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# Intensity and prevalence of some crustacean fish parasites in Turkey and their molecular identification

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**Abstract:** In the present study, intensity, prevalence, and some pathologic effects of *Caligus minimus*, *Nerocila* spp., and *Livoneca punctata* on fishes extending from the eastern Black Sea coast of Turkey were determined. In addition, besides morphological characters of parasites, diagnosis of the species was carried out by using a molecular method (polymerase chain reaction) in characterization. *Caligus minimus* was isolated for the first time from wild sea bass (*Dicentrarchus labrax*) from the eastern Black Sea coast of Turkey. Seasonal distribution of *Nerocila* spp. was determined. The highest prevalence rates of *Nerocila* were determined on snouted sole (*Pegusa nasuta*, syn. *Solea nasuta*) at 26.94%, wrasse or corkwing (*Symphodus* spp.) at 16.77%, rusty blenny (*Parablennius sanguinolentus*) at 11.76%, goby fishes (*Gobius niger* and *Neogobius melanostomus*) at 5.21%, and pipefish (*Syngnathus* sp.) at 3.44%. Conversely, *Nerocila* spp. were not isolated from whiting (*Merlangius merlangus*), horse mackerel (*Trachurus mediterraneus*), Atlantic bonito (*Sarda sarda*), sardine (*Sardina pilchardus*), Black Sea herring (*Alosa immaculata*), or picarel (*Spicara smaris*). *Livoneca punctata* was only isolated from Black Sea herring (*Alosa immaculata*). The most common pathological symptoms were mechanical injury on the skin and fins of the infested fish.

**Key words:** Black Sea, Turkey, infestation, polymerase chain reaction, pathology

## 1. Introduction

The subphylum Crustacea has an important place in Arthropoda. The family Caligidae belongs to that subphylum and exists especially as a parasite in fishes. Caligid species, especially those living in benthic regions, live as parasites on the external surface of different fish (Stewart et al., 2004).

Caligid species are generally defined as sea lice. The most common samples of these parasites are *Caligus rogercresseyi*, *C. minimus*, *C. clemensi*, *C. elongatus*, and *C. fugu*. They cause serious harm to host fishes by feeding on the epidermal tissue, blood, and mucus of fishes (Kabata, 1979). A great number of *C. elongatus* parasites was isolated from adult Atlantic salmon (*Salmo salar*) (Wootten et al., 1982). The intensity and prevalence of these parasites increase in the summer season when water temperature increases. The infestation of caligid parasites in sea and brackish waters causes economic losses, especially in salmonid species; however, there were no serious losses in species other than the salmonid species (Johnson et al., 2004). The number of isolated species belonging to the genus *Caligus* was determined as 23 in reared fishes (Johnson et al., 2004). Studies about caligid fish parasites are relatively scarce in Turkey. Among these species, *Caligus minimus*, *C. apodus*, *C. brevicaudatus*, *C. pageti*, *C. mauritanicus*, *C. temnodontis*, *C. solea*, *C. fugu*, and *C. bonito* were found (Table 1).

Cymothoid parasites belonging to the order Isopoda generally attach to the skin, gills, and oral cavity of their host. Various species of *Nerocila* (Cymothidae; Isopoda) were reported on many marine fish species from different regions all over the world (Brusca, 1978; Öktener et al., 2009; Kayış and Ceylan, 2011). *Nerocila* was reported from the Marmara Sea and the Aegean Sea in Turkey (Geldiay and Kocataş, 1972; Kırkım, 1998). *N. bivittata*, *N. maculata* (accepted status: *N. orbignyi*), and *N. orbignyi* were recorded on Labridae, *Crenilabrus pavo*, *C. tinca*, *C. melops*, *Scorpaena scrofa*, *S. porcus*, *Cottus* sp., *Pagellus erythrinus*, *P. mormyrus*, *Mugil cephalus*, *Spicara maena*, *Meluccius merluccius*, and *Monacanthus setifer* from the western Black Sea in Turkey (Öktener and Trilles, 2004). Six different isopod species (*Anilocra frontalis*, *Anilocra physodes*, *Ceratothoa oestrodes*, *C. steindachneri*, *Nerocila bivittata*, and *Livoneca sinuata*) were listed by Kayış et al. (2009) from 13 different fish species in Turkey.

