

Integrative Taxonomy of Tetraodontiform Fishes (Tetraodontiformes: Percomorpha) From Southwest Coast of India

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Abstract

The taxonomy of the order Tetraodontiformes is not well studied from the Indian coast, hence an integrated approach was attempted in this paper. Forty-two species of tetraodontiform fishes classified under 8 families in 25 genera were identified using morpho-molecular approach from southern west coast of India. Tetraodontidae is the most speciose family with fourteen species, followed by Balistidae and Monacanthidae with seven species each; Ostraciidae, Diodontidae, Triacanthodidae and Tricanthidae harbour three species each and Molidae is represented by two species. Eight species collected during the present study are new records to the Kerala coast. DNA barcoding using mitochondrial CO1 gene confirmed 12 species of tetraodontiform fishes. The molecular data of the present study were compared with sequences from GenBank, and the selected published sequences by expert taxonomists were matched for their maximum identity. The heat map shows low pairwise distances between the nearest species, and the phenetic tree shows that the studied species are nested in clades with closely related species.

Keywords: Taxonomy, morphology, molecular studies, Southwest coast, heat map

Introduction

The systematics of taxonomically and structurally diversified and extensively distributed clade of acanthomorphs, the Tetraodontiformes, have been elucidated recently (Matsuura, 2015), with the persisting nomenclatural challenges. These fishes are widely distributed globally and inhabit the pelagic, deep sea, coastal, reef and freshwater habitats (Tyler, 1980; Matsuura, 2014). Many of the bony structures of the body and head are reduced or fused; reduction in the vertebrae and reduction or losses in fin supporting elements, thick skin covered with spines, prickles, ossicles, scales or bony plates are the key features of this order (Bray, 2020). Four hundred and thirty four extant species represent the order

under ten families and five suborders (Nelson *et al.*, 2016). The diversity and taxonomic works carried out in India for the order Tetraodontiformes include those of Veeruraj *et al.* (2011), Sahayak *et al.* (2015), Sujatha and Padmavathi (2015), Raj and Seshserebiah (2015), Kaleshkumar *et al.* (2015, 2021, 2018), Mohapatra *et al.* (2018), Naranji *et al.* (2016), Kumar *et al.* (2019), Ray and Mohapatra (2020), Mary *et al.* (2021) and Ramachandran (2022).

Mitochondrial DNA is helpful in tracking ancestry as it is maternally inherited, easily isolated and, with its rapid base substitution rate, a powerful molecular marker for evolutionary studies (Avise *et al.*, 1987). An integrated taxonomic approach is always useful in the identification

of complex species which are difficult to identify morphologically alone (Padial *et al.*, 2010; Tornabene *et al.*, 2010). The taxonomy of Tetraodontiformes is not well studied from the Indian coast, and hence in the present study, an integrated approach using the traditional morphometrics and DNA barcoding using the mitochondrial cytochrome oxidase subunit 1 (COI) gene was attempted for selected species. The molecular taxonomic studies conducted on Tetraodontiformes from India include those of Kaleshkumar *et al.* (2015, 2018), Devi (2016), Bemis *et al.* (2020) and Ramachandran (2022).

Here we present a detailed taxonomic account of fishes under the order Tetraodontiformes landed in the major fishing harbours of south India and also DNA barcoding of selected tetraodontiform fishes using COI gene to confirm identification and to understand the phylogeny of identified species.

Materials and methods

The specimens were randomly collected from trawl bycatch, seine nets and gill nets from the major fishing harbours of south India: Neendakara and Sakthikulangara (Kerala) and Jeppiar harbour, Muttom (Tamil Nadu). The collection was performed in 2019 and 2020. The tissue samples from fin clips were taken for molecular analysis and stored in 90 per cent ethanol. For the identification and measurements of members of each family, specific literature was used and compared with the descriptions of species following (Matsuura and Tyler 1997), Matsuura (2001, 2015), Bemis *et al.* (2020) and Froese and Pauly (2023). The specimens were finally preserved in 10 % buffered formalin. Voucher specimens are deposited in the Department of Aquatic Biology and Fisheries museum collections, University of Kerala.

