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Research article

Morphologic and genetic identification of *Pisodonophis semicinctus* (Richardson, 1848) in the Northeastern Levant Basin

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Abstract: On July 27, 2022, a mature female specimen of *Pisodonophis semicinctus* (Richardson, 1848), belonging to the Anguilliformes family Ophichthidae, was captured in the northeastern Levant Basin of the Mediterranean. The observation took place at a depth of approximately 20 meters, off the Seyhan River, in an area characterized by a sandy and muddy bottom. The specimen measured 92.5 cm in total length. The total weight was 683 gr. Notably, it exhibited full ripe gonads, indicating its readiness for reproduction. The stomach of the specimen contained partially digested hard-shelled crustaceans and mollusks. Genetic analysis of the Cytochrome Oxidase-1 (COI) gene provided valuable sequence information, applicable for future molecular identification and phylogenetic studies. The presence of a mature female with a large size and a full stomach suggests the area's potential suitability for *P. semicinctus* in the Levant Basin.

Keywords: Alien Species, Anguilliformes The saddled snake eel, COI, DNA-Barcoding, Ophichthidae, the Eastern Mediterranean, Türkiye.

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Introduction

The Mediterranean Sea is a hotspot for invasion because alien species have been introduced via various ways such as mainly the Suez Canal and Gibraltar. Reporting on the presence and progress of alien species and gathering information about their genetics and key biological aspects such as reproduction is important so that their invasive potential can be assessed.

The family Ophichthidae (snake eels) is a diversified anguilliform family distributed mainly in the coastal areas of tropical and warm-temperate seas (Froese & Pauly, 2023). The family includes 62 valid genera and 360 species around the world (Eschmeyer et al., 2023). To date, seven species of ophichthids have been reported in the Mediterranean (Kovačic et al., 2021), six of which, *Apterichtus caecus* (Linnaeus, 1758), *Dalophis imberbis* (Delaroche, 1809), *Echelus myrus* (Linnaeus, 1758), *Ophichthus rufus* (Rafinesque, 1810), *Ophisurus serpens* (Linnaeus, 1758) and *Pisodonophis semicinctus* (Richardson, 1848) have also been reported in various parts of the Aegean and Mediterranean coasts of Türkiye.

The saddled snake eel, *Pisodonophis semicinctus* (Richardson, 1848) is a rare ophicthyctid fish native to the tropical areas of the eastern Atlantic. It introduced to the Mediterranean via Gibraltar and its first Mediterranean record was from Algeria in 1957 (Dieuziede & Roland 1958). Since then, nine more specimens have been found in the western and central Mediterranean (Bodilis et al., 2012) with several records from France (Escubet et al., 1981, Bodilis et al., 2012), Tunisia (Ragonese and Giusto, 2000, Bradai et al., 2004), Italy (Insacco and Zava,1999; Serena, 2001; Cantone et al., 2003). Its occurrence in the

eastern Mediterranean has first been reported in 2009 by Bilecenoglu et al. (2009) from Ekincik Bay, Türkiye, south-eastern Aegean Sea. Then further records have been presented from Yeşilovacık Bay (Yağlıoğlu & Ayas, 2016) and Antalya Bay (Gökoğlu et al., 2020) in the northern Levant Basin. Apart from these reports, there is no information on the biology of the species.

Information on the sequence of Cytochrome Oxidase-1 (COI) gene region of mitochondrial DNA is important because it is extensively used as a barcode gene in molecular species identification (Hebert et al., 2003). Until now, only one specimen of *P. semicinctus* collected from the eastern Atlantic coast of Nigeria (Bold ID: NIFIB019-22) has been registered in genetic databases. However, no records of this species are available from the Mediterranean region, where it exists beyond its native distribution range. Furthermore, the single available COI sequence is stored in a private database, hindering further analysis and accessibility. This study presents the easternmost occurrence of the species in Mersin Bay, Levant Basin, and also provides essential COI sequence information for the species.

Material and Methods

On July 27, 2022, a specimen of the saddled snake eel was successfully captured by a fisher utilizing shrimp trammel nets. This incident took place at an approximate depth of 20 meters, precisely off the Seyhan River, in the northeastern part of the basin, as indicated in Figure 1. The specific coordinates of the capture site were recorded as 36.64°N latitude and 35.10°E longitude. The immediate surroundings of the area showed a distinctive terrain, featuring a sandy and muddy bottom. The specimen was captured by a local fisher and subsequently reported to our research team. It was then transported to the laboratory at Çukurova University, Fisheries Faculty, with the collection code CUSO001-22. Morphological identification of the specimen was conducted, adhering to the guidelines provided by Bilecenoglu et al. (2009).