There was no report related to *Caligus* species from the coasts of the eastern Black Sea, although various *Caligus* species were reported from the other seas of Turkey. In this study, a report of *Caligus minimus*, a caligid fish parasite found in wild sea bass (*Dicentrarchus labrax*)

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**Table 1.** *Caligus* species reported from Turkish seas.

| Phylum: Arthropoda (Copepoda) | Hosts | Sea | References |
|---|---|---|---|
| *Caligus* sp. | *Sardina pilchardus* | Marmara | Demirhindi, 1961 |
| *Caligus apodus* | *Mugil* sp. | Aegean | Altunel, 1983 |
| | *Solea solea* | Mediterranean | Özak et al., 2013 |
| *Caligus bonito* | *Coryphaena hippurus* | Aegean | Öktener and Trilles, 2009 |
| *Caligus brevicaudatus* | *Solea solea* | Mediterranean | Özak et al., 2013 |
| *Caligus fugu* | *Lagocephalus suezensis* | Aegean | Özak et al., 2012 |
| *Caligus mauritanicus* | *Dentex dentex* | Aegean | Öktener, 2008 |
| *Caligus minimus* | *Dicentrarchus labrax* | Aegean | Tokşen, 1999; Uluköy and Kubilay, 2005 |
| | *Labrus merula* | Aegean | Tanrikul and Percin, 2012 |
| | *Sparus aurata* | Aegean | Akmirza et al., 2010 |
| *Caligus pageti* | *Mugil cephalus*<br>*Liza saliens*<br>*Liza ramada*<br>*Chelon labrosus* | Aegean | Altunel, 1983 |
| *Caligus solea* | *Solea solea* | Mediterranean | Demirkale et al., 2014 |
| *Caligus temnodontis* | *Pomatomus saltatrix* | Mediterranean | Özak et.al., 2010 |

sampled from eastern Black Sea coasts, was presented for the first time from this area. Besides morphological characters, molecular methods were used in the diagnosis of *Caligus minimus* and the mean intensity and prevalence of the parasite in wild sea bass were determined. However, there were two prior scientific studies from the coast of the eastern Black Sea region about *Nerocila orbignyi* and *Nerocila bivittata* (Kayış and Ceylan, 2011; Kayış and Er, 2012).

The Black Sea is a suitable area for aquaculture and new marine fish species will be cultured there. In this context, risk analysis and precautionary steps for new threats are very important issues. Therefore, in the present study, we aimed to determine the mean intensity, prevalence, and pathology of some crustacean parasites in different fish species in the eastern Black Sea, and diagnosis of the species was carried out by using molecular methods in characterization. This study also has importance in presenting the *Caligus* infestation, which can be a potential danger for trout and sea bass farms operating in the Black Sea with the cage system.

## 2. Materials and methods

European anchovy (*Engraulis encrasicolus*), horse mackerel (*Trachurus mediterraneus*), whiting (*Merlangius merlangus*), garfish (*Belone belone*), red mullet (*Mullus barbatus*), bluefish (*Pomatomus saltatrix*), turbot (*Scophthalmus maximus*), bream (*Diplodus vulgaris*), greater weever (*Trachinus draco*), sardine (*Sardina pilchardus*), tub gurnard (*Chelidonichthys lucerna*), brown meagre (*Sciaena umbra*), picarel (*Spicara smaris*), sharpsnout seabream (*Diplodus puntazzo*), Atlantic bonito (*Sarda sarda*), black goby and round goby (*Gobius niger* and *Neogobius melanostomus*), scorpion (*Scorpaena porcus*), snouted sole (*Pegusa nasuta*, syn. *Solea nasuta*), rusty blenny (*Parablennius sanguinolentus*), seahorse (*Hippocampus guttulatus*), pipefish (*Syngnathus* sp.), Labridae (*Symphodus* spp.), flounder (*Platichthys flesus*), stingray (*Raja clavata*), spurdog (*Squalus acanthias*), Black Sea herring (*Alosa immaculata*), shore rockling (*Gaidropsarus mediterraneus*), stargazer (*Uranoscopus scaber*), and sea bass (*Dicentrarchus labrax*) were sampled in the eastern Black Sea from February 2010 to April 2012 (Figure 1). A total of 26,544 fish samples caught by using different methods such as free diving (depth ranges of 0–10 m), fishing (depth ranges of 0–30 m), purse seine (depth ranges of >30 m), and different gillnets (depth ranges of 0–40 m) were examined. After sampling, fish species were described according to Turan (2007).

