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Discovery of a new cyprinid fish, *Osteochilichthys formosus*, from Kerala, India

Mathews Plamoottil^{1*} and Vineeth. K²

¹Department of Zoology, Government College, Kottayam, Kerala- 686013, India

²Baby John Memorial Government College, Chavara, India

*Correspondence: mathewsplamoottil@gmail.com Received 06-02-2022, Revised: 01-07-2022, Accepted: 08-07-2022 e-Published: 09-07-2022

Osteochilichthys formosus, a new teleost fish, is described from Kerala, India. It is a congener species of *Osteochilichthys augraoides* and *O. elegans* of south Indian water bodies. It can be distinguished from its congener species in having a deeper (31.1-33.0 % SL), wider (11.8-14.4 % SL) and silvery body, 37 lateral line scales, 6.5 scales between lateral line and dorsal fin and in its coloration. *Osteochilichthys formosus* is a colorful fish which can be used for ornamental purposes. The new species is a rare freshwater fish residing in the Kazargod district in Kerala. The new species is taxonomically analyzed, scientifically named and compared with its congeners.

Keywords: Discovery, Description, Scientific naming, *Osteochilichthys augraoides*

INTRODUCTION

Osteochilichthys, a freshwater fish genus created by Hora (1942), as a subgenus of the genus *Osteochilus* Gunther (1868), includes moderately sized cyprinid fishes residing in the water bodies of Western Ghats. After detailed examination of specimens described from Western Ghats, he assigned south Indian species of the genus *Scaphiodon* Heckel (1843) in two genera viz. *Osteochilichthys* and *Kantaka*. Hora (1942) inserted *Scaphiodon nashii* Day (1868) and *S. thomassi* Day (1878) in *Osteochilichthys* and *Scaphiodon brevidorsalis* Day (1873) in *Kantaka*. Species of *Osteochilichthys* are characterised by having 11 (rarely 10) branched dorsal fin rays, 5 branched anal fin rays, smooth, weak and non-ossified last simple dorsal fin ray and devoid of barbels. *Kantaka* is characterised by the possession of strong and ossified last simple dorsal fin ray. *Osteochilichthys elegans* Plamoottil (2022) and *O. longidorsalis* Pethiyagoda and Kottelat (1994) are the recent additions to the genus. Currently the name *Kantaka* is no longer used in taxonomic literature and *brevidorsalis* is included in *Osteochilichthys*. '*Osteochilus*' *malabaricus* (Day, 1873) is treated as a synonym of *Osteochilichthys augraoides* and '*Osteochilus*' *godavariensis* Rao (1977) may be a *Labeo* species.

Six specimens of *Osteochilichthys* species procured by these researchers from Kazargod in Kerala, India were found to be unmatched in characters with its relative *Osteochilichthys* species. On careful taxonomic analysis, it was understood that, it bears distinct differences from its congeners; so, it is described here as a new species,

Osteochilichthys formosus.

MATERIALS AND METHODS

In connection with the Major Research Project (CRG) of Science and Engineering Research Board of DST, Govt of India, these authors visited many aquatic bodies of south India for fish procurement and systematic studies. This resulted in collection of many insufficiently known and some forgotten cyprinid fishes from various water bodies of Kerala, Karnataka and Tamil Nadu. Various specimens of *Osteochilichthys* with varied colour patterns and different morphometric characters and meristic counts were procured from Karnataka and Kerala. Six specimens of *Osteochilichthys* collected from Kazargod district in Kerala showed striking variations in rigid taxonomic variables; this led these authors to carry out more taxonomical studies on it. Live specimens of this fish were procured using cast net, anesthetized and fixed in 10 % formaldehyde solution; they were taxonomically analysed. Specimens of other *Osteochilichthys* species which are close congeners, were collected from their type localities in Kerala and Karnataka and compared with the new species. Taxonomic measurements were taken using dial calipers; meristic counts were taken using a stereomicroscope and magnifying lens. Methods used are those of Jayaram (2002); head length and measurements of body parts are given as proportions of standard length (SL). Subunits of the head are presented as proportions of head length (HL); measurements and counts were made on the left side of specimens; distance between two fins were measured from the origin of the fins; distances

between vent and ventral fin was also measured from the origin of the latter. Specimens of the new species are deposited in Zoological Survey of India museum at Shillong & Chennai; specimens of congeners of the new species are now deposited in various Zoological Survey of India museums.

