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Research Article CORRECTED PROOF

# Salinity tolerance of non-native suckermouth armoured catfish (Loricariidae: *Pterygoplichthys* sp.) from Kerala, India

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## Abstract

Loricariid catfishes of the genus *Pterygoplichthys* are native to South America and have been introduced in many localities around the world. They are freshwater fishes, but may also use low-salinity habitats such as estuaries for feeding or dispersal. Here we report results of a field survey and salinity-tolerance experiments for a population of *Pterygoplichthys* sp. collected in Kerala, India. In both chronic and acute salinity-tolerance trials, fish were able to withstand salinities up to 12 ppt with no mortality; however, fish transferred to salinities > 12 ppt did not survive. The experimental results provide evidence that non-native *Pterygoplichthys* sp. are able to tolerate mesohaline conditions for extended periods, and can easily invade the brackish water ecosystems of the state. Further, *Pterygoplichthys* sp. from Kerala have greater salinity tolerance than other congeners. These data are vital to predicting the invasion of non-native fishes such as *Pterygoplichthys* spp. into coastal systems in Kerala and worldwide. This is particularly important as estuarine ecosystems are under threat of global climate change and sea-level rise. In light of the results of the present study and considering the reports of negative impacts of the species in invaded water bodies, management authorities may consider controlling populations and/or instituting awareness programmes to prevent the spread of this nuisance aquatic invasive species in Kerala.

**Key words:** ecophysiology, estuary, invasion, non-native fish, osmoregulation

## Introduction

Trade in aquarium fishes is one of the major pathways for the invasion of potentially harmful aquatic invasions globally (Gozlan et al. 2010; Magalhães and Jacobi 2013; Emiroğlu et al. 2016; Allen et al. 2017; Marr et al. 2017). The ever-increasing trade of ornamental fishes is one of the most important pathways for aquatic invasion in India (Singh et al. 2013; Raghavan et al. 2013; Kumar et al. 2015; Soundararajan et al. 2015; Sandilyan 2016; Muralidharan 2017). Among commonly-traded freshwater ornamental fishes, suckermouth armoured catfishes of the family Loricariidae and genus *Pterygoplichthys* T.N. Gill native to inland water bodies of South America, have established successful invasive populations throughout the world (Fuller et al. 1999; Nico and Martin 2001; Nakabo 2002; Chavez et al. 2006; Joshi 2006; Page and Robins 2006; Armando et al. 2007; Ozdilek 2007; Hossain et al. 2008; Keszka et al. 2008; Nico et al. 2009; Piazzini et al. 2010; Simonović et al. 2010; Wu et al. 2011; Gibbs et al. 2013; Golani and Snovsky 2013; Jones et al. 2013; Rueda-Jasso et al. 2013; Sumanasinghe and Amarasinghe 2013; Zworykin and Budaev 2013; Ishikawa and Tachihara 2014; Jumawan and Herrera 2014; Wei et al. 2017). *Pterygoplichthys* spp. are sold in markets under various common names including armoured catfishes, janitor fish, sailfin catfishes, plecos, glass cleaner catfishes, suckermouth catfishes and others. In India *Pterygoplichthys* spp. have been recorded from several natural water bodies and in many places they have become invasive species within the last two decades (Daniels 2006; Krishnakumar et al. 2009; Knight 2010; Sinha et al.

2010; Singh 2014; Singh et al. 2013, 2014; Kumar et al. 2015; Muralidharan et al. 2015; Panikkar et al. 2015; Meena et al. 2016; Rao and Sunchu 2017).

*Pterygoplichthys* catfishes have many attributes that are thought to have facilitated their invasion around the world. They are able to tolerate wide fluctuations in flow regimes (Welcomme and Vidthayanom 2003; Nico et al. 2012) and resist hypoxia due to structural modifications in the stomach that allow them to breathe atmospheric air (da Cruz et al. 2013). They also have efficient reproductive strategies and deter predation by possessing bony plates that cover their body (Hoover et al. 2004; Wei et al. 2017). Furthermore, invasion success of a non-native species is largely determined by its ability to adapt to the physiochemical parameters in the invaded environment (Kestrup and Ricciardi 2010). For many non-native freshwater fishes, salinity is an important factor that prevents the invasion or acts as a barrier against dispersal (Scott et al. 2008; Gutierre et al. 2014). Salinity may also delimit an invasive species' pattern of dispersal, population structure, abundance and interaction with native species (Cognetti and Maltagliati 2000; Smith et al. 2004; Paavola et al. 2005; Alcaraz and Garcia-Berthou 2007; Alcaraz et al. 2008; Schofield and Nico 2009).

