1	Cryptocentrus steinhardti (Actinopterygii; Gobiidae): a new
2	species of shrimp-goby, and a new invasive to the
3	Mediterranean Sea
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23 ABSTRACT

24	A new species of shrimp goby was collected at depths of 60-80 m, off the southern Israeli
25	Mediterranean coast, revealed by a unique 'DNA barcoding' signature (mtDNA COI and Cytb)
26	that differed from any other previously published goby species. This finding constitutes the third
27	record of an invasive shrimp goby in the Mediterranean Sea, revealing an intriguing ecological
28	consideration regarding possible formation of a fish-shrimp symbiosis in a newly invaded territory.
29	Following a comprehensive morphological and anatomical examinations, this species is being
30	described here as Cryptocentrus steinhardti n. sp., clustered phylogenetically with the silt shrimp-
31	gobies group, in which Cryptocentrus is the most speciose genus. However, a present phylogenetic
32	analysis demonstrates paraphyly of Cryptocentrus hence a generic name for the new species is
33	provisional. This finding constitutes the third record of an invasive shrimp goby in the
34	Mediterranean Sea, revealing an intriguing ecological consideration regarding possible formation
35	of a fish-shrimp symbiosis in a newly invaded territory.
36	Last, describing tropical species in the Mediterranean prior to their discovery in the native
37	distribution is an unusual event, although not the first such case. Several similar examples are

38 provided <u>in the present article below</u>.

Commented [SB1]: No anatomical examination in the description as well as osteological. Description is based on external characetrs.

39 INTRODUCTION

Since the opening of the Suez Canal in 1869 more than 400 multicellular nonnative species of Red 40 Sea origin, including approx. 100 fish species, have been found along the Israeli Mediterranean 41 42 coast (Galil et al., 2020). Out of this diverse invasive fauna, two species are the shrimp-gobies Vanderhorstia mertensi Klausewitz, 1974, (Goren, Stern and Galil, 2013) and Cryptocentrus 43 44 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 45 46 burrowing alpheid shrimps, exchanging tunnel construction capabilities and sentinel services (Karplus and Thompson, 2011). Common throughout the tropics, this unique fish-shrimp 47 48 association is documented from over a 100 fish species that belong to eleven valid genera of 49 gobies: Amblyeleotris Bleeker, 1874; Cryptocentrus Valenciennes (ex Ehrenberg) in Cuvier & Valenciennes, 1837; Cryptocentroides Popta, 1922, Ctenogobiops Smith, 1959, Lotilia 50 51 Klausewitz, 1960; Mahidolia Smith, 1932; Myersina Herre, 1934-; Psilogobius Baldwin, 1972; Stonogobiops Polunin & Lubbock, 1977; Tomiyamichthys Smith, 1956 and Vanderhorstia Smith, 52 1949 (Karplus, 2014; Ray, Mohapatra and Larson, 2018). An additional genus, Flabelligobius 53 Smith, 1956 is considered a synonym of Tomiyamichthys (Hoese et al., 2016; Fricke and 54 Eschmeyer, 2020). 55 During cruises to sample the benthic biota off Ashdod (southern Israel, Mediterranean Sea), three 56 specimens of an unknown shrimp-goby were collected at depths of 60 to 80 m by a bottom trawl 57 net. Integrative examinations of molecular taxonomy and traditional practices indicated that these 58 59 fish belong to an undescribed species of Cryptocentrus genus.

60 MATERIALS & METHODS

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Commented [SB2]: Authors need to add citing to method of count and measurements.

Fish specimens were collected from the southern coast of the Israeli Mediterranean by the commercial 240 hp F/V bottom trawler, *Moty*, under captained by L. Ornoy. The fish were preserved in 70% alcohol and stored at the fish 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.

