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Abstract: *Clarias macrocephalus* Günther, 1864 is a Near Threatened freshwater catfish found in the Philippines and other Southeast Asian countries. Its numbers have dwindled over the past few years because of habitat loss and competition. This study examined the genetic diversity of the remaining viable populations of *C. macrocephalus* in the Philippines. Primers were designed to amplify via polymerase chain reaction (PCR) the complete mitochondrial DNA (mtDNA) control region (870-bp) in 120 specimens collected from three sites: (1) Buguey, Cagayan; (2) Camalaniugan, Cagayan; and (3) Agusan del Sur. Of the 120 sequences generated, only three haplotypes and two polymorphic sites were found. Overall haplotype and nucleotide diversity ($h=0.479$, $\pi=0.00058$) were alarmingly low, consistent with populations of other freshwater fishes that have experienced a genetic bottleneck. The overall F_{ST} value was 0.80050, indicative of large genetic differentiation between populations. The very low genetic variation found in all three *C. macrocephalus* populations calls for conservation and management efforts for the protection of the remaining populations of this economically important species.

Keywords: Aquaculture, fish, genetics, wetland.

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Author Contribution: MTCT was directly involved in laboratory work, data analysis, and in preparation of the manuscript. JCJ was involved in sampling and in preparation of the manuscript. JPQ was involved in designing the study, obtaining funding, sampling, directing and supervising laboratory work and data analysis, and preparation and revision of the manuscript.

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INTRODUCTION

Clarias macrocephalus Günther, 1864, commonly known as Bighead Catfish, is a freshwater catfish native to Thailand, Laos, Cambodia and Vietnam. It was introduced for aquaculture purposes to peninsular Malaysia, China, Guam, and the Philippines (Teugels et al. 1999). Vidthayanon & Allen (2013) also noted that there are records of the species in Myanmar, Japan, China, Indonesia (Sumatra), Guam and the Philippines, which could be either introductions or misidentifications. Teugels et al. (1999) cited Conlu (1986) as the source of information for the introduction of *C. macrocephalus* into the Philippines, although Conlu (1986) considered the species endemic (page 4) and native (p. 72) to the Philippines, which is also erroneous because the species is clearly not endemic to the Philippines. FishBase (Froese & Pauly, 2016) lists the species as native to the Philippines and cites Conlu (1986) as reference. Roxas & Martin (1937), Fowler (1941), and Herre (1953) listed *C. macrocephalus* in their checklist of Philippine fishes and all of them cited Meyer (1885) as reference who reported the presence of the species in Laguna de Bay, the largest lake in the Philippines. Herre (1953), however, doubted its presence in Philippine waters. Herre was able to examine and identify specimens of *Clarias batrachus* but not of *Clarias macrocephalus* from Laguna de Bay (Herre 1934), Taal Lake and Naujan Lake (Herre 1927), municipalities in Cagayan, and in many other places in the Philippines (Herre 1924; Herre 1953). Delmendo and Bustillo (1968), Vallejo (1985), and Aquino et al. (2011) reported the presence of *C. batrachus* in Laguna de Bay, but not of *C. macrocephalus*. Currently, *C. macrocephalus* is widely believed to be a native species in the Philippines (Vallejo, 1985; Conlu, 1986; Juliano et al. 1989). The species was reported in Laguna de Bay (Conlu 1986), Lake Taal (Mercene 1997), Lake Manguao, Palawan (Matillano 2003), Cuyapo, Nueva Ecija (Froese & Pauly 2016), Lake Lanao in Mindanao (Rosagaron 2001), Bicol Region (Southeast Asian Fisheries Development Center 1999), Aurora (Tayamen 2007), and island provinces in Visayas and Mindanao (Tayamen 2007). Juliano et al. (1989) reported an introduction of catfish, identified as *C. batrachus*, from Thailand in 1972. They claimed that since the introduction of the exotic species *C. batrachus*, populations of the native freshwater catfish *C. macrocephalus* had started to decline. Juliano et al. (1989) and also Vallejo (1985) erred in claiming that *C. batrachus* is an exotic species in the Philippines because even before the supposed introduction of the species

in 1972, Herre (1924, 1927, 1934, 1953) had reported its presence in the Philippines. In fact, Herre (1924) included *C. batrachus* in his list of true freshwater fishes of the Philippines; notably, *C. macrocephalus* was not on this list. Assuming misidentification by Juliano et al. (1989), there is a possibility that the catfish introduced from Thailand in 1972 was *C. macrocephalus* and not *C. batrachus*. Assuming also misidentification by Meyer (1885), reports of the presence of *C. macrocephalus* in the Philippines since 1972 (Conlu 1986; Mercene 1997; Southeast Asian Fisheries Development Center 1999; Rosagaron 2001; Matillano 2003; Tayamen 2007; Quilang & Yu 2015; Santos et al. 2015; Froese & Pauly 2016) could be due to this introduction. Recently, studies on DNA barcoding of Philippine catfishes (Quilang & Yu 2015; Santos et al. 2015), reported the presence of the species only in Cagayan Province in North Luzon and in Agusan Marsh, Agusan del Sur in Mindanao despite extensive sampling activities throughout the Philippines. Molecular identification of the specimens through DNA barcoding using the mitochondrial cytochrome c oxidase I (COI) gene was congruent with morphological identification. Mitochondrial COI sequences of specimens from the Philippines clustered with those from Thailand and the computed Kimura 2-Parameter (K2P) genetic distances provided further support that the specimens from the Philippines belong to the same species, i.e., *Clarias macrocephalus*, as those from Thailand. Misidentification of Philippine specimens was therefore ruled out by using both morphological and molecular methods. Hence, the presence of *C. macrocephalus* in the Philippines was established definitively by these studies.

