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First record of the West Atlantic *Ariopsis felis* (Linnaeus, 1766) (Actinopterygii: Siluriformes: Ariidae) in the Mediterranean Sea

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Abstract

A single individual of Ariidae was captured in 2022 in the Palestinian waters off the Gaza Strip, eastern Mediterranean. A comprehensive description of the morphological and meristic characters of the specimen was conducted. Molecular identification was performed through sequence analysis of the Cytochrome Oxidase subunit I (COI). The specimen was identified as *Ariopsis felis*, both morphologically and genetically. The natural range of this species is the western Atlantic and, to the best of our knowledge, the species has never been reported before from Mediterranean waters. The singular occurrence of this fish in the basin prompts further discussion, raising questions about the possible vectors that may be responsible for this new introduction in the Mediterranean Sea.

Keywords: Rare fish; introduced species; cryptogenic species; Mediterranean Sea; Gaza Sea; DNA barcoding.

Introduction

The family Ariidae (Siluriformes) consists of 156 species belonging to 30 genera, widely distributed worldwide, particularly in tropical and subtropical areas, prevalently in marine waters and occasionally in brackish waters of estuaries and coastal lagoons (Daget *et al.*, 1986; Simier *et al.*, 2021; Froese & Pauly 2023). Within the Mediterranean Sea ichthyofauna, only one species of this family is documented, the Guinean sea catfish *Carlarius parkii* (Günther, 1864) (Kovačić *et al.*, 2021). But this record is based on a single specimen, originally reported as *Arius parkii* Günther, 1864, collected in the Mediterranean from the shallow waters of Haifa Bay, Israel, in 1983 (Golani & Ben-Tuvia, 1986; Golani & Sonin, 1996; Golani, 1996, 2021). The natural distribution range of this

species consists of the eastern Atlantic waters extending from Cape Blanc to Angola, sporadically to the Atlantic coasts of Morocco (Acero & Betancur-R., 2016).

In the present study, the occurrence of the hardhead sea catfish *Ariopsis felis* (Linnaeus, 1766) is documented for the first time in the Mediterranean Sea, after the collection of a single specimen in the coastal waters off the Gaza Strip, Palestine. The natural range of this species is based in the western Atlantic. The study provides a detailed account of morphological characters and presents results from genetic analysis, supporting the assigned species identification. The unexpected occurrence of this exotic fish in the eastern part of the Mediterranean Sea is briefly discussed, raising questions into the possible vectors of introduction that may have facilitated this unprecedented range extension.

Material and Methods

On 17 October 2022, a fish unfamiliar to fishermen was found among the catch of a bottom trawler operating in the fishing area off the Gaza Strip, approximately 2 miles from the shore (Coordinates: 31.43167°N, 34.28642°E), at a depth of 25 m, on a muddy bottom (Fig. 1). Bottom trawlers are commonly employed in the region for the catch of demersal and benthic fish (Abudaya *et al.*, 2013). The fishing areas along the Gaza Strip coast have been described by Abudaya *et al.* (2013) and Hussein *et al.* (2022). The mouth of the Gaza Valley river (Ubeid, 2011; Abualhin, 2016) is located 3-5 km from the capture site. Since the fisherman did not recognize the species, he contacted the Department of Fishery (DoF) of the General Directorate of Fisheries of Gaza and brought the sample to its research team. The specimen is now deposited in the collection of the laboratory of DoF, preserved in ethanol under the registration code NS-felis-01, 17-10-2022.

The main measurements were taken with a caliper to the nearest 0.1 cm, meristic characters were counted under a stereoscope and X-rays analysis was performed.

Identification keys of Ariidae from seas all over the world were consulted, among which Fischer & Bianchi (1984), Kailola & Bussing (1995), Kailola (1999), Acero (2002), Daget (2003), Marceniuk (2005), Marceniuk & Menezes (2007), Acero & Betancur-R. (2016) and Marceniuk *et al.* (2017a, 2024).

