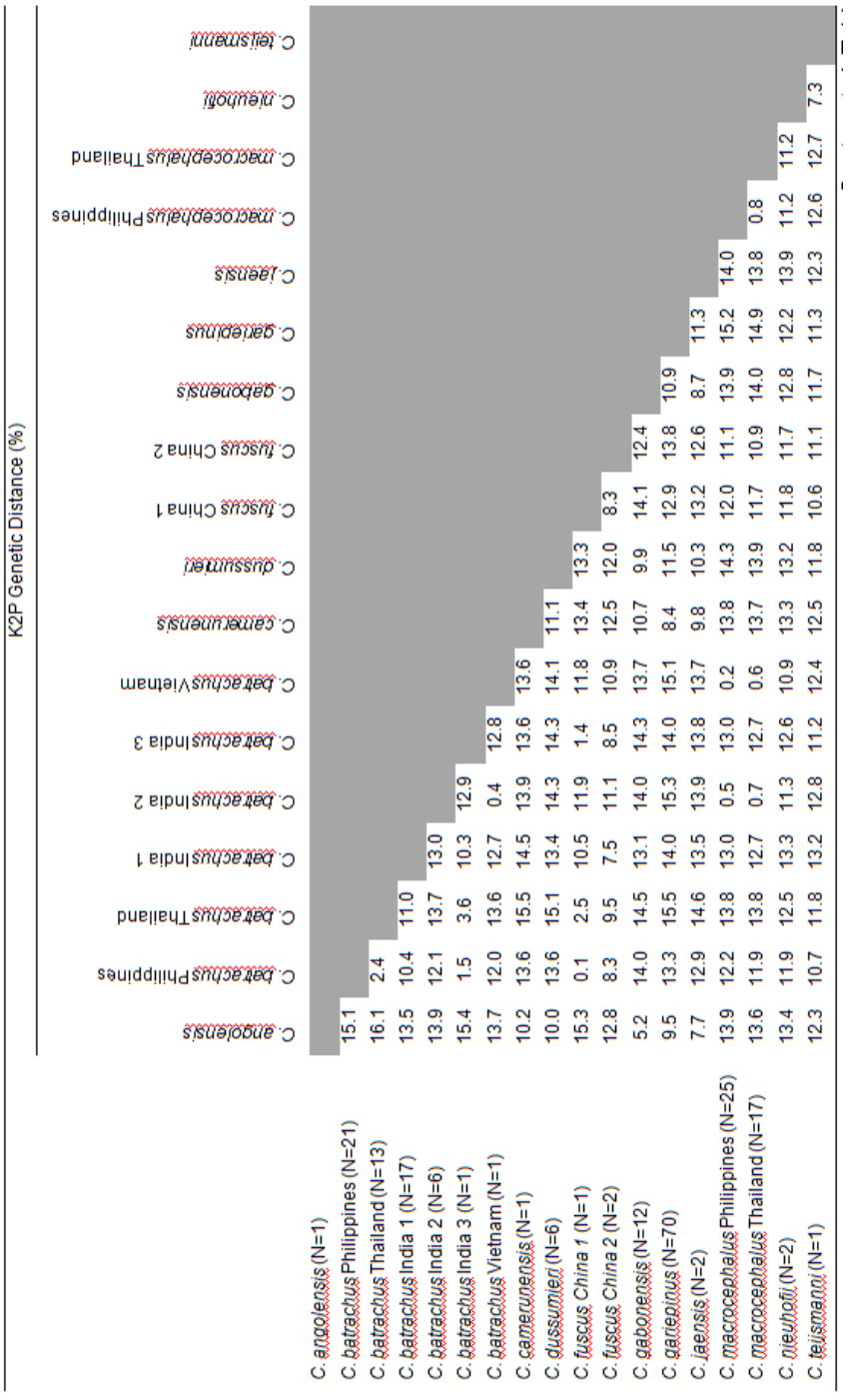
Additional Philippine sequences of C. batrachus

The COI sequences of five *C. batrachus* specimens from Pantabangan Dam, Nueva Ecija obtained by Yambot et al. (unpublished) were included this study. All five sequences clustered with the other Philippine *C. batrachus* specimens. The average within group distance for Philippine *C. batrachus* specimens is 0.09% (Table 4). The *C. batrachus* sequences from Thailand were closest to the Philippine group. The average distance between the two groups is 2.4% (Table 5). The average within group distance for Thai *C. batrachus* specimens is 0.12% (Table 4).

