Cryptocentrus steinhardti **(Actinopterygii; Gobiidae): a new species of shrimp-goby, and a new invasive to the Mediterranean Sea**

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Key words: Integrative taxonomy; Lessepsian migration; Shrimp-goby; New species; Mediterranean Sea

ABSTRACT

A new species of shrimp goby was collected at depths of 60-80 m, off the southern Israeli Mediterranean coast. A unique 'DNA barcoding' signature (mtDNA *COI* and *Cytb*) revealed that it differs from any other previously barcoded goby species clustered phylogenetically with the shrimp-goby group, in which *Cryptocentrus* is the most speciose genus.

A morphological study supported the assignment of the fish to *Cryptocentrus* and differentiated the new species from its congeners. The species is described here as *Cryptocentrus steinhardti* n. sp. However, the present phylogenetic analysis demonstrates a paraphyly of *Cryptocentrus* and

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emphasizes the need for revision of the genus based on integrating morphological and genetic characteristics.

This finding constitutes the third record of an invasive shrimp goby in the Mediterranean Sea, raising an intriguing ecological issue regarding the possible formation of a fish-shrimp symbiosis in a newly invaded territory. Describing an alien tropical species in the Mediterranean prior to their discovery in the native distribution is an unusual event, although not the first such case. Several similar examples are provided below.

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INTRODUCTION

Since the opening of the Suez Canal in 1869 more than 400 multicellular nonnative species of Red Sea origin, including ca. 100 fish species, have been found along the Israeli Mediterranean coast (Galil et al., 2020). Among this diverse invasive fauna are two species of shrimp-gobies: *Vanderhorstia mertensi* Klausewitz, 1974, (Goren et al., 2013) and *Cryptocentrus caeruleopunctatus* (Rüppell, 1830) (Rothman and Goren, 2015). These species are part of a group of near-reef fishes that inhabit sandy and silty habitats and display a remarkable mutualism with burrowing alpheid shrimps, exchanging burrow construction capabilities and sentinel services (Karplus and Thompson, 2011). Common throughout the tropics, this unique fish-shrimp association is documented from over a 100 fish species that belong to eleven valid genera of gobies: *Amblyeleotris* Bleeker 1874; *Cryptocentrus* Valenciennes (ex Ehrenberg) in Cuvier & Valenciennes 1837; *Cryptocentroides* Popta 1922*, Ctenogobiops* Smith 1959, *Lotilia* Klausewitz 1960; *Mahidolia* Smith 1932; *Myersina* Herre 1934 ;*Psilogobius* Baldwin 1972; *Stonogobiops* Polunin & Lubbock 1977*; Tomiyamichthys* Smith 1956 and *Vanderhorstia* Smith 1949 (Karplus, 2014; Ray et al., 2018). An additional genus, *Flabelligobius Smith* 1956 is considered a synonym of *Tomiyamichthys* (Hoese et al., 2016).

During cruises to sample the benthic biota off Ashdod (southern Israel), three specimens of an unknown shrimp-goby were collected at depths of 60 to 80 m by a bottom trawl net. An integrated study using both traditional practices and molecular taxonomy indicated that these fish belong to an undescribed species of *Cryptocentrus*.

MATERIALS & METHODS

Fish specimens were collected from the southern coast of the Israeli Mediterranean by a commercial 240 hp bottom trawler. The fish were preserved in 70% alcohol and stored at the fish

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Deleted: F/V bottom trawler, *Moty*, under captained by L. Ornoy collection of The Steinhardt Museum of Natural History, Tel-Aviv University (SMNHTAU). Muscle tissue samples were taken from fresh specimens for genetic analyses and preserved in 96% alcohol.

For counts and measurements of meristic characteristics we followed Allen et al. (2018).

