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### ARTICLE

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## GENETIC DIVERSITY AMONG THE ENDEMIC BARB *BARBODES TUMBA* (TELEOSTEI: CYPRINIDAE) POPULATIONS FROM MINDANAO, PHILIPPINES

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**Abstract:** *Barbodes tumba* is an important fishery resource in Lake Lanao and nearby areas in Mindanao, Philippines. It is one of only two remaining endemic cyprinids out of 20 endemic species originally reported in the lake. In this study, specimens were obtained from fishermen and fish vendors from the lake and from three other sites in Mindanao, namely, Nunungan Lake in Lanao del Norte, Dagoyanan Lake in Lanao del Sur, and Pulangi River in Bukidnon. Eighty-seven haplotypes were recovered from the 122 complete mitochondrial DNA control region sequences analysed. All four populations showed high levels of haplotype (0.662–0.976) and nucleotide (0.552–2.736 %) diversities. Pairwise  $F_{ST}$  values showed high genetic divergence between populations. Maximum likelihood tree and median-joining network showed geographic separation of Bukidnon population from the three other populations. Lanao Lake specimens also formed a distinct group, which clustered with nine specimens from Dagoyanan Lake. All the other specimens from Dagoyanan clustered with specimens from Nunungan Lake. Despite the high intra- and inter-population genetic variation found in the species, its populations need to be protected and managed so that it will not suffer the same fate as that of the other endemic cyprinids which are now reported to be extinct.

**Keywords:** Control region, cyprinid, Lanao Lake, mitochondrial D-loop, population genetics.

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**Author contribution:** OPA-L was responsible for collection of samples, laboratory work, data analysis, and preparation of the manuscript. JPQ was involved in designing the study, directing and supervising laboratory work and data analysis, and preparation of the manuscript.

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## INTRODUCTION

*Barbodes tumba* is a popular food fish among the natives in communities living along Lake Lanao, the second largest lake in the Philippines. It is one of the two remaining endemic cyprinids of Lake Lanao. The other remaining endemic cyprinid is *B. lindog* (Ismail et al. 2014), which is now rarely caught from the lake and is believed to be on the brink extinction because of overfishing and introduction of non-native species in the lake. *Barbodes tumba* and *B. lindog* were described by Herre (1924) along with 11 other endemic cyprinids in the lake. Seven additional endemic cyprinid species were reported in the lake by Herre (1926, 1932), Fowler (1933), and Wood (1966). These Lanao endemic cyprinids have captured the attention of evolutionary biologists in the past because they were instrumental in the development of species flock concept in fishes and were cited as examples of explosive evolution (Myers 1960; Greenwood 1984; Kornfield & Carpenter 1984).

*Barbodes tumba* has been recorded in Lake Lanao in the different fish surveys reported by Villaluz (1966), Sanguila et al. (1975), Kornfield (1982), Escudero (1994), and Ismail et al. (2014). In 1996, the fish was classified as Vulnerable in the International Union for the Conservation of Nature (IUCN) Red List of Threatened Species (World Conservation Monitoring Centre 1996). Ismail et al. (2014) reported that the population of *B. tumba* in Agus River, the outlet of Lake Lanao, is stable, but the volume of fish caught from the lake in 2008 was only a small fraction (about 0.05%) of the volume recorded in 1990–1991. Ismail & Escudero (2011) assessed *B. tumba* as one of the threatened fishes of the world.

Brooks (1950), Herre (1924, 1953), and Kornfield & Carpenter (1984) reported that *B. tumba* was widely distributed in the Lanao plateau where Lanao Lake, Lake Dapao, Lake Uyaan, Lake Butig, Lake Nunungan and Sigawat River are located. Over the years, the population of *B. tumba* in Lake Lanao has seen a continuous decline. The fish is believed to be locally extinct in some of its original distribution and in Lake Dapao (Kornfield 1982) and Lake Uyaan according to locals living in communities surrounding the lakes.

Other than fish catch statistics from various market surveys, there are no published studies on life history, reproductive biology, and genetics of *B. tumba*. Population genetic studies are important to gather information on the levels of genetic diversity of the species that can be used for the management and conservation of extant populations of the species.

This study aimed to determine intra- and inter-population genetic diversity of *B. tumba*. Specifically, this study aimed to characterize the genetic structure of the different populations of *B. tumba* in Lake Lanao, Dagoyanan Lake, Nunungan Lake and Pulangi River in Bukidnon, in the island of Mindanao, Philippines using the mitochondrial control region.