DNA isolation, PCR and sequencing

DNA isolation, PCR and sequencing of the targeted genetic marker (mtCOI) of *Osteochilichthys formosus* and its relative species followed the standardized protocols. Genomic DNA was extracted from muscle tissue through Kit based method. The extracted DNA was checked in 1% agarose gel electrophoresis using standard protocol. Approximately 648 bp was amplified from the 5' region of the mtCOI gene using the primers described in Ward *et al.* (2005). Thermal profile for PCR was set as per the primer specifications. Approximately, 15 ng of purified PCR product was used for cycle sequencing for both reactions from the outsource services.

The generated sequences were truncated at both ends after alignment in MEGA 6.0 to get a consensus length in the dataset. The evolutionary divergences between sequences of closely related species or sister species among the congeners were estimated using Kimura 2 Parameter (K2P) in MEGA 6.0 (Tamura *et al.*, 2013). Further, the reciprocal monophyly among the targeted congeners were tested through Neighbour Joining (NJ) tree method and Maximum Likelihood (ML) in MEGA 6.0 (Tamura *et al.*, 2013) for species level differentiation.

GenBank accession numbers received for sequences of *Osteochilichthys formosus*, *O. augraoides* (formerly *O. nashii*), *O. brevidorsalis* and *O. longidorsalis* are OK631755, OK285283, OL703027 and MZ428280 respectively.

Abbreviations Used: ZSI/ ANRC- Zoological Survey of India, Andaman & Nicobar Regional Centre, Portblair; DOZ/GCC- Department of Zoology, Government College, Chavara, Kerala; LLS- Lateral line scales; HL- Head length; LLS- Lateral line scales; LL/D- Scales between lateral line and dorsal fin; LL/V- Scales between lateral line and ventral fin; LL/A- Scales between lateral line and anal fin.

RESULTS AND DISCUSSION

Osteochilichthys formosus sp. nov (Fig. 1 & 2; Table 1 & 2). urn:lsid:zoobank.org:act:9FFC22E3-BCCD-42D0-B613-FD83D7807CDF

Type Materials Examined: Holotype: V/F/NERC/ZSI/5482, 196.7 mm SL, a water stream at Chullikkara (Fig. 3 & 4), Kazargod district in Kerala, India, coll. Mathews Plamoottil & Vineeth. K, 26.12.2020; Paratypes: V/F/NERC/ZSI/5483, 2, 132.0-135.1 mm SL, other details same as HT; ZSI/SRS/F9490, 3, 141.5-154.1 mm SL, other details same as HT.

Diagnosis:

Osteochilichthys formosus can be diagnosed from all its relative species in having a deeper and wider body, 37 lateral line scales, 6.5 scales between lateral line and dorsal fin and 4.5 scales between lateral line and anal fin. The new species further differs from all its congeners, except *O. augraoides*, in having a distinct mid lateral black line, a black stripe on the middle of anal fin and a large distinct black blotch on dorsal fin.

Description: Body deep and compressed; both dorsal and ventral profiles equally convex; pre dorsal region gradually ascending to dorsal fin base; head depressed; eyes located on dorso lateral side, far behind gape of mouth, not seen from below ventral side; mouth crescentic and inferior; snout with tubercles, prominent and hard in larger specimens. Nostrils located close to eyes than snout tip.

Dorsal fin located in the middle of snout tip and caudal fin base, considerably in front of ventral fin base, distal margin concave; last simple dorsal fin ray weak and non-ossified; last divided dorsal fin ray divided to root. Dorsal fin shorter than depth of body below it and it is longer than all other fins except caudal fin; dorsal fin base length greater than that of all other fins. Pectoral fin triangular with 1 simple and 13-16 branched rays, its tip never reaches to ventral fin base, reaching 5 scales in front of latter; outer margin concave; ventral fin located 3 scales behind and below dorsal fin origin; its tip never reaches to anal fin base; reaches 2- 3 scales in front of anal fin; outer margin of ventral fin straight; anal fin triangular and its outer margin nearly straight; anal fin with 3 simple and 5 branched rays; simple rays of anal fin slightly thicker than other rays; anal fin tip reaches to caudal fin base in smaller specimens, in larger ones it reaches 1 scale in front of caudal fin base; last branched anal fin ray divided to root and shorter than other rays. Anal fin longer than ventral fin and shorter than caudal fin and its base considerably shorter than that of dorsal fin. Lateral line complete, nearly straight, pass through lower lateral of body and with 37 scales, including 1 unperforated one on caudal fin base; scales distinct, not easily deciduous and compactly arranged; an axillary scale present on either side of ventral fin, less than half length of latter; breast scales are of equal size to other body scales; no scales present on bases of dorsal fin and anal fin. Caudal fin deeply forked.

Color:

Live colors: Body silvery; a distinct black line passes through mid-lateral of body; a black stripe on middle of anal fin; a large distinct black blotch present on dorsal fin; outer edge of dorsal fin orange red. Pectoral fin, ventral fin and anal fin orange red.

