

COMU Journal of Marine Sciences and Fisheries

Journal Home-Page: <http://jmsf.dergi.comu.edu.tr> Online Submission: <http://dergipark.org.tr/jmsf>



RESEARCH ARTICLE

Genetic Confirmation of the Striped Eel Catfish *Plotosus lineatus* (Thunberg, 1787) from Iskenderun Bay (Eastern Mediterranean, Türkiye)

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Received: 09.03.2024 / Accepted: 22.05.2024 / Published online: 10.07.2024

Key words:

Plotosus lineatus
Genetic confirmation
Eel catfish
Invasive species
mtDNA COI

Abstract: The records of the striped eel catfish *Plotosus lineatus* have been assigned based on morphological characters in the Mediterranean Sea. However, molecular and genetic analyses are needed to confirm the morphological taxonomic determination and avoid misidentification. In this study, the mitochondrial cytochrome c oxidase subunit I (COI) gene region was used for genetic confirmation of *Plotosus lineatus* in the Mediterranean Sea. The obtained *P. lineatus* sequences were found to have a 100% identity match with the Red Sea and Mediterranean records. The present molecular genetic study confirms the presence of *P. lineatus* in Turkish marine waters. Also, this study is the first genetic confirmation of *P. lineatus* on the coast of Türkiye and in the Mediterranean Sea.

Anahtar kelimeler:

Plotosus lineatus
Genetik doğrulama
Yılan balığı
İstilacı tür
mtDNA COI

Çizgili Yılan Balığının *Plotosus lineatus* (Thunberg, 1787) İskenderun Körfezi'nden (Dođu Akdeniz, Türkiye) Genetik Doğrulaması

Öz: Çizgili yılan balığı *Plotosus lineatus*'un Akdeniz'deki kayıtları morfolojik karakterlere dayanılarak belirlenmiştir. Bununla birlikte, morfolojik taksonomik belirlemeyi doğrulamak ve yanlış tanımlamayı önlemek için moleküler ve genetik analizlere ihtiyaç duyulmaktadır. Bu çalışmada, Akdeniz'deki *Plotosus lineatus*'un genetik doğrulaması için mitokondriyal sitokrom c oksidaz alt ünitesi I (COI) gen bölgesi kullanılmıştır. Elde edilen *P. lineatus* dizilerinin Kızdikeniz ve Akdeniz kayıtları ile %100 örtüştüğü tespit edilmiştir. Mevcut moleküler genetik çalışma ile *P. lineatus*'un Türk deniz sularındaki varlığını doğrulamaktadır. Ayrıca, bu çalışma *P. lineatus*'un Türkiye kıyılarında ve Akdeniz'de ilk genetik doğrulamasıdır.

Introduction

Marine alien and invasive species have increased in the Mediterranean ecosystem due to the opening of the Suez Canal and the effects of climate change (Turan et al., 2016; Tiralongo et al., 2020; Langeneck et al., 2023). To date, more than 100 non-native fish species from the Indo-Pacific region have entered the Eastern Mediterranean via the Suez Canal (Galil et al., 2018; Turan et al., 2018; Azzurro and D'Amen, 2022; Mutlu et al., 2023).

The family Plotosidae has 10 valid genera and 42 valid species all over the world but only a single species, striped eel catfish *Plotosus lineatus* (Thunberg, 1787), has been reported in the Mediterranean Sea (Golani, 2002; Fricke et al., 2023; Dođdu et al., 2016). *P. lineatus* is distributed throughout the Indo-Pacific, Red Sea and Mediterranean. First recorded in the Mediterranean from the Israeli coast

(Golani, 2002) after which, it was rapidly established in the Mediterranean Sea. Thereafter, it was recorded from Egypt, Syria, Türkiye and Northern Cyprus, respectively (Temraz & Ben Souissi, 2013; Ali et al., 2015; Dođdu et al., 2016; Turan et al., 2022). Monitoring studies have shown that the species is spreading rapidly in the Mediterranean (Turan and Dođdu, 2023; Dođdu & Turan, 2024).