## MATERIALS AND METHODS

### Description of study areas

Thirty (30) to 32 specimens (Image 1) were obtained from fishermen and fish vendors from each of four sites in Mindanao, namely: (1) Lake Lanao; (2) Nunungan Lake; (3) Dagoyanan Lake; and, (4) Pulangi River in Bukidnon province (Fig. 1). Lake Lanao is located in the province of Lanao del Sur, Autonomous Region in Muslim Mindanao (ARMM) with a latitude of 8.000°N, longitude of 124.288°E, and elevation of 702m above sea level (Frey 1969). It is the second largest freshwater lake in the Philippines and covers an area of 35,250ha and has a maximum depth of 112m and a mean depth of 60.2m (Frey 1969). The lake has four large main river tributaries that are located in the municipalities of Ragain, Taraka, Gata, and Masiu; the only outlet of the lake is the Agus River which drains into Iligan Bay. Samples were collected near the source of Agus River in Marawi City. Lake Nunungan, also known as Gadongan, is located in Mount Inayawan Range, Nunungan, Lanao del Norte, Region X with a latitude of 7.819°N, longitude of 123.950°E, and elevation of 830m above sea level. The lake has a surface area of 153ha. The lake outlet drains into the municipalities of Sapad and Lala, Lanao del Norte and then into Panguil Bay. Lake Nunungan is about 6 hours of land travel from Lake Lanao in Marawi City, Lanao del Sur to Barangay Poblacion in the Municipality of Nunungan in Lanao del Norte (about 60km distance from Marawi City). From Barangay Poblacion, one has to walk for 30 minutes to reach lake Nunungan as the road is inaccessible to vehicles. An alternative route is to travel south from Marawi City to the Municipality of Ganasi in Lanao del Sur for 1 hour by land (a distance of about 47km) and then walk up a mountain (Mt. Inayawan) for 4 to 6 hours via the Municipality of Madamba to get to Lake Nunungan. Lake Dagoyanan, also known as Dagianan, is located in the forested area of the municipality of Madalum, Lanao del Sur. It is a small lake that has a surface area of about 3ha. From Marawi City, one can go to the Municipality of Madamba by land transportation for 30 minutes and

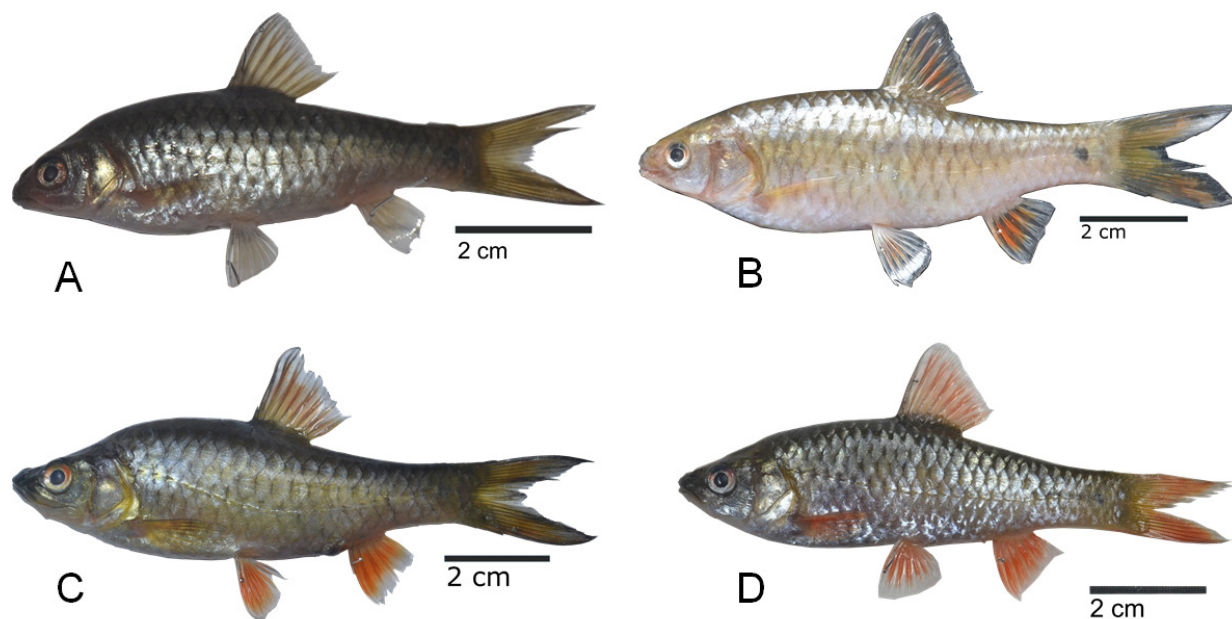


Image 1. Representative specimen from each of the four sites: A - Lake Lanao | B - Bukidnon | C - Dagoyanan Lake | D - Nunungan Lake.