We were first made aware of the presence of non-native suckermouth sailfin catfishes of the genus *Pterygoplichthys* in Kerala state of India by local fishers and ornamental fish traders who collect juveniles for trade. The morphological identification of some of the commonly-traded species in the tropics such as *P. anisitsi, P. multiradiatus, P. pardalis* and *P. disjunctivus* are separated primarily based on the nature of abdominal patterns (Nico et al. 2012). However, the abdominal patterns may overlap in the case of hybrids, making the process of identification difficult or impossible. The species that has invaded Kerala appears to be a hybrid (Kumar et al. 2015).

The invasive populations of *Pterygoplichthys* spp. in many parts of the world threaten indigenous species and in many cases surpass the biomass of native species (Nico et al. 2009; Capps and Flecker 2013). In this study, we determine the salinity tolerance of *Pterygoplichthys* sp., which may help predict the ultimate range in Kerala. Herein, we provide details of a field effort to better survey the geographic range of these fishes in Kerala as well as experimental results from salinity-tolerance tests. Our results are compared to the few previous studies of salinity tolerance in *Pterygoplichthys* spp. and results from field collections in low-salinity waters.

## Methods

### *Field survey and fish collection*

The water bodies of Thiruvananthapuram district of Kerala state, India were surveyed during 2015–2016 to detect the presence of non-native *Pterygoplichthys* sp. Fortnightly collections were made from each water body using cast nets and gill nets. Surveys to collect the fishes were done in the fresh- and brackish-water inland ecosystems of southern Kerala including rivers (Karamana and Neyyar), drainage canals (Amayizhanchan Thodu), Vellayani freshwater lake and Veli brackish-water lake (Figure 1). Though both juveniles and adults were collected during the survey, only adult fishes were used for the salinity-tolerance experiments.

### *Acute salinity tolerance*

Acute (abrupt) salinity tolerance experiments with *Pterygoplichthys* sp. were done following the protocols described by Kefford et al. (2004), Bringolf et al. (2005), Schofield and Nico (2009) and Capps et al. (2011), with some modifications. Fishes were collected from the natural drainage, Amayizhanchan Thodu (0 ppt salinity), Thiruvananthapuram city, Kerala. In total, 200 acclimatized *Pterygoplichthys* were selected for testing.

The experimental setup consisted of 20 (10-L) glass aquaria (30 cm long × 23 cm wide × 15 cm high) filled with 7 L of water. The tanks were allocated to ten different salinity treatments (0 [control], 2, 4, 8, 12, 16, 20, 24, 28 and 35 ppt; n = 2 replicates per treatment). Tanks were randomly arranged to avoid experimental artefacts. The salinity concentrations were prepared using both natural seawater and artificial sea salt (Red Sea© aquarium salt, Israel). After acclimatization, fishes were weighed (+/− 0.1 cm standard length [SL]), measured (+/− 1 g) and then randomly placed in the tanks (one fish per tank) to begin the experiment. Fish ranged in size from 181 to 229 mm SL (mean 208 mm) and 120 to 245 g mass (mean 182 g). Mass did not differ amongst treatments (one-way ANOVA, $F[9,190] = 1.496$; $P = 0.15$; Levene's test $P = 0.89$).

The fish were kept in the experimental tank for a period of 240 hours (10 days) or until death. All the tanks were covered with tight nylon meshes to prevent the fish from jumping out. As the experimental fish were air breathers, separate aeration was not attempted in the experimental tanks. They were not provided food during the experiment, as food could have fouled the water and this hardy fish can survive without food for at least a month (ABK pers. obs.). Survival was recorded every 15 minutes for the

