

Parasite fauna of white-streaked grouper, *Epinephelus ongus* (Bloch, 1790) (Epinephelidae) from Karimunjawa, Indonesia

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(Received 4 March 2016; revised 28 April 2016; accepted 10 May 2016)

SUMMARY

This study provides the first comprehensive information on the parasite fauna of the white-streaked grouper *Epinephelus ongus*. A total of 35 specimens from the archipelago Karimunjawa, Java Sea, Indonesia were studied for metazoan parasites. For comparison, the documented parasite community of 521 *E. areolatus*, *E. coioides* and *E. fuscoguttatus* from previous studies were analysed. A total of 17 different parasite taxa were recognized for *E. ongus*, including 14 new host and four new locality records. This increases the known parasite taxa of *E. ongus* by more than 80%. The ectoparasite fauna was predominated by the monogenean *Pseudorhabdosynochus quadratus* resulting in a low Shannon index of species diversity of the entire parasite community (0.17). By contrast, the species diversity excluding the ectoparasites reached the highest value recorded for Indonesian epinephelids (1.93). The endoparasite fauna was predominated by generalists, which are already known from Indonesia. This demonstrates the potential risk of parasite transmission through *E. ongus* into mariculture and vice versa. One-way analyses of similarity revealed a significantly different parasite community pattern of *E. ongus* compared with *E. areolatus* and *E. fuscoguttatus* as well as minor differences with *E. coioides*. This finding refers to different habitat preferences of these epinephelids within the analysed size range.

Key words: parasite, *Epinephelus ongus*, Epinephelidae, biological indicator, mariculture, Karimunjawa, Indonesia.

INTRODUCTION

Reef fish contribute significantly to food security and income of coastal communities in many developing countries (Donner and Potere, 2007; Hughes *et al.* 2012). Due to its high trade value and increasing demand on the international market, epinephelids belong to the most important fisheries resources, resulting in a continuously growing fishing pressure as well as aquaculture production in Indonesia (Yulianto *et al.* 2015). Indonesia is the second largest grouper producer worldwide (FAO, 2015). Consequently, increasing attention is paid to studies concerning the ecology and biology of wild and cultivated epinephelids. In general, commercially important species are large, such as *Epinephelus coioides* and *E. fuscoguttatus*, with a maximum length up to 120 cm (Craig *et al.* 2011). Both species are relevant for fisheries as well as aquaculture, and have been intensively investigated for diseases and parasites in recent years (e.g. Rückert,

2006; Palm and Rückert, 2009; Kleinertz, 2010; Rückert *et al.* 2010; Palm *et al.* 2011; Kleinertz *et al.* 2014a; Kleinertz and Palm, 2015; Neubert *et al.* 2016). In contrast, the knowledge on the parasite fauna of smaller epinephelids, like the white-streaked grouper *Epinephelus ongus*, is very limited.

E. ongus occurs in the Indo-West Pacific and inhabits coastal reefs as well as brackish water lagoons (Heemstra and Randall, 1993). Ledges and caves in depths of five to 25 m are frequently used as shelter (Myers, 1999). The diet consists of fish and crustaceans and a nocturnal feeding pattern can be assumed (Craig, 2007). With a maximum length of about 40 cm (Craig *et al.* 2011) *E. ongus* is a relatively small member of the Epinephelidae (according to Smith and Craig (2007) the traditional taxon Serranidae is polyphyletic, resulting in the resurrection of the Epinephelidae). *E. ongus* increasingly contributes to the regular catches, and e.g. at the Naha fish market in Okinawa, Japan, it became the most landed epinephelid (Craig, 2007). A similar development can be observed in Karimunjawa Islands, where overfishing of commercially important epinephelid species moves *E. ongus* more and more into the focus of fisheries. Thus, *E. ongus* became the

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most abundant landed epinephelid between 2009 and 2012, and contributed a high proportion to the total weight of landed epinephelids in the Karimunjawa archipelago (Fig. 1).

Karimunjawa is located in the Java Sea, approx. 80 km off the coastal city Jepara, Central Java. This remote archipelago was one of the first areas recognized as being important for the conservation of marine biodiversity in Indonesia (Campbell *et al.* 2013). Consequently, Karimunjawa was declared a National Park in 1999 (Campbell *et al.* 2013). Nevertheless, fishing is still permitted in Karimunjawa, with exception of certain areas such as spawning aggregation sites (Campbell *et al.* 2014) and the fishing pressure has distinctly increased during the last decade (Yulianto *et al.* 2015). This resulted in declining stocks of large epinephelids and a shift towards smaller species, especially *E. ongus* (Fig. 1).