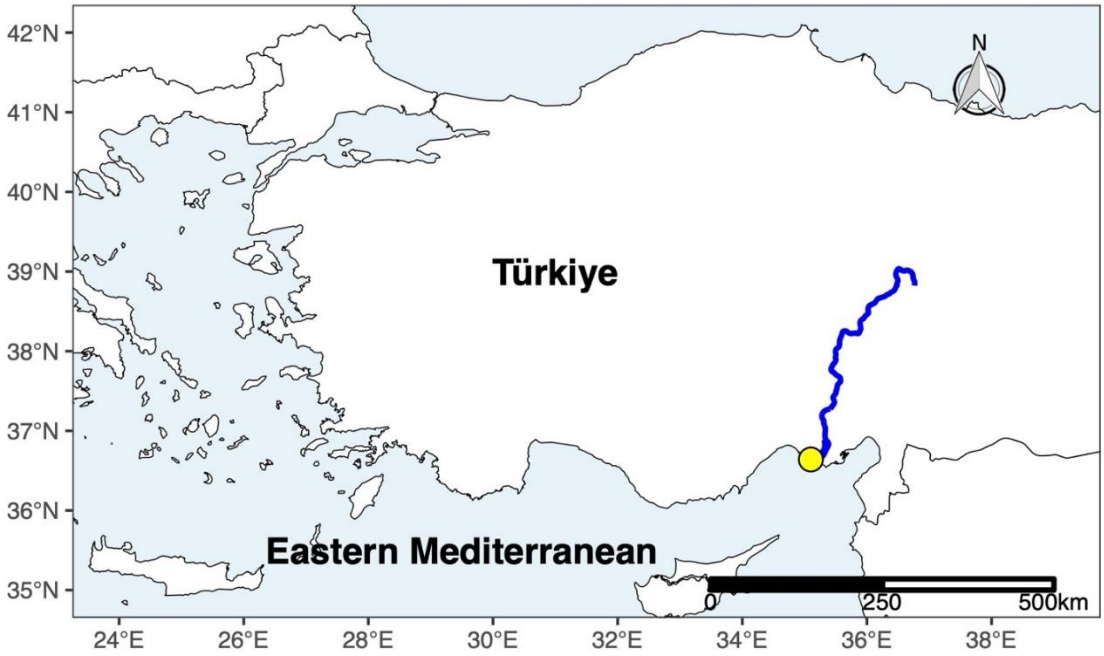

Figure 1. The map of the area where a female specimen of *Pisodonophis semicinctus* was collected in the Mersin Bay. The blue line indicates the Seyhan River, the yellow point represents the sampling location, situated at coordinates 36.64°N, 35.10°E.

A fin clip was extracted from the dorsal fin of the specimen, and subsequently, its COI sequence was analyzed following specific procedures. First, mitochondrial DNA was isolated from the fin clip using the phenol-chloroform method. Then, the targeted gene region was amplified using specific primer pairs fish-F1, R1, or fish-F2, R2 (Ward et al., 2005) and DreamTaq DNA polymerase (Thermo Fisher Scientific Baltics, UAB, Vilnius, Lithuania). A PCR mixture of 20 µL was prepared, consisting of 2 µL of isolated genomic DNA (29 ng/µL), 5 µL of 10X buffer containing 25 mM, 1 µL of 10 mM dNTP mix, 1.4 µL of each primer (10 pmol/µL), 0.25 µL of Taq DNA polymerase (5 U/µL), and 37.95 µL of nuclease-free water. The thermal cycling process was conducted using a Veriti® thermal cycler (Applied Biosystems, Foster City, CA, USA). Once the expected

band was confirmed in gel electrophoresis, PCR products were purified using the NucleoSpin® gel and PCR clean-up kit (Macherey Nagel, Düren, Germany) and then sequenced using the same primers. Following laboratory procedures, the obtained sequences were edited with Geneious Prime 2021.0.3 (https://www.geneious.com) software and subsequently submitted to the GenBank database with the accession code OQ054579.

