Journal of Threatened Taxa | www.threatenedtaxa.org | 26 March 2015 | 7(3): 6987–6995

Communication

ISSN 0974-7907 (Online)
ISSN 0974-7893 (Print)

OPEN ACCESS

Invasion of South American suckermouth armoured catfishes *Pterygoplichthys* spp. (Loricariidae) in Kerala, India - a case study

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Abstract: This paper documents the occurrence of the exotic South American suckermouth armoured catfishes (Loricariidae) of the genus *Pterygoplichthys* spp. in the drainages of Thiruvananthapuram City, Kerala. The morphological taxonomy revealed that the specimens are closely related to *Pterygoplichthys disjunctivus* (Weber, 1991) and *P. pardalis* (Castelnau, 1855), in addition to intermediary forms of unknown identity. DNA barcoding using the mitochondrial DNA cytochrome c oxidase 1 (CO1) also failed to establish the identity of the invaded species. There may be possible hybridisation in aquariums or in fish farms or in the wild, but this needs to be found out aided by detailed studies incorporating different molecular markers and with sequences of topotypes. The possible threats due to *Pterygoplichthys* spp. invasion and management options are discussed in the paper.

Keywords: Hybridisation, invasive alien species, mitochondrial DNA, ornamental fish, taxonomy.

Malayalam Abstract: കേരളത്തിൽ തിരുവനന്തപുരം നഗരത്തിലെ നീർച്ചാലുകളിൽ *Pterygoplichthys* ജനുസിൽപെട്ട വിദേശ അധിനിവേശ ജൈവജാതിയായ ദക്ഷിണഅമേരിക്കൻ സക്കർവായ കവചിത പൂച്ചമത്സ്യങ്ങളുടെ (ലോറിക്കേറിടെ കുടുംബം) സാന്നിദ്ധ്യമാണ് ഈ പ്രബന്ധത്തിൽ രേഖപ്പെടുത്തിയിരിക്കുന്നത്. ബാഹ്യരൂപത്തെ ആധാരമാക്കിയുള്ള വർഗീകരണ ശാസ്ത്രം അനുസരിച്ച് പ്രസ്തുത മത്സ്യങ്ങൾ *Pterygoplichthys disjunctivus* (Weber, 1991) and *P. pardalis* (Castelnau, 1855) എന്ന ജൈവജാതികളിൽപെട്ടവയും തിരിച്ചറിയാനാവാത്ത സങ്കരഇനങ്ങളുമാണെന്ന് വ്യക്തമായി. മൈറ്റോകോണ്ട്രിയയിലെ ഡി.എൻ.എ. തന്മാത്രയിലെ സൈറ്റോക്രോം സി ഓക്സിഡേസ് -1 (CO1) ബാർകോഡിങ്ങും അധിനിവേശ മത്സ്യങ്ങളുടെ തിരിച്ചറിയലിന് സഹായകമായില്ല. അക്വേറിയങ്ങളിലോ, മത്സ്യകൃഷിയിടങ്ങളിലോ, വന്യആവാസവ്യവസ്ഥകളിലോ വച്ച് ഇവയുടെ സങ്കരപ്രജനനം നടന്നിരിക്കാം. എന്നാൽ പല മോളിക്യുളാർ മാർക്കറുകളും ജീനുകളും ടോപ്പോടൈപ്പ് സീക്വൻസുകളും ഉപയോഗിച്ച് ഇത് കണ്ടെത്തേണ്ടതായുണ്ട്. സക്കർ മത്സ്യങ്ങളുടെ അധിനിവേശം സൃഷ്ടിക്കാൻ സാധ്യതയുള്ള പരിസ്ഥിതി പ്രശ്നങ്ങളും പരിപാലന മാർഗങ്ങളും പ്രബന്ധത്തിൽ ചർച്ചചെയ്യുന്നു.

DOI: http://dx.doi.org/10.11609/JoTT.o4133.6987-95

Editor: Anonymity requested.

Date of publication: 26 March 2015 (online & print)

Manuscript details: Ms # o4133 | Received 25 August 2014 | Final received 13 January 2015 | Finally accepted 05 March 2015

Citation: Bijukumar, A., R. Smrithy, U. Sureshkumar & S. George (2015). Invasion of South American suckermouth armoured catfishes *Pterygoplichthys* spp. (Loricariidae) in Kerala, India - a case study. *Journal of Threatened Taxa* 7(3): 6987–6995; http://dx.doi.org/10.11609/JoTT.o4133.6987-95

Funding: None.

