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ORIGINAL ARTICLE

Molecular analysis of the recently described lizardfish *Saurida lessepsianus* (Synodontidae) from the Red Sea and the Mediterranean, with remarks on its phylogeny and genetic bottleneck effect

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ABSTRACT
The opening of the Suez Canal in 1869 led to a massive influx of Red Sea species that invaded the Mediterranean; this was termed 'Lessepsian migration'. Among these species was a species of lizardfish, identified by some authors as *Saurida undosquamis* and by others as *S. macrolepis*. Recently, the Red Sea and the Mediterranean populations were described according to external characteristics as a unique taxon, *Saurida lessepsianus*. Our molecular study confirms this finding and determines that all previous records of *S. undosquamis* and *S. macrolepis* in the Red Sea and the Mediterranean are misidentifications of *S. lessepsianus*. The Mediterranean population of *S. lessepsianus* exhibits a lower genetic variability than that of the Red Sea population, suggesting a bottleneck effect.

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Bottleneck effect; Lessepsian migration; mitochondrial DNA D-loop; *Saurida lessepsianus*

Introduction

Recent developments in genetic analysis have provided a powerful tool for increasing the resolution of fish taxonomy by comparing DNA sequences. In numerous cases, the genetic analysis of different populations of certain species, previously considered as having a wide geographic distribution, were revealed to be of sufficient genetic distance as to be considered different taxa (Tikochinski et al. 2014). These so-called 'cryptic species' were occasionally even elevated later to the specific level.

Genetic techniques also contributed to a better understanding of bioinvasion, by revealing the source of the invading population and indicating whether a reduction of genetic variability of the invading population ('founder effect' or 'bottleneck effect') occurred following the colonization event (Golani et al. 2007; Bernardi et al. 2010).

The genus *Saurida* Valenciennes, 1850 is one of the three genera in the lizardfish family Synodontidae. It consists of *c.* 22 species (Russell 1999; Nelson 2006; Eschmeyer 2015), which are characterized by a cylindrical elongated body and a large mouth equipped with two bands of palatine, vomer and tongue teeth. They have an adipose dorsal fin and nine pelvic fin rays, all of them similar in length.

The taxonomy of the genus was studied by several authors (Matsubara 1955; Tomiyama & Abe 1958; Shindo & Yamada 1972; Machida 1984). In some of these studies, all those species with dotted first dorsal rays and upper caudal rays were considered to be *Saurida undosquamis* (Richardson, 1848). Yamada & Ikemoto (1979) determined morphologically that there are at least two taxa in the western Pacific, in what was previously considered to be *S. undosquamis*, which was later confirmed by genetic analysis (Yamaoka et al. 1989).

Determined to solve the *S. undosquamis* complex, Inoue & Nakabo (2006) conducted a morphological study and concluded that the only species of this complex occurring in the western Indian Ocean and the Red Sea is *Saurida macrolepis* Tanaka, 1917. Although they did not examine specimens from the Mediterranean and since the Red Sea is the likely source of the Mediterranean population, numerous authors have followed Inoue & Nakabo (2006) in calling the Mediterranean population *S. macrolepis* (e.g. Fishelson et al. 2010, 2011; Stern 2010).

The first record of the genus *Saurida* from the Red Sea was made by Norman (1939) and later by Bayoumi (1972) who reported it from the Gulf of Suez as *Saurida undosquamis*. This species invaded the Mediterranean via the Suez Canal; the first specimens were collected in December 1952 and reported by Ben-Tuvia (1953) as *Saurida grandisquamis* Günther, 1864. Several

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years later, the species experienced a population explosion in the Mediterranean and consequently became an important element in the Levantine trawler fishery (Ben-Yami & Glaser 1974; Gücü & Bingal 1994; Golani & Ben-Tuvia 1995).

