

Mistaken by dots: Revealing the misidentification of *Saurida lessepsianus* (Actinopterygii: Aulopiformes: Synodontidae) along the west coast of India (eastern Arabian Sea)

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Abstract

The presently reported study identified *Saurida lessepsianus* Russell, Golani et Tikochinski, 2015 from the west coast of India, which was earlier diagnosed as *Saurida undosquamis* (Richardson, 1848) due to their morphological similarities. *Saurida lessepsianus* is characterized by 48–51 lateral line scales, 44–47 vertebrae, 3–6 rows of teeth on tongue and anterior part of stomach black. The identity of this species was also confirmed by molecular examination (phylogenetic analysis of the COI sequences) and the study revealed a genetic divergence value of 0.08 between *S. lessepsianus* and *S. undosquamis*. Our study confirms the extended distribution of *S. lessepsianus* along the west coast of India (eastern Arabian Sea) from the Red Sea-Mediterranean Sea.

Keywords

DNA barcoding, Indian coast, Lessepsian lizardfish, Misidentification

Introduction

Species of the family Synodontidae, commonly known as Lizardfishes, are commercially important marine/estuarine demersal fishes. The family is represented by 83 valid species under 4 genera: namely *Harpadon* Lesueur, 1825; *Saurida* Valenciennes, 1850; *Synodus* Scopoli, 1777, and *Trachinocephalus* Gill, 1861. Species of genus *Saurida* are widely distributed in the Indo-West Pacific region and presently, 24 valid species are reported (Fricke et al. 2020). The taxonomy of this genus, however, is

confusing because of overlapping morphological characters and there has been a widespread misidentification of species in the published literature.

In India, nine species of *Saurida*, namely *Saurida gracilis* (Quoy et Gaimard, 1824); *Saurida isarankurairai* Shindo et Yamada, 1972; *Saurida longimanus* Norman, 1939; *Saurida micropectoralis* Shindo et Yamada, 1972; *Saurida nebulosa* Valenciennes, 1850; *Saurida pseudotumbil* Dutt et Sagar, 1981; *Saurida tumbil* (Bloch, 1795); *Saurida undosquamis* (Richardson, 1848), and *Saurida wanieso* Shindo et Yamada,

1972 have been reported (Chandra et al. 2020). The identification of *S. undosquamis*, however, is problematic. Inoue and Nakabo (2006) reported *S. undosquamis* to comprise a complex of four species which they called the “*S. undosquamis* group”, covering *Saurida umeyoshii* Inoue et Nakabo, 2006; *Saurida macrolepis* Tanaka, 1917; *S. undosquamis*, and *S. longimanus*, based on the following shared morphological characters: black dots on upper margin of caudal fin, pectoral fin extending beyond origin of pelvic fin, anterior rays of dorsal fin neither elongate nor filamentous, pre-dorsal length greater than distance between dorsal-fin and adipose-fin origins, larger scales on body and 46–55 pored lateral-line scales. Russell et al. (2015), however, showed that the species, previously reported as *S. undosquamis* from the Red Sea and the Mediterranean, and reidentified as *S. macrolepis* by Inoue and Nakabo (2006), in fact, was a new species, *Saurida lessepsianus* Russell, Golani et Tikochinski 2015, that was genetically distinct from specimens of *S. undosquamis* and *S. macrolepis* (see Tikochinski et al. 2016). Thus, the presently reported study was undertaken to validate *S. undosquamis* inhabiting the west coast of India, using morphological and molecular markers. The results showed that the species reported as *S. undosquamis* from the west coast of India (Raje et al. 2012, Chhandaprajnadarsini et al. 2018, 2019) is indeed *S. lessepsianus* and confirms its extended distribution in the western Indian Ocean from the Red Sea–Mediterranean Sea.

