

DNA barcoding of the Walking Catfish, *Clarias batrachus* (Linnaeus, 1758), reveals presence of cryptic species and corrects misconception about its status in the Philippines

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ABSTRACT

Background: The freshwater catfish *Clarias batrachus*, is a food fish that is widely distributed in Southeast Asia. It is also reported in South Asia and China. It is widely believed to be an introduced species in the Philippines going back to a reported introduction from Thailand in 1972.

Results: Five specimens were DNA barcoded from each of four Philippine lakes and two river drainages using cytochrome c oxidase subunit I (COI) and cytochrome b (cyt b) genes. Additional COI and cyt b sequences were mined from GenBank. A Neighbor-joining tree using the Kimura 2-parameter (K2P) method showed that Philippine COI sequences including 28 of the 30 sequences from this study formed a single clade (Cluster 1). The two remaining sequences from this study (CBT37 and CBT46) clustered with Malaysian sequences (Cluster 3). Sequences from Thailand formed a separate clade (Cluster 2). Indonesian samples formed another cluster (4). K2P distances ranged from 0 to 0.2% for Cluster 1, 0 to 0.4% for Cluster 2, 0 to 1.6% for Cluster 3, and 0 to 0.4% for Cluster 4. The distance between the sub-cluster containing samples CBT37 and CBT46 and sequences from Cluster 1 (all other samples from the Philippines) had a range of 4 to 4.7%. The average distance between the Philippine cluster and the Thailand cluster was 2.3%.

Significance: This study revealed the presence of cryptic species within *Clarias batrachus* in the Philippines. The genetic uniqueness of majority of *C. batrachus* from the Philippines is supported by historical records of its presence in the Philippines. A review of literature showed that even prior to the reported introduction from Thailand in 1972, *C. batrachus* was a native and widespread species in the Philippines. Albert Herre even listed it in 1924 as one of the true freshwater fishes of the Philippines.

INTRODUCTION

The Walking Catfish, *Clarias batrachus* (Linnaeus 1758), is a popular food fish throughout Southeast Asia. Owing to a reported importation from Thailand in 1972 (Juliano et al., 1989), *C. batrachus* is widely believed to be an introduced species in the Philippines (Guerrero, 2014). However, records of its presence in the Philippines prior to 1972 show that *C. batrachus* is a species native to the Philippines (Herre 1924, 1927, 1953; Manacop 1937). Herre (1924) even listed it as one of the true freshwater fishes of the Philippines. Within the Philippines, the species has been reported throughout Luzon, Visayas and Mindanao.

This study aimed to DNA barcode *C. batrachus* in the Philippines using mitochondrial cytochrome c oxidase I (COI) and cytochrome b (cyt b) gene sequences. The specific objectives of this study were as follows: (1) to determine whether Philippine populations of *C. batrachus* are genetically distinct from those found in other Southeast Asian countries, and (2) to determine the relationship of Philippine *C. batrachus* populations with populations of *C. batrachus* found in other Southeast Asian countries.

MATERIALS AND METHODS

COI (652 bp) and cyt b (958 bp) sequences were generated from five specimens of *C. batrachus* collected from each of the following sites: Tanay, Rizal, Laguna de Bay; Lake Naujan, Mindoro; Lake Bato, Camarines Sur; Lake Mainit, Surigao del Norte and Agusan del Norte; river drainages in Cabiao, Nueva Ecija and Aparri, Cagayan. For COI, an additional 105 GenBank sequences of *C. batrachus* (n=82), *C. gariepinus* (n=19) and *C. magur* (n=6) were used in the analysis, while an additional 21 cyt b GenBank sequences of *C. batrachus* (n=18) and *C. gariepinus* (n=3) were used in the analysis. Final lengths used for analysis were 493 bp for COI sequences and 309 bp for cyt b sequences.