Competing Interest: The authors declare no competing interests.

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Author contribution: AB did the taxonomic work, compiled the information and wrote the paper. RS conducted the field work along with AB and prepared the morphometry. US and SG were involved in DNA barcoding and analysis of the results.

Acknowledgements: The facilities provided by the Director, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram are gratefully acknowledged. Smrithy Raj thanks University Grants Commission for the Rajiv Gandhi National Fellowship.

INTRODUCTION

The ever increasing global trade of ornamental aquarium fishes is one of the most important and yet poorly documented pathways for aquatic bioinvasion (Rixon et al. 2005; Raghavan et al. 2013); one third of the world's worst aquatic invasive species are ornamental fishes (Padilla & Williams 2004). South American suckermouth armoured catfishes (Loricariidae), are popular throughout the world as aquarium pets because of their characteristic attractive appearance and 'use' in aquarium as a 'cleaner' to remove the algae. Native to the streams, floodplain lakes and marshes of South America, the suckermouth sailfin catfish *Pterygoplichthys* spp. have successfully invaded inland water bodies of various countries across the world, including Philippines (Joshi 2006; Hubilla et al. 2007), Japan (Nakabo 2002), Taiwan (Wu et al. 2011), Vietnam (Zworykin & Budaev 2013), Sri Lanka (Sumanasinghe & Amarasinghe 2013), Indonesia, Malaysia and Singapore (Page & Robins 2006), Israel (Golani & Snovsky 2013), Turkey (Ozdilek 2007), Bangladesh (Hossain et al. 2008), Mexico (Armando et al. 2007), Europe (Keszka et al. 2008; Piazzini et al. 2010; Simonovic et al. 2010) and USA (Nico & Martin 2001).

The suckermouth armoured catfishes reported from India include *Pterygoplichthys anisitsi* from Bihar (Sinha et al. 2010), *P. multiradiatus* from Kerala and Tamil Nadu (Ajithkumar et al. 1998; Daniels 2006; Krishnakumar et al. 2009; Knight 2010), *Pterygoplichthys disjunctivus* and *P. pardalis* from Andhra Pradesh, West Bengal, Bihar and Uttar Pradesh (Singh 2014). *Pterygoplichthys* is one of the fast dispersing species in the invaded countries, introduced primarily through uncontrolled pet trade and their invasion results in serious ecological and economic consequences (Nico et al. 2012). This paper documents the invasion of *Pterygoplichthys* spp. in the drainages of Thiruvananthapuram City, Kerala and the challenges faced during the identification of the invaded species.

MATERIALS AND METHODS

Study area, collection and taxonomy

The suckermouth sailfin catfishes were collected from the natural drainage, Amayizhanchan Thodu (Fig. 1), Thiruvananthapuram city ($76^0$54'–$76^0$58'E & $8^0$44'–$8^0$49'N), Kerala State using cast net of eight feet radius, during various periods in 2013. Out of the 1,023 specimens collected, 102 specimens were separated at random and transported live to the laboratory and the remaining fishes were released back into the water body, for ethical reasons. The fishes brought to the lab were frozen and were preserved in 10% formalin for morphometric studies. All morphometric measurements were taken with digital callipers on the left side of the fish to the nearest 0.1mm, and weight to the nearest milligram (mg) using a digital balance. Sixteen meristic and 37 morphometric characters of the specimens were examined, following the methods suggested by Armbruster (2003, 2004), Ambruster & Page (1996). Names of plate rows follow Schaefer (1997). The morphological identification was done in accordance with the keys of Armbruster (2001, 2004), Armbruster & Page (1996) and Page & Robins (2006).

