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New distributional record of the deep pugnosepony fish, *SecutorIndicius* (Monkolprasit, 1973) (Perciformes: Leiognathidae) from Jakhau coast, Gujarat

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ABSTRACT

A new distributional record of a deep pugnosepony fish belonging to the family Leiognathidae, *Secutorindicius* has been caught for the first time from the Western coast of India at Jakhau landing centre, Kachchh district, Gujarat. The morphometric and meristic characters of the specimen were measured using standard available keys. Further, the DNA barcoding was carried out for the mitochondrial cytochrome oxidase subunit I (COI) gene from the tissues of the paratype specimen and phylogenetic studies using Neighbour joining tree construction to ascertain the relationship and similarity with the specimens of the same species barcoded in other places of South India and authentication of the specimen to *Secutorindicius.*

INTRODUCTION

The Leiognathidae family is also known as the family of ponyfishes**(Woodland *et al.*, 2001),** having a rounded or oblong body, are generally compressed moderately or marked laterally. This family belongs to the order Perciformesconsistingof 10 genera and 51 species. Commonly known as slipmouths, these fishes occupy the habitat of marine and brackish waters with sporadic ingress to freshwater as well **(Nelson, 2016)**.Concerning the Indian coastal waters to the taxonomy of the family Leiognathidae, **Day (1878)** described 14 species of the family. Furthermore, **Munro (1955)** described 12 species of ponyfishes from the neighboring country, Sri Lanka. **(Chakrabarty *et al.*, 2008)** redescribed the ponyfishes of Sri Lanka and resurrected them as Aurigequula**(Fowler, 1918)**. Later, **James (1969)**, **Rani Singh and Talwar (1978a, 1978b)**, **Jayabalan (1985)** and **James and Badrudeen (1990)** added seven more species to the known ponyfish family of India of which four were new to science and three were first reports from India.

The most comprehensive revision of the family Leiognathidae was reported from the Indian seas (**James, 1975)**.**Jayabalan and Ramamoorthi (1977)** gave a specialized key to the genera of Leiognathidae of Porto Novo, and **Talwar and Kacker (1984)** described 15 species of the family. In addition, **Fischer and Bianchi (1984)** described 17 species of ponyfishes from the Western Indian Ocean, including those in India coastal waters as well. The work on ponyfishes has been largely found limited to the Eastern and Southern coasts of India. Though the analysis of taxonomical characters is the simplest way to identify a species **(Nayman, 1965),** molecular studies like DNA barcoding has definitely proved to authenticate the taxonomical data more strongly.

The technique of DNA barcoding has been widely used for species identification as well as species discovery in various groups of organisms **(Hajibabaei *et al*., 2012)**. The effectiveness of DNA barcoding has now been approved for many groups of animals **(Waugh, 2007)**, both invertebrates and vertebrates **(Hajibabaei *et al*., 2006)**, with fishes being one of the most extensively studied groups among them **(Becker, *et al*., 2011**; **Ward, 2012)**. These studies have also generated a large scale of barcode data that are available in BOLD **(Ratnasingham & Hebert, 2007)** and NCBI **(Pruitt *et al*., 2005).**

The present study is focused on deriving the morphological and meristic characters of the specimencollected from the Western part of India. Moreover, it works on developing the genetic information through DNA barcoding, and confirming the molecular data with the database of the species available in NCBI and BOLD.

MATERIALS AND METHODS

A study was carried out along the Western coast of Gujarat between the Kachchh and Saurashtra peninsula. Jakhau (23°13'59.88"N 68°34'35.06"E) situated in Gulf of Kachchh, Gujarat was selected to study fish diversity. Collection of fish specimens from the study area was carried out from December 2013 to March 2015. A specimen of *Secutorindicius* (**Monkolprasit, 1973**) was caught off the coast of Jakhau, a fishing village in the Kachchh district of Gujarat by trawl fishing and was brought to the landing station.The collected specimen was photo-documented on-site, preserved in ice-box, transferred to the laboratory within 24 hours. For molecular analysis, the whole specimen was stored in 95% alcohol **(Sarma & Mankodi, 2017).**

The identification of the collected specimen was carried out till species taxon using standard taxonomic keys using the methods of **Day (1888), Talwar and Kacker (1984)** and **Jhingran (1991).**The accepted English name and scientific name were determined from research papers and Leibniz Institute of Marine Sciences (IFM-GEOMAR) in Kiel, Germany managed website www.fishbase.org, a global species database for fishes **(Froese & Pauly, 2016)**.

