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Short Communication: Helminth fauna of Lutjanidae from Kedonganan, Bali, Indonesia

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Abstract. *Rudianto EA, Wijayanti DP, Haryanti D, Indrayanti E. 2024. Short Communication: Helminth fauna of Lutjanidae from Kedonganan, Bali. Biodiversitas 25: 4986-4993.* The biodiversity of parasites in demersal reef fishes is immense but remains poorly understood and often overlooked. This is also hindered by the lack of survey of parasite communities in reef fishes, especially in Indonesia. Therefore, we surveyed endoparasites of reef-associated and demersal lutjanids in the Kedonganan fish market, Bali. A total of 25 individuals of six lutjanid species: *Lutjanus erythropterus*, *Lutjanus russellii*, *Lutjanus sebae*, *Lutjanus timoriensis*, *Lutjanus vitta*, and *Pristipomoides multidens*, were collected from the fish market. The parasites were collected using a modification of the gut wash method. Two individuals of Philometridae were recovered from *L. vitta*. Other recovered helminth fauna were Acanthocephala, Nematoda, and Digenea, mainly belonging to *Siphoderina* spp. and *Hamacreadium* sp. We also reported for the first time, endoparasites of *L. erythropterus* and *P. multidens* in Indonesian waters. This study highlighted the importance ichthyo-parasitological survey as a part of the biodiversity survey. More surveys and reports on endoparasites in demersal reef fishes in Indonesia are needed, especially on their biology, ecology, diversity, and impact on human health, considering that most of these demersal fishes are consumed by local people.

Keywords: Endoparasite, Kedonganan fish market, Lutjanidae, *Philometra*, snappers

Abbreviations: Indet: Indeterminate, I: Intensity, mA: mean Abundance, mI: mean Intensity, n: number of specimens, n/a: not available, P: Prevalence, SL: Standard Length, TL: Total Length, TW: Total Weight, µm: micrometer

INTRODUCTION

The biodiversity of parasites in reef fishes is immense, especially in the tropical region, but remains poorly understood and often overlooked (Justine 2010; Justine et al. 2012a). Despite their important roles and influence on the marine food web, including reef ecosystems (Lafferty 2013), they are relatively understudied. From the problems mentioned above, it is imperative to include an ichthyo-parasitological survey in the biodiversity survey to discover this “hidden biodiversity” (Carlson et al. 2020).

This problem is more apparent in Indonesia, a center of marine mega biodiversity, where the highest richness of reef fish fauna was found (Allen and Adrim 2003). In Indonesia, there are 63 snapper species of Lutjanidae Gill, 1861 that have been recorded (Allen et al. 2013; Allen and Adrim 2003; Iwatsuki et al. 2015). Based on that number, the family Lutjanidae is one of the most speciose taxa in Indonesian waters (Allen and Adrim 2003). The richness of snapper species (Lutjanidae) would be followed by the richness of its parasitic fauna, as reported by Justine et al. (2012a) in New Caledonia, where host-parasites combination per species of reef-associated and deep-sea snappers reached 10.13 and 12.50 respectively, while the number of parasites species per fish species reached 3.00 and 3.75 respectively.

Although Indonesian ichthyo-parasitological research has expanded and intensified over the past decades, many parasitic fauna and areas remain unexplored, and many of the literatures are scattered (Kleinertz et al. 2022). As an illustration, Theisen (2019) conducted an extensive ichthyo-parasitological survey in Indonesia, where he collected 50,000 parasites individual from 1,531 teleosts of 40 species, with a ratio of 33 parasites individual per fish specimen. Moreover, a literature study conducted by the same author showed that from ichthyo-parasitological publications in Indonesia since 1840 listed 2,326 host-parasite records of 514 parasite species from 266 fish species. This is supplemented by his own ichthyo-parasitological survey that added 678 new parasite records to a total of 3,005 host-parasite records of 621 parasite species from 315 teleost fishes. Therefore, with an estimation of almost 3,400 teleost species in Indonesia and an average of 3.3 up to 4 specific parasite species per sampled fish species, Theisen (2019) concluded that only 5.5% of Indonesian marine ichthyoparasite fauna is currently known to science. For a more historical perspective on Indonesian marine fish parasitology, refer to Theisen et al. (2019) and Kleinertz et al. (2022).

