E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2019; 7(4): 314-320

www.fisheriesjournal.com
Received: 04-05-2019
Accepted: 06-06-2019

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DNA barcoding confirms species substitution of *Parastromateus niger* (black pomfret) using exotic species *Piaractus brachypomus* (Red Bellied Pacu)

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Abstract
Species substitution is a case of fraud practised by the fish dealers for acquiring additional profit. During a fishery survey in the local markets of Kerala state (South India), some 'alien' fishes were encountered which were traded along with *Parastromateus niger* as 'black pomfrets'. Samples of these two groups were collected for morphological and molecular characterization (viz. COI gene). COI sequences of *P. niger* corroborated with the same in NCBI while morphological and molecular characters of 'alien' fishes corroborated with an exotic fish *Piaractus brachypomus*. Molecular analysis carried out using additional COI sequences of 'pomfret' and 'piranha' fishes acquired from NCBI confirmed the speciation of 'alien fishes' as *P. brachypomus*. This study reports the illegal trade of *P. brachypomus* as a species substituent for *P. niger* in Kerala and recommends the enforcement of strict laws for preventing food frauds as it could bring up serious economic breakdowns in fisheries sector.

Keywords: *Parastromateus niger,* COI, *Piaractus brachypomus,* species substitution

1. Introduction

Parastromateus niger (Class Actinopterygii, Order Perciformes, Family Carangidae) or black pomfret is a high valued marine fish inhabiting the shallow inshore waters of the Indian Ocean [1, 2]. Increasing demand for this species has resulted in the reduction of its stock to a larger extent due to over exploitation and lack of proper fishing management programs [3]. Recently its landings in Indian coastal areas declined drastically to the tune of 13924 tonnes in 2016 being only 52.36% of its previous year catch [4]. Higher availability of this species is needed to satisfy its increased demand since other sources of supply like aqua farming has not been in vogue for this species. This indicates possibilities of species substitution and misrepresentation using morphologically similar exotic, non-invasive, protected and low valued species, since seafood mislabelling is a global and significant issue in the present scenario [5-10].

In Kerala (S. India), *P. niger* is having higher market acceptance and is traded in fresh, processed (fillets, brined), sun dried and frozen conditions [11]. During a fishery survey conducted in local markets of Kochi (Kerala, S. India), certain 'alien' fishes were encountered which were traded along with *Parastromateus niger* (black pomfret) as black pomfrets. *P. niger* was identified on the basis of their morphological characters while the 'alien' ones showed a similarity in its body outline and pattern only. Even though, *P. niger* is reported to possess morphological similarity to the white pomfrets, *Pampus argenteus* and Chinese pomfrets, *P. chinensis* [12], little is known about a morphologically similar species substituent for black pomfrets. Hence, whole, fresh samples of these two different groups were collected from local markets of Kochi and a molecular approach was carried out to confirm the case of species misrepresentation. Since mitochondrial cytochrome c oxidase subunit I (COI) gene was used as a well-established molecular marker for identification of bio specimens including invasive and exotic species [13-15], the same were developed from the collected specimens and the results are presented here.

2 Materials and methods

2.1 Sample collection

Whole fish samples of *Parastromateus niger* (n=40) and 'alien' fishes (n=70) were collected from the local markets of Kochi (S. India) and transported to the laboratory in iced condition.

A thorough morphological examination was carried out to identify and confirm the speciation of collected specimens. In order to generate COI sequences, tissue samples were taken from whole fish samples and preserved at -20 ^{o}C.

2.2 Genotyping

Genomic DNA was isolated following the spin column protocol for purification of total DNA from animal tissues provided in the DNeasy Blood and Tissue Kit (Qiagen). For amplifying partial mitochondrial COI gene sequences, the primer pair Fish F1 (5'TCAACCAACCACAAAGACATTGGCAC3') and Fish R1 (5'TAGACTTCTGGGTGGCCAAAGAATCA 3') [16] were used. Temperature profile for amplification included 35 repeats of denaturation at 92 °C for 1 min, annealing at 48 °C for 35-50 s and extension at 68 °C for 90-120 sec [16]. Sigma Aldrich ReadyMix™ Taq with $MgCl^2$ was the PCR reaction mix used for amplifying COI gene sequences in a Corbett gradient thermal cycler. Following the agarose gel electrophoresis using a 1.2% agarose gel, PCR amplicons showing intense bands were chosen for purification and sequencing. Sequences were manually edited, translated online and compiled using BioEdit 7.0.9 [17] followed by alignment using Clustal X [18]. Phylogenetic tree based on Neighbour Joining (NJ) analysis as well as nucleotide sequence distance data were generated (Kimura 2-Parameter model) using MEGA 5 [19].

3. Results and discussion

The collected species were differentiated morphologically as *Piaractus brachypomus* (Class Actinopterygii, Order Characiformes Family Characidae) following [20-22] and *Parastromateus niger* following [23] and the major morphological characters identified are detailed in Table 1.