Over the past few years, a continuous decline in its numbers has been observed. The species was once described by Conlu (1986) as widely distributed in the Philippines, but its distribution has now become limited. *C. macrocephalus* was once a popular food fish but nowadays they are seldom sold in markets. *Clarias macrocephalus* has been experiencing a loss of suitable habitat, which is leading to its dwindling numbers (Vidthayanon & Allen 2013). In the 2012 International Union for Conservation of Nature (IUCN) Red List of Threatened Species, *C. macrocephalus* was designated as Near Threatened (NT) due to the decline in its populations all over Southeast Asia (Vidthayanon & Allen 2013). In the Philippines, *C. macrocephalus* used to be found in abundance in ponds, rice fields and slightly brackish water (Conlu 1986), but most of these areas have become polluted because of improper waste disposal. Urbanization may have also contributed

to habitat loss, since rice fields are increasingly being converted into residential subdivisions. The decline in the abundance of *C. macrocephalus* can also be attributed to competition from other catfish species being introduced in the Philippines for fish farming. Species like *Clarias batrachus* and *C. gariepinus* are now more common and more popular compared to *C. macrocephalus*. *Clarias macrocephalus* is generally smaller in size compared to the other two *Clarias* species. *Clarias* species are also known to hybridize. A hybrid between *C. macrocephalus* and the African catfish *C. gariepinus* has been reported in Thailand (Na-Nakorn et al. 2004a). Studies have been made on the impact of hybrids on *C. macrocephalus* populations. It was found that the hybrids between *C. macrocephalus* and *C. gariepinus* grow faster than *C. macrocephalus* (Na-Nakorn et al. 2004a). There is also a growing fear that the farmed hybrids of *C. macrocephalus* and *C. gariepinus* might cause genetic introgression in populations of *C. macrocephalus* in the wild. This genetic introgression may cause the local extinction of *C. macrocephalus* if the hybrid spreads uncontrollably (Senanan et al. 2004; Na-Nakorn et al. 2004b).

Few studies have been done on *C. macrocephalus* populations in the Philippines, with most studies examining physiology. Genetic diversity studies of *C. macrocephalus* have been done in Thailand (Na-Nakorn et al. 2004b) and Malaysia (Nazia et al. 2010), but not the Philippines. Its declining numbers and limited distribution in the Philippines makes its genetic diversity an important area to study, since genetic diversity is a major factor in the adaptability of species (Ardestani et al. 2014). This study aimed to determine the genetic diversity of *C. macrocephalus* in the Philippines and to check if genetic bottlenecks may have occurred. Data from this study can be used as baseline information for the construction of proper conservation and management strategies to prevent the continuous decline of *C. macrocephalus* and other freshwater fish populations in the Philippines.

MATERIALS AND METHODS

Sample collection and tissue extraction

Forty (40) specimens of *Clarias macrocephalus* were collected from each of three sites (Fig. 1), namely, (i) Paddaya, Buguey, Cagayan (18.26N & 121.89E), (ii) Minanga, Camalaniugan, Cagayan (18.27N & 121.73E), and (iii) San Marcos, Bunawan, Agusan del Sur (8.21N & 125.96E). Specimens were obtained from local

fishermen and fish vendors in these areas. Despite repeated efforts to collect specimens from other major water bodies in the Philippines such as Laguna de Bay and Taal Lake (sites which were previously reported by some authors as having *Clarias macrocephalus*) and provinces such as Quezon, Aurora, Camarines Sur, Albay, Samar, Leyte, Nueva Ecija, and Iloilo, no specimens were collected from these areas. Specimens were identified using morphological descriptions of Conlu (1986), Teugels et al. (1999), Sudarto & Pouyaud (2005) and Ng & Kottelat (2008). One of the morphological features used to distinguish specimens of *Clarias macrocephalus* from other *Clarias* species is the presence of rounded occipital process (Image 1). Morphological identification was also confirmed using DNA barcoding, the results of which were published elsewhere (Quilang & Yu 2015; Santos et al. 2015). Briefly, mtDNA cytochrome c oxidase I (COI) gene sequences of 20 of the specimens from Agusan Marsh (with assigned GenBank Accession nos. KU495710 through KU495729) and of five specimens from Cagayan (GenBank Accession nos. KF604662 to KF604666) were analyzed together with 17 COI sequences of *C. macrocephalus* from Thailand (GenBank Accession nos. JF292321 through JF292337), and all COI sequences of other *Clarias* species available in GenBank. COI sequences of *C. macrocephalus* from the Philippines and Thailand clustered together and had an average intraspecific Kimura 2-Parameter (K2P) genetic distance of 0.5%, a genetic distance that is characteristic of specimens belonging to the same species.

A small piece of epaxial white muscle tissue was excised from each specimen, placed in a microfuge containing absolute ethanol, and stored in the freezer until further use.