Material and methods

A total of 40 individuals of *S. lessepsianus* were collected from the Versova landing center (19°08'N, 72°48'E) and New Ferry Wharf fishing harbor (18°57'N, 72°51'E) of the Mumbai coast (north-western coast of India) and Neendakara fishing harbor (8°56'N, 76°32'E) of the Kerala coast (south-western coast of India) during November–December 2019 (Fig. 1). The specimens were caught by commercial bottom trawls, operating in the region at depths of 150–200 m. Morphometric characters were measured following Russell et al. (2015). Measurements were taken by digital Vernier caliper to the nearest 0.1 mm. The number of vertebrae was counted using X-ray radiographs. Morphometric traits were expressed in percentage of standard length (SL) and head length (HL).

For molecular work, total genomic DNA was extracted by the salting out method (Miller et al. 1988). Partial Mitochondrial COI regions were amplified using reported primers as described by Ward et al. (2005). The PCR was carried out in a 25 µL reaction with 10 × Taq buffer, 200 µM of each dNTPs, 1 unit of Taq-DNA polymerase (Promega, USA), 1 × Taq buffer containing 1.5 mM MgCl₂, 10 picomoles of each primer, and 100 ng of template DNA. The PCR products were purified and sequenced in both directions using primers. The thermal conditions for amplifying COI region were set as an initial denaturation of 5 min at 95°C, followed by 35 cycles of 30 s at 94°C for denaturation, 30 s at 54°C for annealing, 60 s at 72°C for extension, with a final extension at

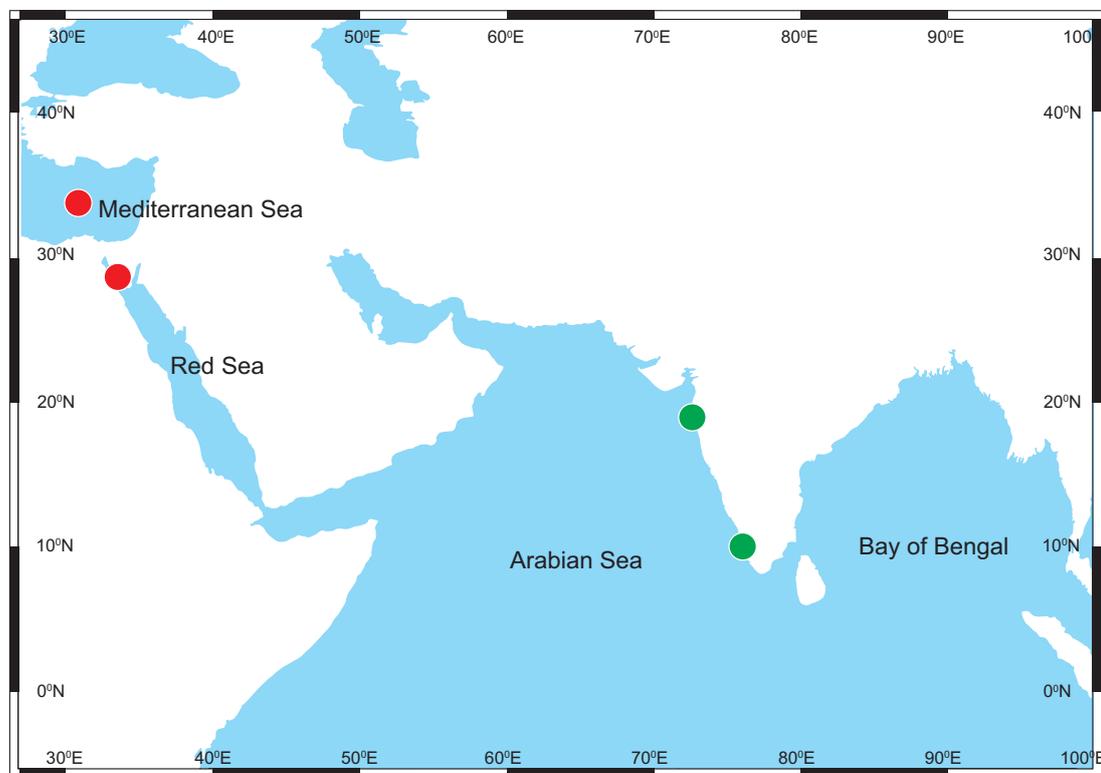


Figure 1. Distribution map of *Saurida lessepsianus*. The red circle indicates previous distribution and green circle indicates present distribution.

