Characterization of genetic diversity of an invasive Lessepsian Migrant, Nemipterus randalli Russell, 1986 from its native and non-native regions using mitochondrial DNA marker

MURUGESAN SRIHARI, SUSANTHI SILPA, ANNAM PAVAN-KUMAR, YARON TIKOCHINSKI, DANIEL GOLANI, BINAYA BUSHAN NAYAK, SHASHI BUSHAN, ZEBA JAFFER ABIDI

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Murugesan SRIHARI[1], Susanthi SILPA[1], Annam PAVAN-KUMAR[2], Yaron TIKOCHINSKI[3], Daniel GOLANI[4], Binaya BUSHAN NAYAK[1], Shashi BUSHAN[1] and Zeba Jaffer ABIDI[1]

[1] Division of Fisheries Resource Management, ICAR-Central Institute of Fisheries Education, Mumbai, 400 061, India
[2] Division of Fish Genetics and Biotechnology, ICAR-Central Institute of Fisheries Education, Mumbai, 400 061, India
[3] School of Marine Sciences, Ruppin Academic Center, 40297 Michmoret, Israel
[4] Hebrew University of Jerusalem, Israel

Corresponding author: pavanannam@gmail.com

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Abstract

This study assessed and compared the genetic diversity of *Nemipterus randalli* across its native and non-native regions analysing the mitochondrial DNA D-loop region. Including all the geographical population samples, 68 haplotypes were observed with an average haplotype diversity value of 0.92±0.04. Relatively, a smaller number of haplotypes was observed in the invasive range of the Mediterranean Sea. All other native geographical samples showed high haplotype and nucleotide diversity values. A significant high genetic differentiation value was observed between the native population samples of India and the invasive samples of the Mediterranean Sea. In the median-joining network tree, *N. randalli* from the Mediterranean Sea and the Red Sea formed a single haplogroup while other samples from India are clustered into two haplogroups.

Keywords: Bio invasion; D-loop; population structure; Mediterranean Sea; Indian Ocean.

Introduction

Biological invasion of the marine ecosystem refers to the spread of species from their native range to another ecosystem and subsequent establishment of a population. This phenomenon poses a potential risk to native fish diversity. Invasive species could alter the structure of the existing ecosystem by competing for food, habitat, predation, and causes genetic disintegration of native species (Wilcove *et al.,* 1998). The success of invasive species in establishing their population depends on their biology, behavior and genetic diversity of the founder population (Sakai *et al.,* 2001). Characterization of the genetic diversity of invasive species from their native and introduced/invaded region would reveal the genetic parameters of the population and is important to better understand the process of biological invasion. Genetic parameters have been used to assess key factors such as intensity of the propagule pressure (number of introduced individuals and the total number of introduction events), hybridization events, and preadaptation of species, that determine the success of the invasion (Romiguier *et al.*, 2014; Tepolt & Palumbi, 2015; Bourne *et al.*, 2018). Mediterranean Sea is the world's most invaded marine ecosystem, and human-made channels that connect seas are the major factors causing bioinvasion in this region. (Edelist *et al.,* 2013; Arndt *et al.,* 2018). Suez Canal has facilitated the migration of fishes from Red Sea to the Mediterranean Sea (Por, 1978). More than 100 fish species present in the Red Sea have also been found in the Mediterranean Sea; of these 100 species, the population of 70 species has been established in the Mediterranean Sea (Fricke *et al.*, 2017; Golani, 2019; Huseyinoglu & Jimenez, 2019).

Nemipterus randalli Russell, 1986 (Order: Perciformes; Family: Nemipteridae), Randall's threadfin bream is a commercially important demersal marine fish. It is widely distributed in the Indian Ocean but is also found in non-native regions such as Mediterranean coast of Israel (Golani & Sonin, 2006), Lebanon (Lelli *et al.*, 2008), Syria (Ali *et al.*, 2013), Turkey (Gulsahin & Kara, 2013), Cyprus (Iglésias & Frotté, 2015) and Egypt (Akel *et al.*, 2020). *Nemipterus randalli* is the most dominant Indo-Pacific alien fish in the Mediterranean Sea with an established population and has became a major catch in artisanal fishery activities and by commercial trawlers, especially in the eastern Mediterranean Sea (Stern *et al.,* 2014; Erguden *et al.*, 2010; Mavruk *et al.*, 2016). Because

of its fast growth, early maturation, and competition for food, *N. randalli* may pose a severe threat to native fish diversity (eg. *Pagellus erythrinus* of the Mediterranean Sea) (Bilge *et al.,* 2019; Yapici & Filiz, 2019). The fish predominantly feeds on crustaceans, cephalopods, molluscs and small fishes (Manojkumar, 2007). Its rapid spread and increasing abundance may affect the diversity of benthic decapod crustaceans and native fish communities feeding on these organisms. Therefore, the fish has been included in the blacklist of marine invasive species (Otero *et al.*, 2013).