DNA extraction, primer design, and PCR amplification

Approximately 20mg of muscle tissue was used for DNA extraction using the Promega Wizard® SV Genomic DNA Purification Kit (Madison, WI). Initially, the universal primers (Meyer et al. 1994; Palumbi et al. 2002) L15995 (5'-AACTCTCACCCCTAGCTCCCAAAG-3') and 12SARH (5'-ATAGTGGGGTATCTAATCCCAGTT-3') were used to generate sequences for the design of specific primers to amplify the entire mtDNA control region as well as the flanking regions coding for tRNA-Proline and tRNA-Phenylalanine. The sequences obtained were then aligned to the sequences downloaded from GenBank of the following closely-related catfish species: *Amblydoras gonzalezi* (family Doradidae; GenBank Accession NC 015745), *Bunocephalus coracoideus* (Aspredinidae; NC 015811), *Centromochlus perugiae* (Auchenipteridae; NC

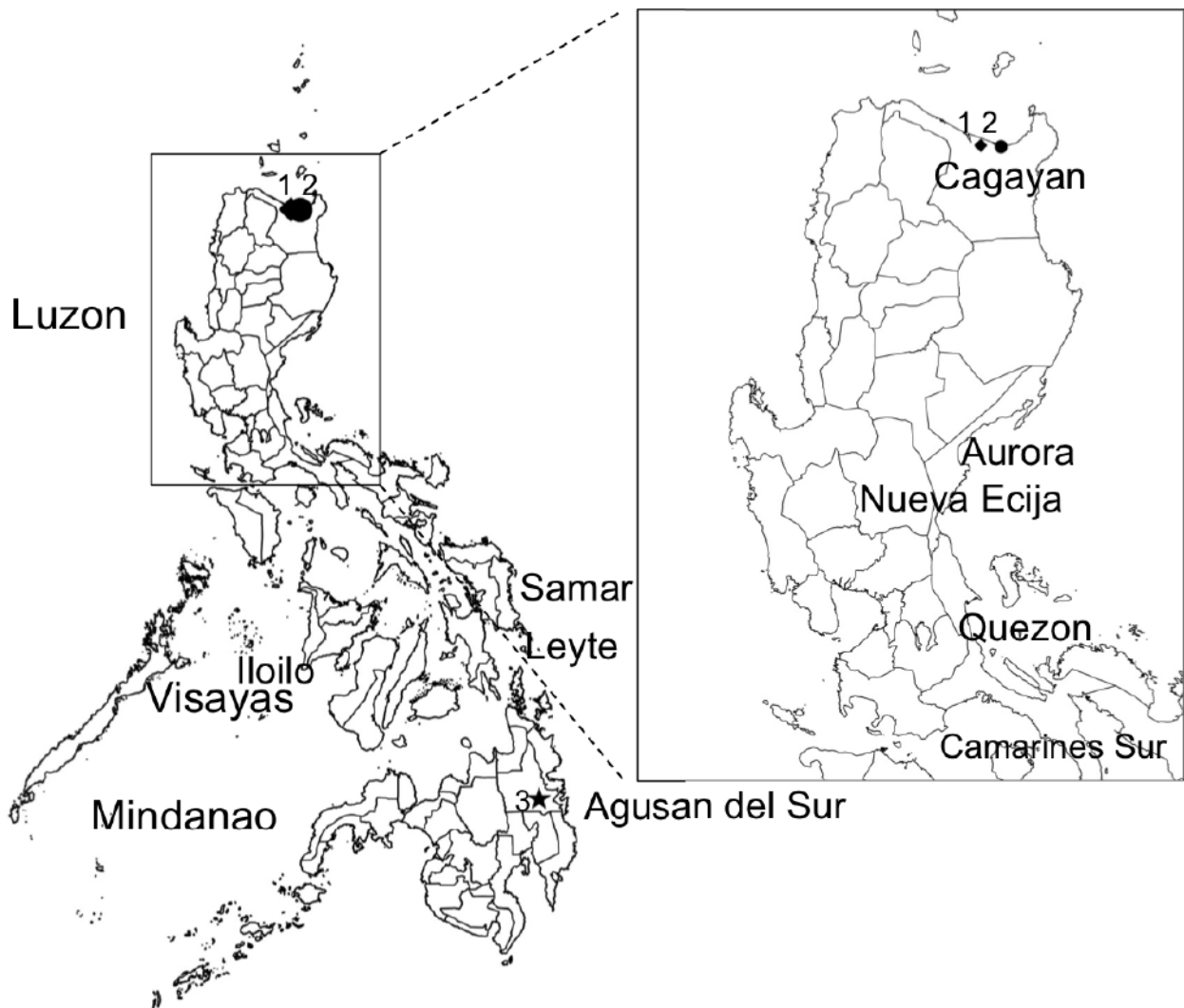


Figure 1. Location of sampling sites: 1 - Camalaniugan, Cagayan; 2 - Buguey, Cagayan; 3 - Bunawan, Agusan del Sur.

015748), *Clarias* sp. (Clariidae; AP 012010), *Hemibagrus macropterus* (Bagridae; NC 019592), *Heteropneustes* sp. (Heteropneustidae; AP 012013), *Pangasianodon gigas* (Pangasiidae; NC 006381), *Pangasius larnaudii* (Pangasiidae; AP 012018), *Silurus glanis* (Siluridae; NC 014261), and *Tetranematichthys quadrifilis* (Auchenipteridae; AP 012025). The aligned sequences were searched for conserved regions for primer design. PCR primers were designed using Primer3Plus software (Untergasser et al. 2007). The specific PCR primers designed for *Clarias macrocephalus* can amplify the whole mitochondrial control region and a part of the flanking tRNA-Proline and tRNA-Phenylalanine regions. The sequences of the primers designed are as follows:

CMCR01H (5'-GGGTTTCATCTTAACATCTTCAGTG-3') and LCMCR01 (5'-AGAGATTTTAACCTCCACCCC-3').

