

Studies on endoparasitic copepods: *Sarcotaces arcticus* (Copepoda: Phylichthyid) infested dotted grouper (*Epinephelus epilistictus*) from the Arabian Gulf water, Saudi Arabia

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Abstract

This study aimed to investigate the presence of *Sarcotaces* sp. in *Epinephelus epilistictus* (the dotted grouper) in Saudi Arabia. So, during our routine clinical examination of fish health conditions at Jubail province in the eastern region of Saudi Arabia (Arabian Gulf), nine grouper fish species were examined for *Sarcotaces* sp. (Copepoda: Phylichthyidae). Only the dotted grouper (*Epinephelus epilistictus*) harbored parasitic Sarcotaces spp. with an incidence of 9.06% of 1600 clinically examined fish samples. The collected *Sarcotaces* sp. was *Sarcotaces arcticus* (*S. arcticus*). The copepods were completely encapsulated cysts with a pyriform or pear-like shape, small to large, white-grayish, fluid-filled with thick black ink beneath the skin, or fully embedded in the muscular layer. The cysts present in the lateral aspect of trunk muscles, flank, caudal peduncle, near the anal opening, or pelvic and pectoral fin with an infestation rate (1–6 cysts) per fish. The cyst histopathology reveals the capsular layer of the cyst consisting of fibrous connective with pressure atrophy of the adjacent muscles associated with dilated blood vessels. The absence of infestation in other kinds of grouper fish species from the water of the Arabian Gulf in Jubail province may indicate parasitic host specificity and geographic locality distribution.

Keywords Copepoda · Dotted grouper · *Epinephelus epilistictus* · Parasitic crustacean · S. *arcticus* · The Arabian Gulf

Introduction

The grouper fish species belonging to the family Serranidae are the most commonly caught fisheries from the Jubail area on the Arabian Gulf. Grouper fish from the water of the Arabian Gulf have nine species, all of which are pricey and of high marketability. Crustacean parasites affecting fishes are mainly divided into three major groups:

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Branchiura, Isopoda, and Copepoda, considered parasitic on both marine and freshwater and tend to cause high mortality (Heckmann 2003; Johnson et al. 2004). Species of the Branchiura and Isopoda are large in both males and females; however, the copepod species are microscopic with a two-stage life cycle (free-living and parasitic stages) (Johnson et al. 2004). Parasitic copepods, of marine fishes, representing the family Philichthyidae, are diverse in their morphology, physiology, and life strategies (Yamaguti 1963; Reimer 1991; Walter 2015).

Piasecki et al. (2020) reported that Sarcotaces sp. infested the Mora with more fish by filling the large galls with black fluid, which also stained the nearest muscles. Inside each gall, a single female of Sarcotaces sp., several "small" males, eggs, and the hatched nauplius stages were present. Nagasawa et al. (2015) found that specimens from Sarcotaces spp. were collected from black cysts formed in the posterior muscles under the skin of a black tip grouper (Epinephelus fasciatus). Sarcotaces sp. belongs to the family Philichthyidae: Copepoda and consists of seven species. The cyst filled with fluid had different life stages of *Sarcotaces* spp. as eggs, larvae, and small males (Nagasawa et al. 2015). Additionally, the Sarcotaces sp. are encysted inside the abdominal cavity and muscles of different fish species (Nagasawa et al. 2015). According to the available literature regarding the infection of Sarcotaces spp. among fish from the water of the Arabian Gulf, Ibrahim (2001) reported the first evidence of the impact of parasitic Copepoda (family: Philichthyidae; genus: Sarcotaces) on brown spotted grouper (Epinephelus chlorostigma) that induced black bag disease collected from El-Qatef province at the eastern region of Saudi Arabia. Later on, Osman et al. (2014) reported Sarcotaces spp. from the grouper (E. chlorostigma), with a prevalence of 6.74% from the same previously mentioned area in Saudi Arabia. Furthermore, Essa (2017) presented a case report on the clinical signs of the black cyst disease of Sarcotaces spp. infestation in E. tauvina (Family: Serranidae) fish in the northwest of the Arabian Gulf of Iraq.