72°C for 10 min. The amplicons were purified and sequenced in both directions using PCR primers.

In addition, the reported COI sequences of *S. undosquamis* ($n = 17$) and *S. lessepsianus* ($n = 3$) and other related species were downloaded from the GenBank to estimate the genetic distance values. The sequences were aligned to their homologous position using the Clustal W program implemented in MEGA7 (Kumar et al. 2016). Nucleotide composition, intra-, and inter-specific genetic divergence values were calculated by Kimura two parameter (K2P) model. A neighbor-joining tree was constructed using K2P distances to represent divergence pattern between species (Saitou and Nei 1987). All the molecular analysis was carried out in Molecular Evolutionary Genetic Analyses (MEGA7) software (Kumar et al. 2016).

Results

Genus *Saurida* Valenciennes, 1850

Saurida lessepsianus Russell, Golani et Tikochinski, 2015

Figs 2–4; Table 1

Lessepsian lizardfish

Description of specimens from west coast of India.

Body elongated and slightly cylindrical; body depth 17.2% of SL; depth of caudal peduncle 6.5% of SL (Table 1); pectoral fins moderately long, reaching or just extending beyond base of pelvic fins; axillary scale long



Figure 2. *Saurida lessepsianus* collected from Arabian Sea, Mumbai coast, India (131.28 mm SL).



Figure 3. Stomach of *Saurida lessepsianus*.

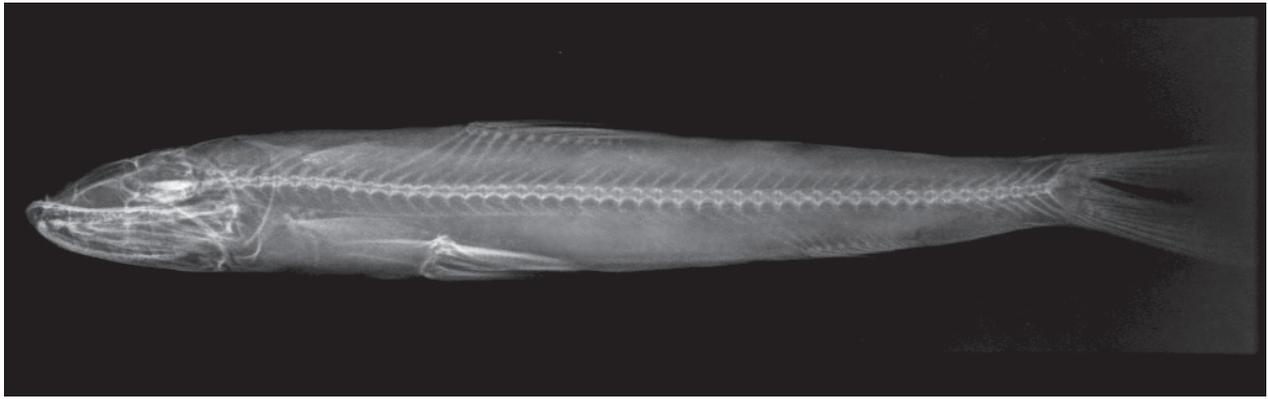


Figure 4. X-Ray radiograph of *Saurida lessepsianus*.