Table 1. Major morphological characters for differentiating *Parastromateus niger* and *Piaractus brachypomus*

Species	*Parastromateus niger*	*Piaractus brachypomus*
Body	Body elevated, compressed and rounded anterior profile	Deep and laterally compressed body
Colour	Deep/greyish brown with blue reflections. Cheeks, opercle and abdomen likely same colour and pale/brownish. Black colour present in pectoral and caudal edge	Deep silvery/grey body with silver shades on sides and dark dorsal portion Red colouration on belly chin and pectoral fins and leading rays of anal fin.
Scales	Small, deciduous, completely covering dorsal and anal fins	The scales are numerous cycloid with smooth caudal margin
Lateral Line	Lateral line very weakly arched anteriorly, with junction of straight and curved parts below posterior third of dorsal fin; straight part of lateral line with 8 to 19 weak scutes. 76 scales in lateral line, 35 of which are in straight portion, and scarcely armed	Lateral line is roughly straight Lateral line scales ranges between 102- 117
Keel	Strong keel present in the belly portion	A serrated keel runs along the belly region and extends upto to anus
Dentition	Teeth in jaws, slender, pointed and inserted on ridge	Two series of molariform incisors located on premaxilla and one row of dentary teeth
Fins	Dorsal – 5 spines, 42-44 rays Pectoral – 1 spine, 22 rays Anal – 3 spines, 33-35 rays Caudal – 19 rays	Dorsal – 2 spines, 15-18 rays Pectoral – 1 spine, 16-19 rays Anal – 3 spines, 24-28 rays Pelvic – 1 spine, 7 rays
Adipose fin	Absent	A small, unrayed adipose fin is present approximately midway between the dorsal and caudal fins with base lined with scales

3.1 Genotyping results

Molecular analysis using the primer pair and temperature profile generated COI gene sequences which were submitted in NCBI (Table 2). These COI sequences were subjected to primary BLAST search [24], and they showed higher identity (>97%) towards homologous COI sequences as *Piaractus brachypomus* and *Parastromateus niger* respectively, present in public database. Molecular analyses were carried out using additional acquired homologous COI sequences [25] of ‘Piranha’ and ‘Pomfret’ fishes from NCBI. The details regarding incorporated COI sequences are detailed in Table 2.

Table 2: Details of COI sequences incorporated for molecular analyses

Sl No	Taxon	Number of COI sequences	Accession numbers	Status
1	*Piaractus brachypomus*	7	MF693914-20	Developed for the present study
		1	KP723356	Acquired from NCBI
2	*Parastromateus niger*	1	MF737196	Developed for the present study
		3	KU943742 HQ560987 HQ560977	Acquired from NCBI
3	*Pampus argenteus*	3	FJ226531-33	Acquired from NCBI
4	*Pampus chinensis*	4	FJ265824-25 FJ226529-30	Acquired from NCBI
5	*Brama brama*	4	JF492979-82	Acquired from NCBI
6	*Brama japonica*	3	JF952690 GU440256 FJ164426	Acquired from NCBI
7	*Brama orcini*	4	KY371231 KF489508 KM366110 KM252676	Acquired from NCBI
8	*Colossoma macropomum*	1	HQ420843	Acquired from NCBI
9	*Eumegistus illustris*	1	KU943868	Acquired from NCBI
10	*Taractes asper*	2	GU440550	Acquired from NCBI

			EU400170	
11	*Taractes rubescens*	4	KY372188-91	Acquired from NCBI
12	*Taractichthys longipinnis*	2	AB639845 EF609476	Acquired from NCBI
13	*Taractichthys steindachneri*	4	KY372194-96 KY372198	Acquired from NCBI
14	*Xenobrama microlepis*	3	KX497160-61 EF609495	Acquired from NCBI
15	*Poecilia reticulata*	1	KR871634	Acquired from NCBI

3.2 Phylogenetic tree and genetic distance data

Phylogenetic tree based on neighbour joining analysis clearly differentiated 'Pomfret' and 'Piranha' fishes as two different clades. Within the pomfrets fishes, individuals belonging to genus *Brama* (*B. brama*, *B. japonica* and *B. orcini*), genus *Eumegistus* (*E. illustris*), genus *Xenobrama (X. microlepis),* genus *Taractichthys* (*T. longipinnis* and *T. steindachneri*) and genus *Taractes* (*T. asper* and *T. rubescens*) arrayed within a major clade. The neighbouring clade was formed by *Parastromateus niger*, *Pampus argenteus* and *P. chinensis* justifying their relationship with each other. *Pampus argenteus* and *P. chinensis* represented closest relatives of *Parastromateus niger* than the other 'pomfrets'. COI sequence developed for *P. niger* in this study also got arrayed with the rest of the same without any confusions. So also in 'Piranhas', COI sequences of *Piaractus brachypomus* sequenced for this study and the acquired nucleotide sequence of the same showed genetic congruency. '*Colossoma macropomum*' arrayed next to *P. brachypomus* with high bootstrap value. At intraspecific level, there existed little genetic divergence within *Piaractus brachypomus* and *Parastromateus niger*. However, their higher interspecific divergence made *Piaractus brachypomus* to array among the 'piranhas' and *Parastromateus niger* among the 'pomfrets' (Fig. 1).

