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Rapid Communication

First record of *Arcania brevifrons* Chen, 1989 (Decapoda; Leucosiidae) and further record of *Macrophthalmus (Macrophthalmus) indicus* Davie, 2012 (Decapoda; Macrophthalmidae) in Hellenic waters

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Abstract

The findings of the crabs *Arcania brevifrons* and *Macrophthalmus* (*Macrophthalmus*) *indicus*, native to the Indo-West Pacific Ocean and Red Sea, and to the Indian Ocean and Red Sea, respectively, in 2021 are described from Rhodes Island, Greece. This first record of the leucosiid *A. brevifrons* in Hellenic waters and the Aegean Sea documents the westward expansion of its Mediterranean distribution. Two individuals of the introduced macrophthalmid *M.* (*M.*) *indicus* were found in the stomachs of silver-cheeked toadfish (*Lagocephalus sceleratus)*, a fish species that itself is invasive. For both species, DNA barcoding was performed using the standard mitochondrial marker cytochrome *c* oxidase subunit I (COI). While the identification of *A. brevifrons* was genetically confirmed, *M.* (*M.*) *indicus* individuals were morphologically identified. There were no *M.* (*M.*) *indicus* genetic samples available in the nucleotide database of GenBank, hindering the ability to genetically confirm species identification. However, *M.* (*M.*) *indicus* had previously been detected 12 years ago in the same area.

Key words: non-indigenous species (NIS), Brachyura, Mediterranean Sea, DNA barcoding, crab, Greece

Introduction

More than 50 non-indigenous species (NIS) of decapod crabs have been introduced in the Mediterranean Sea (Orfanidis et al. 2021), most of Indo-Pacific origin via the Suez Canal (Galil et al. 2015, 2021). In the Hellenic waters of the Aegean, Cretan and Ionian seas, 18 NIS of decapod crabs have been recorded, 14 of which are from the Indo-Pacific region (Zenetos et al. 2018). Most of these species were first recorded from the coastline along Rhodes, Greece and the nearby area in the southeastern Aegean Sea. This is a marine region strongly impacted by biological invasions due to its subtropical environmental characteristics, suitable for native thermophilic

biota and also for tropical or subtropical NIS colonization (Papaconstantinou 2014). This latter phenomenon has been amplified in the last decades by climate change and sea warming (Pancucci-Papadopoulou et al. 2012; Bianchi et al. 2014). This geographically crucial region, close to the coast of Asia Minor, is located along the natural pathway of expansion of Levantine water masses and along the route of intense maritime traffic. It represents a first step for NIS establishment, and, thus, is important for their further northward and westward dispersion into Aegean waters and other Mediterranean sectors (Corsini-Foka et al. 2015, 2017; Kondylatos et al. 2020).

Here, we report the first capture of another Indo-Pacific/Red Sea crab, the leucosiid *Arcania brevifrons* Chen, 1989, in Hellenic waters from the island of Rhodes, Greece in 2021. Furthermore, *Macrophthalmus* (*Macrophthalmus*) *indicus* Davie, 2012 is documented as a prey item for silver-cheeked toadfish (*Lagocephalus sceleratus)* ~ 12 years after its first record in the same area.

Materials and methods

Field and laboratory sampling

In the frame of EXPLIAS project (MIS5049912), experimental fishing was conducted on a monthly basis within April 2021 to March 2022. Two stations, located along the northeastern coast of the island of Rhodes in the Southeastern Aegean Sea, were sampled (Station 1, Traganou beach, 36.309543°N; 28.202262°E; Station 2, Kallithea beach, 36.372956°N; 28.237934°E). Sampling at both stations occurred at 20–25 m of depth, and the bottom was characterized as sandy-muddy scattered with patches of seagrass, *Posidonia oceanica*. Both trammel nets (mesh opening 30 mm, height 1.7 m) and a manual jig (hook size No 10–12) were operated from a commercial 7.35 KW fishing vessel. Both fishing techniques are common in the Hellenic artisanal coastal fishery (Adamidou 2007). In particular, the jig fishing gear, called "katheti" by Greek professional fishermen, is composed of a fishing line with an attached weight on its end and two fixed hooks (e.g., Nédélec and Prado 1999) baited with natural baits.