According to Justine *et al.* (2010), epinephelids harbour an average of ten different parasite species in the Western Pacific. For example, *E. coioides* and *E. fuscoguttatus* harbour 51 and 52 parasite taxa, respectively, in Indonesia alone (Rückert *et al.* 2010; Neubert *et al.* 2016). In contrast, not a single parasite was documented for *E. ongus* from Indonesian waters. So far, only three monogeneans, *Pseudorhabdosynochus summanae* (Young, 1969) (synonym: *Diplectanum summanae* Kritsky and Beverley-Burton, 1986), *P. quadratus* (Schoelincx and Justine, 2011) and *Benedenia fieldsi* (Deveney and Whittington, 2010) as well as two digeneans, *Pearsonellum corventum* (Lester and Sewell, 1989; Overstreet and Køie, 1989) and *Lepidapedoides angustus* (Bray *et al.* 1996), have been recorded for this epinephelid. The present study is the first comprehensive analysis of the parasite fauna of *E. ongus* worldwide, discussing: (1) the infection pattern, (2) the use of the documented parasite community as environmental indicator and (3) the potential risk of parasite transmission into mariculture systems and vice versa.

MATERIALS AND METHODS

Collection of fish

Samples were taken within the framework of SPICE III – MABICO (Science for the Protection of Indonesian Coastal Marine Ecosystems – Impacts of Marine Pollution on Biodiversity and Coastal Livelihoods). A total of 35 *E. ongus* were bought from artisanal fishermen collecting live fish in the vicinity of Karimunjawa. Fish were purchased during May and dissected in August 2013. All fish were directly separated into plastic bags, transported on ice and deep frozen (–20 °C) at the Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Indonesia. Analysed *E. ongus* had a

total length of 25.5 cm (S.E. = 0.4 cm). All available raw data of *E. areolatus*, *E. coioides* and *E. fuscoguttatus* from Indonesian waters (Bali, Java and Sumatra) were used to compare the parasite fauna of *E. ongus* with commercially important larger epinephelids. In detail, these were 60 *E. areolatus* with a total length of 32.7 cm (S.E. = 0.3 cm) by Kleinertz (2010), 356 *E. coioides* with a total length of 28.9 cm (S.E. = 0.3 cm) by Yuniar (2005); Rückert (2006); Kleinertz (2010) and Neubert *et al.* (2016) and 105 *E. fuscoguttatus* with a total length of 26.8 cm (S.E. = 0.3 cm) by Rückert (2006).

Parasitological examination

The parasitological investigation was limited on metazoan parasites and followed the standard protocol by Palm (2011) and Palm and Bray (2014). Skin, fins, nostrils, eyes, gills, gill covers, mouth and gill cavity were examined for ectoparasites by using a Zeiss Stemi DV4 binocular microscope. All fluids from the plastic bag in which the fish was frozen were subsequently studied. Examination for endoparasites included the body cavity and mesentery, followed by internal organs, which were separated into Petri dishes and covered with saline solution (0.9%). The microscopic examination of all organs was conducted using a Zeiss Stemi DV4 under 8–32× magnification. A gut wash was performed according to Cribb and Bray (2010). The musculature was sliced in thin layers and studied using a transmitting light source. The recorded parasites were transferred to saline solution (0.9%), cleaned, fixed and preserved in 70% ethanol for morphological identification using an Olympus BX53 DIC microscope. The parasites were dehydrated in an ethanol series and transferred to 100% glycerine (Riemann, 1988). Selected individuals were stained with acetic carmine, dehydrated, cleared with eugenol and mounted in Canada balsam (Palm, 2004). According to Paladini *et al.* (2011), Monogenea were treated with proteinase K and mounted in Malmberg's Solution to observe skeletonized structures, which are necessary for species identification. The parasite identification was conducted by using taxonomic keys and original descriptions. For the ectoparasitic monogeneans the literature consulted was provided by Whittington *et al.* (2001) and Schoelincx and Justine (2011), for the copepods by Ho and Dojiri (1977); Schmidt and Roberts (1989); Boxshall and Halsey (2004) and Ho and Lin (2004) and for the isopods by Kensley and Schotte (1989). Identification literature of endoparasites was provided by Bray and Cribb (1989) for the digeneans, by Palm (2004) for cestodes, and by Anderson *et al.* (2009); Gibbons (2010) and Dewi and Palm (2013) for nematodes. In addition, molecular identification of eight *Hysterothylacium* and one

