



DNA Barcoding and Phylogenetic Insights into Catfishes (Order: Siluriformes): A Review

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Abstract

Catfishes are ray-finned fish that have barbels, are scaleless, and are bottom feeders. Examples of Catfishes *Clarias batrachus*, *Heteropneustes fossilis*, *Pangasius pangasius*, *Wallago attu*, *Ompak spp.* Catfishes have a wide geographical distribution and are found in North America, Central America, South America, Africa, Eurasia, South-East Asia, Japan, and Australasia. Catfishes belong to the order Siluriformes (Actinopterygii) and the family Bagridae. Due to morphological similarity, many fish look alike, but in reality, they belong to different genera and species. Because of this, fish products can sometimes have mixed or incorrect labelling, which can be harmful. For conservation purposes, it is also important to correctly identify these species. For this, we use a molecular tool called DNA barcoding. Different scientists have applied DNA barcoding in various ways and in different locations. In DNA barcoding, we can use genes like Cytochrome b and the mitochondrial gene Cytochrome c oxidase I (COI). DNA barcoding has helped resolve many doubts about species identification and is a very useful technique. Using this method, we can also construct phylogenetic relationships. With the help of DNA barcoding, the discovery of new species has become easier, and a fish database has been created.

Keywords: DNA barcoding, Phylogenetic relationships, Cytochrome c oxidase I (COI), Cytochrome b

Introduction

Catfishes are a diversified group of fishes that are ray-finned and named for their prominent barbels, which resemble a cat's whiskers (but not all catfish have prominent barbels). Catfish's bodies are naked (scale absent). Most catfish usually live and feed at the bottom of water bodies because they sink instead of floating due to the small size of their air (gas) bladder. Some examples of Catfishes *Clarias batrachus*, *Heteropneustes fossilis*, *Pangasius pangasius*, *Wallago attu*, *Ompak spp.*, *Mystus spp.* etc. Air-breathing catfishes such as

Magur and Singhi live in shallow waters and can survive in low-oxygen conditions, so they are also called “live fishes”, and they are sold alive in the market and at a higher price. Catfishes have a wide geographical distribution and are found in North America, Central America, South America, Africa, Eurasia, South-East Asia, Japan, and Australasia.

Catfishes belong to the order Siluriformes (Actinopterygii) and the family Bagridae. Catfishes have multiple uses, such as human food, a sport of catching fish with a hooked line known as Angling and aquariums, so they are widely exploited globally. North-East India have high abundance of catfishes and has been equally exploited (Bhattacharjee *et al.*, 2012).

Phylogenetics is a branch of biology that deals with the study of evolutionary relationships among different organisms. It aims to understand how various species have evolved from a common ancestor over time.

In phylogenetics, organisms are compared using DNA, RNA, protein sequence and morphological characters. Different research has been conducted through different methods. Like Jondeung *et al.* (2007) conducted phylogenetic analyses through mitochondrial protein and rRNA in Mekong giant catfish (family Pangasiidae), and they conclude that the Mekong giant catfish (family Pangasiidae) is closer in relationship to Siluridae than to Bagridae.

Similarly, a study was also conducted through ATP-Binding Cassette (ABC) Transporter Genes for phylogenetic analysis by Liu *et al.* (2013). And Arce-H *et al.* (201) conducted a study on the phylogeny of the North American catfish family Siluridae (Teleostei: Siluriformes) through combining morphology, genes and fossils.

Classification of catfishes, based on morphological characters, is insufficient and remains controversial due to morphological similarities among clariid catfish. Khedkar *et al.* (2016) explain this by giving the example of *Clarias batrachus* and *Clarias gariepinus* in India. To overcome this, we use DNA barcoding. DNA barcoding based on a fragment of the cytochrome c oxidase subunit I (COI) gene in the mitochondrial genome. It is widely applied in species identification and biodiversity studies. Similarly, Wong *et al.* (2011) differentiated between domestic and imported catfish species in the United States.

DNA Barcoding techniques analysis capacity is rapid and accurate.

Tran *et al.*'s (2017) study discloses that the Cyt b gene region, rather than the COI gene, can be effectively used for differentiating between species. This method is widely accepted as the standard region used for DNA barcoding.

