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Submission date: 02-Sep-2021 11:01AM (UTC+0800)
Submission ID: 1639843746
File name: C1.56_Journal_of_Veterinary_Parasitology_-_Copy.pdf (506.4K)
Word count: 2494
Character count: 13727

Journal of Veterinary Parasitology, 34(2)2020:109-113

Parasitic metazoans infesting cantang groupers from floating net cages in Situbondo Waters, East Java, Indonesia

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Received: 22.07.2020 **Revision: 16.10.2020** **Accepted: 03.11.2020**

Abstract

Most of the cantang grouper (*Ephinepelus fuscoguttatus x E. lanceolatus*) cultivation is conducted in floating net cages, which can predispose to infectious diseases, including parasitic disease. Aim of this study was to identify the parasites in cantang groupers cultivated on floating net cages in Situbondo waters. One hundred and twenty cantang groupers measuring 12.59 ± 1.93 cm were collected from three floating net cages in Situbondo waters. Samples were observed for ectoparasites from skin, fins and gills; and for endoparasites in gastrointestinal tracts of the fish. The result showed twelve (10%) of the fish were infested with *Neobenedenia girellae*. The sites of infection were skin, eye, head and fins. Besides this, intestine of one fish (0.83%) was found infected with *Anisakis typica*. Our finding is the first report of metazoan parasite infection in cantang grouper cultured in Java Sea, especially in Situbondo waters. Moreover, cantang grouper is one of popular fishes consumed by humans and consumption of undercooked fishes could lead to zoonotic infection with *Anisakis*. Therefore, further studies are needed to map the distribution of parasites in cantang groupers.

Keywords: **Situbondo, Cantang grouper, Floating net cage, *Anisakis* sp., *Neobenedenia* sp.**

Introduction

Cantang grouper is a hybridized grouper between female brown-marbled grouper (*Ephinephelus fuscoguttatus*) and male Kertang grouper (*E. lanceolatus*), which is considered to have a high growth rate and disease resistance (Rimmer and Glamuzina, 2019). Most of cantang grouper cultivation is conducted in floating net cages which leads to infectious diseases, such as parasitic diseases. Parasite infection can affect the growth and predispose to secondary infection by bacteria, virus and fungi and cause mortality in fish (Iwanowicz, 2011).

Previous studies reported the occurrence of *Pseudorhabdosynochus ephinepheli, Megalocotylorides epinepheli, Benedenia* and *Neobenedenia* in *Epinephelus coioides* in the grouper cultivated in floating net cages (Seng, 1997); *Benedenia ephinepheli, Neobenedenia melleni*, and *Pseudorhabdosynochus* sp. in *E. coioides* from Lampung Bay, Indonesia (Ruckert *et al.,* 2009) as well as *N. girellae* and *P. seabassi* in Cantang grouper from Lombok (Dewi *et al.,* 2018). Not only ectoparasites, but endoparasites such as *Anisakis* have also been observed from *E. fuscoguttatus-lanceolatus* in floating net cage at Lamong Bay Surabaya Indonesia (Agustina *et al.,* 2017), while *Ephinephelus areolatus* has been identified from Indonesian waters (Kleinertz *et al.,* 2014).

Wiyatno *et al.,* (2012) reported infection of *Neobenedenia*, *Benedenia* and *Pseudorhabdosynochus* in Polka dot groupers (*Cromileptes altivelis)*

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DOI: 10.5958/0974-0813.2020.00018.2

from Situbondo waters. *Camallanus cantangis* and *Echinostoma* infections were reported in Humpback grouper (*Cromileptes altivelis*) on floating net cages in Marine Culture Management Unit Situbondo, East Java (Ulkhaq *et al.*, 2012). Thus, it is important to identify the parasites in cantang groupers cultivated on floating net cages in Situbondo waters in large scale, to prevent mortality caused by parasites and to map the distribution of parasites in Situbondo waters.

Material and Methods

The research was approved by Fisheries and Marine Faculty, Universitas Airlangga (Based on letter of assignment from the Dean of Fisheries and Marine Faculty, Universitas Airlangga, 485/UN3.1.16/KP/2019).