DNA barcoding

Alcohol preserved fin clippings (n=12) from representative specimens sorted based on variations in patterns in abdomen were used to isolate total DNA using Qiagen Dneasy blood and tissue kit according to the manufacturer's instruction. The cytochrome oxidase 1 (CO1) gene of the mitochondrial DNA (mtDNA) was amplified using Fish F1 and Fish R1 primers (Ward et al. 2005) by following thermo cycling condition: Initial denaturation at 95^0C for 5 minutes, followed by 40 cycles of 95^0C for 30 seconds, 52^0C for 40 seconds, 72^0C for 1 minute, and a final extension at 72^0C for 7 minutes. PCR products were visualized on 1% agarose gels and purified using Exo Sap IT (USB). Bidirectional sequencing was performed using the PCR primers and products were labelled with BigDye Terminator V.3.1 Cycle sequencing Kit (Applied Biosystems, Inc.) and sequenced in an ABI 3730 capillary sequencer following manufacturer's instructions.

Twelve sequences from the present study along with 91 sequences retrieved from GenBank were aligned (565 bp) using Geneious Pro (6.0.5; http://www.geneious.com). In order to overcome the growing concern about the utility of both NJ and K2P for DNA barcode analysis (Blanco-Bercial et al. 2014), we used p-Distance to calculate genetic distance as suggested by Collins et al. (2012) for specimen identification and maximum likelihood (ML) tree generated under T92 model as best fitting model for providing a graphic representation of species divergence as implemented in MEGA Version 5 (Fig. 2) (Tamura et al. 2011).

Voucher specimens are deposited in the museum collections of the Department of Aquatic Biology and Fisheries, University of Kerala (DABFUK-FI-211 to DABFUK-FI-222) and COI sequences submitted to GenBank were given accession numbers KJ561586 to KJ561597.

