

Molecular identification of three invasive suckermouth catfish (*Pterygoplichthys* spp.) from the Buriganga River, Bangladesh

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Abstract

Invasive suckermouth catfishes (*Pterygoplichthys* spp.) are widespread in Bangladesh but their species composition is poorly characterized due to previous records relying on morphological identification. The present study aimed to determine the taxonomic status of *Pterygoplichthys* populations of the Buriganga River, Bangladesh, by employing DNA barcoding of the mitochondrial cytochrome c oxidase subunit I (COI) gene. Fourteen specimens were collected from three sampling sites and were first identified by their morphological features. Genomic DNA was isolated from muscle tissue and a 655 bp fragment of the COI gene was amplified and sequenced. The sequences were compared to reference sequences in the NCBI GenBank and Barcode of Life Data System (BOLD) databases. Molecular analysis revealed the presence of three species of *Pterygoplichthys* in the Buriganga River namely, *Pterygoplichthys disjunctivus*, *P. ambrosettii* and *P. pardalis*. Sequence comparisons showed high query coverage ($\geq 99\%$) and sequence identity (99–100%) with authenticated reference sequences, supporting accurate species-level identification. Phylogenetic analysis grouped the specimens into three distinct clades corresponding to the identified species and revealed close genetic relationships with populations from both native and introduced regions worldwide. These findings demonstrate that the sampled specimens revealed the presence of at least three *Pterygoplichthys* species. This study provides the first molecular evidence for the coexistence of *P. disjunctivus*, *P. ambrosettii*, and *P. pardalis* in Bangladesh. The results provide important baseline information for developing management strategies to evaluate the impacts of invasive species.

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Introduction

Suckermouth armored catfishes (commonly known as “plecos,” family Loricariidae) are native to tropical and subtropical freshwater systems of Central and South America including regions such as Costa Rica, Panama, Brazil, the Amazon Basin, and Venezuela, particularly in moderate to fast-flowing streams and rivers (Orfinger & Goodding, 2018). Loricariid catfishes are primarily benthic species, characterized by dorsoventrally flattened bodies covered with protective bony plates, a unique pair of maxillary barbels, and a ventrally located suckorial mouth (Covain & Fisch-Muller, 2007). Members of the genus *Pterygoplichthys* possess additional survival and establishment in novel environments. Suckermouth catfishes, especially those in the genera *Pterygoplichthys* and *Hypostomus*, began appearing outside their native South American range by the mid-20th century, primarily through the aquarium trade and incidental escapes. One of the earliest well-documented non-native records is from Florida, where a loricariid (*Plecostomus* sp.) was reported in Six-Mile Creek (Tampa Bay area) in 1958; subsequent surveys in the 1960s and 1970s confirmed established loricariid populations in Tampa canals and nearby drainages (Ludlow & Walsh, 1991). During the 2000s, introductions expanded rapidly in Asia, for example, in the Philippines (Laguna de Bay and Pasig-Marikina system), where “janitor fish” were confirmed as *P. disjunctivus* and *P. pardalis*, as well as in South Asia and the Middle East/Europe (India, Turkey, Poland, Italy) (Chavez et al., 2006). Suckermouth catfishes (*Pterygoplichthys* spp.) are popular algae-eaters in home aquaria and have been introduced worldwide, primarily via the ornamental aquarium trade. Many non-native populations were founded by intentional releases by aquarists, accidental escapes from fish farms, and flood-mediated dispersal from aquaculture facilities. Their ability to tolerate poor water quality, breathe atmospheric oxygen, and burrowing reproductive behavior once introduced allowed for rapid establishment and expansion throughout diverse freshwater habitats (Orfinger & Goodding, 2018).

The introduction of the suckermouth catfish in Bangladesh can be traced back to the mid-1980s when the species was first seen in Gulshan Lake, Dhaka but not recorded at that time. The first confirmed scientific record of it was in 2008, when *Pterygoplichthys multiradiatus* (Orinoco sailfin catfish) was identified from Kahalu Upazila in Bogura district, Bangladesh (Hossain et al., 2008). Since then, it has been reported by researchers and the media in a variety of freshwater environments across the country. These fish have shown tremendous adaptability in that they thrive in ponds, rivers, lakes, haors, beels, canals, ditches and even in paddy fields. They have been reported from all the four major regions of Bangladesh-greater Dhaka, greater Chattogram, greater Rajshahi and greater Khulna (Rana et al., 2023). Later surveys and reports also confirmed the presence of the Amazon sailfin catfish (*P. pardalis*), the vermiculated sailfin catfish (*P. disjunctivus*) and the common pleco (*Hypostomus plecostomus*), especially from the Brahmaputra River system (Galib, 2015; Hossain et al., 2018; Parvez et al., 2023).

These non-native fish now compete with native fish for food and habitat. Their feeding behavior characterized by widespread grazing of periphytic algae and detrital material reduces the availability of native fishes and invertebrates, destabilizes trophic interactions, and affects the structure and functioning of aquatic ecosystems (Parvez et al., 2023). Suckermouth catfish feed by disturbing and uprooting aquatic vegetation, which is detrimental to the plants; reduces total plant biomass and changes wetland flora, with cascading effects on species that depend on aquatic plants for shelter and reproduction (Capps et al., 2011). These catfish also present serious threats to local fisheries that provide vital food and income. They can support commercially important fish, but also cause lower catches and financial losses for fishing communities, given the native species for space and resources (Hossain et al., 2018; Parvez et al., 2023). They also reduce fish stock productivity by degrading aquatic habitats and water quality, thus endangering the long-term sustainability of both artisanal and commercial fisheries (Capps et al., 2011).

Although the presence of suckermouth catfishes in freshwater habitats of Bangladesh has been reported, detailed information on their species composition and precise taxonomic identity is lacking. The identification efforts were primarily based on visual inspection, morphological features or broad classification, which for morphologically similar species such as *P. pardalis*, *P. disjunctivus* and *Hypostomus plecostomus* (Galib, 2015; Hossain et al., 2018; Parvez et al., 2023). DNA barcoding provides a possible solution to these identification problems, providing accurate species level resolution even between closely related taxa. Barcoding of the suckermouth catfish in Bangladesh is needed for the development of a reliable baseline of invasive species, enhancement of ecological risk assessments and the formulation of national management policies including trade restrictions. DNA barcoding combined with morphological diagnosis enriches the global databases with valuable genetic data in Bangladesh. Thus, the present study was designed to identify the sucker mouth catfish species from the river Buriganga using DNA barcoding technique to confirm the morphological identification.