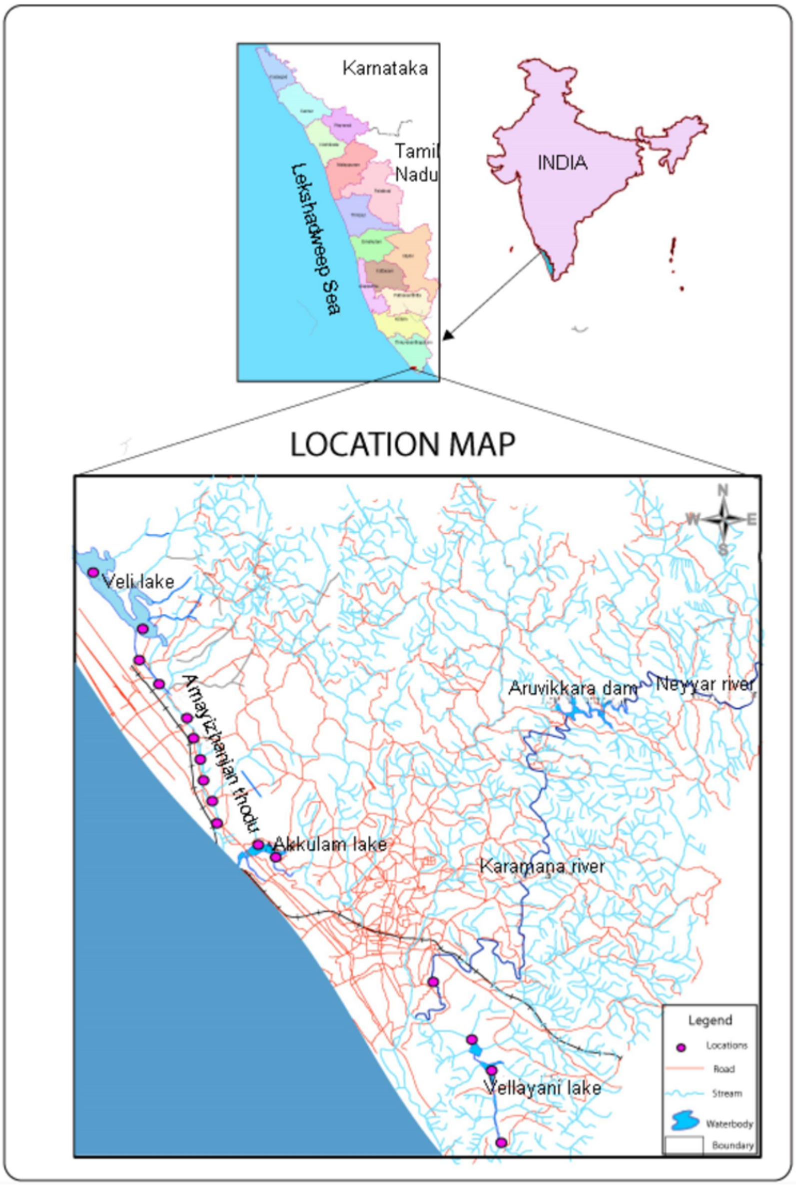


**Figure 1.** Map of sites sampled for *Pterygoplichthys* in Kerala state, India. For details see Supplementary information Table S1.

first hour, every 1 hour for the first 24 hours, every 3 hours for the first 24–48 hours, and every 6 hours for the remaining period of experiment. Water temperature and salinity of each tank was monitored using a thermometer (+/− 0.1 °C) and salinometer (+/− 1 ppt). Water temperatures of all tanks were recorded each day throughout the experimental period. Temperatures ranged from 24.8 to 32.0 °C, but did not differ among salinities. The entire experiment was replicated 10 times, providing a total of 20 replicates for each treatment when data were combined (total = 200 fish).

*Chronic salinity tolerance*

To determine the species' tolerance to gradually-increased salinities, a chronic salinity tolerance experiment was completed. Thirty-two fish were assigned to one of eight treatments (0 [control], 2, 4, 6, 8, 10, 12 or 14 ppt); with four replicates tanks per salinity treatment. Tanks were randomly arranged to avoid experimental artefacts. Fish ranged in size from 76 to 245 mm SL (mean 191 mm) and 115 to 264 g mass (mean 1582 g). Mass did not differ amongst treatments (one-way ANOVA, $F[7,24] = 2.3434$; $P = 0.06$;

**Table 1.** Collection details for *Pterygoplichthys* sp. in various water bodies of Thiruvananthapuram district, Kerala.