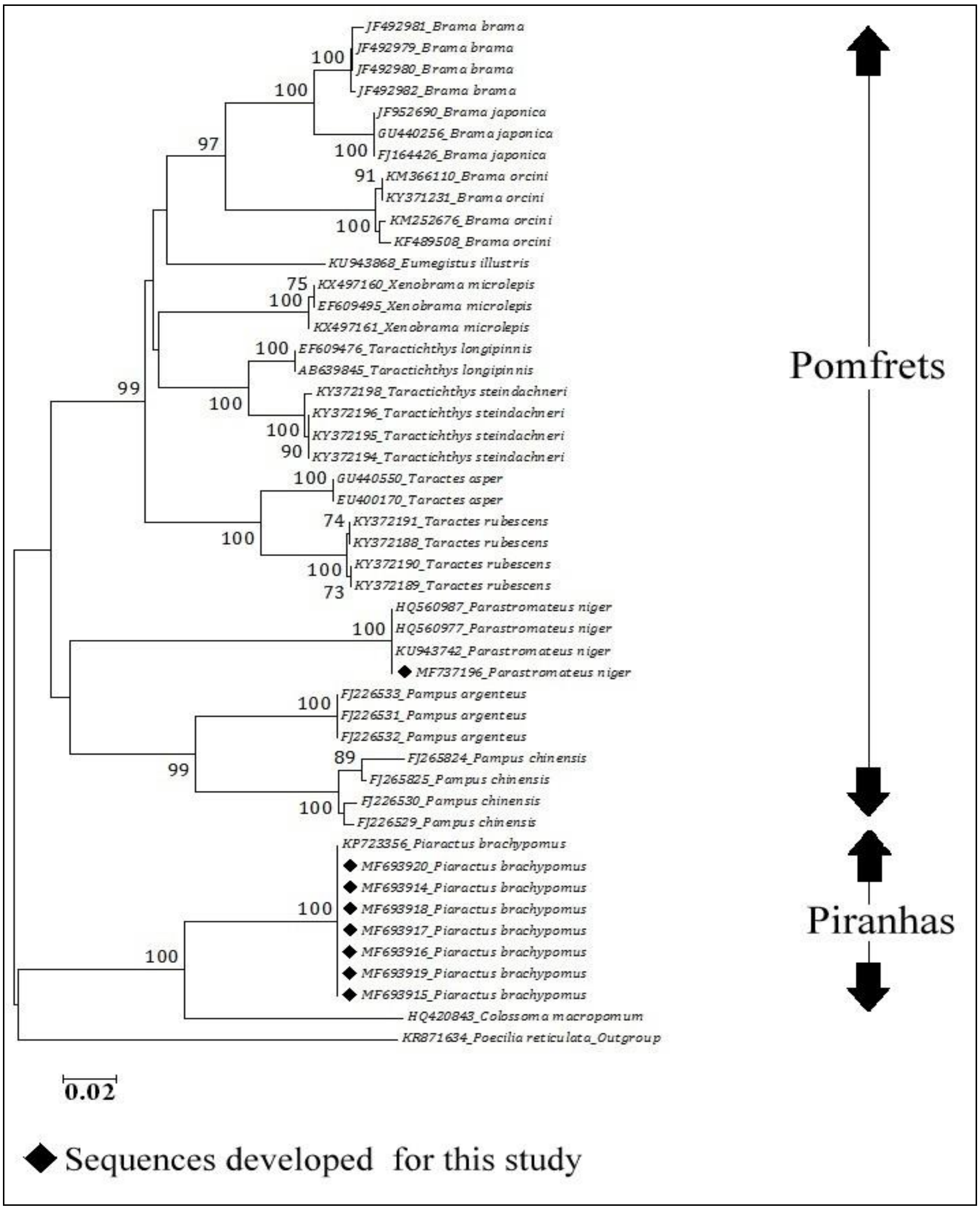

Fig 1: Phylogenetic tree generated using COI sequences with bootstrap values higher than 70

These findings from the phylogenetic tree was further confirmed by pairwise distance data (supplementary material). According to pairwise sequence data, the intraspecific distance within *Piaractus brachypomus* and *Parastromateus niger* was zero. At interspecific level, the divergence rate between *Piaractus brachypomus* and *Parastromateus niger* was very high (25.9%). This justified their differential array (in NJ tree) representing two highly distinct species. Interspecific divergence between other selected individuals (Table 3) also supported the findings of phylogenetic tree.

4. Discussion

Piaractus brachypomus Cuvier, 1818 (Family: Characidae) also known as 'Red Bellied Pacu', is the largest scaled fish inhabiting the Amazon and Orinoco basins of South America [26-28]. It is considered as an important food and aquarium fish in many South American countries including Brazil. Moreover, export of *P. brachypomus* populations from its type localities to different countries across the globe has also been reported. In India, *P. brachypomus* was introduced from Bangladesh during 2003-04 [29-31]. Earlier, occurrence of a huge 'Pomfret' like silvery fish in the north Western Ghats of India, which was identified as *P. brachypomus* was published [32]. Later, inhabitancy of this species was reported from natural waters of other states within the country including Kerala, Tamil Nadu, Telangana and West Bengal [26, 33, 27, 29, 34, 30, 35, 36]. Hitherto, this species is considered as an unofficially introduced exotic fish that could cause serious problems to humans and native fauna [30, 37, 29, 33].

Aquaculture and breeding of this species gained popularity in Kerala and local fish keepers and fish culturists started culturing the same in ponds and tanks across the state [29]. Recent studies reported its occurrence from various lakes like Vembanad [33] and Vellayani [29] and riverine systems including Pamba [26], Chalakudy [32]. Periyar [31] and Muvattupuzha [27] of Kerala. This has been attributed to the accidental release of cultured stocks to open waters [29, 33].