In 10% formalin: Upper lateral dark brown, lower lateral pale brown, fins hyaline.

Etymology:

The specific epithet '*formosus*' is a Latin word meaning beautiful referring to the graceful form and colour of the new species.



Figure 1: A fresh specimen of *Osteochilichthys formosus*, Holotype, V/F/NERC/ZSI/5482.



Figure 2: A preserved specimen of *Osteochilichthys formosus*, Paratype, V/F/NERC/ZSI/5483.



Figure 3: Map showing the type locality of *O. formosus*

Comparisons: *Osteochilichthys formosus* shows distinct differences from its relative species in meristic counts (Table 3) and morphometric features (Table 4).

Osteochilichthys elegans Plamoottil, (2022) (Fig. 5), discovered from Mannarkkad, Kerala, is the recently described *Osteochilichthys* species. *Osteochilichthys*

formosus differs from *O. elegans* in having a higher (31.1- 32.5 vs. 26.5- 28.6) body, 37 (vs. 43-44) lateral line scales, 6.5 (vs. 7.5- 8.5) scales between lateral line and dorsal fin and 4.5 (vs. 5.5) scales between lateral line and anal fin. The new species further differs from *O. elegans* in having (vs. lacking) a black line passing through mid-lateral of body and a black stripe on middle of anal fin.

Osteochilichthys augraoides (Jerdon, 1849; Plamoottil, 2022) (Fig. 6), described from Fraserpett River at the base of the Coorg Hills in Karnataka, is the closest congener of *O. formosus*. Both these have a mid-lateral black stripe, a black band on anal fin and prominent black blotch on dorsal fin.



Figure 4: Chullikkara, the type locality of *O. formosus*



Figure 5: A fresh specimen of *Osteochilichthys elegans*, Holotype, V/F/NERC/ZSI/5420.



Fig 6. A fresh specimen of *Osteochilichthys augraoides*, DOZ/GGC/70

Osteochilichthys formosus differs from *Osteochilichthys*

augraoides in having 37 (vs. 41-42) lateral line scales, 6.5 (vs. 8.5) scales between dorsal fin to lateral line, 3.5- 4.5 (vs. 5.5) scales between lateral line and ventral fin, greater (46.6-48.4 % SL vs. 41.2-43.0) pre dorsal distance, higher (22.6- 25.0 % SL vs. 21.1-21.6) dorsal fin and shorter (15.2-18.0 % SL vs. 20.2-21.1) caudal peduncle.

Osteochilichthys formosus differs from *O. longidorsalis* Pethiyagoda and Kottelat (1994),



Fig. 7. A fresh specimen of *Osteochilichthys longidorsalis*, ZSI/ANRC/M/27238

(Fig. 7), described from Vettilappara of Chalakkudy River in Kerala, mainly in colour of body and fins (In *O. formosus*, body is silvery, a dark line running through mid lateral line, dorsal fin with a black blotch and its tip orange and anal fin with a distinct black band; in *O. longidorsalis* body greenish grey, no mid lateral line, dorsal and anal fin without any significant colour markings). The new species further differs from *O. longidorsalis* in having 37 (vs.38-40) lateral line scales and shorter (22.6-25.0 % SL vs. 29.7- 30.3) dorsal fin.

The new species differs from *O. thomassi* Day (1878), (Fig. 8), described from south Canara,



Fig. 8. A fresh specimen of *Osteochilichthys thomassi*, DOZ/GGC/72

in having 37 (vs. 41-43) lateral line scales, 12-13 (vs. 14) predorsal scales and 6.5 (vs. 7.5) scales between lateral line and dorsal fin. It further differs from *O. thomassi* in

colouration (In *formosus*, body silvery, a dark mid lateral line running through body, dorsal fin with a black blotch and its margin orange red and anal fin with a distinct black stripe; but in *thomassi*, dull silvery colour along the back, an indistinct silvery band along the side, a dull white beneath and a dark band along dorsal fin).

Osteochilichthys brevidorsalis, described by Day (1873) (Fig. 9) from rivers below the Neilgherry Hills, is not a close congener of the new species; the last undivided dorsal fin ray



Fig. 9. A fresh specimen of *Osteochilichthys brevidorsalis*, DOZ/GCC 71

of *brevidorsalis* is very strong and osseous, whereas it is weak and non-osseous in the new species. *Osteochilichthys formosus* further differs from *O. brevidorsalis* in having 37 (vs. 39-40) lateral line scales, 6.5 (vs. 7.5-8.5) scales between lateral line and dorsal fin, shorter (22.6- 25.0 % SL vs. 30.7-32.7) dorsal fin, shorter (29.1-32.4 % SL vs. 32.6-35.3) caudal fin and smaller (31.2-36.2 % HL vs. 36.3-39.3) orbits.