The nearest neighbor phylogenetic tree was generated by using BOLD identification engine's tree-based identification module, employing the Kimura Two Parameter Distance model. Visualization of the tree was accomplished through FigTree v1.4.4 software, accessible at http://tree.bio.ed.ac.uk/software/figtree/.

Results

The captured specimen was thoroughly examined, revealing intriguing details about its characteristics. It was identified as a mature female, measuring 92.5 cm in total length (TL). Furthermore, the specimen exhibited the intriguing feature of full ripe gonads, a critical indicator of its reproductive state (Figure 2). The total weight of the specimen was determined to be 683 gr, with the gonad weight recorded at 29.28 gr. In the gut, there were some digested pieces of unidentifiable hard-shelled mollusks and crustaceans.

The observed specimen exhibited an elongated body with a typical snake-like shape. Notably, the tail's tip lacked fins and displayed a rigid structure. Additionally, well-developed pectoral fins were observed, positioned slightly behind the origin of the dorsal fin. Regarding its coloration, a yellowish hue predominated, adorned with 16 dark patches covering the dorsal region and approximately 75% of the lateral body. The morphometric measurements, expressed as proportions of standard length (SL), were as follows: pre-dorsal length 8.4% of SL, pre-anal length 40%, pre-pectoral length 10%, body depth 4.9%, head width 5.9%, snout length 2.3%, and eye diameter 0.8% of SL (see Figure 2).

According to the BOLD identification engine, our specimen clustered together with the only registered *P. semisinctus* specimen from Nigerian shores, demonstrating a high similarity level of 99.37%. Furthermore, three species of the genus Myrichthys, namely *Myrichthys colubrinus*, *Myrichthys maculosus*, and *Myrichthys breviceps*, showed an almost 85% similarity to our *P. semisinctus* specimen (Figure 3).

Discussion

The results obtained from the captured specimen of the saddled snake eel provide valuable insights into its biology, characteristics and reproductive status, as well as its potential distribution in a new area. These findings could potentially have implications for the species' population dynamics and ecological interactions in the region.

According to the criteria set by CIESMs' (Conservation of the Inland European Seas Monitoring), a fish population can be classified as established if it has been independently recorded at least three times, either in different time periods or in different locations (Golani et al., 2017). Prior to this study, the saddled snake eel had been reported in two other locations along the Turkish coasts of the Mediterranean: Yeşilovacık Bay (Yağlıoğlu and Ayas, 2016) and Antalya Bay (Gökoğlu et al., 2020). By adding our study's findings, which present the species' third recorded occurrence in this area, the CIESMs' establishment criteria for the saddled snake eel population are clearly met.

Additional important evidence supporting the existence of an established population of this species is the presence of full ripe gonads in the captured female specimen. This discovery holds great significance as it indicates, for the first time, the species' reproductive activity in its new distribution area, suggesting that the saddled snake eel is capable of spawning in the northeastern Levant Basin.

The captured specimen in this study measured 92.5 cm, which stands out notably larger than the majority of previously reported sizes for this particular species. Froese and Pauly (2023) have documented that the saddled snake eel can attain a maximum length of 80 cm, TL (Bauchot, 1987), although larger sizes are frequently observed. The first Mediterranean specimen of this species, reported from Algeria, measured 81.2 cm TL as indicated by Bodilis et al. (2012). In 2009, Bilecenoglu et al. (2009) reported an individual measuring 86 cm TL from Sığacık Bay in the eastern Aegean Sea. The largest Mediterranean individual, measuring 94 cm TL, was reported from the Tyrrhenian Sea in the Western Mediterranean (Serena 2001), slightly surpassing the length of the specimen in our study.