Arcania brevifrons Chen, 1989

Trammel nets were set on the night of November 4, 2021 and retrieved the following morning. One male specimen was caught at Station 1 as bycatch. The specimen was transported to the Hydrobiological Station of Rhodes (HSR), measured and photographed. A tissue sample was removed and placed in absolute ethanol for DNA extraction, whereas the crab was preserved in 70% ethanol and deposited at the HSR collection (catalogue number HSR555).

Fragments of a second specimen were found within the stomach of an invasive *Lagocephalus sceleratus* Gmelin, 1789 and photographed. The silver-cheeked toadfish (Total Length TL 51.0 cm, weight 1468.7 g) was caught at Station 2 on the evening of October 11, 2021 via a jig baited with

chops of boneless chicken. All fragments were preserved in 70% ethanol and deposited at the HSR collection (catalogue number HSR557).

Macrophthalmus (*Macrophthalmus*) *indicus* Davie, 2012

Fragments of crab specimens were found in the stomach of two *L. sceleratus* and photographed. The first silver-cheeked toadfish (TL 26.8 cm, weight 242.82 g) was caught at Station 1 on the evening of July 16, 2021, with the use of a jig baited with chops of Atlantic mackerel *Scomber scombrus* Linnaeus, 1758. Crab body parts were extracted from the fish stomach, excluding the legs, were placed in absolute ethanol for DNA extraction. The second *L. sceleratus* (TL 37.2 cm, weight 621.5 g) was caught at Station 1 on the evening of October 11, 2021 by the same method as above. Crab legs from the stomach of the first and all crab part in the second *L. sceleratus* stomach were preserved in 70% ethanol and deposited at the HSR collection with the catalogue numbers HSR556 and HSR558 respectively.

A Nikon SMZ800 stereoscope and a Nikon AW111 camera were used for observations and photographs of the samples.

Genetic analysis

DNA was extracted from the muscle of a walking leg of *A. brevifrons* (collected November 4, 2021; specimen HSR555) and from a cheliped of *M.* (*M.*) *indicus* (collected July 16, 2021; specimen HSR556) using the DNeasy® Blood & Tissue kit (Qiagen). The primers LCO1490 and HCO2198 of Folmer et al. (1994) were used to amplify and sequence the 658 bp COI gene fragment.

Following DNA extraction, the PCR reaction for the *A. brevifrons* sample was performed in a total volume of 12.5 μl consisting of the following: 1 μL of DNA (~ 10 ng), 6.75 μL of ddH_2O, 2.5 μL of MyTaq Red Reaction Buffer (5x), 0.25 μl MyTaq™ Red DNA Polymerase (meridian BIOSCIENCE) and 0.5 μL of each primer (10 μM). PCR amplification was performed using the following program: initial denaturation for 3 min at 94 °C, followed by 38 denaturation cycles at 94 °C for 30 s; primers' annealing at 45 °C for 30 s and extension at 72 °C for 40 s; final extension for 10 min at 72 °C.

The PCR reaction for the *M.* (*M.*) *indicus* sample was performed in a total volume of 12.5 μl consisting of the following: 1 μL of DNA (~ 10 ng), 4.25 μL of dd H_2O, 6.25 μL of PCRBIO Taq Mix (2x) (PCRBIOSYSTEMS) and 0.5 μL of each primer (10 μM). PCR amplification was performed using the following program: initial denaturation for 3 min at 94 °C, followed by 36 denaturation cycles at 94 °C for 30 s; primers' annealing at 50 °C for 30 s and extension at 72 °C for 35 s; final extension for 10 min at 72 °C.