Evolutionary affinity

(Figures 10- 12; Table 5)

The partial coding sequence of mitochondrial cytochrome oxidase subunit 1 (COI) gene of *Osteochilichthys formosus* was PCR amplified using forward primer with DNA sequence 5'-TCAACCAACCACAAAGACATTGGCAC -3' and reverse primer with DNA sequence 5'-TAGACTTCTGGGTGGCCAAAGAATCA -3'. The partial COI sequence of *Osteochilichthys formosus*, collected from Kazargod, Kerala, India, yielded a product with 637p. The DNA sequence was deposited in the GenBank with Accession No. OK631755.1. The DNA sequence, its conceptual translation product and phylogenetic tree are presented in Figures 10, 11 and 12 respectively.

CCGGATAGTCGGAACCGCTTTAAGCCTTCTTATTTCGGGCGGAACCTAAGCCAACCCGG
 ATCCCTCTTGGGCGATGACCAAATTTATAACGTTATCGTTACTGCCACGCCTTTGTG
 ATAATCTTCTTTATAGTAATACCTATTCTTATTGGAGGATTTGGAAACTGACTTGTAC
 CACTAATAATTGGAGCCCCCGATATGGCATTCCCACGCATGAATAACATAAGTTTTT
 GACTGCTACCCCATCATTCTTATTGCTGCTGGCTTCTTCCGGAGTTGAAGCTGGGGC
 CGGAACGGGGTGAACAGTTTACCCTCCACTTGCAGGAAACCTGGCTCACGCAGGAG
 CATCAGTCGACCTAACAATTTTTTCACTTCACCTAGCAGGTGTATCATCAATCCTAGG
 CGCAATTAATTTTATTACTACAACAATTAATATGAAACCCCCAGCTATCTCCCAATA
 TCAGACTCCCTTATTTGTCTGATCCGTACTTGTAAACCGCCGTCCTTCTACTTCTATCA
 CTACCAGTACTAGCTGCCGGAATCACAAATACTTCTCACAGACCGAAACCTTAATACC
 ACATTCTTTGACCCGGCAGGAGGGGGGGACCCAATCCTATATCAACATTTATTCTGA
 TTTTTGGTCACCC

Figure 10: The DNA sequence interpret of CO I gene of *O. formosus*.

Domain:	Data						
UUU (F)	10.0 (1.33)	UCU (S)	1.0 (0.43)	UAU (Y)	3.0 (1.50)	UGU (C)	0.0 (0.00)
UUC (F)	5.0 (0.67)	UCC (S)	4.0 (1.71)	UAC (Y)	1.0 (0.50)	UGC (C)	0.0 (0.00)
UUA (L)	3.0 (0.53)	UCA (S)	6.0 (2.57)	UAA (*)	0.0 (0.00)	UGA (*)	5.0 (3.00)
UUG (L)	2.0 (0.35)	UCG (S)	0.0 (0.00)	UAG (*)	0.0 (0.00)	UGG (W)	0.0 (0.00)
CUU (L)	11.0 (1.94)	CCU (P)	2.0 (0.53)	CAU (H)	1.0 (0.50)	CGU (R)	0.0 (0.00)
CUC (L)	2.0 (0.35)	CCC (P)	5.0 (1.33)	CAC (H)	3.0 (1.50)	CGC (R)	1.0 (1.50)
CUA (L)	12.0 (2.12)	CCA (P)	7.0 (1.87)	CAA (Q)	4.0 (1.60)	CGA (R)	1.0 (1.50)
CUG (L)	4.0 (0.71)	CCG (P)	1.0 (0.27)	CAG (Q)	1.0 (0.40)	CGG (R)	2.0 (3.00)
AUU (I)	9.0 (1.23)	ACU (T)	3.0 (0.86)	AAU (H)	4.0 (0.89)	AGU (S)	1.0 (0.43)
AUC (I)	6.0 (0.82)	ACC (T)	3.0 (0.86)	AAC (H)	5.0 (1.11)	AGC (S)	2.0 (0.86)
AUA (I)	7.0 (0.95)	ACA (T)	7.0 (2.00)	AAA (K)	1.0 (2.00)	AGA (R)	0.0 (0.00)
AUG (M)	3.0 (1.00)	ACG (T)	1.0 (0.29)	AAG (K)	0.0 (0.00)	AGG (R)	0.0 (0.00)
GUU (V)	4.0 (1.07)	GCU (A)	6.0 (1.20)	GAU (D)	2.0 (0.57)	GGU (G)	1.0 (0.21)
GUC (V)	4.0 (1.07)	GCC (A)	7.0 (1.40)	GAC (D)	5.0 (1.43)	GGC (G)	2.0 (0.42)
GUA (V)	6.0 (1.60)	GCA (A)	7.0 (1.40)	GAA (E)	2.0 (2.00)	GGA (G)	12.0 (2.53)
GUG (V)	1.0 (0.27)	GCG (A)	0.0 (0.00)	GAG (E)	0.0 (0.00)	GGG (G)	4.0 (0.84)
Average# codons=	212						