DISCUSSION

The 100% sequence identity between *C. macrocephalus* specimens from Agusan Marsh and those of Cagayan confirms that the two morphotypes belong to the same species. Moreover, the Philippine specimens of *C. macrocephalus* continue to form a group distinct from Thai specimens (Figure 1). The increase in size among specimens from Agusan Marsh may have been an effect of protecting the area as a

Table 5. Pairwise average percent K2P genetic distances between species



wildlife sanctuary. Despite the geographic separation and overall habitat difference between Cagayan and Agusan, the lack of genetic diversity is still a cause of concern. Although COI is not a standard tool for detecting genetic diversity, it still exhibited a relatively high degree of conspecific divergence in other catfish taxa (Wong et al. 2011; Bhattacharjee et al. 2012; Quilang and Yu 2014). While *C. macrocephalus* specimens from the Philippines are genetically diverged from Thailand based on COI sequences, very little diversity was observed within the Philippine samples.

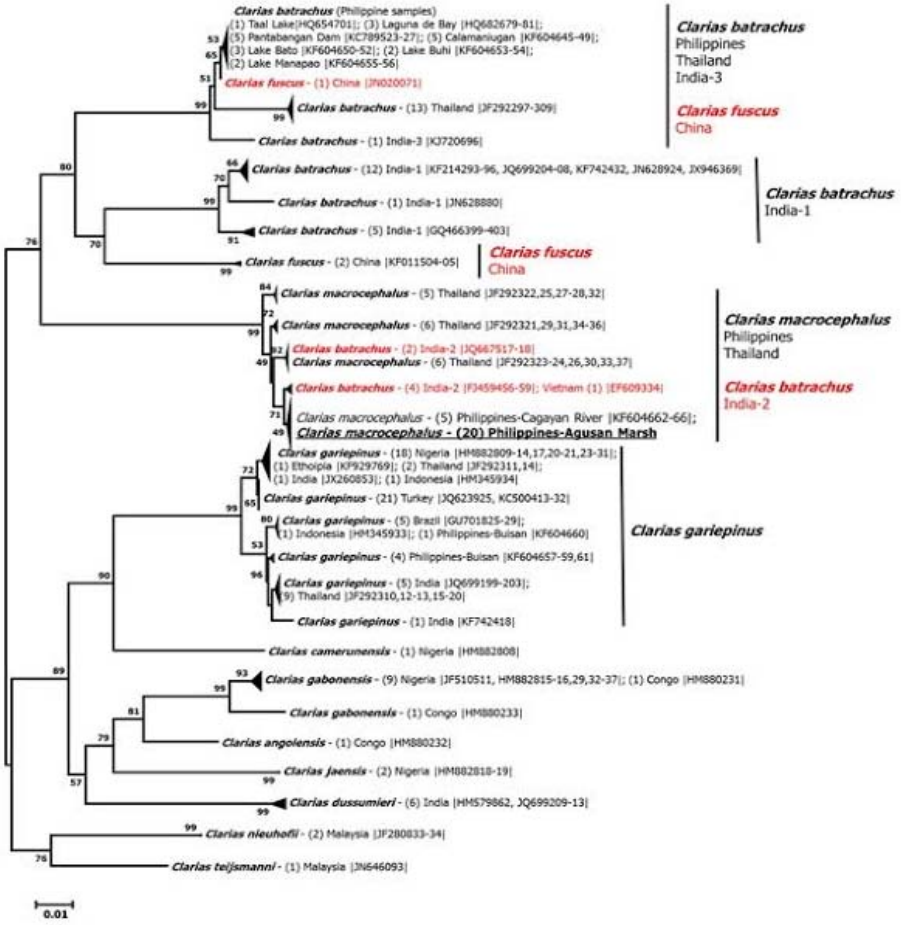


Figure 1.Neighbor-Joining tree of 199 COI sequences of Clarias species computed using the K2P model of nucleotide substitution. Branch lengths are drawn to scale and represent average K2P distances. Numbers in parentheses following the taxon names represent the number of sequences included in the clade. Accession numbers of sequences from GenBank are shown. Bootstrap supports of 1000 replicates are shown. Taxa indicated in red text indicate possible misidentifications.