Genomic DNA was extracted from the tissue of fin clips of twenty fishes using NucleoSpin® Tissue Kit (Macherey-Nagel) following specific instructions. The mitochondrial cytochrome oxidase I (COI) gene was amplified using the Universal primer set Fish-F1 (TCAACCAACCACAAAGACATTGGGCAC) and Fish-R1 (TAGACTTCTGGGTGGCCAAAGAATCA) (Ward *et al.*, 2005) for the mitochondrial cytochrome oxidase I (COI) marker. The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the standard procedures, and Gene sequencing was done at Rajiv Gandhi Centre

for Biotechnology (RGCB), Trivandrum, India and the sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems) and the final sequences and the obtained sequences from GenBank database were edited and aligned using Bio Edit sequence alignment editor V.7.0.9.0. and genetic distance was calculated with the Kimura 2-parameter distance model (K2P) (Kumar *et al.*, 2016). Using MEGA (Version 11). Microsoft Office Excel software (Excel, version 2019 16.0.6742.2048) was used for editing the heatmap of average K2P divergences between COI barcodes. For the phylogenetic analysis, COI sequence data were analyzed using the neighbor-joining (NJ) tree to infer the evolutionary relationships of the studied species with other closely related species. The phylogenetic tree was planned using FigTree v1.4.4 (Rambaut *et al.*, 2018).

Results and discussion

Forty two species of Tetraodontiformes under 8 families and 25 genera were recorded from the fishing harbours of southwest India (Table 1). Tetraodontidae is the most speciose family (14 species), followed by Balistidae and Monacanthidae (7 species each), Ostraciidae, Diodontidae, Triacanthodidae and Tricanthidae (3 species each) and Molidae (2 species) *Mola alexandrini* and *Mola mola* (Table 1.). *Halimochirurgus centriscoides* (Family: Tricanthodidae); *Balistapus undulatus*, *Sufflamen chrysopteron* (Family: Balistidae); *Anacanthus barbatus*, *Pervagor melanocephalus* (Family: Monacanthidae), *Canthigaster petersii*, *Sphoeroides pachygaster*, and *Torquigener brevipinnis* (Family: Tetraodontidae) encountered during the present study are new records to Kerala. Species used for molecular studies except *Mola mola* were deposited in the museum of Department of Aquatic Biology and Fisheries, University of Kerala and provided with voucher numbers for future reference.

Voucher numbers were provided as follows: *Sufflamen franetum* (DABFUK/FI/316), *Cylichthys orbicularis* (DABFUK/FI/317), *Diodon holocanthus* (DABFUK/FI/318), *Arothron stellatus* (DABFUK/FI/319), *Arothron stellatus* (DABFUK/FI/320), *Arothron immaculatus* (DABFUK/FI/321), *Lagocephalus guentheri* (DABFUK/FI/322), *Odonus niger* (DABFUK/FI/323), *Sphoeroides pachygaster* (DABFUK/FI/324), *Lagocephalus scleratus* (DABFUK/FI/325), *Abalistes stellatus* (DABFUK/FI/326), *Canthidermis maculata* (DABFUK/FI/327), *Lactoria cornuta* (DABFUK/FI/328), *Mola mola* (DABFUK/FI/329).

Systematics

1. *Halimochirurgus centriscoides* Alcock, 1899 (Longsnout spikefish)

Materials collected: 2 specimens (SL 120mm, 128 mm), Sakthikulangara harbour, Kerala, Suvarna S. Devi; 21/02/2020.