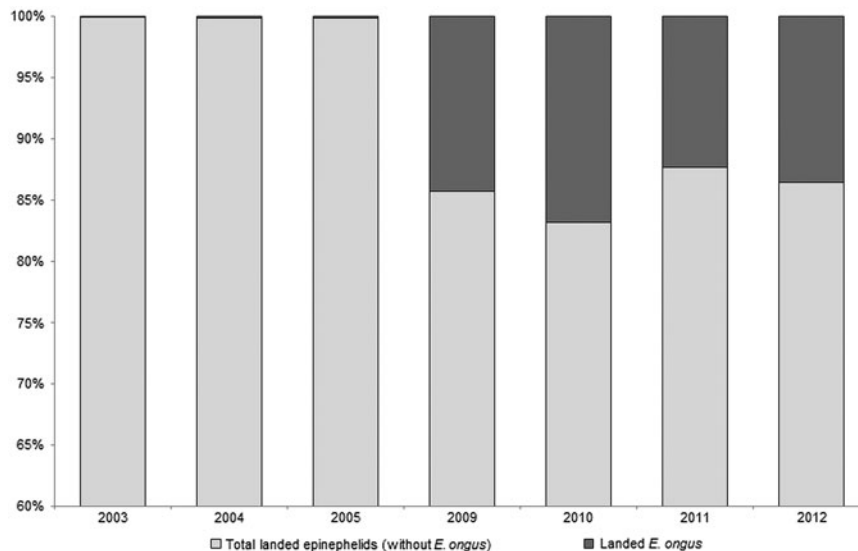


Fig. 1. Development of the contribution of *Epinephelus ongus* to the total landed epinephelids at National Park Karimunjawa, Indonesia between 2003 and 2012 (based on fish weight)

Anisakis specimens was conducted following the protocol by Palm *et al.* (2008). The sequences of the ITS1, 5.8S and ITS2 rDNA are deposited in GenBank under the accession numbers KU705468 for *Hysterothylacium* sp. and KU705469 for *Anisakis typica*.

Quantitative parasite descriptors

The prevalence (P), intensity (I), mean intensity (I_m) and mean abundance (A_m) of all parasites found were calculated following Bush *et al.* (1997). The diversity of the parasite fauna was determined by using the Shannon index of species diversity (Shannon, 1948; Spellerberg and Fedor, 2003) and the Pielou index of evenness (Pielou, 1966). Furthermore, the Berger–Parker index of dominance was used (Berger and Parker, 1970; May 1975). All indices were calculated for the entire parasite fauna as well as for the endoparasite fauna only. Parasites which were only identified to higher taxonomic levels (such as Nematoda indet.) were omitted from these calculations, because they might represent other recorded taxa. The ecto- to endoparasite ratio was calculated (number of ectoparasite species divided by number of endoparasite species) according to Rückert *et al.* (2009a) (Table 1).

Data analysis

Raw data by Yuniar (2005); Rückert (2006); Kleinertz (2010) and Neubert *et al.* (2016) (see above) were included in the present study to compare the recorded parasite fauna of *E. ongus* with previous studied epinephelids from Indonesian waters. Higher taxa as well as records of genera, which were previously identified to the species level were omitted, because they might

represent prior recorded species. Statistical analyses were performed using Primer 6 version 6.1.13. To display the level of similarity between all 556 analysed fish, a similarity matrix was constructed applying Bray Curtis similarity measure. Fish without parasites and outliers, defined by unequal results in the Kruskal stress formula, were omitted from analyses. The relation between samples based on the comparison of similarity matrices was displayed by using multi-dimensional scaling (MDS). One-way analyses of similarity were applied to identify the differences in parasite species composition between the epinephelid species (routine ANOSIM, R values close to 1 indicate high differences and close to 0 indicate high similarity between species compositions). SIMPER analysis was applied to test, which parasite species contributed most to the shown differences of analysed epinephelids. All recorded parasites, which could be identified at least to the genus level, were summarized in a parasite host list for Indonesian waters. In the case of one or more given species identification within a genus, further records of this genus were not listed (Table 2).

RESULTS

Parasite community

The examined *E. ongus* revealed 17 different parasite taxa, seven ecto- and ten endoparasites (ecto- to endoparasite ratio: 0.7). The most speciose parasites were nematodes with seven, followed by crustaceans with four and monogeneans with three species. Less species rich were the cestodes and digeneans with two and one species, respectively. More than 82% (14) of the reported taxa are new host records for *E. ongus* and four represent first locality records for Indonesian waters (Table 1).

