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Research Article **Araştırma Makalesi**

First Gnathia sp. (Gnathiidae) Infestation on Labrus viridis (Labridae) in Türkiye

Türkiye'de Labrus viridis (Labridae)'de İlk Gnathia sp. (Gnathiidae) Enfestasyonu

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Abstract: Gnathiids are ectoparasites of elasmobranchs and teleosts. The adult stage of gnathiids is free-living but has a blood-sucking parasitic form called praniza larvae. Gnathiid praniza infestations have been previously reported in numerous species of fish. In the current study, *Labrus viridis* collected from İzmir Bay, Aegean Sea, was examined for parasite infestation. *Gnathia* sp. was found predominantly in the gills and mouth cavity of fish with a 58.3% prevalence. The samples were collected with fine forceps from the host and preserved in 70% ethanol and 10% formaldehyde solution for observation with a light microscope and scanning electron microscopy to identify parasites. Red and green-blue pranizas were detected, and the hindgut of pranizas showed differences depending on attachment location of the host.

Keywords

- Aegean Sea
- Gnathiid isopods
- Labridae
- *Labrus viridis*
- Ectoparasites

Özet: Gnathiidler, elasmobranch ve teleost balıkların ektoparazitleridir. Ergin aşamada olan Gnathiidler serbest yaşar ancak praniza larvaları adı verilen kan emici bir parazit formu bulunmaktadır. Daha önce birçok balık türünde Gnathiid praniza enfestasyonları rapor edilmiştir. Bu çalışmada, *Labrus viridis* türüne ait balıklar Ege Denizi, İzmir körfezinden örneklenmiş ve parazit enfestasyonu açısından incelenmiştir. Balıkların solungaçlarında ve ağız boşluklarında %58,3 prevelans ile *Gnathia* sp. tespit edilmiştir. Örnekler ince uçlu pens kullanılarak balıktan toplanarak %70 etanol ile %10 formaldehit solüsyonlarında ışık mikroskobunda ve taramalı elektron mikroskobunda incelenmek üzere saklanmıştır. Kırmızı ve Mavi-yeşil pranizalarda, konakçı üzerinde bulundukları bölgelere göre farklılıklar tespit edilmiştir.

Anahtar kelimeler

- Ege denizi
- Gnathiid izopodlar
- Labridae
- *Labrus viridis*
- Ektoparazitler

1. INTRODUCTION

The Labridae family, known as wrasses, are found worldwide in tropical, subtropical, and temperate seas (Parenti and Randall, 2000). Generally, labrids live in the rocks of the upper coastal belt, where species diversity and abundance are high (Hanel et al., 2002). At night, most of the wrasses bury themselves in the sand to sleep, and many simply to take shelter. Most *Labridae* sp. feed on invertebrates, but there are species that feed on several organisms such as zooplankton, fish, coral polyps, crustacean ectoparasites, and fish mucus (Parenti & Randall, 2000).

Isopods can occur from the intertidal to the continental shelf and deep seas in oceans from polar to tropical waters (Schultz, 1969; Brusca & Brusca, 2003). Most parasitic isopods are ectoparasites and feed on the host's blood or hemolymph. To rip the flesh of the host organism and penetrate the blood vessels or sinuses, the mouth part of the parasitic isopods forms a cone with maxillipeds and tiny pointed mandibles (Lester, 2005).

There are three main groups in the Isopoda: cymothoids, epicaridians, and gnathiids (Lester, 2005). Adult gnathiids do not feed and inhabit benthic habitats (Smith, 1904; Monod, 1926) but have a juvenile stage in which blood-sucking praniza larvae feed on blood and tissue fluids from teleosts and elasmobranch hosts and can cause focal lesions at the place of attachment (Lester, 2005; Diniz et al.,

2008; Adday & Khamees, 2022). Adult and larval gnatiids are morphologically different and adults show strong sexual dimorphism (Monod, 1926; Schultz, 1969; Tanaka, 2007). The larval forms consist of three stages of life comprising two shapes called praniza and zuphea (Ferreira, 2011). Furthermore, gnathiids are intermediate hosts for fish blood parasites (Smit & Davies, 2004).

In this study, we report the infestation of *Gnathia* sp. in green wrasse (*Labrus viridis*) in Türkiye for the first time with the morphological characteristics of the praniza larvae and the prevalence values of the infestation.

2. MATERIALS and METHODS

The green wrasse (*Labrus viridis*), caught in İzmir Bay (38° 32′ 9″ N, 26° 45′ 17″ E) in the Aegean Sea, was obtained at İzmir Fish Market, Turkiye between March- April 2022 (Figure 1).