Description: Medium sized stout body, upper jaw prolonged into long snout with a palp/spoon like structure at its end; inferior mouth with a short snout; large eyes; dorsal fin with three spines increasing in size posteriorly with dorsal fin membrane extending between, second dorsal only with rays; pectorals modified into a long sharp spine as lengthy as the first dorsal fin spine; anal fin placed far back on the body long gill opening; scales with spinules; medium sized caudal peduncle with caudal fins with several soft rays.

Colour: Red dorsally and laterally with two white lines, upper thin white line and lower more thick white line; whitish ventrally, long snout and its spoon shaped structure are also red in colour; second dorsal fins last six rays colourless, rest of the dorsal spines and rays red coloured; pectoral spine colourless, caudal fins also red in colour.

Remarks: Only two species, *Halimochirurgus alcocki* M.C.W. Weber, 1913 and *Halimochirurgus centriscoides* Alcock, 1899, are known in this genus. This species is a new record to the Kerala coast.

2. *Balistapus undulatus* (Park, 1797) (Orange-lined triggerfish)

Materials collected: 2 specimens (SL 270 mm, 29 mm); Vizhinjam, Trivandrum, A. Biju Kumar, 09/08/2019.

Description: 3 Dorsal spines; 25 soft rays, 25 Anal soft rays; front of eye without groove; scales above the pectoral-fin base and behind the gill opening to forming flexible tympanum; caudal peduncle scales having 2 longitudinal rows of large spines; rounded caudal fin with narrow peduncle.

Colour: head and body colour greenish brown with diagonal orange lines, large round black blotch covering base of the caudal fin; caudal fin, dorsal, anal and pectoral fins soft rays orange.

Remarks: Mohapatra *et al.* (2020) reported the species from India in their checklist of faunal diversity of the

Indian coast. The species is a new record from Kerala.

3. *Sufflamen chrysopteron* (Bloch & Schneider 1801) (Halfmoon triggerfish)

Materials collected: 2 specimens (SL 212 mm, 224 mm, 225 mm); Sakthikulangara harbour, Kerala, Suvarna S. Devi and A. Biju Kumar, 22/04/2019

Description: Head concave with terminal mouth, cheek fully covered with scales; groove before the eye and beneath nostrils, small row of scales behind tympanum; caudals truncate; black bar running through the pectoral base; large scales all over the body forming small conical spines at the posterior region Dorsal spines 3; Dorsal soft rays 26-27; Anal soft rays: 25.

Colour: A pale brown triggerfish with an orangish yellow bar below the rear of the eye, yellowish caudals with triangle edge of white in tail, slight tinge of blue on lower part especially on chin and belly.

Remarks: Recorded from Lakshadweep (Jones and Kumaran, 1980) and Andaman Nicobar islands (Rajan and Mishra, 2018) and Visakhapatnam (Padmavathi *et al.*, 2017); Mohapatra *et al.* (2020). The species is new record to Kerala.

4. *Anacanthus barbatus* Gray, 1830 (Bearded leatherjacket)

Materials collected: 2 specimens (SL 191 mm, 253 mm, 256 mm); from Vizhinjam harbour, Trivandrum; Suvarna S. Devi and A. Biju Kumar; 25/11/2019.

Description: Body and head elongate and compressed, lower jaw with beard; long based dorsal and anal fins; dorsal spines 1, dorsal soft rays 48-50; anal soft rays: 58 - 60.

Colour: light yellowish in colour, an orange stripe extends from tip of the snout to eye.

Remarks: Reported by Day (1871) and listed by Rajan and Mishra (2018) from Andaman Nicobar Islands. The species is a new record to Kerala.

5. *Pervagor melanocephalus* (Bleeker, 1853) (Redtail filefish)

Materials collected: 2 specimens (SL 153 mm, 151 mm), collected from Vizhinjam, Kerala, A. Biju Kumar; 21/09/2019.

Description: Long and compressed body; first dorsal spine long and strong, second short and hidden; pelvic

rudiment large not attached to posterior margin of ventral flap; scale ridge of male folded.