PCR reactions of 25 μ l volumes were done. The 25 μ l

PCR reaction consisted of the following components: 2 μ l DNA sample, 0.5 μ l dNTP (0.05mM), 1.25 μ l forward primer LCMCR01 (0.1mM), 1.25 μ l reverse primer CMCR01H (0.1mM), 2.5 μ l 1 \times PCR Buffer, 0.125 μ l *Taq* polymerase (Roche *Taq* dNTPack), 17.375 μ l ultrapure water. The samples were subjected to the following PCR conditions: preliminary denaturation at 95 $^{\circ}$ C for 2 min followed by 30 cycles consisting of denaturation at 94 $^{\circ}$ C for 45s, primer annealing at 54 $^{\circ}$ C for 30s, and primer extension at 72 $^{\circ}$ C for 1 min. This was followed by a final extension step at 72 $^{\circ}$ C for 10 min, and storage at 4 $^{\circ}$ C. PCR products were visualized using 1% agarose gel with ethidium bromide. Gel electrophoresis was run for 35 minutes in constant 85-V voltage. The expected PCR product was excised for gel extraction procedures. Gel extraction and PCR product purification was done using QIAGEN QIAquick Gel Extraction kit (QIAGEN, Valencia,

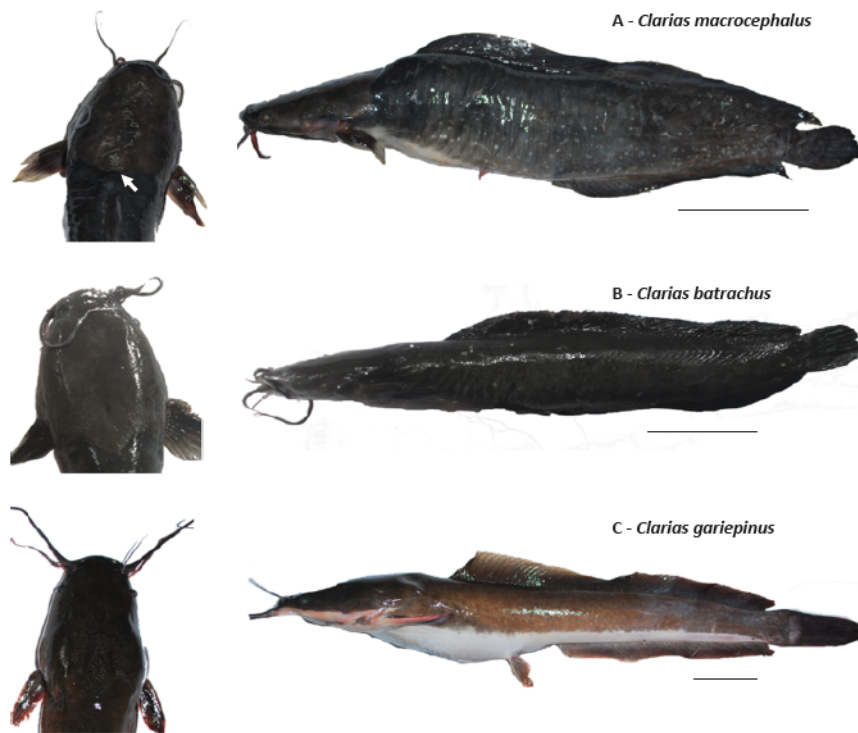


Image 1. Morphological comparisons between *Clarias macrocephalus* and two other common catfishes in the Philippines. Note the rounded occipital process (arrow) of *C. macrocephalus* collected from Cagayan. *C. batrachus* and *C. gariepinus* specimens were collected from Cagayan and Bulacan provinces in the Philippines, respectively. (scale = 5cm). © Jonas P. Quilang

CA) following the manufacturer's protocol. Purified PCR products were sent to First BASE laboratories (Selangor, Malaysia) for bidirectional DNA sequencing.

DNA sequence analysis

DNA sequences were processed using the Staden package (Staden 1996; Staden et al. 2003). The forward and reverse sequences were aligned and a consensus sequence was produced. The consensus sequence was then subjected to nucleotide BLAST (BLASTn) to compare with other sequences in GenBank and to ensure the sequences were from *C. macrocephalus*. MEGA v.6 (Tamura et al. 2013) was used to align all consensus sequences. DNA Sequence Polymorphism (DnaSP) version 5.10 (Librado & Rozas 2009) was used to determine overall DNA polymorphism and to estimate nucleotide diversity (π) and haplotype diversity (h). Computation of pairwise F_{ST} , Analysis of Molecular Variance (AMOVA), Tajima's D, Fu's F, and mismatch distribution tests were performed using Arlequin version 3.5 (Excoffier & Lischer 2010). NETWORK 4.612 (Bandelt et al. 1999) software was used to construct the Median-joining network for the unique haplotypes. All 120 sequences generated from this study have been submitted to GenBank and were assigned accession numbers from KM363261 to KM363380.