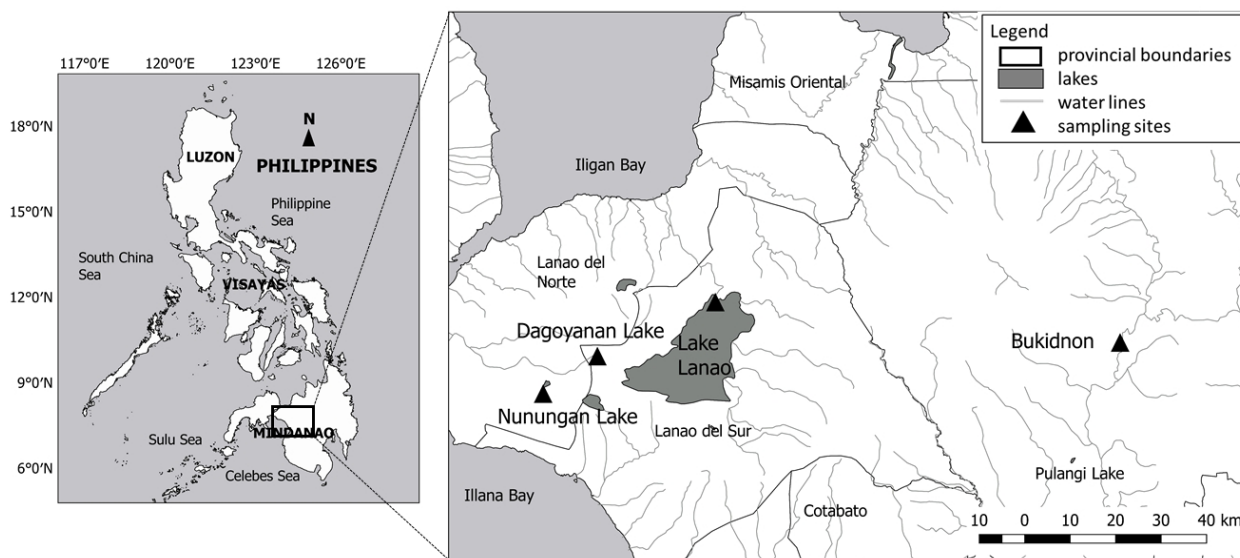


Figure 1. Location of sampling sites (shown in black triangles): Lake Lanao, Lanao del Sur; Dagoyanan Lake, Lanao del Sur; Nunungan Lake, Lanao del Norte; Pulangi River, Poblacion 1, Valencia City, Bukidnon.

then walk for another 30 minutes to Lake Dagoyanan (distance of 36km from Marawi City). The distance between Lake Dagoyanan to the nearest shore of Lake Lanao, which is located in the Municipality of Madalum, is only about 5–6 km. Lake Dagoyanan does not have any outlet. From Lake Dagoyanan, one can walk to the Municipality of Madamba for 30 minutes and then walk up Mt. Inayawan for 4–6 hours to get to Lake Nunungan. On the other hand, Pulangi River is one of the tributary

of Rio Grande de Mindanao river system in Bukidnon province, Region X. It is the longest river in Bukidnon with a total length of 320km and transverses majority of the cities of Bukidnon province. The site where the *B. tumba* samples were collected is the part of Pulangi River that transverses Poblacion 1, Valencia City, Bukidnon. The Rio Grande de Mindanao river system drains into the Illana Bay. Although Bukidnon and Lanao del Sur are adjacent provinces, the distance



between Pulangi River in Bukidnon and Lake Lanao in Lanao del Sur is about 100km. There is also a mountain (Mt. Piyagayongan) between the two provinces so that one has to get around this mountain and travel by land transportation for about six hours to get from Lake Lanao to Pulangi River.

#### DNA extraction, primer design, and PCR amplification

A small piece of white muscle tissue, 1.5cm x 0.75cm x 0.5 cm, from the upper right side of the body was excised from each specimen, placed in a microcentrifuge tube containing 100% ethanol, and stored in the freezer until further use. About 20mg of muscle tissue was subjected to DNA extraction using one of the following: (1) DNeasy Blood and Tissue (QIAGEN, Hilden, Germany) kit; (2) Promega Wizard Genomic DNA Purification (Promega Inc., Madison, WI) kit; (3) InstaGene Matrix (Bio-Rad Laboratories, USA) following the protocol of Hoff-Olsen et al. (1999) with modification; and, (4) simple salting out procedure by Miller et al. (1988) with minor modification. DNA yield was quantified using NanoDrop™ 2000/2000c Spectrophotometer.

The following pair of primers designed using Primer3plus program (Untergasser et al. 2012) were used to amplify the mitochondrial DNA control region of *B. tumba*: forward primer (LPTCR02: 5'-CCCAAAGCCAGAATTCTA-3') and reverse primer (PTCR01H: 5'-GCATCTTCAGTGCTATGCTT-3'). Polymerase chain reactions (PCR) were done in 50-μl volumes. The PCR mix consisted of 1.0μl of dNTP (0.05 mM), 2.5μl of each primer (0.1 mM), 5.0μl of PCR buffer, 0.5μl of (1.25 U) Taq polymerase (Roche Taq dNTPack or Vivantis), 34.5 μl of ultrapure water and 4.0μl of DNA template (4–100 ng/ul). The PCR conditions (Tan et al. 2016) were as follows: initiation for 2min at 95°C followed by 37 cycles of denaturation for 45s at 94°C, primer annealing for 1min at 54°C, and primer extension for 1min at 72°C. A final extension step at 72°C for 10min completed the reaction. PCR products were analysed by electrophoresis using 1% agarose gel with ethidium bromide. Approximately 1,037 bp-sized bands were excised from the gel and purified using QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) or NucleoSpin® Gel and PCR Clean-up (Macherey-Nagel, Germany) following the manufacturer's protocol. Purified DNA products with concentration of 15ng/μl or higher were sent to 1st BASE Laboratories Sdn Bhd, Selangur, Malaysia for bidirectional sequencing.