Colour: Bluish black or light violet on head and anterior portion of body, posterior part of the body orange; black blotch around the gill opening; caudal fin orange with 1/2 blue lines at its tip; first dorsal spine blackish brown; soft dorsal and anal fins yellow.

Remarks: Joshi *et al.* (2016) recorded the species from the Gulf of Mannar, Rao (2003) and Rajan *et al.* (2013) reported it from Andaman Nicobar Islands. The species is a new record for Kerala.

6. *Canthigaster petersii* (Bianconi, 1854) (Peter's toby)

Materials collected: 2 specimens (SL 290 mm, 292.20 mm); Vizhinjam harbour, Kollam, A. Biju Kumar and Suvarna S. Devi, 22/04/2019

Description: Laterally compressed body with spinules on belly; gill opening and nostrils minute; rounded tail; dorsal and anal fin rays 9, pectoral fin rays 15.

Colour: white spots on lateral side of the body reaching upto the caudal fin, abdomen white in colour; around the eye and on the dorsal profile white striations; black spot below the base of dorsal fin.

Remarks: Recorded from Andamans (Allen and Erdmann, 2012) and Tuticorin (Mishra *et al.*, 2019)

7. *Sphoeroides pachygaster* (Muller & Troschel) 1848 (Blunt-head puffer)

Materials collected: 1 specimen (SL 150 mm); Suvarna S. Devi from Sakthikulangara harbour, Kerala; 13/12/2019

Description: Body oblong and devoid of prickles or scales; blunt head, jaws heavy forming a beak of two teeth in both jaws, mouth terminal and small, eye small and ovoid; dorsal and anal fins set far back near caudal fin which is truncated, lacks pelvic fin, dorsal fin usually with 9 soft rays, anal fin with 8 or 9 soft rays, no spines on fins.

Colour: Brown to grey above, ventral side white dorsal and lateral surfaces with arbitrary dark blotches and spots. Pectorals and anal translucent white and without markings, dorsal light dusky grey and caudal dark dusky grey but with tips of rays and lower margin lighter.

Remarks: The species was recorded from eastern

Arabian Sea (Ramachandran *et al.*, 2022). The species is a new record to Kerala.

8. *Torquigener brevipinnis* (Regan, 1903) (Yellow-stripe Toadfish)

Materials collected: 7 Specimens (SL ranging from 39 – 54mm) from Neendakara and Beypore, Kerala; Kerala; A. Biju Kumar; 14/08/2019, 12/03/2019.

Description: Elongated body, round dorsally and flattened ventrally, tapering towards the caudal peduncle; small terminal mouth with thin lips with several tiny papillae; spines over the body is small, less dorsally and moderate ventrally; skin with several longitudinal pleats; three slightly oblique, narrow, creamy-white bands on the cheek; inverted U-shaped band below posterior part of eye; moderate eye; caudal fin truncated with dark brown blotches on rays which looks like six lines.

Colour: Brown dorsally, creamy whitish ventrally, with small round pale yellow spots, outlined by brownish dots. A yellowish-brown band is seen extending from the pectoral fin to the base of caudal fin.

Remarks: The species is similar in many aspects to its congener *T. flavimaculosus*, which lacks the caudal fin bands; whitish bands on the body, below the eyes and yellow spots are lacking in the latter. Biswas *et al.* (2010) and Kaleshkumar *et al.* (2021) recorded the species from the Tamil Nadu coast. The species is a new record for Kerala.