Figure 1. İzmir Bay, Aegean Sea.

The fish were placed on ice (approximately 2 hours) until transferred to the İzmir Katip Celebi University Faculty of Fisheries Fish Disease and Biotechnology Laboratory. The fish samples measured total length (TL) between 32.8 - 34.5 cm and weighted 383 g to 448 g, later examined for parasites according to Smit & Davies (1999) Smit et al. (1999), and Davies & Smit (2001). Parasites were removed from their site of infection (body surface, gills, fins, and mouth) using fine forceps and transferred to petri dishes with a 5% sodium hypochlorite water solution to remove excess debris (Johnson, 1969). The gentle current of this solution was flushed into the samples using a glass dropper and a soft hair brush to activate the removal of host tissue from parasites (Khamees, 1996; Grobler et al., 2003). The parasite samples collected were fixed in 70% ethanol and 10% formaldehyde solution.

Ectoparasites were identified according to morphological descriptions according to Monod (1926) and Hispano et al. (2014) under light microscopy (OLYMPUS, CX22RFS1).For scanning electron microscopy, parasite samples fixed in 10% formaldehyde were dehydrated through a graded series of ethanol up to 100% according to Smit et al. (1999) and Tanrıkul et al. (2019), later mounted and coated with gold using QUORUM Q150 RES (Quorum Technologies, UK), examined in Carl Zeiss

300 VP at İzmir Katip Celebi University Central Research Laboratory. Prevalence values were calculated according to Rozsa et al. (2000) using Quantitative Parasitology 3.0.

3. RESULTS and DISCUSSION

In the examined fish, praniza larvae (Figure 3, A- B) were present in seven total twelve samples, and the parasite was in red and greenish-blue colors. A total of 707 praniza larvae were found in *L. viridis* (Figure 2, A). Larvae of the genus Gnathia Leach, 1814, attach to the body surface, caudal fin, around the eye, gill lamellae (Figure 2, B) (with excess mucus on the gill filaments), around and the mouth cavity (Figure 2, C). The colours of the larvae were greenish blue (280) (Figure 3, C) and reddish (427) as well as few parasites showed both colours in their hindgut (Figure 3, F). Parasites were predominantly collected primarily from the gills, operculum, and mouth of infested fish (Table 1) with 58.3% prevalence. No correlations were detected between fish size and parasite abundance. Furthermore, parasites attached to the fins (Figure 3, E) showed less blood in their hindgut than those attached to the gills, operculum, and mouth (Figure 3, D).

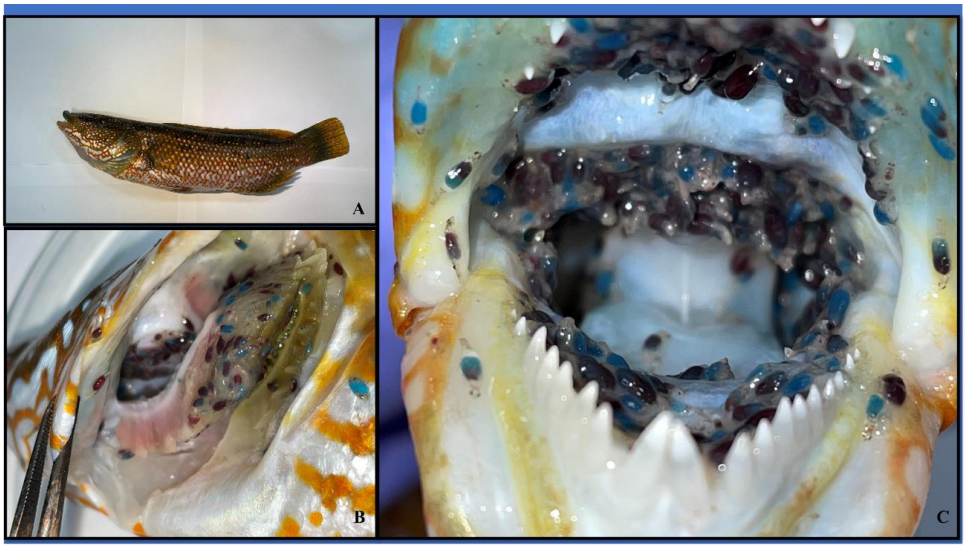

Figure 2. A: *Labrus viridis*, B: Excess mucus on the gills infested with praniza, C: Intensive infestation of praniza in the mouth.

