First record of the Asian sand goby, Favonigobius gymnauchen (Bleeker, 1854) (Teleostei, Gobiidae) from Arabian coast of India and species confirmation through DNA barcoding of COI gene

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Abstract

The Asian sand goby, Favonigobius gymnauchen (Bleeker, 1854) (Teleostei, Gobiidae), is a gobiid fish native to estuarine and marine habitats of Western Pacific waters: Japan, Korean Peninsula, China, Southern Taiwan, Australia, Palau Island, Micronesia, Hong kong and Philippines. In the present study conducted in Jan-March 2021, F. gymnauchen were collected from Kavvayi backwater of Arabian Sea, Kerala, India. The genus and species identity of collected specimens were confirmed by morphological features and by the molecular analysis via. comparison of the mitochondrial COI genome. The molecular sequencing of mitochondrial COI genome yielded a 630 bp product on PCR amplification. The sequenced data were deposited in NCBI GenBank, with accession numbers MZ 505547.1 and OP 236548.1. The deposited COI gene fragments were perfectly matched with specific sequences of the species in NCBI GenBank. This is the first report of Asian sand goby, F. gymnauchen from the Arabian Sea coast of Kerala, India.

Introduction

Gobiidae is the largest marine fish family with valid 264 genera and 1966 species, inhabitant of marine and brackish water environments of most tropical and subtropical areas. They habitually accommodate in diverse and harsh habitats, including intertidal zones, coral reef, shallow coastal estuarine region with hard rocky bottom and also soft muddy or sandy bottom (Larson and Murdy 2001; Thacker 2012; Patzner et al. 2012; Nelson et al. 2016; Eschmeyer et al. 2017; Froese and Pauly 2022; Eschmeyer 2022). They are the smallest fishes in the world with the maximum body size 50 mm and are distinct with wholly or partially fused pelvic fins into an adhesive disc and also have separate spinous and rayed dorsal fins. Most species are cryptic bottom dwelling carnivores of small benthic invertebrates; others are planktivores. Some have symbiotic relationships with invertebrates and others remove ectoparasites from other fishes, thus play unique role in their ecosystem. Many are popular aquarium fishes.The detailed studies on the distribution, diversity, phylogenetic analysis and origin is still lacking from many of the Indo-Pacific areas, so the knowledge on the distribution of gobies along the world is highly disproportional (Kovacic and Patzner 2011). In Indian waters, only limited studies have been carried out on the gobiid diversity; and in Kerala also it has received little attention. It may be due to their small size with many cryptic species, low population and lack of economic value.

Favonigobius is the genera of sand gobies with 9 reported species are native to waters around the Indian and the western Pacific Ocean (Froese and Pauly 2020). Published information have shown that all the species are non-commercial, inhabit in tidal zones of shallow sandy inner reefs, estuaries, marine, brackish water as the demersal forms. As per IUCN Red List status all are not evaluated or least concerned (IUCN 2021). Details of distribution of different species of *Favonigobius* species are given in table 1. *F. gymnauchen* (Bleeker, 1860), Sharp-nosed sand goby or Asian sand goby is a demersal occupant with maximum body size of 13.5 cm and is inhabiting in sandy coastal estuaries and brackish waters (Kim and Park 2002, It is the only *Favonigobies species* inhabited in brackish water and tidal zones of Western and Southern coasts of the South Korean peninsula (Kim et al, 2016). It is a dominant

species of intertidal areas of Japan with an average salinity of 3.8 to 10.2 and is significant in commercial fisheries. *F. gymnauchen* spawns from May to September in bivalve shells, after covering the shell with sand, males are the nest-holders (Yamamoto and Tominaga 2005; Yoshida and Tomiyama 2021).

Sequence analysis of the cytochrome c oxidase I (COI) of the partial mitochondrial gene nucleotide used in DNA barcoding, is the basis of a global molecular level bio-identification system for animals (Hebert et al, 2003). DNAbarcoding has proven particularly successful for species identification in fish. According to Ward et al. 2005 and Ivanova, Zemlak, Hanner, and Hebert (2007) the universal primers designed were extremely efficient for the amplification of the COI sequences of the majority of fish species, The present study is undertaken to report and describe the *F. gymnauchen* from Malabar coast of Kerala, India and species confirmation through COI gene sequencing .