#### DNA sequence analysis

Consensus sequences of each specimen were assembled, edited and trimmed using Staden Package4 (Staden et al. 2000). The resulting consensus sequences were checked if they were the DNA sequences of interest using the online National Center for Biotechnology Information (NCBI) BLASTn program (Altschul et al. 1990). The consensus sequences were submitted to GenBank and were assigned accession numbers MG663328 through MG663449. ClustalW (Thompson et al. 1994) implemented in MEGA 7 software (Kumar et al. 2016) was used to align the 122 sequences.

Nucleotide diversity ( $\pi$ ) and haplotype diversity ( $h$ ) were computed using DNA Sequence Polymorphism version 5 (DNAsp5; Librado & Rozas 2009). To estimate molecular variances within and among populations of *B. tumba*,  $F_{ST}$  values and analysis of molecular variance (AMOVA) were performed using Arlequin version 3.5 (Excoffier & Lischer 2010). Tajima's D and Fu's  $F_s$  tests for neutrality were conducted using Arlequin version 3.5 (Excoffier & Lischer 2010). Maximum likelihood tree was constructed using the model chosen by jModelTest 2.1.9 v20160115 (Darriba et al. 2012). A median-joining network (Bandelt et al. 1999) was constructed using PopArt (Leigh & Bryant 2015) to visualize clustering, connectivity and haplotype history.

#### RESULTS

One hundred twenty-two (122) sequences of the mitochondrial DNA control region were generated from four populations of *B. tumba*. Sequence length ranged from 907 bp to 918 bp. The final alignment length that was used for the analysis was 933 including 42 indels. Of the 933 sites, 191 were variable. The composition of each nucleotide is as follows: C, 17.7%; G, 12.2%; T, 33.2%; and, A, 36.9%. Of the 122 sequences, 87 distinct haplotypes were detected (Table 1). Of the 87 haplotypes, two haplotypes (Hap59 and Hap60) were common to Dagoyanan Lake and Nunungan Lake. There were 28 unique haplotypes found in Lake Lanao population, 28 in Bukidnon population, 15 in Dagoyanan Lake, and 14 in Nunungan Lake. There were no haplotypes shared between specimens from Lake Lanao and those from the other sites. Similarly, no haplotypes were shared between specimens from Bukidnon and those from the three lakes. Overall haplotype diversity and nucleotide diversity values were 0.947 and 3.407%, respectively (Table 1). Haplotype diversity and nucleotide diversity values in each population were also high

**Table 1. Measures of genetic diversity in four populations of *Barbodes tumba* in Mindanao, Philippines.**

Population	n	Number of haplotypes	No. of unique haplotypes	Haplotype Diversity	Nucleotide Diversity (%)
Lake Lanao	30	28	28	0.995	1.075
Bukidnon	32	28	28	0.976	0.552
Dagoyanan Lake	30	17	15	0.864	2.736
Nunungan Lake	30	16	14	0.662	1.248
Overall	122	87	-	0.947	3.407

**Table 2. Analysis of Molecular Variance (AMOVA) within and between the four populations of *Barbodes tumba* in Mindanao, Philippines based on mitochondrial DNA control region.**

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	3	1500.274	16.137*	66.70
Within populations	118	950.440	8.055*	33.31
Fixation Index $F_{ST}$	0.667			

\*p-value &lt; 0.0001

**Table 3. Pairwise  $F_{ST}$  values (below diagonal) and p-values (above diagonal) for four populations of *Barbodes tumba* in Mindanao, Philippines.**

	Lake Lanao	Bukidnon	Dagoyanan Lake	Nunungan Lake
Lake Lanao	-	0.000	0.000	0.000
Bukidnon	0.832	-	0.000	0.000
Dagoyanan Lake	0.487	0.650	-	0.001
Nunungan Lake	0.760	0.822	0.146	-

(0.662–0.976 and 0.552–2.736 %, respectively, Table 1). Results of AMOVA (Table 2) showed that variation among populations (66.70%) was higher compared to variation within populations (33.31%); these results are significant ( $P < 0.01$ ). Both chi-square ( $X^2$ : 334.009,  $df = 258$ ,  $P = 0.0010$ ) and overall  $F_{ST}$  (0.62718) results showed that genetic differentiation was seen among the four populations. Based on pairwise  $F_{ST}$  values (Table 3), there is a significantly high ( $P < 0.01$ ) genetic differentiation between populations. Populations from lakes Nunungan and Dagoyanan are the least genetically diverged, whereas those from Bukidnon and Lanao Lake are the most genetically diverged.

jModelTest 2 (Darriba et al. 2012) indicated that the Hasegawa-Kishino-Yano model (Hasegawa et al. 1985) with gamma distribution (HKY85+ $\Gamma$ ) was the optimal model to use for constructing maximum-likelihood tree. The ML tree (Fig. 2) separated the 87 haplotypes into two major clusters. The first major cluster (Cluster A) consists of specimens from Bukidnon, Lake Lanao and Dagoyanan Lake. The second major cluster (Cluster B) consists of specimens from Dagoyanan and Nunungan lakes. Cluster A is further subdivided into two groups: one group consisting of all the specimens from Bukidnon and the other group consisting of all the specimens from Lake Lanao and nine specimens from Dagoyanan. All the specimens from Lake Lanao formed a separate subgroup from the nine specimens from Dagoyanan. All

the specimens from Nunungan Lake clustered with 21 specimens from Dagoyanan. This grouping is consistent with the results from Median-joining network analysis as shown in Fig. 3.