The mtDNA sequence analysis is extensively used for species identification in taxonomic studies (Hebert et al., 2003; Dođdu and Turan, 2021). In recent years, the issue of invasive alien fish species has become a growing concern in marine ecosystems (Katsanevakis et al., 2014; Watkins et al., 2021; Turan and Dođdu, 2022; Chaikin et al., 2023). The mtDNA sequence analysis can identify and differentiate between species of fish with a high degree of accuracy

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How to cite this article: Turan, C., Ergenler, A., Turan, F. & Dođdu, S.A. (2024). Genetic confirmation of the striped eel catfish *Plotosus lineatus* (Thunberg, 1787) from Iskenderun Bay (Eastern Mediterranean, Türkiye). COMU J. Mar. Sci. Fish, 7(1): 24-29. doi:10.46384/jmsf.1449570

(Landi et al., 2014; Isaacs and Hellberg, 2020; Antil et al., 2023). It can play a key role in the identification and management of alien fish species, allowing for early detection and rapid response to prevent further ecological disruption (Bariche et al., 2015; Turan et al., 2017; Dođdu et al., 2019).

Here, we used mitochondrial cytochrome c oxidase subunit I (COI) for the molecular identification of *Plotosus lineatus*. This is the first genetic confirmation of *P. lineatus* on the coast of Türkiye and in the Mediterranean Sea.

Material and Methods

Four samples of *Plotosus lineatus* were captured by gill net at a depth of 20 m in İskenderun Bay on 20 September 2023 by fishermen. Samples were safely transported to the laboratory and placed on crushed ice for analysis. All tissue samples were stored at -20 °C under 95% ethanol until analysis.

mtDNA were obtained from the muscle samples using phenol-chloroform with minor changes (Sambrook et al., 1989). Polymerase chain reaction (PCR) amplification was performed with the following universal cytochrome c oxidase subunit I (COI) primers (Ward et al., 2005):

Fish1_COI_F1:

5'-TCAACCAACCACAAAGACATTGGCAC-3'

Fish1_COI_R1:

5'-ACTTCAGGGTGACCGAAGAATCAGAA-3'

The PCR reaction was performed in a volume of 50 µl with 0.4 µM of each primer, 0.2 mM dNTP and 0.5 U Taq DNA polymerase in a PCR buffer containing 20 mM Tris-HCl (pH 8.0), 1.5 mM MgCl₂, 15 mM KCl and 1.5 µl template DNA. The denaturation step comprised 30 cycles at 95°C for 45 seconds, 60°C for 30 seconds, and 75°C for 60 seconds, followed by a final extension for 3 minutes at 72°C. Electrophoresis on a 1.5% agarose gel was used to visualise the PCR products. The order of the nucleotides in the mtDNA COI gene region was determined through DNA sequencing. The BigDye sequencer V3.1 and ABI 3130 XL genetic analyzer were applied with the Sanger et al. (1977) chain termination method. COI subsequences aligned using BioEdit (Hall et al., 2011). Genetic diversity and sequence divergence were determined after sequence alignment and phylogenetic trees were constructed using MEGA X (Kumar et al., 2018). Sequences of other *Plotosus* species obtained from GenBank and the BOLD system were used to construct phylogenetic trees. The accession numbers and references of the sequences used are given in Table 1.

Table 1. Sequences of other *Plotosus* species obtained from Genbank and the BOLD system.

Species	Accession Numbers	Database	References
<i>Plotosus lineatus</i>	KR861548.1	GenBank	Bariche et al., (2015)
<i>Plotosus lineatus</i>	BIM263-13	BOLD System	Unpublished
<i>Plotosus lineatus</i>	KM538501.1	GenBank	Shirak et al., (2016)
<i>Plotosus canius</i>	KR086940.1	GenBank	Samani et al., (2016)
<i>Plotosus canius</i>	MK093214.1	GenBank	Kundu et al., (2019)
<i>Plotosus canius</i>	OR271961.1	GenBank	Unpublished
<i>Plotosus canius</i>	KP258655.1	GenBank	Samani et al., (2016)
<i>Plotosus canius</i>	KU692769.1	GenBank	Dahrudin et al., (2017)
<i>Plotosus limbatus</i>	KF824846.1	GenBank	Barathkumar et al., (2020)
<i>Plotosus limbatus</i>	KF824845.1	GenBank	Barathkumar et al., (2020)
<i>Plotosus limbatus</i>	KF824844.1	GenBank	Barathkumar et al., (2020)
<i>Plotosus japonicus</i>	KR270437.1	GenBank	Liu and Zhang (2016)
<i>Plotosus japonicus</i>	NC_027737.1	GenBank	Liu and Zhang (2016)
<i>Plotosus japonicus</i>	AP012020.1	GenBank	Nakatani et al., (2011)
<i>Plotosus japonicus</i>	ILRS001-19	BOLD System	Goren et al., (2020)
<i>Plotosus nkunga</i>	JF494192.1	GenBank	Unpublished
<i>Plotosus nkunga</i>	JF494190.1	GenBank	Unpublished
<i>Plotosus nkunga</i>	KF511562.1	GenBank	Dhar and Ghosh (2015)
<i>Sebastes schlegelii</i>	OR577041.1	GenBank	Yađlıoglu et al., (2023)