Present work focuses on the use of *Piaractus brachypomus* as a substituent to *Parastromateus niger* in local markets of Kochi, Kerala (S. India) and the mode of fish fraud. Accidental species substitutions occur when two different species exhibiting similar morphological characters obstruct proper identification [38-40]. Multiple species procuring the same vernacular name (and *vice versa*) also creates ambiguities and confusions in species identification [38, 39]. Intentional species substitution is the most common type practised by the dealers for acquiring additional profit. It is a clear case of consumer fraud in which species of high commercial value and high consumer acceptance are intentionally replaced with those having less commercial significance and low market value, leading to economic loss [38, 40-42]. Another case of substitution is the trade of vulnerable or overexploited species by masking their exploitation rate, affecting their fishing control and management [6, 38, 41-46]. In addition, substitutions may pose health risk to the consumers when economically important species are replaced by poisonous individuals that can cause serious health hazards [41, 42, 47, 48]. Here, *Parastromateus niger* and *Piaractus brachypomus* were traded in local markets as Black pomfrets. Based on thorough morphological examination, these species could be differentiated. However, consumers lacking knowledge regarding the introduction of this exotic fish could fail to identify the same by morphology. Misrepresentation of *Parastromateus niger* having higher market acceptance with a low valued exotic species like *Piaractus brachypomus* seems like an intentional type of species substitution since there will be considerable increase in profit for the dealer/seller and loss for the customer. A similar case of species substitution in whole fish (*Pampus chinensis*) using *Piaractus mesopotamicus* was published by [49]. Progressively, there may be chances for the use of *P. brachypomus* as a species substituent in processed products (like chunks, fillets, dressed frozen etc.) of high valued fishes.

More than 20 provinces of United States have already legally banned pacu and piranha from trade and aquaculture purposes due to their ruinous reaction on natural fish species and ecosystems [32]. They exhibits out-competing nature on the native species for prey and other resources causing ecological disparity. It over powers the local fish species by its huge size and powerful dentition and by feeding on eggs and fry. It is found to disseminate exotic parasites and diseases which may affect the native fauna gravely [26]. There are reports stating the role of *P. brachypomus* in causing outbreaks of parasitic infestations of Klossinemella and Rondonia in Argentina and Brazil, as well as Spectatus infestations in Argentina, Brazil, and Paraguay [50]. However, its potential impacts have been reported and it is causing enough concern to be listed as a potential threat in some states [51]. Their dentition pattern is a threat to humans as it can cause serious bites [52]. Two human deaths caused due to biting off the testicles of fishermen by Pacu was reported from Papua New Guinea [37].

According to [53], almost half of the 12 pacu species are being marked as traumatogenic, which is a behaviour considered dangerous and exhibited in the piranhas. From India also, there are reports revealing its "traumatogenic" effect on humans in Dimbhe reservoir in Maharashtra [54]. Impacts of introduction of Red bellied pacu in Kerala waters are also extensively discussed on the wake of serious damage to the native aquatic species within the state [32] and is feared to cause the disappearance of many economically important indigenous fishery resources from the state. Since *Piaractus brachypomus* is considered as an exotic species, its negative impacts on biodiversity needs to be monitored and standard quarantine procedures need to be enforced [27].

Table 3: Genetic distance data (Intraspecific divergence within *Piaractus brachypomus* and *Parastromateus niger* (Yellow colour) and interspecific divergence between them (Light blue colour))