Sarcotaces spp. parasites have no zoonotic impact on mammalian fish consumers; however, the infested muscles had bad quality due to the staining ink produced digestion of the blood by the parasite, causing rejection by human consumers (Izawa; 1974).

The Philichthyidae family of parasitic marine fishes is remarkable in appearance, physiology, and life choices. The reciprocal relationship between the fish and the parasite and the inflicted host pathology has been a fascinating topic (Kabata 1979, 1988). Also, the order of Poecilostmatoida includes the Philichthyids. In terms of the evolutionary development of parasitic adaptations, the latter is thought to be inferior to Siphonostomatoida. Interestingly, because they are extensively altered and semi-ectoparasites, the Philichthyids are a remarkable exception. The lateral line canals are a habitat for smaller species. Also, the genus *Sarcotaces* spp. contains the family's most significant species. They live in galls inside the fish muscles or a bodily cavity (Moser et al. 1985) and cause refusal of parasitic fish in a fish shop or even death in the latter cases (Lafferty and Kuris 2009).

Sarcotaces (Copepoda: Philichthyidae) infection causes black bag illness, which may be found anywhere from abyssal depths to tropical reefs. *Sarcotaces* sp. is a strange copepod that causes cysts in the muscle beneath the skin, several centimeters long. Also, when fish are filleted, the cysts are revealed, and the sliced cysts leak a large amount of black fluid over the fillets. This inky black liquid has earned the nickname "Black bag disease" (Piasecki et al. 2020).

So, this study aimed to investigate the cause of the refusable black cysts formed in the muscles under the skin of the dotted grouper (*Epinephelus epilistictus*), as well as, study the prevalence rate of this parasite; the identity of this crustacean with histopathological alteration associated with the infestation in muscle.

Materials and methods

Fish collection

The fishing port at Jubail province on the Arabian Gulf of Saudi Arabia is considered one of the most famous and largest fish landing stations (27° 02′ 20.5″ N 49° 38′ 18.9″ E) in the eastern region. It is considered the leading fisheries trading center where a large commercial quantity of fresh marine fish of different kinds are caught using various methods and directly distributed fresh to fish auctions and fish markets in Jubail. Also, it is the major supplier of fish to other national province localities in the kingdom besides exporting some quantities to the nearby Arabia Gulf countries. Fish inspection is usually performed on fish caught onboard at the landing station, on the vessel boats, during handling and transport to fish auctions, and finally to fish markets and shops in Jubail. All systematic documentary checks, identity checks, and physical and clinical checks are performed by an official from the Veterinarian Office in the Welfare of Fisheries branch at Jubail province, Ministry of Agriculture.

Gross examination of fish

The clinical examination of fish depends mainly on quality control for fish freshness and observations, to any risk in shape, discoloration, ulceration, tumors, swelling, or other infectious fish diseases. Additionally, considerable attention is paid to parasitic infestation either externally or internally, especially the fleshy edible part of the fish, to ensure marine seafood safety to the consumers. Our routine clinical observation checks in the fish landing station, at the fish board, fish auction, and fish market occurred from January 2016 to December 2019. A total of 6500 fresh fish samples of grouper species were grossly examined, and 1600 were dotted grouper fish (*Epinephelus epilistictus*) as previously identified by Carpenter et al. (1997).

Collection of the encysted black bag cysts

In the fish port and auction, only clinically infested dotted grouper fish were packed in separate plastic bags, transferred into an ice pot, and delivered to the laboratory at the Jubail welfare fish branch. They were then subjected to complete necropsy, where careful removal of cysts from the surrounding muscles of the collected infested fish was performed. Since the *E. epilistictus* is a pricey fish, the infested fish were collected and carefully removed parasitic cysts from the adjacent areas of the infested host muscles. Finally, they were collected in plastic bags, put in an icebox, and sent to the laboratory for parasitological identification (Nagasawa et al. 2015).