Table 1. Morphometric parameters of *Saurida lessepsianus* collected during the presently reported study compared with previous studies.

| Morphometric trait | This study (n = 40) | | <i>S. undosquamis</i> (n = 8) (fide Inoue and Nakabo 2006) | | <i>S. lessepsianus</i> (n = 37) (Russell et al. 2015) | |
|---------------------------------|---------------------|--------------|---|------|--|------------|
| | Range | Mean ± SD | Range | Mean | Range | Mean ± SD |
| Standard length [mm] | 112.07–236.58 | | 112.1–358.0 | | 108.0–282.2 | |
| Pre-dorsal length [%SL] | 42.61–46.61 | 44.22 ± 0.38 | 41.1–43.1 | 42.2 | 40.3–45.0 | 42.6 ± 1.2 |
| Pre-adipose length [%SL] | 53.50–84.69 | 79.50 ± 2.93 | 79.5–81.8 | 80.6 | 76.9–83.2 | 80.9 ± 1.4 |
| Pre-anal length [%SL] | 48.67–76.77 | 71.74 ± 2.60 | 70.5–73.4 | 71.8 | 67.2–77.1 | 71.9 ± 1.9 |
| Pre-anal fin length [%SL] | 75.11–79.45 | 77.22 ± 0.51 | 72.9–76.1 | 74.8 | 65.0–79.3 | 74.8 ± 2.5 |
| Pre pectoral length [%SL] | 25.65–27.98 | 26.68 ± 0.20 | 24.1–26.7 | 25.5 | 23.4–28.7 | 25.7 ± 1.2 |
| Pre pelvic length [%SL] | 36.05–41.71 | 39.49 ± 0.58 | 35.8–38.9 | 37.4 | 35.4–42.6 | 38.8 ± 1.6 |
| Head length [%SL] | 19.21–26.05 | 24.40 ± 0.61 | 23.2–25.5 | 24.8 | 22.5–26.5 | 24.4 ± 1.0 |
| Body depth [%SL] | 14.42–20.65 | 17.19 ± 0.53 | 11.7–13.5 | 12.5 | 10.4–17.2 | 13.3 ± 1.5 |
| Body width [%SL] | 11.67–15.05 | 13.72 ± 0.33 | 11.4–14.6 | 13.2 | 10.9–15.6 | 13.0 ± 1.3 |
| Inter-pelvic width [%SL] | 5.06–8.68 | 7.58 ± 0.34 | 7.6–8.6 | 8.2 | 7.6–9.5 | 8.4 ± 0.5 |
| Pectoral fin length [%SL] | 12.77–17.63 | 15.04 ± 0.43 | 13.4–14.5 | 13.9 | 11.5–17.0 | 14.2 ± 1.3 |
| Pelvic fin length [%SL] | 14.81–18.11 | 16.77 ± 0.30 | 16.7–20.0 | 18.1 | 14.2–18.6 | 16.6 ± 0.8 |
| Length of 2nd dorsal ray [%SL] | 15.91–23.17 | 20.14 ± 0.74 | 16.5–20.3 | 18.7 | 16.0–21.5 | 19.8 ± 1.1 |
| Length of last dorsal ray [%SL] | 4.50–6.35 | 5.40 ± 0.22 | 4.6–6.3 | 5.4 | 4.9–6.9 | 5.8 ± 0.5 |
| Length dorsal fin base [%SL] | 12.83–14.54 | 14.03 ± 0.17 | 11.7–13.2 | 12.6 | 12.5–16.6 | 14.4 ± 1.0 |
| Length of 2nd anal ray [%SL] | 7.10–10.75 | 9.64 ± 0.32 | 8.3–10.4 | 9.5 | 7.7–11.1 | 9.5 ± 0.9 |
| Length of last anal ray [%SL] | 4.05–6.96 | 5.18 ± 0.29 | 5.1–7.8 | 6.1 | 3.8–10.5 | 5.8 ± 1.1 |
| Length of anal-fin base [%SL] | 5.94–10.96 | 9.58 ± 0.43 | 8.6–10.3 | 9.6 | 5.5–12.4 | 10.0 ± 1.2 |
| Depth of caudal peduncle [%SL] | 5.57–7.45 | 6.51 ± 0.20 | 5.9–7.2 | 6.4 | 5.7–7.1 | 6.4 ± 0.3 |
| Eye diameter [%HL] | 14.21–25.34 | 18.58 ± 0.92 | 15.3–22.9 | 19.6 | 18.4–25.6 | 21.4 ± 1.9 |
| Pre-orbital length [%HL] | 16.22–29.93 | 19.54 ± 1.28 | — | — | — | — |
| Inter-orbital width [%HL] | 15.38–25.79 | 20.12 ± 0.96 | 16.3–24.8 | 20.6 | 14.5–21.3 | 18.2 ± 1.5 |
| Post orbital length [%HL] | 57.92–82.96 | 62.49 ± 2.31 | 54.5–63.2 | 58.2 | 54.7–63.5 | 59.4 ± 1.7 |
| Upper jaw length [%HL] | 59.58–89.06 | 67.67 ± 2.51 | 66.6–72.0 | 70.0 | 65.3–73.4 | 69.1 ± 1.8 |

