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A bycatch surprise: *Scyllarus subarctus* Crosnier, 1970 (Decapoda: Achelata: Scyllaridae) in the Mediterranean Sea

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ABSTRACT

Scyllarus Fabricius, 1775 includes nine species worldwide, three of which have been recorded from the Mediterranean Sea: *S. arctus* (Linnaeus, 1758), *S. caparti* Holthuis, 1952, and *S. pygmaeus* Bate, 1888. A *Scyllarus* specimen not ascribable to any of the previous species was trawled in November 2020 in unconsolidated substrates in the Gulf of Naples, Italy. The sample was subsequently identified through an integrative taxonomic approach as *Scyllarus subarctus* Crosnier, 1970, a species originally described from southern Angola (Eastern Atlantic Ocean) and apparently expanding northward along Western Africa. It is possible that this taxon may have been already present in the Mediterranean but went overlooked or undetected. Results obtained through molecular analyses underlined the necessity of an extensive update of the supraspecific taxonomy of scyllarids.

Key Words: geographical distribution, Gulf of Naples, slipper lobsters, 16S rRNA gene

The family Scyllaridae Latreille, 1825 includes about 90 species of slipper lobsters distributed in sandy-muddy to rocky substrates, including seagrass meadows and coral reefs, from coastal to upper-slope areas in tropical, subtropical, and temperate regions worldwide (Webber & Booth, 2007; Yang *et al.*, 2012). The family has had a troublesome taxonomic history due to limited sampling and difficulties in establishing significant morphological traits (Holthuis, 1946, 1985, 1991, 2002; Brown & Holthuis 1998; Genis-Armero *et al.*, 2017, 2019). It nowadays includes 22 genera distributed in four subfamilies (WoRMS [https://www.marinespecies.org/aphia.php?p=taxdetails&id=106795]). Among them, *Scyllarus* Fabricius, 1775 is recognizable by developed teeth on the carapace, absence of a carina on antennal segment 4, an arborescent pattern on the dorsal abdominal surface, a simple pereiopod 3 propodus, and the 'U-shaped' anterior part of the thoracic sternum (Holthuis, 2002; Yang *et al.*, 2012). It includes nine species, four of which are distributed in the Western Atlantic [*S. americanus* (Smith, 1869), *S. chacei* Holthuis, 1960, *S. depressus* (Smith, 1881), and *S. planorbis* Holthuis, 1969)] and five in the Eastern Atlantic [(*S. arctus* (Linnaeus, 1758), *S. caparti* Holthuis, 1952, *S. paradoxus* Miers, 1881, *S. pygmaeus* Bate, 1888, and *S. subarctus* Crosnier, 1970]. Two have been commonly reported in the Mediterranean, *S. arctus* and *S. pygmaeus* (Pessani & Mura, 2007; Palero *et al.*, 2011), whereas *S. caparti* mostly extends along the Eastern Atlantic but it is known from two presumably human-mediated records from the Mediterranean: Adriatic and Levantine seas (Holthuis, 1952; Froglia, 1979; Relini & Vallarino, 2016).

Several specimens of *Scyllarus* were found as bycatch of commercial and scientific trawling operations equipped with bottom trawl nets (mouth of 3 × 4 m, 18–40 mm mesh), towed at ~2–2.5 knots on muddy bottoms in the Gulf of Naples, Italy (Crocetta *et al.*, 2020b). Among them, an odd specimen (female, 15.5 g, 32 × 28.3 mm in carapace length × width, 109 mm in total length from the antennal segments to the posterior tip of the telson; Fig. 1A–C) was found off Nisida Island (~40.789346N, 14.143998E), on 9 November 2020, at a depth of ~150–200 m. It was frozen for further examination as it showed similarities with *S. arctus* but subtle differences in carapace width, sculpture, and colour. The specimen showed an irregular arrangement of rostral, pregastric, and gastric teeth (with the distance between pregastric and gastric greater than the one between rostral and pregastric teeth), as well as a difference in the size of the median gastric tooth.

The specimen was defrosted, identified at species level following Crosnier (1970), Fischer *et al.* (1987), Holthuis (1991), Navas & Campos (1998), Dall'Occo (2010), and Genis-Armero *et al.* (2020), and deposited (SZN-B-1224CR121A) in the collection of the Laboratory of Benthos-Napoli, Stazione Zoologica Anton Dohrn, Naples.