DNA Isolation and Sequencing

The genomic DNA was extracted from the stored muscle and gill tissue samples by the standard available QIAGEN DNeasy Blood & Tissue Kit. The COI gene, approximately 650bp length located in the mitochondrial genome, was amplified using two different sets of primers (Table 1).The processed sequencing plate was loaded on an automated 3500xL Genetic Analyzer using POP 7 for sequencing. The sequencing was done both in forward and reverse directions.

Table 1.Primers used for sequencing

FishF2_t1 (Forward)	TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCG GCAC	Ivanova*e t al*.,2007
FishR2_t1 (Reverse)	CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATC AGAA	Ivanova*e t al*.,2007

Sequence Analysis

Sequence analysis was done using sequencing analysis software version 5.4 (Applied Biosystems) and BioEdit, biological sequence alignment editor (Ibis Biosciences). Consensus sequences were generated after aligning gene sequences from forward and reverse primers.

RESULTS

A specimen of the deep pugnosepony fish *Secutorindicius* **(Monkolprasit, 1973)** was caught from the coast of Jakhau, Kachchh district, Gujarat (Figure 1). The genus *Secutor* can be differentiated from all other species in Leiognathidae family by having the mouth angled upwards, located at the level of the centre of the eye, the lower jaw anterior profile almost vertical when mouth closed, and a black line from the anteroventral margin of the orbit to the lower jaw articulation. *Secutorindicius* is distinguishable from its congeners by the following combination of characters: cheek without scales; pre-orbital spine single, not forked; 87-111 lateral line scales; and 14-20 dark vertical markings on the dorsolateral surface of the body **(Froese & Pauly, 2016)** (Table 2).