MF693915_Piaractus brachypomus*		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.023	0.024	0.023	0.024	0.024	0.024	0.024	0.024	0.024	0.023	0.023	0.023	0.023	0.024	0.024	0.024	0.025	0.025	0.025	0.025	0.024	0.024	0.024	0.027	0.028	0.027	0.026	0.026	0.026	0.026	0.026	0.024
MF693919_Piaractus brachypomus*	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.023	0.024	0.023	0.024	0.024	0.024	0.024	0.024	0.024	0.023	0.023	0.023	0.023	0.024	0.024	0.024	0.025	0.025	0.025	0.025	0.024	0.024	0.024	0.027	0.028	0.027	0.026	0.026	0.026	0.026	0.026	0.024
MF693916_Piaractus brachypomus*	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.017	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.023	0.024	0.023	0.024	0.024	0.024	0.024	0.024	0.024	0.023	0.023	0.023	0.023	0.024	0.024	0.024	0.025	0.025	0.025	0.025	0.024	0.024	0.024	0.027	0.028	0.027	0.026	0.026	0.026	0.026	0.026	0.024
MF693917_Piaractus brachypomus*	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.017	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.023	0.024	0.023	0.024	0.024	0.024	0.024	0.024	0.024	0.023	0.023	0.023	0.023	0.024	0.024	0.024	0.025	0.025	0.025	0.025	0.024	0.024	0.024	0.027	0.028	0.027	0.026	0.026	0.026	0.026	0.026	0.024
MF693918_Piaractus brachypomus*	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.017	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.023	0.024	0.023	0.024	0.024	0.024	0.024	0.024	0.024	0.023	0.023	0.023	0.023	0.024	0.024	0.024	0.025	0.025	0.025	0.025	0.024	0.024	0.024	0.027	0.028	0.027	0.026	0.026	0.026	0.026	0.026	0.024
MF693914_Piaractus brachypomus*	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.017	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.023	0.024	0.023	0.024	0.024	0.024	0.024	0.024	0.024	0.023	0.023	0.023	0.023	0.024	0.024	0.024	0.025	0.025	0.025	0.025	0.024	0.024	0.024	0.027	0.028	0.027	0.026	0.026	0.026	0.026	0.026	0.024
MF693920_Piaractus brachypomus*	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.017	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.023	0.024	0.023	0.024	0.024	0.024	0.024	0.024	0.024	0.023	0.023	0.023	0.023	0.024	0.024	0.024	0.025	0.025	0.025	0.025	0.024	0.024	0.024	0.027	0.028	0.027	0.026	0.026	0.026	0.026	0.026	0.024
KP723356_Piaractus brachypomus	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.017	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.023	0.024	0.023	0.024	0.024	0.024	0.024	0.024	0.024	0.023	0.023	0.023	0.023	0.024	0.024	0.024	0.025	0.025	0.025	0.025	0.024	0.024	0.024	0.027	0.028	0.027	0.026	0.026	0.026	0.026	0.026	0.024
HQ420843_Colossoma macropomum	0.138	0.138	0.138	0.138	0.138	0.138	0.138	0.138		0.027	0.028	0.028	0.028	0.026	0.026	0.026	0.026	0.026	0.026	0.026	0.027	0.027	0.027	0.027	0.027	0.025	0.025	0.025	0.025	0.027	0.027	0.027	0.027	0.027	0.027	0.027	0.025	0.025	0.025	0.030	0.030	0.029	0.028	0.027	0.027	0.027	0.027	0.029
JF492981_Brama brama	0.243	0.243	0.243	0.243	0.243	0.243	0.243	0.243	0.270		0.003	0.003	0.003	0.009	0.009	0.009	0.016	0.015	0.016	0.016	0.017	0.017	0.017	0.018	0.018	0.017	0.017	0.017	0.017	0.016	0.018	0.018	0.020	0.020	0.020	0.020	0.026	0.026	0.026	0.026	0.026	0.025	0.024	0.027	0.027	0.027	0.027	0.027
JF492980_Brama brama	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.276	0.004		0.002	0.000	0.008	0.008	0.008	0.015	0.015	0.015	0.015	0.016	0.016	0.016	0.017	0.017	0.017	0.017	0.017	0.017	0.016	0.017	0.017	0.019	0.019	0.019	0.019	0.026	0.026	0.026	0.027	0.027	0.025	0.024	0.027	0.027	0.027	0.027	0.026
JF492982_Brama brama	0.252	0.252	0.252	0.252	0.252	0.252	0.252	0.252	0.279	0.006	0.002		0.002	0.009	0.009	0.009	0.015	0.015	0.015	0.015	0.016	0.016	0.016	0.017	0.017	0.017	0.017	0.017	0.017	0.016	0.017	0.017	0.019	0.019	0.019	0.019	0.026	0.026	0.026	0.027	0.027	0.025	0.024	0.027	0.027	0.027	0.027	0.026
JF492979_Brama brama	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.276	0.004	0.000	0.002		0.008	0.008	0.008	0.015	0.015	0.015	0.015	0.016	0.016	0.016	0.017	0.017	0.017	0.017	0.017	0.017	0.016	0.017	0.017	0.019	0.019	0.019	0.019	0.026	0.026	0.026	0.027	0.027	0.025	0.024	0.027	0.027	0.027	0.027	0.026