All reactions were performed in a T100 Thermal Cycler (Bio-Rad). Amplified COI gene products were checked by electrophoresis on agarose gel, and the PCR products were excised from the gel and cleaned using the

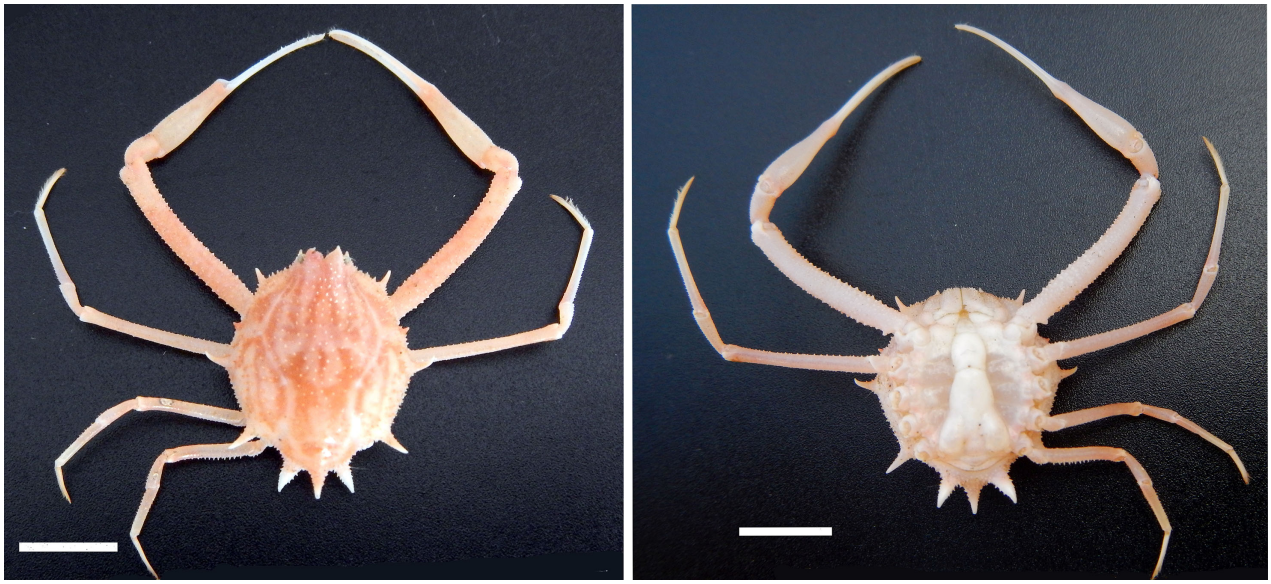

Figure 1. *Arcania brevifrons*, male, collected on November 5, 2021 at Traganou beach, Rhodes, Greece, (scale bar = 10 mm). Photos by Gerasimos Kondylatos.

NucleoSpin Gel and PCR Clean-up XS kit (Macherey-Nagel). Sanger sequencing reactions were performed using BigDye™ Terminator v3.1 Cycle Sequencing Kit and were electrophoresed on an ABI 3730xl DNA Analyzer (Applied Biosystems™). All resulting sequences were confirmed by sequencing both strands (forward and reverse directions) where possible. The obtained sequences were compared with sequences in GenBank using NCBI's BLAST tool and were deposited in GenBank under accession numbers OP382628 for *A. brevifrons* and OP382629 for *M.* (*M.*) *indicus.* For the latter species, there were no available sequences in GenBank, and a phylogenetic tree was constructed to infer its relationship with other species of the genus (i.e., creating a neighbor-joining tree using Kimura-2P distances and 1000 bootstrap replicates with MEGA v6 software; Tamura et al. 2013).

