

Rapid Communication**New records of the Indo-Pacific shrimp *Urocaridella pulchella* Yokeş & Galil, 2006 from the Eastern Mediterranean Sea**Markos Digenis^{1,2,3}, Michail Ragkousis³, Katerina Vasileiadou², Vasilis Gerovasileiou^{2,*} and Stelios Katsanevakis³¹Department of Biology, University of Crete, P.O. Box 1470, 71110, Heraklion, Crete, Greece²Hellenic Centre for Marine Research (HCMR), Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), P.O. Box 2214, 71003, Heraklion, Crete, Greece³Department of Marine Sciences, University of the Aegean, University Hill, 81100 Mytilene, GreeceAuthor e-mails: markosdigenis@gmail.com (MD), ragkousis.diving@gmail.com (MR), kvasileiadou@hcmr.gr (KV), vgerovas@hcmr.gr (VG), stelios@katsanevakis.com (SK)

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Citation: Digenis M, Ragkousis M, Vasileiadou K, Gerovasileiou V, Katsanevakis S (2021) New records of the Indo-Pacific shrimp *Urocaridella pulchella* Yokeş & Galil, 2006 from the Eastern Mediterranean Sea. *BioInvasions Records* 10(2): 295–303, <https://doi.org/10.3391/bir.2021.10.2.07>

Received: 25 November 2020**Accepted:** 1 February 2021**Published:** 17 February 2021**Handling editor:** Vadim E. Panov**Copyright:** © Digenis et al.

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OPEN ACCESS**Abstract**

Urocaridella pulchella Yokeş & Galil, 2006 is a palaemonid cleaner-shrimp of Indo-Pacific origin that was first described from the Mediterranean Sea in 2006. However, limited information is available about its distribution and ecology due to the small size and cryptic habit of the species. We hereby report the first records of *U. pulchella* in marine caves and crevices of Greece, updating the species distribution that now spreads from the Levantine Sea to the South Aegean. Species identification was based on morphological examination and DNA barcoding of collected samples and *in situ* photographs. We also report on the fish cleaning behaviour of the species (based on observations of cleaning behaviour to the native Mediterranean moray eel, *Muraena helena*), the finding of an ovigerous-female and a wide distribution range, attesting to the establishment of the species in the area.

Key words: non-indigenous species, alien species, decapods, cleaner shrimps, marine caves, Aegean Sea**Introduction**

Shrimps of the family Palaemonidae include more than 1000 described species which belong to 150 genera (Horká et al. 2018); however, information about the palaemonid genus *Urocaridella*, which thrives in the Indo-Pacific Ocean, is limited (Ďuriš 2017). The cleaner-shrimp *Urocaridella pulchella* Yokeş & Galil, 2006 was first described from the southwestern coasts of Turkey, in the eastern Mediterranean Sea. Yokeş and Galil (2006) suggested that it should be a non-indigenous species (NIS), as it was the first representative of its genus found in the Mediterranean Sea. One decade later, indeed, the species was found in Saudi Arabia and Jordan, Red Sea (Ďuriš 2017; Horká et al. 2018) confirming its origin. Therefore, it has been suggested to be a Lessepsian migrant in the Mediterranean Sea (Ďuriš 2017). Until now, it constitutes the only species of genus *Urocaridella* in the Mediterranean Sea and its published records are scarce. Recently,

Katsanevakis et al. (2020a) published four records of *U. pulchella* from Israel, which represented the first report of the species in the country and the second Mediterranean location where *U. pulchella* was found. Sequences of COI for the species, collected in the Red Sea, Egyptian waters, were published by Horká et al. (2018) (GenBank entry for the sequence with accession number KY197952.1 and personal communication with I. Horká). A recent survey in marine caves of Greek Islands, coupled with observations from diving professionals, brought new records of the species (including the first records in the Aegean Sea), revealing that it is more widespread in the eastern Mediterranean basin than previously thought.

