Indian J. Fish., 69(4): 115-118, 2022
DOI: 10.21077/ijf.2022.69.4.124310-15

Note

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

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ABSTRACT

Invasive suckermouth armoured sailfin catfishes of *Pterygoplichthys* genus is a threat to natural fish biodiversity. We attempted to identify suckermouth armoured sailfin catfishes, *Pterygoplichthys* spp. which have successfully invaded and established in the East Kolkata Wetland, using morphological and molecular techniques. Based on the colouration pattern/spot on the belly region they were categorised into three groups *viz., Pterygoplichthys pardalis*, *Pterygoplichthys disjunctivus* and an intermediate group. In total, fin clips from 32 individuals were collected from the above three groups and a portion of mitochondrial cytochrome oxidase subunit-I (COI) gene was sequenced using Sanger's method. Out of the 32 individuals, good quality sequences were obtained for 30 individuals. Surprisingly, mtDNA COI gene sequence analysis revealed that a sequence length of 581 bp was identical among all individuals. Species level barcode records from BOLD database revealed the identity of specimens in all categories as *P. pardalis.* Results of the study suggest that *Pterygoplichthys* established in East Kolkata Wetland might have originated from a single reproducing entity.

Keywords: Cytochrome oxidase subunit1, East Kolkata Wetland, Mitochondrial DNA, *Pterygoplichthys,* Suckermouth sailfin catfish

Presence of invasive alien species (IAS) in natural habitats is a sign of loss of biodiversity leading to decline in native fish species globally (Gurevitch and Padilla, 2004; Vila *et al.,* 2011). Ornamental suckermouth armoured catfish such as *Pterygoplichthys multiradiatus* (Hancock, 1828) are high in demand in south India (Knight, 2010). However, problems arise when they grow in size and become difficult to maintain in aquaria. At this point, the traders and hobbyists release them into natural water bodies. This happens due to lack of clear-cut guidelines or policies on disposal and propagation of exotic ornamental fish species especially those having detrimental effect on indigenous biodiversity. Krishnakumar *et al.* (2009) reported *P. multiradiatus* in Vylathur and the Chackai Canal of Kerala, India. Further, it has also been reported from other places like wetlands of Chennai (Knight, 2010). Similarly, *Pterygoplichthys anisitsi* (Eigenmann and Kennedy, 1903) has been reported from Bihar by Sinha *et al.* (2010). *Pterygoplichthys disjunctivus* (Weber, 1991) and *Pterygoplichthys pardalis* (Castelnau, 1855) were recorded from Andhra Pradesh, West Bengal, Bihar and Uttar Pradesh (Singh, 2014). The invasive catfish species such as *P. disjunctivus* and *P. pardalis* were reported from Gomokpota Beel under East Kolkata Wetlands (Hussan *et al*., 2016). Further, the author also witnessed spreading of these species in the pond embankments of East Kolkata Wetland (EKW).

The South American suckermouth armoured catfishes are well known for causing substantial harm in terms of competition for food and increasing water turbidity (Chavez *et al*., 2006; Nico, 2010; Orfinger and Goodding, 2018). One of the major threats to aquaculture by these species is collapse of pond embankment erosion caused by nest burrows (Nico, 2010). Though various species of suckermouth sailfin catfishes were reported from EKW, proper taxonomic identification through DNA barcoding have not yet been attempted, a dearth we seek to address in the current study.

Pterygoplichthys specimens were collected from East Kolkata Wetlands (EKW, 22°25'-22°40' N; 88°20'-

88°35' E), West Bengal, India as described earlier and grouped into three broad groups *viz.,* PP (*P. pardalis*), PD (*P. distinctive*) and PI (intermediary) (Fig. 1) (Hussan *et al.,* 2019). Handling of fish was complied with the guidelines for control and supervision of experiments on animals by the Government of India and approved by Institutional Animal Ethics Committee (AEC) of ICAR-CIFA, Bhubaneswar. In total, 32 fin clips comprising 7 from PP group, 8 from PD group and 15 from PI group were collected and intact genomic DNA was isolated following standard phenol-chloroform method (Russell and Sambrook, 2001). The quantity of isolated DNA was checked on 0.8% agarose gel and quantified using nanodrop spectrophotometer.