Examination of the content of the encysted black bag cysts

The freshly collected encysted black bag cysts were transferred into a Petri dish, dissected with sterile scapulae to remove black fluid content, and carefully examined under a stereoscopic microscope. Also, the cyst wall was slightly scarped and kept directly on a glass slide covered with a cover slide and then directly examined under a light microscope to separate the microscopic-size parasite stages (eggs and nauplius at different stages of development). Finally, all the parasitic stages were collected and preserved in 70% ethanol and then examined under a stereoscopic microscope (Reimer 1991; Osman et al. 2014).

Male collection

The freshly collected closed cysts were gently squashed or pressed from the wide anterior end of the cyst toward the narrow posterior end. A thin light serous fluid came out of the cyst with very minute small dots (males of 5-10) in number that was used to fertilize the huge numbers of eggs of the unstructured female (males of about 1 ml in length) and then collected in a glass slide for microscopical examination (Osman et al. 2014).

Female collection

Additionally, on the freshly collected closed cysts, a small incision on the wall of the cysts was gently made using forceps to take off the parasitic female directly under the cystic wall and then put in a glass jar filled with fresh water to examine the female structures (Piasecki et al. 2020).

Identification of the collected Copepoda

The collected parasites were fixed in 70% ethanol and then examined under a stereoscopic microscope in the Parasitology Laboratory, Faculty of Veterinary Medicine, Cairo University. The collected Copepoda was identified according to Yamaguti (1963). The identification was based on male and female length and width, the length of the caudal furca with male body length (Reimer 1991).

Histopathological examination

A small section of 1×1 cm of infested muscles with cysts was fixed in 10% neutrally buffered formalin. The fixed samples were embedded in paraffin (Bancroft and Layton 2012). The paraffin blocks were sectioned in 5 μ m thickness, stained with hematoxylin and eosin (H & E), and finally, examined under a light microscope (Olympus BX50, Japan).

Results

Prevalence study of the collected Sarcotaces sp.

A gross examination of 6500 fresh fish samples from the different grouper spp. was performed. One hundred and 45 among 1600 samples of the dotted grouper fish (*E. epilistictus*) were infested with the cysts of *Sarcotaces* sp with an incidence of 11.3%, as indicated in Table 1. The total collected cysts were 435 cysts of *Sarcotaces* sp. with different intensities of varying sizes on the dotted grouper (*E. epilistictus*), ranging from 35 in the pectoral fin to 95 cysts in the caudal fin. In contrast, relative to the examined fish's length, it ranged from 20 to 50 cm, grouped into four with the cyst intensity ranging from 1–6. Dealing with the seasonal dynamics of *Sarcotaces* sp., it reveals that the Copepoda was high in spring with a 65 (10%) prevalence rate and low in winter with a 20 (9.5%) prevalence rate as indicated in Tables 2 and 3. The infested dotted grouper fish (*E. epilistictus*) ranged from 15 to 45 cm with an average weight ranging from (350 to 3.5 kg \pm 150 g.). It also showed complete encapsulated pyriform cysts or pear-shaped filled with black inky fluid with a highly vascular capsular gray white thick wall of (2–3 mm). The cysts found in different sites of the infested fish (near the pectoral and pelvic fin, lateral trunk muscle, at the right and left area of the caudal peduncle, and near the anal opening) with infestation rate (1–6 cysts in number) per fish (Table 3).