Materials And Methods

1.Collection of the specimen

In the present study five specimens of *F. gymnauchen* were collected from Kavvayi backwaters of Arabian Sea during a fish survey in Jan- March 2021. The Kavvayi backwater (12.0929° N, 75.1677° E) is situated between the border of Kannur and Kasargod District in Northern Kerala. The fishes were collected by netting and were washed and stored in 70 % alcohol for further use. The intact specimens were kept in 10% formalin in the Zoology museum of Government College, Kasaragod for further references.

2. Morphological studies:

The specimens were examined in detail using a hand lens and the species identification was done by following FAO species Identification Sheet 2022, Eschmeyer et al. 1979 and related literature. The phenotypic characterization of fishes were analysed and morphometric measurements were taken to the nearest 0.1 mm using digital calipers. Morphometric characters were expressed as % standard length (SL) and % head length (HL) by the methods followed Miller (1988); meristic methods followed Chen and Fang (2006) and Chen and Miller (2008). Meristic abbreviations are as : D1 = First dorsal fin; D2 = Second dorsal fin; V = Ventral fin; A = Anal fin; P = Pectoral fin; PSD = Predorsal scales; LSS= Longitudinal scales series; and TSS = Transverse scales series.

For comparison of morphometric character of 5 specimens, all values except for total length (TL) and standard length (SL) are proportions. Total length (TL, mm), Standard length (SL, mm), Standard length (SL) /TL, Head length (HL) /SL, Body depth at pelvic origin (BDP) /SL, Head width (HW) /SL, Pre first dorsal length (Pre-FDL) /SL, First dorsal fin base length FDFL) /SL, Second dorsal fin base length SDFL)/SL, First dorsal fin length/SL, Second dorsal fin length/SL, Pectoral fin length (PcFL) /SL, Pelvic fin base depth /SL, Pre pelvic length /SL, Anal fin base length (AFL) /SL, Anal fin length /SL, Pre anal fin

length (PAL) /SL, Caudal peduncle length CPL) where measured and tabulated. Simultaneously tissue specimens were also collected and stored with 90% ethanol at -10°C for further molecular level analysis.

3.Molecular analysis

a.DNA extraction and amplification: Genomic DNA was isolated from the muscle tissues using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions. DNA amplification was done by PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems).

b. Sequencing: Sequencing reaction was done from RGCB Trivandrum, in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems , USA) following manufacturer's protocol. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al., 2010). The aligned and analysed sequences of *F. gymnauchen* were deposited in GenBank; NCBI .

c. Phylogenetic analysis and evolutionary lineage: The search for species identification or comparison of sequences was done at the GenBank database using the BLASTn tool. The sequences were blasted and the percentage of identity with the sequences of the same species or species with close identity were compared. The genetic divergences, phylogenetic reconstruction and evolutionary lineage was done by MEGA 11 (Kumar et al. 2016). The bootstrapping of evolutionary trees was costructed as per Felsenstein (1985) and Zharkikh and Li (1995).

Results

The species identity of the Asian sand goby, *F. gymnauchen* was done by analysis of morphological characters and DNA barcoding of COI gene, and the distribution pattern collected from the published research papers were plotted in the map (Fig. 1)

1. Morphological features of *F. gymnauchen* (Bleeker, 1860).

a.Phenotypic characterization: Max length: 13.2 cm TL. Dorsal spines: 6 - 7; Anal spines: 1; Anal soft rays: 9, First dorsal fin rays: 4-5; Second dorsal fin rays: 9-11; Anal fin rays: 13; Pectoral fin rays: 16- 17; Pelvic fins (joined) total rays:12; caudal fin rays: 9+8= 17. General appearance is shown dorsal view in figure 2 and ventral view in figure 3.

b. **The morphometric measurements**

Morphometric characters of 5 specimens of *F. gymnauchen* collected from the Kavvayi backwater of Arabian Sea are given in Table 2. .