Analysis of the population genetic structure of invasive species from their native and non-native regions by using neutral genetic markers may help in determining the population bottlenecks/founder effects, range expansion, gene flow restriction and genetic relationship between the populations (Chiesa *et al.,* 2019). Detailed knowledge of the population genetic structure of invasive species is essential for understanding the evolutionary significance of invasive events. The mitochondrial control region (D-loop) is a non-coding region with a high rate of tolerable mutations, rendering it a suitable marker for studying intraspecific-level evolutionary studies (Domingues *et al.,* 2006). In the present study, we assessed and compared the genetic diversity between the native and non-native populations of *N. randalli* by using the mitochondrial DNA partial D-loop region.

Materials and Methods

A total of 66 individuals of *N. randalli* were collected from four locations of its native range along the Indian coast, Mumbai (*n*= 18), Mormugao (*n*=13), Kochi (*n*=16) and Thoothukudi (*n*=19) during September to November 2018 (Fig. S1). The fish were identified up to the species level according to morpho-meristic characters, reported earlier. The fin tissue was collected aseptically and preserved in absolute ethanol. Total genomic DNA was isolated from the fin tissue by using the salting-out method (Miller *et al*., 1988). For DNA barcoding, a representative DNA sample from each location was subjected for PCR amplification and sequencing of partial cytochrome *c* oxidase subunit I gene as reported by Ward *et al*. (2005) to confirm the specimen identification. Based on the sequence similarity (99-100%) with the reference sequences (KU499702-KU499704), the fishes were confirmed as *N. randalli,* and the barcodes were submitted to the NCBI GenBank (Accession numbers MT502344-MT502348). The mitochondrial partial D-loop region (~390 bp) from all the samples was amplified using the primers reported by Tikochinski *et al*. (2019). PCR amplification was performed in 12.5 µL reaction volume containing 100 ng template DNA, 10 pmol of each primer, 200 µM of each dNTPs, 1 unit of Taq DNA polymerase and 1x Taq buffer containing 1.5 mM MgCl$_2$ and the following thermal regime was used: initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, extension at 72°C for 60 s and a final extension for 7 min on a thermal cycler (S1000™,

Bio-rad, USA). The PCR product was purified using the Gel extraction kit (Thermo Fisher Scientific, USA) according to the manufacturer's guidelines. The amplicons were sequenced in both forward and reverse directions by using the PCR primers (Agrigenome Labs, Kochi). The quality of the sequences was verified by observing the Phred score (Q>30) of each base using FinchTV (http://www.geospiza.com/Products/finchtv.html). The sequences were submitted to the NCBI GenBank with accession numbers MT502122-185, MW027133-MW027134.

Additionally, D-loop sequences of *N. randalli* (MH142087-2142) from its invasive range, the Mediterranean Sea (*n*=42) and the Red Sea (*n*=14), recently reported by Tikochinski *et al*. (2019) were included in the analyses. All the sequences (*n*=122) were aligned to their homologous positions using the Clustal W program inbuilt in the MEGA7 software (Kumar *et al*., 2016). The number of segregating sites (*S*), haplotypes (*nh*), haplotype diversity (*hd*) and nucleotide diversity (π) values were calculated using DnaSP6 (Rozas *et al.,* 2017). The population genetic structure was analysed by estimating the genetic differentiation (Φ_{ST}) values and analysis of molecular variance (AMOVA) using the program Arlequin with 1000 permutations (Excoffier *et al.,* 2005). For AMOVA, stocks of *N. randalli* from the Mediterranean and Red Sea were considered as "region 1", whereas stocks from the Indian coast were considered as "region 2". Tajima's D and Fu's F values were estimated to detect the deviation from the neutrality expectations and population demographic history was inferred by performing the mismatch distribution analysis using DnaSP6 (Rozas *et al.,* 2017). A coalescent simulation algorithm with a bootstrap value of 1000 was used to test the significance of all the statistical tests. To determine the relationship between haplotypes, a median-joining network was constructed using Popart (http://popart.otago.ac.nz/).

Results and Discussion

A total of 66 sequences of mitochondrial partial D-loop region of *N. randalli* were generated from the present study and merged with previously available data to form a total of 122 individual sequences. Out of 385 bp analyzed, the number of constant and singleton sites was 351 and 6, respectively. Of 34 variable nucleotides, 28 sites were parsimony informative sites. The frequency of nucleotides was as follows: A (38.9%); T (34.4%); C (13.9%) and G (12.8%) and the A+T content value was found to be 73.3%. Due to reduced selection pressure, the mitochondrial D-loop region displays high polymorphism and this region has been used as a marker to study phylogeography and population genetics of several fishes (Viñas *et al.,* 2010).

