Report of Nerocila bivittata (Risso, 1816) (Isopoda: Cymothoidae) Parasitic on Alien Fish, Pterois miles (Bennett, 1828) from the Aegean and Mediterranean Sea

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Report of *Nerocila bivittata* (Risso, 1816) (Isopoda: Cymothoidae) Parasitic on Alien Fish, *Pterois miles* (Bennett, 1828) from the Aegean and Mediterranean Sea

Alper Yanar [1*], Cemal Turan [1], Servet Ahmet Doğdu [2]

[1] Department of Marine Sciences, Faculty of Marine Sciences and Technologies, Iskenderun Technical University, 31220, Iskenderun, Hatay, Turkey

[2] Iskenderun Technical University, Maritime Vocational School of Higher Education, Underwater Technologies, 31220, Iskenderun, Hatay, Turkey

Abstract

Since 2014, an invasive alien fish species, lionfish, *Pterois miles* (Bennett, 1828) has been started to distribute in the Turkish Mediterranean Sea coasts and spread out to the Aegean Sea. In the present study, a Cymothoid fish parasite, *Nerocila bivittata* (Risso, 1816) is reported from the pectoral fin of *P. miles* caught from the Gulf of Iskenderun (Northeastern Mediterranean) and Bodrum, Turkey (Aegean Sea Coast). This study is the first record of *N. bivittata* on lionfish *P. miles* caught from Gulf of Iskenderun. Key diagnostic characters of *N. bivittata*, some morphological differences observed between the newly collected material of *N. bivittata* and its previous descriptions, in addition to newly observed additional characters were presented.

Keywords:
Lionfish, cymothoid isopod, Gulf of Iskenderun, Aegean Sea

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Introduction

For nearly a century and a half, the Suez Canal has been mediating the migration of Indian Ocean and Red Sea biota to the Mediterranean and Black Sea (Ergüden et al., 2012; Yağlıoğlu et al., 2014; Ünal et al., 2015; Turan et al., 2015; Doğdu et al., 2016; Stamouli et al.,2017; Galanidi et al., 2019). In particular, some fish species, belonging to the families Tetraodontidae, Nemipteridae, and Haemulidae have been successfully adapted to the whole Mediterranean environment after their immigration and now, these fish species are treated as invasive alien immigrant fish species (Turan et al., 2011; Yağlıoğlu & Turan, 2012; Ergüden et al., 2013; Turan et al., 2018). Twenty years ago, lionfish, *Pterois miles* (Bennett, 1828) was reported for the first time in the southeastern

*Corresponding Author: Alper YANAR, E-mail: alper.yanar@iste.edu.tr

Mediterranean waters, off the Israel coasts (Golani & Sonin, 1992; Gürlek et al., 2016; Turan, 2020). During the following years, this poisonous fish species maintained its distribution towards the northeastern Mediterranean Sea and was reported for the first time in Turkish Mediterranean waters (Turan et al., 2014). Since its first discovery, the lionfish has successfully inhabited the Turkish coasts of Mediterranean and Aegean Sea.

In addition to the occurrence of invasive alien fish species in the Mediterranean, some species of fish parasites, carried on these alien fishes, have also been reported. For example, a species of Caligid copepod, *Caligus lagocephali* Pillai, 1961 (reported as *Caligus fugu* Yamaguti & Yamasu, 1959) and a Teaniacanthid copepod, *Taeniacanthus lagocephali* Pearse, 1952 were first discovered in the Mediterranean on two Red Sea immigrant puffer fishes, *Lagocephalus spadiceus* (Richardson, 1845) and *L. suezensis* Clark & Gohar, 1953. (Özak et al., 2012).

However, in addition to carrying their own parasites, immigrant fishes can also be a new host for native or already existing parasite species. In parallel with this case, a short time ago, two Cymothoid parasites, *Nerocila milesensis* Öktener, Tuncer & Trilles, 2020 (Isopoda: Cymothoidae) and *Nerocila bivittata* (Risso, 1816) were reported from the Red Sea immigrant lionfish, *Pterois miles* caught in south Aegean Sea coasts of Turkey (Öktener et al., 2020). The first parasite, *N. milesensis*, was a newly discovered species, the latter was previously known and one of the most abundant species of the Cymothoid parasites.