1. 22. Gene barcoding of COI gene and Phylogenetic analysis of *F. gymnauchen*

The DNA barcoding by partial sequencing of mitochondrial COI genome of *F. gymnauchen* yielded a 630 bp product on PCR amplification, and was provided with an accession number of MZ 505547.1, upon deposition in NCBI GenBank. The sequence shows 100 percentage identities with *F. gymnauchen*, deposited in Genbank with an accession numbers: MW498616.1 and MW498615.1 from Malaysia. GenBank deposition indicated the novel and first time records of *F. gymnauchen* in Arabian sea coast of India. The phylogenetic tree was constructed by Fast Minimum Evolution method(Fig.4) and also by neighbour joining method (Fig.5).

Discussion

Favonigobius gymnauchen is an euryhaline fish and has reported from Western Pacific water: Japan, Korean Peninsula, and China also in southern Taiwan, Hongkong, Australia, Palau Island and Micronesia (Masuda et al, 1984; Kuo and Shao, 1999; Donaldson and Myers, 2002; Froese and Pauly, 2022). This species is found over muddy or sandy bottoms, of the intertidal zone, in mangroves, estuaries, etc, seems to play an important role in the food web around tidal flats as a predator of the larva and juveniles of many fishery resources like crab, prawn and Japanese founder etc, (Imada and Namba 1981, Harada et al. 2015, Noichi et al. 1993) and as the prey of many commercial fishes (Miyahara et al. 1995, Yamamoto and Tominaga 2014, Kwak et al. 2005) . In Hiroshima Bay, Western Japan, it is the most dominant species in all habitats (estuary, seagrass bed, and sandy beaches). Recently Thuy et al.2021, found out its occurrences with temporal and spatial variations in larval and juvenile communities as dominant taxa at the Kalong estuary ecosystem of Vietnam.

NCBI Blast shows 100.00% identity with accession number MW 498616.1 and MW498615.1 deposited from Malaysia (Abidin et al. 2021), 99.84% identity (accession no. MK 902731.1) from East coast of India by Laskar et al. 2019 (Unpublished). The molecular analysis of sequence of COI gene confirms the species identity of *F. gymnauchen* in the Arabian coast of India. It is the first report from Kerala and Indian water.

Conclusion

The study of morphological characters and sequence analysis of COI gene confirms the species identity of *F. gymnauchen* in the Arabian coast of India.The present record is a new addition to the backwater fish species list from this region. The discovery of *F. gymnauchen* in Indian waters indicates that a suitable habitat for this species occurs along the Arabian Sea coast. Distributional, ecological and economic significances of Asian Sand gobies and other members in the subfamily Gobiinae and genus Favonigobius may be explored in the future through intensive research.

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Tables

Table 1. Distribution of different species of *Favonigobius*

Sl No	Species	Distribution	Authors
1	*F. aliciae* (Herre, 1936)	Southeast Asian waters of Malay and Indo-chinese peninsula; Indonesia, the Mekong delta Cambodia	Rainboth W.J., 1996.
2	*F. exquisitus* Whitley, 1950 (Exquisite sand-goby)	Subtropical Southwest Pacific: New South Wales, Australia	Kuiter 1993; Chargulaf 2011; Froese, Pauly 2022 .
3	*F. lateralis* (Macleay, 1881) (Southern Longfin goby or Spotted Goby)	Western Pacific coast: from the southern coast of the Australia (Victoria) to Western Australia (Tasmania); eastern Australia including Tasmania and New Zealand and from the northern Great Barrier Reef, Queensland south to the southern coast of New South Wales	Paulin, 1989; Kuiter, 1993.
4	*F. lentiginosus* (Richardson, 1844) (Eastern Long fin Goby or Sandy Brown Goby)	Western Central Pacific and Southwest Pacific, to the Australia and New Zealand coastal waters	Larson and Murdy, 2001; Francis et al. 2005.
5	*F. melanobranchus* (Fowler,1934) (Black throat goby)	Indo-West Pacific ocean: South Africa; Mediterranean Sea (the first report); Gulf of Thailand and Red sea; Western Australia , the Arabian Gulf to northern Australia, Papua New Guinea, and South China Sea .	Hoese 1986; Kovacic, Golani 2007; Bogorodosky 2011; Vilasri 2018; Gill 1992; Randall 1995; Randall, Lim 2000; Allen, Adrim 2003; Hoese, Larson 2006.
6	*F. opalescens* (Herre, 1936) (Soft bottom goby) The most abundant goby	Western Pacific: South China Sea, (an usual bay species) collected from NhaTrang and Van Phong bays (Vietnam).	Froese, Pauly (Eds) FAO 2022.
7	*F.punctatus* (Gill & Miller, 1990) (Yellow spotted sand goby)	Eastern Indian Ocean: known only from the Swan Estuary in Western Australia Reported from temperate, and restricted to shallow sand flats.	Neira, Miskiewicz, Trnski 1998; Hoese et al. 2006.
8	*F. reichei* (Bleeker 1854) (Indo-Pacific tropical sand goby)	Indian and Pacific Ocean: Eastern and western Pacific, Andaman and Nicobar Islands, East Africa to the Philippines , North to Japan , south to northern Australia. Western and eastern Indian Ocean and	Kottelat et al. 1993; Masuda et al. 1984; Fricke