Table 1. Attachment places of pranizas collected from *Labrus viridis*

	Reddish praniza				Green-blue praniza			
Labrus viridis	Gills	Skin	Mouth	Fins	Gills	Skin	Mouth	Fins
Sample 1	173	7	199	5	92	12	161	3
Sample 2	2	-	1	-	1	-	2	-
Sample 3	2	2	4	1	3	-	1	1
Sample 4	4	-	3	-	-	-	-	-
Sample 5	3	-	2	-	3	-	-	-
Sample 6	5	-	2	-	1	-	-	-
Sample 7	9	-	3	-	-	-	-	-
Sample 8	-	-	-	-	-	-	-	-
Sample 9	-	-	-	-	-	-	-	-
Sample 10	-	-	-	-	-	-	-	-
Sample 11	-	-	-	-	-	-	-	-
Sample 12	-	-	-	-	-	-	-	-
Total:	198	9	214	6	100	12	164	4

In Labridae, cleaning behaviour is well represented, and 46% of the fish are known to be cleaner in the wild (Cote, 2000). In marine fish cleaning mutualism, the family Labridae are known to be

predators of parasites and decrease the ectoparasite loads of the infected host species through cleaning behavior (Grutter, 1999) and separated into two groups; obligate cleaner (eight species) diet contains almost entirely parasites, and facultative cleaners (41 species) which were only cleaning as juveniles (Cote, 2000). This study supports evidence from observations by Cote (2000) and Arnal et al. (2006) who pointed out cleaning behaviour among the Labridae species related to fish size, colour patterns, and body shape. Arnal et al. (2006) stated the emergence of cleaning behaviour and the presence of a dark lateral stripe on the body surface of cleaning species by phylogenetic traits among the Labridae family, where *L. viridis* is not present.

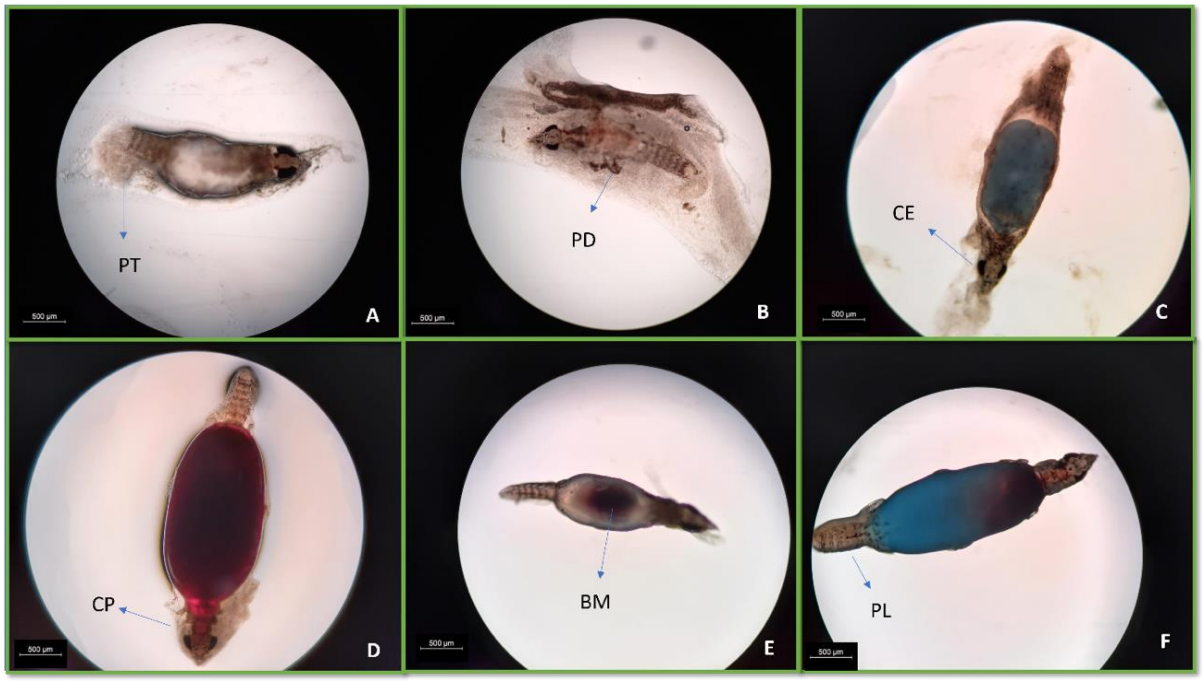

Figure 3. A: Female *Gnathia* sp., B: Male *Gnathia* sp., C: Green-blue praniza, D: Reddish praniza collected from the mouth, E: Reddish praniza collected from base of the fins, F; Praniza with both colours. Note: BM: blood meal; CE: Compound eye; CP: Cephalosome; PD: Pereopod; PL: Pleon; PT: Pleotelson.