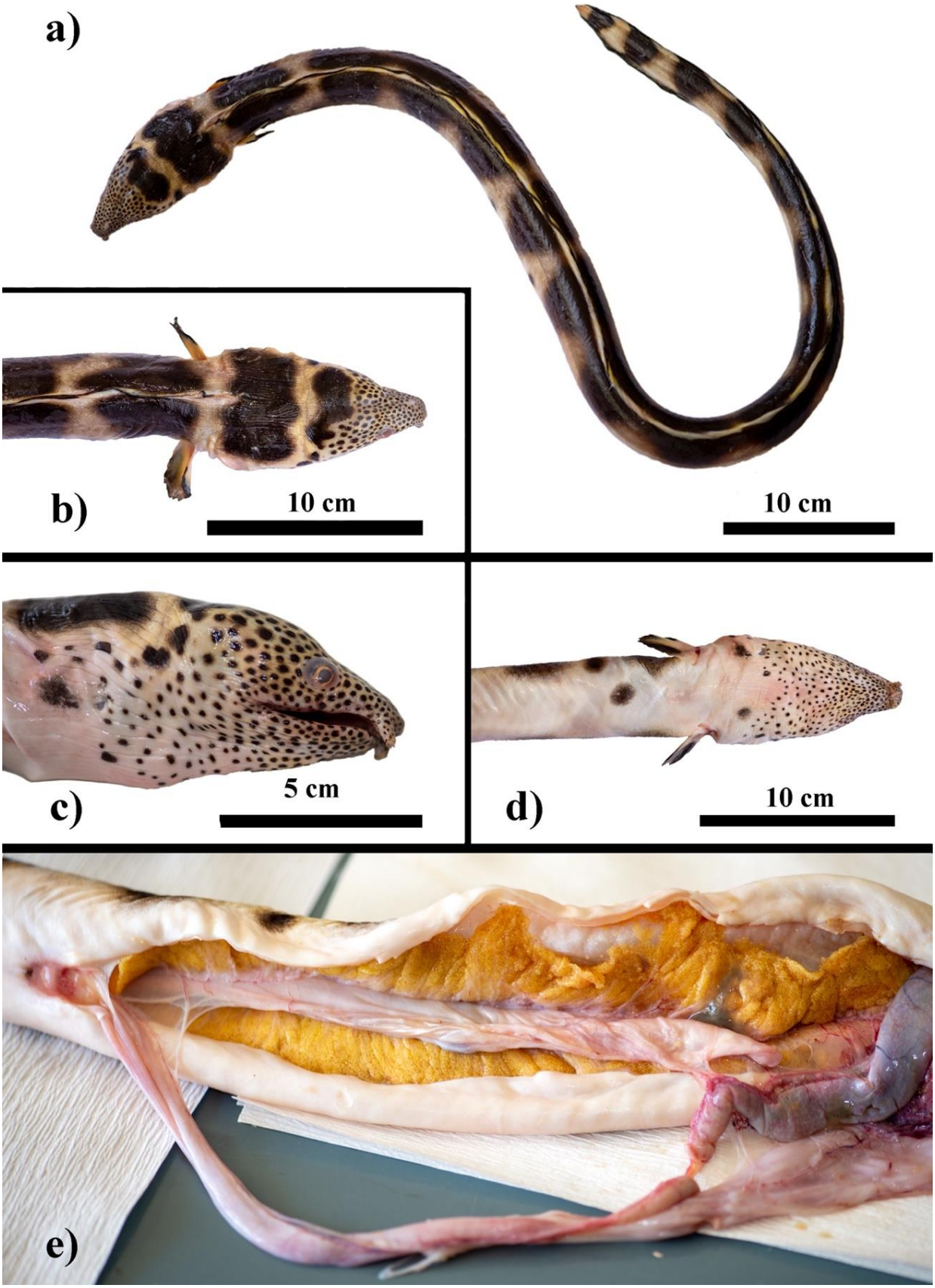

Figure 2. Body shape (a), dorsal (b), lateral (c) and ventral (d) view of the head of *Pisodonophis semicinctus* sampled off the Seyhan River, Mersin Bay, Türkiye. Gonads of the specimen (e).