Genetic analysis

Total genomic DNA was extracted from the three specimens using a micro tissue genomic DNA isolation kit following the manufacturer's protocol (AMBRD Laboratories, Turkey). Next, ca. 50 ng of template DNA was used to amplify a 651 bp fragment of the mitochondrial cytochrome c oxidase subunit I gene (*COI*) and 467 bp of the mitochondrial Cytochrome b (*Cytb*). Primers and PCR reactions are detailed in supplementary table S1. The contiguous sequences of both genes, including measurements, photos and trace files, were uploaded to BOLD system at www.v4.boldsystems.org under the BIM project (Biota of the Israeli Mediterranean) with BOLD Sample IDs: BIM769-20 for the holotype and BIM534-17 and BIM770-20 for the two paratypes. Due to the absence of *Cytb* sequences for other shrimp-associated gobies, only the *COI* gene was used to explore the phylogeny of this group. For this purpose, 107 previously published sequences belonging to ten putative genera were mined from BOLD and NCBI and aligned using ClustalW, with a single sequence of *Gobius niger* as an outgroup (Supplementary Table S2). The genetic vouchers were included in the dataset only if they indicated a precise information on the sampling localities and an unambiguous association with a Barcode Index Number (BIN) of their corresponding taxonomic identifications. Sequences of *Cryptocentrus yatsui*, for example, were excluded from the analyses since they shared a BIN with the gobies *Oligolepis formosanus* and *Redigobius bikolanus* (BIN:BOLD:ADB4723).

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The best model test for nucleotide substitution was verified for the aligned dataset using jModelTest ver. 2.1.10 (Darriba et al., 2012) under the Akaike Information Criterion (AIC). Finally, Maximum Likelihood phylogenetic reconstruction was computed using the online program NGPhylogeny.fr (Lemoine et al., 2019) and the model HKY85+G+I with 500 replicates.

Nomenclatural acts

The electronic version of this article in Portable Document Format (PDF) will represent a published work according to the International Commission on Zoological Nomenclature (ICZN), and hence the new name contained in the electronic version is effectively published under that Code from the electronic edition alone. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/. The LSID for this publication is: urn:lsid:zoobank.org:pub:B5279F4D-F5BC-454D-9ED8-3E2A13C69EAE. The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central, and CLOCKSS.

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RESULTS

Cryptocentrus steinhardti **n. sp**

Figures 1,2

Holotype: SMNH P-16037, Ashdod, Israel (31°44.835 N, 34°24.787 E), depth 80 m, January 8, 2018, 19:45. Col. N. Stern, Total length (TL) 81.9 mm, BOLD voucher BIM769-20.

Paratypes: SMNH P-14556 Ashdod, Israel (31°45.202 N, 34°27.036 E), depth 60 m, February 12, 2012, night. Col. N. Stern, TL 71.5, BOLD voucher BIM534-17; SMNH P-16038, Ashdod, Israel (31°45.589 N, 34°27.282 E), depth 60 m, December 11, 2016, 19:45. Col. N. Stern, TL 72.8 mm, BOLD voucher BIM770-20.

Diagnosis

A *Cryptocentrus* species with 58-61 rows of cycloid scales along the body, 20-21 pre-dorsal scales, reaching ca. 3/4 of the distance to eye (Figure 2) and 19-21 transverse rows. Scales cover abdomen and pre-pelvic region. No scales on pectoral fin base. First dorsal fin with six spines; second dorsal fin with a single spine and 10-11 segmented rays (last one branched). Anal fin with one spine and 9-10 segmented rays (last one branched). Pectoral fins with 14-15 rays. Pelvic fins completely united with a well-developed fraenum. Caudal fin with 17 segmented rays, 13 of them branched.

Gill rakers: 10-11 on outer gill arch, two on upper arch, one at the angle, and 7-8 on lower limb. Head sensory papillae in transverse pattern (*sensu* Miller, 1986).

Description

Body elongate and compressed. Upper profile of head convex. Mouth oblique. Maxilla to below a vertical from posterior margin of eye. Upper jaw with outer row of 16 caniniform teeth (eight on each side of the jaw) curved backward. Internal teeth in 1-2 rows small, pointed, curved backward. Lower jaw with outer 2-3 rows of small caniniform teeth, curved backward. Internal teeth in a single row of six large canines (three on each side of the jaw). No teeth on vomer. Tongue rounded.

Gill opening moderate, extending forward to below posterior margin of pre-opercle, restricted by a membrane on lower part (Figure 2). The membranes of left and right sides are completely separate. Lower margins of opercle intersect at isthmus. Gill membrane connected to side of isthmus. Gill rakers short, 10-11 rakes on outer arch, two on upper arch, one at the angle, and 7-8

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on lower arch. Anterior nostril, a tube, close above upper lip. Posterior nostril, a pore, in front of eye.