and pointed; dorsal-fin base length comparatively longer than anal-fin base.

In fresh condition, body dark dorsally and light colored below the lateral line with a series of 9 small blotches along the lateral line; 6–10 indistinct black spots on the first two dorsal rays; 6–8 distinct black spots on the dorsal margin of the caudal fin; adipose fin whitish with black blotches anterodorsally; pelvic and anal fin whitish; lower lobe of the caudal fin and upper part of the pectoral fin blackish (Fig. 2); stomach black anteriorly and white posteriorly, with white intestine (Fig. 3).

Counts and proportional measurements of the specimens are provided in Table 1. The mode and range of meristic traits of *S. lessepsianus*: Dorsal-fin rays 11 (11–12);

pelvic-fin rays 9; pectoral-fin rays 14 (13–15); anal-fin rays 11 (11–12); lateral-line scales 49 (48–51); transverse scales above and below lateral line 4½ and 5½, respectively; pre-dorsal scales 15 (15–19); pre-adipose scales 14 (14–16); vertebrae 47 (44–47) (Fig. 4); two rows of palatine teeth; no teeth on vomer; 4–6 tooth rows on tongue.

Around 650 bp of COI gene were amplified and sequenced using primers. The sequence quality was verified by observing the Phred score of each nucleotide using Finchtv software. The sequences were submitted to the GenBank with accession numbers of MN853856 and MT139594–MT139596 (COI). The ends of the COI sequences were trimmed to include all reported sequences which resulted in a sequence length of 544 bp. The genet-

ic distance values found in the presently reported study and reported sequences from India were 0; accordingly, sequences were clustered into a single clade. The mean genetic distance values between *S. lessepsianus* of the Mediterranean/Red Sea and the Arabian Sea was 0.008 ± 0.002 . These sequences formed separate branches of the clade. The mean genetic divergence value between *S. lessepsianus* and *S. undosquamis* of Australian waters was 0.079 ± 0.012 (Table 2). The sequences of *S. lessepsianus* were clustered distinctly from *S. undosquamis* reported from Australia (Fig. 5).

Discussion

Saurida lessepsianus is a recently described species of the genus *Saurida*, which was misidentified as *S. undosquamis* in the Red Sea and the Mediterranean Sea. Russell et al. (2015) described the species with the following combination of characters: elongated, cylindrical body with a depressed head; caudal peduncle slightly compressed; mouth very large with 2 rows of teeth on outer palatines; 0–2 rows of teeth on vomer; pectoral fin long, reaching line between origin of pelvic fins and dorsal fin; vertebrae