Yağhoğlu & Turan (2012) conducted a genetic study of *S. undosquamis* (= *S. lessepsianus*) from the Red Sea and the Mediterranean using PCR-RFLP of the mitochondrial 16S gene. Due to the different methods used in their study and our study the results of the two studies cannot be evaluated in the same manner.

Recently, Russell et al. (2015) described the Red Sea and the Mediterranean population as a distinct species, *Saurida lessepsianus* Russell, Golani & Tikochinski, 2015, and claimed that neither *S. undosquamis* nor *S. macrolepis* occur in this region.

The objectives of this study were to genetically barcode the Red Sea and the Mediterranean *S. lessepsianus* and compare them to specimens of *S. undosquamis* and *S. macrolepis* from Japan. We also wanted to assess whether the Mediterranean population has experienced a reduction in genetic variability as a result of a bottleneck effect.

Materials and methods

Sample collection and DNA extraction

Fifty-eight specimens of *Saurida* spp. were collected from different locations. Eighteen specimens of *S. lessepsianus* came from the southern Red Sea (Eritrea) and 40 from the Mediterranean Sea: 10 from Turkey (Antalya) and 30 from Israel (Jaffa and Haifa). One specimen of *S. golanii* Russell, 2011 was collected in Eilat, the Red Sea; two specimens of *S. macrolepis* were collected in Japan; three specimens of *S. undosquamis* were collected in Australia; two specimens of *Saurida* sp. were collected in Taipei, Taiwan. Specimens were deposited in the Fish Collection of the Hebrew University of Jerusalem, Jerusalem (HUJ) and the Museum and Art Gallery of the Northern Territory, Darwin (NTM). Adult fish muscles (about 50 mg) were used for DNA sample preparation using the AccuPrep® genomic DNA extraction kit (Bioneer, Daejeon, Korea).

PCR and sequencing

Approximately 650 bp were amplified from the 5′ region of the mitochondrial cytochrome *c* oxidase subunit I gene (COI) using the following primers (Ward et al. 2005):

FishF1: 5′ TCAACCAACCACAAAGACATTGGCAC 3′

FishR1: 5′ TAGACTTCTGGGTGGCCAAAGAATCA 3′

PCR reactions were carried out in 25 µl reaction volumes containing 1× PCR buffer (including 1.5 mM $MgCl_2$), 0.2 mM of each dNTP, 1 µM of each primer, 1 unit of Super-Term Taq polymerase (Hoffmann-La Roche), and about 100 ng of template DNA. PCR reactions were processed in an MJ Research thermal cycler with the following thermal regime: an initial step of 2 min at 95°C followed by 35 cycles of 0.5 min at 94°C, 0.5 min at 57°C and 1 min at 72°C, followed by 3 min at 72°C and then held at 15°C. PCR products were visualized on 1.5% agarose gels and sequenced bidirectionally using the PCR primers on an ABI 377 DNA Sequencer (Applied Biosystems, Foster City, CA) following the manufacturer's instructions.

Approximately 709 bp were amplified from the mtDNA D-loop sequences of *Saurida* samples using newly designed primers from the flanking cytochrome *b* and 12S genes:

SA-D-15581 F: 5′ CTCTACCACTGACTCCCAAAGC 3′
SA-D-31 R: 5′ CAGTGTTATGCTTTGTTTAAGCTACGC 3′

PCR reactions were carried out as described above with the following thermal regime: an initial step of 2 min at 95°C followed by 32 cycles of 45 s at 94°C, 45 s at 55°C and 45 s at 72°C, followed by 3 min at 72°C and then held at 15°C. PCR products were visualized and sequenced as described above.

Data analysis

BioEdit Sequence Alignment Editor ver. 7.0.9.0 (Hall 1999) was used to align the different haplotypes. Neighbour-joining analysis was carried out using PHYLIP version 3.69 (Felsenstein 2009). Trees were constructed using the neighbour-joining approach. Bootstrap values were obtained using MEGA6 software (Tamura et al. 2013). Principal coordinates analysis was carried out using the Multi-Variate Statistical Package (version 3.22) by Kovach Computing Services. All sequences were sent to GenBank (accession numbers KX096944–KX096980).