Tajima's D and Fu's  $F_s$  tests were done to infer the demographic history of each population. The results of Tajima's D and Fu's  $F_s$  tests including associated p-values are shown in Table 4. Tajima's D values were negative for Lake Lanao and Bukidnon populations, indicating an excess of rare nucleotide site variants compared to what would be expected under a neutral model of evolution. The results of Fu's  $F_s$  test, which is based on the distribution of haplotypes, showed negative values for Lake Lanao and Bukidnon populations, indicating an excess of rare haplotypes over what would be expected under neutrality. The hypothesis of neutral evolution was rejected for Lake Lanao and Bukidnon populations. Dagoyanan and Nunungan lake populations had neutral mutations.

Mismatch distribution analyses were performed to infer historical demographic expansions (Rogers & Harpending 1992; Ray et al. 2003). The results revealed that the mismatch distribution plot of Lake Lanao population has unimodal and ragged shape, Bukidnon population has bimodal and smooth shape plot, while Dagoyanan and Nunungan populations exhibited multimodal and ragged shape plots (Fig. 4).

Sum of squared deviations (SSD) (Schneider &

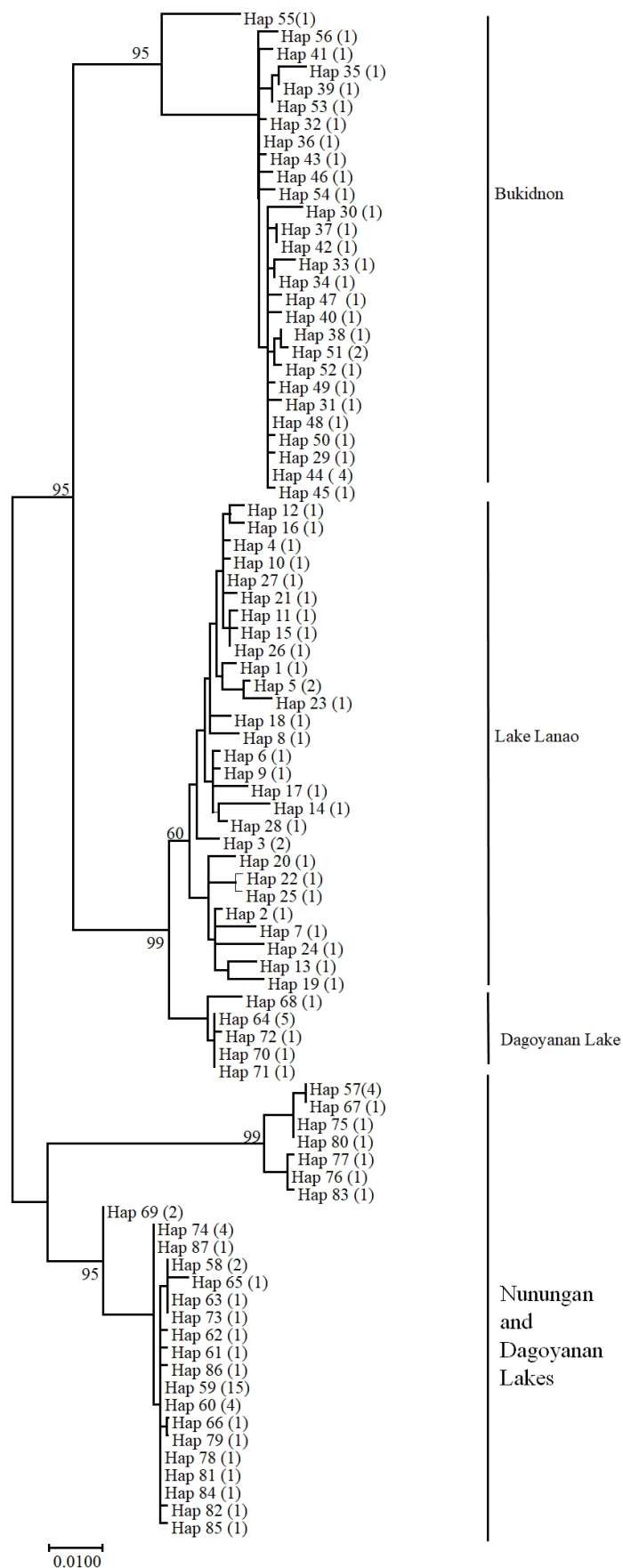


Figure 2. Unrooted HKY +  $\Gamma$  distance maximum-likelihood tree of 87 haplotypes of *Barbodes tumba* from four localities in Mindanao. Number in parenthesis indicates the number of specimens in each haplotype. Values on the nodes represent bootstrap support (1000 replicates). Bootstrap support less than 50% are not shown. The scale bar represents 1 substitutional change per 100 nucleotide positions.