Figure 11: The conceptual translation product of the CO I gene of *Osteochilichthys formosus*

>OK631755.1 *Osteochilus formosus* | cytochrome oxidase subunit I gene | voucher MP-2021 | partial cds, mitochondrial | 637bp

The COI sequence of *Osteochilichthys formosus* showed bias to nucleotide AT, with following composition of nucleotides T=29.4%, C=27.2 %, A=25.7% and G=17.7% and the average value of nucleotide composition of 9

nucleotides shows T=29.2 %, C=26.3 %, A=26.6% and G=17.9 % (Table 5).

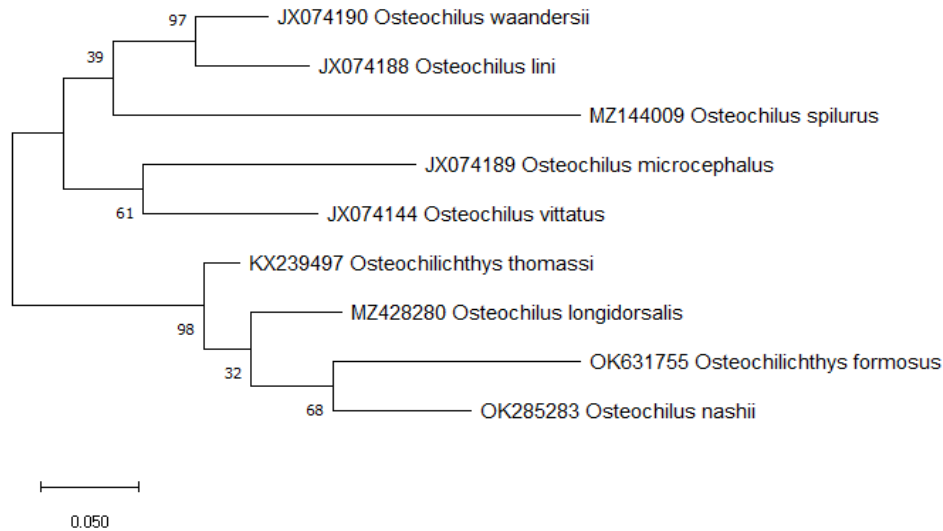


Figure 12: Molecular Phylogenetic tree of *Osteochilichthys formosus* inferred by ML- Tamura- Nei Model

Table 1: Morphometric characters of *Osteochilichthys formosus* (n=6)

Measurement	Range	mean	S.D
Total length	168.5-252.1	192.4	28.0
Standard Length (mm)	132.0-196.7	150.4	21.8
Head Length(mm)	31.3-47.3	35.9	5.26
% SL			
Head length	23.3-25.1	23.8	0.59
Head depth	18.1-22.1	19.8	1.27
Head width	14.2-14.7	14.4	0.19
Body depth at dorsal origin	31.1-33.0	32.0	0.66
Body depth at ventral origin	31.0-32.5	31.8	0.63
Body depth at anal origin	21.6-23.8	22.8	0.79
Body width at dorsal origin	11.8-14.4	13.2	0.76
Body width at ventral origin	15.9-18.0	16.8	0.71
Body width at anal origin	7.4-8.5	7.9	0.39
Pre-dorsal length	46.6-48.4	47.1	0.65
Post-dorsal length	56.0-59.1	57.4	1.24
Pre-pelvic length	51.2-54.7	52.8	1.14
Pre- anal length	75.2-79.1	76.3	1.38
Length of dorsal fin	22.6-25.0	23.6	0.74
Length of pectoral fin	20.2-23.1	21.2	1.03
Length of pelvic fin	17.6-22.6	19.1	1.69
Length of anal fin	20.1-25.1	23.3	1.94
Length of caudal fin	29.1-32.4	31.0	1.02
Length of base of dorsal fin	20.7-21.9	21.2	0.40
Length of base of pectoral fin	4.2-4.8	4.5	0.18
Length of base of pelvic fin	4.6-5.5	5.0	0.28
Length of base of anal fin	9.8-11.1	10.5	0.48
Length of caudal peduncle	15.2-18.0	16.9	0.87
Depth of caudal peduncle	12.4-13.0	12.5	0.20
Width of caudal peduncle	5.0-6.6	5.8	0.54
Distance between pectoral fin and pelvic fin	31.1-33.7	32.3	0.79