66 Genetic analysis

Total genomic DNA was extracted from the three individuals using a micro tissue genomic DNA 67 68 isolation kit following the manufacturer's protocol (AMBRD Laboratories, Turkey). Next, approx. 50 ng of template DNA was used to amplify a 651 bp fragment of the mitochondrial cytochrome 69 70 c oxidase subunit I gene (COI) and 467 bp of the mitochondrial Cytochrome b (Cytb). Primers and 71 PCR reactions are detailed in supplementary table S1. The contiguous sequences of both genes, including measurements, photos and its trace files, were uploaded to BOLD system at 72 73 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. 74 75 In order to investigate the total genetic divergence of shrimp-associated gobies complex, 101 76 previously published sequences belonging to ten putative genera were mined from BOLD and NCBI to comprise an aligned dataset, with a single sequence of Gobius niger as an outgroup 77 (Supplementary Table S2). The genetic vouchers were included in the dataset only if they indicated 78 a precise information on the sampling localities and an unambiguous association with a Barcode 79 Index Number (BIN) of their corresponding taxonomic identifications. In this regard, sequences 80 of Cryptocentrus yatsui for example, were excluded from the analyses since they shared a BIN 81 with the gobies Oligolepis formosanus and Redigobius bikolanus (BIN:BOLD:ADB4723). The 82 best model test for nucleotide substitution was checked for the aligned dataset using Mega X 83

(Kumar et al., 2018) prior to further analyses. Last, phylogenetic reconstruction and genetic
distances between species were computed for the dataset using the model HKY+G+I with 5,000
replicates.

87 Nomenclatural acts

The electronic version of this article in Portable Document Format (PDF) will represent a 88 89 published work according to the International Commission on Zoological Nomenclature (ICZN), 90 and hence the new name contained in the electronic version is effectively published under that 91 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 92 93 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 94 this publication is: urn:lsid:zoobank.org:pub:B5279F4D-F5BC-454D-9ED8-3E2A13C69EAE. 95 96 The online version of this work is archived and available from the following digital repositories: PeerJ, PubMed Central, and CLOCKSS. 97 RESULTS 98

99 Cryptocentrus steinhardti n. sp.

100 Figures 1,2

- 101 Holotype: SMNH P-16037, Ashdod, Israel (31°44.835 N, 34°24.787 E), depth 80 m, January 8,
- 102 2018, $19:45_{\overline{x}}$ Col. N. Stern, Total length (TL) 81.9 mm, BOLD voucher BIM769-20.
- 103 Paratypes: SMNH P-14556, Ashdod, Israel (31°45.202 N, 34°27.036 E), depth 60 m, February
- 104 12, 2012, night_a: Col. N. Stern, TL 71.5, BOLD voucher BIM534-17; SMNH P-16038, Ashdod,
- 105 Israel (31°45.589 N, 34°27.282 E), depth 60 m, December 11, 2016, 19:45₂, Col. N. Stern, TL 72.8
- 106 mm, BOLD voucher BIM770-20.

107 Diagnosis

A Cryptocentrus species with 598-61 rows of cycloid scales along the body, 20-21 pre-dorsal 108 109 scales, reaching ca. 3/4 of the distance to eye (Figure 2) and 1920-21 transverse rows. Scales cover 110 abdomen and pre-pelvic region. First dorsal fin with six spines; second dorsal fin with a single spine and ten segmented rays (last one branched). Anal fin with one spine and nine segmented rays 111 (last one branched). Pectoral fins with 14-15 rays. Pelvic fins completely united, with a well-112 developed fraenum. Caudal fin with 17 segmented rays, 13 of them branched. Gill rakers: 7-8 on 113 114 outer gill arch, two on upper archlimb, one at the angle, and 4-5 on lower archlimb. Head sensory 115 papillae in transverse pattern.

116 Description

Body elongate and compressed. Upper profile of head convex. Mouth oblique. Maxilla <u>reaching</u> to below a vertical <u>from-at</u> 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, <u>extending forward</u> to below posterior <u>margin of pre-opercleculum</u>, restricted by a membrane at lower part. Lower margins of opercleula intersect at isthmus. Gill membrane connected to side of isthmus. Gill rakers short, 7-8 rakes on outer arch, two on upper archlimb, one at the angle, and 4-5 on lower <u>archlimb</u>. Anterior nostril, a tube, close above upper lip. Posterior nostril, a pore, in front of eye.