Materials and methods

In summer 2020 several marine caves and rocky reefs of the Aegean Sea were surveyed with SCUBA diving in the framework of the research program ALAS “ALiens in the Aegean – a Sea under siege” which aims at filling knowledge gaps on the impacts of marine alien species in the Aegean Sea (Katsanevakis et al. 2020b). The project focuses on understudied alien-native interactions as well as on impacts on priority and vulnerable habitats (e.g. marine caves).

Urocaridella pulchella was observed and photographed in three marine caves (Table S1). Two of the photographed specimens were collected from Kastelorizo and Rhodes islands and preserved in 96% ethanol. The specimens were deposited in the Natural History Museum of Crete (NHMC) under the reference codes NHMC.82.03.18104 and NHMC.82.03.18105, respectively. The samples were examined and photographed in the lab under a Zeiss Discovery V12 stereo-microscope equipped with a digital camera. Additional records were provided by two diving professionals from Crete (*personal communication*).

Genomic DNA was extracted from the two specimens collected. Extractions were accomplished with Nucleospin Tissue kit (MACHEREY NAGEL GmbH & Co., Duren, Germany). To verify morphological identification of the two specimens, the COI gene was used. The gene was amplified with Polymerase Chain Reaction method (PCR) using the primers LCO1490 (5' GGTCACAAATCATAAAGATATTGG 3') and HCO2198 (5' TAAACTTCAGGGTGACAAAAAATCA 3') (Folmer et al. 1994). Reactions were in final volumes of 20 µl containing 20 ng of DNA template, 1x Taq Buffer, 2.0 mM MgCl₂, 0.2 mM dNTPs, 0.25 µM of each primer, 0.5 Units of KAPATaq DNA Polymerase (KAPA BIOSYSTEMS, Boston, USA). The PCR reactions were carried out with an initial step at 95 °C for 3 min, followed by 35 cycles at 94 °C for 20 sec, 45 °C for 30 sec, 72 °C for 1 min, followed by a final extension at 72 °C for 5 min. The PCR products were sequenced in ABI3730 at the Genetics Lab of the Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC) of the Hellenic Centre for Marine Research (HCMR). The new sequences were deposited to GenBank with accession numbers: MW287623 and MW287624.

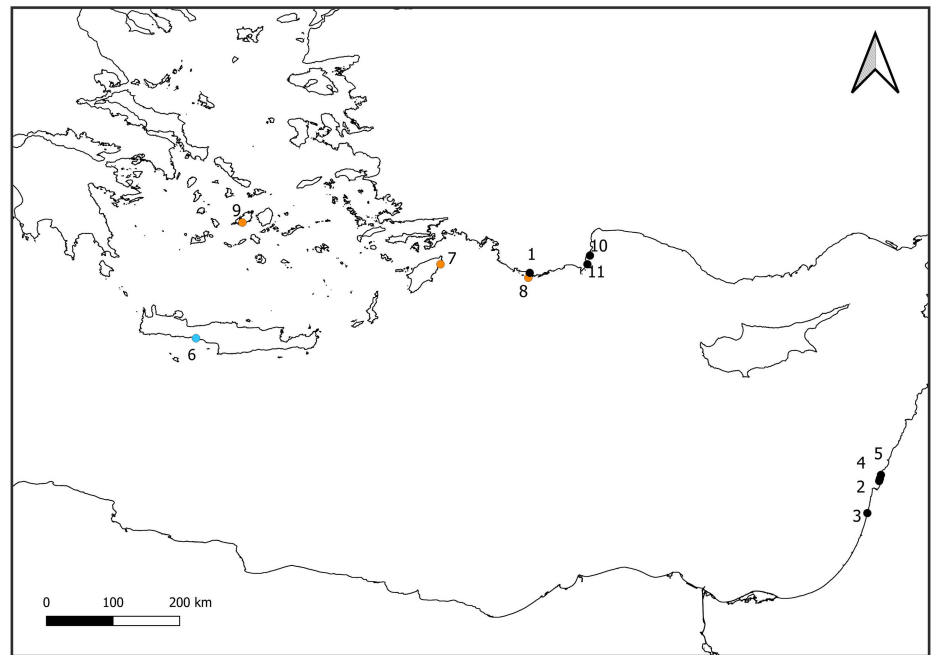


Figure 1. Distribution of *U. pulchella* records in the Mediterranean Sea. Published records from literature are indicated with dark dots while new records are indicated with blue (citizen science photographs) and orange dots (scientific observations and collected samples). Details on the records are provided in Supplementary material Table S1.