Materials and Methods

Study area: The study was conducted in the tide-influenced Buriganga River, which runs through Dhaka City to the west and south and emerges from the Dhaleshwari in the south of Savar, close to Dhaka. This river is only 27 kilometers long and has a maximum depth of 18 m, with a mean depth of 7.6 m (Banglapedia, 2014). It receives municipal runoff, sewer outlets, and untreated industrial waste from urban housing and nearby industrial belts (Fig. 1).

Sample collection and sorting of *Pterygoplichthys* spp.: A total of fourteen (14) suckermouth sailfin catfish specimens were collected using cast nets from three distinct locations (Upstream: Aminbazar; Midstream: Sadarghat; Downstream: Fatulla) of the Buriganga River; with each location at least 9 km apart from the nearest sampling location (Fig. 1). The sampling was conducted along the river in June, 2025 After collection, the specimens were

kept in sterilized plastic bag and stored in insulated iceboxes. Immediately after sampling, all specimens were transferred to the laboratory and stored under refrigeration. Species identification of individuals was carried out using morphological descriptions (Armbruster & Page, 2006). Preliminary identification of all collected specimens was conducted based on established morphological keys, primarily focusing on diagnostic features described in the literature (Page & Robins, 2006; Wu et al., 2011). All specimens were first confirmed to belong to the genus *Pterygoplichthys* by their characteristic tall, sail-like dorsal fin, which contains 10 or more rays (I/10–12). This key feature distinguishes them from the often-confused *Hypostomus plecostomus*, which possesses a shorter dorsal fin with fewer rays (I/7). Following confirmation of the genus, specimens were identified to the species level (*P. pardalis*, *P. disjunctivus*, and *P. ambrosettii*) using the key diagnostic features (Table 1). The most reliable feature for separating these species was the distinct patterning on the ventral (belly) surface (Fig. 2). The morphological identification was based on the observation of two independent observers. The confusion for identification was resolved by discussion with third observers.

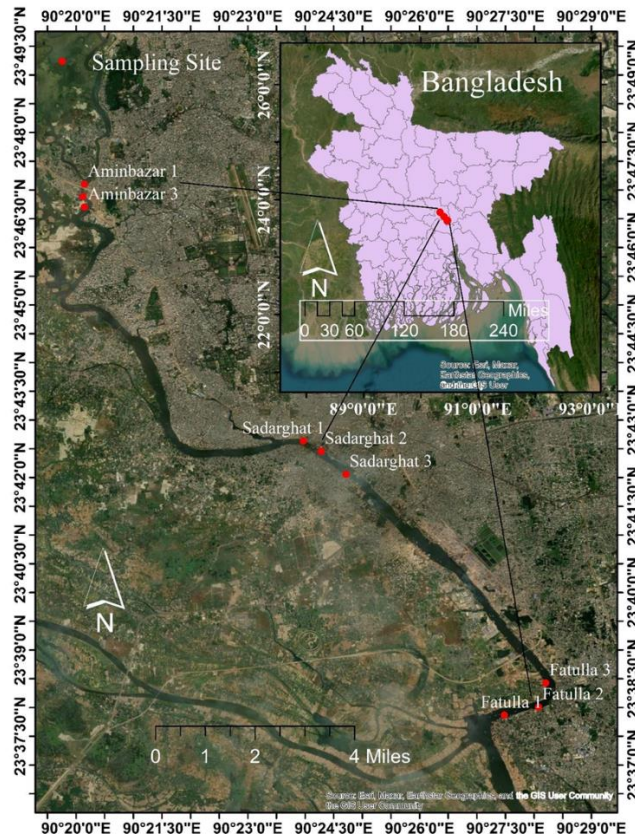
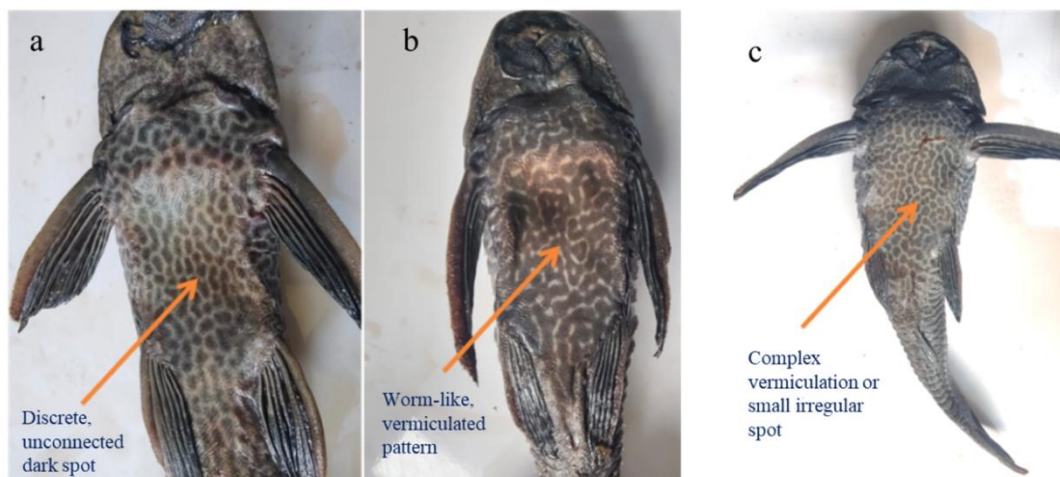


Fig. 1. Map showing sampling locations along the Buriganga River, Bangladesh.

Table 1. Key morphological features used in this study to distinguish the three *Pterygoplichthys* spp.

Features	<i>Pterygoplichthys pardalis</i>	<i>Pterygoplichthys disjunctivus</i>	<i>Pterygoplichthys ambrosettii</i>
Ventral (Belly) Pattern	Discrete, unconnected dark spots on a pale background.	Worm-like, vermiculated pattern (interconnected, broken lines) on a pale background.	Complex vermiculation or small irregular spots; belly is fully plated (armored) in adults.
Head Pattern	Pale, reticulate (net-like) lines radiating outward.	The head is spotted but lacks a reticulate (net-like) pattern.	Small, irregular spots.
Body Armor / Keels	Weak lateral keels (ridges).	Weak lateral keels.	Four strong, distinct lateral keels (ridges) running along the body.