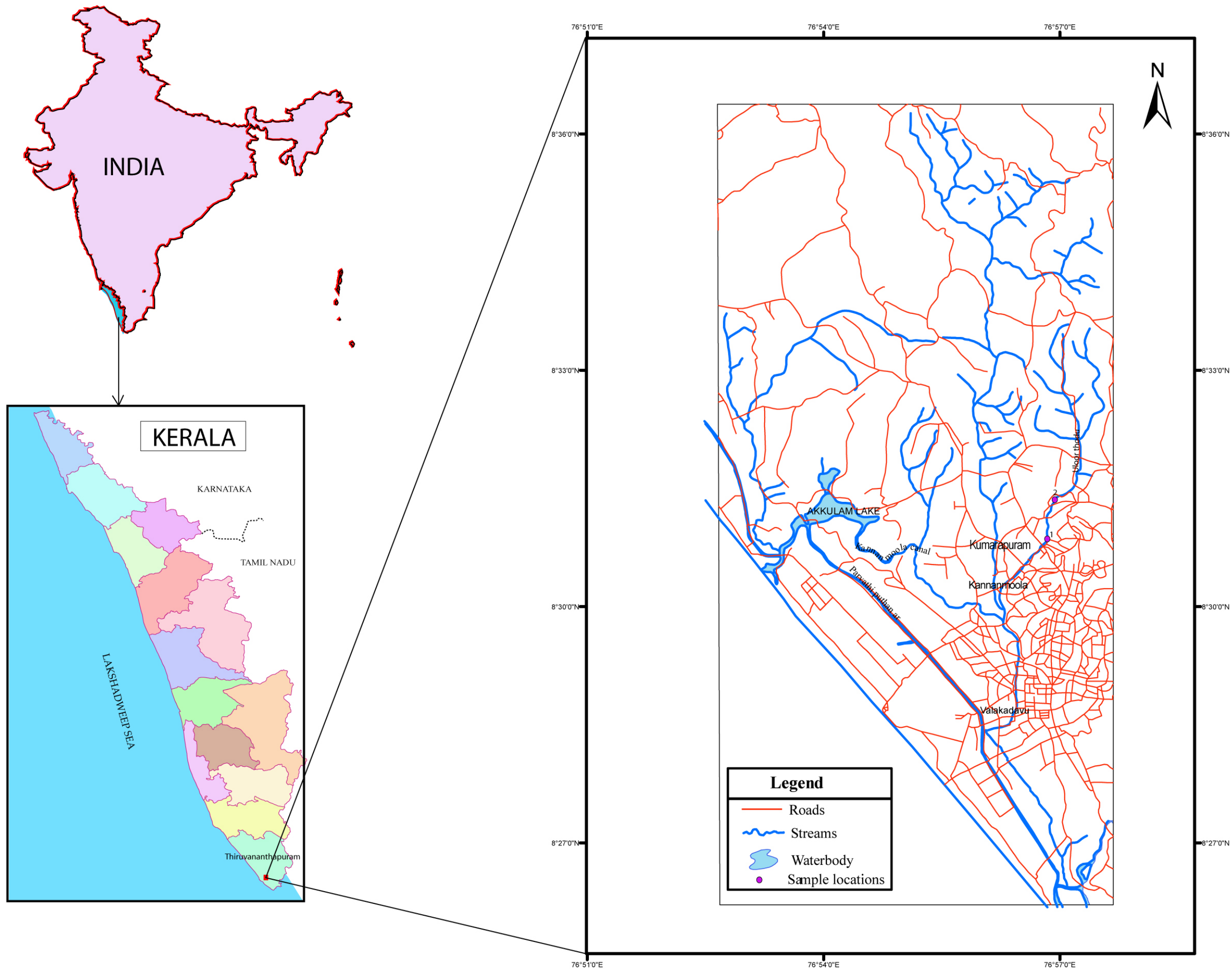

Figure 1. Drainages of Thiruvananthapuram City, Kerala showing collection sites

RESULTS AND DISCUSSION

The examination of 102 specimens based on colouration and morphometric and meristic characters (Tables 1, 2) revealed the presence of two putative species *Pterygoplichthys disjunctivus* (Weber, 1991) (Images 1, 3 i, j) and *P. pardalis* (Castelnau, 1855) (Images 2, 3a,b) and the inter-grade morphological forms (Images 3c–h). The specimens classified under these two species share similar morphometric measurements and show slight variations from the range of measurements given by Weber (1992). The number of fishes collected during each cast net operation varied from 3–27 (average 12), indicating their abundance in the aquatic ecosystem (Images 4, 5). Young fishes were recorded round the year from the drainages, indicating their successful invasion. The juveniles were not collected in the cast net as they prefer to hide among marginal vegetation and crevices along the side walls of the drainages. Further, we found that the crevices along the lateral granite walls of the drainages were used by the fishes for laying their eggs.

Image 1. *Pterygoplichthys disjunctivus* (Weber, 1991)

Although there is considerable variation in body shape and dentition, as a group loricariids are characterized by a depressed body covered by large bony plates, a unique pair of maxillary barbels, and a ventral suctorial mouth (Covain & Fisch-Muller 2007). The

Table 1. Meristic characteristics of *Pterygoplichthys disjunctivus* and *P. pardalis*

	Characters	Counts (n=102)
1	Dorsal fin rays (DFR)	11–13
2	Anal fin rays (AFR)	4–5
3	Caudal fin rays (CFR)	13–14
4	Pectoral fin rays (PCFR)	6–7
5	Pelvic fin rays (PLFR)	5
6	Lateral line plates (LLP)	27–30
7	Abdominal plates (AP)	5–7
8	Dorsal plates (DP)	3–4
9	Plates on dorsal interradial membrane (PDIM)	11–13
10	Plates on anal interradial membrane (PAIM)	2–3
11	Plates on pectoral interradial membrane (PPCIM)	1
12	Plates on pelvic interradial membrane (PPLIM)	2
13	Plates on adpressed pectoral fin (PAPCF)	6–9
14	Plates on adpressed pelvic fin (PAPLF)	7–10
15	Postanal plates (PAP)	12–16
16	Plates between dorsal fin base and adipose fin (PBDFAF)	6–7

Image 2. *Pterygoplichthys pardalis* (Castelnau, 1855),

species delineation within the genus *Pterygoplichthys* remains in chaos primarily because the four closely related species such as *P. anisitsi, P. multiradiatus, P. pardalis* and *P. disjunctivus* are separated only based on the nature of their abdominal patterns (Nico et al. 2012). All the specimens collected during the study lacked an elevated supraoccipital process and have the supraoccipital bone bordered posteriorly by three scutes. These traits are shared by four species - *P. anisitsi, P. multiradiatus, P. pardalis* and *P. disjunctivus*. *P. anisitsi* can be distinguished by the presence of light spots on a dark background in abdomen, while *P. multiradiatus* has a pattern of uncoalesced dark spots on a light background. Both *P. disjunctivus* and *P. pardalis* possess a pattern of coalesced dark spots on a light background in the abdominal region (Weber 1991, 1992).