The study of endoparasitic fauna of snappers (Lutjanidae) in Indonesia has a long history. It began with a report in 1886 by Brock (1886) that described a digenean, possibly *Prosogonometra* Vigueras, 1940 (Synonym:

Eurycoelum sluiteri) in *Lutjanus erythropterus* Bloch, 1790 at Northern Java waters (Kleinertz et al. 2022). Yamaguti (1952, 1953) also described several digenean species from several lutjanid hosts in Sulawesi/Celebes during 1940s. Many ichthyo-parasitological survey for endoparasites of lutjanid in and adjacent of Indonesia focused on nematode taxa, especially Anisakidae (Yuniar 1999; Palm et al. 2008, 2017; Setyobudi et al. 2010; Kuhn et al. 2013; Muttaqin and Abdulgani 2013; Anggraeni 2014; Paremme et al. 2018; Puspitarini et al. 2018; Ulkhaq et al. 2019; Fiqhi 2020; Muttaqin et al. 2021). This is due to the medical importance of Anisakidae, which posed zoonotic potential against humans (Adroher-Auroux and Benítez-Rodríguez 2020). An extensive ichthyo-parasitological study of Indonesian marine teleost, especially with a compilation of lutjanid host-parasite lists had been conducted by Theisen (2019) that reported 22 digenean species, 5 acanthocephalan species, 22 nematodes species, and 4 cestodes species from 21 Lutjanidae species. Other studies such as Zainurrahman (2013); Pradipta et al. (2014); and Hartini et al. (2019) reported other endoparasitic fauna aside from nematodes, such as Digenea, Acanthocephala, and Cestoda. However, further ichthyo-parasitological research of snappers in Indonesia is crucial to uncover endoparasitic fauna that have yet to be discovered.

Kedonganan fish market is the biggest fish market in Bali (Hariyanto et al. 2015). The fish sold here are sourced not only from the local fishermen in the adjacent water but also from neighboring districts and even other provinces (Hariyanto et al. 2015). Several parasitological surveys had been conducted on fish in this location or the fish were obtained from this market, such as Palm et al. (2008) to molecular barcoding of *Anisakis*; Pradipta et al. (2014) to survey parasitic worms in *Pterocaesio diagramma* and *Caesio cuning* (Bloch, 1791); Palm et al. (2017) to survey *Anisakis* in several marine fishes; Suratma et al. (2017) to survey *Anisakis* in swordfish (*Triuchurus lepturus*); Komariah et al. (2020) to survey *Anisakis* in *Decapterus* sp.; Kandouw et al. (2023) to survey endoparasites in *Rastrelliger* sp. Some of the parasites identified in these studies are potentially zoonotic to human. Therefore, conducting endoparasite surveys are also important to assess the risk posed by the parasite from the commercial fishes.

The economic importance of snappers (Lutjanidae) in Indonesia, especially as an export commodity and consumption for local communities, made ichthyo-parasitological survey and analysis of its zoonotic potential important. Therefore, during the DNA barcoding project on Lutjanidae, we conducted an ichthyo-parasitological survey for helminth fauna in Lutjanidae. In this study, we reported the first record of the occurrences of Philometridae from *Lutjanus vitta* (Quoy & Gaimard, 1824) collected from the Kedonganan fish market and to bring more information about the endoparasite Lutjanidae species, especially *L. erythropterus* and *Pristipomoides multidens* (Day, 1871) that previously had never been surveyed in Indonesia.

MATERIALS AND METHODS

Study area

A total of 25 Lutjanidae individuals of six species were collected at the Kedonganan fish market, Bali, Indonesia (8°45'27.15"S, 115°10'6.91"E) on 16 August 2023 (Figure 1).