**Fig. 2.** Variation in ventral spots of sucker mouth catfish (*Pterygoplichthys* spp.) observed in the studied fish specimens from the Buriganga River, Bangladesh. (a) Discrete, unconnected dark spots in *Pterygoplichthys pardalis*; (b) worm-like, vermiculated pigmentation pattern in *Pterygoplichthys disjunctivus*; and (c) complex vermiculation with small irregular spots in *Pterygoplichthys ambrosettii*. Arrows indicate representative pattern types in each specimen.

Genomic DNA extraction, PCR amplification, and sequencing: Genomic DNA was extracted from approximately 20–25 mg of muscle tissue from each specimen using the TIANamp® Genomic DNA Kit (TIANGEN Biotech, Beijing, Co., Ltd., China). The extracted

genomic DNA was then subjected to PCR to amplify a target fragment of the mitochondrial cytochrome c Oxidase I (COI) gene (655 bp) using primers, FishF1: 5'-TCAACCAACCA CAAAGACATTGGCAC-3' and FishR1: 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3' (Ward et al., 2005). The PCR amplifications were performed in a total volume of 25 μ L, containing the following components: 12.5 μ L 2x PCR Master Mix, 1.0 μ L FishF1 primer (10 μ M), 1.0 μ L FishR1 primer (10 μ M), 2.0 μ L Template DNA, and 8.5 μ L nuclease-free water. The thermal cycling was performed on a Thermal Cycler (Bio-Rad). The amplification procedure included an initial denaturation step at 95 °C for 5 min followed by 35 cycles of: (i) denaturation at 95 °C for 30 s, (ii) primer annealing at 58 °C for 30 s, and (iii) extension at 72 °C for 45 s, with a final extension at 72 °C for 7 min to ensure complete extension of all amplicons. A positive (previously confirmed) control and a negative control (no template control) were used in PCR. Agarose gel electrophoresis was used to confirm the successful PCR amplification on 1.5% (w/v) agarose gel. The presence of one bright and distinct band with a size of about 650 bp, and no indication of the same band in the negative control lane, indicated successful amplification. Samples with positive PCR results were sequenced through Sanger sequencing upon purification using a PCR purification mini kit (Favorgen Biotech Corp., Taiwan). Each raw sequence chromatogram was subjected to stringent quality control, and low-quality reads were trimmed to retain only high-confidence sequences.

Molecular identification: Following successful sequencing, the raw forward and reverse chromatogram files for each specimen were analyzed using Geneious Prime (v.2025.2) (<https://www.geneious.com/updates/geneious-prime-2025-2>). The raw traces were visually inspected for quality, and low-quality or ambiguous bases at the 5' and 3' ends of each sequence were manually trimmed. For each specimen, the trimmed forward (FishF1 primer) and reverse (FishR1 primer) sequences were then reverse-complemented and assembled into a consensus sequence, with nucleotide ambiguities resolved by checking the corresponding chromatogram peaks.

The resulting consensus sequences were compared with two major molecular databases to determine the taxonomic identity of each specimen. First, each sequence was analyzed using the BLASTn (nucleotide-nucleotide) algorithm at the National Center for Biotechnology Information (NCBI) and searched against the NCBI non-redundant nucleotide (nr) database. Second, each sequence was submitted simultaneously to the Barcode of Life Data Systems (BOLD). We used the 'Species Level Barcode Records' identification engine, which compares the query sequence to the curated FISH-BOL (Fish Barcode of Life) reference library, a database validated for fish species identification. We also verified the sequences using SnapGene software (<https://www.snapgene.com/>) to confirm the reading frame and check for any unexpected insertions, deletions or stop codons. In this process, a single ORF (open reading frame) without internal stop codons or frameshifts was identified for partial COI sequences, and these sequences were finally submitted to NCBI GenBank. The GenBank accession numbers for all sequences are given in Table 4.

Phylogenetic analysis: To infer the evolutionary relationships among the Buriganga *Pterygoplichthys* populations, phylogenetic analysis was carried out using MEGA11. First, a detailed reference dataset was built. The dataset contained COI sequences obtained from specimens in this study. The sequences were combined with representative sequences of *Pterygoplichthys* downloaded from the NCBI GenBank database. Reference sequences were selected based on the highest scoring BLAST hits and relevant literature to ensure the inclusion of validated reference sequences for all three identified species: *P. disjunctivus*, *P. ambrosettii*, and *P. pardalis*. Reference sequences from key locations within their native range in South America (e.g., Argentina, Brazil) and other invaded regions (e.g., India, Japan, USA, Thailand) were included to provide a global context. Evolutionary relationships were inferred using the Maximum Likelihood method based on the Kimura 2-parameter (K2P) model, and bootstrap support values (%) based on 1,000 replicates are shown above branches.

Results and Discussion

Species identification by morphometric and meristic observations and DNA barcoding: Catfish specimens were first morphologically examined for primary characters and then cross-verified with DNA barcoding to confirm species identity. Table 2 shows a generally high agreement between morphological identification and COI DNA barcoding results for most samples. Morphology and COI sequences matching *P. pardalis* in the genetic databases were used to identify specimens with fully plated bellies and isolated spots as *P. pardalis*. Barcoding was used to confirm specimens with vermiculated abdominal patterns as *P. disjunctivus*. The congruent DNA barcode identification confirmed the morphological diagnosis for the organisms exhibiting the reticulate light-on-dark pattern of *P. ambrosettii*. Crucially, the comparison side-by-side shows how differences in belly pattern and armor between samples were used to reliably assign species. Overall, the combination of unique abdominal patterns (spots vs. vermiculations), armor traits (plating coverage and number of keels), and genetic barcodes, allowed for accurate species identification of the samples of suckermouth catfish, with *P. pardalis*, *P. disjunctivus*, and *P. ambrosettii* each identified by their characteristic features and DNA sequences.

To identify the specimens, the 597–642 bp COI sequences were blasted against the NCBI GenBank and BOLD (Barcode of Life Data) databases. The BLAST analysis data demonstrated the invasive population of the Buriganga River suckermouth catfish is not a common homogenous population, but rather a co-invasion of three species, including *P. disjunctivus*, *P. ambrosettii* and *P. pardalis*. The identification was not ambiguous, and all the samples were identified with high query coverage (99–100%), and identity (99–100%) with the reference sequences in the databases (Table 3). *P. disjunctivus* was the most dominant species that was present in 10 of the 14 samples while three samples were identified as *P. ambrosettii* (Table 3). Only one sample was identified as *P. pardalis* by barcode sequencing.