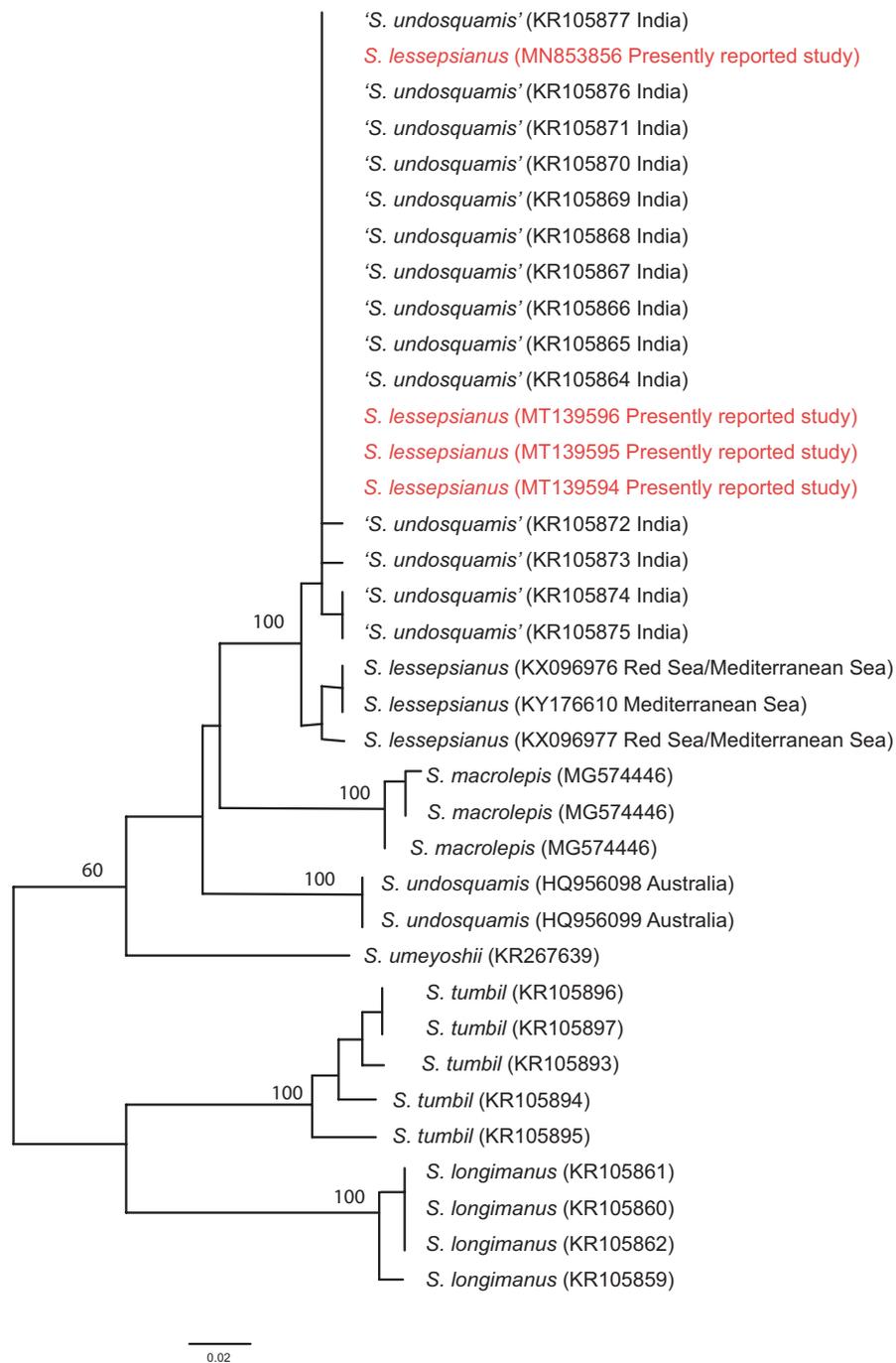


Figure 5. Neighbour-joining tree of the genus *Saurida* constructed using COI gene.

Table 2. Genetic distance values of *Saurida* species.

| Species | PS | Su1 | SLRS | SUAU | St | Sm | Sl | Su |
|---|--------------|--------------|--------------|--------------|--------------|--------------|--------------|----|
| <i>S. lessepsianus</i> (PS) | 0.000 | | | | | | | |
| <i>S. 'undosquamis'</i> (India: Su1) | 0.000 | 0.000 | | | | | | |
| <i>S. lessepsianus</i> (Red Sea: SLRS) | 0.008 | 0.008 | 0.004 | | | | | |
| <i>S. undosquamis</i> (Australia: SUAU) | 0.081 | 0.081 | 0.075 | 0.000 | | | | |
| <i>S. tumbil</i> (St) | 0.206 | 0.206 | 0.205 | 0.199 | 0.003 | | | |
| <i>S. macrolepis</i> (Sm) | 0.088 | 0.091 | 0.098 | 0.093 | 0.215 | 0.002 | | |
| <i>S. longimanus</i> (Sl) | 0.216 | 0.216 | 0.213 | 0.205 | 0.167 | 0.206 | 0.001 | |
| <i>S. umeyoshii</i> (Su)* | 0.119 | 0.119 | 0.119 | 0.107 | 0.188 | 0.124 | 0.193 | |