N. bivittata is reported from numerous fish hosts from Mediterranean Sea (Trilles, 1991; Samn et al., 2014; Castelló et al., 2020). Also *N. bivittata* was recorded from the Mediterranean (Charalambos et al., 2019; Çelik et al., 2020), Aegean (Demir, 1952; Geldiay & Kocataş, 1972; Kırkım et al., 2008; Öktener et al., 2009, 2010; Akmirza, 2014), Marmara (Kırkım, 1998) and Black Sea (Öktener, 2007; Öktener et al., 2009; Kayış & Er, 2012; Er & Kayış, 2015; Oğuz & Öktener, 2007) coasts of Turkey.

In this paper, we present the general morphology and the key diagnostic characters of *N. bivittata* based on the newly collected material from lionfish caught from the Gulf of Iskenderun and the Aegean Sea waters, off Bodrum, Turkey. In addition, we present the morphological differences we observed between the presently reported material of *N. bivittata* and it previous re-descriptions. In this study, the Gulf of Iskenderun constitutes a new distribution area for *N. bivittata* on lionfish.

Materials and Method

Nerocila bivittata (Risso, 1816) (n=2) were collected from lionfish, *Pterois miles* (Bennett, 1828) (n=15, total body length 15–32 cm) caught from Bodrum in August 2021 and from the Gulf of Iskenderun in July 2021 (n=18, TBL 16–34 cm). *N. bivittata* was removed from the surface of the left pectoral fin of fish samples and preserved in 70% ethanol. Collected specimens were cleared in lactic acid for two hours and photographed using an Axiocam ER 5S camera attached on

Olympus SZX7 stereo microscope. Zeiss Axio Scope A1 light microscope was used to examine the dissected parts placed in temporary slides. Identifications and comparisons were performed according to Trilles, (1975); Bruce (1987), Öktener et al., (2020), and Aldık et al., (2022).

Results

Family: Cymothoidae Leach, 1818

Genus: Nerocila Leach, 1818

Species: *Nerocila bivittata* (Risso, 1816)

Two non-ovigerous females; collected from the pectoral fin of lionfish, *Pterois miles* (Bennett, 1828) caught from eastern Mediterranean waters of the Gulf of Iskenderun and the Aegean Sea waters, off Bodrum province (Figure 1). Specimens were deposited in the collections of the Department of Marine Sciences in Iskenderun Technical University (Registration Number: 2021-PC-008).

Figure 1. *Nerocila bivittata* attached to the pectoral fin of *Pterois miles*

The total body length of the Aegean Sea specimen was 15.28 mm, and 10.45 mm in width (Figure 2) whereas the Mediterranean specimen from the was 15.41 mm in length and 10.83 mm in width. Pointed lateral margins of pereonites extended to posterior. Along the longitudinal axis, width of pereonites (from 1 to 5) increased gradually and slightly. Cephalon, extending onto first pereonite, has slightly rounded posterior margin. Pereonite 1 is the narrowest part of the pereon, while pereonite 5 is the widest. Posterior margins of pereonite 5 to pleon 5 has bow like concave shape and lateral margins of pereonite 7 extending to pleon 5.

Figure 2. Dorsal view of *Nerocila bivittata* (scale bar: 5 mm)

Pereonite 7, extending to posterior, overlies and cover some part of pleonite 1. Pleonite 1 shorter than others, 2 to 4 almost equal and shorter than 5. Lateral margins of pleonites are narrowest in pleonite 1, widest in pleonite 2.