Fish were examined for the crustacean parasites and then the parasites were fixed in 70% alcohol for molecular studies or 4% formalin. Stereomicroscope was used for determination of morphological characteristics of the parasites and these characters were used for the description of parasites (Brusca, 1978; Williams and Williams, 1978; Kabata, 1979; Brusca, 1981; Kabata, 1992; Kırkım, 1998; Masahiro and Ho, 2013). Prevalence and mean intensity values of parasites were calculated according to Margolis

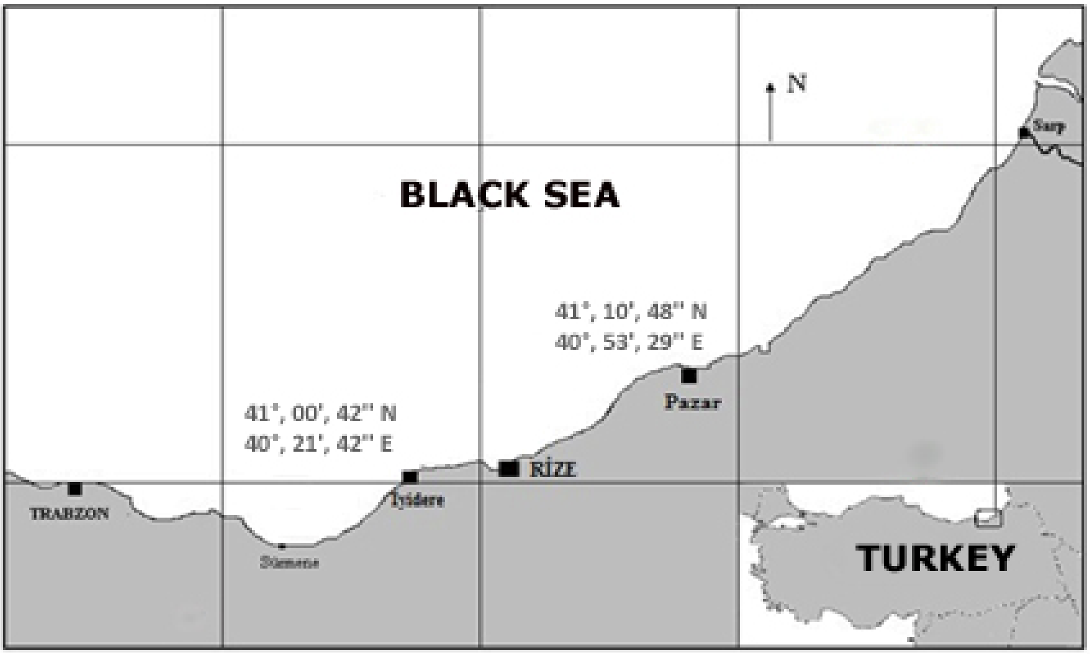


**Figure 1.** Sampling area.

et al. (1982). The study was approved by the local ethics committee of Rize University (reference no. 2010/35)

DNA extraction of parasites was carried out for diagnosing the species with the molecular method. For this purpose, sufficient samples were taken from parasites and DNA extraction was carried out using the QIAGEN DNA extraction kit (Düsseldorf, Germany). The DNA samples obtained from parasites were processed using mtDNA cytochrome oxidase gene universal primers (LCO14905'-GGTCAACAAATCATAAAGATATTGG-3', HCO21985'-TAAACTTCAGGGTGACCAAAAAAT-CA-3') in all crustaceans. The obtained DNA samples were studied according to the polymerase chain reaction (PCR) conditions indicated in Tables 2 and 3 and the results were sent to Macrogen for sequencing (Amsterdam, the Netherlands). The sequence information was matched with GenBank information and the diagnosis of the parasite was achieved.

## 3. Results

*Caligus minimus* was found in the mouth, on the upper palate, and especially on the tongue of sea bass (Figure 2). According to the morphological and molecular diagnoses (94%), the parasite was determined as *Caligus minimus* (Figures 3 and 4). The prevalence, intensity, and male-to-female ratio of the parasite were estimated as 90%, 2.5, and 8:15, respectively. It was observed that there were no parasites on the skin and fins.