Genetic analysis

Total genomic DNA (gDNA) was extracted from approximately 20 mg of tissue preserved in 99% ethanol using the Wizard® SV Genomic DNA Purification System by Promega, according to the manufacturer's instructions. The quality of the extracted gDNA was subsequently assessed on a 1% agarose gel electrophoresis.

The Cytochrome Oxidase subunit I (COI) gene was amplified with the M13-tailed primer cocktail (COI-3; C_FishF1t1-C_FishR1t1) described by Ivanova *et al.* (2007). The PCR reaction was performed in 25 µL total volume containing 3 µL gDNA template, 5 µL 1x PCR buffer, 2.5 µL of MgCl₂ (25 mM), 0.5 µL dNTPs (25 mM), 0.25 µL of each primer cocktail (10 µM) and 0.25 U GoTaq G2 Flexi DNA polymerase (Promega). Amplification was performed in a T-gradient thermocycler (Biometra) with an initial denaturation of 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 52°C and 60 s at 72°C and a final extension step for 10 min at 72°C. The PCR product was evaluated on 2% agarose gel. Purification and sequencing were performed at MacroGen Europe (Milan, Italy), using the M13F and M13R primers (Messing *et al.*, 1983; Ivanova *et al.*, 2007).

Trace files were edited with the software MEGA 11 (Tamura *et al.*, 2021) and the sequence was compared against published sequences through GenBank BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 20 October 2023) and the Barcode of Life Data System (BOLD, <http://www.boldsystems.org>, accessed on 20 October 2023) Identification Engine. Available COI se-



Fig. 1: Map of the eastern Mediterranean showing the sampling locations of Ariidae records: *Ariopsis felis*, Gaza Sea, Palestine, present study (red star); *Carlarius parkii*, Haifa Bay, Israel (Golani & Ben-Tuvia, 1986; Golani & Sonin, 1996) (yellow triangle).

quences were retrieved for 12 species of Ariidae from both BOLDsystems v.4 (Ratnasingham & Hebert 2007; <http://www.boldsystems.org>) and NCBI databases (<https://www.ncbi.nlm.nih.gov>). A Neighbour-joining (NJ) tree (Saitou & Nei, 1987) was computed with MEGA using p-distance (Collins & Cruickshank, 2013) with pairwise deletion. Bootstrap analysis with 1000 replicates (Felsenstein, 1985) was performed to assess statistical support to nodes of the NJ tree topologies.

Results

Morphological characterization

The fish, 224 mm in total length, 230 g in weight, showed the following characteristics (Fig. 2A, B): body elongated and cylindrical; head rounded and slightly flat; front and rear nostrils close together on each side of snout, the posterior pair partly covered by a flap of skin; humeral process pointed, triangular (Fig. 2B); rugose bony shield on head, with supraoccipital process extending posteromedially to meet the semilunar separate bone at base of dorsal-fin spine (named nuchal plate, pre-dorsal plate) (Fig. 3A); a keel on supraoccipital process; a groove in median depression of head, extending forward to opposite rear halves of eyes and preceded by a fontanel; mouth large and inferior; three pairs of barbels (1

maxillary and 2 mental) around mouth, maxillary barbels the longest, reaching pectoral-fin bases (Fig. 2A, 3A); on the roof of the mouth, premaxillary teeth conical forming a plate slightly curved; conical palatal teeth arranged in 2 patches on each side (2 small irregular median patches associated with vomere, the right smaller than the left, and 2 larger oval shaped lateral patches), the median adjacent to the lateral in each side, the two lateral well separated one from the other (Fig. 3B). A sharp spine, frontally and posteriorly serrated, in the dorsal fin, preceded by a very short one (Fig. 3A); a sharp serrated spine in pectoral fin; adipose fin moderately developed opposite to anal fin; caudal fin forked with pointed lobes, the dorsal lobe longer than the ventral lobe. Dorsal fin rays: I+I+7, Pectoral fin rays: I+9; Anal fin rays: 15; Ventral fin rays: 6. Anterior gill rakers on first arch 13 (including 2 damaged), on second arch 11. A number of at least 46 vertebrae were distinguishable after the X-rays were applied (Fig. 2C). Colour: grey-brown dorsally, silvery whitish ventrally, adipose fin dark, other fins reddish-brown with dark tips. Main morphometric measurements and their proportions as % of standard length are presented in Table 1.

