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First record of the phyllosoma larva of the pygmy locust lobster *Scyllarus pygmaeus* (Crustacea, Decapoda) in the eastern Mediterranean sea

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Marine Record

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Abstract

Taxonomic intricacies and high interspecific similarity have hampered the identification of scyllarid phyllosoma larvae to the species level. The pygmy locust lobster, *Scyllarus pygmaeus*, is distributed across the Mediterranean Sea and in the eastern Atlantic; however, its phyllosoma larvae were previously recorded only from the western Mediterranean. We employed DNA barcoding using the mitochondrial COI gene to identify *S. pygmaeus* phyllosoma collected from the offshore waters of the southeastern Mediterranean Sea and described its morphology. We further discuss the lack of genetic structure in S. *pygmaeus* with potential implications for species connectivity and conservation.

Introduction

Slipper lobsters of the family Scyllaridae are widespread in shallow temperate and tropical seas, including the Mediterranean Sea (Lavalli and Spanier, 2007). Among scyllarids, the pygmy locust lobster, *Scyllarus pygmaeus* (Bate, 1888), is the smallest in the Mediterranean and therefore was initially considered to be a juvenile of the co-occurring small European locust lobster, *S. arctus* (Pessani and Mura, 2007), probably due to the similar serrated flat antennae. The distribution of *S. pygmaeus* spans across the Mediterranean Sea (but according to Holthuis, 1991, had not yet been reported from the North African coast east of Morocco) and extends to the islands in the eastern Atlantic Ocean, including Madeira, the Canary Islands, and Cape Verde (Figure 1), at depth of 5 to 1200 m (Holthuis, 1991; Pessani and Mura, 2007). Although adults of *S. pygmaeus* have been observed in the eastern Mediterranean Sea (Levantine Basin) (Lewinsohn, 1974; Lewinsohn and Holthuis, 1986), scyllarid larvae previously found in this region could not be confidently identified as *S. pygmaeus*.

Scyllarid development consists of a short-lived 'prelarval' naupliosoma that lasts only minutes, followed by planktonic phyllosoma larval stages (instars) lasting weeks to months (Booth *et al.*, 2005). The final phyllosoma instar eventually metamorphoses into the nektonic postlarva, known as a nisto or pseudibacus, which settles and initiates the benthic phase (Williamson, 1969). Using phyllosoma morphological characters to identify the scyllarid species is challenging, as not all stages have been described or correctly assigned (Pagliarino *et al.*, 2013). Furthermore, determining the exact phyllosoma developmental stage can be difficult, because many authors disagree on the discriminating features (Pessani and Mura, 2007; Pagliarino *et al.*, 2013). The fragile, long-lived phyllosoma larvae are hard to maintain in laboratory conditions, and therefore the complete life cycle of only a few scyllarid lobsters has been documented (Satoshi and Kuballa, 2007; Wakabayashi *et al.*, 2012). Employing integrative taxonomy by combining DNA barcoding and morphological characterization is therefore imperative for establishing the phyllosoma larval stages of scyllarid species.

Using laboratory-hatched eggs, Mura and Pessani (1994) described the first phyllosoma stage of *S. pygmaeus*. The last phyllosoma stage, collected in the northwestern Mediterranean Sea, was described by employing DNA barcoding to authenticate the species identity (Palero *et al.*, 2008). An intermediate stage of *S. pygmaeus* phyllosoma was recorded in the Balearic Sea, western Mediterranean, using DNA barcoding, and further phyllosomata were identified using morphological features (Mallol *et al.*, 2014). In Sicily, the central Mediterranean Sea, scyllarid phyllosomata were found and speculated to be intermediate stages of *S. arctus* or *S. pygmaeus*; however, the exact taxon was not assigned (Pagliarino *et al.*, 2013). Here we used integrative taxonomy to describe an intermediate stage phyllosoma of *S. pygmaeus*, recorded for the first time in the eastern Mediterranean Sea.