Results

There were observed 5 variable and 630 conservative nucleotides over 635 bp DNA barcode of four *Plotosus lineatus* samples. With the four barcodes obtained from our study, eleven haplotypes were found in a total of 22 sequences belonging to the species of the genus *Plotosus*, and no common haplotype was found between the species (Table 2. See Supplementary Material for further details). The mtDNA COI gene region sequence of four *Plotosus lineatus* samples was obtained at 635 bp and into the Genbank with accession number: PP434659-PP434662 and BOLD with sample number: PLIN001-PLIN004 (BIN: ACG8821). The Genbank and BOLD databases were used

for comparison with sequences of other *Plotosus* species with a worldwide marine distribution. The accession numbers of the sequences used are given in Table 1.

The analyses of Neighbour-Joining (NJ) and Maximum Parsimony (MP) trees of the sequences created within the scope of our study and the sequences obtained from Genbank and BOLD databases are given in Figure 1 and Figure 2. Both NJ and MP phylogenetic trees were observed with similar tree topologies. The bootstrap consensus tree inferred from 1000 replicates was taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985).

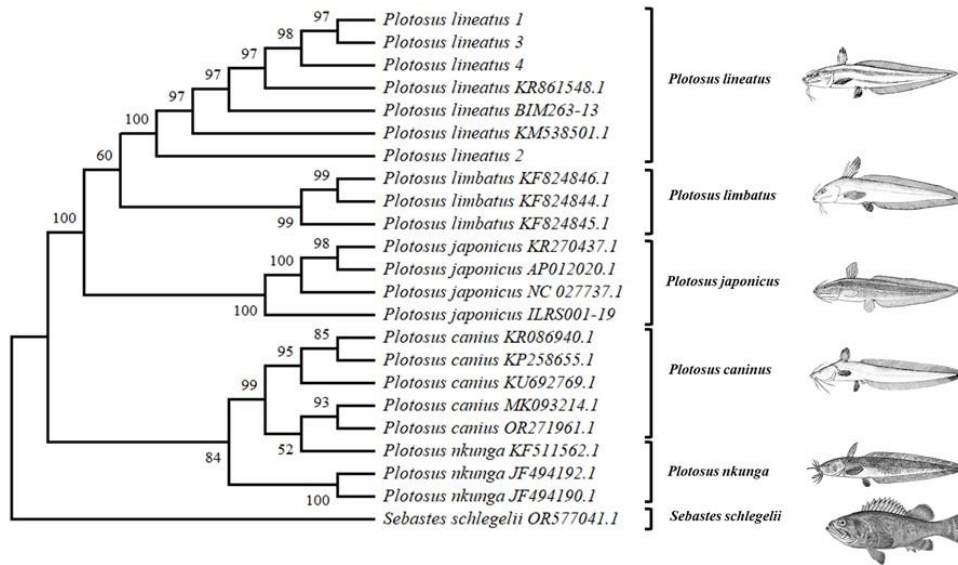


Figure 1. Neighbour-Joining (NJ) phylogenetic tree based on COI sequences. Fish drawings from Froese & Pauly (2023). The NJ tree was constructed by using the outgroup species *Sebastes schlegelii*. In the tree created with the bootstrap test (1000 replicates), branches with a bootstrap value of less than 50% were reduced.