JF952690_Brama japonica	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.260	0.040	0.036	0.038	0.036		0.000	0.000	0.015	0.015	0.015	0.015	0.016	0.016	0.016	0.018	0.018	0.018	0.018	0.018	0.017	0.016	0.017	0.017	0.019	0.019	0.019	0.019	0.026	0.026	0.026	0.028	0.028	0.026	0.025	0.028	0.028	0.028	0.028	0.027
GU440256_Brama japonica	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.260	0.040	0.036	0.038	0.036	0.000		0.000	0.015	0.015	0.015	0.015	0.016	0.016	0.016	0.018	0.018	0.018	0.018	0.018	0.017	0.016	0.017	0.017	0.019	0.019	0.019	0.019	0.026	0.026	0.026	0.028	0.028	0.026	0.025	0.028	0.028	0.028	0.028	0.027
FJ164426_Brama japonica	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.248	0.260	0.040	0.036	0.038	0.036	0.000	0.000		0.015	0.015	0.015	0.015	0.016	0.016	0.016	0.018	0.018	0.018	0.018	0.018	0.017	0.016	0.017	0.017	0.019	0.019	0.019	0.019	0.026	0.026	0.026	0.028	0.028	0.026	0.025	0.028	0.028	0.028	0.028	0.027
KM366110_Brama orcini	0.233	0.233	0.233	0.233	0.233	0.233	0.233	0.233	0.253	0.113	0.108	0.110	0.108	0.113	0.113	0.113		0.003	0.000	0.004	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.017	0.019	0.019	0.020	0.020	0.019	0.019	0.024	0.024	0.024	0.026	0.026	0.025	0.024	0.026	0.026	0.026	0.026	0.026
KM252676_Brama orcini	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.253	0.110	0.106	0.108	0.106	0.110	0.110	0.110	0.006		0.003	0.003	0.018	0.018	0.018	0.018	0.018	0.019	0.019	0.019	0.018	0.018	0.019	0.019	0.020	0.020	0.019	0.019	0.025	0.025	0.025	0.026	0.026	0.025	0.025	0.027	0.027	0.027	0.027	0.027
KY371231_Brama orcini	0.233	0.233	0.233	0.233	0.233	0.233	0.233	0.233	0.253	0.113	0.108	0.110	0.108	0.113	0.113	0.113	0.000	0.006		0.004	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.018	0.017	0.019	0.019	0.020	0.020	0.019	0.019	0.024	0.024	0.024	0.026	0.026	0.025	0.024	0.026	0.026	0.026	0.026	0.026
KF489508_Brama orcini	0.235	0.235	0.235	0.235	0.235	0.235	0.235	0.235	0.256	0.113	0.108	0.110	0.108	0.113	0.113	0.113	0.008	0.006	0.008		0.018	0.018	0.018	0.018	0.018	0.019	0.019	0.019	0.019	0.017	0.019	0.019	0.020	0.020	0.019	0.019	0.025	0.025	0.025	0.026	0.026	0.025	0.025	0.026	0.026	0.026	0.026	0.027
KX497161_Xenobrama microlepis	0.240	0.240	0.240	0.240	0.240	0.240	0.240	0.240	0.269	0.125	0.120	0.122	0.120	0.125	0.125	0.125	0.151	0.151	0.151	0.151		0.002	0.002	0.015	0.015	0.015	0.015	0.015	0.015	0.017	0.018	0.018	0.016	0.016	0.016	0.016	0.023	0.023	0.023	0.023	0.023	0.022	0.022	0.023	0.023	0.023	0.023	0.025
KX497160_Xenobrama microlepis	0.240	0.240	0.240	0.240	0.240	0.240	0.240	0.240	0.269	0.127	0.122	0.125	0.122	0.127	0.127	0.127	0.154	0.154	0.154	0.154	0.002		0.000	0.015	0.015	0.015	0.015	0.015	0.015	0.016	0.018	0.018	0.016	0.016	0.016	0.016	0.023	0.023	0.023	0.023	0.023	0.022	0.022	0.023	0.023	0.023	0.023	0.025
EF609495_Xenobrama microlepis	0.240	0.240	0.240	0.240	0.240	0.240	0.240	0.240	0.269	0.127	0.122	0.125	0.122	0.127	0.127	0.127	0.154	0.154	0.154	0.154	0.002	0.000		0.015	0.015	0.015	0.015	0.015	0.015	0.016	0.018	0.018	0.016	0.016	0.016	0.016	0.023	0.023	0.023	0.023	0.023	0.022	0.022	0.023	0.023	0.023	0.023	0.025
EF609476_Taractichthys longipinnis	0.237	0.237	0.237	0.237	0.237	0.237	0.237	0.237	0.266	0.149	0.144	0.144	0.144	0.152	0.152	0.152	0.143	0.145	0.143	0.145	0.103	0.105	0.105		0.000	0.009	0.009	0.009	0.009	0.015	0.017	0.017	0.018	0.018	0.018	0.018	0.020	0.020	0.020	0.021	0.021	0.020	0.020	0.023	0.023	0.023	0.023	0.026
AB639845_Taractichthys longipinnis	0.237	0.237	0.237	0.237	0.237	0.237	0.237	0.237	0.266	0.149	0.144	0.144	0.144	0.152	0.152	0.152	0.143	0.145	0.143	0.145	0.103	0.105	0.105	0.000		0.009	0.009	0.009	0.009	0.015	0.017	0.017	0.018	0.018	0.018	0.018	0.020	0.020	0.020	0.021	0.021	0.020	0.020	0.023	0.023	0.023	0.023	0.026
KY372196_Taractichthys steindachneri	0.226	0.226	0.226	0.226	0.226	0.226	0.226	0.226	0.249	0.135	0.130	0.130	0.130	0.142	0.142	0.142	0.146	0.151	0.146	0.156	0.113	0.115	0.115	0.040	0.040		0.000	0.000	0.003	0.016	0.018	0.018	0.019	0.019	0.019	0.019	0.021	0.021	0.021	0.023	0.023	0.022	0.022	0.025	0.025	0.025	0.025	0.024