128	Scales: Body covered with cycloid scales, including abdomen and pre-pelvic region; 59-61 scales
129	in longitudinal series; 20-21 mid-pre-dorsal scales reaching ca. 3/4 of the distance between dorsal
130	fin and interorbital; 19-21 series of scales from origin of first dorsal fin to mid-abdomen.
131	Fins: First dorsal fin with six spines, third and fourth spines elongate reaching the third ray of
132	second dorsal fin. Second dorsal fin with a single spine and ten segmented rays (last one branched).
133	Rays long, the last three reach the caudal fin. Anal fin with one spine and nine segmented rays
134	(last one branched). Pectoral fins with 14-15 rays. Pelvic fins completely united to form of disc,
135	with a well-developed fraenum. Caudal fin with 17 segmented rays, 13 of them branched.
136	Selected meristic characteristics and proportions are given in Table 1.
137	Cephalic sensory system: The skin of the head of all three type specimens was severely damaged
138	in the commercial trawl net, hindering detection of the cephalic canal and papillae. Figure 2
139	presents the cephalic system of the best preserved specimen in the best condition (holotype).
140	Nasal pores (pair) in front of eye, close to second posterior nostril poreopening. Anterior
141	interorbital pore (single) is above anterior margin of eye. Posterior interorbital pore is above 1/6
142	posterior of eye. Post-orbital pores (pair) are above posterior margin of eye. Three pores in anterior
143	oculoscapular canal. Posterior canal could not be detected (or does not exist). Two pre-opercular
144	pores. Papillae on head arranged in a transverseal pattern (Fig. 2).
145	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 <u>parts of 2nd</u> dorsal fin.

148 Genetic analysis

- 149 Comparing the genetic sequences of both COI and Cytb with previously published data have
- shown great differences with any known gobies, with minimum distances of 17.41% of nucleotide

151	diversity between the new species and Cryptocentrus atolaorsus and C. higrocentatus (BOLD
152	vouchers GBGCA2109-13 and GBGCA1963-13, respectively) in COI (Table 3), and 12.8%
153	differences between C. cinctus in Cytb (NCBI voucher MT199211). Although clustered with
154	relatively low bootstrap values, phylogenetic analysis of available COI sequences has shown
155	monophyletic relationship for Cryptocentrus species, including the newly described species, as
156	well as clustering with all species of silt shrimp gobies (Thacker and Roje, 2011) from the genera
157	Lotilia, Myersina and Stonogobiops, and sister-grouping with Mahidolia spp. (Fig. 3). Other
158	phylogenetic studies regarding these taxa, have also related Stonogobiops and Mahidolia spp.
159	within the Cryptocentrus species complexclade, while Lotila and Myersina were absent from their
160	dataset (Thacker and Roje, 2011; Thacker, 2015; McCraney, Thacker and Alfaro, 2020). In fact,
161	to the best of our knowledge this present study is the first to incorporate these genera, as well as
162	Psilogobius spp. in a phylogenetic evaluation of shrimp-associated gobies.

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163 Moreover, the cluster of reef shrimp gobies has revealed two possible misidentifications: (1)

164 Tomiyamichthys lanceolatus, which may be regarded as a Vanderhorstia species (see Fig. 1 in

165 Thacker, Thompson and Roje 2011), (2) and Vanderhorstia mertensi, which is shown here based

166 on a single sequence from its invasive population in the Mediterranean Sea. Both putative species

167 in this case are suspected to be a result of a wrong assignment, considering the weak diagnostic

168 characteristics of the genus (Shibukawa and Suzuki, 2004).

169 Etymology

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

Commented [SB3]: This is not true. With inclusion in analysis Lotilia, Mahidolia, and Psilogobius, the present analysis confirms proposal by Hoese & Larson (2004) that the genus Cryptocentrus is paraphyletic.

The generic name Cryptocentrus is available for C.cryptocentrus and close related species. Hence generic name for C.steingardti is provisional and more species of "Cryptocentrus" should be included in further analysis. More likely group of species with white midpredorsal band forms a monophyletic group and may be form a separate genus. A low bootstrap support of Lotilia and C.reingardti cannot show a real relationships of them with other Cryptocentrus previously assigned to that genus (i.e. from C.leptocephalus to C.maudae).

i.e. authors need to add more details in discussion of the result of phylogenetic analysls.