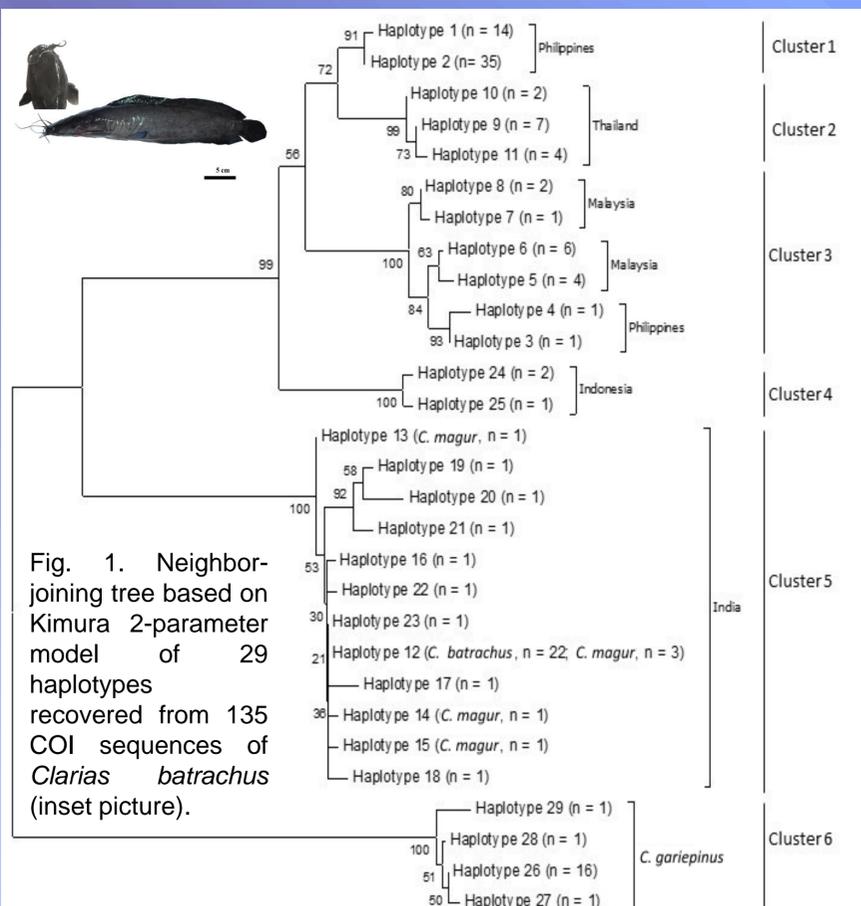


Fig. 1. Neighbor-joining tree based on Kimura 2-parameter model of 29 haplotypes recovered from 135 COI sequences of *Clarias batrachus* (inset picture).

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RESULTS AND DISCUSSION

Thirty (30) COI and 30 cyt b sequences were generated from the six sampled populations. Additional sequences were mined from GenBank and were used in the analysis. The Neighbor-joining (NJ) trees and the number of specimens for each haplotype are shown in Figs. 1 & 2. The average Kimura 2-parameter (K2P) genetic distances are 0.083% for Cluster 1 (Fig. 1), 0.149% for Cluster 2, 0.648% for Cluster 3, 0.267% for Cluster 4. Cluster 5, which consisted of both *C. magur* and *C. batrachus* sequences from India, had an average distance of 0.316%. The distance between the subcluster containing samples CBT37 and CBT46 from Laguna de Bay and sequences in Cluster 1 (all other samples from the Philippines) had a range of 4 to 4.7%, and an average of 4.38%. Between this subcluster (CBT37 and CBT46) and the rest of Cluster 3 (Malaysian sequences), the distances ranged from 0.6 to 1.6%, with an average of 1.03%. The average K2P distances between clusters are as follows: 2.27% for clusters 1 and 2, 4.82% for clusters 1 and 3, 5.64% for clusters 1 and 4, 10.59% for clusters 1 and 5, and 14.2% for clusters 1 and 6.

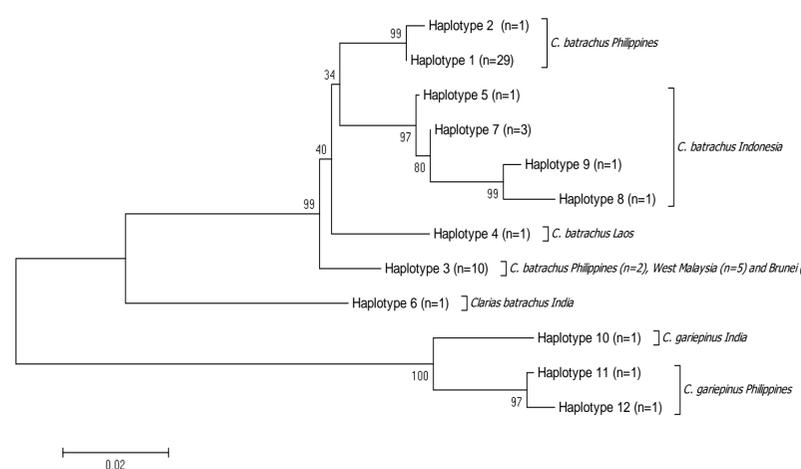


Fig. 2. Neighbor-joining tree (based on Kimura 2-parameter model) of 12 haplotypes recovered from 51 cyt b sequences. Bootstrap support values using 1000 replicates are shown. Scale bar represents two nucleotide substitutions for every 100 nucleotides. The tree was constructed using MEGA 6 (Tamura et al., 2013).

For cyt b (Fig. 2), 28 of the 30 sequences generated from this study clustered with two sequences mined from GenBank, which also came from the Philippines. Sequences from both Java and Sumatra, Indonesia formed a separate cluster. As with COI, cyt b sequences of specimens CBT 37 and CBT46 from Laguna de Bay clustered with sequences from West Malaysia and Brunei.

The computed K2P distances show that there are populations of *C. batrachus* in the Philippines that are genetically distinct from those found in other countries. Based on DNA barcoding, these populations are of the majority (found in all lakes and rivers sampled). A smaller portion of the Philippine populations (two specimens from Laguna de Bay) grouped with those from Malaysia.

In conclusion, DNA barcoding supports the claim of Herre (1924) that *C. batrachus* is a true freshwater species of the Philippines and it is thus, a native species in the country. Because of the large average K2P distance (2.3%) between *C. batrachus* specimens from Thailand and the Philippines, it is doubtful whether the catfish specimens that were reported to have been imported from Thailand in 1972 were those of *C. batrachus*.

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