Distance between pelvic fin and anal fin	23.7-25.6	24.7	0.74
Distance between anal fin and caudal fin	21.8-23.1	22.6	0.42
Distance from ventral to vent	21.7-24.6	23.1	1.07
Distance from anal to vent	0.9-2.1	1.5	0.47
% HL			
Head depth	74.7-92.1	82.6	5.08
Head width	57.3-63.1	61.1	1.89
Eye diameter	31.2-36.2	34.1	1.64
Pre-orbital distance	64.4-69.4	66.8	1.62
Post-orbital distance	35.2-38.9	36.8	1.48
Pre-occipital distance	77.0-88.3	81.5	3.48
Post-occipital distance	115.8-124.6	120.5	3.14
Inter orbital width	37.9-42.7	39.9	1.67
Inter narial width	27.0-29.7	28.3	1.10
Snout length	34.7-39.2	36.6	1.40
Width of gape of mouth	31.7-39.7	34.2	2.83

Table 2: Meristic counts of *Osteochilichthys formosus* (n =6)

Sl. No	Characters	Holotype	Range
1	Lateral line scales	37+1	37+1
2	Pre-dorsal scales	12	12-13
3	Dorsal fin origin to lateral line	6.5	6.5
4	Ventral fin origin to lateral line	3.5	3.5-4.5
5	Anal fin origin to lateral line	4.5	4.5
6	Circumpeduncular scales	7	6-7
Fin ray count			
7	Dorsal fin rays	iii.11	iii.11
8	Pectoral fin rays	i.14	i.13-16
9	Pelvic fin rays	i.8	i.8
10	Anal fin rays	iii.5	iii.5
11	Caudal fin rays	iii.17.iii	iii.17.iii

Table 3: Meristic counts of *Osteochilichthys formosus* and relative species

Sl.no	Characters	<i>O. formosus</i>	<i>O. elegans</i>	<i>O. nashii</i>	<i>O. longidorsalis</i>	<i>O. brevidorsalis</i>	<i>O.thomassi</i>
1	Dorsal fin	iii.11	iii.10-11	iii, 11	iii.10	iv.11	iii.11
2	Anal fin	iii.5	iii.5	iii, 5	iii.5	iii.5	iii.5
3	Pectoral fin	i.13-16	.i, 13-14	i, 14	i.13-14	i.14	i.13
4	Pelvic fin	i.8	i.8	i, 8	i, 8	i.8	i.8-9
5	Caudal fin	iii.17.iii	iii.17.iii	iii.17.iii	iii.17.iii	iii.17.iii	iii.17.iii
6	LLS	37	43-44+1	41-42	38	39-40	41-43
7	Predorsal scales	12-13	13-14	11-12	11-12	13-14	14
8	LL/D	6.5	7.5-8.5	8.5	6.5-7.5	7.5-8.5	7.5
9	LL/V	3.5-4.5	4.5	5.5	3.5	4.5	5.5
10	LL/A	4.5	5.5	5.5	4.5-5.5u	6.5	5.5

Table 4: Comparison of morphometric features of *O. formosus* and relative species