With the help of DNA barcoding, we have obtained a large amount of mitochondrial genome data, and we are still actively working on it. Recently, Huynh *et al.* (2025) reported the first complete mitochondrial genome of *M. albolineatus* (Roberts, 1994) (Bagridae) and its phylogenetic relationship.

Methodology

For this study, online databases such as Google Scholar, Springer Link, PubMed, Science Direct, ResearchGate and Scopus were carefully searched. Keywords like catfishes, DNA barcoding, and phylogenetic relationships were used. The information collected from these sources about DNA barcoding and phylogenetic studies of catfishes is summarised below.

Result and Discussion

Rognon *et al.* (1998) studied physical characteristics and enzyme-based genetic differences in African populations of *C. gariepinus* and *C. anguillaris*. They found that both methods could separate some groups based on where the fish came from. However, the allozyme data also showed complicated genetic differences within *C. gariepinus* populations.

Carvalho *et al.*'s (2011) study shows that DNA barcoding is an effective method to identify fish species correctly in markets. A high level of mislabelling was found, especially in processed fish products like fillets, where visual identification is difficult. These results indicate that fraud is more common in processed fish than in whole fish. Therefore, there is an urgent need to improve monitoring systems and to create an official list of approved common names for freshwater fish and seafood to protect consumers and ensure fair trade.

Van der Bank *et al.* (2012) found that in tropical regions, less than 40% of fish species are properly identified, and many species are extinct before recognition. At the time, people considered that species can survive climate change only by moving to new areas. To understand this, they studied *Schilbe intermedius* (African silver catfish) because Africa has different climatic conditions. They hypothesised that micro-evolutionary changes help this fish survive in different African climates. Researchers used the COI gene for the DNA barcoding. They analysed genetic diversity and relationships among seven populations of *S. intermedius* from different African river systems, and they found that Fish populations formed three main genetic groups: Southern Africa, Eastern Africa, and Western Africa. South African fish were genetically distinct from those in Namibia and Botswana, and when combined with Nigerian populations, two sub-clusters emerged, which originated from two isolated river systems. They concluded that African silver catfish populations exhibit significant genetic differences across different river systems, likely due to microevolution occurring locally.

Quilang and Yu (2015) studied DNA barcoding on commercially and economically important catfishes in Philippine *via* mitochondrial cytochrome c oxidase I (COI) gene on commercially and economically important Philippine catfishes using 75 specimens from 11 species and 5 families. After that, genetic distances were measured, and Neighbour-Joining (NJ) trees were constructed based on the Kimura 2-Parameter method. Then, the average K2P distances within species, genus, family and order were 0.2, 8.2, 12.7 and 21.9%, respectively. Using COI gene sequences, fish samples grouped correctly according to species for 7 out of 11

catfish species. Their result showed that *Arius dispar* and *A. manillensis*, and between *Pterygoplichthys disjunctivus* and *P. pardalis*, were not genetically distinguishable but morphologically distinguishable. *Clarias batrachus* of the Philippines is different from the species found in India and Thailand. This supports that the Indian populations should be called *Clarias magur* and the Southeast Asian populations should be called *Clarias aff. Batrachus*.

Kushwaha *et al.* (2015) reported some differences in the mitochondrial genome of *Clarias batrachus* when it was compared with 24 other catfish species. Using whole genome sequencing (WGS) to obtain the complete mitochondrial genome is faster and more reliable than Sanger sequencing because cells contain many mitochondria. In this study, they successfully assembled a complete mitochondrial genome with very high data coverage (~284×). The gene arrangement, gene content, and conserved regions in *C. batrachus* matched well with known fish classification. This shows that the mitochondrial genome evolution agrees with fish taxonomy.

Moran *et al.* (2016) examined how the introduction of new predator fish into an ecosystem can strongly affect native fish species. To understand this impact, researchers need to know what these predators are eating. But prey fish found in a predator's stomach are often partially or completely digested, making visual (morphological) identification hard. This study tested whether DNA barcoding can help identify fish prey from the stomachs of North American catfishes. Fish prey items of non-native Blue Catfish *Ictalurus furcatus* and Flathead Catfish *Pylodictis olivaris* were obtained *via* gastric lavage and ranked as lightly, moderately, or heavily digested. Researchers extracted DNA from the prey using universal fish primers to amplify a specific mitochondrial gene (the COI gene) and compared it with GenBank to identify the species. The result shows that 86% of lightly or moderately digested prey were identified to species, and 66% of heavily digested prey were identified to species. Traditional visual identification alone identified only 65% of prey to species level, and combining morphology + DNA barcoding increased identification success to 88%. DNA barcoding revealed that catfish were eating important fish species, such as Striped Bass (*Morone saxatilis*) and Alosa species. These species are targets of fishery restoration programs, so predation by non-native catfish is a conservation concern.