Sequences obtained were corrected by MEGA X software (Kumar et al. 2018) and uploaded in Blastn (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) of GenBank to retrieve similar sequences. Moreover, COI sequences of other *Urocaridella* species were downloaded from GenBank (accession numbers: MN393102.1; MN393096.1; KY197951.1; KY197953.1) to be included in the analysis. All sequences were aligned with MUSCLE algorithm (Edgar 2004) embedded in MEGAX software. Aligned sequences were processed with Maximum Likelihood analysis under GTR+I model. Selection of the model was done by jModelTest software (Guindon and Gascuel 2003; Guindon et al. 2010; Darriba et al. 2012). The Maximum Likelihood analysis was carried out using PhyML 3.0 provided by the Montpellier Bioinformatics Platform (<http://www.atgc-montpellier.fr>).

Results

Distribution

Urocaridella pulchella was recorded in cryptic habitats (i.e. marine caves and crevices) in four locations in Greece (Figure 1 and Supplementary material Table S1): the dark zone of the Blue cave on Kastelorizo Island at 5 m depth; the semi-dark zone of Seal's cave, Rhodes at 12 m depth (Figure 2A); several small caves and crevices in Plakias area on the southern coasts of Crete at 1 to 20 m depth; and the semi-dark zone of a cave on Pantieronisi Island, close to Paros Island, Cyclades at 14 m depth (Figure 2B). One of the two individuals observed in the latter site was an ovigerous female (Figure 2B). Identification of *U. pulchella* from Rhodes and Kastelorizo