Scales: Body covered with cycloid scales, including abdomen and pre-pelvic region; pectoral fin base naked; 59-61 scales in longitudinal series; 20-21 mid-pre-dorsal scales reaching ca. 3/4 of the distance between dorsal fin and interorbital; 19-21 series of scales from origin of first dorsal fin to mid-abdomen.

Fins: First dorsal fin with six spines, third and fourth spines elongate, reaching the third ray of second dorsal fin. Second dorsal fin with a single spine and 10-11 segmented rays (last one branched). Rays long, the last three reach the caudal fin. Anal fin with one spine and 9-10 segmented rays (last one branched). Pectoral fins with 14-15 rays. Pelvic fins completely united with a well-developed fraenum. Caudal fin with 17 segmented rays, 13 of them branched.

Selected meristic characteristics and proportions are given in Table 1.

Cephalic sensory system: The skin of the head of all three type specimens was severely damaged in the commercial trawl net, hindering detection of the cephalic canal and papillae. Figure 2 presents the cephalic system of the best preserved specimen (holotype).

Nasal pores (pair) in front of eye, close to second nostril pore. Anterior interorbital pore (single) is above anterior margin of eye. Posterior interorbital pore is above 1/6 posterior of eye. Post orbital pores (pair) are above posterior margin of eye. Three pores in anterior oculoscapular canal. Posterior canal could not be detected (or does not exist). Two pre-opercular pores. Papillae on head arranged in a transversal pattern (Figure 2).

Color (preserved): Body yellow with dark brown pigmentation that becomes denser on back and head. Three irregular wide darker bars on each side of body: the first bar under 1st dorsal fin and second and third bars under anterior and posterior 2nd dorsal fin. Brown scattered spots on side of

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body in between broad bars. Chin with dark dense pigmentation. Upper half of first dorsal and anal fins' membranes are black.

Genetic analysis

Comparing the genetic sequences of both *COI* and *Cytb* with previously published data have shown great differences with any known gobies, with minimum distances in *COI* of 18.77% and 18.54% of nucleotide diversity between the new species and *Cryptocentrus albidorsus* and *Stonogobiops xanthorhinica* (BOLD vouchers GBGCA2109-13 and GBGCA2095-13, respectively) (Table 2), and 12.85% in *Cytb* differences between *C. cinctus* (NCBI voucher MT199211).

Phylogenetic reconstruction of all available shrimp-associated gobies, incorporating for the first time representatives from the genera *Lotilia*, *Myersina* and *Psilogobius,* has revealed a basal separation between two groups of shrimp-gobies, in accordance with the suggestion by Thacker and Roje (2011): silt shrimp-gobies, which include our newly described species, and reef shrimp-gobies. Nevertheless, the poorly supported internal nodes within the tree emphasizes a systematic conundrum within this group (Figure 3). *C. steinhardti* shares a branch with four other genera: *Mahidolia*, *Myersina*, *Psilogobius* and *Stonogobiops*, with no support for its generic assignment in terms of mtDNA phylogeny (Figure 3).

Finally, the cluster of reef shrimp-gobies reveals two possible misidentifications: (1) *Tomiyamichthys lanceolatus*, which may be regarded as a *Vanderhorstia* species (see Figure 1 in Thacker et al. 2011), (2) and *Vanderhorstia mertensi*, which is shown here based on a single sequence from its invasive population in the Mediterranean Sea. Both putative species in this case are suspected to be the result of a wrong assignment, considering the weak diagnostic characteristics of the genus (Shibukawa and Suzuki, 2004).

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Etymology

The new species is named after Michael H. Steinhardt in recognition of his immensely important contribution to the establishment and construction of the Steinhardt Museum of Natural History at Tel Aviv University, Israel.

DISCUSSION

As evident from the genetic results of this study, as well as from the findings of Thacker and Roje (2011), Thacker (2015) and McCraney et al. (2020), the generic status and validity of some shrimp-associated gobies are yet to be settled, and required further revisional examinations with more species involved and more genetic markers. In the present study, we followed the status of the genera and species as presented by Fricke et al. (2020).