| Site | Habitat type | Salinity (ppt) | N | Length range (mm SL) |
|---|---|---|---|---|
| Neyyar River | Freshwater | 0 | 0 | |
| Karamana River | Freshwater | 0 | 4 | 154–173 |
| Vellayani lake | Freshwater | 0 | 11 | 165–211 |
| Veli lake | Brackish-water | 0–8 | 3 | 188–194 |
| Amayizhanchan Thodu | Freshwater | 0 | 314 | 181–229 |
| | | Total | 332 | |

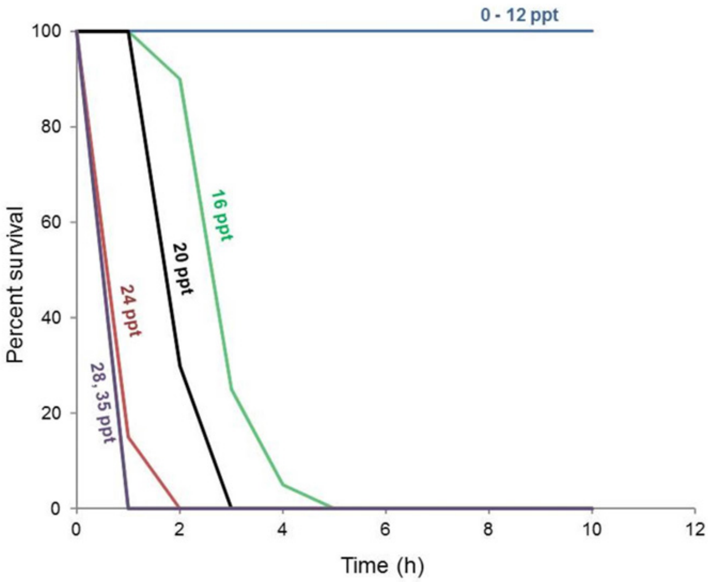


**Figure 2.** Acute salinity tolerance of *Pterygoplichthys* sp. from Kerala, India. Salinities tested include: 0 (control), 2, 4, 8, 12, 16, 20, 24, 28 and 35 ppt. Twenty replicates were tested for each salinity; total number of fish tested = 200. Fish were transferred directly from 0 ppt to test salinities and held there for 10 days (240 hours).

Levene's test $P < 0.001$). Fishes were held in 100-L aquaria and all started at 0 ppt. Salinities were then gradually increased (a change of 2 ppt every 2 days) for all treatments except the control until the treatment attained its target salinity. Survival was measured daily and dead fish, if any, were removed. At the end of the experiment, fish had been exposed to the control salinity for 23 days, 2 ppt for 20 days, 4 ppt for 17 days, 6 ppt for 14 days, 8 ppt for 11 days, 10 ppt for 8 days and 12 ppt for 5 days.

*Data analysis*

For both acute and chronic experiments, fish mass was compared across treatments with one-way Analysis of Variance (ANOVA) to ensure fish size did not differ amongst treatments. Levene's test was used to check for heteroscedasticity. Life expectancy was estimated with the Kaplan-Meier product-limit estimator (Kaplan and Meier 1958) and the log-rank test was used to compare survivorship curves (Savage 1956; Cox and Oakes 1984). For the acute-salinity challenge, all treatments began at the same time (time = 0). However, for the chronic-salinity experiment, fish reached their target salinities sequentially (i.e., staggered over time). Thus, for the chronic-salinity experiment the day the fish reached their target salinity was designated as time = 0 for that treatment. We set our alpha level for statistical significance at 0.05. All data were analysed using NCSS version 11.

## Results

*Distribution of* Pterygoplichthys *sp.*

The field surveys detected the presence of non-native *Pterygoplichthys* spp. from Karamana River, Amayizhanchan Thodu, Veli Lake and Vellayani Lake in the Thiruvananthapuram district of Kerala, India. We captured 332 individuals of *Pterygoplichthys* sp. from the water bodies of Thiruvananthapuram district, indicating the spread of species into various water bodies (Table 1). The survey results showed that Amayizhanchan Thodu, the natural drainage channels of Thiruvananthapuram District of Kerala, is heavily invaded by *Pterygoplichthys* sp., and the presence of this species in Veli Lake shows the ability of species to invade brackish waters of the state.

*Acute salinity tolerance*

No fish died in treatments ranging from 0–12 ppt during the 240 hrs (10 days) of the experiment. However, above 12 ppt survival decreased significantly (Figure 2, Table 2). Individuals lived about three hours at 16 ppt, two hours at 20 ppt and one hour at 24 ppt. At salinities of 28 and 35, fish died within one hour. Log-rank tests showed that each treatment of 16–35 ppt were distinctly different from all other treatments (Table 2).