Antennula 8 segmented and longer than antenna. Article 1-2 slightly larger than others. Articles 6 to 8 carry aesthetascs (Figure 3A). Antenna consists of 11 articles (Figure 3B). Article 5 to 9 with aesthetascs on distolateral corners of articles. Article 10 and 11 carry much more aesthetascs and aesthetascs are also located on distal corner of article 11. Mandible 3 segmented (Figure 3C); proximal segment largest, middle segment shortest, distal segment armed with 7 setae along outer distal margin. Outermost seta longest, lengths of adjacent 6 setae decreased gradually. Inner distal margins of outermost 7 setae are ornamented with finely serrations (Figure 3C-inset). Maxilla bilobed, 2 spines on lateral lobe and 2 spines on median lobe. Also rows of finely denticles aligned along outer margin of the maxilla (Figure 3D). Maxilliped without oostegial lobe, a palp with 6 recurved lateral spine (innermost 2 spine are smaller than others) (in specimen from Bodrum with 5 recurved lateral spine) on ventral margin of article 3 (Fig 3E). Maxillula carries 4 apical spines, 3 of which slightly curved (Figure 3F).

Pereopod 1 shorter than others. Pereopod 5 longest. Pereopod 7 ischium carrying 1 seta, merus with 2 setae, carpus with two rows of 2 and 3 setae, posterior margin of propodus armed with 2 and 3 pairs of setae (Figure 4) opposite to each other.

Pleopods not visible in dorsal view. Sizes of pleopods slightly decreasing from 1 to 5. All exopods longer than endopods belonging to its own pleopods. All pleopods bearing coupling hooks on their protopods. Pleopod 2 with appendix masculina about 0.6 length of endopod. 3–5 endopod with proximo-medial lobe well developed and folded. Endopod of uropod shorter than exopod, and not reach up to posterior margin of pleotelson.