Results

Arcania brevifrons

The morphological characteristics of the HSR555 *Arcania brevifrons* specimen from Rhodes Island, Greece, collected in on November 4, 2021 were in agreement with the detailed descriptions given by Chen (1989) and Galil et al. (2017) (Figure 1). The carapace is granulose both dorsally and ventrally, with 11 spines on its borders (the two anterior ones are the smallest and the posterior five are larger, almost of same size). The frontal teeth on the carapace are relatively short, with dispersed acuminate spinules covering the dorsal surface and borders. The chelipeds are long, and the merus is cylindrical and almost as long as the carapace length. The carpus is small and granulate near its borders, with slender fingers that bear fine denticles along the cutting edges. The ambulatory legs are slender, and covered with

Figure 2. Left photo: *Macrophthalmus (Macrophthalmus) indicus* fragments found in the stomach of *Lagocephalus sceleratus*, collected on July 16, 2021, at Traganou beach, Rhodes, Greece. Right photo: enlargement of eyestalk "a" with black arrow showing detail of the apical "style" at the end of the cornea, (scale bar = 1 mm). Photos by Gerasimos Kondylatos.

minute conical granules, while the dactylus lacks granules but has hairs along its anterior and dorsal borders. The abdomen in the male consists of five segments (3rd–5th fused) that are sparsely granulate. In life, the carapace was dark pinkish to pale orange with scattered dark reddish lines arranged bilaterally. The chelipeds are pale orange with a whitish dactylus, and the ambulatory legs are pale orange (Figure 1). This *A. brevifrons* specimen measured 2.14 cm in carapace length (CL) and 1.83 cm in carapace width (CW) and weighed 2.6 g (four ambulatory legs were missing).

The second specimen of *A. brevifrons* was found in the stomach of *L. sceleratus* collected on October 11, 2021. While the individual was partially consumed, the specimen was identified to species using the cheliped (whole), pieces of the carapace showing the lateral, anterior spines and frontal spines, and pieces of ambulatory legs. The coloration was also in agreement with the live specimen, previously caught, as the crab was consumed only a short time before *L. sceleratus* was caught.

The nucleotide database of GenBank contained only COI sequences from two specimens of *A. brevifrons* from Israel (Galil et al. 2017). The obtained sequence for our *A. brevifrons* specimen had a length of 591 bp and the BLAST search showed similarity over 98.98% with the available sequences from Israel.

Macrophthalmus (*Macrophthalmus*) *indicus*

A total of 8 intact or fragmented eyestalks, two walking legs and fragments of three chelipeds were retrieved from the stomach of the *L. sceleratus* caught on July 16, 2021 (Figure 2). The crab ocular peduncles were assigned to *Macrophthalmus* (*Macrophthalmus*) *indicus* since a developed distinct "style"