Table 2. Morphometric characteristics of *Pterygoplichthys disjunctivus* and *P. pardalis*

	Characteristics	Range (mm)	Mean	Standard deviation
1	Total length (TL)	270.2–574.4	407.2	21.25
2	Standard length (SL)	220.8–430.8	310.4	10.22
3	Pre-dorsal length (PDL)	81.1–164.6	114.2	20.51
4	Head length (HL)	37.2–80.3	55.6	9.76
5	Head dorsal length (HDL)	27.1–61.2	45.3	8.85
6	Cleithral width (CW)	18.4–25.2	20.3	2.32
7	Head pectoral length (HPL)	13.8–29.7	19.8	6.54
8	Thorax length (THL)	31.7–75.7	51.6	11.63
9	Pectoral spine length (PCSL)	45.4–86.1	65.2	10.96
10	Abdominal length (AL)	34.3–75.0	51.7	11.41
11	Pelvic spine length (PESL)	60.0–110.2	83.3	12.50
12	Post-anal length (POAL)	63.3–139.5	92.2	20.25
13	Anal fin spine length (AFSL)	33.8–71.4	52.4	9.31
14	Dorsal pectoral distance (DPCD)	18.6–33.4	26.2	3.09
15	Dorsal spine length (DSL)	49.3–84.1	67.3	11.57
16	Dorsal pelvic distance (DPED)	18.4–32.3	25.9	3.04
17	Dorsal fin base length (DFBL)	82.4–134.3	105.7	18.61
18	Dorsal adipose distance (DAD)	24.3–54.3	41.9	17.20
19	Adipose spine length (ASL)	13.0–26.6	20.30	3.63
20	Dorsal adipose caudal distance (DACD)	9.1–19.3	13.3	2.72
21	Caudal peduncle distance (CPD)	13.4–26.0	19.3	3.582
22	Adipose anal distance (AAD)	21.3–53.5	29.1	6.031
23	Dorsal anal distance (DAND)	10.0–12.7	11.1	0.56
24	Pre-anal distance (PRAD)	128.2–269.5	205.6	36.98
25	Length of first caudal fin ray (LFCFR)	57.3–114.1	91.6	16.58
26	Length of last caudal fin ray (LLCFR)	54.1–107.0	88.2	88.16
27	Head eye length (HEL)	30.5–66.6	46.8	8.28
28	Orbit diameter (OD)	6.0–9.1	7.6	0.83
29	Snout length (SNL)	21.0–45.3	30.0	5.26
30	Internares width (INW)	14.0–30.0	22.6	4.53
31	Interorbital width (IOW)	32.4–59.9	40.7	6.38
32	Head depth (HD)	40.2–71.6	54.1	10.84
33	Mouth length (ML)	22.4–40.1	33.4	3.98
34	Mouth width (MW)	26.6–48.0	35.8	6.43
35	Barbel length (BL)	13.1–25.0	18.9	2.63
36	Dentary tooth cup length (DTCL)	17.1–20.4	18.6	0.95
37	Premaxillary tooth cup length (PTCL)	11.5–19.0	14.2	2.43