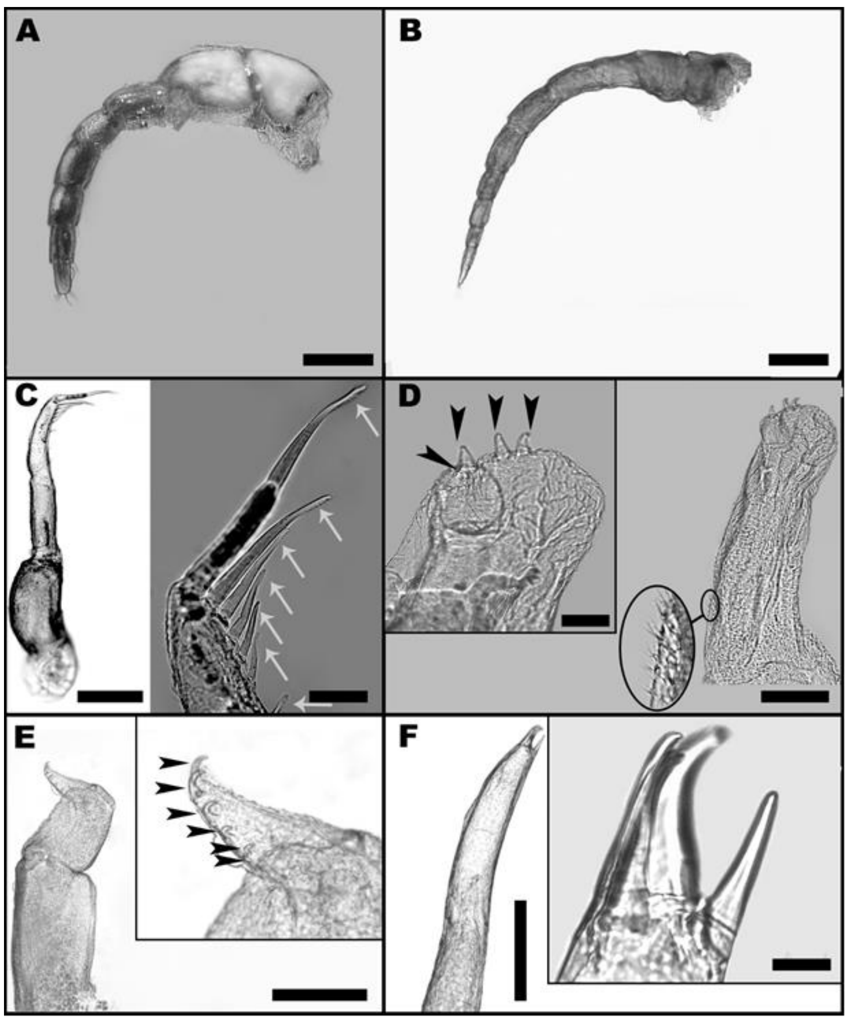

Figure 3. *Nerocila bivittata* (specimen from Gulf of Iskenderun) A; Antennula, B; Antenna, C; Mandible, C-inset; Setae on distal margin of mandible (arrows; serrated setae) , D; Maxilla, D-inset; Tip of maxilla (arrow heads; 4 spines on maxilla), E; Maxilliped, E-inset; article 3 of

Maxilliped (arrowheads; recurved spines), F; Maxillula, F-inset; 4 apical spines on Maxillula. Scale bars: A-B=400 µm, C= 250 µm, C-inset=50 µm, D= 200 µm, D-inset= 30 µm, E= 400 µm, F= 200 µm, F-inset= 15 µm

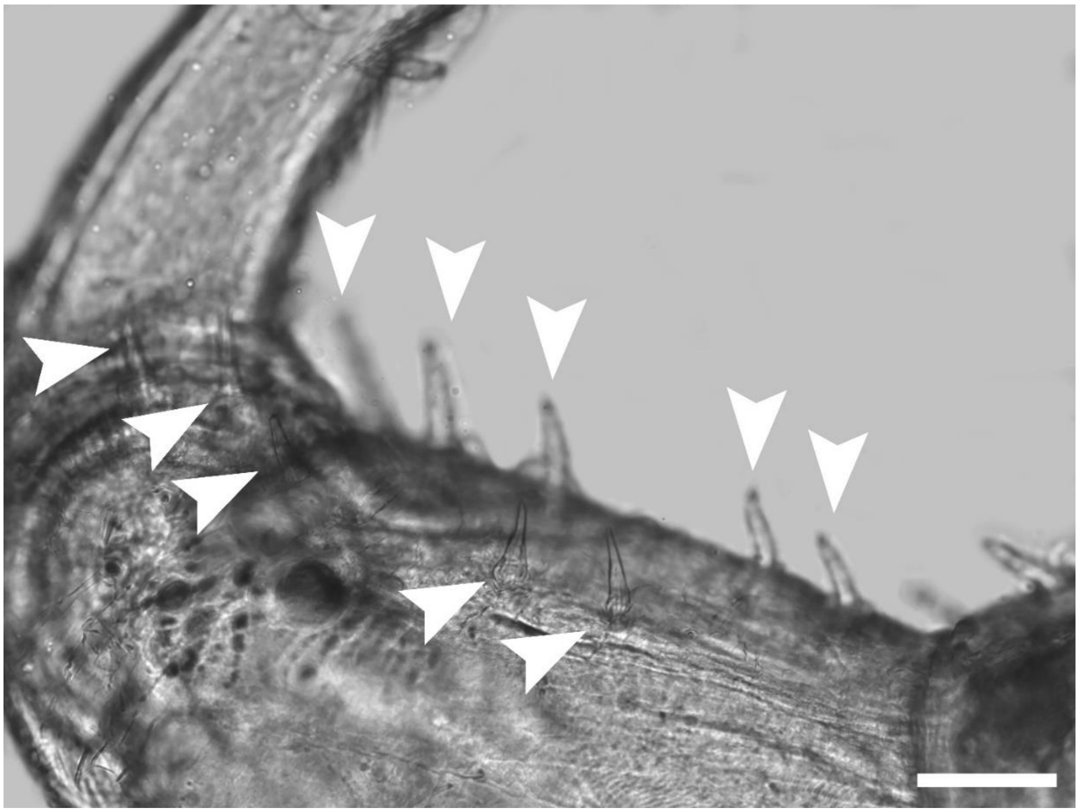

Figure 4. *Nerocila bivittata* (specimen from Gulf of Iskenderun), propodus of Pereopod 7 (Arrowheads; setae), scale bar: 100 µm

Remarks

The morphological features of our specimens revealed similarities to *N. bivittata* reported by Trilles, (1975), Kırkım, (1998), Öktener et al., (2020), Aldık et al., (2022), in having identical coloration, similar body length, body proportions, general shape and body structure. However some morphological differences and additional characters were observed. These are as follows:

(1) Antennula of our specimens carrying aesthetascs on article 6 to 8 (Figure 3A). However, Trilles (1975) and Kırkım (1998) observed that aesthetascs exist on article 5 to 8. Öktener et al., (2020) reported that their specimens carrying aesthetascs on article 8.