The study's findings present the main molecular identification of the invasive suckermouth catfish population in the Buriganga River, one of the most economically significant and ecologically degraded waterways in Bangladesh. Morphologically, these catfishes are extremely similar, distinguished only by subtle traits such as belly patterning, dorsal fin ray counts, and the presence of a nuchal crest (Wu et al., 2011). Such subtle differences are prone to misinterpretation. For instance, *P. ambrosettii* and *P. anisitsi* were historically confused synonyms, and *P. disjunctivus* was long misidentified as *P. multiradiatus* by aquarium hobbyists (Wu et al., 2011). The most important finding of this study is that the Buriganga River is not invaded by a single invasion species but is experiencing a complex co-invasion of at least three different invasion species, i.e., *P. disjunctivus*, *P. ambrosettii* and *P. pardalis*. The discovery of *P. disjunctivus* as the dominant organism in the given habitat adds a very important element to the rapidly evolving template of loricariid introductions in South Asia. This is also in line with the recent literature that suggests the increasing threat of various loricariid species in the wetlands of Bangladesh, where their real diversity and distribution are just starting to be known (Rana et al., 2023; Sarkar et al., 2023).

The co-occurrence of species is a known finding in other areas, for instance, in Taiwan, *P. pardalis* and *P. disjunctivus* were both discovered, but its establishment in Bangladesh highlights the importance of molecular-level surveillance in order to fully understand the extent of the issue. This contrasts with other regional studies, where the genetic lines were less distinct. For example, Sahoo et al. (2022) reported that East Kolkata Wetland specimens visually classified as *P. pardalis*, *P. disjunctivus*, or intermediates all shared an identical COI haplotype identified as *P. pardalis*, suggesting a single introduced lineage. This finding, and the presence of individuals with intermediate morphology, highlights the significant challenge of potential hybridization. If *P. pardalis* and *P. disjunctivus* interbreed (as has been suggested in other regions, such as Indonesia and the Philippines), mitochondrial COI barcodes, which reflect only the maternal lineage, will be insufficient to resolve the issue (Patoka et al., 2020). A hybrid could carry *P. pardalis* mtDNA even if it has mixed morphology, effectively hiding the genetic signature of *P. disjunctivus*. Jumawan et al. (2011) found evidence consistent with introgressive hybridization in an invasive population in the Philippines, leading them to call for a taxonomic re-evaluation of these nominal species. While our study successfully and clearly delineated three species, the lack of COI divergence between morphotypes in other studies (like Sahoo et al., 2022) suggests either a single species introduction in those regions or ongoing hybridization blurring the species boundaries.

Table 2. Comparative morphological and genetic identification of sucker-mouth catfish samples. The table provides an integrated view of traditional taxonomy and molecular identification for the invasive catfish samples, highlighting the diagnostic features of each species and the reliability of combined approaches in confirming species-level identification

ID	A	B	C	D	E	F	G	H	I	J	K	L	Suggested Species	Species by sequencing	Species by consensus
1H	1/12	1/4	1/5	1/5	14	27	Complex vermiculation	Small irregular pattern	Present	Vermiculated body	Fully plated	FSLK	<i>Pterygoplichthys ambrossetii</i>	<i>P. ambrossetii</i>	<i>P. ambrossetii</i>
2H	1/12	1/4	1/5	1/5	14	28	Vermiculation	no reticulate line	Present	vermiculated body	Vermiculated	WK	<i>Pterygoplichthys disjunctus</i>	<i>P. disjunctus</i>	<i>P. disjunctus</i>
3H	1/12	1/4	1/6	1/5	1/14	27	Complex vermiculation	No reticulate line	Present	Vermiculated body	Fully plated	WK	<i>P. disjunctus</i>	<i>P. ambrossetii</i>	<i>P. ambrossetii</i>
S1	1/12	1/4	1/6	1/5	1/14	28	Vermiculation	no reticulate line	Present	vermiculated body	vermiculated	WK	<i>P. disjunctus</i>	<i>P. disjunctus</i>	<i>P. disjunctus</i>
S3	1/12	1/4	1/5	1/5	1/14	28	Vermiculation	No reticulate line	Present	Vermiculated body	vermiculated	WK	<i>P. disjunctus</i>	<i>P. disjunctus</i>	<i>P. disjunctus</i>
S4	1/12	1/4	1/5	1/5	1/14	29	Vermiculation	No reticulate line	Present	Vermiculated body	vermiculated	WK	<i>P. disjunctus</i>	<i>P. disjunctus</i>	<i>P. disjunctus</i>
S5	1/12	1/4	1/5	1/5	1/14	28	Vermiculation	Reticulate line	Present	Vermiculated body	Vermiculated body	WK	<i>P. disjunctus</i>	<i>P. disjunctus</i>	<i>P. disjunctus</i>
S6	1/12	1/4	1/5	1/5	1/14	28	Complex vermiculation	Small irregular pattern	Present	Vermiculated body	Fully plated	FSLK	<i>P. ambrossetii</i>	<i>P. ambrossetii</i>	<i>P. ambrossetii</i>
S8	1/12	1/4	1/5	1/5	1/14	28	Vermiculation	Reticulate line	Present	Vermiculated body	Vermiculated	FSLK	<i>P. disjunctus</i>	<i>P. disjunctus</i>	<i>P. disjunctus</i>
S9	1/12	1/4	1/5	1/5	1/14	29	Vermiculation	No reticulate line	Present	Vermiculated body	Vermiculated	FSLK	<i>P. disjunctus</i>	<i>P. disjunctus</i>	<i>P. disjunctus</i>
S10	1/12	1/4	1/5	1/5	1/1	27	Vermiculation	No reticulate line	Present	Vermiculated body	Vermiculated	FSLK	<i>P. disjunctus</i>	<i>P. disjunctus</i>	<i>P. disjunctus</i>
S12	1/12	1/4	1/5	1/5	1/14	26	Vermiculation	Reticulate line	Present	Vermiculated body	Vermiculated	FSLK	<i>P. disjunctus</i>	<i>P. disjunctus</i>	<i>P. disjunctus</i>
S13	1/12	1/4	1/5	1/5	1/14	28	Vermiculation	No reticulate line	Present	Vermiculated body	Vermiculated	FSLK	<i>P. disjunctus</i>	<i>P. disjunctus</i>	<i>P. disjunctus</i>
S14	7/12	7/4	x	1/6	1/5	26	Discrete spot	No reticulate line	Present	Belly spotted	Discrete spot	FSLK	<i>P. pardalis</i>	<i>P. pardalis</i>	<i>P. pardalis</i>

Note: A: Dorsal Fin Ray: P/D/A = 1/10-12(> = 10 tall sail); Pleco = 1/7; B: Anal Fin Ray: 1/4; C: Pectoral Fin Ray: 1/5; D: Pelvic Fin Rays: 1/5; E: Caudal Fin Rays: 14; F: Lateral Line Plates:

Par = 26-32; Dis = 28-30; Ambro = 27-30; Placos = 5 row per line; G: Ventral Markings; H: Head pattern; I: Adipose fin; J: Diagnostic feature; K: Pattern, Pard = discrete spot, Dis = vermiculated/continuous, Ambro = fully plated, Plecos = variable spotting; L: Keels: WK = weak keels, FSLK = four strong lateral keels line.