We observed a total of 68 haplotypes with an average haplotype diversity value of 0.92±0.04. This observation confirms the presence of considerable genetic diversity among the fishes collected from different geographical regions. In comparison with the native populations, the Mediterranean populations showed relatively less num-

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ber of haplotypes and moderate haplotype diversity values (0.74 vs 0.9). This could be because of the recent colonization of the *N. randalli* by a few individuals from the source population (founder effect). We also observed haplotype sharing between the Red Sea and the Mediterranean Sea populations (Fig.1). This migration might have occurred recently and thus, the founder population exhibited lesser genetic diversity than the native populations. Similar to our results, Ogwang *et al.* (2020) reported using COI gene that genetic diversity in the Mediterranean population is lower than the Red Sea population. Several studies have also reported a decrease in the genetic diversity in the invasive marine fish populations compared with that in native populations (Sanna *et al.*, 2015; Dimitriou *et al.*, 2019).

Other native populations were found to have large number of haplotypes and high nucleotide diversity values (0.9 & >0.5%) (Table 1). These high values can be attributed to the abundance, stability and long evolutionary history of the populations. Studies have reported similar observations in angelfishes (Hobbs *et al.*, 2013). *Nemipterus randalli* has prolonged spawning season periodicity with an average fecundity (number of ova) of approximately 30,000 (Raje, 1996). Fishes with high fecundity and maturity at early age ("r" strategists) exhibit high genetic diversity (Romiguier *et al.*, 2014; Martinez *et al.*, 2018). *N. randalli* can be categorized under r-selected fish and its reproductive characteristics of the fish could be the reason for large population size with notable genetic diversity.

A significantly high genetic Φ_{ST} value (>0.60) was observed between the samples collected from the Indian Ocean and Mediterranean Sea (Table. S1). Similar high Φ_{ST} values were also observed between the Indian coast and the Red Sea populations. The AMOVA analysis indicated a majority of the molecular variance (54.78%) between the regions 1 and 2, suggesting the presence of two groups of *N. randalli* and lack of gene flow between them. It could be due to the restricted larval dispersal from the Indian Ocean to the Red Sea through the Straits of Bab-el-Mandab (Siddall *et al.,* 2003). The Red Sea

was partially disconnected from the Indian Ocean during the Pleistocene period, and *N. randalli* might have established a distinct population in the Red Sea during that period. Limited gene flow was reported between the Red Sea and the Indian Ocean during the last major glacial period (DiBattista *et al.,* 2013). Further, upwelling along the Somalian coast and the turbid waters along the Pakistan and eastern Indian coast could also be a potential barrier for gene flow (Roberts *et al.,* 1992).

The Φ_{ST} value between the Red Sea and Mediterranean Sea populations was low but significant. Less genetic differentiation was observed among the native Indian population, with a Φ_{ST} value of 0.001 (Mormugao-Kochi/Mumbai) to 0.08 (Kochi-Thoothukudi); however, the values were non-significant (Table. S1). This might be due to the pattern of ocean currents and lack of geographical barriers along the Indian coast (Shenoi, 2010). The AMOVA analysis indicated low variation (3.31%) among the samples of each region, confirming the gene flow between the different geographical locations within each group. A total of 42% of genetic variation was observed among the individuals of each geographical location, which confirms the existence of notable genetic variation within the populations.

In the median-joining network tree, the Mediterranean Sea and the Red Sea populations formed a single haplogroup (haplogroup1). The populations from India formed two haplogroups (Haplogroups 2 and 3) with a star-like topology (Fig.1). Haplogroup 1 and 2 differed in four mutations, whereas haplogroup 2 and 3 differed in a single mutation. Population demographic analyses showed statistically non-significant Tajima's D and Fu's F values in the native and invasive population (Table. 1). This observation confirms that these populations are evolving as per the mutation-drift equilibrium and according to the neutral theory of molecular evolution. In the invasive population, both Tajima's D and Fu's F values were found to be positive that indicates the lack of rare alleles. Bimodal mismatch distribution observed in the invasive population is suggestive of a stable population; however, it may also signify the presence of two
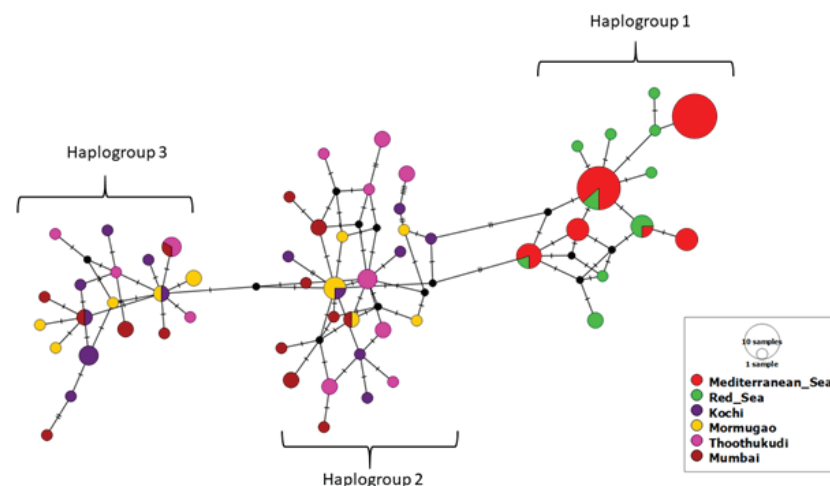