Characters	<i>O. formosus</i>	<i>O. elegans</i>	<i>O. augraoides</i>	<i>O. longidorsalis</i>	<i>O. brevidorsalis</i>	<i>O. thomassi</i>
Total length	168.5-252.1	144.5-160.5	168-176	137.0-204.3	112.0-160.0	101.0-103.0
Standard Length	132.0-196.7	117.1-133.2	137-143	105.9-156.2	84.0-123.5	80.3-86.4
Head Length	31.3-47.3	----	-----	26.0-36.0	21.6-30.0	20.0
Head length	23.7-25.1	-----	20.9-21.8	-----	-----	----
Head depth	19.6-22.1	-----	18.1-18.2	17.6-18.8	-----	17.7-17.9
Head width	14.3-14.7	13.1-14.1	12.4-12.5	----	---	12.7-12.9
Body depth at dorsal origin	31.1-32.5	26.5-28.6	28.6-29.9	28.1-28.5	----	27.7-29.1
Body depth at anal origin	21.6-22.5	18.3-20.8	20.4-20.9	20.0-21.2	----	19.0-19.7
Body width at dorsal origin	11.8-14.4	8.73-11.5	---	---	8.09-10.4	8.84-10.9
Body width at anal origin	7.7-8.6	-----	9.5-10.4	6.1-7.4	5.9-6.4	6.2-6.9
Pre-dorsal length	46.6-48.4	43.7-45.0	41.2-43.0	-----	-----	----
Pre-pelvic length	52.4-54.7	48.1-50.4	49.6-50.3	48.5-52.4	46.9-49.4	51.0-51.7
Pre-anal length	75.2-79.1	71.1-72.4	72.9-75.5	--	72.3-73.0	----
Length of dorsal fin	23.3-25.0	20.7-22.6	21.1-21.6	29.7-30.3	30.7-32.7	25.5-26.2
Length of pectoral fin	20.7-23.1	19.5-20.8	18.1-18.9	23.6-25.0	----	19.6-20.7
Length of pelvic fin	18.9-22.6	-----	17.4-18.2	24.3-24.7	22.6-24.4	---
Length of caudal fin	29.1-31.9	-----	27.9-29.1	32.7-33.3	32.6-35.3	----
Length of base of dorsal fin	21.1-21.5	-----	19.5-20.4	17.2-18.4	---	--
Length of base of anal fin	10.0-11.1	-----	----	8.6-9.2	----	9.8-10.0
Length of caudal peduncle	15.2-18.0	18.8-20.0	20.2-21.1	---	19.8-20.9	19.9-20.2
Depth of caudal peduncle	12.4-12.6	11.1-12.0	10.9-11.1	---	----	----
Width of caudal peduncle	5.0-6.7	-----	4.19-4.3	--	---	4.7-4.9
Distance between pectoral & pelvic	32.5	26.6-28.0	30.0-30.6	28.2-29.4	24.4-28.9	28.0-29.5
Distance between pelvic & anal	23.7-25.4	-----	----	26.4-28.0	---	----
Distance between anal & caudal	22.5-23.1	23.0-25.6	---	21.6-22.0	24.0-26.4	24.3-25.5
Distance from ventral to vent	21.7-23.3	-----	23.3-25.8	24.7-25.2	---	----
Head depth	82.3-92.1	71.8-76.6	---	76.9-81.4	---	72.0-76.5
Head width	57.3-61.5	-----	---	--	--	52.0-55.0
Eye diameter	31.2-35.8	-----	36.6	---	36.3-39.3	40.5-41.5
Pre-orbital distance	64.4-68.2	-----	63.3	---	---	69.5-70.0
Post-orbital distance	35.5-38.4	38.0-42.8	---	32.5-35.3	---	35.0
Pre-occipital distance	79.0-82.0	-----	---	90.8-94.1	---	81.0-87.0
Post-occipital distance	115.8-124.6	100.6-109.6	--	---	---	--
Inter orbital width	38.3-42.7	-----	---	---	---	34.0-37.5
Inter narial width	29.1-29.7	23.3-26.3	30.0	---	---	22.5-25.0
Snout length	36.7-37.0	---	33.3	---	30.3-31.6	30.0-32.5
Width of gape of mouth	31.9-39.7	21.6-25.0	20.0	---	---	20.0

Table 5 Comparison of nucleotide frequencies of CO I gene sequence of *Osteochilichthys formosus* with its kin species

	T(U)	C	A	G	Total	T-1	C-1	A-1	G-1	Pos #1	T-2	C-2	A-2	G-2	Pos #2	T-3	C-3	A-3	G-3	Pos #3
KX239497 <i>Osteochilichthys thomassi</i>	29.2	26.5	26.8	17.4	678	18.1	26.5	24.3	31.0	226	41.6	27.9	15.5	15.0	226	27.9	25.2	40.7	6.2	226
MZ428280 <i>Osteochilus longidorsalis</i>	29.2	26.2	26.7	17.9	599	17.5	25.5	25.5	31.5	200	41.5	30.0	14.0	14.5	200	28.6	23.1	40.7	7.5	199
OK285283 <i>Osteochilus nashii</i>	28.7	26.7	27.6	17.0	630	17.6	26.7	24.8	31.0	210	41.4	29.0	15.2	14.3	210	27.1	24.3	42.9	5.7	210
MZ144009 <i>Osteochilus spilurus</i>	28.9	26.6	26.0	18.6	655	19.7	25.7	23.4	31.2	218	41.3	28.9	15.1	14.7	218	25.6	25.1	39.3	10.0	219
JX074189 <i>Osteochilus microcephalus</i>	28.6	26.5	25.7	19.2	678	19.9	24.8	24.8	30.5	226	41.6	27.9	15.5	15.0	226	24.3	27.0	36.7	11.9	226
JX074144 <i>Osteochilus vittatus</i>	29.4	26.4	26.4	17.8	678	19.9	24.8	24.3	31.0	226	41.6	27.9	15.5	15.0	226	26.5	26.5	39.4	7.5	226
JX074190 <i>Osteochilus waandersii</i>	29.1	26.0	27.4	17.6	678	19.9	24.3	24.8	31.0	226	41.6	27.9	15.5	15.0	226	25.7	25.7	42.0	6.6	226
OK631755 <i>Osteochilichthys formosus</i>	29.4	27.2	25.7	17.7	637	18.9	26.9	24.5	29.7	212	42.0	28.3	15.1	14.6	212	27.2	26.3	37.6	8.9	213
JX074188 <i>Osteochilus lina</i>	30.2	24.9	27.3	17.6	678	21.2	23.0	24.3	31.4	226	41.6	27.9	15.5	15.0	226	27.9	23.9	42.0	6.2	226
Avg.	29.2	26.3	26.6	17.9	656.8	19.2	25.3	24.5	30.9	218.9	41.6	28.4	15.2	14.8	218.9	26.7	25.3	40.1	7.9	219