Molecular identification

The COI marker was successfully amplified and the resulting 652 bp sequence has been submitted to GenBank

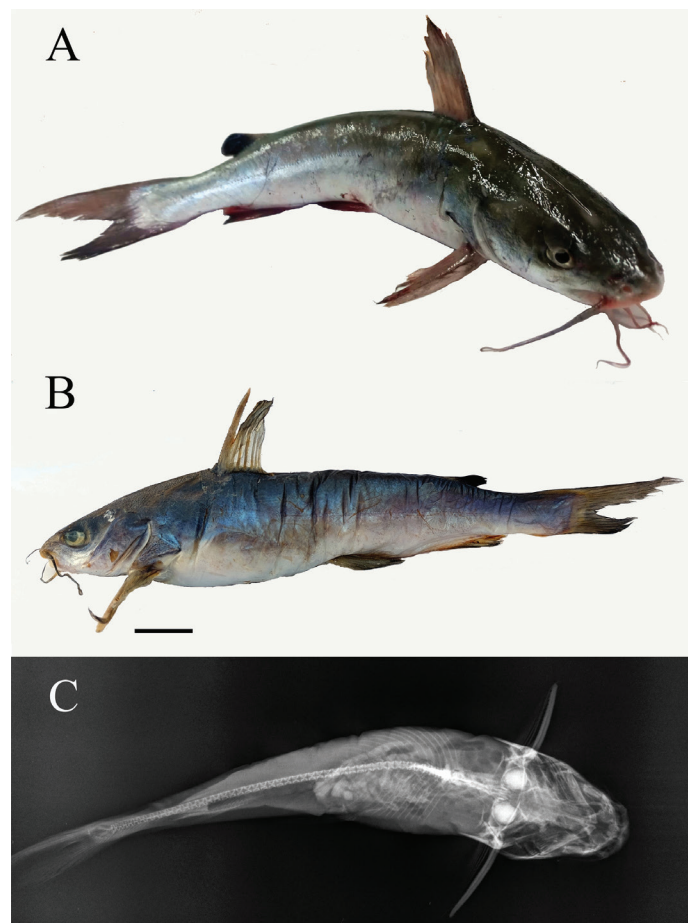


Fig. 2: The specimen of *Ariopsis felis* from Gaza Strip waters, Palestine (A: freshly caught; B: preserved in liquid, scale bar= 20 mm; C: X-rays).