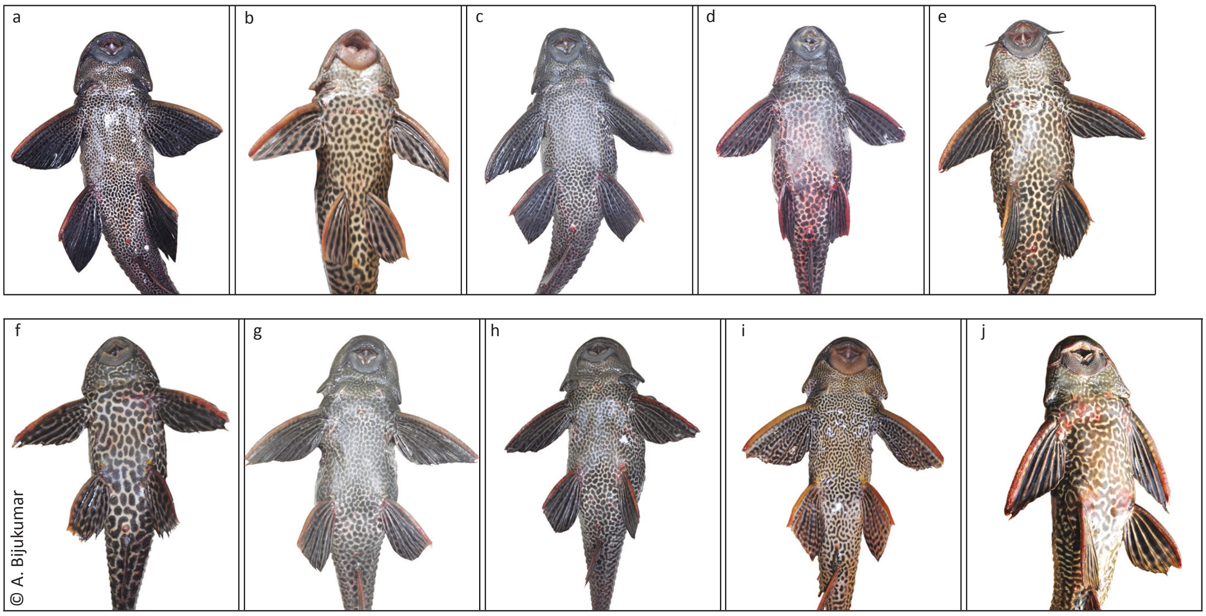

Image 3. Abdominal patterns of various specimens of *Pterygoplichthys* spp.:
a–b - *P. pardalis* species group; c–h - Inter-grades; i–j - *P. disjunctivus* species group

Image 4. Catch of suckermouth catfish in cast net

Image 5. Total catch of suckermouth catfish from a single cast net operation from the drainages of Thiruvananthapuram City, Kerala

Pterygoplichthys disjunctivus is a native of Rio Maderia drainage of Brazil and Bolivia (Page & Robins 2006). Out of the 102 specimens examined, six specimens exhibited the morphological characters of *P. disjunctivus*, which include abdominal colour pattern consisting of light and dark vermiculations formed as a result of coalescence of spots (Images 3i,j). *P. pardalis* occurs naturally in the Amazon River basin of Brazil and Peru (Weber 2003). It has discrete black spots on the ventral side of the body (Images 3a,b). The abdominal spots of the inter-grades were joined together, beginning to form vermiculations (Images 3c–h). Both *P. disjunctivus* and *P. pardalis* possessed a radiating pattern of light lines on the head. Absence of specific taxonomic characters to morphologically delineate species was a challenge and therefore the sequencing of mitochondrial gene CO1 was attempted for confirming the taxonomy of invaded fish.

A 565-bp CO1 fragment was used, including 12 sequences from the present study and 91 sequences of *Pterygoplichthys* spp. from GenBank. Among the 565 bp, 490 sites were conserved and 75 sites were variable. The genetic distance between the query sequences in the present study and that of the four putative species (*P. ambrosettii, P. pardalis, P. disjunctivus* and *P. joselimaianus*) was in the range of 0.0 to 0.007 (0%) and with that of *P. etentaculatus* was 0.03 (3%) (Appendix 1: attached as excel file).

Species level match queries using our *Pterygoplichthys* CO1 sequences in the GenBank showed conflicting matches with other loricariids. For example, the specimens identified as *P. disjunctivus* (Images 3i,j) in the present study showed 100% sequence similarity with *Pterygoplichthys pardalis, P. disjunctivus* and *P. joselimaianus*. All the other specimens in our collection showed 100% match with *P. ambrosettii, P. pardalis* and *P. disjunctivus.* However, the genetic distance between these species are 0%, indicating a severe taxonomic ambiguity among the species or a possible hybridisation in aquariums or fish farms or in the wild. The results show the possibility that the gene sequences uploaded by various researchers may be misidentifications or they may represent hybrid forms. Sequencing of topotypes of loricariids and use of nuclear as well as microsatellite markers would help resolving the issues on the identification of invaded loricariids.

The comparison of specimens using mitochondrial DNA of sequence data of putative species *Pterygoplichthys disjunctivus* and *P. pardalis* from Philippines (Jumawan et al. 2011) and Taiwan (Wu et al. 2011) also showed low resolution. Wu et al. (2011) also hypothesized possible hybrid origin of the introduced *Pterygoplichthys* exhibiting "hybrid superiority" which might have increased their fitness during invasions. According to Nico et al. (2012) some of the introduced populations of *Pterygoplichthys* show such extensive variations among individuals that they might best be described as "hybrid swarms".