Among crustacean groups, isopods are one of the most morphologically divergent (Bayoumy et al., 2013), and *Gnathia* Leach, 1813 is the widest genus among the family Gnathiidae, which comprises 12 genera, and more than 190 species distributed worldwide (Cohen, 1994; Hadfield & Smit, 2008; Hispano et al., 2014). Due to their body plan being different from that of other isopods, gnathiids have been an incomprehensible taxon (Tanaka, 2007).

To feed on blood and plasma, these isopods attach the host's skin and gills with piercing mouthparts (Figure 4). and use a muscular oesophagus and a grooved paragnath (Monod, 1926). After praniza feeds on blood, it goes to the benthos for meal digestion and is molten into females or males (Tanaka, 2007; Ferreira, 2011; Öktener & Tuncer, 2020). Gnathiids can affect the host by inflaming and destroying mucosal tissue (Honma & Chiba, 1991), decreasing blood volume (Jones & Grutter, 2005), transmitting blood parasites (Curtis et al., 2013), increasing stress hormones (Triki et al., 2016), and reducing juvenile performance and growth (Jones & Grutter, 2008), or by killing the host (Paperna & Por, 1977; Mugridge & Stallybrass, 1983). Smit et al. (2003) reported that the length of the feeding period of Zuphea larvae was different by attachment to the area of the host fish. The larvae attached to the body of the host fish completed feeding faster than those attached to the fish. This study supports evidence from clinical observations by Smit et al. (2003) and Hispano et al. (2014) that the amount of blood in the intestinal tract of larvae is associated with host attachment. In our findings, the larvae attached to the fins showed less blood than those attached to the mouth, gills, and body surface.

Praniza attached to *Labrus bergylta* and *Anguilla anguilla* was also observed in green-blue colour by other authors (Monod, 1926; Mouchet, 1928; Hispano et al., 2014).

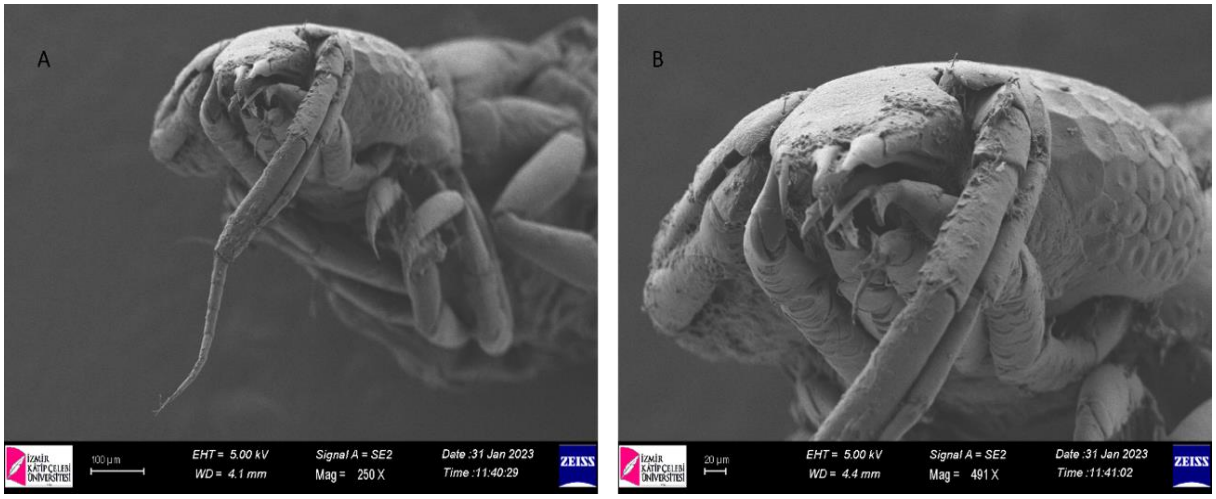

Figure 4. Scanning electron micrograph of Gnathia praniza larvae. A-B: view of the cephalosome.