Results

COI sequences of the 58 *Saurida* spp. specimens, 624 bp long, were successfully obtained, comprising altogether four different haplotypes. *Saurida lessepsianus* specimens are located on a distinct branch of the neighbour-joining tree that was constructed by the COI sequences (Figure 1), and form a distinct cluster in a principal coordinates graph (Figure 2).

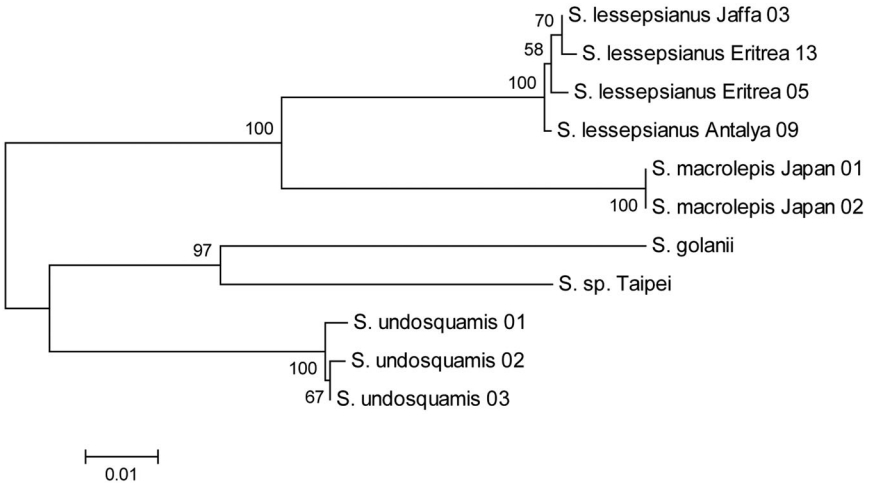

Figure 1. Neighbour-joining tree of *Saurida* species, based on COI differences. Node values represent bootstrap results (1000 iterations).

The average distances between *S. lessepsianus* and its close neighbours, *S. macrolepis* and *S. undosquamis*, are 8.7% and 15%, respectively – well above the 3% threshold widely accepted for specific level distinction (Hebert et al. 2003; Ward et al. 2009). When all the Red Sea and the Mediterranean specimens of *S. lessepsianus* are assembled on a tree (Figure 3) they have a maximum difference of 0.48%, clearly falling under the same genetic species definition.

A neighbour-joining tree based on the mtDNA D-loop (control region) 709 bp sequences (Figure 4) supports our findings from the previous trees – *S. lessepsianus* specimens are all clustered and differ from other *Saurida* species. The minor differences between *S. lessepsianus* specimens divide them into three clades: one which consists only of Mediterranean specimens (from Israel and Turkey) (Clade I); another which consists only of Eritrean specimens (Clade II); and a third branch which consists of both Mediterranean and Eritrean specimens (Clade III). The distances between the clades are given in Table I. The mean distance within clades is 2.851×10^{-3} ($n = 1322$), and not surprisingly, is significantly smaller ($P < 0.0001$) than the mean distance between clades, which is 23.821×10^{-3} ($n = 992$). Since distances between pairs are not independent, the significance level was estimated using computer simulations (10,000 random permutations).