Armoured catfishes have established natural populations across its invasive range (Zworykin & Budaev 2013) and there are several factors that contribute to the successful invasion of loricariids world over. The ability to withstand water pollution (Welcomme & Vidthayanom 2003), toleration of poor oxygen content in water by means of accessory respiration with diverticula of the gastrointestinal tract (Armbruster 1998), lower number of predators and less vulnerability to predation due to spiny fins and hard external 'armour' (Zworykin and Budaev 2013), occasional migrations across land and low current velocity (Nico et al. 2012), peculiarities in reproduction and development such as prolonged reproductive period, batch spawning, development of eggs at very low water levels and active parental care (Hoover et al. 2004; Liang et al. 2005), coupled with greater feeding opportunities in smaller streams (Nico et al. 2012) help them to invade a variety of ecosystems and to establish successfully. A survey conducted among the ornamental fish traders and hobbyists in Thiruvananthapuram City revealed an interesting fact: when the specimens grow in size, the traders and hobbyists find it difficult to keep them in tanks and therefore release them into the natural drainages (Bijukumar, unpublished information).

The effects of invasive armoured catfishes on the ecosystem and biodiversity are well documented (Hoover et al. 2004; Zworykin & Budaev 2013). Mortality of piscivorous birds due to the strong spines of the loricariid catfishes that have been reported (Bunkley-Williams et al. 1994; Hoover et al. 2004). Disruption of the food chain caused by the grazing behaviour of this fish has also been documented; nutrients are prematurely diverted from the "consumer" components of food webs and transformed into faeces available only to scatophags and decomposers (Smith 1981). The 'plowing' of the bottom substrate by these catfishes may also uproot and change the composition of aquatic vegetation (Hoover et al. 2004). The smaller sized herbivorous fishes with comparatively short life spans, low fecundity, and limited resistance to hypoxia and desiccation, would not compete with the more competitive loricariids established in the ecosystems (Hoover et al. 2004). The bottom feeding suckermouth catfishes may also incidentally eat demersal eggs of autochthonic species while consuming the bottom periphyton (Hoover et al. 2004).

Invasive *Pterygoplichthys*, being large and bewilderingly resilient species, is likely to out-compete the native algae consumers, aggressively drive them away, and consume the eggs of those species and others (Hoover et al. 2004); there are reports of invasive sailfin fish impacting local fisheries through these processes (Keszka et al. 2008, Mendoza-Carranza et al. 2010). In Sri Lanka, *P. pardalis* introduced in Pologolla reservoir is reported to make a significant negative impact on cichlid fisheries as this armoured catfish damages gillnets of the fishery (Sumanasinghe & Amarasinghe 2013). Further, *Pterygoplichthys* spp. use the larger crevices in the granite wall along the drainage canals for the purpose of nest making. Presence of young fishes throughout the year during the study is an indication that they breed all year round.

Pterygoplichthys species and putative hybrids have been extensively introduced outside their natural ranges and have established successful reproducing populations in inland waters in tropics and subtropics (Hoover et al. 2004; Mendoza-Carranza et al. 2010;