KY372195_Taractichthys steindachneri	0.226	0.226	0.226	0.226	0.226	0.226	0.226	0.226	0.249	0.135	0.130	0.130	0.130	0.142	0.142	0.142	0.146	0.151	0.146	0.156	0.113	0.115	0.115	0.040	0.040	0.000		0.000	0.003	0.016	0.018	0.018	0.019	0.019	0.019	0.019	0.021	0.021	0.021	0.023	0.023	0.022	0.022	0.025	0.025	0.025	0.025	0.024
KY372194_Taractichthys steindachneri	0.226	0.226	0.226	0.226	0.226	0.226	0.226	0.226	0.249	0.135	0.130	0.130	0.130	0.142	0.142	0.142	0.146	0.151	0.146	0.156	0.113	0.115	0.115	0.040	0.040	0.000	0.000		0.003	0.016	0.018	0.018	0.019	0.019	0.019	0.019	0.021	0.021	0.021	0.023	0.023	0.022	0.022	0.025	0.025	0.025	0.025	0.024
KY372198_Taractichthys steindachneri	0.229	0.229	0.229	0.229	0.229	0.229	0.229	0.229	0.251	0.132	0.127	0.127	0.127	0.139	0.139	0.139	0.141	0.146	0.141	0.151	0.117	0.120	0.120	0.040	0.040	0.004	0.004	0.004		0.015	0.018	0.018	0.019	0.019	0.019	0.019	0.022	0.022	0.022	0.023	0.023	0.022	0.022	0.025	0.025	0.025	0.025	0.025
KU943868_Eumegistus illustris	0.226	0.226	0.226	0.226	0.226	0.226	0.226	0.226	0.278	0.129	0.124	0.127	0.124	0.139	0.139	0.139	0.138	0.143	0.138	0.141	0.126	0.124	0.124	0.110	0.110	0.117	0.117	0.117	0.115		0.017	0.017	0.017	0.017	0.018	0.018	0.024	0.024	0.024	0.024	0.024	0.022	0.022	0.025	0.025	0.025	0.025	0.026
GU440550_Taractes asper	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.261	0.151	0.146	0.146	0.146	0.146	0.146	0.146	0.161	0.156	0.161	0.158	0.144	0.146	0.146	0.118	0.118	0.130	0.130	0.130	0.135	0.133		0.000	0.011	0.011	0.011	0.011	0.024	0.024	0.024	0.025	0.026	0.025	0.024	0.025	0.025	0.025	0.025	0.026
EU400170_Taractes asper	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.234	0.261	0.151	0.146	0.146	0.146	0.146	0.146	0.146	0.161	0.156	0.161	0.158	0.144	0.146	0.146	0.118	0.118	0.130	0.130	0.130	0.135	0.133	0.000		0.011	0.011	0.011	0.011	0.024	0.024	0.024	0.025	0.026	0.025	0.024	0.025	0.025	0.025	0.025	0.026
KY372191_Taractes rubescens	0.235	0.235	0.235	0.235	0.235	0.235	0.235	0.235	0.262	0.164	0.159	0.161	0.159	0.161	0.161	0.161	0.168	0.168	0.168	0.171	0.126	0.124	0.124	0.131	0.131	0.144	0.144	0.144	0.149	0.133	0.059	0.059		0.000	0.002	0.002	0.024	0.024	0.024	0.024	0.025	0.023	0.023	0.025	0.025	0.025	0.025	0.026
KY372188_Taractes rubescens	0.235	0.235	0.235	0.235	0.235	0.235	0.235	0.235	0.262	0.164	0.159	0.161	0.159	0.161	0.161	0.161	0.168	0.168	0.168	0.171	0.126	0.124	0.124	0.131	0.131	0.144	0.144	0.144	0.149	0.133	0.059	0.059	0.000		0.002	0.002	0.024	0.024	0.024	0.024	0.025	0.023	0.023	0.025	0.025	0.025	0.025	0.026
KY372190_Taractes rubescens	0.235	0.235	0.235	0.235	0.235	0.235	0.235	0.235	0.259	0.166	0.161	0.164	0.161	0.163	0.163	0.163	0.165	0.165	0.165	0.168	0.128	0.126	0.126	0.131	0.131	0.144	0.144	0.144	0.149	0.135	0.061	0.061	0.002	0.002		0.000	0.024	0.024	0.024	0.024	0.025	0.023	0.023	0.025	0.025	0.025	0.025	0.026
KY372189_Taractes rubescens	0.235	0.235	0.235	0.235	0.235	0.235	0.235	0.235	0.259	0.166	0.161	0.164	0.161	0.163	0.163	0.163	0.165	0.165	0.165	0.168	0.128	0.126	0.126	0.131	0.131	0.144	0.144	0.144	0.149	0.135	0.061	0.061	0.002	0.002	0.000		0.024	0.024	0.024	0.024	0.025	0.023	0.023	0.025	0.025	0.025	0.025	0.026
FJ226532_Pampus argenteus	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.240	0.234	0.234	0.234	0.234	0.245	0.245	0.245	0.215	0.223	0.215	0.221	0.216	0.219	0.219	0.184	0.184	0.193	0.193	0.193	0.195	0.232	0.229	0.229	0.232	0.232	0.232	0.232		0.000	0.000	0.017	0.017	0.015	0.015	0.022	0.022	0.022	0.022	0.026
FJ226533_Pampus argenteus	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.240	0.234	0.234	0.234	0.234	0.245	0.245	0.245	0.215	0.223	0.215	0.221	0.216	0.219	0.219	0.184	0.184	0.193	0.193	0.193	0.195	0.232	0.229	0.229	0.232	0.232	0.232	0.232	0.000		0.000	0.017	0.017	0.015	0.015	0.022	0.022	0.022	0.022	0.026
FJ226531_Pampus argenteus	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.224	0.240	0.234	0.234	0.234	0.234	0.245	0.245	0.245	0.215	0.223	0.215	0.221	0.216	0.219	0.219	0.184	0.184	0.193	0.193	0.193	0.195	0.232	0.229	0.229	0.232	0.232	0.232	0.232	0.000	0.000		0.017	0.017	0.015	0.015	0.022	0.022	0.022	0.022	0.026