DNA barcoding has been done by Abdullah *et al.* (2017) on Ariidae species in Malaysia. The study showed that most species were clearly distinct and formed their own genetic groups. On average, the genetic difference between species (K2P distance) was 9.6%. However, two species (*Arius venosus* and *Nemapteryx caelata*) showed very little genetic difference, making them hard to separate. When the DNA sequences were compared using BLAST, only two species matched, even though eight fish species were identified. This mismatch may be due to wrong species identification, mistakes in the GenBank database, hybridisation between species, or incomplete evolutionary separation.

The Asian Redtail Catfish (*Hemibagrus nemurus*) is a very important fish for income and food in South Sumatra (Syarifudin *et al.*, 2017). Studying its DNA is important to help protect the species and to select good fish for breeding. DNA analysis showed that the Asian Redtail Catfish is closely related to other fishes of the Bagridae family. It formed a separate genetic group from *Pangasius* species and from *Oreochromis niloticus* (tilapia).

Fish from the family Pangasiidae are very important for aquaculture and are highly valued as food in Vietnamese markets. To correctly identify these fish, Tran *et al.* (2017) studied the DNA barcoding and evolutionary relationships of nine catfish species from the Mekong River basin in Vietnam. They found that the largest genetic difference was between *Pangasius larnaudii* and *Pangasius bocourti* using the COI gene, and between *Pangasius macronema* and *Pangasianodon hypophthalmus* using the cyt b gene and the smallest difference was observed between *Pangasius macronema* and *Pangasius conchophilus* for both the COI gene as well as cyt b gene. Phylogenetic tree analysis showed two main genetically distinct groups, corresponding to two different genera. The study also confirmed that, along with the COI gene, the cyt b gene is reliable and can be used as a standard DNA barcoding marker.

Rhamdia quelen is the most commonly farmed native freshwater fish in South Brazil. Earlier, *Rhamdia branneri* and *Rhamdia voulezi* were considered the same species as *R. quelen*, due to their morphological similarity and can interbreed easily in fish farms, so all of them were sold with the common name, silver catfish. Using DNA barcoding, Scaranto *et al.* (2018) proved that fish farms actually contain a mixture of different *Rhamdia* species. The result shows the presence of at least three species: *R. branneri*, *R. voulezi*, and two genetic types of *R. quelen* (*R. quelen 1* and *R. quelen 2*). The genetic difference between species ranged from 1.1% to 5.1%, while the genetic variation within the same species was very low, and the detection of farmed fish in natural rivers such as the Uruguay and Benedito Novo Rivers indicates genetic pollution of wild populations.

Catfish are an important source of protein in Malaysia and are harvested throughout the year. However, species identification, particularly within the family Ariidae, is often difficult due to the presence of cryptic characteristics, which frequently leads to mislabelling. Therefore, Nasihin-Seth *et al.* (2019) applied DNA barcoding for accurate species identification. In this study, nine species of Ariidae and two species of Plotosidae from Malaysia were successfully barcoded.

Wachirachaikarn and NaNakorn (2019) used microsatellite markers to study genetic diversity in hatchery populations of *C. gariepinus* in Thailand. They found clear genetic differences among the different hatchery populations.

Zou *et al.* (2020) studied the mitochondrial COI gene sequences from 20 species belonging to four genera. These sequences were used to examine the barcoding gap (genetic differences between species) and

to construct phylogenetic relationships, which will aid in understanding species identification and their evolutionary relationships. The phylogenetic tree revealed that the monophyly of the genera *Pelteobagrus*, *Leiocassis*, and *Pseudobagrus* did not exist in China; instead, they are distributed in a single genus.