The isolated DNA was subjected to polymerase chain reaction (PCR) amplification using primers, Fish F1-5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR 2-5'ACTTCAGGGTGACCGAAGAATCAGAA-3' (Ward *et al.,* 2005) to amplify a portion of mtDNA COI gene of 658 bp size at 54^{0}C of annealing temperature. Amplification reaction was performed in 25 µl reaction volume containing 50 ng of template DNA, 2.5 µl 10x PCR buffer with $MgCl_2$, 1 µl of each forward and reverse primer (10 pmol ml^{-1}), 2 µl dNTP mix (2.5 mM each) and 0.25 U of taq DNA polymerase. PCR was carried out in HiMedia Prima-96™ thermal cycler for 35 cycles. The PCR conditions used were: Initial denaturation at 95^{0}C for 5 min followed by denaturation at 94^{0}C for 30 s, annealing at 54^{0}C for 30 s, extension at 72^{0}C for I min and final extension at 72^{0}C for 10 min (Mohanty *et al.,* 2013). The PCR products were purified using Qiagen PCR purification kit and sequenced on an ABI 3130 xl automated capillary sequencer (Foster City, CA, USA). The raw sequences were edited manually using Bioedit ver. 7.5.0.3 (Hall, 1999) and consensus sequences were generated using the program Lasergene 8 (DNASTAR). The consensus sequences were aligned using ClustalW (Thompson *et al.,* 1994) as implemented in BioEdit ver. 7.2.5 (Hall, 1999). Nucleotide diversity haplotype and base pair differences were determined using the program DnaSP ver. 6.11.01 (Rozas *et al.,* 2017) and nucleotide saturation was tested by plotting number of transitions and transversions against Kimura-2-parameter distance using K80 model (Kimura, 1980) using DAMBE ver. 5.3.15 (Xia and Xie, 2001). The program MEGAX (Kumar *et al.,* 2018) was used to construct Neighbor Joining, Maximum Likelihood and Maximum parsimony consensus tree using Kimura-2-parameter model with 1000 replicates for bootstrap analysis. Species identification was performed by searching the sequences against species level barcode records of BOLD database.

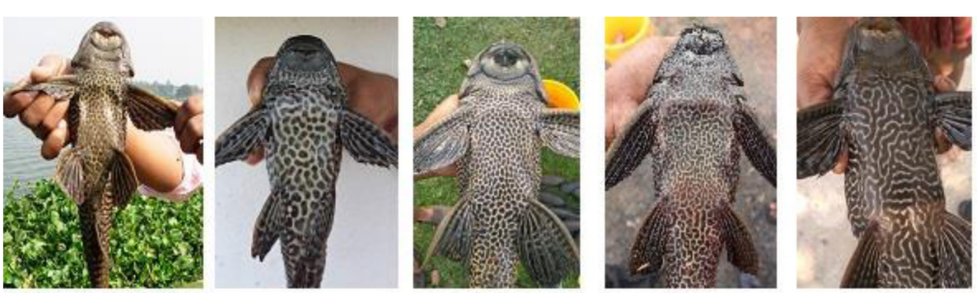
During the study period, frequent catch of suckermouth catfishes was observed in the ponds in EKW area. The average length and weight of the 32 sampled specimens ranged from 320 to 411 cm and 34.93 to 37.36 g, respectively (Table 1).

MtDNA COI gene partial sequence information of 30 individuals from 3 groups as classified based on the external characteristics and colouration/spots on the belly were obtained and submitted in the NCBI GenBank (Accession. No OM283619-OM283648). The aligned sequence data was found to be 581 bp in size. Unexpectedly, all the 581 sites were identical in all 30 individuals with nucleotide composition as A: 26.7, T: 31.5, G: 15.8 and C: 26.0% and G+C content was observed to be 41.8%. The *Pterygoplichthys* COI sequences were observed to be A+T rich. Species identification was performed by searching against species level barcode records of BOLD database and observed to match 100% with *P. terygoplichthys pardalis.*

In contrast to other studies, here we observed that all the 30 sequences were identical. Therefore, genetic distance and population genetic parameters were observed

Table 1. Average weight, length and the calculated values for the total length-weight relationship for three different groups of *Pterygoplichthys* sp. collected from EKW

Group	n	Average length (cm)	Average weight (g)
PP	09	37.36±2.49	411.62±72.51
PD	08	35.34±2.04	321.50±46.32
PI	15	34.93±1.59	320.80±35.66



G1 G2 G3 G4 G5

Fig. 1. Differentiation of *Pterygoplithchys* species based on abdominal colouration/spot pattern

to be zero. All the individuals were observed to be similar with respect to partial COI gene sequence variation.