Total genomic DNA was extracted from the ventral part of the abdomen as described by Crocetta *et al.* (2020c). Because the 16S rRNA gene shows a higher amplification rate than the COX1 gene in Achelata (Genis-Armero *et al.*, 2020), and almost all *Scyllarus* species were already sequenced for this gene

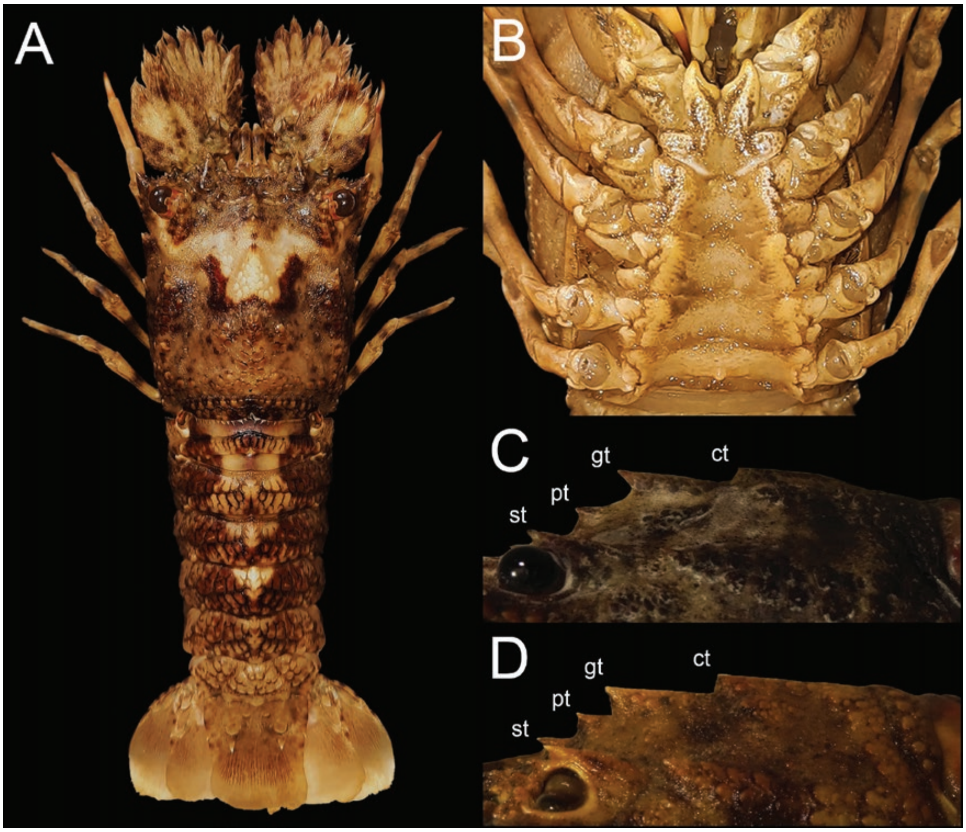

Figure 1. Scyllaridae from the Gulf of Naples, Mediterranean Sea. *Scyllarus subarctus* [SZN-B-1224CR121A] (**A**–**C**). *Scyllarus arctus* [SZN-B-227CR8B] (**D**). Dorsal view (total length 109 mm) (live colour) (**A**). Ventral view (live colour) (**B**). Arrangement of median teeth in *S. subarctus* (carapace length × width 32 × 28.3 mm) and *S. arctus* (carapace length × width 29.7 × 24.9 mm) (preservation in ethanol; rostral tooth hidden by supraorbital tooth) (**C**–**D**). ct, cardiac tooth; gt, gastric tooth; pt, pregastric tooth; st, supraorbital tooth. Specimens not to scale.

fragment, a partial sequence of the 16S rRNA gene was amplified using the universal primers developed by Palumbi (1996): 16Sar_forward 5'-CGCCTGTTTATCAAAAACAT-3'; 16Sbr_reverse 5'-CCGGTCTGAACTCAGATCACGT-3'. The polymerase chain reaction (PCR) was conducted in 25 µl volume reaction, containing 2.5 µl (10×) of Roche buffer (all reagents from Sigma-Aldrich, Darmstadt, Germany), 2.5 µl (2 mM) of Roche dNTPack Mixture, 1 µl each of forward and reverse primer, 0.25 µl (5 U/µl) of Roche Taq DNA polymerase, 1 µl of template DNA, and sterilized distilled water to 25 µl. Amplification was performed with an initial denaturation at 95 °C (5 min), followed by 39 cycles of denaturation at 95 °C (1 min), annealing at 48 °C (1 min), extension at 72 °C (1 min), with an extension at 72 °C (5 min). The PCR product was purified and Sanger sequenced at the Molecular Biology and Sequencing Service of SZN through an Automated Capillary Electrophoresis Sequencer 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA), using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Renfrew, UK). The chromatograms for each sequence obtained were checked, assembled, and edited using Sequencher v.5.0.1 (GeneCodes, Ann Arbor, MI, USA) and compared with reference sequences from the NCBI nucleotide (NT) database using BLASTn (Morgulis *et al.*, 2008). The obtained sequence was deposited in GenBank under the code MW721842.