RESULTS

The entire mitochondrial control region (870-bp) was used for analysis. Only two variable sites were found from the 870 aligned sites. Out of the 120 control region sequences, only three distinct haplotypes were found. Two haplotypes were found in Agusan specimens, two in Buguey and one in Camalaniugan. Only haplotype 2 was common to all sites. Haplotype 1 was found only in Buguey, while haplotype 3 was found only in Agusan specimens. Table 1 shows the frequencies of each haplotype per population. Haplotype 2 was the most common, haplotype 1 the least common. The dorsal head view and left body side of representative specimens from each haplotype are shown in Image 2. No detectable morphological differences were observed between haplotypes.

The Median-joining network analysis for the three haplotypes is shown in Fig. 2. Haplotype 2 was determined to be the parent haplotype and was common to all three sites. Haplotypes 1 and 3 are shown to be exclusive to Buguey and Agusan specimens, respectively. Fig. 2 also shows that haplotypes 1 and 3 diverged from the parent haplotype.

The measures of genetic diversity in populations of *C. macrocephalus* are shown in Table 2. Overall haplotype diversity (h) and nucleotide diversity (π)

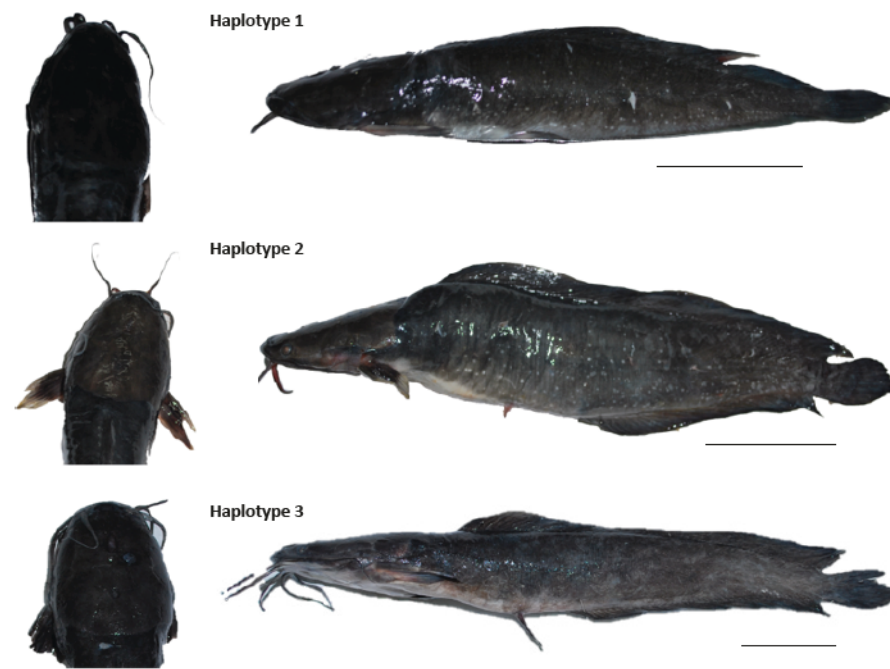


Image 2. Representative specimen from each *Clarias macrocephalus* mitochondrial DNA control region haplotype. Haplotype 1 specimen came from Buguey, Cagayan while specimens for haplotypes 2 and 3 came from Camalaniugan and Agusan del Sur, respectively (scale = 5 cm)

Table 1. Frequencies of haplotypes in each of three populations of *Clarias macrocephalus* in the Philippines. $n = 40$ per population.

Haplotype	Agusan	Buguey	Camalaniugan	Total
1	0	5	0	5
2	4	35	40	79
3	36	0	0	36

for all specimens in all the three sites were 0.479 and 0.00058, respectively. Specimens from Buguey had the highest haplotype and nucleotide diversity values. On the other hand, specimens from Camalaniugan had zero values for both haplotype and nucleotide diversity due to the presence of only one haplotype. Overall F_{ST} value was computed to be 0.80050. Results of AMOVA (Table 3) show that variation among populations (80.05%) was higher compared to variation within populations (19.95%) and that these results are significant ($P=0.00$). Tajima's D and Fu's F tests for neutrality were also done (Table 4). Mutations in the mitochondrial control region were determined to be neutral ($D = -0.12393$, $P = 0.52700$; $F = 0.17894$; $P = N.A.$).

Results of the mismatch analysis (Table 5, Fig. 3) showed that both Agusan and Buguey populations conformed more to the spatial expansion model rather than to the sudden expansion model. Mismatch distribution analysis cannot be done for the Camalaniugan population because of the presence

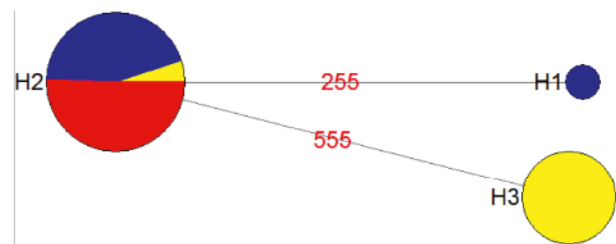


Figure 2. Median-joining network analysis for the three unique haplotypes of *Clarias macrocephalus* from the Philippines. The analysis was generated using NETWORK 4.612 software. Each node represents a single haplotype (H1, H2, H3, for haplotypes 1, 2, and 3, respectively). The mutated nucleotide positions are shown along the lines connecting the haplotypes. Divisions of each node are proportional to the frequency of each haplotype. Designated colors for each population: yellow—Agusan, blue—Buguey, red—Camalaniugan.

of only one haplotype. Pairwise F_{ST} values between populations were also computed (Table 6). The highest F_{ST} value was between populations from Agusan and Buguey, while the lowest F_{ST} value was between populations from Buguey and Camalaniugan.