Table 3. Species identification and sequence similarity of 14 *Pterygoplichthys* spp. from the Buriganga River

Specimen ID(s)	Species Outcome	Sequence size (bp)	Query Coverage (%)	Percent identity	Gene Bank Accession Number (Submitted)	Accession Number (Top Match) and country of origin
1H	<i>P. ambrosettii</i>	616	100	99.68	PX447864	MN854573.1 (Switzerland)
2H	<i>P. disjunctivus</i>	629	100	99.68	PZ023956	OQ932828.1 (India)
3H	<i>P. ambrosettii</i>	637	100	99.22	PZ406945	MN854573.1 (Switzerland)
S1	<i>P. disjunctivus</i>	624	100	99.84	PZ408586	PX672219.1 (Russia)
S3	<i>P. disjunctivus</i>	627	100	99.84	PZ408587	OQ932828.1 (India)
S4	<i>P. disjunctivus</i>	642	100	99.69	PZ408589	PX672219.1 (Russia)
S5	<i>P. disjunctivus</i>	627	100	99.84	PZ408588	PX672219.1 (Russia)
S6	<i>P. ambrosettii</i>	597	100	99.83	PZ408590	GU701545.1 (Canada)
S8	<i>P. disjunctivus</i>	624	100	99.84	PZ408592	OQ932828.1 (India)
S9	<i>P. disjunctivus</i>	627	100	99.84	PZ408591	OQ932828.1 (India)
S10	<i>P. disjunctivus</i>	634	99	99.84	PZ408593	PX672219.1 (Russia)
S12	<i>P. disjunctivus</i>	618	100	100	PZ408594	OQ932828.1 (India)
S13	<i>P. disjunctivus</i>	642	100	99.69	PZ408595	PX672219.1 (Russia)
S14	<i>P. pardalis</i>	624	100	99.84	PZ407626	PX107360.1 (Indonesia)

DNA barcode properties and nucleotide composition: Successful PCR amplification of the mitochondrial cytochrome c oxidase I (COI) gene was achieved for all 14 samples. The nucleotide composition of the 597-642 bp fragments were analyzed. The sequences exhibited a characteristic A+T bias (56.3%), which is a common feature of the mitochondrial genome in fish. The average Guanine (G) content (18.0%) was the lowest of the four nucleotides across all three species (Table 4).

Table 4. Average nucleotide composition (%) of the COI Gene for the identified *Pterygoplichthys* spp.

Species	A (%)	T (%)	C (%)	G (%)	A+T (%)	C+G (%)
<i>P. disjunctivus</i> (<i>n</i> = 10)	27.07 ± 0.28	30.89 ± 0.10	25.45 ± 0.24	16.28 ± 0.13	57.96 ± 0.30	41.72 ± 0.30
<i>P. ambrosettii</i> (<i>n</i> = 3)	26.71 ± 0.16	30.89 ± 0.09	25.75 ± 0.17	16.28 ± 0.19	57.61 ± 0.09	42.03 ± 0.18
<i>P. pardalis</i> (<i>n</i> = 1)	26.87	30.99	16.34	16.34	57.86	41.84
Overall average	26.98 ± 0.29	30.89 ± 0.013	25.52 ± 0.23	16.28 ± 0.16	57.87 ± 0.26	41.8 ± 0.26

Genetic distances: The genetic divergences among and between the identified three species were determined using the Kimura 2-Parameter (K2P) model. The comparison revealed an apparent barcoding gap, in which the genetic distance between species was far greater than the distances within species. Within-species variation, *P. disjunctivus* showed little divergence, with a mean K2P distance of 0.009 (0.000–0.022) (Fig. 3 and Table 5). This means that there exist great genetic similarities among these individuals. The three samples of *P. ambrosettii* (1H, 3H and S6) were separated by 0.031 (Fig. 3 and Table 5). The distance within the species could not be used in the case of *P. pardalis* due to the small sample (only one has been found).

The difference between species was extremely high. Mean distance between *P. disjunctivus* and *P. pardalis* were 0.017 (1.7%). *P. disjunctivus* and *P. ambrosettii* were found at a distance of 0.025 (2.5%), *P. ambrosettii* and *P. pardalis* were found at a distance of 0.026 (2.6%). This genetic separation is very obvious, and it demonstrates that they are three different species.

Table 5. Mean genetic divergence (Kimura 2-Parameter) within and between identified *Pterygoplichthys* species

Species	<i>P. Disjunctivus</i>	<i>P. ambrosettii</i>	<i>P. pardalis</i>
<i>P. disjunctivus</i>	0.009		
<i>P. ambrosettii</i>	0.025	0.031	
<i>P. pardalis</i>	0.017	0.026	N/A

N/A (Not Applicable): Intra-species divergence could not be calculated as there is only one specimen of *P. pardalis* (S14) found.

The extreme degree of intra-species genetic discrepancy in the enormous *P. disjunctivus* population (a prevailing K2P range of 0.009) is low by population standards, a common attribute of a recent introduction event, most likely a founder effect. This means the Buriganga species might have arisen from a few released individuals, creating a genetic bottleneck. This genetic diminution in the invasive populations, despite their remarkable expansion in geographical ranges, has been documented all over the world in this genus; a large-scale examination of invasive *P. pardalis* in Mexico, as an example, revealed an

exemplary lack of mitochondrial DNA variability in their introduction, despite their dispersion to a variety of environments (Vargas-Rivas et al., 2023).