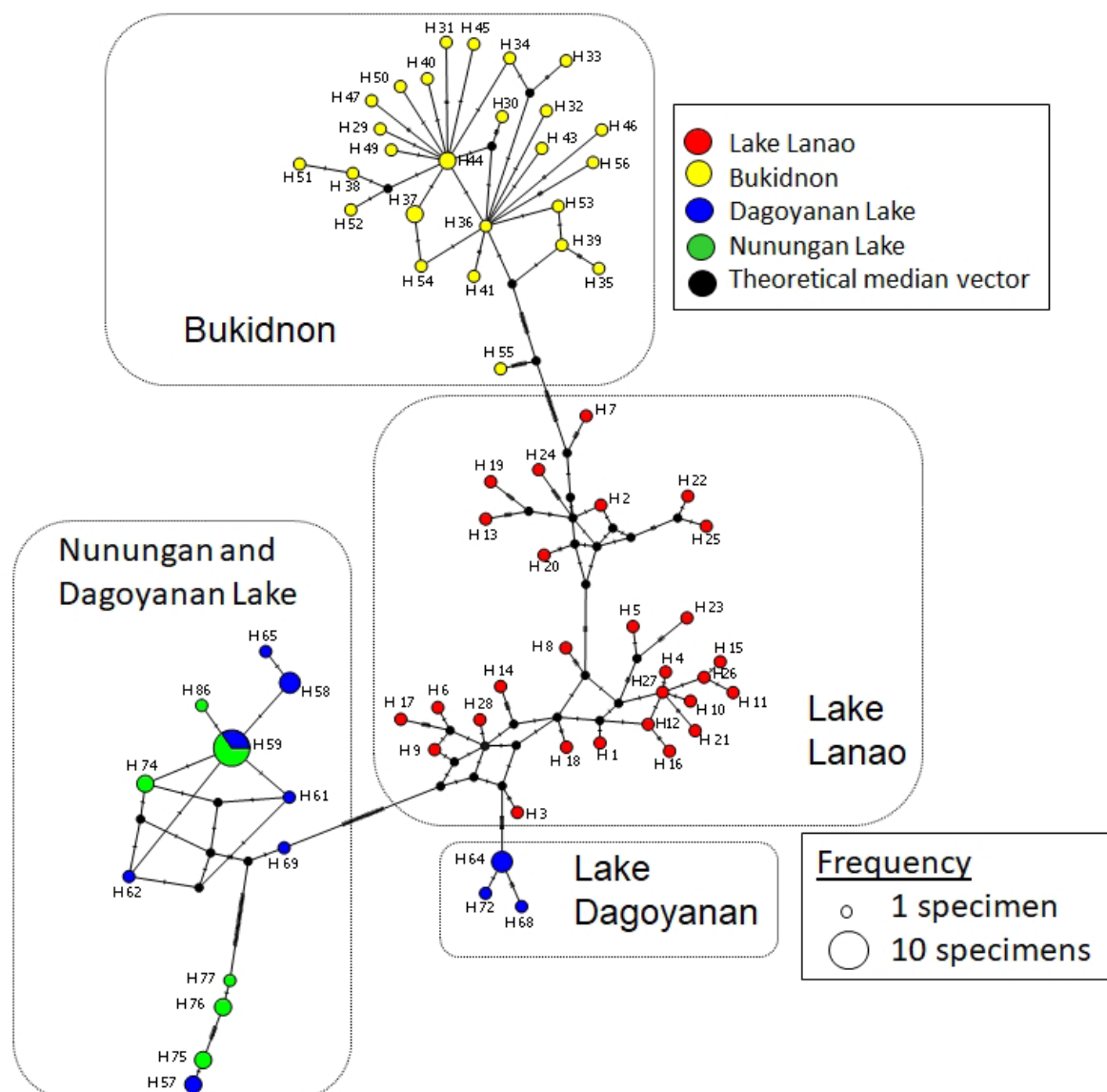


Figure 3. Median-joining haplotype network of four populations of *Barbodes tumba* in Mindanao, Philippines. Hatch marks on the branches represent the number of nucleotide changes. Each circle represents a haplotype, and the sizes of haplotype circles are proportional to the haplotype frequencies. The black dots represent theoretical median vectors introduced by the network software.

Excoffier 1999) and the raggedness index ( $r$ ) (Harpending 1994) under both demographic and spatial expansion models were also calculated for each population. All populations had nonsignificant SSD and raggedness index, except for the SSD of Nunungan and Dagoyanan Lakes under sudden expansion model, which indicates that the dataset has relatively good fit to a model of population expansion (Harpending 1994; Table 4).

## DISCUSSION

High values for haplotype and nucleotide diversities were observed in all populations of *B. tumba*. This can be interpreted as stable populations with long evolutionary history or secondary contact between differentiated lineages (Grant & Bowen 1998). High haplotype and high nucleotides diversities were also observed in other freshwater fishes such as *Channa striata* in a river basin in Central Thailand (Boonkusol & Tongbai 2016) and *Acrossocheilus paradoxus* in Taiwan (Wang et al. 2000). Populations that exhibit both high haplotype and nucleotide diversities may be due to the combination



Table 4. Neutrality tests and mismatch distribution analysis for four populations of *Barbodes tumba* in Mindanao, Philippines.