Sampling and identification of Lutjanidae

Fishes' biometric data, such as Total Length (TL), Standard Length (SL), and Total Weight (TW), were measured (Klimpel et al. 2019). Fishes were then photographed for further morphological identification (Strauss and Bond 1990) using snapper identification books (Allen 1985, 1987; Anderson 1987; Satria et al. 2022).

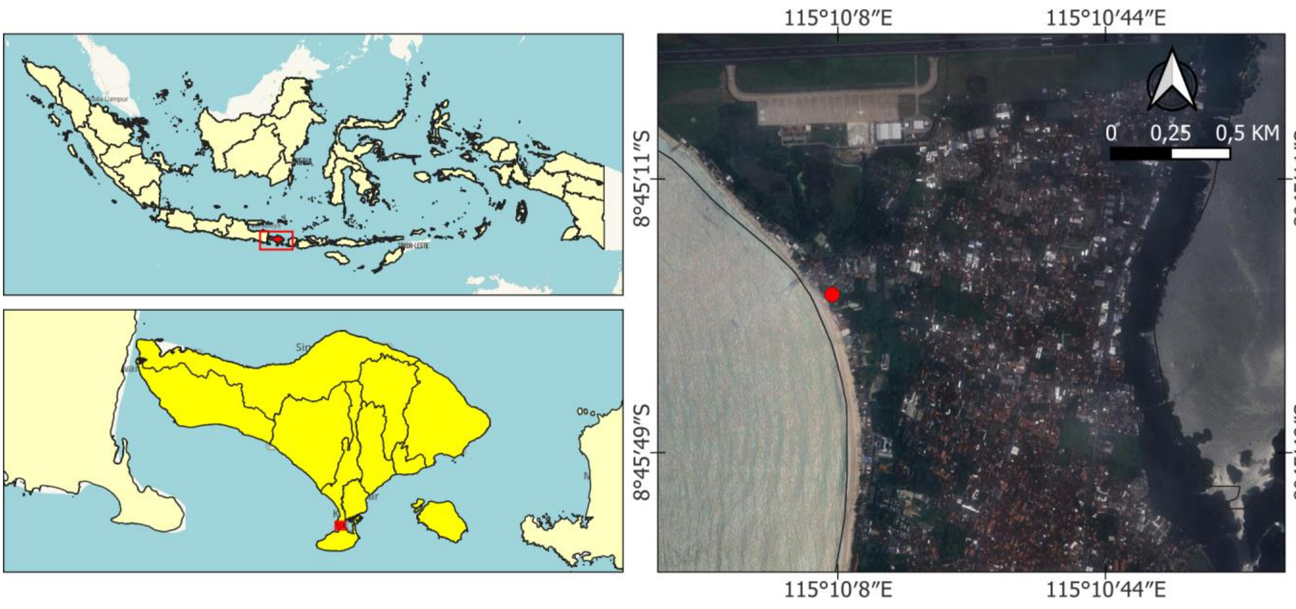

Figure 1. Sampling site in Kedonganan fish market, Bali, Indonesia

Parasitological examination and identification

The fish viscera were collected by modification of gut wash method (Cribb and Bray 2010; Justine et al. 2012b), then were brought to Marine Biology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Diponegoro, for further parasitological examination. This method only targeted parasites in the body cavity and inner organs (Justine et al. 2012b).

Parasitological observation was conducted under binocular stereomicroscope (Olympus CZ61). Endoparasites that were isolated then preserved in 96% ethanol. Endoparasites were identified morphologically using binocular microscope (Olympus CX23 and Motic X_3 BA120) and were compared with identification books, annotated checklist, and literatures (Kabata 1985; Grabda 1991; Arai and Smith 2016; Theisen 2019; Kleinertz et al. 2022).