Table 1. Prevalence in % (P), mean intensity (I_m), intensity (I) and mean abundance (A_m) of parasites from *Epinephelus ongus* from Karimunjawa, Indonesia, with additional parasitological indices

Parasite/parasitological index	P (%)	I_m	I	A_m
Ectoparasites				
<i>Benedenia hawaiiensis</i> (M) ^{a,b}	5.7	1.0	(1)	0.06
<i>Pseudorhabdosynochus quadratus</i> (M) ^b	77.1	260.1	(1–1166)	200.69
<i>Pseudorhabdosynochus</i> sp. (M)	14.3	2.6	(2–4)	0.37
<i>Alcirona</i> sp. (Cr) ^a	14.3	1.6	(1–3)	0.23
<i>Caligus</i> sp. Larvae (Chalimus) (Cr) ^a	60.0	3.0	(1–9)	1.83
<i>Lepeophtheirus epinepheli</i> (Cr) ^{a,b}	11.4	1.0	(1)	0.11
Gnathiidae indet. Larvae (Praniza) (Cr) ^a	22.9	3.5	(1–8)	0.80
Endoparasites				
<i>Macvicaria macassarensis</i> (D) ^a	8.6	1.3	(1–2)	0.11
<i>Nybelinia</i> sp. (C) ^a	2.9	1.0	(1)	0.03
Tetraphyllidea indet. (<i>Scolex pleuronectis</i>) (C) ^a	2.9	2.0	(2)	0.06
<i>Anisakis typica</i> (N) ^a	2.9	1.0	(1)	0.03
<i>Capillaria</i> sp. (N) ^a	5.7	2.0	(1–3)	0.11
<i>Hysterothylacium</i> sp. (N) ^a	22.9	2.3	(1–6)	0.51
<i>Philometra</i> cf. <i>lateolabracis</i> (N) ^{a,b}	14.3	2.0	(1–4)	0.29
<i>Philometra epinepheli</i> (N) ^a	5.7	1.5	(1–2)	0.09
<i>Philometra ocularis</i> (N) ^a	22.9	2.0	(1–4)	0.46
Nematoda indet. (N)	2.9	7.0	(7)	0.20
Parasitological indices				
Shannon index of species diversity (total)			0.17	
Shannon index of species diversity (endoparasites)			1.93	
Berger-Parker index of dominance (total)			0.97	
Berger-Parker index of dominance (endoparasites)			0.27	
Pielou index of evenness (total)			0.06	
Pielou index of evenness (endoparasites)			0.84	
Ecto-/endoparasite ratio			0.7	

C, Cestoda; Cr, Crustacea; D, Digenea; M, Monogenea; N, Nematoda.

^a Recorded for the first time for *E. ongus*.

^b New locality record (Indonesia).

Ectoparasites

The gill infecting monogenean *Pseudorhabdosynochus quadratus* was the predominating ectoparasite and represents a core species for *E. ongus* in Karimunjawa (prevalence > 60%). It differs from another recorded *Pseudorhabdosynochus* species (*Pseudorhabdosynochus* sp.) by a wider and elongated tube of the skeletonized vagina. The second most prevalent ectoparasite was the copepod *Caligus* sp. (larval chalimus stage), infecting the gills (second core species). The third most prevalent ectoparasite were gnathiid praniza larvae. These isopods were found on the gills, mouth cavity and operculum. *Alcirona* sp. (Isopoda), *Lepeophtheirus epinepheli* (Copepoda) and *Benedenia hawaiiensis* (Monogenea) were also detected from the gills. Numeric information on the prevalence, intensity, mean intensity and mean abundance of documented ectoparasites are given in Table 1.

