Research Article

DNA barcoding of *cheimerius nufar* (valenciennes, 1830) (perciformes: sparidae) from the sultanate of Oman

Abstract

Santer sea bream *Cheimerius nufar* is one of the most important commercial fish of the Oman. Presented work aims to use DNA barcoding techniques to identify *Cheimerius nufer*. Five representative samples were taken from three locations in the Sultanate of Oman (Ashkarah, Lakbi and Salalah). Species identification is done by extracted DNA which used for PCR amplification of COI gene and sequencing the mitochondrial gene cytochrome oxidase COI gene. Then the species identification compared the generated sequence with the databases at NCBI and BOLD. Neighbour Joint (NJ) clustering analysis showed that all samples formed single monophyletic clade further suggesting that they belonged to *Cheimerius nufer* with percent identity more than 98%.

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Introduction

The Santer seabream is a widely distributed economically important fish that is highly consumed all over the World. Lot of concerns have been raised due to decline in the population of this fish. In fact a report by friends of the Sea has mentioned that *C. nufar* is heavily exploited fish on the Oman coast Al-Marzouqi et al.[1] Due these reasons, many times immature adults and small sized individuals were also collected. Al-Marzouqi[2] also feared that due to such heavy exploitation the fishery is in state of decline in Oman. Further *C. nufar* is also a victim of fish adulteration due its huge demands from all over the World. Studies have mentioned that many other species of fishes are sold in the market under the labelling of *C. nufar* Barendse & Francis[3] and in such scenario identification of these samples is of prime importance. However, identification based on morphological characters would be highly difficult for the consumers and even for the end users as it shares morphological similarity with its adulterants. DNA barcoding offers a reliable and rapid method of species identification as it is independent of the morphological characters and species identification would be easier for non-taxonomist as well. In this technique species identification is done by sequencing the mitochondrial gene cytochrome oxidase I and then comparing the generated sequence with the databases at NCBI and BOLD.[4,6] Despite the possible threats for the populations of *C. nufar* in the Oman due to heavy exploitation till now no attempts have been made to identify this fish using DNA barcoding. Therefore in this study we aimed to identify *C. nufar* collected from different localities of Oman using DNA barcoding.

Materials and methods

Sample Collection and Morphological Data

Tissue samples of *C. nufar* were collected from three different locations in Oman. The locations in Oman were Ashkarah, Lakbi and Salalah (Figure 1) were used for this study. Five representative samples were taken from each site. The samples were caught from local fishermen market under the Ministry of Agriculture and Fisheries Wealth confirmation. The samples were transported to the laboratory in Muscat under cool box with ice and stored at -20°C

till further examination. Samples were taken out, photographs were taken and size of each sample was recorded. Once the morphological examination was done, each sample was cut from and a tissue of size 1 cm x 1 cm was cut and stored in a tube containing what man filter paper and silica gel. The sample was dried under room temperature and stored at -20°C till further analysis was done.
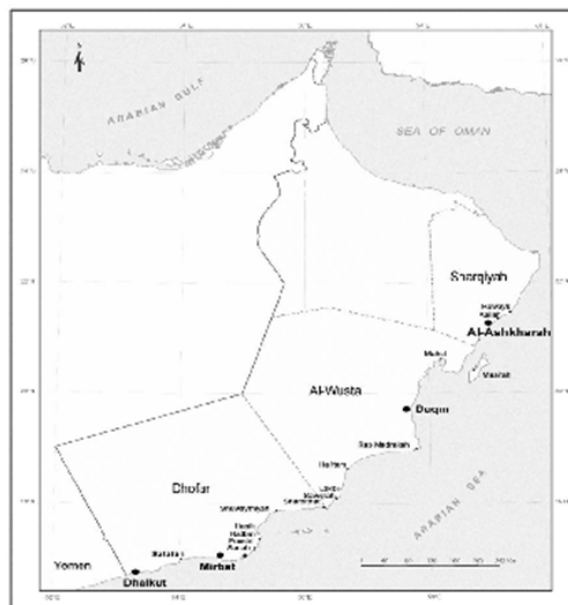
Figure 1 Map of Oman.