*Chronic salinity tolerance*

*Pterygoplichthys* sp. survived all treatments fom 0 to 12 ppt with no mortality. After reaching 14 ppt, individuals died in 24–36 hrs (mean = 30 hrs). It was not possible to compare treatments with the log-rank test as there was only mortality in one treatment (14 ppt).

**Table 2.** Results from acute salinity exposure experiments for *Pterygoplichthys* sp. A: Mean survival times (h) for each salinity and standard deviation (SD). * = unable to compute mean or SD as all fish survived the treatment for the entire length of the experiment (240 hrs). B: Log-rank tests comparing survival between treatments. Statistic given is *P*. Only treatments with mortality are compared (16–35 ppt).

A

| Salinity | Mean survival time (h) | SD |
|---|---|---|
| 0 | * | |
| 2 | * | |
| 4 | * | |
| 8 | * | |
| 12 | * | |
| 16 | 3.2 | 0.7 |
| 20 | 2.3 | 0.47 |
| 24 | 1.11 | 0.39 |
| 28 | 0.5 | 0 |
| 35 | 0.5 | 0 |

B

| Salinity | 16 | 20 | 24 | 28 | 35 |
|---|---|---|---|---|---|
| 16 | * | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| 20 | | * | < 0.001 | < 0.001 | < 0.001 |
| 24 | | | * | < 0.001 | < 0.001 |
| 28 | | | | * | NS |
| 35 | | | | | * |

## Discussion

Many native and introduced freshwater fish species occasionally use estuarine and brackish water areas for feeding or dispersal (Loftus 1988; McIvor and Odum 1988; Brown et al. 2007). Fishes are limited to suitable salinities because of the stress exerted on osmoregulation by the surrounding water medium. This becomes an important aspect of biogeographic distribution (Myers 1949). Loricariids are a family of freshwater fishes (Nelson 1994) but have invaded estuarine environments in several countries beyond their native range due to their ability to tolerate low levels of salinity, as shown in this report.

*Pterygoplichthys* spp. possess several characteristics that enable them to establish invasive populations in their non-native range (Douglas et al. 2002; Ebenstein et al. 2015; Geerinckx et al. 2011; German and Bittong 2009; Harter et al. 2014; Jumawan and Herrera 2015; Villalba-Villalba et al. 2017; Zworykin and Budaev 2013). Some of these include: 1) the ability to survive anoxic conditions by means of accessory respiration with diverticula of the gastrointestinal tract (Armbruster 1998); 2) the presence of bony armor plates and spines, which makes them relatively difficult to consume; 3) reproductive advantages that facilitate invasion 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); and 4) the ability to exploit ecologically-degraded aquatic habitats, which favor invasive species such as *Pterygoplichthys* spp. that can withstand poor water quality conditions (da Cruz et al. 2013; Parente et al. 2010).

We have shown that *Pterygoplichthys* sp. is able to tolerate low levels of salinity, and that is yet another factor potentially explaining the invasion success of this group. In India, dumping of ornamental fishes is the primary route for introduction of non-native species into aquatic ecosystems (Kumar 2000; Singh and Lakra 2011; Soundararajan et al. 2015; Sandilyan 2016). The armoured catfishes of the genus *Pterygoplichthys* have been reported from various natural water bodies of India in the last decade, and in later stages as invasive populations in many major rivers and lakes (Knight 2010; Sinha et al. 2010; Singh and Lakra 2011; Singh et al. 2014; Kumar et al. 2015; Panikkar et al. 2015; Meena et al. 2016; Rao and Sunchu 2017). According to Kumar et al. (2015) the ornamental fish trade could be the reason for the introduction of *Pterygoplichthys* sp. In the water bodies of Kerala. This study shows that these catfishes have the potential to spread to other freshwater and brackish water ecosystems, possibly using brackish water “salt bridges” to reach new freshwater drainages.