**Table 2.** PCR cycle procedures.

| | Temperature (°C) | Time (min) | Number of cycles |
|---|---|---|---|
| Denaturation | 94 | 3 | 1 |
| | 94 | 1 | 36 |
| Annealing | 48 | 1 | |
| Extension | 72 | 1 | |
| | 72 | 10 | 1 |
| | 4 | ∞ | |

**Table 3.** PCR cycles reactions.

| Components | Final concentration | Amount (μL) |
|---|---|---|
| 10X PCR buffer | | 10 |
| dNTP mix | 10 mM | 4 |
| $MgCl_2$ | 0.25 mM | 3.5 |
| Primer | 40 mM | 0.5 |
| Taq polymerase | | 0.3 |
| Template DNA | 50 mM | 5 |
| $dH_2O$ | | 26.7 |
| Total | | 50 |


**Figure 2.** *Caligus minimus* on mouth cavity (A) and tongue (B) of *Dicentrarchus labrax*.

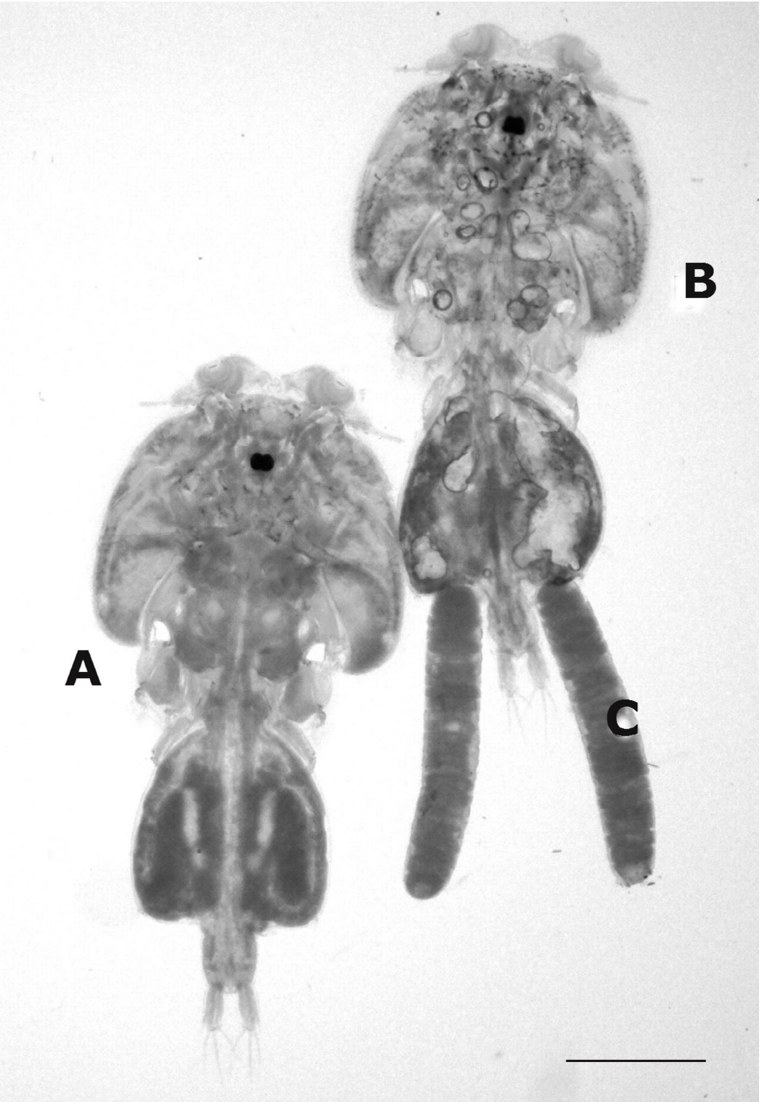


**Figure 3.** *Caligus minimus*: A) male, b) female, c) eggs. Scale bar = 1 mm.

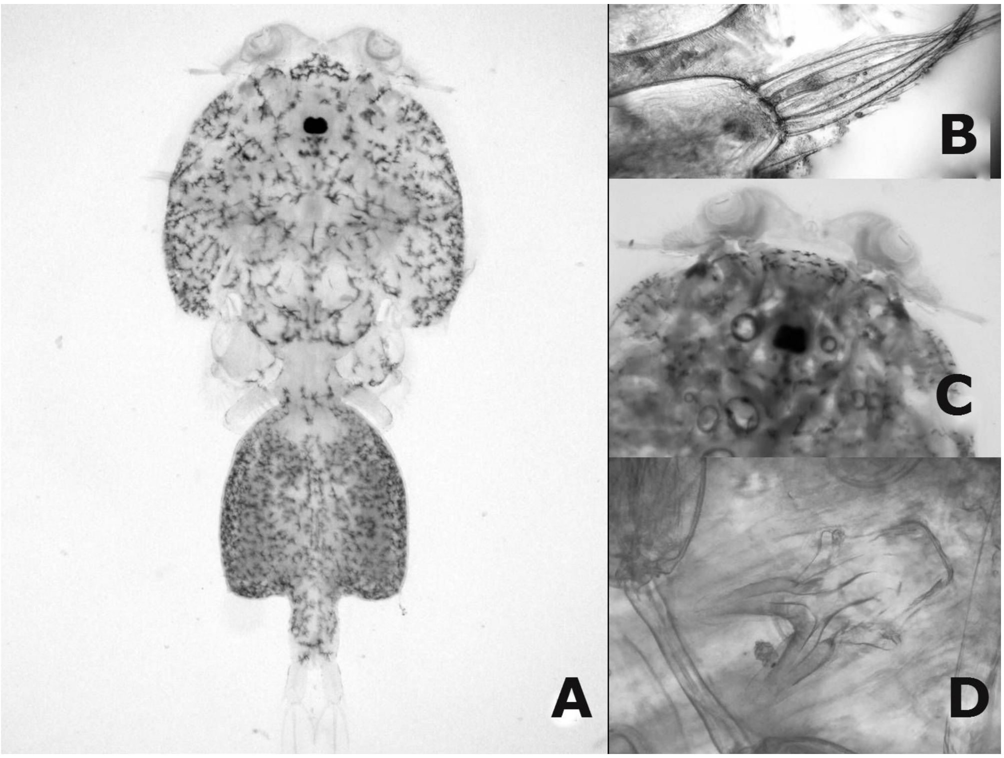

**Figure 4.** *Caligus minimus* male (A), caudal rami (B), lunules (C), sternal furca (D).

A total of 688 isopods from 15 different fish species from a total of 28 sampled fish species were recovered from the external surfaces of fishes, except for gills, and the parasites were identified as *Nerocila acuminata* (159) by molecular diagnoses (95%), *N. bivittata* (434) by molecular diagnoses (96%), and *Nerocila* spp. (95). Prevalence and mean intensity of parasites for each fish species are expressed in Table 4.

The highest prevalence was observed on *Platichthys flesus*, *P. nasuta* (Figures 5A and 5B), *Parablennius sanguinolentus*, *Symphodus* spp., *Gobius niger, Neogobius melanostomus*, and *Syngnathus* spp.

All parasites were recovered from fish living at depths between 0 and 30 m. The highest intensity and variety of parasites were observed in summer, whereas the least infestation was observed in winter. Some pathological symptoms were observed on the infested fish. The most common problem was mechanical injury to fish skin (Figures 5C and 5D).

*Livoneca punctata* was only isolated from the gills of Black Sea herring (*Alosa immaculata*) (n = 80 and infested fish = 24) (Figure 6). Prevalence and intensity of the parasites were found to be 30% and 2.