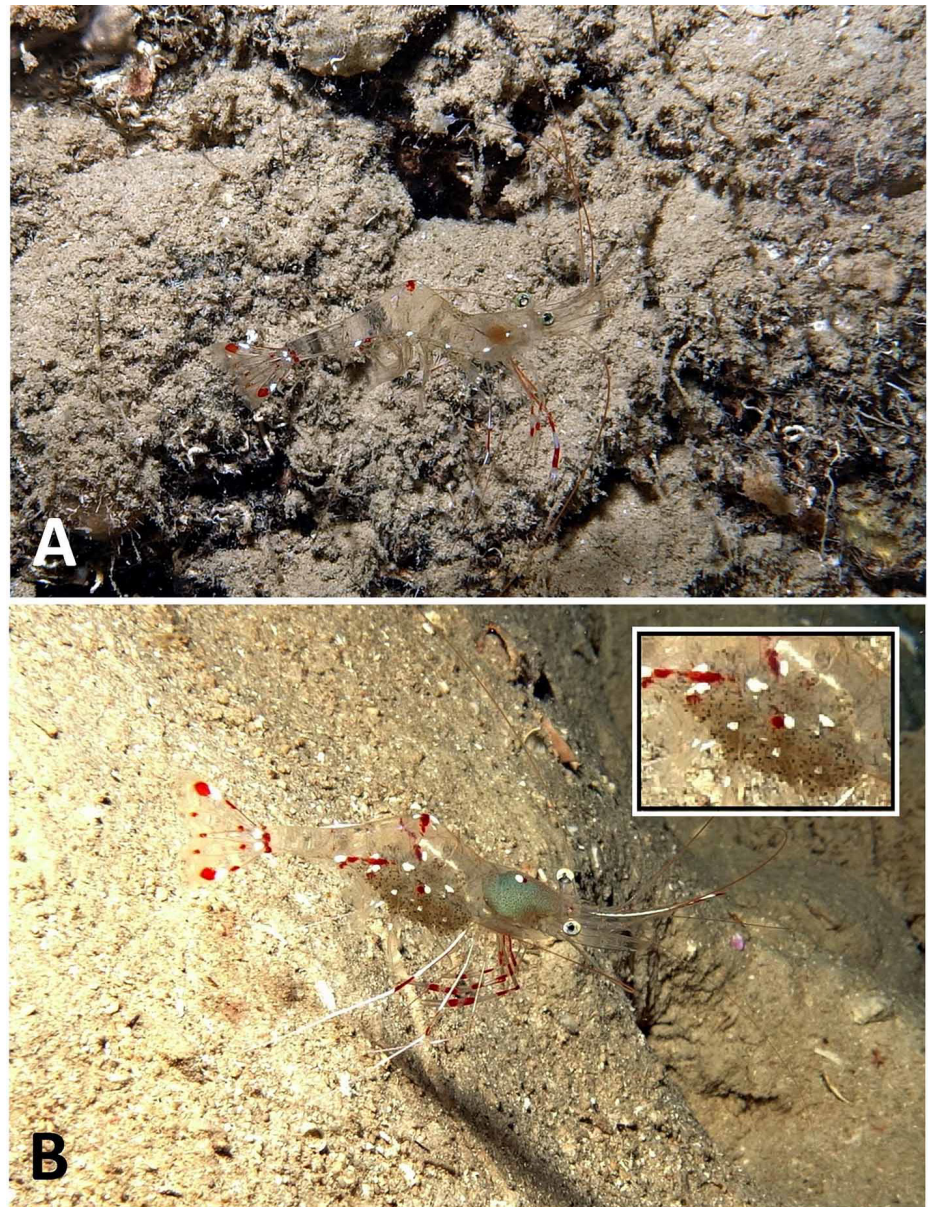


Figure 2. *Urocaridella pulchella* photographed (A) in Seal's cave, Rhodes Island and (B) in Pantieronisi cave, Cyclades, carrying eggs (close up photo). Photos by Michail Ragkousis.

Islands was based on morphological examination and DNA barcoding of collected samples and *in situ* photographs. Records from Crete and Pantieronisi were based on visual observations and photographic evidence. Notably, the specimens reported from Plakias (Figure 3), were captured on video actively exhibiting their fish cleaning behaviour on the Mediterranean moray eel, *Muraena helena* Linnaeus, 1758.

Morphological identification

Morphological features of the collected specimens were in accordance with the original species description by Yokeş and Galil (2006). In both collected specimens and individuals photographed, characteristic morphological features could be easily observed. The carapace was laterally compressed, transparent and smooth with small red spots at the abdomen. A red bar



Figure 3. Two *U. pulchella* individuals cleaning the Mediterranean moray *Muraena helena* at the depth of 15 m in a crevice of Plakias area, southern Crete in 2020. Clip of a video filmed by Jérôme Smeets.