The Red Sea is the main origin for over 400 alien species reported from the Mediterranean coast of Israel, among them five goby species (Galil et al. 2020). In the Red Sea the number of shrimp-gobiy species is 23, as featuring in the latest checklist of the Red Sea fishes (Golani and Fricke, 2018). These species belong to eight genera: *Amblyeleotris* Bleeker 1874, (6 spp.) *Cryptocentroides* Popta 1922 (1 sp.), *Cryptocentrus* Valenciennes 1837 (4 spp.), *Ctenogobiops* Smith 1959 (3 spp.), *Lotilia* Klausewitz 1960 (1 sp.), *Psilogobius* Baldwin 1972 (1 sp.), *Tomiyamichthys* Smith 1956 (3 spp.) and *Vanderhorstia* Smith 1949 (4 spp.).

Cryptocentrus steinhardti differs from the species of the genera *Vanderhorstia, Ctenogobiops*, *Cryptocentroides* and *Tomiyamichthys* in possessing transversal sensory papillae on the head vs. longitudinal sensory papillae on the head (Larson and Murdy, 2001; Bogorodsky et al., 2011).

Psilogobius spp. differ from the new species in possessing ctenoid scales on the posterior part of the body, lacking pre-opercular pores (Watson and Lchner, 1985) and the presence of thin vertical white lines on side of the body (Larson and Murdy, 2001).

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Thacker et al. (2011) recognized two different clades in this group: one clade contains the genera *Amblyeleotris, Ctenogobiops* and *Vanderhorstia* and the other contains *Cryptocentrus, Mahidolia,* and *Stonogobiops*. McCraney et al. (2020) assigned the species of the genera *Amblyeleotris*, *Ctenogobiops, Vanderhorstia* and *Tomiyamichthys latruncularius* (Klausewitz 1974) to the lineage *Asterropteryx* (together with non-shrimp associated genera) and the other shrimp-goby genera including *Tomiyamichthys oni* (Tomiyama 1936) to the lineage "*Cryptocentrus*". Hoese & Larson (2004) suggested, after examining 28 species of *Cryptocentrus,* that this genus is not monophyletic. This approach was supported by the generic dendrogram of McCraney et al. (2020, Fig. 6), although their "*Cryptocentrus*" lineage contains only ten species of *Cryptocentrus*. Our findings also show that "*Cryptocentrus*" is a polyphyletic group (Fig. 3) and includes species of the genera *Stonogobiops, Mahidolia, Myersina, Psilogobius* and *Lotilia*. Thus, the status of the new species among its congeners and closely-related genera should be examined.

In light of morphological characteristics and genetic analyses, we allocate the new species to the genus *Cryptocentrus*, despite the *COI* phylogenetic tree that has appeared to have positioned it within a different clade of genera (Fig. 3).

This genus currently comprises 36 species (Froese and Pauly, 2020). Allen and Randall (2011) distinguished a group of four species characterized by possessing fewer than 70 scales in longitudinal series along the body. They included the following four species in this group: *C. caeruleomaculatus* (Herre, 1933), *C. cyanospilotus* Allen & Randall, 2011, *C. insignitus* (Whitley, 1956) and *C. strigilliceps* (Jordan & Seale, 1906). The group was then expanded with the descriptions of *C. epakros* Allen, 2015 (Allen, 2015) and *C. altipinna* Hoese, 2019 (Hoese, 2019). Two of these species, *C. caeruleomaculatus* and *C. strigilliceps* are known from the

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western Indian Ocean (Froese and Pauly, 2020), but none of these have been reported to date from the Red Sea (Golani and Fricke, 2018).

Cryptocentrus steinhardti differs from all other members of this group, except *C. insignitus* and *C. epakros* in possessing cycloid scales only. It differs from *C. insignitus* in possessing a higher number of scales along the body (50-55 vs. 59-61), the presence of mid-predorsal scales (Table 3) and no ocellus on the first dorsal fin. *C. epakros* differs from *C. steinhardti* by possessing a lower number of scales along the body (47 vs. 59-61) and fewer transversal scales (12 vs. 19-21, Table 3).