The Gnathiidae family has free-living adults and parasitic juveniles that feed on the blood and tissue fluids of the host organism. Taxonomic descriptions depend on the non-feeding benthic adult male gnathiids, upon the identification of gnathiid juveniles or female adults might be difficult (Smit & Davies, 2004). Gnathiids were described as both free-living and juvenile (parasitic stage) in the Turkish coasts. The first description of Gnathiids in Turkiye Seas was by Geldiay & Kocataş (1972), who identified *Gnathia vorax* from benthos in the Aegean Sea (Balıklıova, İzmir). Later, *Gnathia vorax* was reported from benthos and fish (undescribed fish sp.) from different parts of the Aegean Sea (Kırkım, 1998). *Paragnathia formica* was described from fish (undescribed fish sp.) for the first time by Kırkım (1998) in the Aegean Sea and Türkiye. Furthermore, Kırkım et al. (2008) reported *P. formica* from *Mugil cephalus* and *Pagellus erythririus*.

Parasitic stage of Gnathiids was reported by several authors from different parts of the Türkiye in various fish species such as *Diplodus annularis* (Akmirza, 2000, Akmirza, 2001; Akmirza, 2010), *Diplodus vulgaris* (Akmirza, 2000; Akmirza, 2001; Alaş et al. 2009; Akmirza, 2010; Koyuncu et al. 2013), *Diplodus sargus* (Akmirza, 2000), *Dentex dentex* (Akmirza, 2000), *Lithognathus mormyrus* (Akmirza, 2000), *Pagrus pagrus* (Akmirza, 2000), *Symphodus tinca* (Akmirza, 2001), *Scorpaena porcus* (Akmirza, 2001), *Scoepaena scrofa* (Akmirza, 2001; Alaş et al., 2009; Akmirza, 2010), *Gaidropsarus mediterraneus* (Akmirza, 2001), *Umbrina cirrosa* (Akmirza, 2001), *Epinephelus aeneus* (Genç et al., 2003), *Epinephelus marginatus* (Genç, 2007), *Epinephelus costae* (Erol, 2007; Genç et al., 2011), *Mugil cephalus* (Alaş et al., 2009), *Gaidropsarus mediterraneus* (Alaş et al. 2009), *Serranus cabrilla* (Alaş et al., 2009), *Trachurus mediterraneus* (Alaş et al., 2009), *Sarpa salpa* (Alaş et al., 2009), *Sciaena umbra* (Alaş et al., 2009; Akmirza, 2014), *Pagellus erythrinus* (Alaş et al., 2009; Akmirza, 2010), *Spicara maena* (Akmirza, 2010), *Coris julis* (Akmirza, 2010), *Stephanolepis diaspros* (Akmirza, 2010), *Sparus aurata* (Akmirza, 2010), *Dicentrarchus labrax* (Akmirza, 2010), *Conger conger* (Akmirza, 2012); *Sargocentrun rambrum* (Öktener & Tuncer, 2020), *Upenus moluccensis* (Öktener & Tuncer, 2020), *Parapeneus forsskali* (Öktener & Tuncer, 2020). Previously, *Gnathia maxillaris* was isolated from *L. viridis* in the Saronicos gulf, Greece (Papoutsoglou, 1975). This study is the first documented record of parasitic infestation of blood-sucking praniza larvae of *L. viridis* in Türkiye.

Within marine fishes, parasitism caused by isopods can cause significant problems in cultured fish. In addition, they can also affect wild populations. Infestations caused by larvae of the genus *Gnathia* Leach, 1814 have been reported throughout the world. Bayoumy et al. (2013) reported infestations of

gnathiids in *Epinephelus tauvina* with a prevalence of 58.3% prevalence in the Saudi Arabian Coastal Water of Dammam. Öktener & Tuncer (2020) stated infestations of praniz along the southern Turkish coast of the Aegean Sea in *Parapenus forskali*, *Upeneus moluccensis*, *Sargocentron rabrum* with a prevalence of 63%, 47%, and 58%, respectively. Adday & Khames (2022) pointed out the gill lamellae of *Chiloscyllium arabicum* infested with gnathiid larvae with 69% in the coastal waters of Iraq (latitudes 48°44′ to 48°46′ and longitude 29°46′ to 29°47′). In this study, *L. viridis* collected from the Aegean Sea of Turkiye were infested with larvae of the genus *Gnathia* with 58.3% prevalence.

4. CONCULUSION

Various fish species have been reported with Gnathiid infestations, and the occurrence patterns demonstrated for larval gnathiids. The taxonomy of Gnathiidae based on the morphology of adult males and the description of larvae is deficient for most. Therefore, this makes identification of gnathiid larvae hardly possible. Mortality of gnathiid larvae can affect benthic individuals and interactions with each other.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

ETHICAL STATEMENTS

Ethics committee approval is not required.

FUNDING

The authors received no financial support for the research.

DATA AVAILABILITY STATEMENT

Data supporting the findings of the present study are available in the supplementary material to this article.

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