Megarani *et al.* (2020) studied 26 catfish collected from 9 rivers in different parts of Indonesia. They extract DNA by using a tissue isolation method, then Cytochrome b is copied using PCR with special primers (CytBF and CytBR). After that DNA sequences were then analysed to understand genetic differences and evolutionary relationships using MEGA X software. They found that the Cyt B gene had 1139 DNA bases, which coded for 379 amino acids in all fish samples, and when sequences were compared, then 395 DNA positions were different among the fish, which coded for 63 amino acid positions. Thus, the result shows that some catfish (BB, PM, MP, KR2, KR3) showed no amino acid differences among themselves, so they are very closely related. Others showed differences, such as MS: 1 amino acid change, KR1 and KS: 2 amino acid changes, BF: 38 changes, EM: 31 changes, and BSBJ: 26 changes. The highest difference was observed between EM and BF, meaning they are genetically very distant. Finally, they conclude that Indonesian catfish were grouped into five major genetic clades: Fish samples KR and MP from Sumatra, MS and BB from Kalimantan, and PM from Java were found to be closely related to *Hemibagrus nemurus* and *Hemibagrus wyckioides*, which belong to the Bagridae family. Fish samples KS from Kalimantan and one sample (KR1) from Sumatra were closely related to *Sperata seenghala* and *Hemibagrus spilopterus*, also from the Bagridae family. Fish samples BSBJ from Java were closely related to *Pseudolais pleurotaenia*, which belongs to the Pangasiidae family. Fish samples EM from Java were closely related to *Mystus cavasius* of the Bagridae family. Fish samples from West Papua were closely related to *Potamosilurus latirostris*, which belongs to the Ariidae family.

The silver butter catfish (*Schilbe intermedius*) is found in many river systems across Africa. However, there is still limited information about its mitochondrial genetic diversity, population structure and past population history. A study by Nneji *et al.* (2020) used DNA barcoding to examine the mitochondrial genetic diversity of this species and showed clear patterns of genetic variation across different African rivers.

Chand *et al.* (2021) compared the mitochondrial genomes of two catfish species, *Clarias batrachus* and *Clarias gariepinus*. They found differences in 12 out of the 13 mitochondrial proteins produced by these species. The product of these proteins affects many characteristics. Thus, *C. gariepinus* survive better than *C. batrachus*, especially under stressful conditions.

Because there are weak rules and poor enforcement in some countries, like Thailand, food fraud happens, especially when one fish species is replaced with another. So, fish product labels need to be carefully checked. Thus, they use DNA barcoding for accurate identification of fish (Panprommin and Manosri, 2022). They studied the COI gene, about 650 base pairs long, and result shows that almost all samples were correctly

identified up to the species level, showing 98–100% match with known records in the GenBank and BOLD databases. Only one sample could be identified up to the genus level because the database did not have enough information to identify the exact species. In total, the products contained 25 different species belonging to 18 genera. Among them, only *Pangasianodon hypophthalmus* is listed as endangered according to the IUCN. The researchers used FishBase to check the scientific names that matched the common or market names used on the labels. The result showed that 18 out of 54 samples (33.33%) were mislabelled, and among the products that already used scientific names, only 1 out of 9 was mislabelled.

Hemibagrus hoevenii, a tropical freshwater catfish, is found in Asian waters. Bagrid catfishes are commonly called Old World pimelodids. Until now, DNA barcoding has not been used to identify bagrid catfish species in the Muar River, Johor. Therefore, Rais *et al.* (2022) carried out this study to update the list of Baung (bagrid catfish) species found in the Muar River. The results showed that different phylogenetic methods produced the same result: all DNA sequences from Baung Lawi samples grouped in one cluster. This confirmed that the species present in the Muar River is *Hemibagrus hoevenii*.

Chalermwong *et al.* (2023) evaluated a comparative study of DNA barcoding for 37 clariid catfish by using 2,970 barcode sequences from different methods, such as mitochondrial cytochrome c oxidase I (COI), cytochrome b (Cyt b) genes and D-loop sequences. Results show that the distances were 85.47%, 98.03%, and 89.10%, respectively. Thus, the Cyt b gene showed the clearest genetic differences (barcoding gap) between species (98.03%, distance). A clear barcoding gap was observed between genetic variation within species (less than 4.4%) and between species (more than 66.9%) in the Cyt b dataset, but not in the COI and D-loop datasets. So, Cyt b serves as a standard region for DNA barcoding. They also found that in walking catfish, what appears to be one species may actually comprise more than one genetically distinct species. Similarly, in North African catfish, there was high genetic variation within the same species, which makes identification challenging.