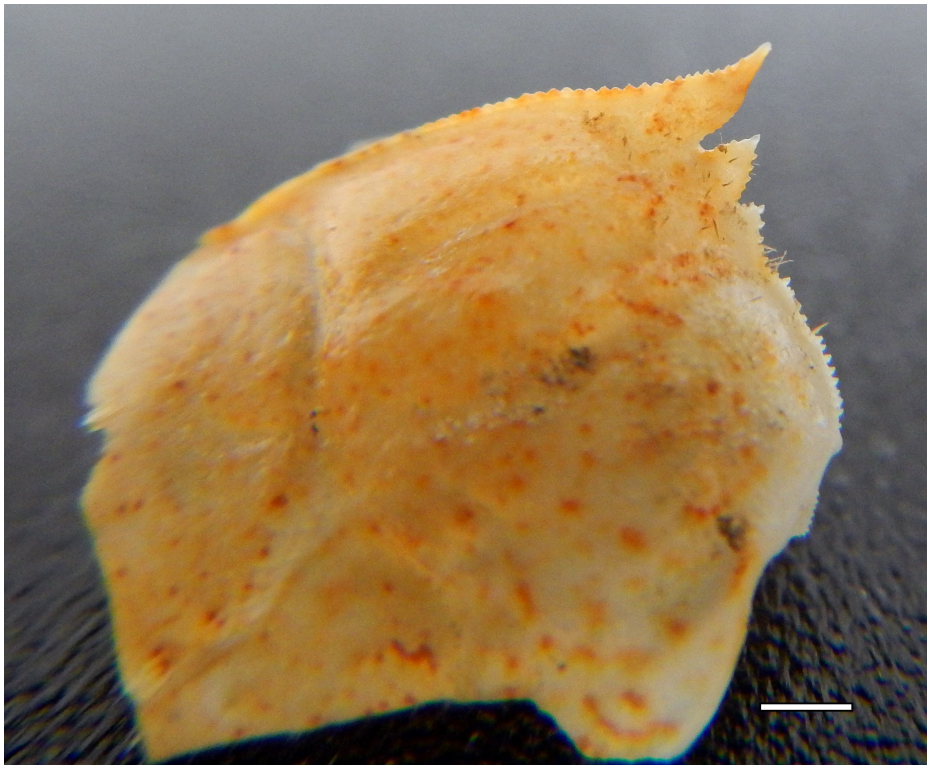

Figure 3. The supraorbital margin and the exorbital tooth of *Macrophthalmus* (*Macrophthalmus*) *indicus* visible in a fragment of carapace found in the stomach of *Lagocephalus sceleratus* collected on October 11, 2021 at Traganou beach, Rhodes, Greece, (scale bar = 1 mm). Photo by Gerasimos Kondylatos.

was observed in all corneas (Figure 2), in agreement with Davie (2012, Figures 11a, b, c) and Pancucci-Papadopoulou et al. (2010, Figures 2, 3). The developed "style" of the cornea is one of the features distinguishing *M.* (*Macrophthalmus*) *indicus* from *M.* (*Macrophthalmus*) *graeffei* A. Milne Edwards, 1873 (Davie 2012). In two of the available fragments of chela, the dactylus was strongly curved inward and its upper margin had denticles, while the cutting edge on the fixed finger was elevated medially (Figure 2). The longest eyestalk of *M.* (*M.*) *indicus* measured 10.8 mm.

From the second *L. sceleratus* individual collected on October 11, 2021, 8 eyestalks and 12 pieces of the cephalothorax bearing anterolateral spines (Figure 3) were retrieved. The shape of the supraorbital margin and of the exorbital tooth agree with the species identifications via Davie (2012, Figures 11a, b, c and 12a) and Pancucci-Papadopoulou et al. (2010, Figures 3, 4). These pieces together correspond to at least 6 individuals, of which 3 were identified as male and 1 as female based on pieces of the abdominal cavity.

In the nucleotide database of GenBank, there were no available sequences for *M.* (*M.*) *indicus*. There were, however, genetic sequences for several closely related species – *M.* (*M.*) *serenei* Takeda and Komai, 1991, *M.* (*M.*) *milloti* Crosnier, 1965, *M. convexus* Stimpson, 1958, *M.* (*M.*) *depressus* Rüppell, 1830 and *M.* (*Mareotis*) *japonicus* (De Haan, 1835). The sequence obtained for the *M.* (*M.*) *indicus* collected here had a length of 496 bp but less than 93% similarity with the COI sequences of any of the other