Commented [SB4]: Change name to Vanderhorstia lanceolata in the tree to avoid confusion. Authors need to add Tomiyamichthys oni, included in Thacker et al. (2011).

174	As evident from the genetic results of this study, as well as from the findings of Thacker and Roje
175	(2011), Thacker (2015) and McCraney et al. (2020), the generic status and validity of some shrimp-
176	associated gobies are yet to be settled, and required further revisional examinations with more
177	species involved. In the present study, we followed the status of the genera and species as presented
178	by Fricke et al. (2020).

179 Thacker et al. (2011) recognized two different clades in this group: one clade includes the genera

180 *Amblyeleotris, Ctenogobiops* and *Vanderhorstia* and the other includes *Cryptocentrus, Mahidolia,*

181 *Tomiyamichthys* and *Stonogobiops*. Later studies by Thacker (2015) and McCraney et al. (2020)

182 followed this approach and assigned the genera Amblyeleotris, Ctenogobiops and Vanderhorstia

to the lineage *Asterropteryx* and the eight other genera to the lineage "*Cryptocentrus*".

184 The shrimp-associated gobies belonging to the Asterropteryx lineage are all characterized by

185 longitudinal suborbital papillae rows (sensu Miller, 1986), while the species of the lineage

186 *Cryptocentrus* are characterized by transverse rows (with the exception of *Tomiyamichthys*).

Cryptocentrus- stein#hardti differs from species of Cryptocentroides spp. by its wide gill opening, 187 188 reaching to below the pre-opercular margin, while Cryptocentroides species are characterized by 189 a restricted gill opening, extending to below pectoral-fin base. Lotilia spp. and Mahidolia spp. differ from the new species by their naked nape and lower scale count along the body (less than 190 53 in Lotilia spp. and less than 40 in Mahidolia spp.). Myersina spp. differ from C. steinhardti by 191 their lack of scales on mid nape. Psilogobius spp. differ from the new species in possessing ctenoid 192 scales on body, at least on its posterior part. Stonogobiops spp. differ from the new species by their 193 194 large vomerine teeth.

The genus *Cryptocentrus* currently comprises 36 species (Froese and Pauly, 2020). Allen and Randall (2011) distinguished a group of four species characterized by possessing fewer than 70 Commented [SB5]: Authors incorrectly interpreted Thacker, Thompson & Roje 2011 where theses authors focused on shrimpassociated gobies. They clearly showed and in other articles too (e.g. McCraney) two clades. Asterropteryx is not associated with shrimps, and it is clear from analysis in all these articles. So unclear why authors of present article decided that Amblyeleotris, Ctenogobiops and Vanderhorstia belongs to lineage Asterropteryx. This is a wrong conclusion.

Commented [SB6]: Should be re-written, see comment above

Commented [SB7]: Authors omitted important article Hoese & Larson (2004) where authors noted that the genus paraphyletic and proposed species groups.

series of 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 expanded
with the descriptions of *C. epakros* Allen, 2015 -(Allen, 2015) -and *C. altipinna* Hoese, 2019
(Hoese, 2019). None of these species have been reported to date from the Red Sea (Golani and
Fricke, 2018).

203 C<u>ryptocentrus</u>- steinhardti differs from all other members of this group, except *C. insignitus*, in
 204 possessing cycloid scales only. It differs from *C. insignitus* in possessing a higher number of scales
 205 along the body and the presence of mid-pre-dorsal scales (Table 2).

206 The finding of a new Indo-Pacific invasive species in the Mediterranean prior to its discovery in 207 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 208 209 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 210 211 Mediterranean and later on also reported from India (Galil, Kumar and Riyas, 2013); the flounder 212 Arnoglossus nigrofilamentosus Fricke, Golani and Appelbaum-Golani 2017 (Fricke, et al. 2017) that is probably a Red Sea species, and the jellyfish Rhopilema nomadica Galil, Spanier & 213 Ferguson, 1990 (Galil et al., 1990) was described on the basis of types from the Mediterranean 214 215 although it is an Indo-Pacific species.