(2) Trilles (1975), Öktener et al., (2020) and Aldık et al., (2022) informed that mandible article 3 with 8, 8 and 9 setae, respectively. However our specimen's mandible with 7 setae. In addition, inner distal margins of these 7 setae ornamented with fine serrations.

(3) Shape and spines of the maxilla were found similar to the maxilla presented in previous descriptions (Trilles, 1975; Öktener et al., 2020; Aldık et al., 2022). However, we observed additional rows of fine denticles aligned along the outer margin of the maxilla (Figure 3D).

(4) Trilles (1975) and Öktener et al., (2020) reported that their specimen's maxilliped with 4 recurved spines. Aldık et al., (2022) observed that 5 recurved spines on maxilliped. Our specimens carries 6 recurved spines (Figure 3E); adjacent 2 spine located proximally are smaller than other 4 spine, on article 3 of the maxilliped.

(5) Preopod 7 propodus of our specimens carries 2 and 3 pairs setae (Figure 4). Numbers and positions of these setae compatible with (Kırkım, 1998). However (Öktener et al., 2020) reports two rows of 5 and 3 setae and (Aldık et al., 2022) reports 10 setae but different in positions.

Discussion

Cymothoid parasites are often attached to the body surface, buccal cavity, fins, and gills of their fish hosts. As they feed on tissues on their attachment site, they cause severe lesions and give physical damage to their fish hosts. Cymothoid parasites are also vectors of secondary bacterial and viral infections which lead mortalities particularly in finfish culture (Hadfield & Smit, 2019). These direct and indirect effects give rise to economic losses. However, although it is known to have negative effects on their hosts, it is possible to obtain useful information about their environment and benefit from them. For example, they are used as biological tags in stock assessment (Cuyás et al., 2004), bioindicator in environmental pollution (Pérez-del-Olmo et al., 2019) and alternative medicament in medicine where treatment of diabetic foot ulcers and chronic wounds (Meimeti et al., 2019; Vitsos et al., 2019). Because of these ecological and economical reasons, it is important monitoring the geographical distribution and prevalence of Cymothoid isopods.

Cymothoid isopods are protandrous hermaphrodites and have a diphasic life cycle. In early stages of their life cycle they attached to temporarily teleost hosts (Brusca, 1978a). When they encounter appropriate host, they change the host and undergo to male stage. Unless the male leaves the final host and re-attaches to a new hosts, the male remains as male (Lester, 2005). If it changes the host, then transforms into a female (Adlard & Lester, 1995). If they do not encounter the specific host, they leave the host and transform into another form (Brusca, 1978b). Every stages of this are complicated and depend on many circumstances life cycle, including moulting phases. At each moulting phase, body proportions, number of setae, size and shape of appendages change (Jones et al., 2008). Therefore, it can be thought that this phenomenon may give rise to appearing of some taxonomic disparities among studies, including the present study.

In some cases that parasite is not able to find its specific or appropriate host, the parasite may feed temporarily to survive on any other taxon, until encounter one of the specific hosts

(Lindsay & Moran, 1976; Hoberg & Brooks, 2008; Jones et al., 2008). Sometimes, the parasite may exist on a non-specific host accidentally. Following the reports of Charalambos et al., (2019) from Cyprus and Öktener et al., (2020), the present study, as the third report of *N. bivittata* on lionfish from different locations, is important in terms of being an evidence of that lionfish is not temporarily or accidentally host.

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Author Contributions

A.Y., C.T. and S.A.D. performed all the experiments and drafted the main manuscript text.

Conflict of Interest

The authors declare there is no conflict of interest in this study.

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