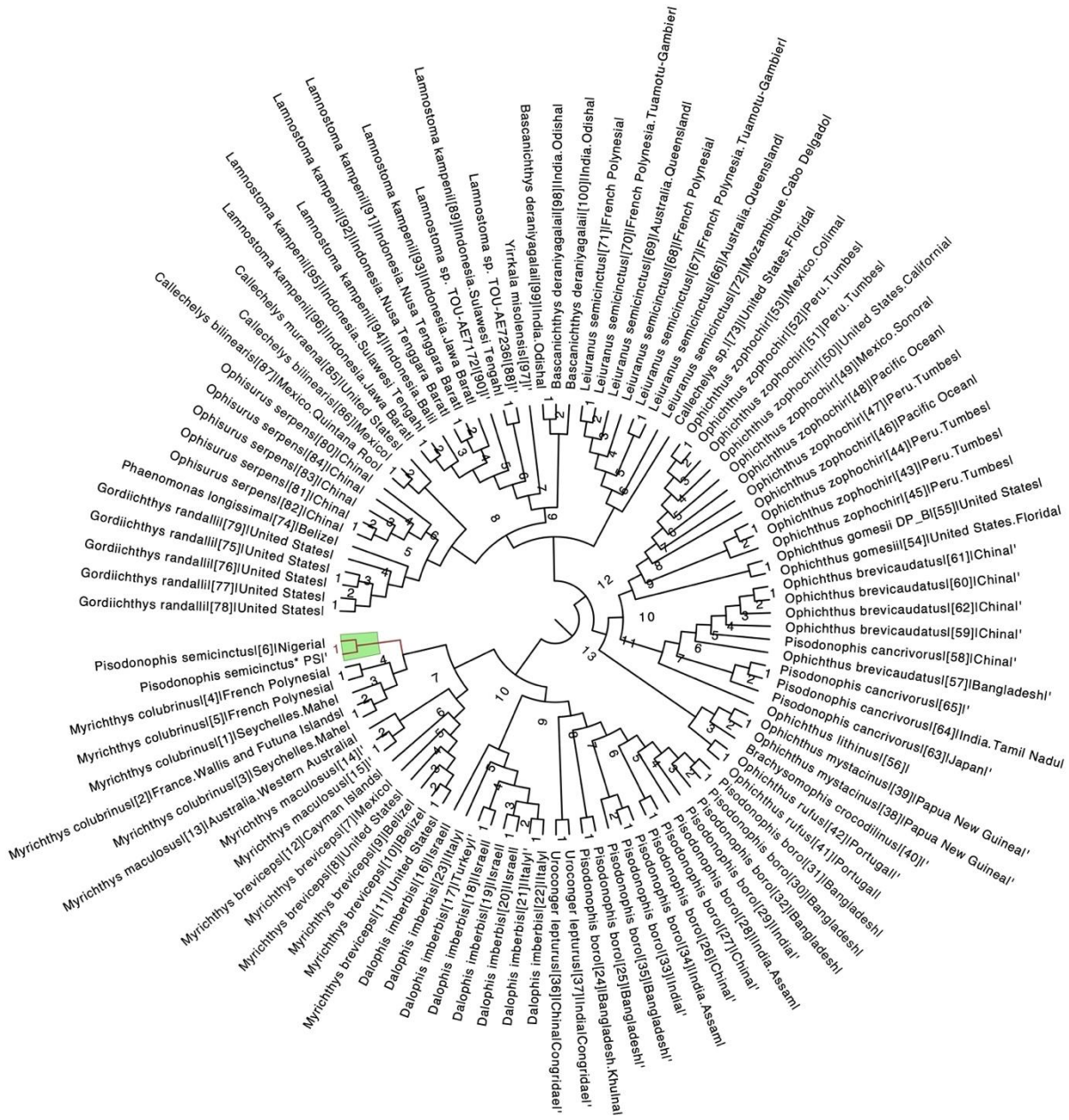

Figure 3. The phylogenetic tree of *Pisodonophis semicinctus*, with the cluster of *P. semicinctus* specimens identified by a green polygon. The specimen from our present study is denoted by "PS*".

The size distribution within a population is a crucial indicator of its overfishing status; populations experiencing lower fishing pressure tend to achieve larger sizes (Beverton and Holt, 1957). Despite being recognized as an economically important fish within its native range (Froese and Pauly, 2023), the saddled snake eel remains unfamiliar to Turkish fishers. Consequently, the lack of fishing pressure may account for the large body sizes observed in this study.

The knowledge of the feeding habits of the saddled snake eel relies on the taxonomic synthesis by Leiby (1990), which suggests that the species primarily consumes small benthic invertebrates, particularly crustaceans and mollusks. While our study was not explicitly focused on investigating the feeding behavior of the species, our findings align with and further support this existing information.

For the first time in the Mediterranean, the COI gene sequence of the saddled snake eel is presented in this study. The COI gene is widely utilized in taxonomic and population genetics studies. Therefore, our findings will serve to advance future research, facilitating a deeper

understanding of the spreading dynamics of this species in the Mediterranean.

In summary, the capture of a fully mature female saddled snake eel, exhibiting full ripe gonads, a large size close to its maximum achievable length, and a full stomach, suggests the area's potential suitability for the species. However, additional research is necessary to investigate its ecological role and population dynamics in the Levant Basin.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical Approval

No need to ethical approval for this study.

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