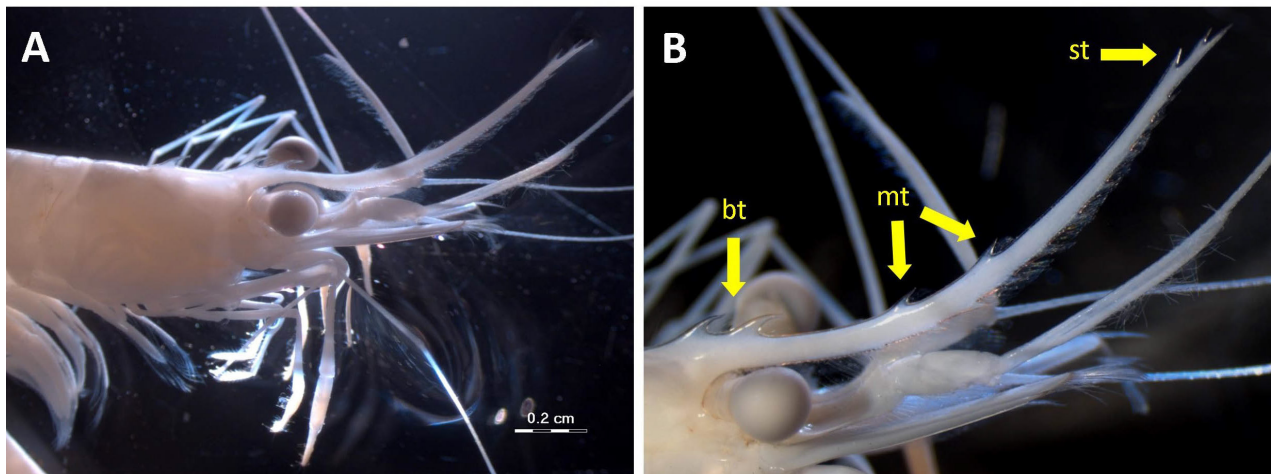


Figure 4. (A) Side view of the specimen from Kastelorizo; (B) Rostrum with three pairs of teeth: basal teeth (bt), midrostral teeth (mt) and subterminal teeth (st) are shown with yellow arrows. Photos by Markos Digenis.

was across the third abdominal segment and the uropodal exopods striped red and white, banded with red subterminally. Pereopods were also white, banded with red. More specifically, the first two pereopods had bright-red palms and carpo-propodal joints and the last three pereopods had red carpi and propodi (Figures 2A, 2B and 3).

The collected specimen from Kastelorizo had a slender rostrum, prominently upcurved, tapering, 1.5 times the length of carapace (Figure 4A); the rostrum of the specimen from Rhodes broke during sampling. The distalmost tooth of the basal pair of teeth was larger and underneath each curved tooth, a row of three or four long plumose setae protruded (Figures 4B, 5A). Acute, midrostral pair of teeth was smaller than the first pair, set wider apart and two or three long plumose setae protruded from the rostrum underneath each tooth (Figures 4A, 5B).

Subterminal pair of teeth appeared acute, glabrous and non-serrate (Figures 4B, 5C). Ventral margin of rostrum bore ten acute, non-serrate teeth,

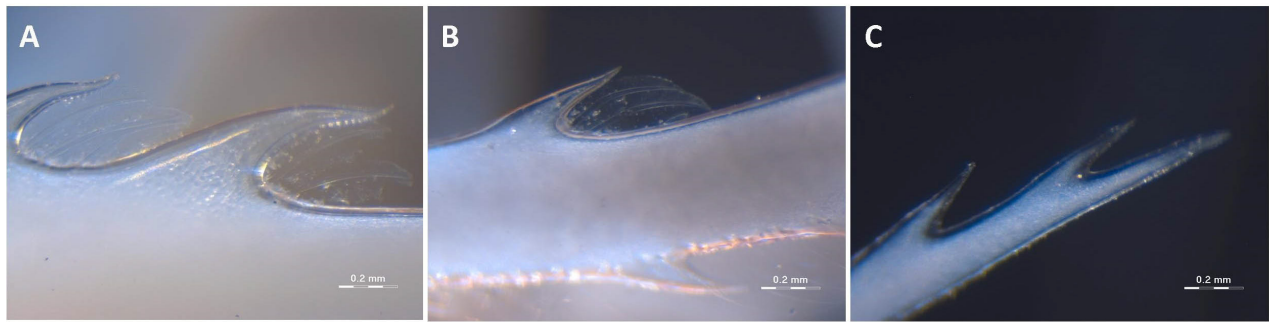


Figure 5. (A) First pair of rostrum dorsal teeth with two and three plumose setae underneath; (B) Tooth of the second pair of teeth with two plumose setae underneath; (C) Rostrum subterminal non-serrate pair of teeth. Scale: 0.2 mm. Photos by Markos Digenis.