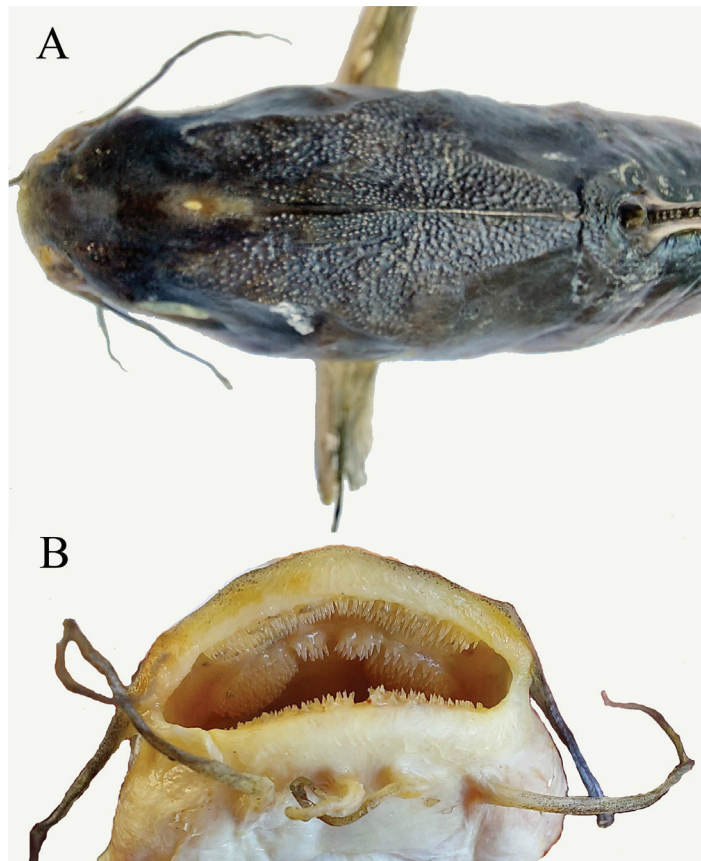


Fig. 3: *Ariopsis felis* from Gaza Sea, Palestine: dorsal view of bony shield on the head (A) and premaxillary and palatal teeth (B).

Table 1. Main morphometric measurements (mm), expressed also in percent of standard length (SL), of the *Ariopsis felis* specimen collected in the Gaza Sea, Palestine, in October 2022.

Measurements	mm	% SL
Total length	224	
Fork length	193	
Standard length	181	
Head length	40.7	22.5
Snout length	13	7.2
Eye diameter	9	5.0
Interorbital distance	21.4	11.8
Body depth	40	22.1
Snout to dorsal fin	57.3	31.7
Snou to ventral fin	93	51.4
Snout to adipose fin	134	74.0
Snout to anal fin	130	71.8
Distance between dorsal fin end and adipose fin origin	55.7	30.8
Caudal peduncle depth	13.3	7.3
Dorsal fin spine length	30.0	16.6
Pectoral fin spine length	28.5	15.7

under Accession Number OR923365. Molecular identification through BLAST and BOLD yielded high percent identity matches for *A. felis* at 99.81% and 99.85%, respectively, confirming the morphological identification. The NJ tree (Fig. 4) was obtained by comparing our sequence with 12 available sequences from *A. felis* and 9

from the sister species *Ariopsis assimilis* (Günther, 1864) retrieved from BOLD. Additionally, one sequence from each of 10 other species within the Ariidae family was included in the analysis. The generated sequence clustered within the *A. felis* clade with a fully-robust bootstrap support (BP: 100%).