DNA isolation

Tissue samples from the samples collected were taken out from the storage and thawed at room temperature. The samples were washed with PBS buffer, dried and approximately 25-50 mg of tissue material was homogenized in 200 µl of the buffer. To the homogenized sample, lysis buffer 500 µl was added and proceeded for DNA extraction. Further steps were carried out using the phenol: chloroform organic extraction method Taggart et al.[5] The DNA was checked using 0.8% agarose gel running at 60 V for 45 min.

PCR amplification and DNA sequencing

Extracted DNA was used for PCR amplification of COI gene using primers: LCO 1490F - GGT CAA CAA ATC ATA AAG ATA TTG G and HCO 2198R - TAA ACT TCA GGG TGA CCA AAA AAT CA as detailed by DNA barcoding protocol led down by Canadian Centre for DNA barcoding (CBOL- Inova N & Grainger[7]). The PCR reactions were carried out using CCDB protocol. For each reaction of total volume 10.5 µl 10% trehalose was added (6.25µl), 10X PCR buffer (1.25µl), 50 mM MgCl2 (0.625 µl), 10 µM of each primer (0.125 µl each), 10 mM dNTPs (0.0625µl), 5U/µl platinum Taq polymerase (0.06µl) and 2 µl of ddH2O. To this mixture 2µl of extracted DNA was added. The thermal cycling was carried out according to the standard protocol laid by CBOL, which consisted of an Initial denaturation at 95°C for 10 minutes followed by first 5 cycles of denaturation, annealing and chain extension at 94°C (30 secs), 45-50°C (30 secs), 72°C (60 secs) respectively. The second stage consisted of 30 cycles of denaturation, annealing and chain extension at 94°C (30 secs), 51-54°C (30 secs), 72°C (60 secs) respectively The final chain extension step was for 10 minutes at 72° C and a final hold at 4° C. The PCR products were checked on 2% Agarose gel followed purification using Exo-SAP. DNA sequencing was carried out with forward as well as reverse primers of the universal primer. Sequencing was performed according to standard protocol provided for Big Dye Terminator kit® V 3.1 (Applied Bio systems) using ABI automated DNA sequencer (ABI 310 Genetic Analyzer). 3 µl of cleaned purified PCR product was used for each 10 µl reaction.

Bioinformatics analysis

Obtained sequences were edited to remove ambiguous base calls. The forward and the reverse sequences were assembled using Chromas Pro (http://www.technelysium.com.au/chromas_lite.html). FASTA format of these sequences were used for species identification using BLAST search at NCBI (http://blast.ncbi.nlm.nih.gov/) and species identification tool at BOLD (http://www.barcodinglife.com). The samples is said to be indentified if it showed sequence match of 97% or more than that with other *C. nufar*sequences submitted in the databases. And if not then it is considered to show high intraspecies nucleotide divergence. All the obtained sequences were aligned using Bio Edit Hall TA.[8] The phylogenetic tree were constructed using MEGA6 software Tamura K et al.[9] The phylogenetic analysis was done using NJ method in MEGA6. The Kimura 2 parameter (K2P) model of base substitution was used to calculate pair wise genetic distances in MEGA 6 software. The distance matrix was also constructed to calculate intraspecies and interspecies nucleotide divergences.

Results and discussion

DNA was obtained from all the 30 samples and obtained DNA was used for PCR amplification COI gene. All the samples were positive for amplification of expected size (Figure 2). Species identification using BOLD database was shown in Table 1. Our results revealed that all the samples showed sequence similarity match with *Cheimerius nufar* with percent identity more than 98%. No case was observed where our query sequence matches with species under different species name. Further suggesting that all samples studied here indeed belonged to *Cheimerius nufar*. We also calculated interspecies nucleotide divergences of the samples studied here. The intraspecies nucleotide divergence calculated was 0%, thus suggesting that all samples studied here indeed belonged to same species. NJ clustering analysis also showed that all samples formed single monophyletic clade further suggesting that they belonged to same species (Figure

3). Thus our analysis using similarity search and NJ clustering analysis showed that samples collected from two localities of Oman indeed belonged to *Cheimerius nufar*. This is the first study from Oman that showed utility of DNA barcoding for species identification of commercially important species, *C. nufar*. We believe that efforts should be made to collect samples of *C. nufar* from different localities of Oman to establish the intraspecies divergence between them. And once such database is ready it would be easier for anybody to identify this species if sold under different species name.