## 4. Discussion

Sampling methods of hosts in fish disease are the most important part of diagnosis. Mistakes can cause incorrect results during the sample collection in the disease diagnosis procedure. The sample methods dealing with *Nerocila* spp. are not clearly stated in most papers. In this study, host susceptibility of *Nerocila* spp. extending from the eastern Black Sea coast was determined with different sampling techniques (gillnet, free diving, fishing line, and purse seine). Mean intensity and prevalence of *Nerocila* spp. were determined by different sampling methods. In this context, the free diving technique was observed as the most effective sampling method. However, likely infestations were observed in the gillnet sampling. For example, *Nerocila orbigny* (Kayış and Ceylan, 2011) and *Nerocila bivittata* infestations were observed on *Mullus barbatus*, *Belone belone*, *Hippocampus guttulatus*, and *Uranoscopus scaber* in the gillnet operations, but *Nerocila* spp. infestation was not determined from these fish in the other sample techniques. Therefore, the most accurate approach for the sampling of parasites is to consider mutual data obtained from all sample methods. The present study showed that *Symphodus* spp., *Pegusa nasuta*, *Gobius niger*, and *Neogobius melanostomus* were observed as common hosts for *Nerocila* spp. in all sample methods.

Cymothoid isopods generally breed twice a year, in February and August (Aneesh, 2013). Female parasites with eggs were accordingly observed in February and August in the present study. Therefore, the highest levels of intensity and variety of parasites were observed in summer, whereas the least infestation parasites was observed in winter. This can be explained by parasites that usually prefer the coastal area (0–30 m), while fish migrate to deeper waters in the winter as a result of changes of water temperatures in the sea.