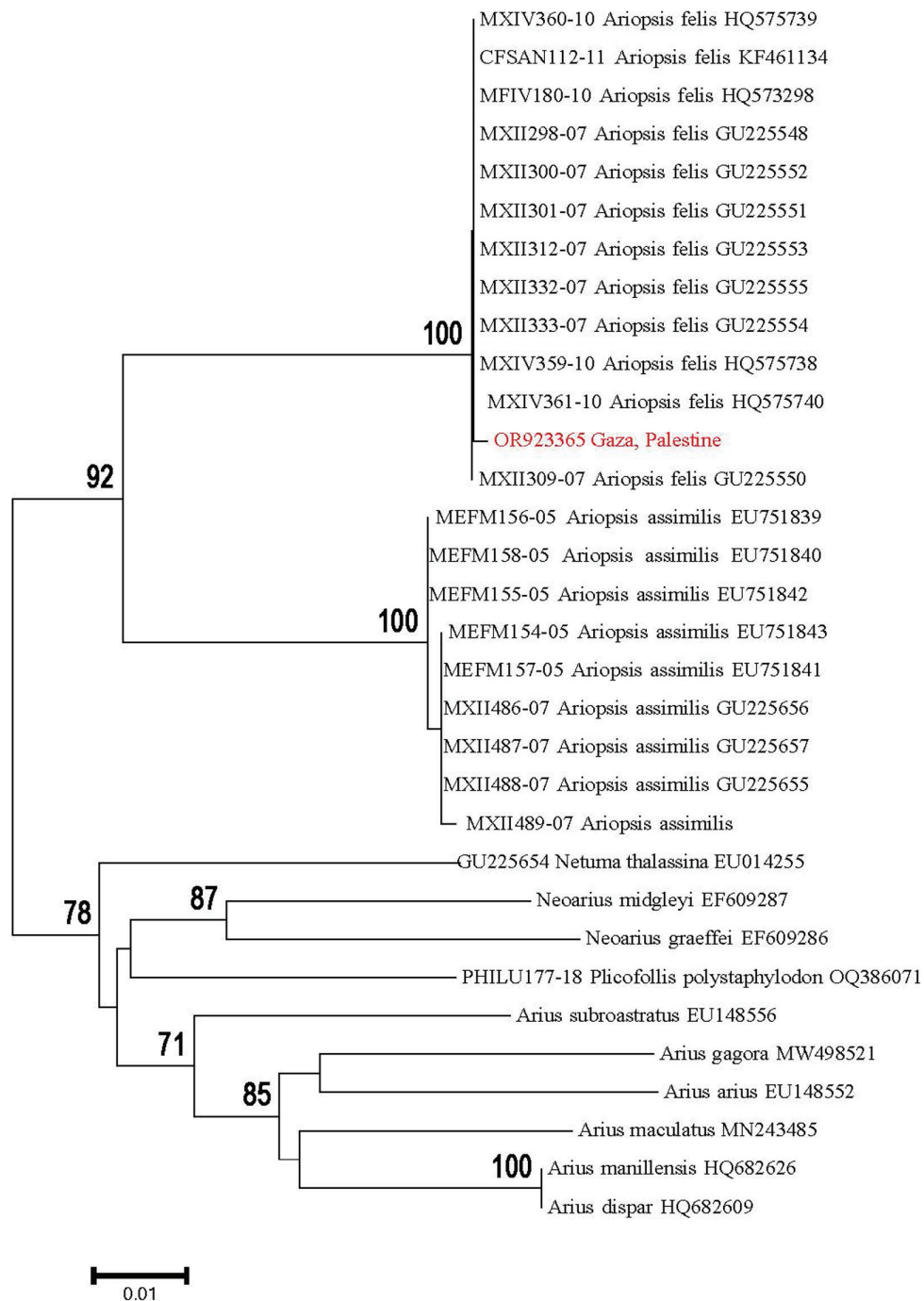


Fig. 4: Neighbor-Joining tree based on COI genetic p-distances of 12 Ariidae taxa. The sequences of *Ariopsis felis* and *Ariopsis assimilis* used in the tree reconstruction were originally recorded on BOLD and GeneBank as *Sciades felis* and *Sciades assimilis*, respectively; nevertheless, based on information from the World Register of Marine Species (WoRMS Editorial Board, 2023) and FishBase (Froese & Pauly, 2023), the correct genus designation for these sequences is recognized as *Ariopsis*. Numbers near nodes indicate bootstrap values ($\geq 70\%$). A distance scale bar is given.

Discussion

The morphological and morphometric characters observed in the specimen from the waters of the Gaza Strip led us to identify it as *A. felis*, with a primary reliance on the criteria outlined by Acero (2002) and Marceniuk *et al.* (2017a). Subsequent genetic analysis confirmed the accuracy of this assignment. The molecular identification provided crucial support to the morphological assignment, particularly due to the highly unusual and out-of-

native-range collection of the specimen. The family Ariidae benefits from numerous publicly available sequences (e.g., 655 public records on BOLD in 54 species), which contributed to a more precise specimen identification within the family. All sequences comprising the well-supported clade of *A. felis*, which includes the sequence of the Gaza sample, are sourced from published papers (Valdez-Moreno *et al.*, 2010; Deeds *et al.*, 2014) supporting the robustness of the clade (Antil *et al.*, 2023).