Concentrating only on the Mediterranean population, and comparing the Israeli (Jaffa or Haifa) and the Turkish (Antalya) samples, we found no significant difference ($P = 0.3743$) in their genetic heterogeneity

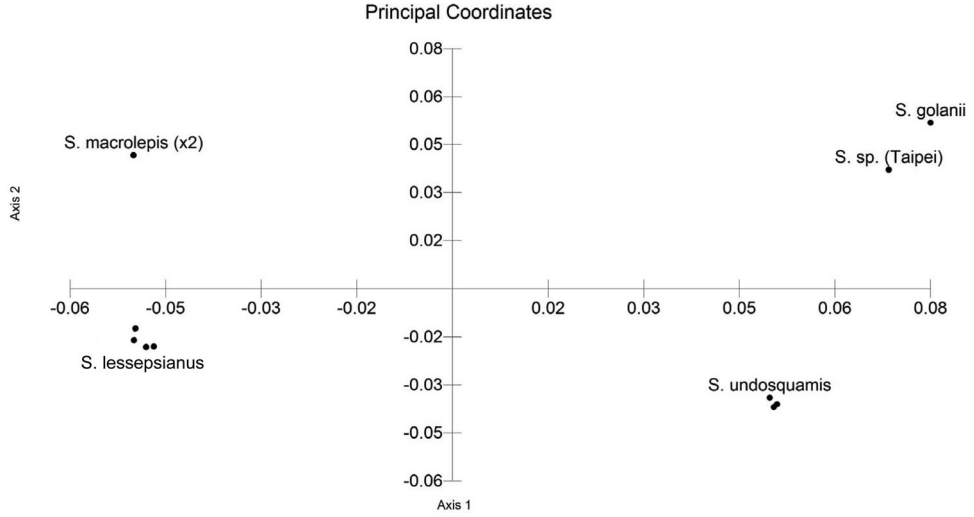

Figure 2. Principal coordinates analysis of *Saurida* species, based on COI differences. The first two axes are drawn, which together explain 80.6% of the variance.

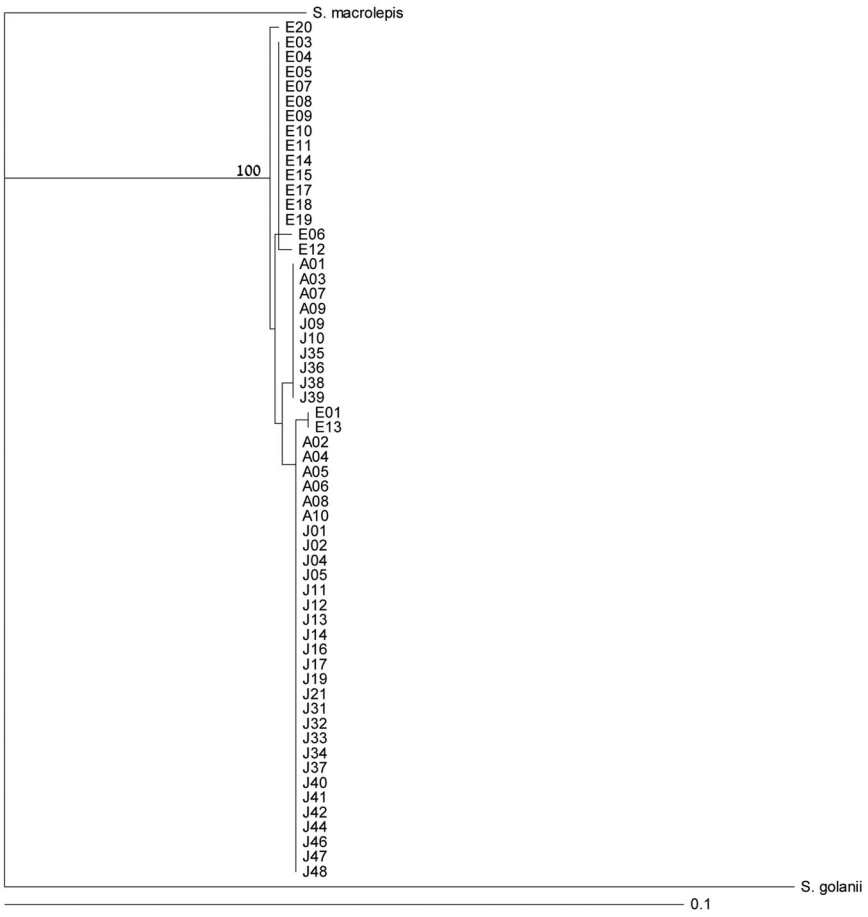

Figure 3. Neighbour-joining tree of *Saurida lessepsianus* from the Mediterranean (A = Antalya, Turkey; J = Jaffa or Haifa, Israel) and the Red Sea (E = Eritrea), based on COI differences (*S. macrolepis* and *S. golanii* are given for comparison). Node values represent bootstrap results (1000 iterations).