**Table 4.** Prevalence and mean intensity of *Nerocila* spp. on some fishes. n: fish numbers, in: infested fish numbers. [•]*Nerocila bivittata*, [*]*N. acuminata*, [▲]*Nerocila* spp.

| Fish species | n | in | Prevalence (%) | Mean intensity |
|---|---|---|---|---|
| *Pegusa nasuta*[*,•,▲] | 219 | 59 | 26.94 | 2.74 |
| *Symphodus* spp.[*,•,▲] | 1585 | 254 | 16.02 | 1.54 |
| *Parablennius songuinolentus*[•] | 17 | 2 | 11.76 | 1 |
| *Gobius niger*[*,•], *Neogobius melanostomus*[•] | 211 | 11 | 5.21 | 2.18 |
| *Syngnathus* spp.[•,▲] | 232 | 8 | 3.44 | 3.87 |
| *Platichthys flesus*[•] | 9 | 3 | 33.33 | 3.5 |
| *Scophthalmus maximus*[•] | 91 | 2 | 2.19 | 3.5 |
| *Sciaena umbra*[▲] | 85 | 3 | 3.52 | 2.33 |
| *Uranoscopus scaber*[*,•] | 622 | 17 | 2.73 | 1.35 |
| *Mullus barbatus*[▲] | 2743 | 4 | 0.14 | 1 |
| *Hippocampus guttulatus*[•] | 138 | 1 | 0.72 | 1 |
| *Diplodus puntazzo*[▲] | 251 | 1 | 0.39 | 1 |
| *Scorpaena porcus*[*,•] | 1035 | 18 | 1.73 | 1.33 |
| *Dicentrarchus labrax*[•] | 18 | 1 | 5.55 | 1 |
| *Belone belone*[•] | 140 | 1 | 0.71 | 1 |