Common descriptors

Prevalence (P), Intensity (I), Mean Intensity (mI), Abundance (A), and Mean Abundance (Ma) of parasites were measured according to Bush et al. (1997) and Klimpel et al. (2019):

Prevalence = $[P\%] = \frac{n_i}{n_\Sigma} \times 100$

Where: ni: number of hosts infected with a specific parasite i; n∑: total number of observed hosts

Prevalence was expressed as a percentage (Klimpel et al. 2019):

Intensity $(I) = I_{min} - I_{max}$

Where: I_{min}: The lowest number of specific parasite species found in a single host; I_{max}: The highest number of specific parasite species found in a single host

Intensity was expressed as a range (Klimpel et al. 2019):

Mean Intensity $(\mathrm{mI}) = I_{\Sigma i}/n_{\Sigma i}$

Where: $I_{\Sigma i}$: Total number of a specific parasite species i; $n_{\Sigma i}$: Number of hosts infected with the specific parasite species i

Mean Abundance $(\mathrm{mA}) = I_{\Sigma i}/n_{\Sigma}$

Where: $I_{\Sigma i}$: Total number of a specific parasite I; n_{Σ}: Total number of observed hosts

RESULTS AND DISCUSSION

Identification and biometric of snappers

Twenty-five individuals Lutjanidae were collected from the fish market, including *L. erythropterus*, *Lutjanus russellii* (Bleeker, 1849), *Lutjanus sebae* (Cuvier, 1816), *Lutjanus timoriensis* (Quoy & Gaimard, 1824), *L. vitta*, and *P. multidens* (Figure 2). The average total length and weight were 27.83 cm and 285.44 g, respectively. The median value of the total length and weight were 28 cm and 290 g, respectively. Based on the sizes, all the individuals were classified as the juvenile (<34.0 cm) (Freitas et al. 2011).

Endoparasites found in snappers

All fish examined in this study were infected by at least one endoparasite taxon. The parasitic taxa identified consisted of Nematoda, Digenea, and Acanthocephala (Table 1), with a total of 213 endoparasites. Nematoda (n = 118) and Digenea (n = 94) were the most prevalent endoparasitic groups, representing 54 and 44% of the total, respectively). A three-year parasitological survey conducted by Villalba-Vasquez et al. (2022) also found that Digenea was the most abundant endoparasites group in *Lutjanus peru* (Nichols & Murphy, 1922). Compared with other studies conducted in Indonesia (Pradipta et al. 2014; Hartini et al. 2019; Theisen 2019) and other regions (Justine et al. 2012; Villalba-Vasquez et al. 2022), we did not observe infection by Cestoda in this study. The prevalence of Nematoda ranged from 25% (*Cucullanus* sp. and Philometridae) to 100%, while the prevalence of Digenea ranged from 12.5% (*Hamacreadium* sp.) to 100% (*Siphoderina* sp.). These relatively high number could be explained by the small sample sizes for each snapper species.

