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Lack of mitochondrial differentiation between Red Sea and Mediterranean populations of the Lessepsian rabbitfish, *Siganus rivulatus* (Perciformes: Siganidae)*

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SUMMARY: A lack of genetic differentiation between Red Sea and Mediterranean populations of the Lessepsian rabbitfish, *Siganus rivulatus,* was evidenced using mitochondrial DNA (cytochrome b). This result implies that Mediterranean populations were not founded by a few individuals, but rather that immigration of *S. rivulatus* into the Mediterranean is a continuous process.

Key words: Lessepsian migrants, *Siganus*, genetic differentiation, mitochondrial DNA.

RESUMEN: FALTA DE DIFERENCIACIÓN MITOCONDRIAL ENTRE POBLACIONES DEL MAR ROJO Y MEDITERRÁNEAS DEL MIGRADOR LESEPSIANO *SIGANUS RIVULATUS* (PERCIFORMES: SIGANIDAE). – La falta de diferenciación genética entre poblaciones del Mar Rojo y mediterráneas de sigano jaspeado, *Siganus rivulatus*, ha sido evidenciada utilizando ADN mitocondrial (citocromo b). Este resultado implica que las poblaciones mediterráneas no fueron fundadas por unos pocos individuos, sino que la inmigración de *S. rivulatus* es un proceso continuo.

Palabras clave: migrador lesepsiano, *Siganus*, diferenciación genética, ADN mitocondrial.

INTRODUCTION

Many fish species from the Red Sea are known to have established populations in the Mediterranean following the opening of the Suez Canal (Golani, 1996). One question which arises with these so-called Lessepsian species is whether they have undergone some demographic bottleneck, if colonisation is the result of a few pioneer individuals, or whether the influx of migrants has been a steady process.

We address this question by testing mitochondrial DNA variability on one siganid species (rabbitfish), *Siganus rivulatus* (Forsskål, 1775) sampled in the Red Sea and the Mediterranean.

Siganus rivulatus is one of the first Lessepsian migrants to be found in the Mediterranean, having been first recorded in the 1920s (Steinitz, 1929). It

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has established breeding populations for a long time and is currently one of the most abundant species in the Levantine rocky shore area, contributing considerably to the local artisanal fishery (Golani and Ben-Tuvia, 1995). *Siganus rivulatus* is a herbivorous fish, feeding mainly on green algae (Lundberg and Golani, 1995). Its spawning season in both the northern Red Sea and the eastern Mediterranean lasts from April to July (Popper, 1979).

MATERIALS AND METHODS

Forty-four individuals were collected from the Israeli coast of the Mediterranean (Haifa) in March 1997, and 39 individuals from the Israeli coast of the Red Sea (Eilat) in February 1997. Samples were transported in alcohol (90% ETOH) to Sète, France for genetic analysis.

Total DNA was extracted according to the phenol_chloroforme method (Sambrook *et al.,* 1989) and a fragment of approximately 350 bp of the gene cytochrome b was amplified by PCR using universal primers (L14841 and H15149) (Kocher *et al.,* 1989). Each 25 µl PCR mixture contained 0.15 U of *Taq* DNA polymerase (Promega, Madison WI, USA), 2.5 µl buffer (Promega, Madison WI, USA), 0.8 mM dNTPs, 2.5 mM MgCl2, 0.48 µM of each primer and 30-50 ng template DNA. PCR was carried out with an initial denaturation at 95°C for 1 min, followed by 35 cycles of amplification (denaturation at 94°C for 12 sec, annealing at 52°C for 12 sec, and extension at 72°C for 20 sec, with a final extension at 72°C for 3 min). The SSCP technique (Orita *et al.,* 1989) was used to detect sequence polymorphism. PCR products were mixed with a 0.5 volume of formamide dye (95 % of Formamide, 20 mM EDTA, 42‰ xylene cyanol, 42‰ bromophenol blue) and 7 µl was loaded on a 10% non-denaturating polyacrylamide gel. The migration of the denatured DNA was carried out to 12 W/gel for 6 hours at 10 °C in TBE 1 X buffer placed in a tank with vertical migration. Haplotypes were revealed with ethidium bromide. For each haplotype detected, two individuals were sequenced over 262 bp using the dideoxynucleotide termination method of Sanger.