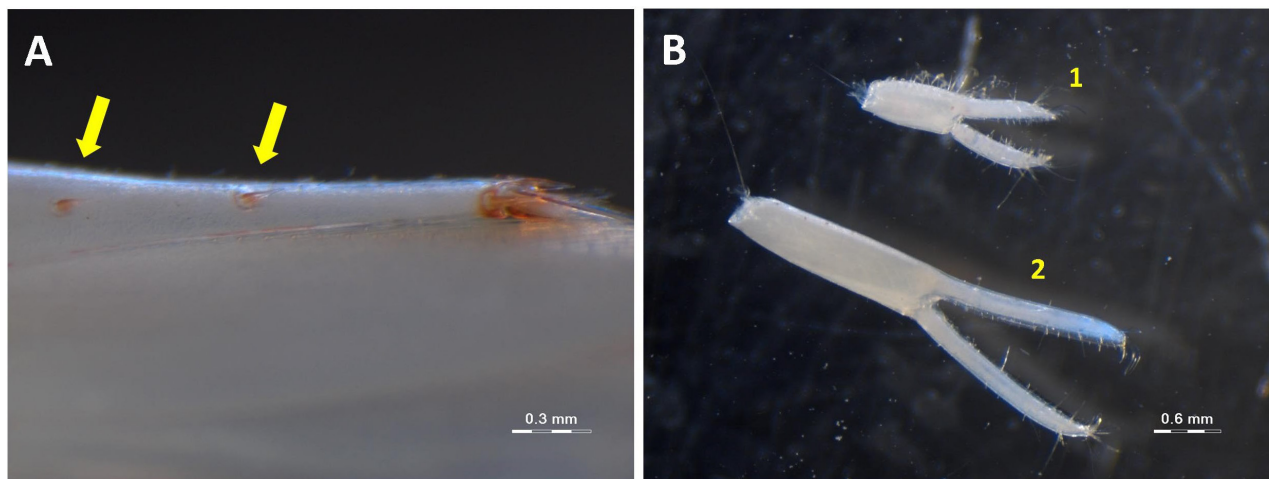


Figure 6. (A) Telson with the left minute spine of each pair (arrows), (B) Chela of the first (1) and second (2) pereopod. Photos by Markos Digenis.

placed closer together distally; proximalmost ventral tooth was situated posteriorly to midrostral pair, distalmost ventral tooth slightly posterior to subterminal dorsal teeth.

The third abdominal segment was bearing prominent, rounded, ridge medially and subrectangular in profile. Tapering telson was shorter than uropods and slightly shorter than the sixth segment, bearing dorsally on distal half two pairs of minute spines (Figure 6A). The first pereopod had fingers, as long as palm, distally setose and hooked with tips crossing when closed (Figure 6B). The inner margin of the palm had long hooked setae, and short serrate setae. Carpus was subcylindrical, longer than chela, with transverse row of short setae near inner distal margin, while merus was cylindrical, longer than carpus. The ischium was stout and its length was one third of that of merus; the second pereopod was far exceeding the length of the first pereopod with a chela more than double in size. The carpus was 1.5 times as long as the palm and distally thickened; merus and ischium were slender, cylindrical and nearly as long as the carpus (Figures 2A, 2B and 4A).

DNA Barcoding

Genetic processing of the two specimens provided approximately 550 bp of COI sequences. The Blastn search gave the highest similarity (> 99%) with

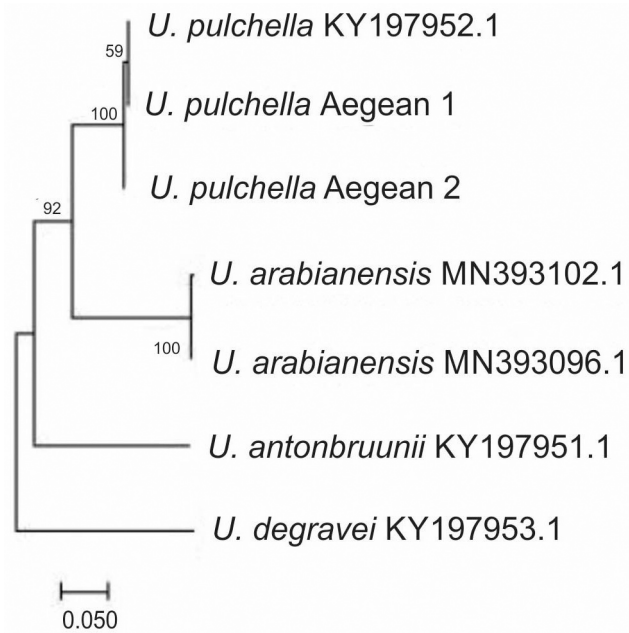


Figure 7. Maximum Likelihood phylogenetic tree of COI gene from *Urocaridella* species.