Only three haplotypes were observed from 25 sequences (π = 0.036) in Philippine samples compared to that of Thailand where ten haplotypes are present from 17 sequences (π = 0.600). It is thus important to further assess the genetic diversity of *C. macrocephalus* in these areas. This is especially true since *C. macrocephalus* is now very seldom caught in areas where they were previously found. Tan et al. (unpublished) noted the absence of *C. macrocephalus* among the catchments in several major water bodies in the country despite repeated sampling. These areas include Laguna de Bay, Taal Lake, and the provinces of Quezon, Camarines Sur, Albay, Samar, Leyte, Nueva Ecija, and Iloilo.

The addition of *C. batrachus* COI sequences from Pantabangan, Nueva Ecija, increased the number of Philippine *C. batrachus* samples. This group now represents seven localities all over Luzon, which adds support to the distinct genetic lineage formed by *C. batrachus* from the Philippines. Philippine and Thai specimens of *C. batrachus* may actually represent different species due to the >10-fold higher genetic distance between them compared to the average distance within groups (Hebert et al. 2004). Sequences of *C. batrachus* from India were even more diverged. One group of sequences formed a distinct clade (India-1) with 99% bootstrap support. The average distances of this clade with Philippine and Thai sequences were 10.4% and 11.3%, respectively. The second group (India-2), together with the COI sequence from Vietnam, clustered with *C. macrocephalus* sequences with 99% bootstrap support. Two of the sequences from India were identical with some *C. macrocephalus* specimens from Thailand, while the others, including the sequence from Vietnam, were closer to the *C. macrocephalus* specimens from the Philippines (Figure 1). One *C. batrachus* sequence from India (India-3) did not cluster with either of the first two Indian subgroups, but was closer to Philippine and Thai sequences with average distances of 1.5% and 3.6%, respectively.

Ng and Kottelat (2008) observed that *C. batrachus* from Northeastern India had the same head shape and pectoral spine serration as *C. magur* and should be designated to the latter species. Eight *C. batrachus* sequences from India have map coordinates data from GenBank. Two of the India-1 sequences had map coordinates (JN628880, 924), and both were from Northeast India. Four out of the six India-2 sequences (FJ459456-59) were from Central India, while the other two India-2 sequences (JQ667517-18) were from West India. Based on these data, the India-1 clade in this study may actually be the *C. magur* described by Ng and Kottelat (2008) from Northeast India. This subgroup formed a distinct clade with 99% bootstrap support (Figure 1). The average genetic distances between India-1 specimens and other species or subgroups ranged from 7.5% to 13.5%. None of the India-2 specimens

were obtained from Northeast India, and their average distance from India-1 sequences was 13.0%.

India-2 specimens clustered with *C. microcephalus*, which has not yet been reported in India (Figure1; Table 2). It is possible that the lack of reports on *C. macrocephalus* is due to the widespread mislabeling of catfish species as *C. batrachus*, as in the case of other fish species (Smith et al. 2008; Maralit et al. 2013). The *C. batrachus* sequence from Vietnam was likewise grouped with *C. macrocephalus*. Kottelat (1998) claimed that the *C. batrachus* found in Vietnam were also misidentified (Table 2). Kottelat (2001) listed *C. fuscus* as the only *Clariidae* species in Vietnam, citing Pellegrin (1907) who identified the species as *C. macrocephalus*. Although *C. fuscus* is native to Vietnam, it is possible that Pellegrin (1907) also examined specimens correctly identified as *C. macrocephalus*.