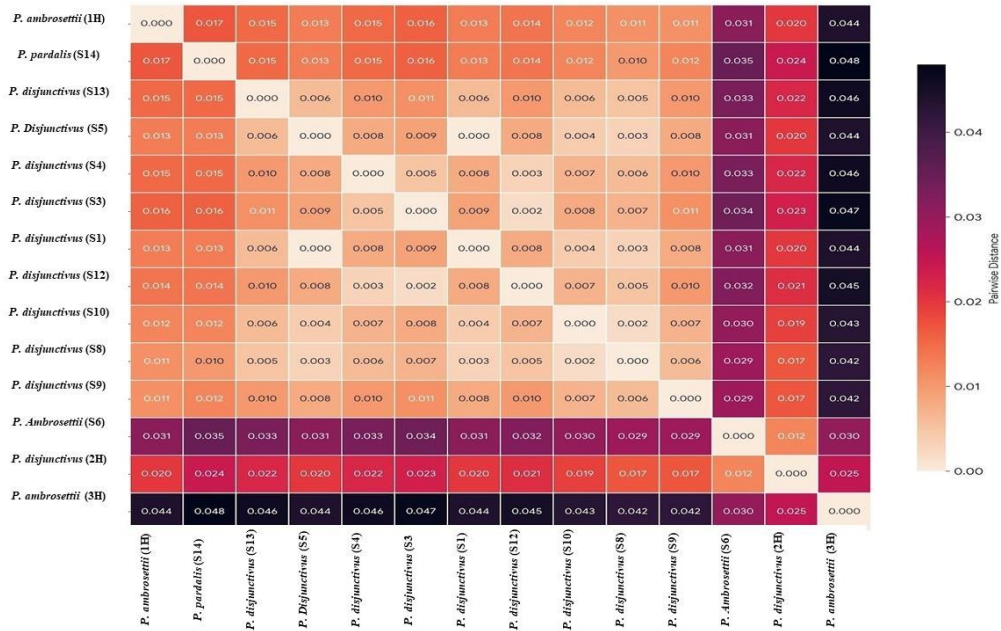


Fig. 3. Pairwise genetic distance heatmap based on COI (cytochrome c oxidase I) sequences of sucker mouth catfish (*Pterygoplichthys* spp.). The matrix illustrates intra- and interspecific nucleotide divergence among sampled individuals, with values representing Kimura 2-Parameter (K2P) genetic distances. Lower values (light coloration) indicate close genetic similarity, while higher values (dark coloration) reflect greater evolutionary divergence among taxa. Phylogenetic analysis: Fourteen high-quality COI sequences from this study were compared with global reference sequences from GenBank, representing both native South American and worldwide invasive populations of *Pterygoplichthys* (Fig. 4).

The majority of the Buriganga River specimens clustered within the *P. disjunctivus* lineage together with reference sequences originating from the Philippines, Russia, Japan, South Africa, and India. Several nodes within this clade received high bootstrap support values (95% to 100%) suggesting that the grouping of these specimens is highly supported (Figure 4). The close clustering of Bangladeshi samples with geographically distant reference sequences indicates low genetic divergence in the COI region and supports the identification of *P. disjunctivus* as the dominant invasive species in the studied area.

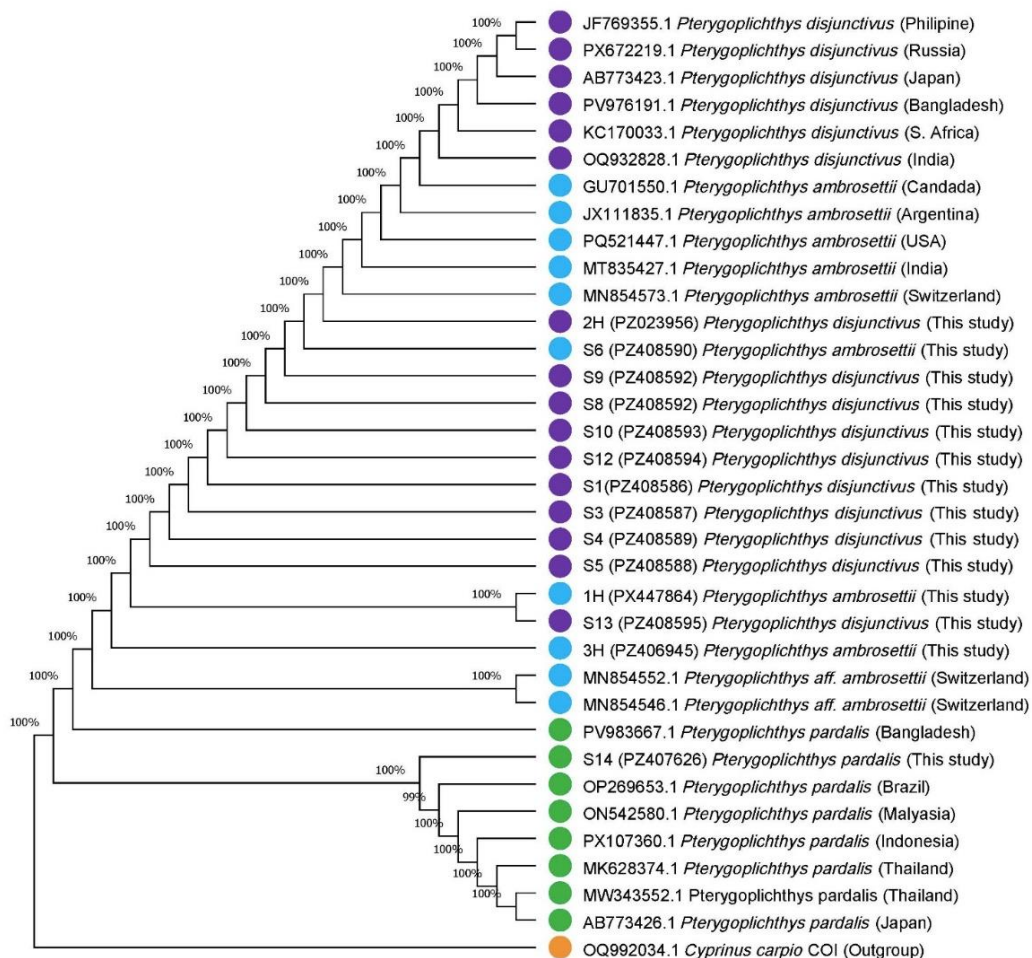


Fig. 4. Maximum Likelihood phylogenetic tree inferred from mitochondrial cytochrome c oxidase subunit I (COI) sequences of *Pterygoplichthys* spp. collected from the Buriganga River, Bangladesh, and reference sequences retrieved from GenBank. Sequences generated in the present study are marked as “This study”. *Cyprinus carpio* (OQ992034.1) was used as the outgroup.