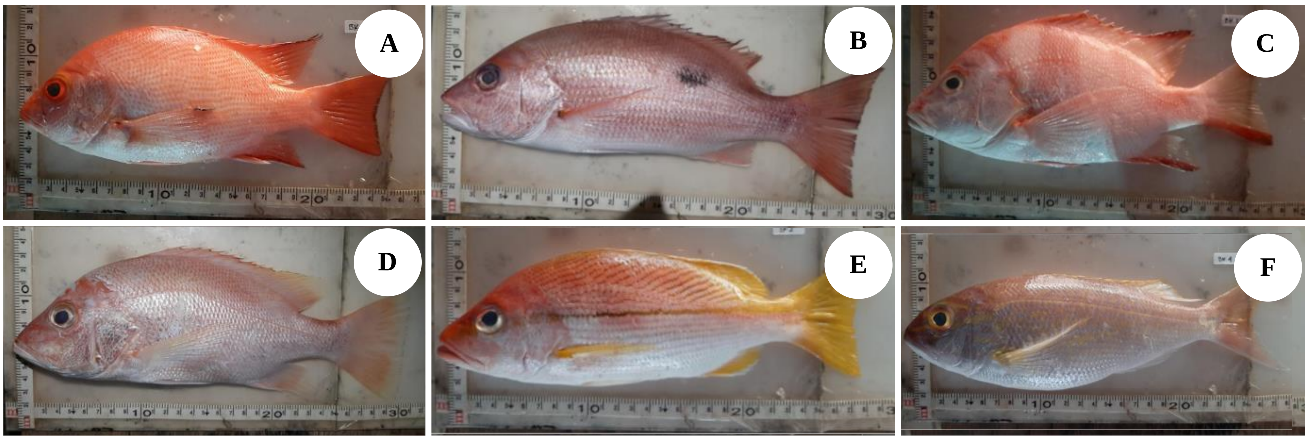

Figure 2. Six species of snappers that were collected in Kedonganan fish market, Bali, Indonesia. A. *Lutjanus erythropterus* Bloch, 1790; B. *Lutjanus russellii* (Bleeker, 1849); C. *Lutjanus sebae* (Cuvier, 1816); D. *Lutjanus timoriensis* (Quoy & Gaimard, 1824); E. *Lutjanus vitta* (Quoy & Gaimard, 1824); and F. *Pristipomoides multidens* (Day, 1871)

Table 1. List of endoparasites found inside snappers in Kedonganan, Bali, Indonesia, their common descriptors and zoonotic potential

Host species	n host	Endoparasites	Microhabitat	P	I	mI	mA	Zoonotic
Lutjanus erythropterus Bloch, 1790	2	Nematoda	Intestine, liver, stomach	100%	4-7	5.5	5.5	n/a
		Siphoderina sp. (Digenea)	Intestine	100%	1-12	6.5	6.5	-
Lutjanus russellii Bleeker, 1849	4	Nematoda	Gonad, intestine, stomach	75%	3-9	5.67	4.25	n/a
		Siphoderina sp.	Pyloric caeca	50%	7-8	7.5	3.75	-
Lutjanus sebae Cuvier, 1816	4	Nematoda	Intestine, liver, stomach	100%	4-11	6.75	6.75	n/a
Lutjanus timoriensis Quoy & Gaimard, 1824	1	*Siphoderina* sp.	Pyloric caeca	100%	9	9	9	-
		Hamacreadium sp.	Intestine	100%	2	2	2	-
		Nematoda	Intestine	100%	25	25	25	n/a
Lutjanus vitta Quoy & Gaimard, 1824	8	Acanthocephala	Intestine	12.5%	1	1	0.125	n/a
		Cucullanus sp. (Nematoda)	Intestine	25%	1-2	1.5	0.375	-
		Siphoderina sp. (Digenea)	Pyloric caeca	50%	2-9	4.75	2.375	-
		Hamacreadium sp. (Digenea)	Pyloric caeca	12.5%	1	1	0.125	-
		Nematoda	Intestine	37.5%	1-2	1.67	0.625	-
		Philometridae (Nematoda)	Gonad (ovary)	25%	1	1	0.25	-
Pristipomoides multidens Day, 1871	6	Nematoda	Intestine, liver, stomach, body cavity	66.67%	3-13	5.75	3.83	n/a
		Siphoderina sp. (Digenea)	Pyloric caeca	50%	2-24	15.67	7.83	-

Note: n: Number of specimens; P: Prevalence; I: Intensity; mI: mean Intensity; mA: mean Abundance; n/a: Information not available; -: No zoonotic potential

Table 2. Microhabitat/sites distribution of endoparasites in snappers in Kedonganan, Bali, Indonesia

Microhabitats/sites	Endoparasite		
	Nematoda	Digenea	Acanthocephala
Heart	-	-	-
Body cavity	✓	-	-
Stomach	✓	-	-
Intestine	✓	✓	✓
Pyloric caeca	✓	✓	-
Liver	✓	-	-
Gonad/Ovary	✓	-	-