Gross and histopathological examination of the infested fish

Each cyst often contains one female of *Sarcotaces* sp and a few males engorging themselves near the fish blood vessels. The female *Sarcotaces* sp. feeds on the blood, leaving a thick, inky fluid by-product (digested oxidizing blood). The narrow pointed end of the cysts, usually toward the skin surface (Fig. 1) were small to large (3-6 cm in length and 1-3 cm in diameter) and located beneath the skin or completely unrecognizable through gross appearance. The unrecognizable cysts appeared as a buffy swelling on the skin surface of the infested host (Fig. 1), where the cysts completely penetrated deeply into the muscular tissues. This cystic gall may explode or accidentally rupture during the filleting process and taint the muscular layer with a thick black inky fluid stain (Fig. 1), reducing the fish marketability value. In some cases, cystic gall causes vertebral deformity if embedded deeply in muscular tissues near the vertebral column. After completely evacuating the content of the endoparasitic cyst of Sarcotaces sp. actions in the surrounding water, the cyst wall bulged outside on the skin of the infested fish. Additionally, there was no indication of parasitic host-induced mortality, except that the fish marketability value was affected by the S. arctitus. Furthermore, the histopathological finding revealed the cyst wall consists of fibrous connective tissues with pressure atrophy of the adjacent muscles with dilated blood vessels (Fig. 1).

Taxonomy of the collected Sarcotaces sp.

The collected Copepoda belongs to the order: Poecilostomatoida; family philichthidae; Genus: *Sarcotaces* (Olsson; 1872). Species: *S. arcticus*.

Grouper sp.	English name	Maximum size	No. examined	No. infested	%	
E. areolatus	Areolate grouper	40 cm	600	-ve	0	
E. bleekeri	Dusky tail grouper	76 cm	550	-ve	0	
E. caeruleopunctatus	White spotted grouper	60 cm	450	-ve	0	
E. coioides	Orange spotted grouper	95 cm	460	-ve	0	
E. episitictus	Dotted grouper	80 cm	1600	145	11.03	
E. latifasciatus	Striped grouper	157 cm	800	-ve	0	
E. multinotatus	White blotched grouper	100 cm	650	-ve	0	
E. octofasciatus	Eight bar grouper	80 cm	500	-ve	0	
E. polylepis	Small scale grouper	50 cm	790	-ve	0	
Total			6500	145	4.4	
P value				0.0001		

 Table 1
 The prevalence of S. arcticus infection in different grouper fish

Total no. of examined dotted grouper (E. epilistictus) 1600	Spring		Summe	Summer		Autumn		Winter				
	No. ex	No.inf	%	No. ex	No.inf	%	No. ex	No. inf	%	No. ex	No. inf	%
	650	65	10	420	35	8.3	320	25	7.8	210	20	9.5

 Table 2
 Frequency distribution of S. arcticus cysts infestation based on the seasonal variation of the dotted grouper (E. epilistictus)

Table 3 Frequency distribution of *S. arcticus* cysts infestation based on the size of the dotted grouper (*E. epilistictus*)

No of infected fish	Body length of the groups in (cm)	Numbers of study of infected fish in each group	Numbers of cysts	Prevalence of infec- tion in each group %
145	20–25 cm	30	2–4	7.25–3.62
	25–35 cm	25	1–6	14.5–2.4
	35–50 cm	60	1–3	14.5–4.8
	> 50 cm	30	1–2	14.5–7.25
Total		145		



Fig. 1 A Clinically observed cysts on the host and unrecognized cysts; arrow revealed presence of bulged cyst on the exterior. **B**, **C**, **D**, **E** Careful removal of cysts from the host. Arrow revealed that presence of cyst inside muscle of dotted grouper with presence of blood vessels supported the cyst. **F** Histopathological section on the thick wall of *Sarcotacse* cyst wall consist of fibrous connective with pressure atrophy of the adjacent muscles Stained by H&E; magnification lens; × 20 times × 100

Description of male S. arcticus

Males were smaller than females, owing to structural differences. The body was elongated, cylindrical, with a segmented body. Thoracic appendages were decreased in form and quantity, whereas the cephalothoracic appendages were highly developed (two thoracopods). Males (n = 10) had lengths of 1.00–1.50 mm (1.35 mm±0.3 mm) and total width of 0.25–0.55 mm (0.45±0.15 mm), excluding the caudal rami. The cephalothorax, the first two (pedigerous) somites of the thorax (Fig. 2), and the legless trunk formed a semi-triangular anterior section of the body in the dorsal view. Also, the caudal rami were reduced to a single short seta and the first and second male antennae consisted of four segments.