		Indo-West Pacific from South Africa west to Ponape (Micronesia) and north to Japan; it has a wide distribution in the Red Sea, Indo-West Pacific, East Africa, South Africa, Seychelles and Mauritius (Mascarenes) east to Marshall Islands and New Guinea, north to southern Japan, and south to northern Australia and New Caledonia.	R., 1999; Patzner et al. 2012.
9	*F. gymnauchen* (Bleeker, 1860) (Sharp- nosed Asian goby)	Western Pacific water: Japan, Korean Peninsula, China, southern Taiwan, Australia, Palau Island, Micronesia, Hongkong and Philippines	Kimand, Nakaya 2013; Chae et al. 2019; Yoshida, Tomiyama 2021; Jin et al. 2021.

Table 2. Morphometric characters of 5 specimens of *F. gymnauchen* collected from the Kavvayi backwater of Arabian Sea.

Morphometric measurements	Min.	Max.	Mean
Total length (TL, cm)	11.3	13.2	12.5
Standard length (SL, cm)	8	9	8.5
Standard length (SL) /TL	0.65	.70	0.68
Head length (HL)	2	3	2.5
Head length (HL) /SL	0.28	0.31	0.29
Body depth at pelvic origin	2	3	2.5
Body depth at pelvic origin (BDP) /Sl	0.27	0.31	0.29
Head width (HW)	1.5	1.9	1.7
Head width (HW) /Sl	0.1	0.3	0.2
Pre first dorsal length (Pre-FDL)	2.4	2.8	2.6
Pre first dorsal length (Pre-FDL) /Sl	0.29	0.33	0.31
First dorsal fin base length (FDFL)	1.2	1.6	1.4
First dorsal fin base length (FDFL) /Sl	0.15	0.17	0.16
Second dorsal fin base length (SDFL)	1.0	1.5	1.2
Second dorsal fin base length (SDFL) /Sl	0.2	0.6	0.4
First dorsal fin length	2.3	2.5	2.4
First dorsal fin length /Sl	0.26	0.30	0.28
Second dorsal fin length	0.8	1.6	1.2
Second dorsal fin length /Sl	0.11	0.17	0.14
Pectoral fin length (PcFL)	2.5	2.9	2.7
Pectoral fin length (PcFL) /Sl	0.30	0.34	0.32
Pelvic fin base depth	0.7	1.1	0.9
Pelvic fin base depth /Sl	0.09	0.12	0.11
Pre pelvic length	1.2	1.6	1.4
Pre pelvic length /Sl	0.17	0.19	0.17
Anal fin base length (AFL) /Sl	3.1	3.7	3.4
Anal fin base length (AFL) /Sl	0.2	0.7	0.4
Anal fin length	1.2	1.4	1.3

Anal fin length /Sl	0.14	0.16	0.15
Pre anal fin length (PAL) /Sl	0.2	0.5	0.4
Pre anal fin length (PAL) /Sl	0.3	0.7	0.5
Caudal peduncle length (CPL)	0.7	1.1	0.9
Caudal peduncle length (CPL) /Sl	0.10	0.12	0.11