Population	Tajima's D		Fu's Fs		Sudden expansion model				Spatial expansion model			
	D	p-value	F	p-value	SSD	p-value	r	p-value	SSD	p-value	r	p-value
Lake Lanao	-1.767	0.016	-15.327	0.000	0.003	0.700	0.009	0.529	0.004	0.524	0.009	0.400
Bukidnon	-2.317	0.000	-23.023	0.000	0.002	0.489	0.017	0.636	0.002	0.550	0.017	0.679
Dagoyanan Lake	1.825	0.974	4.038	0.926	0.025	0.027	0.018	0.053	0.016	0.833	0.018	0.848
Nunungan Lake	0.112	0.600	0.560	0.603	0.228	0.005	0.033	0.999	0.029	0.582	0.033	0.905

SSD, sum of squared deviations; r, raggedness index

of high mutational rate and large population size of the species as in the case of *A. paradoxus* (Wang et al. 2000), or to gene flow between regional populations due to fish dispersal by flood, aquaculture (Boonkusol & Tongbai 2016), and anthropogenic transportation. In the case of *B. tumba*, high haplotype and nucleotide diversities may be due to large effective population sizes as a result of their reproductive characteristics. Cyprinids were reported to have high fecundity (500–5,500 eggs per female; Ismail & Escudero 2011), mature in a short period of time of about 112 days to one year (Cek et al. 2001), breed all year round (Ismail & Escudero 2011), and the interval between successive spawning is about 20 to 40 days (Targonska & Kuchaczyk 2012). Despite the reported drastic drop in fish catch for *B. tumba* in Lake Lanao in a survey conducted from 1990 to 1991 (Ismail et al. 2014), genetic diversity of population of *B. tumba* in the lake remains high.

The chi-square test for genetic differentiation, AMOVA, pairwise and overall  $F_{ST}$  values indicate that there is genetic differentiation in the four populations of *B. tumba* in Mindanao. Genetic divergence is expected among the different populations of *B. tumba* for being freshwater fishes that thrive in geographically isolated bodies of water compounded by their limited dispersal capacity (Wang et al. 2000).

The maximum-likelihood tree and median-joining network separated the 87 haplotypes into two major clusters and subgroups within each cluster. The first major cluster consists of specimens from Bukidnon, Lanao and Dagoyanan lakes. The second major cluster consists of specimens from Dagoyanan and Nunungan lakes. Within the first cluster, *B. tumba* specimens from Bukidnon formed a distinct group, which separated from the group formed by the specimens from Lanao and Dagoyanan lakes. The tree shows that *B. tumba* population from Lake Lanao is distinct from the Nunungan Lake population. Some haplotypes from Dagoyanan Lake clustered with either Lake Lanao or Nunungan Lake. No haplotypes were shared between Bukidnon population and the other populations from the three lakes. These findings are supported by the  $F_{ST}$  values. The separation of the Bukidnon population may be explained by the geographic distance and barrier (Mt. Piyagayongan) between Bukidnon and Lanao provinces. While the relative lower genetic differentiation among the Lanao del Norte and Lanao del Sur populations (i.e., specimens from Lanao, Dagoyanan and Nunungan lakes) may be attributed to a secondary contact between the populations due to the close proximity of their distances. Secondary contact among the three Lanao populations