According to Golani and Fricke (2018) four species of *Cryptocentrus* have been reported from the Red Sea: *Cryptocentrus caeruleopunctatus* (Ruppell 1830, *Cryptocentrus cryptocentrus* (Valenciennes 1837), *Cryptocentrus fasciatus* (Playfair 1867) and *Cryptocentrus lutheri* Klausewitz 1960. *C. steinhardti* differs from these four species in lower scale count along the body (59-61 vs. 77-108), lower transverse scale series (19-21 vs. 29-43) and lower number of gill rakers on the lower limb of first arch (8-9 vs. 11-13, including angle's raker; Table 4).

The finding of a new Indo-Pacific invasive species in the Mediterranean prior to its discovery in the Indo-Pacific Ocean or Red Sea is an unusual event, although other such events have been documented. The snapping shrimp *Alpheus migrans* Lewinsohn & Holthuis, 1978, which belongs to an Indo-Pacific species group, was first described from the Mediterranean (Lewinsohn and Holthuis, 1978); the jellyfish *Marivagia stellata* Galil and Gershwin, 2010 was described from the Mediterranean and later on also reported from India (Galil et al., 2013); The flounder *Arnoglossus nigrofilamentosus* Fricke, Golani and Appelbaum-Golani 2017 (Fricke, et al. 2017) that is probably a Red Sea species; the goby *Hazeus ingressus* Engin, Larson and Irmak 2018, which belong to an Indo-Pacific genus, was discovered in the Mediterranean (Engin et al,

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2018) and later was found in Abu Dabab, Red Sea, Egypt (Bogorodsky, pers. Comm.), and the jellyfish *Rhopilema nomadica* Galil, Spanier & Ferguson, 1990 (Galil et al., 1990) was described on the basis of types from the Mediterranean although it is an Indo- Pacific species.

Finding this shrimp-associated goby, however, which is also the third such goby to be documented as an invasive species in the Mediterranean (after *Vanderhorstia mertensi* and *Cryptocentrus ceruleopunctatus*) raises the question of its possible symbiosis with an alpheid shrimp. Since this taxon of gobies possesses either an obligatory or facultative association with shrimps (Lyons, 2013), its pairing with one of the ca. twenty candidate species of alpheids shrimp from the Mediterranean and the Red Sea (Karplus, 2014) can be a key factor for its survival and population establishment success in the invaded territory. Unfortunately, as the catch of *C. steinhardti* in this study was not associated with any shrimp species, the question of its possible symbiosis in the Mediterranean remains open and in need for further observations.

Last, *C. steinhardti* was collected during the night and at depths of 60 to 80 m. Finding this species during the period of dark and below the depth limits of recreational diving could be an additional reason for overlooking this species and its possible shrimp associates in its native origin.

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Captions for figures and tables

Figure 1. Holotype of *Cryptocentrus steinhardti* n.sp. (SMNHTAU P-16037, Total length 81.9 mm)

Figure 2. Cephalic sensory system *Cryptocentrus steinhardti.* NP -nasal pore; AIO - anterior interorbital pore; AO- anterior oculoscapular canal; PIO - posterior interorbital pore; PO - post orbital pore; POP- preopercolar pores. GO - Lower margin of gill opening.

Figure 3. ML phylogenetic analysis of all available *COI* sequences of shrimp-gobies. Numbers above nodes are >50 bootstrap values; In red – the new species described in this study; In parentheses – number of sequences for each species. Further information for this dataset is provided in Table S2. *Myersina* is misspelled in this figure.

Table 1: Selected meristic characteristics and proportions (Measurements in mm; proportion in %).

Table 2: Selected meristic counts of “low scale count group” *Cryptocentrus* (*sensu* Allen and Randall, 2011).

Table 3: Compression of selected counts of Red Sea species of *Cryptocentrus*.

Table 4. Genetic relationships, in %, across all available COI sequences of shrimp-associated gobies. In parentheses, no. of sequences for each species; Below diagonal, pairwise genetic distances; above diagonal its standard errors. In red, values for *Cryptocentrus steinhardti.*

Table S1. Information for the primers used for PCR and sequencing in this study.

Table S2 – BOLD information for COI sequences of all available shrimp-associated gobies used for the phylogenetic analysis in this study (n=111)

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