Female S. arcticus

After carefully removing the adult female from the encapsulated cyst, it appeared as a soft white, shapeless mass of thin wall (Fig. 3). It also bulged after being immersed in a glass jar with water, appearing as a ball or bag with tubercles on the body surface of the female body elongated and drop-form. The anterior end is rounded while the posterior part is narrowed (Fig. 3). The female's body was elongated oval-shaped, rounded at the anterior end, and pointed to narrow in the posterior part (Fig. 3). It ranged from 25–45 mm $(35.8 \pm 8.1 \text{ mm})$ with its width from 12–20 mm $(16.9 \pm 4.0 \text{ mm})$.

Additionally, the female body was covered with lobated mamillation or small papillae (Fig. 3), somatically segmentation with protrusions in the anterior and posterior parts strongly developed. The cephalon was linked to the opening of the mouth, and a very long first abdominal segment, with a diameter comparable to its height. The appendages were also either decreased or absent Fig. 3.

Eggs

The eggs present between the female and the membrane were minute and spherical-shaped. It measured 0.15-0.35 (0.15 ± 0.5) mm in diameter, yellow-colored, and contained well-developed embryos (nauplii) (Fig. 4).

Larval stages of S. arcticus

The larval stage, known as the nauplius, was oval with the rounded anterior and posterior end having two caudal setae carrying three pairs of appendages (first, a second antenna, and mandible) and an antenna with three segments (Fig. 4).

Discussion

The females are huge, soft, and delicate, and they float inside their chamber, separated from the host by a connective tissue membrane that lines the vacuum in the fish muscles. Females grow to be about 2–5 cm long. Their gall (also known as a cyst) is filled with black digested blood and includes parasite eggs, larvae, and "dwarf" males. Sarcotaces females are filled with a black fluid that resembles ink. In most cases, mechanical processing (filleting process) of

infested fish causes damage to the female and spills the ink. The ink stains the fillet, rendering it unsellable. Tintenbeutel [ink bag] was given to German fishermen due to this occurrence (Gonzales and Tanzola 2000; Piasecki et al. 2020). The point of a female projecting through such a skin breach was shown by Amlacher (1958).

Although *Sarcotaces* spp. is not hazardous to humans, the staining effect of the ink diminishes the value of fish landings. Therefore, regardless of infection standards, their existence disqualifies all consignments under EU regulations. Izawa (1974) produced a diagrammatic drawing, illustrating the comparative morphology of males of six Philichthyiidtaxa. Delamare Deboutteville (1962) published a diagrammatic illustration showing the comparative morphology of the males of six Philichthyiid genera, including *Sarcotaces* spp. Species of the genus *Sarcotaces* are poorly known, with a few publications related to individual species, especially from the water of the Arabian Gulf (Ibrahim 2001; Osman et al. 2014; Essa 2017). Different species of *Sarcotaces* had been studied before by Olsson (1872), *S. verucosus* and Gonzales and Tanzola (2000); *S. arcticus* by Priebe (1963) and *S. pacificus* by Komai (1924), Ezpeleta Herce (1974), and Izawa (1974); *S. komaii* by Shiino (1953).

This study identified *Sarcotaces* sp. males which were found in all cysts of infested *E. epilistictus*; however, the female was not found in any cyst tested in their study, while the male in their study was elongated, slender, and flattened dorsoventrally, with no discernible segmentation. Additionally, only lateral notches distinguish body segments. The head was tiny, rounded, and broader than long, distinguishable from the thorax by a distinct constriction. Laterally, the third segment develops into two wings, and finally, but not least, the abdomen is represented by the last section; Osman et al. (2014) reported that the contents of the cysts were tested for adults and larval stages of copepods; therefore; numerous copepod nauplii were found in the smear preparations of the gall inky fluid in our study. The nauplius has three pairs of appendages and is oval-shaped, with the body's caudal end rounded and possessing two setae. Additionally, groups of eggs were found and organized on the inner wall of the cyst in histological sections of the infested fish's cyst. Therefore, these results from our study are similar to the research approach according to Izawa (1973) and Moser et al. (1985).