Materials and methods

A plankton sample was collected in 12 December 2022 at 20:53–21:13 using a horizontal tow of a Bongo net (65–200 µm mesh size, Sea-Gear, USA) at a depth of 0.5–1 m in the offshore

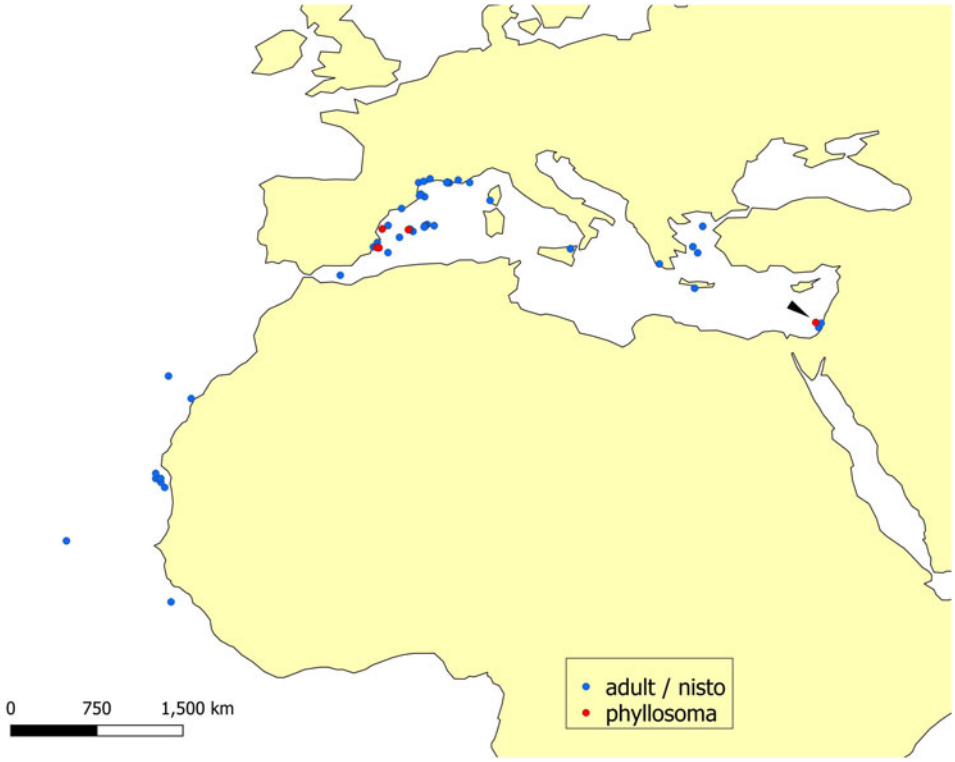

Figure 1. The distribution map of *Scyllarus pygmaeus*. Adult (including nisto) and phyllosoma records are presented in blue and red circles, respectively. Occurrences were downloaded from GBIF.org (https://doi.org/10.15468/dl.2haffv), OBIS https://obis.org/ and BOLD https://www.boldsystems.org/ on 18 February 2024. The arrowhead is directed towards the new phyllosoma record described in this study.

waters (bottom depth 1100 m) of the central Israeli Exclusive Economic Zone, southeastern Mediterranean Sea (start: 32.2103641°N, 34.224159°E; end: 32.215101°N, 34.232902°E, Figure 1). The 65 and 200 µm samples were merged together and stored in −20°C pending analysis.

In the lab at the National Institute of Oceanography, Israel Oceanographic and Limnological Research (IOLR), the thawed sample was examined under a stereomicroscope (SZX16, Olympus, Japan). A phyllosoma larva was identified morphologically following Pessani and Mura (2007) and Palero *et al.* (2008), and the developmental stage was determined following Santucci (1925). One pereiopod was cut for molecular barcoding, and the specimen was stored in 70% ethanol and deposited in the zooplankton collection, the National Natural History Collections, Hebrew University of Jerusalem (NNHC, HUJI).