(as measured by the mean pairwise distance within each sample). Again, the significance level was estimated using computer simulations (10,000 random permutations).

The Mediterranean population of *S. lessepsianus* exhibits a more limited genetic heterogeneity than the original Red Sea population. This is demonstrated by the distribution of different mtDNA D-loop haplotypes: 10 different haplotypes in a sample of 40 specimens from the Mediterranean, compared with 17 different haplotypes in a sample of 18 specimens from the Red Sea. Simpson's index of diversity is 0.6333 ± 0.0845 and 0.9935 ± 0.0082, respectively (estimate ± SE).

Discussion

The most important finding of this study, as shown in the phylogenetic tree constructed by the sequence differences of the COI (Figures 1a and 1b), is the clear demonstration that *Saurida lessepsianus* from both the Red Sea and the Mediterranean constitutes a single taxon that is neither *S. undosquamis* nor *S. macrolepis*.

The formation of a distinct species in this area could be the result of the geological history of the Red Sea (DiBattista et al. 2013; Fernandez-Silva et al. 2015; Jackson et al. 2015). The Red Sea was formed by the divergence of the Arabian Plate from the African Plate during the Oligocene Era *c*. 20–30 Mya. The present connection with the Indian Ocean, which is the source of Red Sea fauna and flora, occurred much later, during the Pliocene Era *c*. 5 Mya. Since this connection, via the shallow straits of Bab-el-Mandab, the Red Sea has undergone various periods of separation and alternating isolation from the West Indian Ocean that continued even after the last glacial maximum of the Pleistocene Era some 20,000 years ago. Limited gene flow was found between Red Sea and Indian Ocean populations since this last major glacial period (DiBattista et al. 2013). Present-day barriers to gene dispersal between the Red Sea and the Indian Ocean still exist, resulting from upwelling along the Horn of Africa