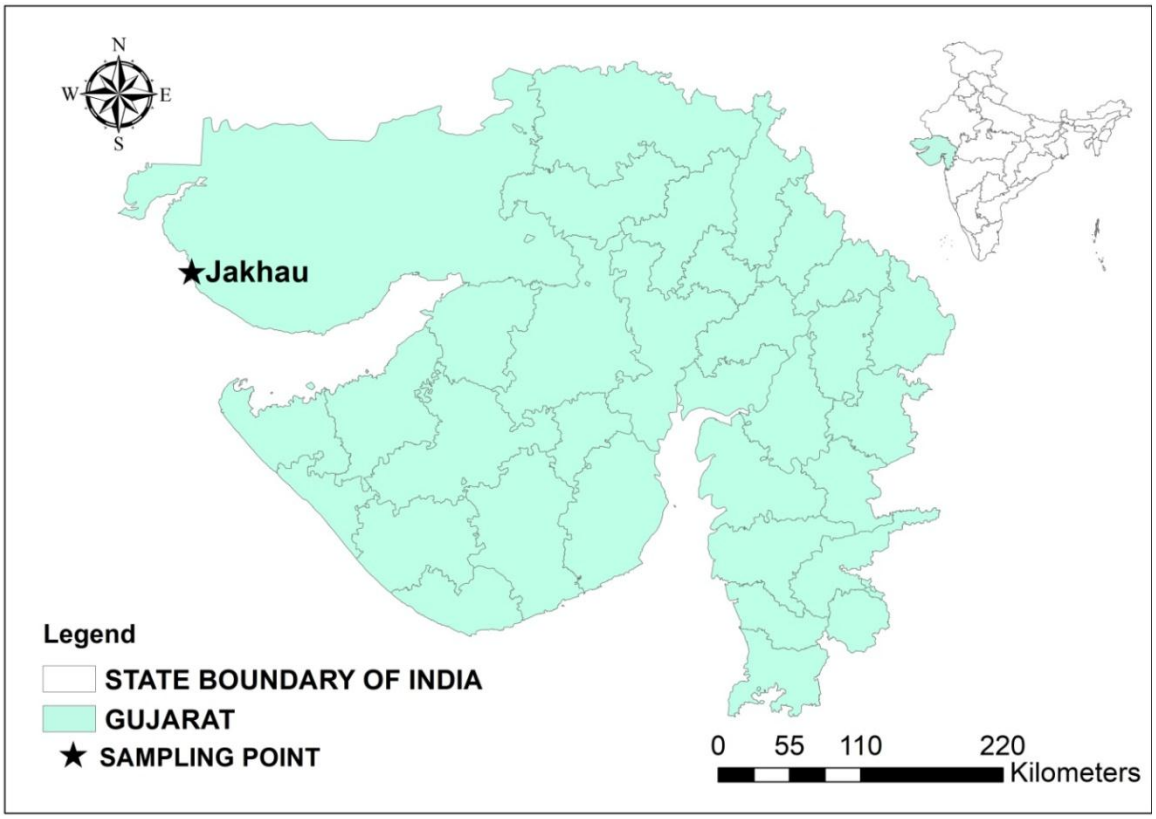

Figure1. A map of the study area showing collection site of *Secutorindicius* specimen.

Table 2. Morphometric and biometric characteristics of the *Secutorindicius* specimen.

Factors	Morphometric measurements
Total Length	93 mm
Standard Length	87 mm
Body Length	74 mm
Snout Length	18 mm
Head Length	7 mm
Head Width	16 mm
Eye Diameter	4 mm
Anal Fin Length	34 mm
Dorsal Fin Length	39 mm
Caudal Fin length	19 mm
Total weight	115 g

The body of Deep pugnoseponyfish, *SecutorIndicius*is is oval, deep and very compressed with a depth of 2 – 2.4 times its standard length. The head is strongly concave with the mouth pointing outwards. Small body scales are found throughout the silvery coloured body having 18 – 21 dark, vertical markings in the form of dashes or dots on the dorsal and ventral side (Figure 2).

Figure 2. A specimen of Deep pugnoseponyfish, *Secutorindicius*caught from Jakhau coast, Kachchh, Gujarat.

A narrow black line runs from the edge of orbit to chin with small black spots on the upper part of operculum **(Froese & Pauly, 2019)**. The occurrence of the Deep pugnoseponyfish, *Secutorindicius* in the western coast of Gujarat in the Arabian Sea has been attributed to its large eco-physiological plasticity. The reason for the new distribution of the species can be attributed to its adaption to the feeding regime of new environmental conditions, but also might have high competitive potential to the present indigenous species which should be evaluated.

The COI gene of the specimen Deep pugnoseponyfish, *Secutorindicius* in this study was successfully barcoded, and the barcode was submitted to Barcode of Life Data Systems (BOLD Systems V3) with the sample ID LQDWL-TIS-30-12-2013-015 with record details under LGEN077-14. The data for creating the neighbour joining tree using phylogeny.fr **(Dereeper *et al.*, 2008)** suggests the genetic difference between the specimens of *Secutorindicius* collected from various locations throughout the world. The base 0.006 suggests the nucleotides per sites in the alignment. The paratype specimen, found in this study, showed no genetic difference from the specimens caught and

molecularly examined from the Eastern and Western coast of India suggesting the same parent population. A similarity percentage of 99% was recorded between the samples collected and derived from Tamil Nadu, India and Indonesia. The samples found in Indonesia and Taiwan showed the lesser percentage similarity of 91%. The molecular data extracted and study truly confirms that the specimen found in this study is of *Secutorindicius*, belonging to the family of Leiognathidae (Figure 3).