Figures

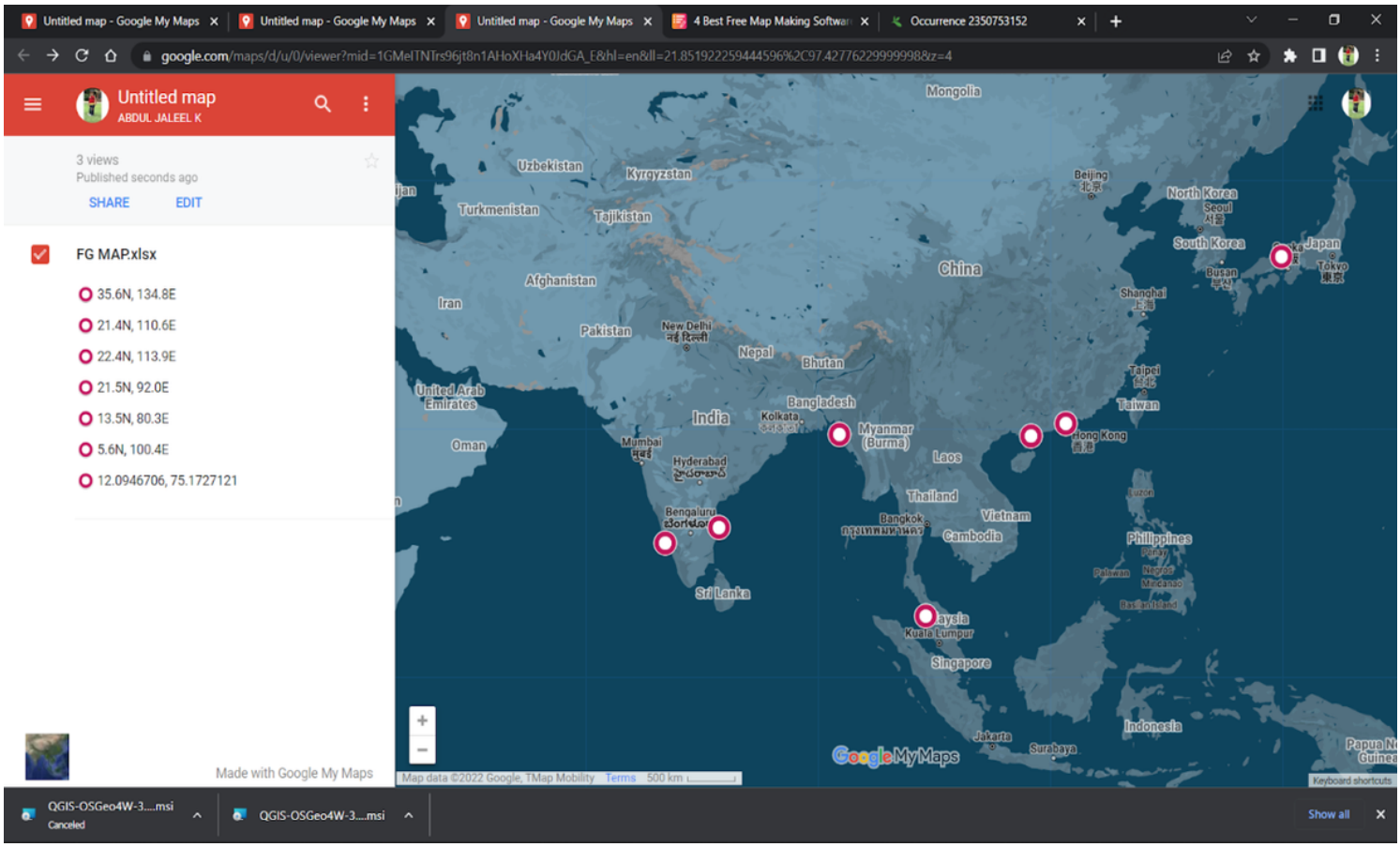

Figure 1

Distribution map of *F. gymnauchen*, including new record from Kavvayi back water of Kerala, India in the present study