The second group included specimens clustering with the reference sequences of authentic *P. ambrosettii* from Argentina, Canada, the United States, India and Switzerland. These specimens clustered with previously identified *P. ambrosettii* sequences, offering additional support for the presence of this species in the Buriganga River. The high bootstrap support within this lineage suggests that the COI marker is well-resolved enough to differentiate *P. ambrosettii* from other closely related members of the genus, despite their morphological similarity.

Specimen S14 represented another separate third lineage and grouped with *P. pardalis* reference sequences from Bangladesh, Malaysia, Indonesia, Thailand, Brazil and Japan. The position of this specimen within the *P. pardalis* clade, furnishes molecular evidence for the presence of this species in Buriganga River and indicates its genetic affinity to the populations reported from both native and introduced locations. The pattern of clustering suggests that *P. pardalis* is less common than *P. disjunctivus* in the sampled population but still contributes to the taxonomic diversity of invasive *Pterygoplichthys* spp. in the river system.

The phylogenetic relationships of the present study indicated that the Buriganga River population is not a single invasive lineage but a group of multiple species of *Pterygoplichthys*. Such a pattern could suggest multiple introductions, multiple source populations, or the release of mixed stocks through the ornamental fish trade, which has been widely recognized as the main pathway for the global spread of sailfin catfishes. The presence of three genetically distinct species in a relatively small sample area underscores the importance of DNA barcoding for species identification and for understanding the invasion dynamics of morphologically similar loricariid catfishes. The COI tree indicates mainly that the Buriganga River population is taxonomically heterogeneous. This point is significant because invasive loricariids are often grouped under general common names like “common pleco” or “sucker fish,” despite research from Asia indicating that multiple *Pterygoplichthys* species may inhabit the same invaded waters. Page & Robins (2006), studying in South-East Asia, recorded the presence of *P. pardalis*, *P. disjunctivus*, and *P. multiradiatus* in non-native environments and highlighted how the ventral patterns are useful for distinguishing the first two species: distinct ventral spots in *P. pardalis* compared to vermiculate or merged ventral markings in *P. disjunctivus*. They further posited that the most probable route of introduction in the area was through aquarium releases or escapes. The Buriganga material generally fits that diagnostic framework, indicating clear occurrences of *P. pardalis* and *P. disjunctivus*, whereas the added presence of *P. ambrosettii* implies that urban waters in Bangladesh might be harboring a more diverse propagule pool than typically expected. The present results closely resemble the southern Vietnam study by Stolbunov et al. (2021), which employed morphological and molecular data to identify *P. pardalis*, *P. disjunctivus*, and their interspecific hybrid across major river systems in southern Vietnam. The comparison is useful as it shows that multi-taxon invasions in a tropical Asian freshwater ecosystem are not uncommon. It aids in understanding why any remaining specimen-level discrepancies between external morphology and mitochondrial placement in the Buriganga material should be openly addressed instead of being concealed as a mistake. In invaded *Pterygoplichthys*, taxonomic ambiguity has been associated with phenotypic similarity and potential introgression/hybridization. The study in Mexico conducted by Vargas-Rivas et al. (2023) clearly indicated that, despite the phenotypic differences observed across basins, their COI and CYTB data showed a singular mitochondrial haplotype, with the invasive fish being classified as *P. pardalis* or as hybrids

resulting from one or a limited number of introduction events. In relation to that pattern, the Buriganga tree aligns better with either successive introductions of various lineages or the distribution of mixed ornamental varieties. The probable route of introduction is the trade of ornamental fish. This interpretation closely aligns with the South-East Asian synthesis by Page & Robins (2006), who observed that these catfishes are prevalent in the pet trade and were likely released by fish keepers, as well as with wider invasion discussions referenced in subsequent studies from Vietnam and Mexico. For an urban river like the Buriganga, aquarium releases and escapes from captivity are more plausible explanations than natural range growth. Ecologically, the discovery of the Buriganga is significant as the introduced *Pterygoplichthys* are not just taxonomic oddities. Page & Robins (2006) outlined various impact mechanisms already recorded elsewhere: tunneling and burrowing may enhance siltation, sizable herbivore populations can change food web dynamics, and competition with indigenous fish species has been proposed in Florida. They also contended that established populations can diminish the energy accessible to native herbivores and the fish that feed on associated arthropods. These pathways are closely connected to a stressed river system like the Buriganga, where a plentiful, benthic, armored, air-breathing grazer could exert additional pressure on already affected littoral and benthic habitats.

Conclusion

This research offers the first molecular based evidence of invasive species of suckermouth armored catfish (*Pterygoplichthys* spp.) in the Buriganga River, Bangladesh, through COI DNA barcoding. Morphological and molecular studies of the sampled specimens revealed the presence of at least three *Pterygoplichthys* species including *P. disjunctivus*, *P. ambrosettii*, and *P. pardalis*. Elevated sequence similarity and clustering in phylogenetics validate the efficiency of DNA barcoding for identifying species. The presence of three species signifies invasion through several introductions from the ornamental fish trade. Their presence in a disrupted urban river emphasizes their flexibility and ability to invade. This genetic foundation allows for the tracking of invasive loricariid catfish in Bangladesh. Wider surveys, genetic examinations, and ecological research are essential to comprehend their distribution and guide the management of indigenous biodiversity.

Authors' Contribution

Saykot Sawjal: Investigation, Methodology, Writing original draft; **Md. Al Zahid:** Supervision, Funding acquisition, Conceptualization, Methodology, Writing review & editing; **Md. Habibullah-Al-Mamun:** Methodology, Writing review & editing; **Anwar Hossain:** Methodology, Writing review & editing; **Md. Mizanur Rahman:** Methodology, Validation, Writing review & editing; **Nasir Uddin:** Methodology, Validation, Writing review & editing; **Md. Hasan Faruque:** Methodology, Validation, Visualization, Writing review & editing; **Nilufa Yeasmin:** Methodology, Validation, Writing review & editing;

Fida Chowdhury: Methodology, Validation, Visualization, Writing review & editing;
Shankar Chandra Mandal: Supervision, Conceptualization, Methodology, Validation, Visualization, Formal analysis, Writing review & editing. All authors read and approved the manuscript.

Conflict of interest

The authors declare that they have no financial or non-financial interests to disclose.