Fig. 1: Medium Joining network of *Nemipterus randalli* (each vertical bar intersecting the joining line represents on mutation).

Table 1. Details of population samples of *Nemipterus randalli* analyzed in this study: Sampling locations, number of samples, number of haplotypes, haplotype diversity and associated standard error, nucleotide diversity and associated standard error, neutrality tests among geographical samples of *Nemipterus randalli* (* Sequences from Tikochinski *et al.,* 2019).

S.no	Sampling locations	n	Number of haplotypes (*nh*)	Haplotype diversity (*hd*)	Nucleotide diversity (π)	Tajima's D-Value	Fu's F_s-value
1	Mediterranean Sea*	42	6	0.74±0.040	0.004±0.0003	0.863	0.63
2	Red Sea*	14	10	0.96±0.045	0.006±0.0004	-0.88	-5.11[#]
3	Mumbai (Arabian Sea)	18	15	0.96±0.024	0.016±0.0003	-0.280	-8.62
4	Mormugao (Arabian Sea)	13	11	0.97±0.039	0.008±0.0003	-0.296	-6.34
5	Kochi (Arabian Sea)	16	14	0.97±0.035	0.010±0.0002	-0.510	-9.09
6	Thoothukudi (Bay of Bengal)	19	12	0.95±0.028	0.013±0.0008	-0.105	-2.72

[#] statistical significance: P< 0.05

distinct lineages or secondary contact between differentiated lineages or spatial expansion with low migration rates (Rogers & Harpending, 1992; Alvarado-Bremer *et al*., 2005). It could be because of the multiple invasions of *N. randalli* to the Mediterranean Sea from their native regions.

Unimodal mismatch distribution, negative but nonsignificant Tajima's D value, and significant Fu's F value indicated that populations from the Red Sea could have a propensity for expansion because of founder/bottleneck effects. However, the populations from the Indian coast have a constant size, and this theory is supported by their high haplotype and nucleotide diversity values (>0.5%) and multimodal mismatch distributions (Fig. S2).

Elucidating the mechanisms underlying the success of invasion is the major aspect of ecology (Kolar & Lodge, 2001). The results of the present study on the genetic characterization of *N. randalli* in its native and non-native regions may help in gaining insights into these mechanisms and hence may contribute to the field of population genetics.

Larval dispersal is a crucial factor that determines the invasive spread in non-native regions. (Jackson *et al.,* 2015). The Indian populations display a high level of connectivity, with a lack of structuring between the populations, which would be consistent with the rapid dispersal of *N. randalli* in the Mediterranean Sea. By analyzing the genetic diversity and by studying the demography of the fish population in the Indian Ocean, we predict that *N. randalli* might establish a larger population in the Mediterranean Sea in the near future. It may significantly contribute to commercial fishery in the Mediterranean area by replacing native species such as *Pagellus erythrinus*. Thus, maintaining a healthy ecological community with top predators of *N. randalli* might be an effective strategy to control its spread.

In conclusion, a considerable genetic diversity across the stocks of *N. randalli* was found. Our study confirms the presence of two groups of *N. randalli* populations, one inhabiting the Red Sea and Mediterranean Sea and the other inhabiting the Indian Ocean. Further studies with nuclear markers and large sample size should investigate other factors such as effective population size, inbreeding coefficient, and other population genetic parameters of *N. randalli* pertaining to both native and invasive populations.

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Supplementary data

The following supplementary information is available online for the article:

Table S1. Pairwise Φ_{ST} between geographical samples of *Nemipterus randalli* collected from different locations (* Statistical significance, P< 0.05).

Fig. S1: Sampling locations used in the present study. *Diamond indicates the samples from Tikochinski *et al.,* 2019, circles indicate samples from the present study.

Fig. S2: Mismatch distribution curves for Nemipterus randalli from sampling locations.

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