Figure 2

F. gymnauchen dorsal view collected from Kavvayi back water of Kerala, India

Figure 3

F. gymnauchen lateral view from Kavvayi backwater of Kerala, India

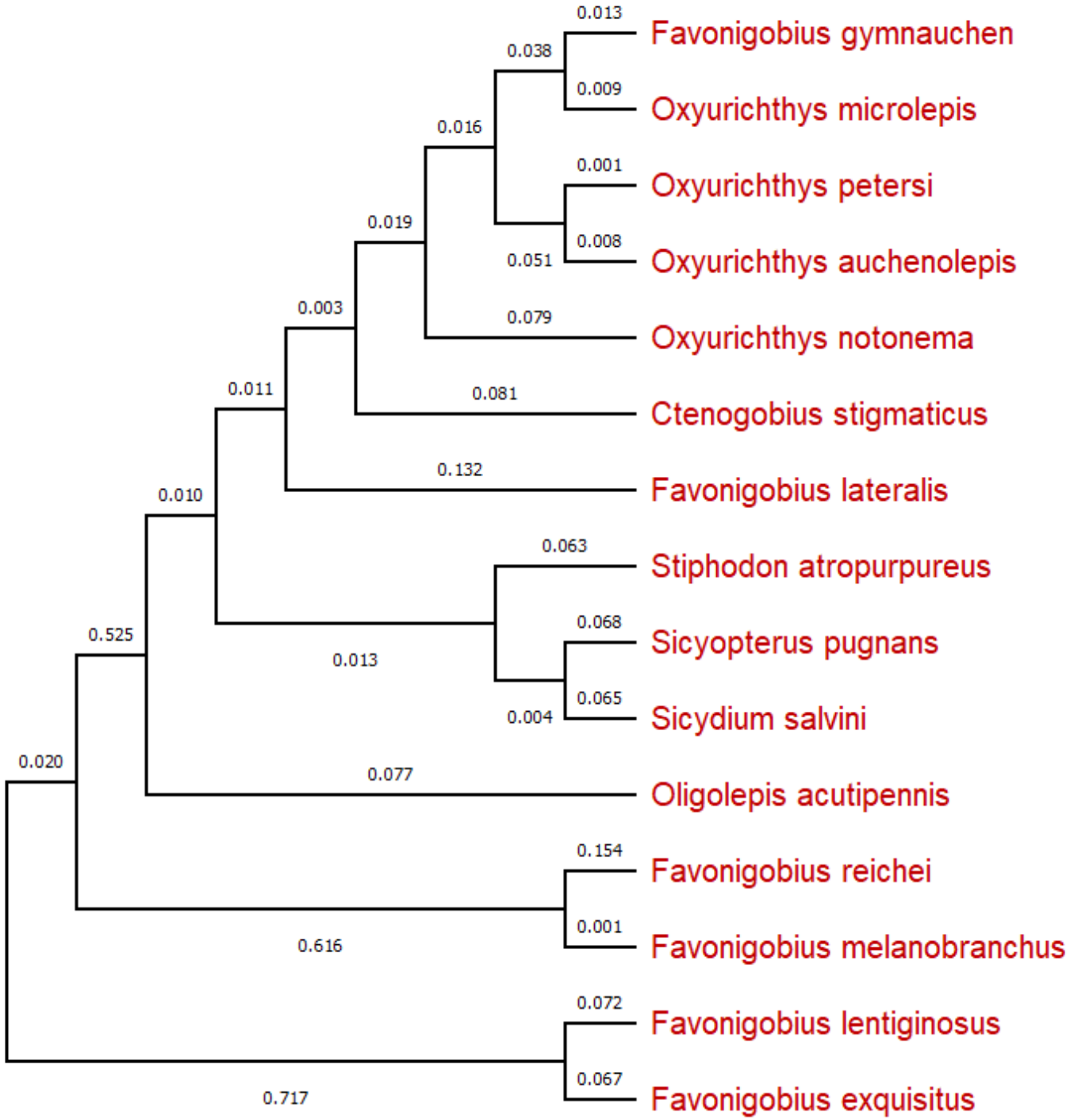