One sequence from India (India-3) grouped more closely to *C. batrachus* from the Philippines and Thailand than to India-1 and India-2 sequences. This observation suggests that actual *C. batrachus*is present in India, apart from those reported as misidentifications. The 1.5%- and 3.6%-distance of this sequence from the Philippine and Thai samples indicate geographic divergence.

Ng and Kottelat (2008) also suggested three provisional species making up what is widely recognized as *C. batrachus*. They designated a neotype of *C. batrachus* from Java and species from mainland Southeast Asia, including Thailand, as *C.* aff. *batrachus* "Indochina". The species from the rest of Sundaic Southeast Asia were assigned as *C.* aff. *Batrachus* "Sundaland." Ng and Kottelat (2008) were unable to analyze Philippine samples. However, they were able to analyze specimens from Insular Malaysia and Borneo and assigned them to *C.* aff. *Batrachus* "Sundaland." Based on the geographic proximity of the Philippines to Borneo, the Philippine samples may be more similar to *C.* aff. *Batrachus* "Sundaland." The calculated average distance supports the distinction of Philippine samples from those of Thailand.

Of the three *C. fuscus* specimens from China, two formed a distinct clade with 99% bootstrap support (Figure 1), while one sequence grouped more closely with *C. batrachus* from the Philippines. The average distance between the two subgroups is 8.3%. Although Herre (1953) lists *C. fuscus* as native to the Philippines, Ng (1999) and Sudarto et al. (2004) claimed that this is most likely a case of misidentification, since *C. fuscus* is a known Chinese taxon. Because the diverging sequence showed close similarity with Philippine samples, it is possible that the accession from the Yunnan province is actually a *C. batrachus* specimen introduced

from Sundaic Southeast Asia. Importation of *C. batrachus* to China has been documented by Brummett (2008).

Among the *Clarias* species included in this study, the African catfish *C. gariepinus* has the most sequences available in GenBank. A total of 70 COI sequences of the species from eight countries were used (Table 1). Among these, *C. gariepinus* is native to Nigeria, Ethiopia, and Turkey, and is exoticto the rest. All 70 sequences clustered together with 99% bootstrap support and the average within-species distance is 0.98%. No case of misidentification was observed in this study. There is also no case of misidentification documented in Fishbase (Froese and Pauly 2014). The large-scale importation of *C. gariepinus* to many countries, including the Philippines, is due to its superior size (Juliano et al. 1989; Vitule et al. 2006).

In contrast to *C. gariepinus,* the distribution of *C. macrocephalus* is limited to Southeast Asia with little reports of importation. It is inferior in size and is threatened by exotic larger-sized competitors (Vidthayanon and Allen 2013). Apart from the 20 generated in this study and the five of Quilang and Yu (2015), only 17 COI sequences were available from GenBank for this species. Sequences from the Philippines formed a clade distinct from the Thai sequences. The average within-group distances for the Philippine and Thai sequences were 0.04% and 0.60%, respectively (Table 4), while the average distance between the two subgroups was 0.8%. These values show that the two subgroups belong to the same species. The *C. macrocephalus* sequences from Thailand show greater diversity compared to *C. macrocephalus* from the Philippines. As discussed earlier, it is important to assess the genetic diversity of this threatened species for management and conservation purposes.

The other *Clarias* species, namely *C. angolensis, C. camerunensis, C. dussumieri, C. gabonensis, C. jaensis, C. nieuhofii,* and *C. teijsmanni*, were also delineated based on COI sequences. The average within-species distances ranged from 0.0% to 0.64% (Table 4), while the average between-species distances ranged from 5.2% to 13.9% (Table 5). Thus, DNA barcoding can be used for the identification of the above mentioned species, as well as *C. gariepinus* and *C. macrocephalus* as previously discussed. Further taxonomic review is necessary for *C. batrachus* and *C. fuscus*.

Status of Philippine Clarias Species

Six *Clarias* species have been reported in the Philippines (Table 6): five of these were reported as native species (Herre1953), while one is introduced (Juliano et al.