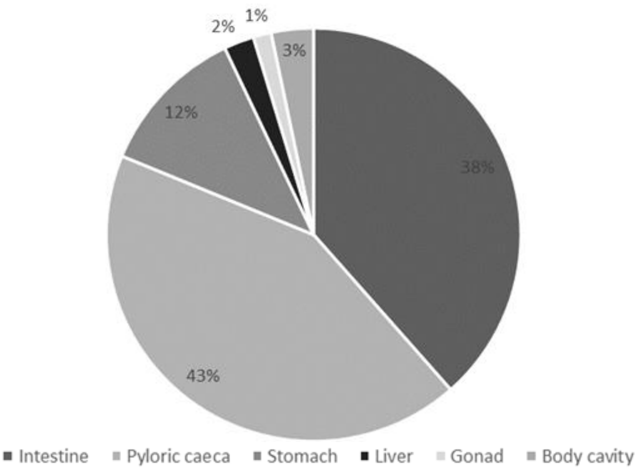

Figure 3. Microhabitat/sites distribution of endoparasites in snappers in Kedonganan, Bali, Indonesia

Microhabitats preferences of endoparasites

Microhabitats that were preferred by endoparasites were mainly in the stomach, intestine, and pyloric caeca (Table 2). Among these, the most colonized microhabitat by endoparasites was pyloric caeca (43%), followed by intestine (38%), and stomach (12%) (Figure 3). Other organs such as the body cavity, liver, and gonad are only represented 3, 2, and 1%, respectively. In the endoparasites community, each individual fish host provides a distinct microhabitat for endoparasites to inhabit (Price 1990). Usually, many endoparasites tend to colonize specific and predictable sites within these microhabitats (Price 1990). This specialization results from endoparasites adapting to exploit complex environments characterized by steep gradients in environmental conditions and available resources (Price 1980). For instance, the family Cryptogonimidae, particularly the genus *Siphoderina*, which was found in this study, is commonly found in the intestine or pyloric caeca of Lutjanidae (Miller and Cribb 2008a, 2008b; Martin and Cutmore 2022).

The infections of Philometridae in *L. vitta* from Kedonganan, Bali

In this study, we recorded 2 philometrid nematodes isolated from *L. vitta* (BK2 and BK3) (Figure 4). Some studies have recorded the occurrences and description of this parasites in Indonesia from several hosts, such as Moravec et al. (2012) who described 5 new philometrid species, *Philometra lobotidis* Moravec, Walter & Yuniar, 2012 from Atlantic tripletail (*Lobotes surinamensis* Bloch, 1790), *Philometra javaensis* Moravec, Walter & Yuniar, 2012 from immaculate puffer (*Arothron immaculatus* Bloch & Schneider, 1801), *Philometra psettoditis* Moravec, Walter & Yuniar, 2012 from Indian spiny turbot (*Psettodes erumei* Bloch & Schneider, 1801), *Philometroides indonesiensis* Moravec, Walter & Yuniar, 2012 from hound needlefish (*Tylosurus crocodilus* Péron & Lesueur, 1821), and *Philometroides trichiuri* Moravec, Walter & Yuniar, 2012 from largehead hairtail (*Trichiurus lepturus* Linnaeus, 1758) and savalai hairtail (*Lepturacanthus savala* Cuvier, 1829); Dewi and Palm (2013) described two philometrid species, *Spirophilometra endangae* Dewi & Palm, 2013 and *Philometra epinepheli* Dewi & Palm, 2013 from *Epinephelus coioides* Hamilton, 1822 in Kedonganan fish market, Bali; Neubert et al. (2016) reported *Philometra* spp. from white-streaked grouper (*Epinephelus ongus* Bloch, 1790); and Koepper et al. (2021) reported *Philometra* sp. from white-streaked grouper (*E. ongus*); and Dewi and Palm (2017) reported several philometrid nematodes, such as *Philometra nemipteri* Luo, 2001 from *Nemipterus japonicus* Bloch, 1791; *Philometra otolithi* Moravec & Manoharan, 2013 from *Otolithes ruber* Bloch & Schneider, 1801; *P. lobotidis* from *Lobotes surinamensis* Bloch, 1790; *Philometra ocularis* Moravec, Ogawa, Suzuki, Miyaki & Donai, 2002 from *Variola louti* Forsskål, 1775; *Philometra* sp. from *Cephalopholis sexmaculata* Rüppell, 1830; and *Philometroides marinus* Moravec & Buron, 2009 from *Rachycentron canadum* Linnaeus, 1766 in Kedonganan fish market, Bali. Dewi and Palm (2017) also reported a new philometrid species, *Philometra damriyasai* Dewi & Palm, 2017 isolated from *Tylerius spinosissimus* Regan, 1908 from Kedonganan fish market.