Molecular Taxonomy

The present study included 38 nucleotide sequences of different Tetraodontiformes belonging to 8 different families, out of which 13 sequences (accession numbers are provided in brackets) from the present study were included for the current analysis, and 1 nucleotide sequence of *Pterois volitans* (Perciformes; Scorpaenidae) was used as an outgroup. The neighbor-joining phenetic tree showed deep divergences between all families of Tetraodontiformes and relatively small differences within members of each family (Figure1). Furthermore, *Odonus niger* (OQ916876) and *Abalistes stellatus* (OQ918289), the two species belonging to Balistidae nested in two clades with 79.9% bootstrap value. Moreover, there are two separate clades comprising *Lactoria cornuta* (OQ918269) (Ostraciidae), *Mola mola*

(OQ918272) (Molidae), with 100% bootstrap value. On the other hand, *Cylichthys orbicularis* (OQ916400) and *Diodon holocanthus* (OQ916419) (Diodontidae) nested in one clade with 99.6% bootstrap value. Also, *Arothron immaculatus* (OQ916460) and *Arothron stellatus* (OQ916427, OQ916435) (Tetraodontidae) nested in clade with 99.5% bootstrap value. Furthermore, the remaining species belonging to Tetraodontidae, *Sphoeroides pachygaster* (OQ918225), *Lagocephalus scleratus* (OQ918252) and *Lagocephalus guentheri* (OQ916833), are nested in three separate clades with 100% bootstrap value (Figure1).

The heatmap describing average K2P divergences between COI barcodes of different Tetraodontiformes species in the present study is given in Figure 2. The overall genetic distance average recorded was 0.20. The highest interspecific genetic distance observed was 0.29 from different localities. The lowest genetic distance was recorded between the sequences of the same species in the present study and the matched sequences from NCBI.

Molecular genetics and DNA analyses helped in the estimation of similarities and differences in genes among organisms (Antoniou and Magoulas, 2014). Genetic data are widely used in fisheries management, including identification, discrimination, and species conservation (Basheer *et al.*, 2016; Victor, 2016; Alzahaby and Biju Kumar, 2023). The heat map exhibits genetic distances between species where the smaller genetic distances between species indicate a close genetic relationship (deep blue), while the large genetic distances indicate a more distant genetic relationship (deep violet) (Figure 2). Genetic diversity within species were calculated as zero between members of each family from the 8 families.

Even though the taxonomy and systematics of Tetraodontiformes are thoroughly studied by Matsuura (2014), several taxonomic problems persist in various families, especially in the phylogenetic positions of Triodontidae and Molidae, which is obscure. In India, under the order Tetraodontiformes, 70 species under 8 families are reported in Fish Base (Ferreira and Pauly, 2023), which needs revision, as Gopi and Mishra (2015) have listed 101 species under the order.

The records of the order Tetraodontiformes reported

from Kerala till now comprised 8 families with 41 species (Biju Kumar *et al.*, 2019). In addition to this, *Lagocephalus spadiceus* (Devi, 2016), *Mephisto fraserbrunneri* (Bemis *et al.*, 2020) and *Mola alexandrini* (Mohan *et al.*, 2006; Kishore *et al.*, 2013) were also recorded. The present study identified 42 species of fishes in the order Tetraodontiformes from the southwest coast of India, and eight new records to Kerala *Halimochirus centriscoides* Alcock, 1899 (Tricanthodidae); *Balistapus undulatus* (Park, 1797), *Sufflamen chrysopterum* (Bloch & Schneider 1801) (Balistidae); *Anacanthus barbatus* Gray, 1830, *Pervagor melanocephalus* (Bleeker, 1853) (Monacanthidae); *Canthigaster petersii* Richardson 1845, *Torquigener brevipinnis* (Regan, 1903) and *Sphoeroides pachygaster* Muller & Troschel, 1848 (Tetraodontidae), making the total number of tetraodontiforms recorded so far from Kerala as 52 species. This short-term survey reflects the higher diversity of these fish taxa on the southwest coast of India.