Composite Likelihood model. Substitution pattern and rates were estimated under the Tamura-Nei (1993) model (+G). Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics. Relative values of instantaneous r should be considered when evaluating them. For simplicity, sum of r values is made equal to 100. The nucleotide frequencies are A = 26.6%, T/U = 29.2%, C = 26.3%, and G = 17.9%. For estimating ML values, a tree topology was automatically computed. The maximum Log likelihood for this computation was -2589.86. This analysis involved 9 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 678 positions in the final dataset. Evolutionary analyses were conducted in MEGA X

The phylogenetic tree generated using ML (Tamura-Nei) method showing the phylogenetic position of *Osteochilichthys formosus* (Accession No. OK631755.1) isolated from Kazargod, Kerala, India. Phylogenetically *Osteochilichthys formosus* (Accession No. OK631755.1) showed to be the closest relatives of *Osteochilichthys augraoides* (formerly *O. nashii*) (OK285283.1) from Mysore in Karnataka.

Here we have done molecular identification of *Osteochilichthys formosus* using DNA based taxonomy. The result confirmed that the molecular identification method is strictly correlated with classical taxonomy. The cytochrome oxidase I gene of *Osteochilichthys formosus* yielded a product having 637bp amplified DNA sequence. Nucleotide BLAST results proves that *Osteochilichthys formosus* isolated from Kazargod have only 96.59% nucleotide similarity to *Osteochilichthys augraoides*

(formerly *O. nashii* (OK285283.1)). So *Osteochilichthys formosus* is different from *Osteochilichthys augraoides* and can be treated as a new species.

CONCLUSION

Osteochilichthys formosus is the major *Osteochilichthys* species of water bodies of Kannur and Kazargod in Kerala; *Osteochilichthys longidorsalis* is a species restricted to Trichur district; *O. brevidorsalis* is distributed in the hill streams of Palakkad Mountain ranges and water bodies below Nigiris. *Osteochilichthys augraoides* is restricted to Bhavani River and its tributaries in Karnataka; *Osteochilichthys* fishes with mid lateral black band and anal fin stripe were commonly regarded as *O. augraoides*; it is now confirmed that fishes with these characters in Kerala are *O. formosus* and those in Karnataka are *O. augraoides*. Compared to the latter, *O. formosus* is deep bodied and with fewer lateral line scales. The new species is edible and consumed by native people; as it is colorful, it can also be utilized for ornamental purposes. It is expected that more taxonomic studies may be conducted on this beautiful fish in days to come.

Comparative materials examined:

Osteochilichthys elegans: Holotype: V/F/NERC/ZSI/5420, 133.2 mm SL, a water stream at Mannarkkad, Palakkad district in Kerala, India, coll. Mathews Plamoottil, 20.12.2020. Paratypes: ZSI/ANRC/M/27755, 5, 117.1-126.6 mm SL, other details same as Holotype.

Osteochilichthys augraoides: DOZ/GCC 70, 2,137 & 143 mm SL, a water stream at Mysore, Karnataka, India, coll.

Mathews Plamoottil and Vineeth, K, 25/01/2020.

Osteochilichthys longidorsalis: ZSI/ANRC/M/27238, 3, 105.9-156.2 mm SL, Athirappally, Trichur, coll. Mathews Plamoottil&Vineeth. K, 10.04.2021.

Osteochilichthys brevidorsalis: DOZ/GCC 71, 6, 76.7-123.5 mm SL, The Bhavani River in Palakkad, coll. Mathews Plamoottil, 28/12/2020.

Osteochilichthys thomassi: DOZ/GCC 72, 6, 76.7- 123.5 mm S, The Bhavani River in Palakkad, coll. Mathews Plamoottil, 28/12/2020.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Mathews Plamoottil (MP) and Vineeth, K (VK) collected fishes; MP designed and performed the experiments and also wrote the manuscript. MP read and approved the final version.

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