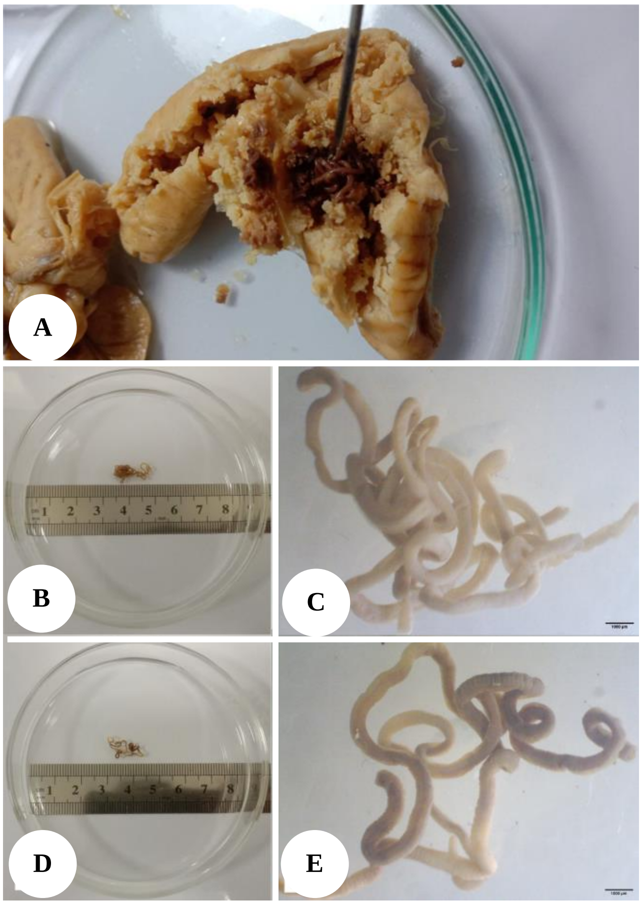

Figure 4. Philometrid fauna found in *Lutjanus vitta* specimens: A. Philometrid nematode found in the ovary of *L. vitta* (BK2). 2 isolated philometrid individuals from *L. vitta* hosts; B. From BK2 specimen; C. Magnified view; D. From BK3 specimen; E. Magnified view

Figure 5. Other helminth fauna found in snappers in Kedonganan, Bali, Indonesia. A. *Siphoderina* sp. from *P. multidens* (BK9) specimen, scale bar= 1000 µm; B. *Cucullanus* sp. from *L. vitta* (BK25) specimen, anterior par; C. Acanthocephala from *L. vitta* (BK2) specimen, anterior part

Philometrid nematodes had been recorded in various Lutjanidae hosts. For instance, *Philometra parabrevicollis* Moravec, Mizher & Ali, 2021 was reported from *Lutjanus lutjanus* Bloch, 1790 (Moravec et al. 2021) from Iraq. *Philometra* sp. was reported from *Lutjanus synagris* Linnaeus, 1758 by Cavalcanti et al. (2010) from the coast of Brazil. *Philometra* sp. was isolated from *L. russellii* from the southeast coast of India by Selvakumar et al. (2016). Several philometrid species were described such as *Philometra arafurensis* Moravec & Barton, 2018 and *Philometra papillicaudata* Moravec & Barton, 2018 from *L. sebae*, *Philometra mawsonae* Moravec & Barton, 2018 and *Dentiphilometra malabarici* Moravec & Barton, 2018 from *Lutjanus malabaricus*, and *Philometra* sp. from *P. multidens* from Australian coast (Moravec and Barton 2018). Ghafar et al. (2023) reported the occurrence of *P. kolachii, P. lutjani* and *P. kemarii* from *Lutjanus argentimaculatus* Forsskål, 1775. As for the report of the occurrences of *Philometra* in *L. vitta*, Moravec and Justine (2011) described 2 species, *P. brevicollis* and *P. mira* from *L. vitta* in New Caledonia.