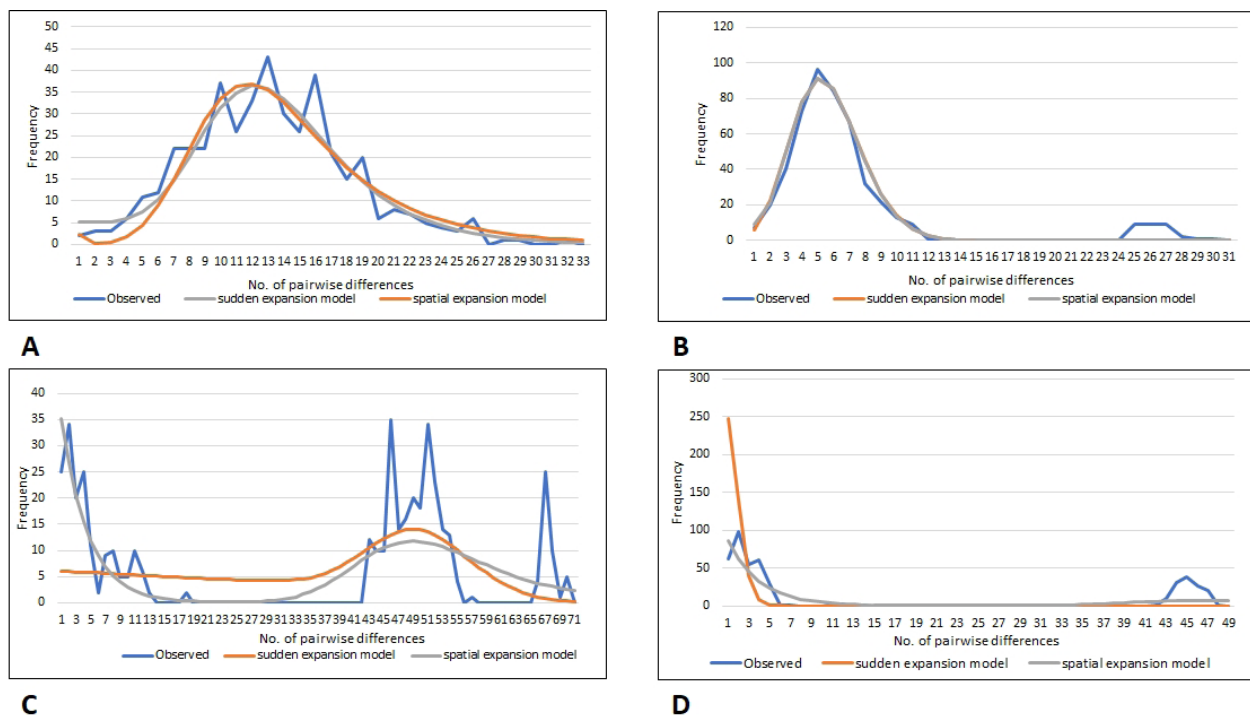


Figure 4. Mismatch analysis plots for four populations of *Barbodes tumba* based on the mitochondrial control region: A - Lake Lanao | B - Bukidnon | C - Dagoyanan Lake | D - Nunungan Lake.

can be brought about by flooding and human-mediated translocation either intentionally or unintentionally. This could explain why two haplotypes were shared between populations from Nunungan and Dagoyanan lakes and why they are the least genetically diverged based on  $F_{ST}$  values. Mixing between populations from these two lakes could have taken place in the past, which could have been brought about by human-mediated translocation since one can just walk from one lake to another for 5 to 7 hours. Lake Lanao population does not share any of its 28 unique haplotypes with any other population. These 28 haplotypes formed a single group which in turn clustered with five haplotypes from Lake Dagoyanan (Fig. 2). Lake Lanao and Lake Dagoyanan are the closest to each other based on geographic distance and because of this there may have been secondary contact between populations from these two lakes in the past.

Nunungan Lake and Dagoyanan Lake populations were found to have neutral mutations based on Tajima's D test and Fu's  $F_s$  values (Table 4). As such, these two populations were at demographic equilibrium with no selection pressure acting on them. *Barbodes tumba* in Nunungan and Dagoyanan lakes face no pressure as of now from overexploitation by humans, predation by introduced species, or habitat destruction. People in

Nunungan and Dagoyanan do not prefer *B. tumba* for food because of the availability of larger fishes (such as tilapia, common carp, mudfish and catfish). In addition, there are only a few households that live in the vicinities of the two lakes. Predatory fishes such as *Glossogobius* spp. and *Giuris margaritaceus*, which were blamed for the extinction of other cyprinid species in Lake Lanao basin, are absent in these lakes. *Barbodes tumba* populations from Bukidnon and Lanao Lake showed significant negative Tajima's D and Fu's  $F_s$  values (Table 4), a signature of population expansion after a recent bottleneck. According to Fu (1997), however, natural selection is only one of potentially many possible causes of significantly negative Tajima's D and Fu's  $F_s$  values. Negative value can also be seen in a population undergoing recent growth (Fu 1997).

Historical demographic expansions can be interpreted as follows: Unimodal mismatch distribution indicates a range expansion in populations with high deme size and gene flow (Ray et al. 2003) or past demographic expansion (Rogers & Harpending 1992), while a multimodal (including bimodal) mismatch distribution indicates recent demographic expansion after the population size diminished due to fragmentation (Ray et al. 2003). Smooth with peak mismatch distribution suggests that population has been growing, while a

ragged and erratic mismatch distribution suggests that the population has been stationary for a long time or in equilibrium (Rogers & Harpending 1992; Harpending 1994). Ragged mismatch distributions can also result from fragmentation of the population's habitat that leads to contraction of their effective size (Ray et al. 2003). Non-significant values for SSD and raggedness index signify that the data do not deviate from that expected under the model of expansion.

Mismatch distribution analysis on the Lake Lanao population revealed a unimodal distribution that conformed to both demographic and spatial expansions (Fig. 4A and Table 4). In 1996, *B. tumba* was classified as vulnerable to extinction by International Union for Conservation of Nature due to decline in abundance in Lake Lanao (World Conservation Monitoring Centre 1996). This population decline, however, did not result in low genetic diversity. Mismatch analyses of populations from Bukidnon, Dagoyanan and Nunungan lakes showed multimodal distributions (Fig. 4B) with non-significant SSD and *r* values (Table 4), except the SSD values for Nunungan and Dagoyanan populations under sudden expansion model. These suggest that Bukidnon population has undergone spatial expansion, fragmented and then followed by demographic expansion. *Barbodes tumba* populations in Dagoyanan and Nunungan lakes, on the other hand, are expanding as they conform to the spatial expansion model.

In summary, there is high genetic diversity in each of the four populations of *B. tumba* in Mindanao and high genetic divergence between populations. Despite the reported drastic decline in fish catch for this species in Lake Lanao in the early 1990s, this did not result in low genetic diversity. Although the genetic diversity is high in each of the four populations, the species still merits management and conservation plan because of its limited distribution or endemism (limited to Mindanao region in the Philippines), its vulnerability to predation, and its utility as food species. In addition, Bukidnon population should be managed separately because it is distinct from the three other populations from the Lanao provinces. Local government units in areas where this endemic species is found should enact measures to prevent overfishing of the species, the introduction of exotic species that can prey on this endemic cyprinid and destruction of their habitat.

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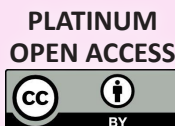
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