The presence of deformities such as truncated caudal fins and abnormal body shapes, was also noted. Two specimens from Agusan, three from Buguey, and two from Camalaniugan had deformities.

Table 2. Measures of genetic diversity in three populations of *Clarias macrocephalus* in the Philippines.

Population	<i>n</i>	<i>H</i>	<i>h</i>	π	Number of segregating sites
Agusan	40	2	0.185	0.00021	1
Buguey	40	2	0.224	0.00026	1
Camalaniugan	40	1	0.000	0.00000	0
Overall	120	3	0.479	0.00058	2

n, sample size; *H*, number of haplotypes; *h*, haplotype diversity; π , nucleotide diversity

Table 3. Analysis of Molecular Variance (AMOVA) within and between three populations of *Clarias macrocephalus* in the Philippines

Source of variation	<i>df</i>	Sum of squares	Variance components	% of variation	<i>P</i>
Among populations	2	22.017	0.27350	80.05	0.00
Within populations	117	7.975	0.06816	19.95	
Overall	119	29.992	0.34167		

Table 4. Results of Tajima's *D* and Fu's *F* tests

Population	Tajima's <i>D</i>		Fu's <i>F</i>	
	<i>D</i>	<i>P</i> value	<i>F</i>	<i>P</i> value
Agusan	-0.30658	0.27700	0.12935	0.31000
Buguey	-0.06522	0.30400	0.40747	0.33600
Camalaniugan	0.00000	1.00000	0.00000	N.A.
Overall	-0.12393	0.52700	0.17894	N.A.

DISCUSSION AND CONCLUSION

In this study, *Clarias macrocephalus* was found to have low values for both haplotype diversity ($h = 0.479$) and nucleotide diversity ($\pi = 0.00058$). These results indicate that *C. macrocephalus* populations have experienced genetic bottlenecks. Large overall genetic differentiation ($F_{ST} = 0.80050$) was also observed, which indicates that Agusan populations have diverged from the two populations from Cagayan. The high genetic differentiation can be explained by the physical isolation of Agusan populations from the two Cagayan populations (Buguey and Camalaniugan). The longer the separation of the two populations, the more variation can be observed (Ardestani et al. 2014). Agusan is found in Mindanao, which is at the Southern part of the Philippines, while Cagayan province is found in Luzon, which is at the Northern part of the country. Barring human intervention, it is not possible for gene flow to

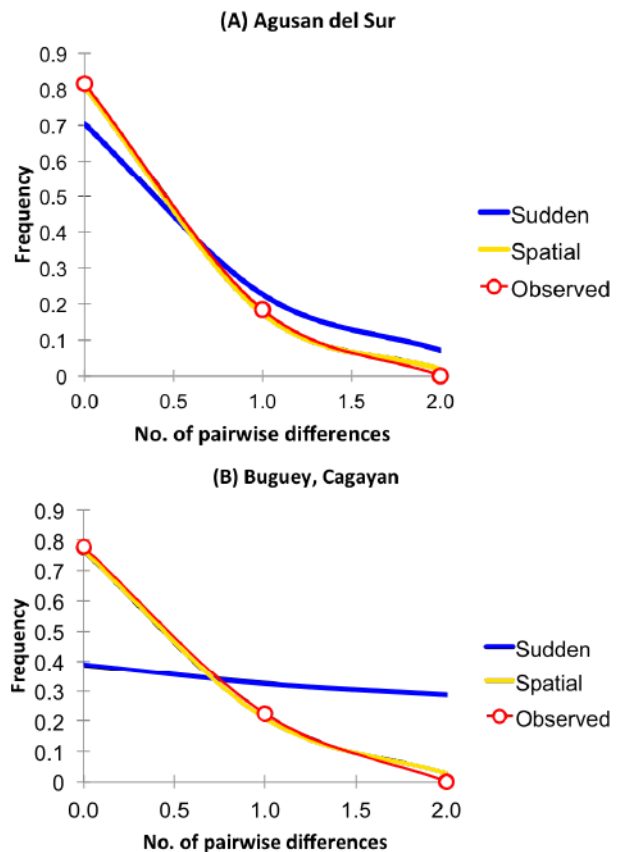


Figure 3. Mismatch distribution analysis showing observed values (red) and expected values under sudden expansion model (blue) and spatial expansion model (yellow) for *Clarias macrocephalus* populations in Bunawan, Agusan del Sur and Buguey, Cagayan. There was no model generated for Camalaniugan, Cagayan population due to the presence of only one haplotype.

occur between the southern and northern populations since they are separated by both land and water barriers. Populations of freshwater fishes that have experienced genetic bottleneck resulting in low values for both haplotype and nucleotide diversities include those of Cape Hake *Merluccius paradoxus* from Namibia and South Africa ($h = 0.53$, $\pi = 0.0014$; von der Heyden et al. 2010), *Sinocyclocheilus graham* (Cyprinidae) from Fumin, China ($h = 0.197$, $\pi = 0.002$; Chen et al. 2009), and *Poecilia orri* and *Gambusia yucatana* (Poeciliidae) from South-eastern Mexico ($h = 0 - 0.50$, $\pi = 0 - 0.003$; Vasquez-Dominguez et al. 2009).