Out of the twenty samples given for DNA barcoding, only 13 yielded results, revealing 12 different species from five families: Balistidae (*Odonus niger*, *Canthidermis maculata*); Ostaciidae (*Lactoria cornuta*); Tetraodontidae (*Arothron hispidus*, *Arothron immaculatus*, *Arothron stellatus*, *Lagocephalus guentheri*, *Lagocephalus scleratus*, *Sphoeroides pachygaster*); and Diodontidae (*Cylichthys orbicularis*, *Diodon holocanthus*); Molidae (*Mola mola*). Species in the same family formed distinct clades in the phylogenetic tree (Figure2.). Kaleshkumar *et al.* (2015) analysed mitochondrial COI gene of *Chilomycterus reticulatus*, *Arothron hispidus* and *Lagocephalus guentheri* and confirmed that the three species had a dichotomous relationship with their ancestor species.

In conclusion, the present study has strongly authenticated the efficacy of COI in identifying different tetraodontiform species with designated barcodes. The present results also suggest that COI barcoding can be used as a practical method for resolving unequivocal identification of the collected species from south Indian waters of India with applications in its management and conservation.

Further in-depth studies of coral reefs and long-term integrative taxonomic research are recommended to consolidate India's marine fish fauna database.

Table 1. List of Tetraodontiformes obtained from the southwest coast of India in the current study

Sp No	Family	Species	Samples collected
1	Triacanthodidae	<i>Halimochirus gusalcocki</i> Weber, 1913	1
2		<i>Halimochirus guscentriscoides</i> Alcock, 1899	4
3		<i>Mephistofraser brunneri</i> Tyler 1966	5
4	Triacanthidae	<i>Triacanthus biaculeatus</i> (Bloch, 1786)	11
5		<i>Triacanthus nieuhoftii</i> Bleeker, 1852	2
6		<i>Pseudotriacanthus strigillifer</i> (Cantor, 1849)	2
7	Balistidae	<i>Abalistes stellatus</i> (Anonymous 1798)	7
8		<i>Balistapus undulatus</i> (Park, 1797)	2
9		<i>Canthidermis maculata</i> (Bloch, 1786)	2
10		<i>Pseudobalistes flavimarginatus</i> (Rüppell 1829)	2
11		<i>Sufflamen chrysopterum</i> (Bloch & Schneider 1801)	2
12		<i>Sufflamen fraenatum</i> Latrielle 1804	10
13		<i>Odonus niger</i> Rüppell 1836	6
14	Monacanthidae	<i>Aluterus monoceros</i> (Linnaeus, 1758)	7
15		<i>Aluterus scriptus</i> (Osbeck, 1765)	2
16		<i>Anacanthus barbatus</i> Gray, 1830	3
17		<i>Cantherhines pardalis</i> (Rüppell, 1837)	2
18		<i>Paramonacanthus frenatus</i> (Peters, 1855)	3
19		<i>Paramonacanthus pusillus</i> (Rüppell, 1829)	2
20		<i>Pervagor melanocephalus</i> (Bleeker, 1853)	2
21	Ostraciidae	<i>Lactoria cornuta</i> (Linnaeus, 1758)	4
22		<i>Ostracion cubicus</i> (Linnaeus, 1758)	4
23		<i>Tetrasomus gibbosus</i> (Linnaeus, 1758)	2
24	Tetraodontidae	<i>Arothron hispidus</i> (Linnaeus, 1758)	8
25		<i>Arothron immaculatus</i> Bloch and Schneider 1801	9
26		<i>Arothron nigropunctatus</i> Bloch and Schneider 1801	3
27		<i>Arothron reticularis</i> (Bloch & Schneider, 1801)	9
28		<i>Arothron stellatus</i> (Bloch & Schneider, 1801)	7
29		<i>Canthigaster bennetti</i> Bleeker, 1854	2
30		<i>Canthigaster petersii</i> Richardson 1845	2
31		<i>Chelonodon patoca</i> (Hamilton, 1822)	2