The pattern of palatal teeth in particular is one of the

distinctive characters used for the identification of species in the family Ariidae. The palatal teeth in our sample were arranged in two pairs of irregularly rounded patches, well-separated by a space smaller than their diameter and the vomere-associated teeth were absent. These features allowed us to separate our sample from *C. parkii* described from the nearby Israel waters, in which the palatal teeth are in single pair of rounded patches (cf. Günther, 1864; Golani & Sonin, 1996; Daget, 2003; Marceniuk & Menezes 2007; Acero & Betancur-R., 2016). The disposition of the palatal teeth of the Gaza specimen showed some similarity to that described for the Indo-west Pacific species *Hexanematachthys sagor* (Hamilton, 1822) (Fischer & Bianchi, 1984; Kailola, 1999; Froese & Pauly, 2023) and to those of some Ariidae of the west-central Pacific ocean, such as *Arius armiger* De Vis, 1884, *A. leptaspis* (Bleeker, 1862), *A. (Hemiarius) stormii* (Bleeker, 1858), *A. utarus* Kailola, 1990 and other species (Kailola, 1999), as well as from Ariidae of the eastern-central Pacific (Kailola & Bussing, 1995). Palatal teeth of the Ariidae *Netuma thalassina* (Rüppell, 1837) and *Plicofollis dussumieri* (Valenciennes, 1840) reported from the Red Sea (Golani & Fricke 2018) differ clearly from the teeth of the Gaza specimen (Fischer & Bianchi, 1984; Marceniuk *et al.*, 2017b). Conversely, the palatal teeth of the sample studied in the present work were especially similar to those of the west Atlantic *A. felis* (Acero, 2002; Marceniuk *et al.*, 2017a).

Meristic counts taken agreed with those cited by Marceniuk *et al.* (2017a), apart from the anal fin rays count (15 vs 17-19) and the number of gill rakers in the second arch (11 vs 13-17).

Proportions of main morphometric measurements of our specimen fell within the ranges given for *A. felis* by Marceniuk *et al.* (2017a), except that obtained for body depth (22.1% of standard length) that exceeded the maximum value of the range of 16.4-20.3% of standard length reported by the same authors.

The subtropical marine species *A. felis* is common, and sometimes locally abundant, on muddy bottoms of shallow coastal waters, brackish waters of estuaries and coastal lagoons, only occasionally entering fresh waters; it is distributed from Cape Cod, Massachusetts, to Florida, USA, and Gulf of Mexico, including Louisiana, USA, to Yucatan (Acero, 2002; Pensinger *et al.*, 2021).

This benthic fish is caught mainly with bottom trawls, seines, and through hook-and-line; it reaches a maximum total length of 70 cm, common 25 cm, and it feeds prevalently on decapods, gastropods and fish (Acero, 2002; Osowski *et al.*, 2023). The hardhead sea catfish constitutes part of the diet of bullsharks and alligators in the western Gulf of Mexico (Marsaly *et al.*, 2023); occasionally, it is source of food for bottlenose dolphins in the northern Gulf of Mexico (Ronje *et al.*, 2017). Generally, *A. felis* is known to show a longevity extending over 20 years, exhibiting large oocytes and low fecundity and males which incubate eggs and juveniles in their mouths (Acero, 2002; Pensinger *et al.*, 2021). The sharp dorsal and pectoral fin spines can inflict painful wounds (Gordon *et al.*, 2021). The hardhead sea catfishes are not com-

mercially or recreationally targeted fishes in their natural range (Pensinger *et al.*, 2021), and are difficult to keep in ordinary aquaria (<https://saltwater.aqua-fish.net/?hardhead-sea-catfish>).

The record of *A. felis* described in the present study from the eastern Levant waters of Palestine is the first for the whole Mediterranean Sea and constitutes the second member of Ariidae found in the basin, after *C. parkii*, the capture of which was only at about 170 km of distance from the Gaza sample location (Fig. 1) (Golani & Ben-Tuvia 1986; Golani & Sonin, 1996).