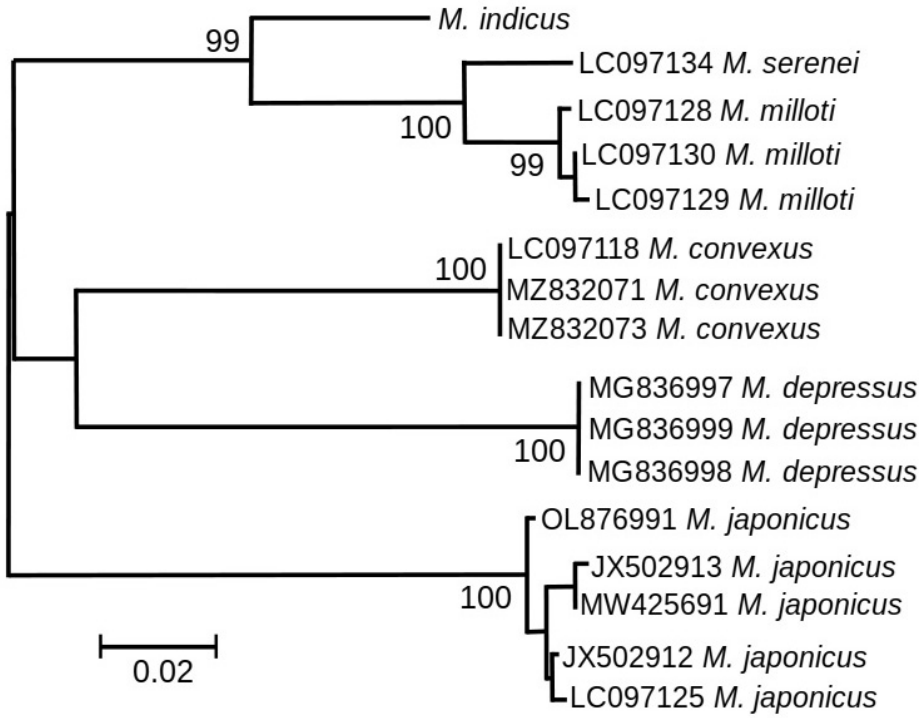

Figure 4. The phylogenetic position of the *Macrophthalmus* (*M.*) *indicus* specimen in relation to the four *Macrophthalmus* (*Macrophthalmus*) species and *Macrophthalmus* (*Mareotis*) *japonicus* with available COI sequences in GenBank (Neighbour-joining tree using Kimura-2P distances and 1000 bootstrap replicates).

Macrophthalmus species. The phylogenetic analysis showed that this species is placed within other *Macrophthalmus* species and closer to *M.* (*M.*) *serenei* and *M.* (*M.*) *milloti* (Figure 4).

Discussion

The leucosiid crab *Arcania brevifrons* is native to the Indo-West Pacific/Red Sea waters (Galil 2001). It was first detected in the Mediterranean Sea off Ashdod, Israel, in 2016 at 60 m depth. It has been hypothesized to have been introduced via the Suez Canal, similar to the other three invasive leucosiids, *Myra subgranulata* Kossmann, 1877, *Ixa monodi* Holthuis & Gottlieb, 1956 and *Coleusia signata* (Paul'son, 1875), established in the basin (Galil et al. 2017) and southeastern Aegean waters (Zenetos et al. 2018). In 2019, *A. brevifrons* was recorded from Antalya Bay, Turkey (Bariche et al. 2020), only ~ 300 kilometers away from the present study. The collection of two adult *A. brevifrons* specimens from a localized area within a one-month period, and one of them in the stomach of an invasive *L. sceleratus*, could indicate an established population near the island of Rhodes. Its occurrence in these waters indicates that the species may be colonizing the Levantine coasts, following the Lessepsian migration process, a pattern reminiscent of the three other invasive leucosiid species (cf. Galil et al. 2017 and references therein).

The native range of *M.* (*M.*) *indicus* is the Indian Ocean, from the Red Sea, Gulf of Oman, and Persian Gulf to Indonesia (Davie 2012). This