The NCBI data mining revealed the presence of sixty-eight 16S rRNA partial sequences of seven *Scyllarus* species, thus encompassing all known species except *S. planorbis* and *S. paradoxus*. All were downloaded, together with a sequence of *Crenarctus bicuspidatus* (De Man, 1905) as outgroup, based on its sister relationship with *Scyllarus* taxa (Yang *et al.*, 2012; Genis-Armero *et al.*, 2020) (Supplementary material Table S1). A sequence of *Acantharctus posteli* (Forest, 1963) was also included as the taxon fell in the *Scyllarus* clade in recent phylogenetic works (Yang *et al.*, 2012; Genis-Armero *et al.*, 2020). Sequences were aligned using ClustalW (2.1) on the CIPRES Science Gateway (Miller *et al.*, 2010) using default parameters. The alignment was trimmed and phylogenetic analyses were performed as in Crocetta *et al.* (2020c).

The specimen was first screened for the main diagnostic characters of the three *Scyllarus* species native or recorded from the Mediterranean, but it lacked the: 1) median teeth arrangement on the carapace diagnostic for *S. arctus*, consisting of a gastric somewhat similar to the other teeth and a regularly-spaced arrangement of rostral, pregastric, and gastric median teeth (Fig. 1C, D); 2) transverse hairy ridge on the smooth anterior part of abdominal somites, typically present in *S. pygmaeus*; 3) median crests with tubercles on the dorsal part of abdominal somites 2–4, typically present in *S. caparti*. Congeners can be distinguished as follows: 1) *S. posteli* has two median teeth on the anterior third of carapace anterior to the cervical groove (three in our specimen); 2) *S. paradoxus* has an evident sculpturing on the anterior portion of the abdominal somites (smooth in our specimen); 3) *S. americanus* has a bilobed pregastric median teeth (non-bilobed pregastric median teeth in our specimen); 4) *S. planorbis* lacks the median tubercle on the last thoracic sternite (present in our specimen); 5) *S. chacei* has rounded second (pregastric) and third (gastric) median teeth (acuminate in our specimen); 6) *S. depressus* median teeth on the carapace are regular and generally similar to *S. arctus* (irregular in our specimen) (Crosnier, 1970; Fischer *et al.*, 1987; Holthuis, 1991; Navas & Campos, 1998; Dall'Occo, 2010; Genis-Armero *et al.* 2020). The external morphology nevertheless resembled that of *S. subarctus*, originally described from southern Angola (Eastern

Atlantic Ocean) and more recently expanding northward along the western coast of Africa (Dall'Occo, 2010; Genis-Armero *et al.*, 2020).

BLASTn queries of a 695 bp (base pair) partial sequence of the 16S rRNA gene showed a 98.71–99.76% similarity with *S. subarctus* and a 97.67–97.85% similarity with *S. depressus*, thus leaving some doubts the molecular identification of our specimen. The alignment was trimmed to a shorter length (511 bp) as to include as much samples as possible in the subsequent phylogenetic analyses. The DNA substitution model selected according to the corrected Akaike Information Criterion method was the Hasegawa-Kishino-Yano model (Hasegawa *et al.*, 1985) with discrete Gamma model (Yang, 1994). The Bayesian Inference (BI) ($-lnL$ = 2168.51 for run 1; $-lnL$ = 2168.10 for run 2) and maximum likelihood (ML) ($-lnL$ = 1998.37) analyses arrived at similar tree topologies with well-defined clades often showing high or maximal support, although some relationships between species were not clear (Supplementary material Fig. S2). The overall topology showed one clade (A) with maximal support (BI = 1, ML = 100), including the nominal species *S. chacei* and *S. americanus*, which resulted a sister group of the rest of the taxa. The remaining five species branch off successively (B) (BI = 1, ML = 95), with all nodes in the BI tree being highly supported except for the position of *S. posteli* (F) as sister species of *S. arctus* (H) and *S. subarctus*/*S. depressus* (I) (BI = 0.86). Three clades showed moderate support, with: 1) (F) *S. posteli* as sister species of *S. arctus* (H) and *S. subarctus*/*S. depressus* (I) (ML = 65); 2) (G) *S. arctus* (H) as a sister species of *S. subarctus*/*S. depressus* (I) (ML = 63); 3) (I) with *S. subarctus* and *S. depressus* (ML = 63). All the sequences of *S. pygmaeus* formed a monophyletic group (C) (BI = 1, ML = 98) with sister species (D) (BI = 1, ML = 91). All sequences of *S. caparti* and *S. arctus* also formed monophyletic groups (E) (BI = 1, ML = 91) and (H) (BI = 1, ML = 100), respectively. Relationships between *S. depressus* and *S. subarctus* were unresolved, as they formed a paraphyletic group (I) (BI = 1, ML = 63). The obtained sequence, however, was well-nested within the *S. subarctus* sequences downloaded from GenBank, and thus molecular results confirmed the morphological identification.