1989). As was dicussed earlier, the status of *C. batrachus, C. macrocephalus*, and *C. gariepinus* were confirmed in recent collections. The three other species, namely *C. nieuhofii, C. fuscus,* and *C. meladerma*, were not present in recent collections in various freshwaters of Luzon (Quilang and Yu 2015), thus raising questions regarding the past reports. FishBase (Froese and Pauly 2014) lists these species as native to the Philippines citing Herre (1953), but these were not listed by Conlu (1986). Herre (1953) reported the presence of *C. nieuhofii*, under the synonyms *C. nieuhofii* and *C. gilli*, from Luzon and Mindanao. Although there are no recent reports of this species in Luzon, its presence in Mindanao freshwaters is yet to be verified. Sudarto et al. (2004) were able to examine a type specimen of this species from Mindanao. The morphological features of this type specimen were characteristic of one belonging to the species *C. nieuhofii*. This finding confirms that the early reports of this species in the country are correct, but whether or not this species is still present in the country today remains to be verified. Herre (1953) reported the presence of *C. fuscus* in the country, citing Fowler (1941). Ng (1999), on the other hand, suggested that the *C. fuscus* identified by Fowler (1941) might actually be a

Table 6. List of reported *Clarias* species in the Philippines and their current status

	Previous report	Current status
C. batrachus	Native (Herre 1924; Herre 1953)	Verified native; currently present (Quilang and Yu 2015; this study)
C. macrocephalus	Native (Herre 1953)	Verified native; currently present (Quilang and Yu 2015; this study)
C. fuscus	Native (Fowler 1941; Herre 1953)	Questionable Probable introduction (Cui and Zhao 2013) Probable misidentification (Ng 1999) Not listed (Conlu 1986)
C. meladerma	Native (Herre 1953)	Questionable Listed as present in the country (Rainboth 1996) Not listed (Conlu 1986; Ng 2013)
C. nieuhofii	Native (Herre 1924; Herre 1953; Fowler 1941)	Verified native; current presence not verified (Sudarto et al. 2004) Not listed (Conlu 1986)
C. gariepinus	Introduced (Juliano et al. 1989)	Verified introduced; currently present (Quilang and Yu 2015; this study)

case of misidentification. Interestingly, IUCN lists *C. fuscus* as an introduced species in the country (Cui and Zhao 2012). This report is also questionable because Cui and Zhao (2012) cited no reference for the Philippines, but they did so for the introduction of *C. fuscus* to Hawaii and Japan. With no other information available, the presence of *C. fuscus* in the Philippines could not be confirmed. Herre (1953) also noted previous reports of *C. meladerma* in the Philippines, particularly in Laguna de Bay. He indicated that the actual presence of the species in the country is "doubtful." No *C. meladerma* specimen was observed in the catfish samples obtained from Laguna de Bay in recent years (Quilang and Yu 2015). Rainboth (1996) included the Philippines in the geographic distribution of *C. meladerma*, but did not further specify the actual location nor cite any previous reports. IUCN, on the other hand, did not list *C. meladerma* as present in the Philippines (Ng 2013). As such, the presence of *C. meladerma* in the Philippines could not be confirmed.

CONCLUSIONS

Based on the COI sequences generated from this and past studies, it is clear that the Philippine specimens of *C. batrachus* and *C. macrocephalus* form distinct lineages, thus confirming their native status, despite reports of introductions of the former. The Philippine sample of *C. gariepinus* clustered with *C. gariepinus* from other countries, where the species was also introduced and somewhat divergent from native populations. With the questionable status of *C. fuscus* and *C. meladerma*, it is best to limit the list of native *Clarias* species to three, namely *C. batracus, C. macrocephalus,* and *C. nieuhofii*, until concrete evidence of the presence of the former two species is reported. The use of highly polymorphic markers like mitochondrial control region and microsatellite DNA is recommended to further assess the seemingly low genetic diversity of *C. macrocephalus*, which is currently listed as threatened. Likewise, the current presence of *C. nieuhoffi* in the Philippines still has to be confirmed, and if so, the genetic diversity of this will have to be assessed. The COI barcodes of Philippine and Thai samples of *C. batrachus* should also be compared with those from Java and the rest of Southeast Asia in order to confirm their species identities.

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