The Guinean sea catfish *C. parkii* is widely distributed in the east Atlantic waters of Africa, entering estuaries and freshwater of rivers (Froese & Pauly, 2023). It was initially considered a species expanding its range into the Mediterranean through the Strait of Gibraltar, with no relation to human activity (Golani *et al.*, 2002, 2015; Psomadakis *et al.*, 2012). Nevertheless, the absence, for decades, of intermediate records for the species from the basin, have led some authors, including Evans *et al.* (2020) and Zenetos *et al.* (2022), to suggest that the Guinean sea catfish is a cryptogenic species of the basin, that is, a species not demonstrably native or introduced, sensu Carlton (1996).

Explaining the singular occurrence of *A. felis* in the Gaza Sea presents a formidable challenge, given that its native distribution spans from the northwestern Atlantic to the Gulf of Mexico. As a result, assigning a natural range expansion of *A. felis* from the Atlantic into the waters off Gaza is not a plausible hypothesis. In addition, scattered observations in Mauritania, Brazil and Australia have been reported in GBIF (iNaturalist contributors, 2023a, b, c), adding complexity to the understanding of its current geographic distribution.

Other western Atlantic fish species have been detected in the Mediterranean. For instance, the holocentriform *Holocentrus adscensionis* (Osbeck, 1765), identified in Malta waters (Vella *et al.*, 2016), is considered cryptogenic according to Evans *et al.* (2020). The acanthuri-form *Acanthurus coeruleus*, Bloch and Schneider, 1801, a species frequently recorded in the basin, is speculated to have entered the Mediterranean either naturally through the Strait of Gibraltar or by shipping or as a result of aquarium escapes (Golani *et al.*, 2015, 2021). Similarly, the pomacentrid *Stegastes variabilis* (Castelnau, 1855), reported twice, is thought to have reached the Mediterranean via ballast waters or as an aquarium escapee (Vella *et al.*, 2015; Golani *et al.*, 2023).

Human activity, particularly the possibility of introduction via ballast waters, seems more plausible. The site of capture off Gaza lies between and in close proximity to major shipping ports, including Port Said, Tel Aviv, Haifa and Beirut. This complex interplay between human-related factors and environmental dynamics emphasizes the need for further investigation to unravel the mechanisms behind the unexpected presence of *A. felis* in the eastern Mediterranean.

In the wider region, there are rivers in Israel and in the Gaza Strip, emptying into the Mediterranean Sea; also, the brackish waters of Bardawil Lagoon along the north

coast of Sinai and the Nile Delta and its channels in Egypt, are located not far from the Mediterranean sites where the two single records of Ariidae, *C. parkii* (Israel) and *A. felis* (Gaza Sea, Palestine), were made (Fig. 1). While these sites might offer suitable environmental conditions for supporting small populations of marine Ariidae, there is currently no information regarding the potential establishment of both species in the broader region.

Therefore, in our opinion, it is premature to suggest an eventual pathway for the introduction of *A. felis* into the southeastern Levantine waters since the current understanding we have of the species is based solely on our recent singular discovery. This underscores the need for further monitoring of exotic/alien fish species' distributions within the eastern Mediterranean, so as to provide conclusive insights into the potential pathways and mechanisms of introduction for the same species in the region.

The present record significantly contributes valuable information to the interesting but poorly-known ichthyofaunal diversity of the Gaza coast. The Palestinian marine region is characterized by more than 128 bony fish species reported in fishery activities, including Lessepsian migrants and newcomers originating from the Atlantic (Abuamrah, 2018; Abd Rabou, 2019; Bariche *et al.*, 2019; Abd Rabou *et al.*, 2020; Hussein *et al.*, 2022; Azzurro, 2023; Department of Fisheries Gaza, Palestine, unpublished data). The ongoing exploration of this marine environment enhances our comprehension of the intricate dynamics underlying the ichthyofaunal composition of this area.

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