Total genomic DNA was extracted from the pereipod using the DNeasy Blood and Tissue kit (QIAGEN, Germany) according to the manufacturer's specifications. Following the DNA extraction, the cytochrome *c* oxidase subunit I (COI) gene was amplified using PCR with universal primers LCO1490 and HCO2198 (Folmer *et al.*, 1994). Reaction conditions were as follows: 94°C for 2 min, followed by 5 cycles of 94°C for 40 s, 45°C for 40 s, and 72°C for 1 min, and followed by 30 cycles of 94°C for 40 s, 51°C for 40 s, and 72°C for 1 min, and a final elongation step of 72°C for 10 min. Obtained PCR products were purified and sequenced by Hylabs (Rehovot, Israel).

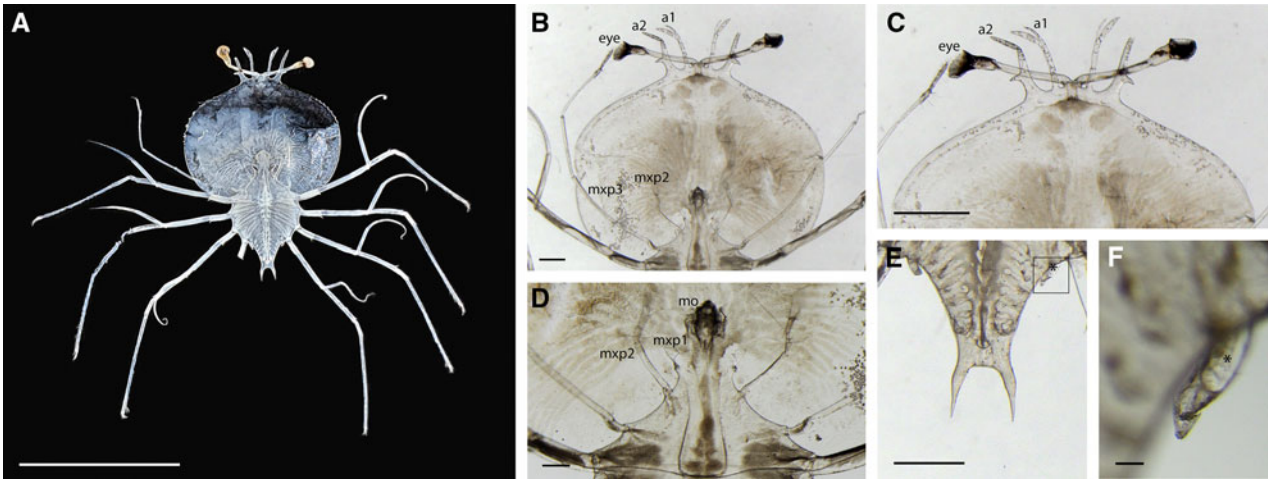

Figure 2. *Scyllarus pygmaeus* phyllosoma stage VII (following Santucci, 1925). **A**. dorsal view. **B–D**. Cephalic shield with antennule (A1), antenna (A2), eyes, and maxillipeds 1–3 (mxp1, mxp2, mxp3). **E**. Abdomen with forked telson. **F.** Pereipod 5 (P5) bud (marked by *). Scale bars: A – 1 cm, B – 1 mm, C – 1 mm, D – 0.5 mm, E – 1 mm, F – 0.1 mm.