Haslawati *et al.* (2023) have studied the DNA barcoding using the COI gene, which is a useful method for identifying fish species, especially invasive pangasiid catfish that may threaten native species. The researchers studied the diversity and evolutionary relationships of Pangasiidae catfishes found in Peninsular Malaysia. They created a family tree (neighbour-joining tree) that grouped the fishes into five main clusters, each representing a different genus. The genus *Pangasius* was further divided into two smaller groups: one group included *Pangasius bocourti* and *P. djambal*, and the other included *P. nasutus* and *P. conchophilus*. The study also applied a technique called Automatic Barcode Gap Discovery (ABGD), which compares genetic variation within species and between different species. This method clearly identified gaps separating individuals of the same species from those of different species. Using ABGD, the DNA sequences were grouped into five distinct clusters that defined species boundaries more accurately than the neighbour-joining tree alone.

Encheloclarias tapeinopterus is a vulnerable species found exclusively in Bangka Island, Indonesia (Valen *et al.*, 2023). During our field trip on 25–26 January 2023, they collected two fish specimens of *E. tapeinopterus*. Their study is the first ever report of DNA barcoding for *E. tapeinopterus*, using the COI gene, and the sequence has been submitted to GenBank. The phylogenetic analysis showed that *E. tapeinopterus* is closely related to the genera *Clarias* and *Heterobranchus*, forming a separate evolutionary group.

Catfish are one of the most heavily overfished groups of fish worldwide and are widely exploited in the eastern region of Nepal. Therefore, it is important to assess the true diversity of catfish in this region using both morphological characters and molecular DNA barcoding techniques (Limbu, 2024). The study recorded two species, *Amblyceps arunachalense* and *Erethistoides sicula*, for the first time in Nepal. According to the IUCN Red List (2022), one species was categorised as Endangered (EN), two species as Near Threatened (NT), one species as Vulnerable (VU), one species as Data Deficient (DD), and twenty-four species as Least Concern (LC).

Patil *et al.* (2024) investigated the molecular phylogenetics of ornamental catfishes (Siluriformes) from Northeast India using DNA barcoding. They found a maximum intraspecific genetic distance of 0.03 and a minimum interspecific distance of 0.14. This clear barcoding gap enabled the accurate identification of the species.

To identify species from the Chinese market, Zhao *et al.* (2024) by using integrated DNA barcoding method. The molecular identification results revealed that 36.4 % of the samples were wrongly labelled, and commercial fraud was observed in all six types of processed fish products.

The mtDNA COI gene sequences were generated for the first time to facilitate the correct identification of species in *Mystus cavasius*, along with their biometric characters, from the upper reaches of the Beas River basin in Himachal Pradesh (Thakur *et al.*, 2025).

Yaswanthkumar *et al.* (2025) noted that the classification of Bagridae catfish in the Western Ghats was often confusing because earlier studies relied primarily on external body measurements (morphometric characters). This led to mistakes in identifying species and even the naming of some invalid species. To solve this problem, the researchers studied freshwater Bagrid catfish from the Western Ghats, especially the Cauvery River basin, using multiple approaches such as Morphological studies, Molecular analysis, Osteological studies, and evolutionary analysis. By combining these methods, the study provided a more accurate understanding of species diversity, correct taxonomy, and evolutionary relationships of Bagrid catfish in the region. The results improved the understanding of species diversity, taxonomy, and evolutionary relationships of Bagrid catfish in the Western Ghats.

Fish maws, derived from the swim bladders of fish, contain high collagen content and nutritional benefits, leading to significant market demand, especially in Asia. Xing *et al.* (2025) investigate the species authenticity

of fish maw products using mini-DNA barcoding technology to address issues of mislabelling and substitution. Mini-DNA barcoding has proven crucial in identifying species accurately, revealing a high mislabelling rate of 51.01% among 347 samples.

Conclusion

DNA barcoding is a very effective and reliable technique used to correct product mislabelling. It is a fast and rapid method commonly applied to identify species accurately. For DNA barcoding, mitochondrial genes such as Cytochrome c oxidase subunit I (COI) and Cytochrome b (cyt b) are commonly used. Different scientists have used DNA barcoding in various places to correctly identify fish because many fish look very similar morphologically but actually belong to different species. For accurate identification, DNA barcoding, a molecular tool, is being used. DNA barcoding also helps determine their phylogenetic relationships.

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