Sp No	Family	Species	Samples collected
		<i>Chelonodontops leopardus</i> (Day, 1878)	2
33		<i>Lagocephalus inermis</i> (Temminck& Schlegel, 1850)	12
34		<i>Lagocephalus scleratus</i> Gmelin, 1789	9
35		<i>Lagocephalus guentheri</i> Miranda Ribeiro, 1915	12
36		<i>Sphoeroides pachygaster</i> (Muller&Troschel) 1848	1
37		<i>Torquigener brevipinnis</i> (Regan, 1903)	7
38	Diodontidae	<i>Cyclithys orbicularis</i> Bloch 1785	9
39		<i>Diodon holocanthus</i> Linnaeus 1758	8
40		<i>Diodon hystrix</i> Linnaeus, 1758	8
41	Molidae	<i>Mola alexandrini</i> (Ranzani 1839)	1
42		<i>Mola mola</i> (Linnaeus, 1758)	1

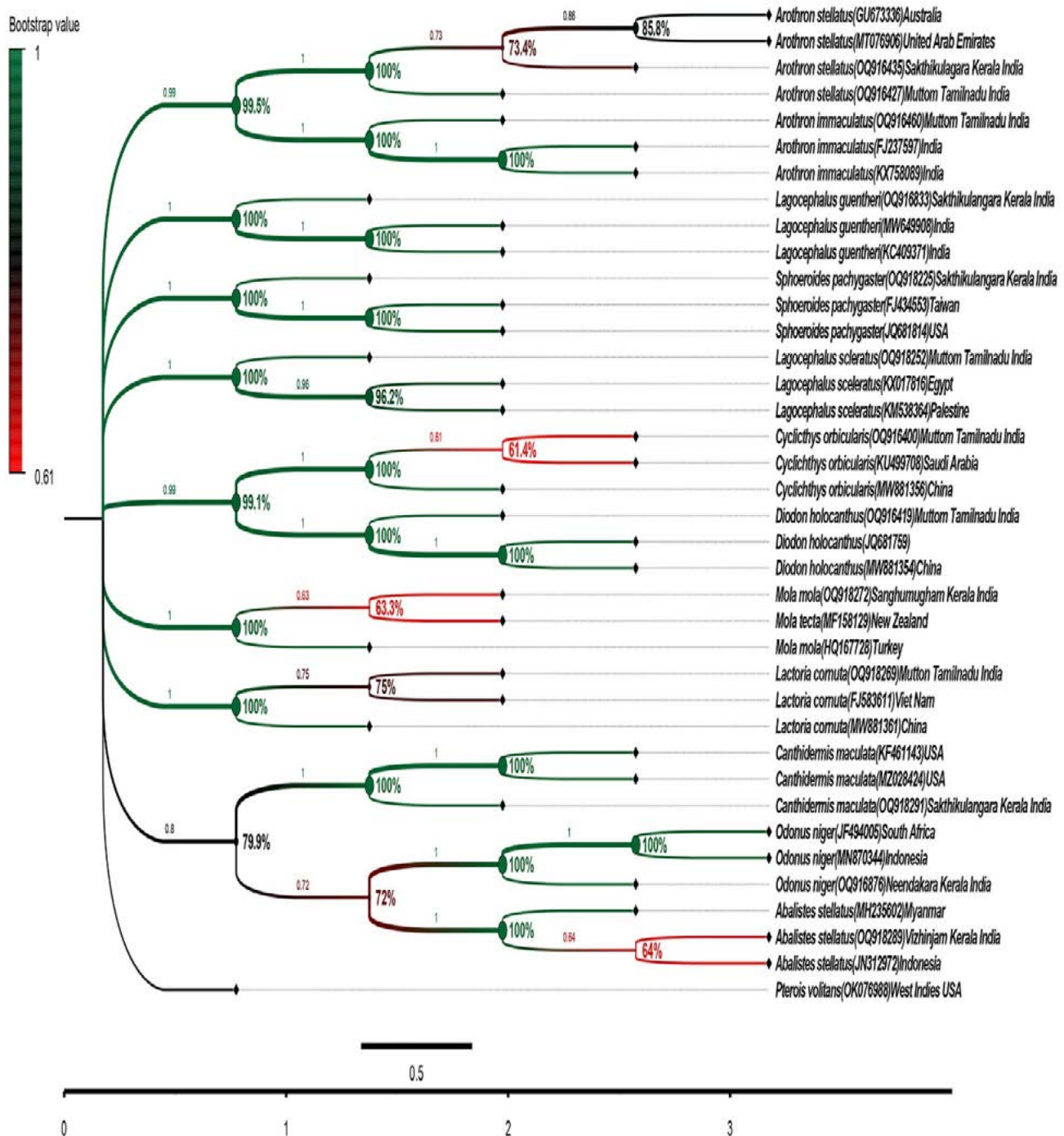