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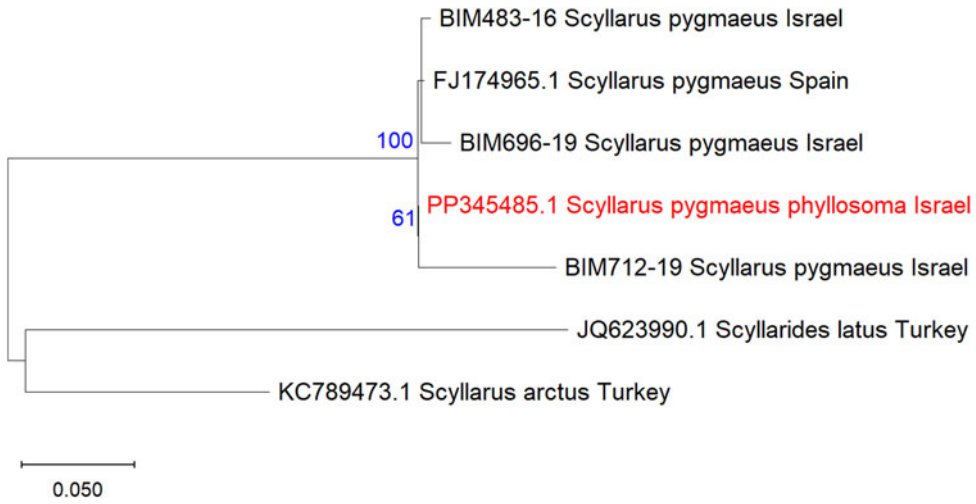

Figure 3. Maximum-likelihood phylogenetic tree of *Scyllarus pygmaeus* based on the COI gene, using the T92 + G substitution model. The outgroups *Scyllarus arctus* and *Scyllarides latus* were used as a root node. The numbers in blue indicate the percentage of ML bootstrap support (1000 replicates) for nodes that received at least 60% support. The scale bar denotes the estimated number of nucleotide substitutions per site.

A total of seven COI sequences of Scyllaridae were analysed, including one sequence of the phyllosoma of *S. pygmaeus* obtained in this study, three sequences of adult *S. pygmaeus* from Israel obtained from BOLD (BIM483-16, BIM696-19, BIM712-19), one sequence of *S. pygmaeus* phyllosoma from western Mediterranean (Spain) obtained from NCBI GenBank (FJ174965.1). Sequences of *Scyllarus arctus* (KC789473.1) and *Scyllarides latus* (JQ623990.1) from Turkey were used as an outgroup. Sequence alignment was conducted using ClustalW embedded in MEGA v11.0 (Tamura *et al.*, 2021). The best-fitting substitution model was selected according to the Bayesian information criterion using maximum-likelihood (ML) model selection in MEGA. ML analysis was performed using the T92 + G model with 1000 bootstrapping replicates.

Results

Morphological description

Scyllarus pygmaeus (Bate, 1888): phyllosoma, stage VII (following Santucci, 1925) (Figure 2).

Dimensions: TL (total length) 12.96 mm; CL (cephalic length) 8.06 mm; CW (cephalic width) 9.84 mm; TW (thorax width) 4.16 mm; EL (eye length) 3.30 mm; A1L (antennular length) 2.02 mm; A2L (total antennal length) 1.96 mm; AbdL (abdomen length) 3.90 mm.

Cephalic shield subrectangular, 1.6 times wider than long and three times wider than thorax; stalked eye, longer than antennule and antenna. Antennule biramous, penduncle 3-segmented; inner ramus unsegmented. Antenna forked, flat. First maxilliped bud flat, unarmed and large with wide anterior and posterior lobes. Second maxilliped of three segments. Third maxilliped long, of five segments. Periopods 1–3 bearing ventral coxal and subexopodal spines. Periopod 5 bud. Pleopod buds present. Uropods absent. Gills absent. Telson forked.

DNA barcoding

The DNA barcode consisting of a fragment of 638 bp of the COI gene was sequenced from the phyllosoma larva of *S. pygmaeus* and assembled from forward and reverse sequences. The sequence was deposited in NCBI GenBank under the accession number PP345485. NCBI blastn yielded 99.68% identity. Maximum likelihood analysis of Scyllaridae sequences obtained from GenBank and BOLD (Figure 3) did not show a clear separation between the *S. pygmaeus* phyllosoma obtained in this study, and the adult *S. pygmaeus* previously collected in the Israeli Mediterranean or the *S. pygmaeus* phyllosoma from the northwestern Mediterranean (Spain).