(Below the diagonal (values in bold): genetic distance values; PS: presently reported study samples; Su1: Reported sequences of *Saurida 'undosquamis'* from India; SLRS: Reported *S. lessepsianus* from Red Sea / Mediterranean Sea; SUAU: Reported *S. undosquamis* from Australia).

*Only one sequence used for analysis.

47–51; pored lateral-line scales 44–47; stomach grey to black anteriorly and white posteriorly.

During the presently reported study, the specimens of *Saurida* species examined from the west coast of India, differed from *S. undosquamis* (Table 3) by having 47–51 pored lateral-line scales (vs. 54–58), 44–47 vertebrae (vs. 52), 3–6 rows of teeth across the tongue (vs. 7–12), and stomach greyish or black anteriorly with intestine pale whitish (vs. stomach and intestine both pale whitish). The recorded morphological characteristics of the specimens collected along the west coast of India match with that of *S. lessepsianus* described from the Red Sea and Mediterranean Sea by Russell et al. (2015) (Table 3). Some morphological characters like dots on the dorsal fin and caudal fin were found to overlap between the *S. undosquamis* and *S. lessepsianus*. These characters are similar and are a major cause of misidentification.

DNA barcoding has been successful in resolving taxonomic ambiguity and validating many fish species (Pavan-Kumar et al. 2018, Mary et al. 2019). This method delimits the species based on the degree of COI sequence similarity or divergence value among individuals (Hebert et al. 2003). Accordingly, conspecific individuals show lower divergence values than congeneric

Table 3. Comparison between two species *Saurida lessepsianus* and *Saurida undosquamis*.

| Characters | <i>Saurida lessepsianus</i> (This study) | <i>Saurida undosquamis</i> (Russell et al. 2015) |
|---|---|---|
| Alimentary tract | Stomach greyish or black anteriorly; intestine pale whitish | Stomach and intestine pale whitish |
| Number of spots on upper margin of caudal fin | 6–8 | 5–12 |
| Pored lateral-line scales | 47–51 | 54–58 |
| Vertebra | 44–47 | 52 |
| Rows of teeth on tongue | 3–6 | 7–12 |

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individuals, irrespective of their geographic locations. In the presently reported study, COI sequences showed low divergence values with reported sequences of *S. lessepsianus* from the Red Sea/Mediterranean Sea and the Arabian Sea. Thus, the specimens from India, previously reported in the literature and in public sequence databases as *S. undosquamis*, are misidentifications of *S. lessepsianus*. Furthermore, the growing problem of incorrect species identification in public sequence databases such as GenBank, highlight the necessity for taxonomic verification of published sequence data by referencing voucher specimens.

The presently reported study reports the extension of the known distribution range of *S. lessepsianus* from the Mediterranean/Red Sea region to the west coast of India, eastern Arabian Sea. Most likely, *S. lessepsianus* is widespread in the western Indian Ocean and extended into the Red Sea, subsequently disconnected from its original population and then further migrated into the Mediterranean Sea through the Suez Canal. The connection between the Red Sea and the Indian Ocean is a shallow strait (Bab-el-Mandab) and the turbid waters of the southern Red Sea, combined with the cold nutrient-rich waters of the Gulf of Aden, act as a potential barrier for gene flow (Roberts et al. 1992, DiBattista et al. 2013), which may explain the intraspecific divergence in the populations of *S. lessepsianus* between these regions.

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