Deformities were observed in several *C. macrocephalus* specimens. Five out of the total 80 specimens (6.25%) from Cagayan and two out of the 40 specimens (5%) from Agusan del Sur had deformities including truncated tail fins and abnormally-shaped bodies. Deformities in the skeleton, dorsal and caudal fins and sexual organs have been observed in other

Table 5. Results of Mismatch Distribution for Sudden Expansion Model and Spatial Expansion Model

Population	Sudden Expansion Model							Spatial Expansion Model					
	SSD	P (SSD)	R	P (R)	T	θ_o	θ_t	SSD	P (SSD)	R	P (R)	T	θ
Agusan	0.01667	0.23300	0.48195	0.445	2.96484	0.450	0.450	0.00022	0.17800	0.43195	0.534	0.17701	0.03969
Buguey	0.30449	0.12500	0.35425	0.137	2.92969	0.900	3.600	0.00048	0.13800	0.35425	0.467	0.27023	0.00072
Camalaniugan	0.00000	0.00000	0.00000	0.000	0.00000	0.000	0.000	0.00000	0.00000	0.00000	0.000	0.00000	0.00000

Table 6. Pairwise F_{ST} values (below diagonal) and P values (above diagonal) for three *Clarias macrocephalus* populations

	Agusan	Buguey	Camalaniugan
Agusan	-	0.00000	0.00000
Buguey	0.89744	-	0.06348
Camalaniugan	0.80050	0.10256	-

fish species, including Kirrikuchi Charr (*Salvelinus leucomaenis japonicus*; Sato, 2006), White-spotted Charr (*Salvelinus leucomaenis*; Yamamoto et al. 2013), Mosquitofish (*Gambusia affinis*; Leberg & Firmin 2008), Three-spined Sticklebacks (*Gasterosteus aculeatus*; Mehlis et al. 2012), *Poecilia reticulata* (Zajitschek & Brooks 2010), Westslope Cutthroat Trout (*Salmo clarki lewisi*; Leary et al. 1985), Rainbow Trout (*Salmo gairdneri*; Kincaid 1976), and Atlantic Salmon (*Salmo salar*; Gjerde et al. 2005). Such deformities are a manifestation of inbreeding depression caused by several generations of inbreeding in a population with low genetic diversity. Inbreeding depression can cause low resistance to parasites, higher vulnerability to environmental pressures, body deformations and higher mortality rates (Mehlis et al. 2012). Fitness-related traits (i.e., body size, body condition, growth rate, fecundity, and survival rate) may also be affected by high inbreeding rates as a result of low effective population size (Sato 2006). The deformities observed in the *C. macrocephalus* specimens are a clear indication of several generations of inbreeding. *C. macrocephalus* populations in the Philippines may be experiencing inbreeding depression as a result of the very low genetic diversity. Aside from the deformities, relatively smaller body sizes of *C. macrocephalus* were also observed among Cagayan populations compared to the Agusan population.

Similar genetic diversity analyses were done in *C. macrocephalus* populations from Malaysia and Thailand. Nazia et al. (2010) analyzed sequences from cytochrome *b* (Cyt *b*) and partial D-loop (control region) from

Malaysian populations of *C. macrocephalus*. Out of the total 1047-bp region amplified on 57 individuals, their analysis revealed 21 haplotypes and 81 polymorphic sites. Nucleotide diversity (π) was 0.003 for all populations, while haplotype diversity varied between 0.657–0.765. High *h* and low π values are characteristic of populations which have experienced periods of low effective populations followed by rapid population growth (Grant & Bowen 1998). Nazia et al. (2010) observed high levels of within-population diversity, but limited between-population variations. They also observed low genetic diversity among sub-populations. Their results indicate that although the populations are geographically isolated, a common origin or ongoing gene flow from human-mediated translocations may have caused low between-population variation. Generally, there was no genetic differentiation observed among the three populations studied in Malaysia, but local adaptations were observed through exclusive haplotypes that can be explained by independent evolution (Nazia et al. 2010).

Thailand populations of *C. macrocephalus* also experienced fluctuations in population size. Na-Nakorn et al. (2004b) found only eight polymorphic loci out of 18 isozyme loci analyzed. Their analysis revealed that variation among Thailand populations of *C. macrocephalus* is relatively small compared to averages for other bony fishes. They found that *C. macrocephalus* has lower levels of genetic variation compared with other *Clarias* species. They also found significant differences between northern and southern populations. The low diversity values for Thailand populations were attributed to geographical isolation, low effective population sizes, and genetic bottlenecks. Also, genetic introgression may also contribute to low diversity values since *C. macrocephalus* females can interbreed with *C. gariepinus* males to form hybrids, which already existed for several generations. These genetic introgressions were also observed not only in central Thailand but also near the Mekong River basin in southern Thailand (Na-Nakorn et al. 2004a). Genetic introgression may go either way for the species, as it may

be beneficial or harmful to the organism as it may cause extinction by replacement of genes or genetic mixing. Most introgressions are due to anthropogenic factors, which can also cause problems in aquaculture ventures that require pure broodstocks of fish. Introgression must be minimized and prevented to ensure that *C. macrocephalus* populations can survive.