Discussion

The pygmy locust lobster, *Scyllarus pygmaeus*, was first described in 1888 by Bate from material collected in the Canary Islands during the HMS *Challenger* expedition (Bate, 1888). It was further recorded from the Mediterranean Sea, Cape Verde Islands, and Madeira at depths from 5 to 1200 m (Forest and Holthuis, 1960; Holthuis, 1991). In the Mediterranean Sea, the presence of *S. pygmaeus* was overlooked for decades due to the confusion with *S. arctus*, whose immature form was mistakenly identified as *S. pygmaeus* (Lewinsohn, 1974). The juvenile form was described separately as the species *Nisto laevis* (Sarato, 1885). Only more than a century later, DNA analysis revealed it to be juvenile *S. pygmaeus* (Palero *et al.*, 2009). Lindley *et al.* (2004) hypothesized that the intermediate phyllosoma specimens from the eastern Mediterranean described by Stephensen (1923) belong to *S. pygmaeus* given its prominence in the region. However, the high interspecific larval similarities and inconsistent delineation of stages by different authors hampers the validation of these records.

Mura and Pessani (1994) described the morphology of the first phyllosoma larval stage of *S. pygmaeus* from eggs hatched in the laboratory. Palero *et al.* (2008) described the morphology of the final phyllosoma larval stage of *S. pygmaeus* collected in the northwestern Mediterranean Sea, identified by DNA barcoding. Similarly, Mallol *et al.* (2014) used DNA barcoding to identify the phyllosoma of *S. pygmaeus* collected from the Balearic Sea. Pagliarino *et al.* (2013) reported the finding of six Scyllaridae phyllosoma larvae from the waters around Sicily. Nonetheless, since their identification was only based on morphological characters, and as the specimens were of earlier stages than those reported by Palero *et al.* (2008), they could not attribute the phyllosomata to a specific taxon. One of the two phyllosoma morphotypes they described, 'specimen e', was similar in size (TL: 16 mm) to the phyllosoma described in this study, but the shapes of the first maxilliped and the telson differ. Based on total length and further morphological characters, we can assign the *S. pygmaeus* phyllosoma to stage 6–7 (following Stephensen, 1923), stage 7

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(following Santucci, 1925), or stage 9 (following Fiedler and Spanier, 1999). These stages were identified by the three previous authors as *S. arctus* but were likely *S. pygmaeus* (see Pessani and Mura, 2007 for further explanation).

While ovigerous females of *S. pygmaeus* have been recorded peaking between May and July (Pessani and Mura, 2007), the final phyllosoma stage described by Palero *et al.* (2008) was collected in May, suggesting phyllosoma larval period of about 10 months. We can therefore estimate the age of the phyllosoma larva described here to 6 months. This estimation should be treated with caution since Lewinsohn and Holthuis (1986) reported a prolonged reproductive season in the eastern Mediterranean, lasting until December. Although the available information is scarce, our molecular analysis, based on the mitochondrial COI gene, did not indicate a population level genetic structure. Faria *et al.* (2013) studied the population genetics of *S. latus* in a wide distribution range in the northeastern Atlantic and western Mediterranean and found that it is panmictic across its distributional range. They attributed this to high fecundity and long-lived pelagic larvae. It is plausible that *S. pygmaeus* individuals also belong to a single panmictic population. Such a pattern could have implications for future conservation efforts as populations that are genetically panmictic may be more susceptible to the effects of genetic drift, demographic fluctuations, and stochastic events (Lacy, 1987). Further studies on the population genetics and larval development of the pygmy locust lobster *S. pygmaeus* are needed to reveal its recruitment dynamics and connectivity patterns.

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Competing interests. None.

Data Availability Statement. The data underlying this article are available in the GenBank Nucleotide Database at https://www.ncbi.nlm.nih.gov/genbank/, and can be accessed with accession number PP345485.1.

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