During the rainy season in 2019, one hundred and twenty samples of cantang grouper (*Ephinepelus fuscoguttatus x E. lanceolatus*) measuring 12.59 ± 1.93 cm long were collected from three floating net cages in Situbondo waters. Samples were packed live in the aerated plastic bags and transported to laboratory. Examination of skin, fins and gills were performed for ectoparasites and thorough examination of gastrointestinal tract was made for endoparasites. Nematode specimens were fixed in 10% normal buffer formalin and preserved in 70% ethanol. Monogenean parasites were fixed in alcohol glycerin (5%) and all parasites were stained by Acetic Carmine method, dehydrated in graded ethanol series, cleared with xylene and mounted in entellan (Heil, 2009; Kleinertz *et al.,* 2014). Morphological identification of parasites was done as per Ogawa *et al.,* (1995, 2006 and 2014), Nada and Amany (2011), Arai and Smith (2016) and Sardella and Luque (2016). Diagrams were made using binocular microscope and drawing tube (Nikon, Japan).

Results and Discussion

Twelve (10%) of the Cantang groupers were found to be infested with *Neobenedenia girellae* (Hargis, 1955, Yamaguti, 1963) a monogenean ectoparasite (Fig. 1). The parasites were found in the skin, eye, head and fins of the fish. One sample (0.3%) was infected with *Anisakis typica* (Diesing, 1860; Baylis, 1920), a nematode, in the intestine (Fig. 2).

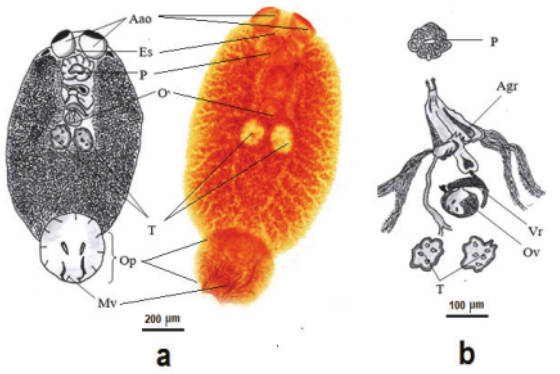

Fig. 1. Whole body of *N. girellae* (a) from cantang grouper with drawing tube (left) and acetocarmine stained (right) showing the anterior attachment organ (Aao), eye spot (Es), pharynx (P), ovarium (Ov), testis (T), opisthaptor (Op) and marginal valve (Mv). Magnified figure (b) showing organs of *N. girellae* including pharynx (P), accessory gland reservoir (Agr), vitelline reservoir (Vr), ovary (Ov) and testis (T).

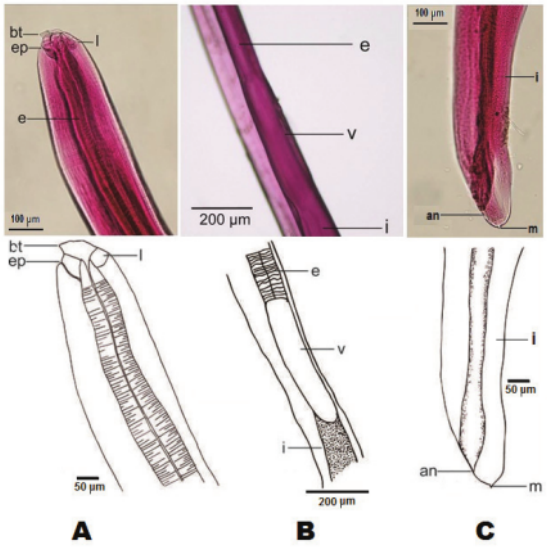

Fig. 2. Morphology of *Anisakis typica* from cantang grouper taken with binocular microscope (top row) (acetocarmine) and drawing tube (bottom row). Anterior end (A) showing boring tooth (bt), excretory pore (ep) and lips (l). Mid portion (B) showing the ventriculus (v), oesophagus (e) and intestine (i). Posterior end (C) with anus (an) intestine (i) and mucron (m).