the COI sequence of *U. pulchella* (GenBank accession number KY197952.1). The phylogenetic tree from Maximum Likelihood analysis among *Urocaridella* species is shown in Figure 7. The *U. pulchella* sequences from Greece formed a clear clade with the *U. pulchella* retrieved from GenBank, providing evidence on the validity of the taxonomic identification of the species.

Discussion

This work reports on the first occurrence records of *U. pulchella* from Greek waters and the Aegean Sea (Zenetos et al. 2018, 2020; Katsanevakis et al. 2020c). The herein reported new records extend from Kastelorizo Island (very close to the type locality of the species description in southwestern Turkey by Yokeş and Galil 2006) to the Cyclades Islands in the South Aegean Sea. The earliest records of the species were reported in 2018 from Crete by SCUBA diving professionals, based on visual observations and photographs. However, in the absence of collected specimens for thorough morphological examination and molecular identification, there was a time lag in the reporting of the species, as is the case for several NIS (Zenetos et al. 2019). Based on the new observations from the 2020 survey in caves, in addition to the photographic evidence, positive identification was verified based on morphological examination and DNA barcoding of collected samples. All records were from cryptic habitats at a depth range of 1–20 m in agreement to previous records (Table S1).

The broad distribution range, number of records and reported individuals as well as the finding of ovigerous female in one of the surveyed sites in Greece (Figure 3) and previous records from Israel (Katsanevakis et al. 2020a) indicate the reproductive success and possible establishment of

U. pulchella in the Eastern Mediterranean Sea. The absence of regular reports regarding the species presence can be attributed to its small size, mostly transparent body and preference for cryptic habitats. Nearly all specimens reported in this work, as well as previous records, have been observed in caves, crevices (Table S1) and/or during night dives (Yokeş and Galil 2006; Katsanevakis et al. 2020a). In addition, the cleaning behaviour captured in this work (Figure 3), testifies to this role of *U. pulchella* in the Mediterranean ecosystem that was documented by Yokeş and Galil (2006) and seems to be similar to the one reported from the Red Sea and Indo-Pacific Ocean (Horká et al. 2018).

Despite the fact that the majority of NIS reported from marine caves are known from the entrance and semi-submerged zones of caves (Gerovasileiou et al. 2016), *U. pulchella* thrives even in dark cave zones (Öztürk et al. 2020; present study). The potential impacts of NIS on the native cave fauna remains unknown (Gerovasileiou et al. 2016). Possible competition of *U. pulchella* with native decapods (e.g. *Lysmata* spp.) that exhibit a similar fish-cleaning behaviour is also unknown. In this context, further investigations of NIS biota in marine caves are already under way.

Acknowledgements

We are grateful to Dimitris Miriokefalitikis from Evelin Dive Center and Jérôme Smeets from Kalypso Dive Center for providing underwater photographs and videos of *Urocaridella pulchella* from Crete. We thank three anonymous reviewers for their useful comments.

Funding Declaration

The study was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the “First Call for HFRI Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant” (Project ALAS – “ALiens in the Aegean – a Sea under siege”; Project Number: HFRI-FM17-1597).

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Supplementary material

The following supplementary material is available for this article:

Table S1. *Urocaridella pulchella* recordings from the Mediterranean Sea up to date.

This material is available as part of online article from:

http://www.reabic.net/journals/bir/2021/Supplements/BIR_2021_Digenis_etal_SupplementaryMaterial.xlsx