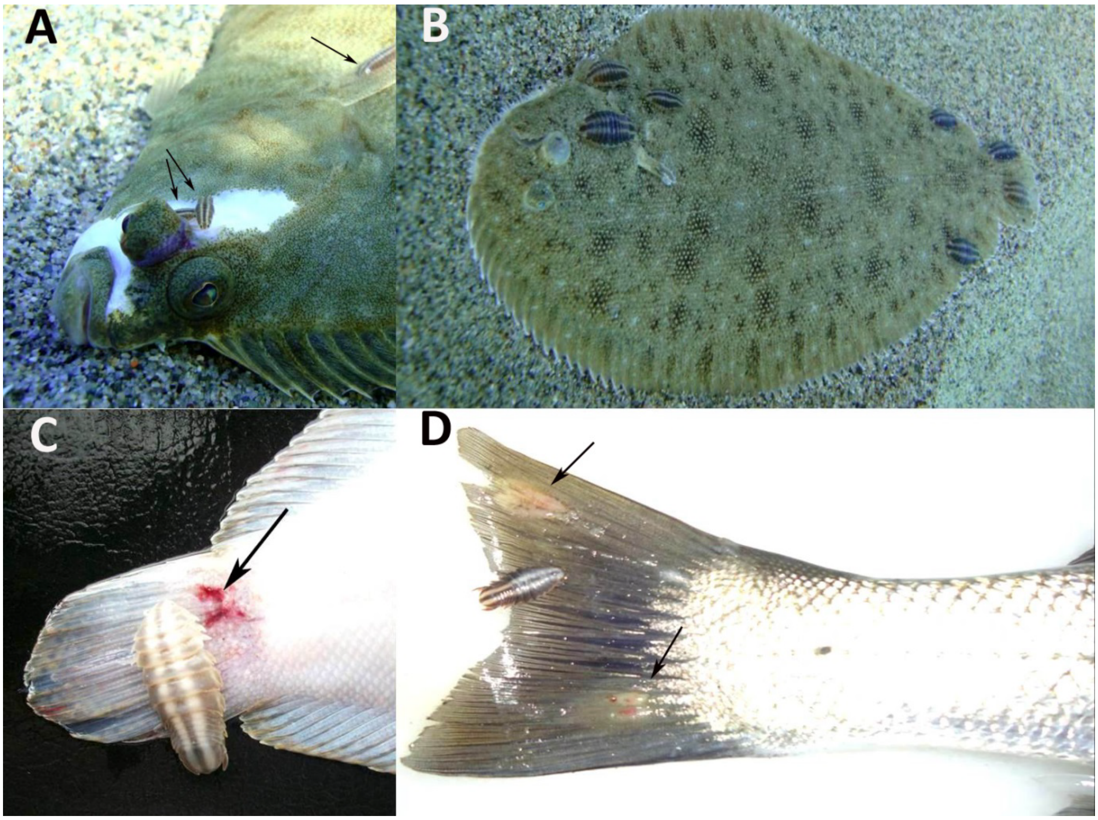


**Figure 5.** Infestation of *Nerocila* spp. on *Platichthys flesus* (A) and infestation of *Nerocila bivittata* on *Pegusa nasuta* (B), mechanic injury on caudal peduncle of sole (C), clear lesions on caudal fin of sea bass (*D. labrax*) (D).

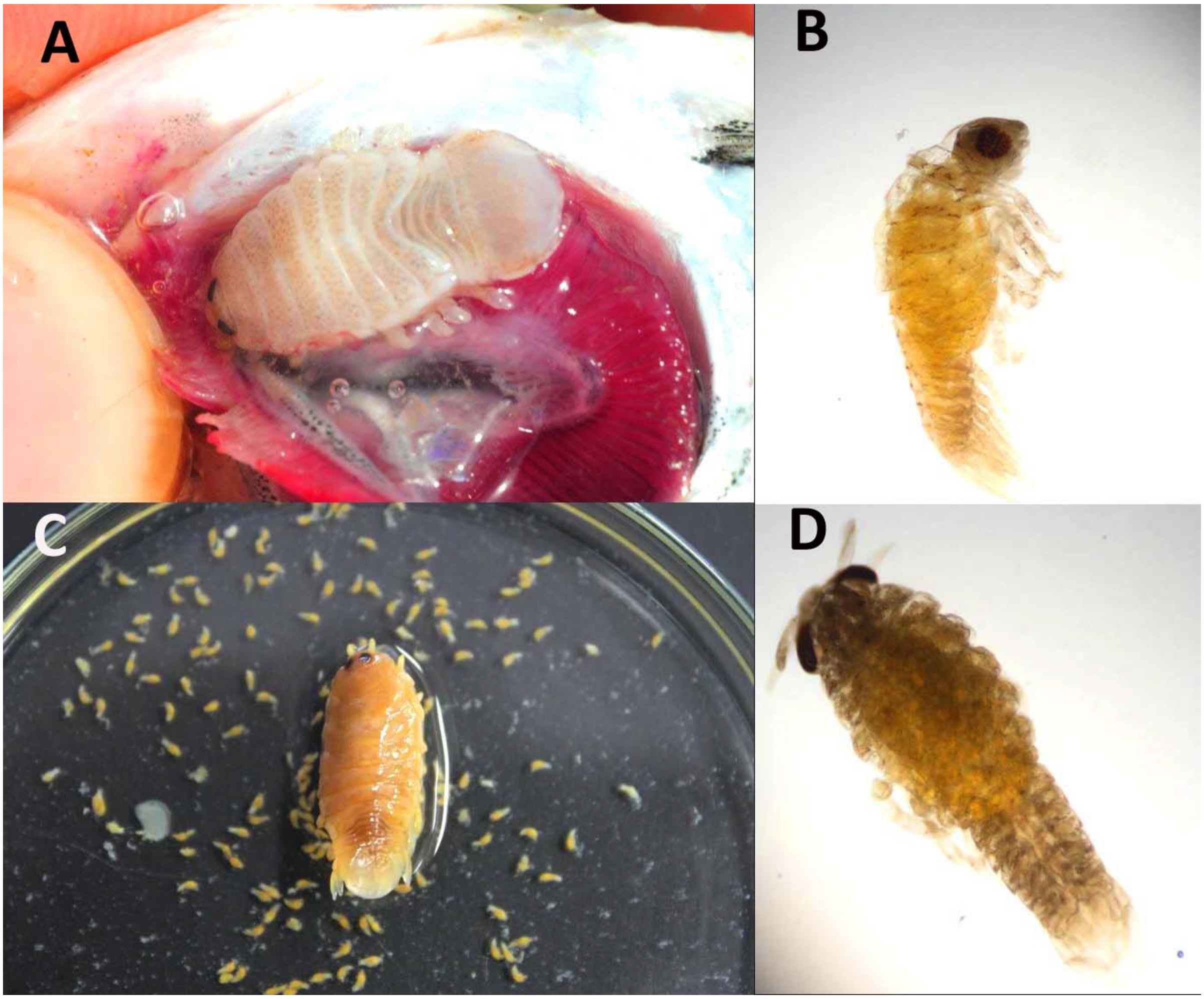


**Figure 6.** *Livoneca punctata* on gill of *Alosa immaculata* (A), manca (B, D), adult female *L. punctata* and its juvenile manca (C).