The monogenean *N. girellae* had a body length of 3.7-5.5 mm, width 1.6-2.1 mm. Anterior attachment organs of parasite had a length of 0.29-0.39 mm and width of 0.36-0.48 mm. Opisthaptor (posterior attachment organ) was 0.89-1.2 mm long and 0.78-1.42 mm wide. Opisthaptor was equipped with an anterior hamulus, posterior hamulus, hooklet, accessory sclerite, and 14 small hooks on the edge.

According to Ogawa *et al.* (1995), *N. girellae* had dorsoventrally flattened, transparent, elongated round body with length 3.6-5.6 mm and width of 1.8-3.7 mm. They had two pairs of eyes on the anterior part above the pharynx. Pharynx was flower shaped, measuring 0.36-0.55 mm. A pair of attachment organs was located on the anterior part of the body measuring 0.28-0.48 mm. Opisthaptor measured 0.8-1.3 mm and was equipped with accessory sclerites measuring 0.12-0.21 mm in length. Anterior hamuli measured 0.18-0.37 mm and posterior hamuli 0.08-0.14 mm.

N. girellae is a species that has distinctive features, the shape of the fine jagged testes and the shape of accessory sclerites which exhibit similarity with already described species. In addition, *N. girellae* had a pair of testes post-ovarian in location. The ovary and vitelline reservoir were connected by vas deferens and were connected to the seminal vesicle in the cirrus on the anterior part of the body. *N. girellae* does not have a vagina, and fertilization is by insemination of sperm using the anterior organ of attachment either alone or between other worms to fertilize each other (Ogawa *et al.,* 2014). The organ of attachment was used to attach to the host's body. It had a pharynx shaped like a flower, two testicles which are located in the middle part of the body and have follicular vitellaria which is spread on the body.

Colorni and Diamant (2014) stated that capsalidae monogeneans were commonly found on skin, scales, and gills. These parasites can move actively on the body surface and feed on epithelial cells and mucus which was causes haemorrhages, inflammation and excessive mucus production (Brazenor and Hutson, 2015). These parasites mostly infest the host around the eyes, damage the cornea and cause blindness (Ogawa *et al.,* 2006). Infested fish swim erratically and rubbing the body into the net, which causes skin ulceration and secondary bacterial infection (Colorni and Diamant, 2014).

The nematode *A. typica* had a body length of 14.17 mm and width of 0.35 mm similar to the study of Nada and Amany (2011), who recorded a body length of 14.14-21.85 mm and width of 0.34-0.36 mm. Boring tooth was prominent and located in anterior end. There were dorsal and ventrolateral lips around boring tooth. Excretory pore was located between the dorsal and ventrolateral lips (Fig. 2).

In the body cavity of *A. typica*, the oesophagus, ventriculus and intestines were found. The esophagus was divisible in two parts, the anterior part measuring 1.15 mm length and a ventricular part measuring 0.50 mm length. Ventriculus was oval and connected directly to the intestine. Mucron of *A. typica* was short with rounded posterior tip. The length of ventriculus is one of the important parameters to identify the species of *Anisakis* morphologically (Setyobudi *et al.,* 2011). Ventriculus length in this study was 0.50 mm. According to Nada and Amany (2011), ventriculus of *A. typica* has a size range of 0.49-0.79 mm. Furthermore, the length of the oesophagus of *A. typica* is 1.120-1.681 mm (Sardella and Luque, 2016). *A. typica* does not have intestinal caecum in body cavity. The key to identify parasites from the family Anisakidae in the genus *Anisakis* is the absence of intestinal cecum (Arai and Smith, 2016). Posterior end of *A. typica* in this study was rounded as was observed in other *Anisakis* spp. (Nada and Amany, 2011; Serrano *et al.*, 2017).

The presence of *A. typica* has been recorded in fish in Bali Strait (Palm *et al.,* 2008; Kuhn *et al.,* 2013) and in the Makassar Strait (Anshary *et al.,* 2014), indicating that these parasites live in the tropical areas (Hafid and Anshary, 2016). Further studies are needed to map the distribution of parasites in Cantang grouper.

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Acknowledgement

Authors are grateful to Study Program of Aquaculture, University Airlangga Campus Banyuwangi for providing all facilities and equipment for this study.

Conflict of interest

The authors declare that there were no conflicts of interest in this study.

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