Table 1 Identification of the samples studied here using BOLD database

No	Sample name	BOLD identification	Similarity (%)
1	*Cheimerius nufar* Lakbi 1	*Cheimerius nufar*	98.26
2	*Cheimerius nufar* Lakbi 2	*Cheimerius nufar*	98.26
3	*Cheimerius nufar* Lakbi 3	*Cheimerius nufar*	98.26
4	*Cheimerius nufar* Lakbi 4	*Cheimerius nufar*	98.26
5	*Cheimerius nufar* Lakbi 5	*Cheimerius nufar*	98.26
6	*Cheimerius nufar* Lakbi 6	*Cheimerius nufar*	98.26
7	*Cheimerius nufar* Lakbi 7	*Cheimerius nufar*	98.26
8	*Cheimerius nufar* Lakbi 8	*Cheimerius nufar*	98.26
9	*Cheimerius nufar* Lakbi 9	*Cheimerius nufar*	98.26
10	*Cheimerius nufar* Lakbi 10	*Cheimerius nufar*	98.26
11	*Cheimerius nufar*Salalah 11	*Cheimerius nufar*	98.26
12	*Cheimerius nufar*Salalah 12	*Cheimerius nufar*	98.26
13	*Cheimerius nufar*Salalah 13	*Cheimerius nufar*	98.26
14	*Cheimerius nufar*Salalah 14	*Cheimerius nufar*	98.26
15	*Cheimerius nufar*Salalah 15	*Cheimerius nufar*	98.26
16	*Cheimerius nufar*Salalah 16	*Cheimerius nufar*	98.26
17	*Cheimerius nufar*Salalah 17	*Cheimerius nufar*	98.26
18	*Cheimerius nufar*Salalah 18	*Cheimerius nufar*	98.26
19	*Cheimerius nufar*Salalah 19	*Cheimerius nufar*	98.26
20	*Cheimerius nufar*Salalah 20	*Cheimerius nufar*	98.26
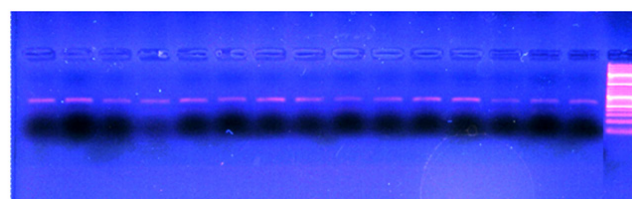
Figure 2 Some samples of fish PCR amplified after purification as a single band isolated from each plus a 100 bp ladder.
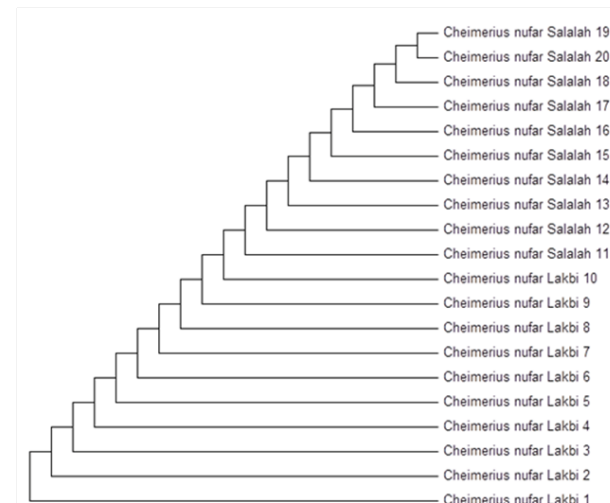
Figure 3 NJ clustering analysis of the samples studied here. Clustering analysis was based on Kimura 2 parameter using MEGA6 software.

Acknowledgments

Conflicts of interest

References

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