Indian Ocean species was described for the first time by Davie (2012), who provided the characters that distinguish *M.* (*M.*) *indicus* from the west Pacific *M.* (*M.*) *graeffei*, including the distinct "style" at the end of the cornea and the shape of the supraorbital margin and lateral teeth, all of which were observed in the specimens from Rhodes. In the Mediterranean, *M.* (*M.*) *indicus* was first recorded as *M. graeffei* from southern Turkey (Enzenross and Enzenross 1995). The species colonized the eastern Levant (Ksiunin and Galil 2004; Gerovasileiou et al. 2017), reaching the southeastern Aegean waters of Gökova Bay, Turkey (Ateş et al. 2007) and Rhodes Island, Greece, where it was collected in 2009 on soft bottom (Pancucci-Papadopoulou et al. 2010; Davie 2012). Probably due to the small size of this crab and scattered sampling on soft bottoms over the last decade, no further records had been documented before the present report. This crab is the prey of the invasive Randall's threadfin bream *Nemipterus randalli* Russell, 1986 in Israel waters (Gilaad et al. 2017). The presence of several digested specimens in the stomach of the invasive tetraodontid *L. sceleratus* suggests that *M.* (*M.*) *indicus* may be established and integrated into the local food web, similar to other crab NIS in the area (Corsini-Foka et al. 2015; Katsanevakis et al. 2020; Kondylatos et al. 2020).

Our findings support previous observations of a generalist feeding behaviour of the invasive *L. sceleratus*, adding another piece of information to the current knowledge on the food web of both the silver-cheeked toadfish and the two NIS of brachyuran crabs. Documented NIS prey of *L. sceleratus* within the Mediterranean Sea include the bluespotted cornetfish *Fistularia commersonii* Rüppell, 1838, *L. sceleratus* juveniles (in adult specimens), the devil firefish *Pterois miles* (Bennett, 1828), the yellowspotted puffer *Torquigener flavimaculosus* Hardy & Randall, 1983 and other non-indigenous Osteichthyes (e.g. the marbled spinefoot *Siganus rivulatus* Forsskål & Niebuhr, 1775), gastropods (e.g. *Cerithium scabridum* Philippi, 1848), the echinoderm *Diadema setosum* (Leske, 1778) and decapods (e.g. *Thalamita poissonii* (Audouin, 1826)) (Ulman et al. 2021 and references therein; Kondylatos et al. 2020; G. Kondylatos *unpublished data*). Predators of *L. sceleratus* in the basin are the loggerhead turtle, the white grouper *Epinephelus aeneus* (Geoffroy Saint-Hilaire, 1817), the garfish *Belone belone* (Linnaeus, 1760), the dolphinfish *Coryphaena hippurus* Linnaeus, 1758 and larger sized *L. sceleratus* (Ulman et al. 2021). However, the incorporation of alien species in the food web of the Mediterranean Sea is mostly uncharted. Available data come from the stomach analysis of abundant NIS fish such as *F. commersonii*, *L. sceleratus*, the Red Sea goatfish *Parupeneus forsskali* (Fourmanoir & Guézé, 1976), *P. miles* and other (Corsini-Foka 2010; Golani 2010; Gilaad et al. 2017 and references therein; Zannaki et al. 2019; Ulman et al. 2021 and references therein; G. Vagenas *unpublished data*; G. Kondylatos *unpublished data*). As of now, there is no

other available information on the incorporation of *A. brevifrons* in the food web of the basin.

Genetic analysis of an *Arcania* specimen from Rhodes confirmed its morphological identification as *A. brevifrons*. On the other hand, the genetic analysis of the *Macrophthalmus* individual cannot confirm its morphological identification as *M.* (*M.*) *indicus* due to the lack of reference sequences for most of the *Macrophthalmus* species listed in WoRMS (2022), including *M.* (*M.*) *indicus* in question. This lack of representation of species in genetic databases has been emphasized (Hering et al. 2018) and is definitely one of the major setbacks in genetic analyses, especially for confirming the presence of newly detected NIS.

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Authors’ contribution

GK contributed in research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, ethics approval, funding provision, roles/writing – original draft, writing – review and editing; DM contributed in data analysis and interpretation, roles/writing – original draft, writing – review and editing; EG contributed in data analysis and interpretation, roles/writing – original draft, writing – review and editing; PK contributed in data analysis and interpretation, roles/writing – original draft, writing – review and editing; MC-F contributed in investigation and data collection, data analysis and interpretation, funding provision, roles/writing – original draft, writing – review and editing; DK contributed in roles/writing – original draft, writing – review and editing.

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