Our histopathological finding revealed the cyst wall of fibrous connective tissues with pressure atrophy of the adjacent muscles with dilated blood vessels. In contrast, other research



Fig. 2 S. arcticus male; A male smaller than females. The body is elongated, cylindrical, with segmented body; B anterior end of male showing cephalothorax; C posterior end of male. A, B, C magnification lens of $\times 20$



Fig.3 *S. arcticus* female; **A**; **B**; **C** newly removed female from their cyst; **D** and **E** female after put in the water revealed presence of lobated protrusions or small papillae covering the body; the body had somatic segmentation. **D** Female had protrusions in the front and posterior parts which are strongly developed

recorded no sign of bleeding in the sac wall or inflammation associated with *Sarcotaces* sp infestation. Furthermore, histological sections of the infested fish's cyst revealed masses of eggs placed on the inner wall of the cyst, similar to the findings of Bullock et al. (1986), who concluded that all *Sarcotaces* sp. swellings were elevated, soft, and up to 2 cm in diameter with fibrous wall contain masses of eggs.

Sarcotaces spp. findings are rare, indicating the species' restricted specialization. Since the females within the species are generally similar, despite their variety in size and other traits, certain reports based only on female morphology are less credible. As a result, *S. arcticus* identified in Scorpaeniformes fishes in the Pacific might be distinct from the Atlantic *S. arcticus* that infects gadiform fishes. *S. verucosus*, which is similar to *S. arcticus*, is also identical to our results. The bulk of the records from the Atlantic belong to the Lophiiformes and Perciformes families, with both male re-descriptions representing two distinct species (Gonzales and Tanzola 2000).

However, the argument of Moser et al. (1985) on host specificity is dissimilar to the conclusion that *S. komaii* and *S. arcticus* are junior synonyms of *S. verucossus* after performing considerable morphological research. Heegaard (1947) also proposed that *S. verucosus*, *S. arcticus*, and *S. pacificus* are the same species.

Osman et al. (2014) recorded that *Sarcotaces* spp. in Arabian Gulf; Qatef, Eastern Province of Saudi Arabia, infested the *E. chlorostigma*, with a low prevalence rate of 6.74%, almost similar to our study, although the research did not identify the species recorded in Saudi Arabia in grouper. Therefore, our study focused on (*E. epilistictus*) in Jubail province.



Fig. 4 *S. arcticus* eggs and larvae which reveals; **A** rounded; yellow eggs (arrow); **B** newly hatched larva with legs and rounded posterior end (arrows); **C** and **D** the larva hatched from their eggs (arrows); **A**, **B**, **C**, **D** with magnification lens of $\times 10$

Conclusion

Our study revealed that of 1600 samples of the dotted grouper (*E. epilistictus*), 145 were infested with *Sarcotaces* sp. cysts with an incidence level of 11.3%. The collected cysts were 435 cysts of *Sarcotaces* sp., with intensity ranging from 35 to 95. In conclusion, after carefully inspecting any gross lesion or cyst to the parasitic cyst, the grouper is still usable by the consumer.

Author contribution Mustafa M. Ibrahim: design of the study and collection of the samples apply the pathological reports of the fish. Marwa M. Attia: identify the parasites; photographed the samples. Mustafa M. Ibrahim and Marwa M. Attia: analysis and interpretation of the data. The two authors drafting, revising the manuscript, and approved the final manuscript.

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Data availability All data from our work and available in this manuscript.

Code availability This manuscript is presenting a rare disease present in grouper not use special software.

Declarations

Ethics approval All studies were applied on fish markets.

Conflict of interest The authors declare no competing interests.

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