The suckermouth sailfin catfishes are the most acclaimed ornamental fish among the pet trader community and considered as invader alien species worldwide (Hussan *et al.,* 2016). Different species under the genus *Pterygoplicthys* have been reported from lentic as well as lotic water bodies from different parts of India (Muralidharan *et al.,* 2014; Bijukumar *et al.,* 2015; Reenamole and Ambili, 2016; Rao and Sunchu, 2017). However, reports on details of the species abundance of the genus *Pterygoplicthys* from EKW are scanty except for the report by Hussan *et al.* (2019). Based on the morphological characteristics and belly colouration/spot patterns Hussan *et al.* (2019) reported two species of *Pterygoplicthys i.e. P. pardalis* and *P. disjuctivus*. In addition to these, the authors also reported another intermediate group having mixed characteristics.

P. pardalis and *P. disjuctivus* are endemic to Amazon River basin and Madeira River basin respectively (Orfinger and Goodding, 2018). In contrast to other fish species, overlapping of natural habitats is observed in these two species in their natural range. Identification of species of *Pterygoplicthys* genus outside its native tract has been explained by many hypotheses (Wu *et al.,* 2011; Nico *et al.,* 2012; Orfinger and Goodding, 2018). Intermediate morphotypes observed are suggested to be hybrids of *P. pardalis* and *P. disjuctivus* and known as "hybrid swarms" (Wu *et al.,* 2011; Nico *et al.,* 2012). According to another school of thought, variation in colouration patterns between *P. disjunctivus* and *P. pardalis* is suggested as variation within a species and therefore *P. disjunctivus* is considered to be synonym of *P. pardalis* (Jumawan *et al.,* 2011; Wu *et al.,* 2011). In the present study, though abdominal colouration was distinct, molecular data revealed that all the sampled individuals belong to a single species and species identification using BOLD database revealed the identity to be *P. pardalis.*

Analysis of COI sequences revealed that all the 30 *Pterygoplicthys* individuals collected from EKW are identical. Decline in diversity of native species is considered to be mostly caused by invasion of non-indigenous species like *Pterygoplicthys*. Invasive species often exhibit low genetic diversity due to founder effect or population bottleneck (Allendorf and Lundquist, 2003). This might be the reason why no sequence variation was observed in the present investigation among 30 *Pterygoplicthys* individuals collected from different regions of EKW exhibiting different abdominal colouration patterns. Our molecular data suggests that, the *Pterygoplicthys* present in the EKW might have established from a single reproducing entity, either escaped from aquaria or introduced occasionally by the aquarium traders or hobbyists. Further, the possibilities of hybridisation among the species can not be ruled out. However, hybridisation among *Pterygoplicthys* might result in variation among the collected samples. Therefore, further investigations by taking nuclear markers is needed to resolve the identity issue of *Pterygoplicthys* present in EKW.

In conclusion, three groups of *Pterygoplicthys* were identified morphologically based on the pigmentation patterns observed on the belly region. Out of the total samples collected, 30 individuals were randomly selected and subjected to DNA barcoding by sequencing a portion of mtDNA COI gene using universal primers. Analysis of COI sequences revealed no sequence variation. Species identification using BOLD data revealed that, *Pterygoplicthys* samples collected belong to the species *P. pardalis.* This might be due to the fact that the *Pterygoplicthys* individuals might have originated from a single reproducing entity. However, further investigations using large number of individuals and both nuclear and mtDNA markers may be required to support the present findings.

Acknowledgements

The authors are thankful to the Director, ICAR-CIFA, Bhubaneswar for providing laboratory facilities. This work has been supported by the Science and Engineering Research Board (SERB), Department of Science and Technology (DST), Govt. of India (SERB-CRG/2018/001909).

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Date of Receipt : 28.05.2022
Date of Acceptance : 06.12.2022