Two studies have previously evaluated the salinity tolerance of *Pterygoplichthys* sp. using an experimental approach. In Mexico, Capps et al. (2011) recorded collections of *Pterygoplichthys* sp. from the field at salinities up to 8 ppt. The acute salinity tolerance of field-collected samples was tested, and it was found that the species was able to survive abrupt (acute) exposure to salinities up to 9 ppt with little mortality over 10 days. Interestingly, the *Pterygoplichthys* sp. tested in Mexico appear slightly more sensitive to salinity than the ones in this study. Mexican *Pterygoplichthys* sp. survived an average of 58 hrs at 11 ppt and all fish at 12 ppt were dead by 40 hrs, whereas in our study all fish in salinities up to 12 ppt survived with no mortality for the entire length of the experiment (240 hours). Brion et al. (2013) reported non-native *Pterygoplichthys* sp. collected from the Philippines at salinities of 5–8 ppt. They conducted acute-salinity-tolerance tests on specimens purchased from a pet store, and found the $LC_{50}$ was 10.6 ppt for a 96-hour period. Interestingly, fish in salinity treatments of 15 and 20 ppt survived for an average of 47 and 24 hours, respectively. That was longer than the fish in the present study survived at these higher salinities. Because of difficulties in identification of *Pterygoplichthys* species, it is unclear whether the work of Brion et al. (2013) and Capps et al. (2011) was on the same species we have studied in Kerala. It is possible that different species have different tolerances to salinities; however, without better species discrimination it is not possible to address that question at this time. A few additional studies have recorded collections of non-native *Pterygoplichthys* sp. in low-salinity waters: Chavez et al. (2006) collected the fish in waters to 2 ppt in the Philippines. Golani and Snovsky (2013) collected *Pterygoplichthys pardalis* and *P. disjunctivus* in Israel at salinities from 0.5–2.0 ppt. Arévalo-Frías and Mendoza-Carranza (2015) reported *Pterygoplichthys* sp. in Mexico from 1.6–5.6 ppt. Finally, Idelberger et al. (2011) reported *Pterygoplichthys* spp. from Charlotte Harbor estuary (Florida, USA) at salinities up to at least 5 ppt and possibly as high as 12 ppt.

While *Pterygoplichthys* sp. are tolerant of low levels of salinity (to 12 ppt), higher salinities could limit migration through estuarine waters and confine the fish to its current distribution. Salinity may also limit further dispersal of this fish to other freshwater drainages through the marine route, although during monsoon flooding, when the estuaries, mangrove wetlands and rivers of India are flooded with freshwater, temporary aquatic connections may emerge (Selvam 2003; Martin et al. 2008). The major hydrological variable in the backwaters of Kerala is salinity, with a gradual decline in salinity from 30 ppt at the entrance of the estuary to 0.2 ppt at the confluence of the tributary and the rivers (Menon et al. 2000). Upstream of the river entry are farms built below-sea-level (Koohafkan and Altieri 2011), where traditional rice-fish culture and prawn filtration are have long been practiced (Subramanian 1988; Kurup and Ranjeet 2002). In the estuaries and backwaters of Kerala during both southwest and northeast monsoons there is considerable flooding with freshwater, rapid decline in salinities across the habitat, and low salinities or even freshwater conditions in river mouths. These conditions may facilitate *Pterygoplichthys* sp. survival and dispersal in these regions.

The experimental results provided here show that non-native *Pterygoplichthys* sp. in Kerala are able to tolerate salinities up to 12 ppt for extended periods, and can invade brackish-water ecosystems of the state. The salinity tolerance of Kerala *Pterygoplichthys* sp. is greater than many typical freshwater fishes that can tolerate low levels of salinity (to about 8 ppt; Peterson and Meador 1994). These data are vital to predicting the invasion success and potential geographic spread of non-native fishes such as *Pterygoplichthys* spp. into coastal systems in Kerala and worldwide. Knowing salinity tolerance levels and predicting invasions are particularly important because invasive *Pterygoplichthys* catfishes have been reported to have negative effects on native fauna and local fisheries (Hoover et al. 2004; Sumanasinghe and Amarasinghe 2013) and management authorities may be able to control populations (e.g., Nico et al. 2012; Gibbs et al. 2013; Hill and Sowards 2015) or provide awareness programmes to prevent the spread of this nuisance species in both freshwater and estuarine habitats.

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**Supplementary material**

The following supplementary material is available for this article:

**Table S1.** Records of *Pterygoplichthys* sp. from Kerala, India.

This material is available as part of online article from:

http://www.reabic.net/journals/mbi/2018/Supplements/MBI_2018_Kumar_etal_Table_S1.xlsx