Figure1. The neighbor-joining (NJ) phenetic tree of various Tetraodontiformes species from South India developed using Kimura two-parameter model (K2P) distance among 38 COI sequences. The scale bar represents a 0.2 sequence difference. The numbers along the nodes are bootstrap values based on 1000 iterations. Species' names, collection locations, and Gene Bank accession numbers are shown for each taxon. *Pterois volitans* (OK076988) is the outgroup sequence.

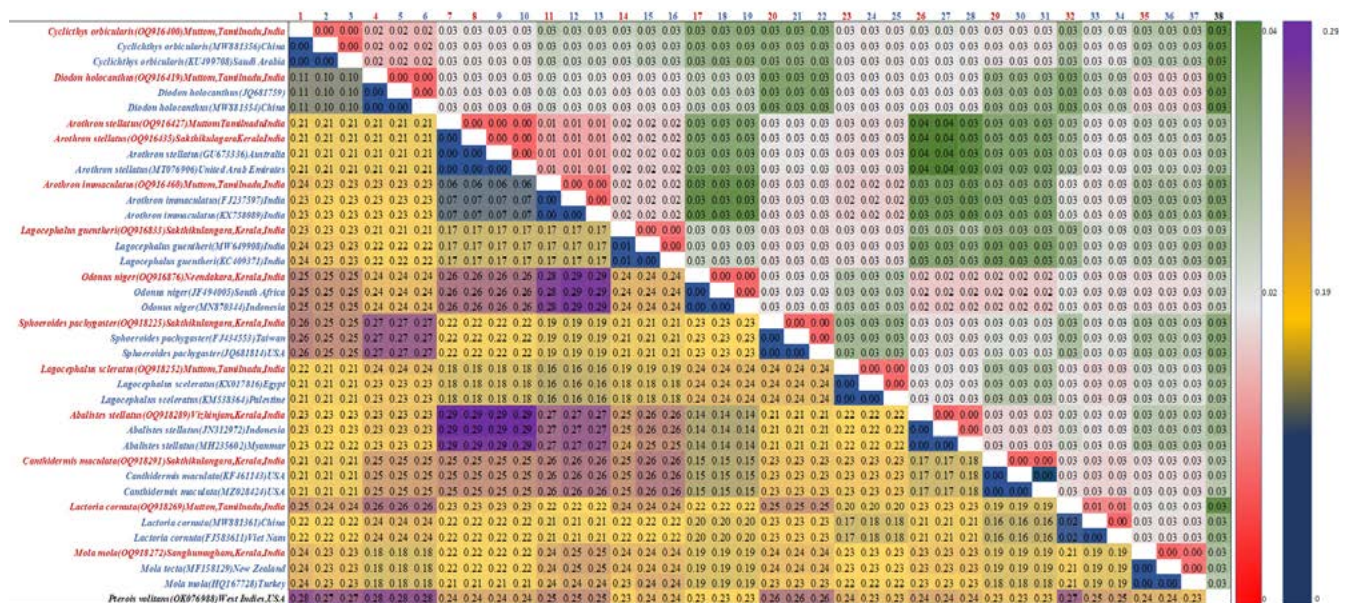


Figure2. Heatmap describing average K2P divergences between COI barcodes of different Tetraodontiformes species in the present study.

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