RESULTS

Five haplotypes were detected by SSCP among all fishes analysed and sequenced over 262 bp (Genebank accession n° AY167612; AY167613; AY167614; AY167615; AY167616). One common haplotype (A) was equally frequent in samples from both the Mediterranean and the Red Sea. Some rare haplotypes were encountered either in the Mediterranean fishes or in the Red Sea or in both (haplotype C). Pairwise nucleotidic divergence was estimated according to the Kimura 2-parameter model with the software MEGA2 (Kumar *et al.,* 2001). Fig. 1 shows the Neighbour-Joining tree of haplotypic divergence encountered in both samples. Note that the D haplotype diverged by 6 mutations from A, indicating that the coalescence tree of *S. rivulatus* is quite deep. Table 1 provides the haplotype frequencies. From these, a between sample divergence of Theta = – 0.001 was estimated by the F_{ST} estimator of Weir and Cockerham (1984). This value was not significantly different from zero (p> 33.6%) according to a permutation test (Genetix 4.02, Belkhir *et al.,* 1996-2001).

Our results show that the mitochondrial diversity has been preserved during the colonisation process, which is probably not the result of the settlement of a few successful individuals only. If that were the case, the most common haplotype A would have probably been fixed in the Mediterranean and the rare variant C would not have been found at the same frequency in both samples. Thus, the colonisa-

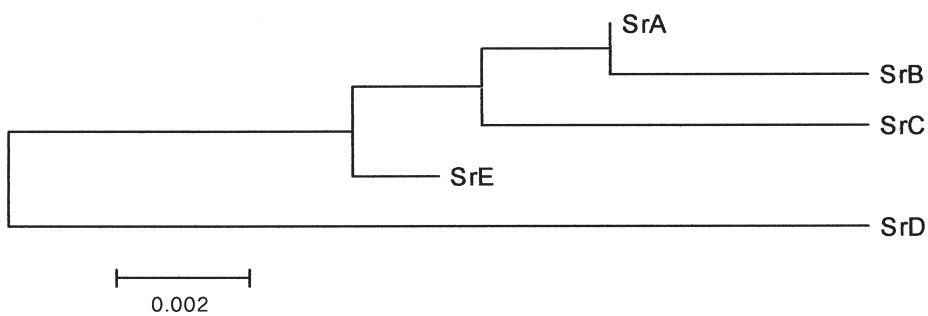

FIG 1. – Neighbour-joining tree of *Siganus rivulatus* cytochrome b haplotypes. Scale bar: 0.002% nucleotide divergence.

TABLE 1. – Frequency of mitochondrial cytochrome b SSCP haplotypes in *Siganus rivulatus.*

Haplotypes	Mediterranean N=44	Red Sea N=39
A	0.92	0.92
B	0.06	-
C	0.02	0.02
D	-	0.04
E	-	0.02

tion was probably not accompanied by a bottleneck, suggesting that this initial migration was either a continuous process or involved a sufficiently high number of individuals at the same time before the large subsequent population expansion. Note that present-day migrants could not significantly alter haplotype frequencies given the large present-day Mediterranean population size, so most of the variability was there from the beginning of the process. Many migrant individuals from *S. rivulatus* were thus able to thrive and develop in their new Mediterranean environment. This hypothesis is supported by the comparative study of the parasitofauna of *S. rivulatus* in both the source and target populations (Diamant, 1998); this study indicated that migration of this species was undertaken by adult active swimmers. A large adaptive plasticity of this species must be postulated given the large trophic and biotic differences between the two water bodies. These results are in essence similar to the recently published case of *Atherinomorus lacunosus* from the same places (Bucciarelli *et al.*, 2002), in which mitochondrial genetic diversity was preserved between Mediterranean and Red Sea populations.

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