Pairwise F_{ST} values between Agusan and Buguey ($F_{ST} = 0.89744$), and Agusan and Camalaniugan ($F_{ST} = 0.80050$) indicate that Agusan populations are genetically diverged from Camalaniugan and Buguey populations. As mentioned earlier, it is impossible for genetic exchange to occur between Agusan and Cagayan (both Buguey and Camalaniugan) populations, since both terrestrial and aquatic barriers separate Northern and Southern Philippines. On the other hand, the low pairwise F_{ST} values between the two populations from Cagayan indicate presence of gene flow that could be attributed to anthropogenic and natural causes. This is consistent with the geographic locations of the two populations from Cagayan. There is no clear geographical barrier that could preclude gene flow between Buguey and Calamaniugan populations. A similar occurrence can also be seen in *Gambusia holbrooki* in the Greater Melbourne Area, Australia, wherein only a single haplotype existed. Bunyip River populations experience occasional flooding which allows unrestricted dispersal and gene flow between populations. Founder effect can also explain the lack of genetic diversity in *G. holbrooki* (Ayres et al. 2010). This case can also be observed among *C. macrocephalus* populations in the Chaophraya river, since rainy season allows the different tributaries of the Chaophraya to form vast flood plains, there is no barrier to gene flow between different subpopulations in the Chaophraya River (Na-Nakorn et al. 2004b).

The lack of genetic diversity among *C. macrocephalus* populations in the Philippines has serious consequences. Reduced genetic diversity can be disadvantageous as it can make a species more prone to extinction. Efforts should be concentrated on making strategies for conservation and management of these threatened species. Management options for species with low genetic diversity include translocations, habitat rehabilitation or protection, captive breeding and reintroductions. Translocations are effective as these increase population size as well as genetic diversity among different populations. Habitat rehabilitation or protection allows the fish stocks to recover from anthropogenic disturbances. Captive breeding programs allow controlled breeding environments favorable to the growth and propagation of the species. Reintroduction

to areas where populations previously existed can also help in the survival of the species (Faulks et al. 2008).

The data from this study support the claim that habitat rehabilitation can help in the recovery of genetic diversity. For *C. macrocephalus*, the existence of the Agusan Marsh Wildlife Sanctuary should have provided a source for alternative copies of genes and of genetic variability, which could have been tapped for translocations, captive breeding or reintroductions. However, low genetic diversity is observed for the Agusan population despite it being a protected area. Agusan Marsh was declared a protected area and a wildlife sanctuary in 1999. The low genetic diversity values indicate that it might have been too late because the population has already experienced bottleneck effects at the time of the implementation of the wild life sanctuary. The protection should have been implemented earlier, before a bottleneck occurred.

Since each population is unique, different management strategies must be employed for different populations so that each population can be maximized (Nazia et al. 2010). In situ conservation measures such as establishment of nature reserves are done to preserve genetic diversity, while *ex situ* measures such as breeding programs are done to increase genetic diversity (Zheng et al. 2005). Different management options should be studied well before being enacted. It was observed that captive individuals may have lower diversity levels compared to wild populations, which may be caused by founder effects and low effective population sizes (Jiang et al. 2005). Vrijenhoek (1998) suggested that in situ breeding is more ideal compared to captive breeding, which requires the fish to be moved out of their natural habitats. Captive breeding should be done as a last resort for endangered species in the management of fish stocks. Breeders must also take extra precaution to prevent population bottlenecks. Poorly-managed breeding programs may result in inbreeding depression and diversity loss, which, in turn, will result in low performance, survival and reproduction rates. To prevent low reproduction rates, natural breeding systems must be taken into account. Manual translocations can also be done to facilitate gene flow between sites, especially between geographically isolated populations.

Owing to the very low genetic diversity values obtained in this study, populations of *C. macrocephalus* must be managed and conserved at the soonest possible time. The *death valley* model proposed by Vrijenhoek (1998) can determine if manual translocation is an ideal strategy for Cagayan and Agusan *C. macrocephalus*

populations. Since natural barriers separate Cagayan and Agusan populations, management of each population must be done separately. In situ strategies (e.g., habitat rehabilitation, establishment of protected areas, etc.) must be done first for all populations as a preliminary conservation effort. If these strategies fail to increase genetic diversity for *C. macrocephalus*, then ex situ strategies such as translocations, captive breeding and reintroductions must be exercised with caution and should be done only as a last resort when the species is nearing extinction. All efforts must be focused on the conservation and management of, not just *C. macrocephalus*, but also other freshwater fishes in danger of facing extinction. Increasing local awareness through education of the locals in the vicinity can relieve the pressures being exerted by anthropogenic factors.

In summary, genetic diversity analysis of *Clarias macrocephalus* populations in the Philippines showed that they have already experienced genetic bottleneck as indicated by the very low haplotype and nucleotide diversity values. The lack of genetic diversity is a serious threat to the species as it makes the species more prone to extinction. Analysis also showed that the Agusan population is genetically diverged from Buguey and Camalaniugan populations, even if one haplotype is shared by the three populations. The observed low genetic diversity in *C. macrocephalus* calls for management and conservation efforts to be mobilized as soon as possible in order to ensure the recovery of populations in the wild. Genetic diversity studies only provide a snapshot of the current situation for *Clarias macrocephalus* populations in the Philippines. What happens next would ultimately be based on what actions and plans will be enacted for the survival of *C. macrocephalus* in the Philippines.

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