Finding <u>this the new shrimp</u>-associated goby, however, which is also the third such goby to be documented as an invasive species in the Mediterranean, raises the question of its current association with an alpheid shrimp. Since this taxon of gobies possesses either an obligatory or facultative association with shrimps (Lyons, 2013), pairing with one of the approx. twenty Commented [SB8]: Another example is Hazeus ingressus Engin, Larson & Irmak, 2018 was described from Turkey but later was found by reviewer (i.e. me) from Abu Dabab, Egypt.

220	candidate species of alpheids shrimp from the Mediterranean and the Red Sea (Karplus, 2014) is
221	a key factor for its survival and population establishment success in the invaded territory.
222	Unfortunately, the catch of C. steinhardti in this study was not associated with any shrimp species,
223	thus the question of its possible symbiosis in the Mediterranean remains open and required further
224	observations

Cryptocentrus- <u>s</u>Steinhardti was collected at depths of 60 to 80 m during night trawls. Finding this
 species during the period of dark and beyond the depth limits of recreational diving, despite this
 possibly having been accidental, could be an additional reason for overlooking this species and its
 possible shrimp associates to date in its native origin.

229 ACKNOWLEDGMENTS

We thank Mr. O. Rittner for the photographs of the fish and to Ms. N. Paz for editing themanuscript.

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	Fish catalogue number (SMHTAU)			
Measurements and counts	16037	16038	14556	
Total length	81.9	72.8	71.46	
Standard length	58.5	51.1	49.95	
Head length	14.8	14.2	12.9	
Body depth	8.9	7.8	7.6	
Head width	5.8	5.4	5.1	
Eye diameter	3.44	4	3.6	
Interorbital	1.1	1.1	1	
Distance snout to origin of first dorsal fin	18	16.9	14.8	
Distance snout to origin of second dorsal fin	30.2	29.2	25.8	
Distance snout to origin of anal fin	33.4	30.5	28	
No. of scale series along the body	61	59	60	
No. of scale in transversal series	20	21	19	
No. pre-dorsal scales	21	20	20	
No. of spines in first dorsal fin	6	6	6	
No. of spines/ segmented rays in second dorsal fin	I <u>.</u> =+10	I <u>_</u> =+10	I <u>_</u> =+10	
No. of spines/ segmented rays in anal fin	I <u></u> +9	I <u>.</u> =9	I <u>_</u> +9	
No. of rays in pectoral fin (left side)	15	15	15	
No. of caudal rays	17	17	17	
Count of gill rakers on upper archlimb	2	2	2	
Count of gill rakers on upper archlimb	5	4	4	
Count of gill rakers at arch angle	1	1	1	
Total count of gill rakers	8	7	7	
Proportions (in %)				
Standard length of total length	71.4	70.2	69.9	
Head length of standard length	25.3	27.8	25.8	
Body depth of standard length	15.2	15.3	15.2	
Eye diameter of head length	23.2	28.2	27.9	
Interorbital space of head length	7.4	7.7	7.8	
Distance snout to origin of first dorsal fin	30.8	33.1	29.6	
Distance snout to origin of second dorsal fin	51.6	57.1	51.7	
Distance snout to origin of anal fin	57.1	59.7	56.1	

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

 Table 2 Selected meristic counts of "Cryptocentrus low scale count" group.

Species	LL	$2^{nd}D$	А	PreD-Mid line
Cryptocentrus steinhardti n. sp.	59-61 ¹	I,+10	I, + 9	20-21 Formatted: Font: Not Italic
Cryptocentrus cyanospilotus	49-59 ²	I <u>.</u> =10	I <u>,</u> ≠9	10-13 Formatted: Font: Not Italic
Cryptocentrus caeruleomaculatus	60 ²	I <u>.</u> =10	I <u>.</u> ≠9	none
Cryptocentrus strigilliceps	50-71 ²	I <u>.</u> +10	I <u>.</u> ≠9	"Predorsal midline and sides scaled to a point
				just before to just behind posterior preopercular
				margin" (Hoese, 2019)
Cryptocentrus insignitus	52-55 ¹	I <u>,</u> +12	I_+11	Nape and shoulders incompletely scaled
				(Whitley, 1956).
Cryptocentrus altipinna	56-65 ²	I <u>.</u> +10	I <u>.</u> ≠9	none
Cryptocentrus epakros	47 ²	I <u>,</u> +10	I <u>.</u> =+9	19