Philometrid nematode is capable of severely damaging the host gonad, in this case ovary, but the presence of philometrids in the gonad is usually only found in mature hosts (Moravec and de Buron 2013). There were some suggestions whether the immature fish are insusceptible to philometrid nematode or the possibility of a synchrony between the host and philometrid maturation (Moravec and de Buron 2013). This parasite also poses a risk to human health, where humans can be an accidental host if they eat and handle uncooked or raw fish infected by philometrid nematode (Moravec and de Buron 2013).

Other helminth fauna infections in snappers from Kedonganan, Bali

Another noteworthy helminth fauna in this study was digenean *Hamacreadium* sp. family Opecoelidae Ozaki, 1925 and *Siphoderina* spp. family Cryptogonimidae Ward, 1917 (Figure 5) recovered from *L. vitta* and *P. multidens* hosts respectively (Table 2). *Hamacreadium* and *Siphoderina* are some of the common digenean found in lutjanid hosts. Several studies have reported the occurrences of *Siphoderina* in several lutjanid hosts in Indonesian waters, such as *Siphoderina acanthostomus* (Yamaguti, 1934) Miller & Cribb, 2008 from *Lutjanus* sp. by Yamaguti (1953), *Siphoderina* sp. from *Lutjanus fulviflamma* Forsskål, 1775 by Theisen (2019), and *Siphoderina* sp. from *Lutjanus* sp. by Hartini et al. (2019). *Siphoderina elongata* (Gu & Shen, 1979) Miller & Cribb, 2008 (now assigned as *Adlardia elongata* (Gu & Shen, 1979) Miller, Bray, Goiran, Justine & Cribb, 2009) was reported in Indonesia by Machida (2009) from *Nemipterus* sp. host. *Hamacreadium* sp. had been reported by Zainurrahman (2013) and Theisen (2019) from *L. vitta* host and *Hamacreadium lethrini* Nagaty & Abdel Aal, 1962 nec Yamaguti, 1834 (now assigned as *Hamacreadium morgani* Baz, 1946) by Hartini et al. (2019) from *Lutjanus* sp. host. In Indonesia, the morphological study of Cryptogonimidae Ward, 197 and Opecoelidae, Ozaki 1925 was still limited (Machida 2009). Currently, there is no molecular work or data that available from Indonesia. In contrast, Queensland (Australia) region, have been subjected to more intensive morphological and molecular research on cryptogonimids (Martin and Cutmore 2022).

The trematode fauna in the coral triangle, including Indonesia, are poorly known, hence it is impossible to understand the true trematodes richness in Indonesia (Cribb et al. 2016). For example, Cribb et al. (2016) pointed out that in the Sunda Shelf and Java Transitional Province of Indo-West Pacific combined, only 27 trematodes species have been recorded. In contrast, in the Northeast Australian Shelf province alone, 340 trematodes species have been recorded.

This study highlighted the importance of including a parasitological survey in the biodiversity survey, where we uncover a new parasite record (Philometridae) in *L. vitta* that was never recorded previously. Although the sample size was small, we found numerous number of parasites. We also contributed the parasite list of goldband snapper (*P. multidens*) in Indonesian waters, which previously had never been recorded. More morphological, ultrastructural, and molecular studies should be conducted for more precise identification of philometrid nematodes, and other endoparasite fauna. Proper parasite collection, preservation, and fixation are paramount for identification and morphological studies.

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