FJ265824_Pampus chinensis	0.274	0.274	0.274	0.274	0.274	0.274	0.274	0.274	0.295	0.264	0.264	0.264	0.264	0.282	0.282	0.282	0.250	0.253	0.250	0.256	0.229	0.231	0.231	0.198	0.198	0.215	0.215	0.215	0.215	0.224	0.254	0.254	0.239	0.239	0.239	0.239	0.133	0.133	0.133		0.006	0.008	0.008	0.026	0.026	0.026	0.026	0.031
FJ265825_Pampus chinensis	0.269	0.269	0.269	0.269	0.269	0.269	0.269	0.269	0.278	0.246	0.246	0.246	0.246	0.261	0.261	0.261	0.231	0.234	0.231	0.238	0.214	0.217	0.217	0.187	0.187	0.205	0.205	0.205	0.205	0.215	0.240	0.240	0.228	0.228	0.228	0.228	0.112	0.112	0.112	0.017		0.005	0.006	0.026	0.026	0.026	0.026	0.033
FJ226530_Pampus chinensis	0.261	0.261	0.261	0.261	0.261	0.261	0.261	0.261	0.278	0.240	0.240	0.240	0.240	0.254	0.254	0.254	0.229	0.232	0.229	0.235	0.214	0.216	0.216	0.184	0.184	0.208	0.208	0.208	0.209	0.209	0.238	0.238	0.224	0.224	0.224	0.224	0.111	0.111	0.111	0.032	0.011		0.004	0.026	0.026	0.026	0.026	0.030
FJ226529_Pampus chinensis	0.261	0.261	0.261	0.261	0.261	0.261	0.261	0.261	0.272	0.233	0.233	0.233	0.233	0.247	0.247	0.247	0.229	0.232	0.229	0.234	0.213	0.216	0.216	0.182	0.182	0.206	0.206	0.206	0.206	0.209	0.238	0.238	0.226	0.226	0.226	0.226	0.112	0.112	0.112	0.036	0.015	0.008		0.026	0.026	0.026	0.026	0.030
MF737196_Parastromateus niger*	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.267	0.256	0.250	0.250	0.250	0.262	0.262	0.262	0.243	0.249	0.243	0.246	0.200	0.202	0.202	0.210	0.210	0.221	0.221	0.221	0.227	0.241	0.233	0.233	0.232	0.232	0.232	0.232	0.209	0.209	0.209	0.256	0.230	0.236	0.236		0.000	0.000	0.000	0.028
KU943742_Parastromateus niger	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.267	0.256	0.250	0.250	0.250	0.262	0.262	0.262	0.243	0.249	0.243	0.246	0.200	0.202	0.202	0.210	0.210	0.221	0.221	0.221	0.227	0.241	0.233	0.233	0.232	0.232	0.232	0.232	0.209	0.209	0.209	0.256	0.230	0.236	0.236	0.000		0.000	0.000	0.028
HQ560987_Parastromateus niger	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.267	0.256	0.250	0.250	0.250	0.262	0.262	0.262	0.243	0.249	0.243	0.246	0.200	0.202	0.202	0.210	0.210	0.221	0.221	0.221	0.227	0.241	0.233	0.233	0.232	0.232	0.232	0.232	0.209	0.209	0.209	0.256	0.230	0.236	0.236	0.000	0.000		0.000	0.028
HQ560977_Parastromateus niger	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.259	0.267	0.256	0.250	0.250	0.250	0.262	0.262	0.262	0.243	0.249	0.243	0.246	0.200	0.202	0.202	0.210	0.210	0.221	0.221	0.221	0.227	0.241	0.233	0.233	0.232	0.232	0.232	0.232	0.209	0.209	0.209	0.256	0.230	0.236	0.236	0.000	0.000	0.000		0.028
KR871634_Poecilia reticulata_Outgroup	0.242	0.242	0.242	0.242	0.242	0.242	0.242	0.242	0.301	0.264	0.258	0.258	0.258	0.270	0.270	0.270	0.258	0.264	0.258	0.267	0.245	0.245	0.245	0.259	0.259	0.244	0.244	0.244	0.250	0.268	0.256	0.256	0.259	0.259	0.259	0.259	0.254	0.254	0.254	0.315	0.307	0.299	0.301	0.285	0.285	0.285	0.285	

5. Conclusion

In India, limited quality assessment techniques employed for evaluating the authenticity of locally marketed fish and fishery products are a major problem. Therefore, sensitive and reliable methods should be adopted for the correct identification of traded species, thus preventing species substitutions and market fraud [55]. Present work strongly recommends the need for DNA based studies as a harmonised tool for fishery resource management [42, 56]. Implementation of strict laws for monitoring the release of alien species into natural waters and aquaculture systems are to be done by government according to the scenario persisting within the country/state or else our indigenous fishes and fishery resources will be eradicated by the non-indigenous fishes [36].

6. Acknowledgements

We gratefully acknowledge the Director, School of Industrial Fisheries, Cochin University of Science and Technology for providing necessary facilities and support for conducting this research.

7. Declaration of interest

The authors declare responsibility for the entire contents of this paper and have no conflicts of interest.

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