*Nerocila* spp. attach to the external surface of the host fish and feed on their epithelial tissue and blood. Common pathological symptoms in *Nerocila* infestations are skin lesions and hemorrhages. Some pathological effects of *Nerocila depressa* on *Sardinella albella* were reported by Printrakoon and Purivirojkul (2011). We observed skin lesions and hemorrhages on the fish. However, the most clear pathological effects were observed on the caudal peduncle of sole and the caudal fin of sea bass.

There are some papers regarding host susceptibility to *Nerocila* spp. in the Turkish seas (Öktener, 2003; Öktener and Trilles, 2009; Kayış et al., 2009). Only *Nerocila bivittata* and *N. orbignyi* were reported from different fish species in these papers (Horton and Okamura, 2001; Alaş et al., 2008; Öktener and Trilles, 2009). However, a few studies reported from the coast of the eastern Black Sea dealt with *Nerocila* spp. The present study reveals that *Nerocila* spp. infest 15 different fish species in that area. *Syngnathus* sp., *Mullus barbatus*, *Scophthalmus maximus*, and *Uranoscopus scaber* were also recorded for the first time as hosts for the genus *Nerocila*.

Due to its low salinity and different bottom structure, the Black Sea is different from other seas in terms of its biological diversity. In comparison to other aquatic systems, because of factors such as diversity of fish species and the depths in which the fish species live, it was observed to be an environment open to differences in terms of its parasite–host relations. For instance, the depth of sampling was stated as 60 m in a study conducted to investigate the existence of *Nerocila bivittata* in syngnathid species in the Black Sea (Kayış and Er, 2012). However, the living space of this species in the literature was at 40 m (Kırkım, 1998).

Research conducted about copepod diversity in the Black Sea were primarily focused on planktonic species. However, three copepod species were reported as parasites on fishes in the Black Sea: *Lernanthropus kroyeri* from *Dicentrarchus labrax* (Öktener et al., 2010), *Ergasilus lizea* from *Liza aurata* (Öztürk, 2013), and *Caligus minimus* from *Dicentrarchus labrax* (Özer and Öztürk, 2011). Copepod species found on fishes as parasites were generally observed on external surfaces, skins, and fins of the fishes. The species belonging to the genus *Caligus* were notably isolated from the buccal cavity of fishes (Özak et al., 2012). Similar to previous studies, *C. minimus* was isolated from the buccal cavity of natural sea bass in this study. Sea bass shows migration, usually in river mouths and lagoons, due to its temperature and salinity tolerance (Dando and Demir, 1985). Reared sea bass is cultured in pelagic water at a distance from the coast. It was reported that *Lernanthropus kroyeri* was the only species on cultured sea bass in the region. There was no other report apart from this. In this study, the isolation of *C. minimus* from wild sea bass could be explained by differences in the living space and diet of the fishes.

Özak (2007) stated in a study about the biological requirements of *Caligus minimus* that this species could complete its life cycle in at 10‰–36‰ salinity and at temperatures of 15–21 °C. The average salinity rate of the Black Sea is 17‰. The average temperature value in the depth space of 0–15 m was 12 °C in the months during which this study was conducted. These two species (*C. minimus* and *D. labrax*) shared common zones in terms of their biological needs; therefore, it could be readily assumed that there was a host–parasite relationship between them. It has been stated that the infestation of caligid fish parasites occurred both in wild and cultured trout species (MacKenzie et al., 1998; McCarney et al., 2002). The culture of different fish species (*Oncorhynchus mykiss*, *Salmo coruhensis* (Turan et al., 2009), and *Dicentrarchus labrax*) in cage systems is very common on the coasts of the Black Sea in Turkey. The isolation of *Caligus minimus* from wild fishes in the Black Sea ecosystem indicates a potential danger for fish species reared in cage systems in the future. Moreover, the treatment of *Caligus* parasites is quite difficult and expensive.

*Livoneca punctata* was only isolated from the gills of Black Sea herring (*Alosa immaculata*). Similarly, the parasite was isolated from Black Sea herring (prevalence 30% and intensity 1) on the western coast of the Black Sea (Özer and Olguner, 2013).

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