Results obtained are in agreement with previous studies: 1) *S. chacei* and *S. americanus* formed a single clade, with these species already nested with species of *Eduarctus* Holthuis, 2002 rather with species of *Scyllarus* (Yang *et al.* 2012); 2) *Acantharctus posteli* nested with *Scyllarus* species in the phylogenies of Yang *et al.* (2012) and Genis-Armero *et al.* (2020); 3) the relationships between *S. depressus* and *S. subarctus* apparently remained unresolved (Genis-Armero *et al.*, 2020). These two nominal taxa may account for a single species with an amphi-Atlantic distribution, with *S. depressus* recently colonizing the Western Atlantic, thus being the only such species clustering with Eastern Atlantic species (Genis-Armero *et al.*, 2020). Another possibility is that the 16S rRNA gene fragment used by Genis-Armero *et al.* (2020) and by us is not able to well-discriminate between the two species. Yang *et al.* (2012) included both species in their phylogeny, but failed to amplify several gene fragments of *S. subarctus*, thus leaving the question open.

Many studies have been undertaken on the marine biota of the Mediterranean basin (Bianchi & Morri, 2000; Coll *et al.*, 2010) and the Gulf of Naples (Gambi *et al.*, 2013; Fasulo *et al.*, 2019); the decapods of the Naples area have been reviewed by Moncharmont (1981), and studied subsequently by Gambi *et al.* (2003), Soppelsa & Crocetta (2004), Soppelsa *et al.* (2005), Thessalou-Legaki *et al.* (2012), and Giacobbe *et al.* (2018). Notwithstanding these studies, *S. subarctus* had never been recorded from the Gulf of Naples and even the entire Mediterranean Sea. Genis-Armero *et al.* (2020) speculated that a recent increase in the distribution of slipper lobsters in the northeastern Atlantic is due to climate warming. The phyllosoma larva is well-adapted to planktonic life and long-distance dispersal, as it may last week or months in the plankton before settlement, and the large size of the nisto stage also enhances dispersal (Kaestner, 1980; Felder *et al.*, 1985; Booth *et al.*, 2005; Palero *et al.*, 2014). *Scyllarus subarctus* may thus have entered the Mediterranean during its earliest life history stages facilitated by the Atlantic Current, which reaches the western Mediterranean, then spreads along the North African coastline, before bifurcating when it reaches the Strait of Sicily, generating the Tyrrhenian Current that allows Atlantic waters to enter the Tyrrhenian Sea (El-Geziry & Bryden, 2010; Menna *et al.*, 2019). Such current transports larvae that could later spread in the basin, thus connecting Atlantic and Mediterranean populations of several species, including decapods (García-Merchán *et al.*, 2012). A similar dispersal pathway in the Mediterranean has been speculated for other taxa (García-Valdés *et al.*, 2013; Bazzicalupo *et al.*, 2018; Crocetta *et al.*, 2020a). The nearest known populations of *S. subarctus*, however, inhabits the Atlantic Morocco and the Canary Islands (Genis-Armero *et al.*, 2020), which may be to be too far away for this mechanism to operate.

Genis-Armero *et al.* (2020) also noted that the apparent increase in slipper lobster records could be the result of an improved sampling or taxonomic effort. Another possibility is that other *S. subarctus* specimens or even populations may have been already present in the Mediterranean, but specimens were confused with the very similar *S. arctus*, or live in habitats not widely monitored, like the unconsolidated substrates of the Gulf of Naples. It is also possible that *S. subarctus* may have arrived as larvae with the help of human vectors such as shipping and ballast water. The Naples seaport, one of the largest in the Mediterranean basin (Aveta & Romano, 2020), is a hub for invasive marine species of all phyla (Gambi *et al.*, 2016; Servello *et al.*, 2019), but no environmental DNA studies or even faunal surveys have ever been conducted in local harbours and marinas, or in nearby areas.

SUPPLEMENTARY MATERIAL

S1 Table. GenBank 16S rRNA partial sequences of *Scyllarus* and *Crenarctus bicuspidatus*.

S2 Figure. Phylogenetic relationships in *Scyllarus* based on the 16S rRNA partial sequences downloaded from GenBank (codes in Table S1) and the specimen from the Gulf of Naples (highlighted in green). Numbers above/below branches represent posterior probabilities (BI) and bootstrap values (ML). Scale bar represents nucleotide substitution.

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