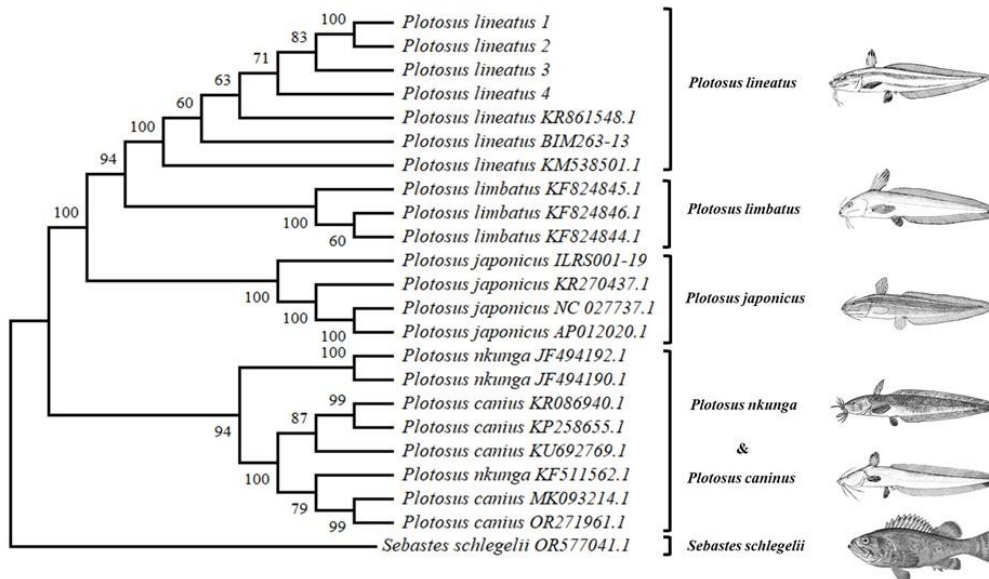


Figure 2. Maximum Parsimony (MP) phylogenetic tree based on COI sequences. Fish drawings from Froese & Pauly (2023). The tree was constructed by using the outgroup species *Sebastes schlegelii*. In the MP tree created with the bootstrap test (1000 replicates), branches with a bootstrap value of less than 50% were reduced.

Discussion

The majority of Lessepsian fish entering the Mediterranean Sea probably originate from populations in the Red Sea (Gollani et al., 2021; Azzurro et al., 2022). In this study, *Plotosus lineatus* specimens distributed in Turkish marine waters were genetically analysed and *P. lineatus* were identified by the NJ and MP trees with a high bootstrap value. At the species level, the obtained *P. lineatus* sequences have a 100% identity match with KR861548.1 (Bariche et al., 2015) and KM538501.1 (Shirak et al., 2016) from the Red Sea according to the blast results obtained from the Genbank database. Moreover, according to the NJ and MP phylogenetic trees, *Plotosus lineatus* were found as genetically distinct from other *Plotosus* species. Goren et al. (2020) recorded *Plotosus japonicus* from the Gulf of Aqaba using the COI gene region and reported that *P. lineatus* and *P. japonicus* showed a sympatric distribution. Therefore, they concluded that the records of these species should be genetically confirmed. Species identification studies, combining both morphological and molecular tools, are becoming increasingly common, allowing clear taxonomic identity to be established (Vella et al., 2016; Karan et al., 2019; Deidun et al., 2020; Turan et al., 2020).

In conclusion, this study proves the presence of *Plotosus lineatus* in Turkish marine waters and in the Mediterranean Sea. Genetic analyses showed that *P. lineatus* is the species that entered the Mediterranean Sea and showed genetic differences from other *Plotosus* species at the species level.

Conflicts of Interest

The authors declare that there are no conflicts of interest or competing interests.

Author Contributions

Cemal TURAN: Designing of the study, identification of species, data analysis, checking-original draft preparation. Ayşegül ERGENLER: Supported the laboratory study, sample collections, and checking-original draft preparation. Funda TURAN: Data analysis, checking-original draft preparation. Servet Ahmet DOĞDU: Designing of the study, identification of species, data analysis, writing-original draft preparation, submission, writing-review and editing, visualization.

Ethics Approval

Ethics committee approval is not necessary for this study.

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