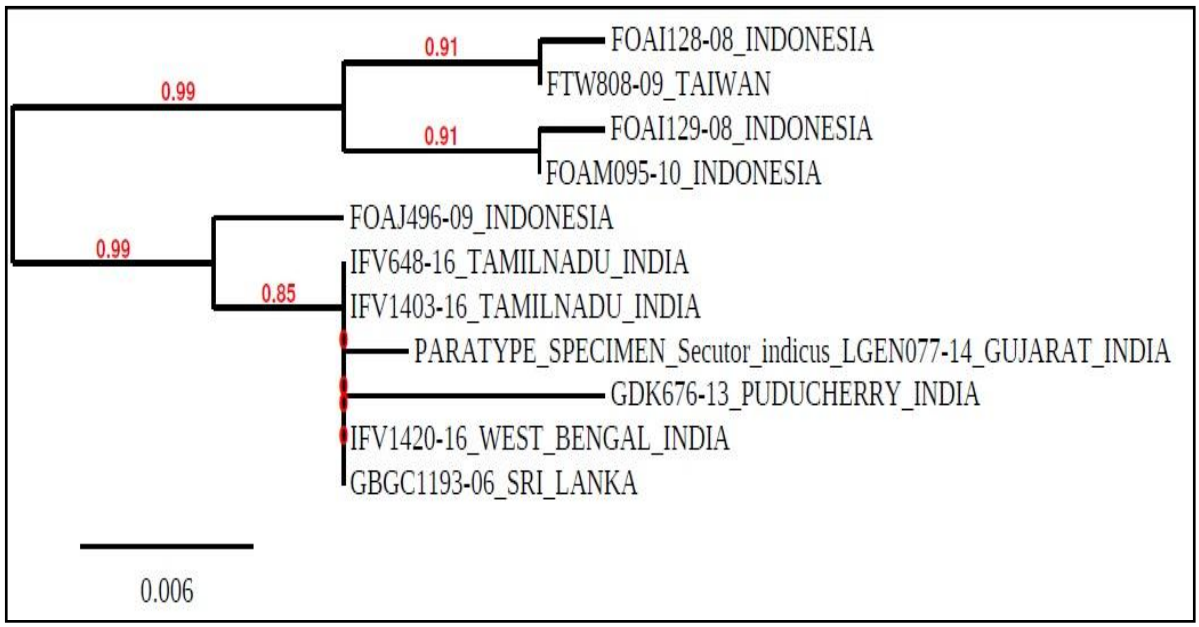

Figure 3. .Summary of neighbour joining tree of cytochrome oxidase I gene sequences derived from 10 samples of *Secutor indicius* found in various locations constructed using phylogeny.fr.

DISCUSSION

In this study, the specimen found was addressed for its morphometric and meristic characters matching them with the molecular data and phylogeny. Consequently, it is confirmed that the specimen is Deep pugnoseponyfish, *Secutor Indicius* **(Monkolprasit, 1973)**. The species was first misnamed as *Secutor insidiator* **(Froese & Pauly, 2016)** but later the *SecutorIndicius* **(Monkolprasit, 1973)** has been accepted as a different species with differences like irregular arranged rows of spots present in *S. Insidiator* **(Jayabalan, 1988),** while 14 – 20 had horizontally arranged row of spots in the dorsal side of *S. Indicius.*

The distribution revealed that *Secutor indicius* has been previously reported from the southern part of India, Sri Lanka, Taiwan, and even Japan **(Kimura *et al.*, 2008),** but for the first time it has been caught off the western part of India. This shows the presence of a complete population of these species in this specific area that lacks investigation due to less ichthyofauna survey and fishery expedition. Another species, *Secutor mazavasaoka*

(Baldwin & Sparks, 2011) has been reported from the Western Indian Ocean with striking morphometric differences with *Secutor indicius*. This new distribution can also be attributed to factors like food and feeding habits, and the changes in the demography of the species in this area as well.

Further work of studying and comparing the molecular data with the other related species of this genus is required to determine whether the species has its population in the region or has separated itself from another species, and hence, developed with new characteristics. Moreover, the species frequency of occurrence should be resolved to determine whether it has established a sustainable population in its new surroundings or not.

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