Ethical statement

The study was reviewed and approved by the Ethical Review Committee of the Faculty of Biological Sciences, University of Dhaka (Ref. No. 314/Bio.Scs)

Declaration of generative AI and AI-assisted technologies

During the preparation of this work the authors used QuillBot in order to paraphrase. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

References

- Armbruster, J. W., & Page, L. M. (2006). Redescription of *Pterygoplichthys punctatus* and description of a new species of *Pterygoplichthys* (Siluriformes: Loricariidae). *Neotropical Ichthyology*, *4*(4), 401–410.
- Banglapedia. (2014). Buriganga River. In Banglapedia: National Encyclopedia of Bangladesh. Asiatic Society of Bangladesh.
- Capps, K. A., Nico, L. G., Mendoza-Carranza, M., Arévalo-Frías, W., Ropicki, A. J., Heilpern, S. A., & Rodiles-Hernández, R. (2011). Salinity tolerance of non-native suckermouth armoured catfish (Loricariidae: *Pterygoplichthys*) in south-eastern Mexico: implications for invasion and dispersal. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *21*(6), 528–540.
- Chavez, J. M., De La Paz, R. M., Manohar, S. K., Pagulayan, R. C., & Vi, J. R. C. (2006). New Philippine record of south american sailfin catfishes (Pisces: Loricariidae). *Zootaxa*, *1109*(1), 57–68.
- Covain, R., & Fisch-Muller, S. (2007). The genera of the Neotropical armored catfish subfamily Loricariinae (Siluriformes: Loricariidae): a practical key and synopsis. *Zootaxa*, *1462*(1), 1–40.
- Galib, S. M. (2015). Fish fauna of the Brahmaputra River, Bangladesh: richness, threats and conservation needs. *Journal of fisheries*, *3*(3), 285–292.
- Hossain, M. Y., Rahman, M. M., Ahmed, Z. F., Ohtomi, J., & Islam, A. B. M. S. (2008). First record of the South American sailfin catfish *Pterygoplichthys multiradiatus* in Bangladesh. *Journal of Applied Ichthyology*, *24*(6).

- Hossain, M. Y., Vadas Jr, R. L., Ruiz-Carus, R., & Galib, S. M. (2018). Amazon sailfin catfish *Pterygoplichthys pardalis* (Loricariidae) in Bangladesh: a critical review of its invasive threat to native and endemic aquatic species. *Fishes*, **3**(1), 14.
- Jumawan, J. C., Vallejo, B. M., Herrera, A. A., Buerano, C. C., & Fontanilla, I. K. C. (2011). DNA barcodes of the suckermouth sailfin catfish *Pterygoplichthys* (Siluriformes: Loricariidae) in the Marikina River system, Philippines: Molecular perspective of an invasive alien fish species. *Philippine science letters*, **4**(2), 103–113.
- Ludlow, M. E., & Walsh, S. J. (1991). Occurrence of a South American armored catfish in the Hillsborough River, Florida. *Florida Scientist*, 48–50.
- Orfinger, A. B., & Goodding, D. D. (2018). The global invasion of the suckermouth armored catfish genus *Pterygoplichthys* (Siluriformes: Loricariidae): annotated list of species, distributional summary, and assessment of impacts. *Zoological Studies*, **57**, e7.
- Page, L. M., & Robins, R. H. (2006). Identification of sailfin catfishes (Teleostei: Loricariidae) in southeastern Asia. *The Raffles Bulletin of Zoology*, **54**(2), 455–457.
- Parvez, M. T., Lucas, M. C., Hossain, M. I., Chaki, N., Mohsin, A. B. M., Sun, J., & Galib, S. M. (2023). Invasive vermiculated sailfin catfish (*Pterygoplichthys disjunctivus*) has an impact on highly valued native fish species. *Biological Invasions*, **25**(6), 1795–1809.
- Patoka, J., Takdir, M., Yonvitner, Aryadi, H., Jerikho, R., Nilawati, J., Tantu, F.Y., Bohata, L., Aulia, A., Kamal, M.M. and Wardiatno, Y. (2020). Two species of illegal South American sailfin catfish of the genus *Pterygoplichthys* well-established in Indonesia. *Knowledge & Management of Aquatic Ecosystems*, (421), 28.
- Rana, M. M., Mahmud, S., Siam, K. H. H., Chowdhury, H. A., Das, S. R., Islam, M., ... & Naser, N. (2023). Invasive Suckermouth catfishes (Siluriformes: Loricariidae) in Bangladesh wetlands: A review on the present status and challenges. *Bangladesh Journal of Zoology*, **51**(3), 371–396.
- Sahoo, L., Swain, P. P., Hussan, A., Mohapatra, A., & Sundaray, J. K. (2022). DNA barcoding delineates the identity of invasive South American sucker mouth armoured catfishes of genus *Pterygoplichthys* of East Kolkata Wetland, West Bengal, India as single species. *Indian Journal of Fisheries*, **69**(4), 115–118.
- Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, **4**(4), 406–425.
- Sarkar, A., Rana, S., Bhowmik, P., Hasan, M.N., Shimul, S.A. and Nahid, S.A.A. (2023) 'A Review of Suckermouth Armoured Catfish (Siluriformes: Loricariidae) Invasion, Impacts and Management: Is Its Invasion a Threat to Bangladesh's Fisheries Sector?', *Asian Fisheries Science*, **36**(3), 128–143.
- Stolbunov, I. A., Dien, T. D., & Karabanov, D. P. (2021). Taxonomic composition and distribution of alien suckermouth armored catfish (Siluriformes: Loricariidae) in Southern Vietnam. *Inland Water Biology*, **14**(3), 263–273.

- Vargas-Rivas, A. G., Barba-Macias, E., Sánchez, A. J., & Castellanos-Morales, G. (2023). Lack of mtDNA genetic diversity despite phenotypic variation and environmental heterogeneity in the exotic suckermouth armored catfish (*Pterygoplichthys pardalis*). *Biological Invasions*, **25**(4), 1035–1056.
- Ward, R. D., Zemlak, T. S., Innes, B. H., Last, P. R., & Hebert, P. D. (2005). DNA barcoding Australia's fish species. *Philosophical transactions of the royal society B: biological sciences*, **360**(1462), 1847–1857.
- Wu, L. W., Liu, C. C., & Lin, S. M. (2011). Identification of exotic sailfin catfish species (*Pterygoplichthys*, Loricariidae) in Taiwan based on morphology and mtDNA sequences. *Zoological studies*, **50**(2), 235–246.

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