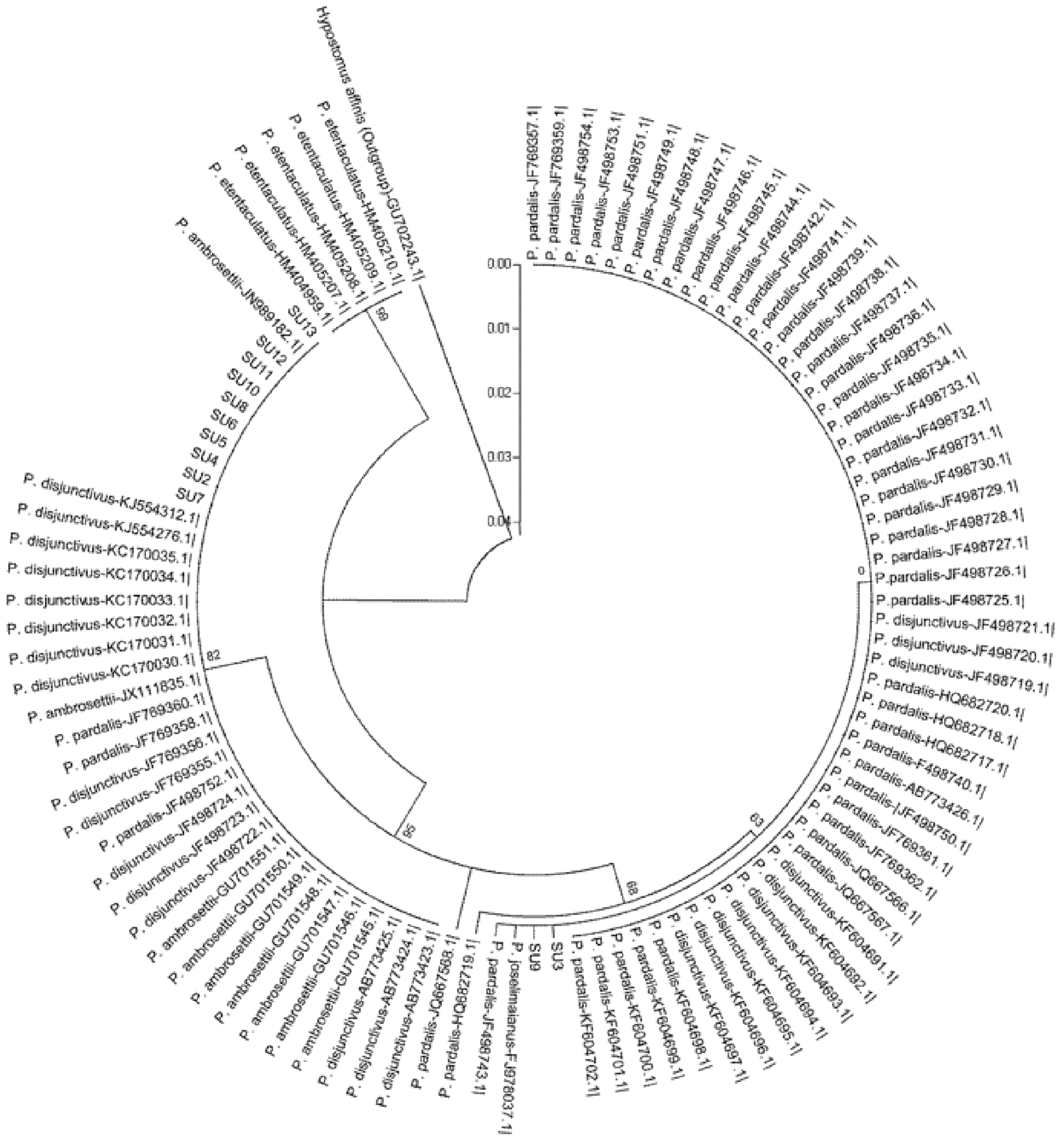

Figure 2. Maximum Likelihood (ML) tree of cytochrome oxidase I sequences of *Pterygoplichthys* spp. (SU 2 to SU 13) with similar sequences retrieved from NCBI-GenBank (54 species).

Nico et al. 2012; Sumanasinghe & Amarasinghe 2013; Zworykin & Budaev 2013). The effect of these invading armoured catfishes on the indigenous fish stock in India is not clear and therefore warrants further studies. Therefore, more systematic studies/risk assessments are warranted in India to document the socio-economic and environmental effects due to the invasion. In South America this fish and the value added products from them have been used extensively as a high quality food (Rueda-Jasso et al. 2013). As the first step in the process of eradication of this nuisance species, exploiting these fish as food can be tried in India, especially in those regions where they have established successful populations.

Controlling invasive species can be difficult, but the best method to prevent introduction is through policy making and education. There is an urgent need for a legislation to prevent the release of ornamental fish and potentially damaging cultivable alien species into the natural bodies and to formulate programmes to return the unwanted pets to the aquarium traders as a precautionary principle (Ishikawa & Trachihara 2014). Further, awareness material should be developed in local languages, explaining the ecological implications of this species, specifically targeting students, aquarium hobbyists and fish breeders.

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