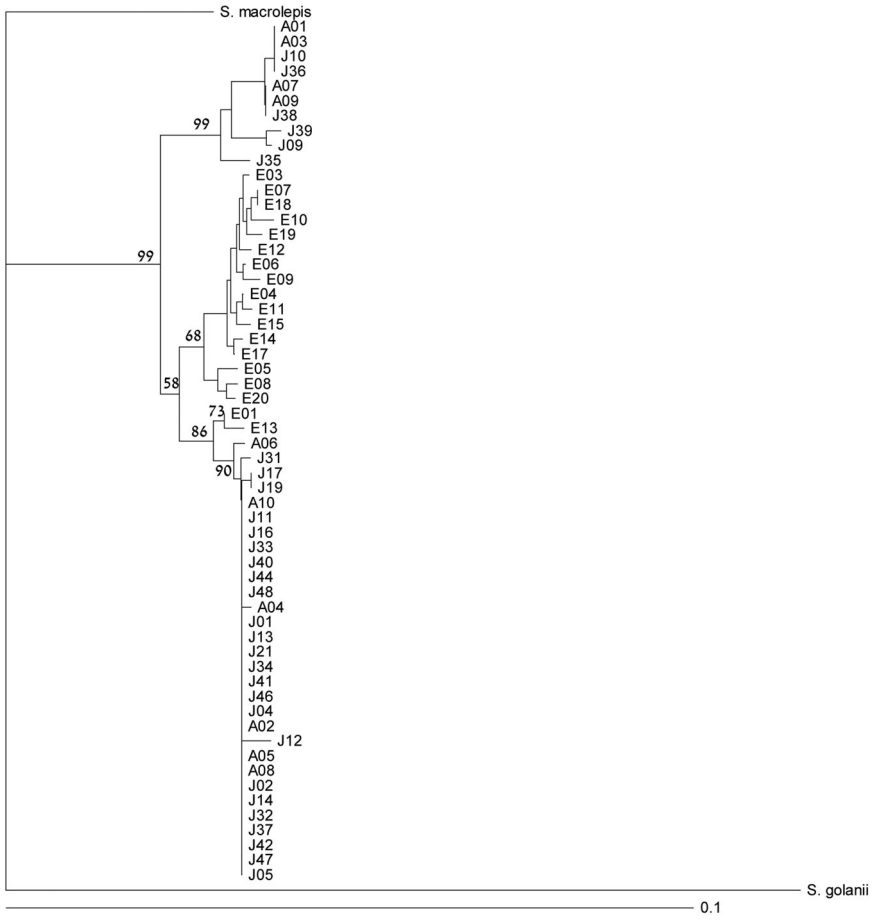

Figure 4. Neighbour-joining tree of *Saurida lessepsianus* from the Mediterranean (A = Antalya, Turkey; J = Jaffa or Haifa, Israel) and the Red Sea (E = Eritrea), based on mtDNA D-loop differences (*S. macrolepis* and *S. golanii* are given for comparison). Node values represent bootstrap results (1000 iterations).

at Somalia and the turbid waters of the coast of Pakistan and western Indian coast (Roberts et al. 1992). This long period of separation with no gene flow between the original population of the Indian Ocean and those of the Red Sea could cause allopatric speciation (Ayala 1999; Helfman et al. 2009). Similar cases were revealed regarding *Atherinomorus forskali* (Rüppell, 1838) (Bucciarelli et al. 2002) and *Sillago suezensis* Golani, Fricke & Tikochinski, 2014 (Tikochinski et al. 2013; Golani et al. 2014).

Table I. Mean mtDNA D-loop pairwise distances within and between the three Mediterranean clades of *Saurida lessepsianus* (number of pairs in parentheses).

	Clade I	Clade II	Clade III
Clade I	6.204×10^{-3} ($n = 90$)	29.129×10^{-3} ($n = 160$)	27.718×10^{-3} ($n = 320$)
Clade II		6.591×10^{-3} ($n = 240$)	19.727×10^{-3} ($n = 512$)
Clade III			1.641×10^{-3} ($n = 992$)

A comparison of the specimens from Israel with those from Antalya, Turkey (*c.* 600 km from Israel) did not reveal significant differences in the genetic composition of the two populations, and they are therefore jointly referred to as the Mediterranean population. These findings join previous studies that did not find a tendency of certain mitochondrial DNA haplotypes of the colonizing species to spread further than others (Azzurro et al. 2006; Golani et al. 2007; Golani & Bernardi 2012).

The results of this study show significant differences between the Red Sea and the Mediterranean *Saurida lessepsianus* populations, and therefore suggest a bottleneck effect. A similar case was found in the cornetfish, *Fistularia commersonii* Rüppell, 1838. However, in other Lessepsian migrants, such as *Siganus rivulatus* Forsskål & Niebuhr, 1775, *S. luridus* (Rüppell, 1829), *Upeneus moluccensis* (Bleeker, 1855), *U. pori* Ben-Tuvia & Golani, 1989 and *A. forskali*, a reduction of genetic variability was not found (Bernardi et al. 2010). Despite a reduction of its genetic variability, the

Mediterranean population of *S. lessepsianus* has been very successful in establishing a huge stock in its new habitat, expressed in its large portion (226 tonnes) in the trawl catch of Israel only one year after this species reached the Levant.

Disclosure statement

No potential conflict of interest was reported by the authors.

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