Figure 4

Phylogenetic tree of ***F. gymnauchen*** **-** Fast Minimum Evolution method

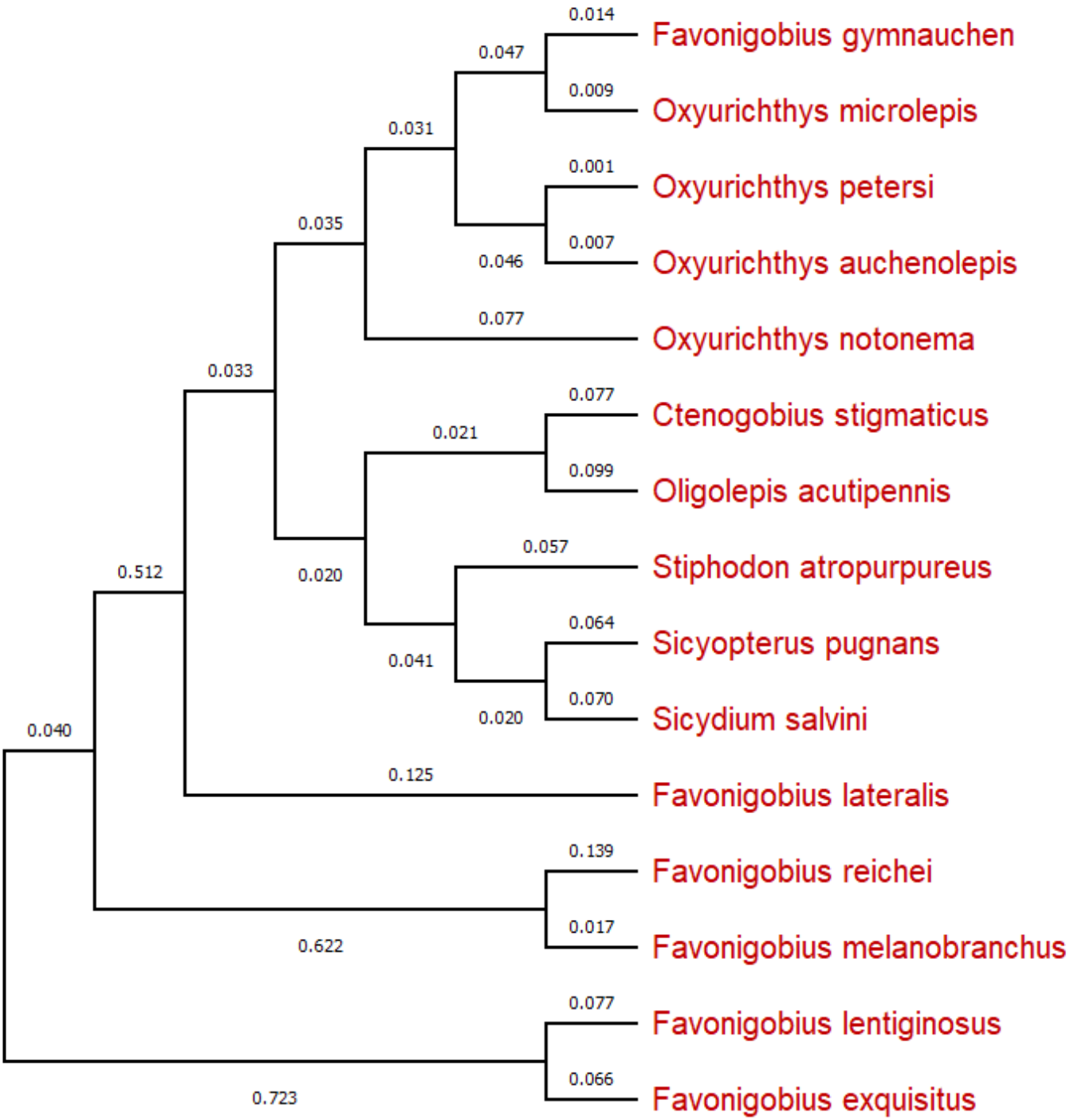

Figure 5

Phylogenetic tree of ***F. gymnauchen*** by neighbour joining method