Endoparasites

Philometra ocularis as adult females and *Hysterothylacium* sp. as third stage larvae were the predominating endoparasites. *P. ocularis* was

recorded under the eyes of examined fish, whereas *Hysterothylacium* sp. occurred in the liver, pyloric caeca, intestine and body cavity. The morphology of *Hysterothylacium* sp. is similar to *Hysterothylacium* sp. I as described by Rückert (2006) and Palm and Rückert (2009). The genetic identification of *Hysterothylacium* sp. revealed highest similarity to *H. deardorffoverstreetorum* (identity 97%, e.g. GenBank accession number JF730200.1) described from a flounder in Brazil (Knoff *et al.* 2012). However, the specimens found in this study differed in 24 base pairs of the ITS1, 5.8S and ITS2 rDNA from *H. deardorffoverstreetorum*, requiring more detailed analyses in terms of species identification. *Philometra* cf. *lateolabracis* (Nematoda) was the third most prevalent endoparasite and was recorded from the gonads of female fish. The digenean *Macvicaria macassarensis* was recorded from the pyloric caeca and intestine and the nematode *Capillaria* sp. in the stomach of the analysed fish. Beside *P. ocularis* and *Philometra* cf. *lateolabracis*, a third member of Philometridae was found. *P. epinepheli* was isolated from the tissue under the skin of the opercula. *Anisakis typica* (Nematoda), which occurred in the stomach, was identified by DNA analysis [identity 100%,

Table 2. Comparison of the metazoan parasite fauna of *Epinephelus areolatus*, *E. coioides*, *E. fuscoguttatus* and *E. ongus* from Indonesian coastal waters (+ present, – absent)

Epinephelid species	<i>Epinephelus coioides</i>	<i>Epinephelus fuscoguttatus</i>	<i>Epinephelus areolatus</i>	<i>Epinephelus ongus</i>
Parasite species				
Ectoparasites				
<i>Piscicola</i> sp. (H) ¹	–	+	–	–
<i>Zeylanicobdella arugamensis</i> (H) ^{2,8}	+	–	–	–
<i>Benedenia epinepheli</i> (M) ^{3,4,8}	+	+	–	–
<i>Benedenia hawaiiensis</i> (M) ⁰	–	–	–	+
<i>Benedenia hoshinai</i> (M) ⁵	+	–	–	–
<i>Diplectanum grouperii</i> (M) ⁶	+	–	+	–
<i>Diplectanum</i> sp. (M) ⁷	–	+	–	–
<i>Haliotrema cromileptis</i> (M) ⁵	+	–	–	–
<i>Neobenedenia melleni</i> (M) ^{3,4}	+	+	–	–
<i>Pseudorhabdosynochus coioidesis</i> (M) ⁶	+	–	+	–
<i>Pseudorhabdosynochus epinepheli</i> (M) ^{2,3}	+	+	–	–
<i>Pseudorhabdosynochus lantauensis</i> (M) ^{2,3,6,8}	+	+	+	–
<i>Pseudorhabdosynochus quadratus</i> (M) ⁰	–	–	–	+
<i>Alcirona</i> sp. (Cr) ^{0,3,5,8}	+	+	+	+
<i>Argathona rhinoceros</i> (Cr) ^{3,5}	+	+	–	–
<i>Caligus</i> cf. <i>epinepheli</i> (Cr) ⁸	+	–	+	–
<i>Caligus</i> sp. larvae (Chalimus) (Cr) ^{0,5}	+	–	–	+
<i>Cymothoa elegans</i> (Cr) ³	–	+	–	–
<i>Hatschekia cernae</i> (Cr) ⁵	+	–	–	–
<i>Hatschekia</i> sp. (Cr) ^{8,9}	+	–	+	–
<i>Lepeophtheirus epinepheli</i> (Cr) ⁰	–	–	–	+
<i>Lepeophtheirus</i> sp. (Cr) ^{10,11,12}	+	+	–	–
<i>Sagum epinepheli</i> (Cr) ^{3,5,8}	+	+	–	–
Endoparasites				
<i>Allonematobothrium epinepheli</i> (D) ^{2,3,5,8,9,a}	+	–	+	–
<i>Allopodocotyle epinepheli</i> (D) ^{2,3,5,8}	+	+	–	–
<i>Allopodocotyle</i> sp. (D) ^{8,9}	–	–	+	–
<i>Cainocraedium epinepheli</i> (D) ⁸	+	–	–	–
<i>Lecithochirium magnaporum</i> (D) ^{2,3,8}	–	+	–	–
<i>Lecithochirium neopacificum</i> (D) ³	–	+	–	–
<i>Lecithochirium</i> sp. (D) ⁵	+	–	–	–
<i>Macvicaria macassarensis</i> (D) ⁰	–	–	–	+
<i>Podocotyloides stenometra</i> (D) ⁵	+	–	–	–
<i>Prosorhynchus</i> cf. <i>crucibulum</i> (D) ^{2,3}	–	+	–	–
<i>Prosorhynchus luzonicus</i> (D) ^{3,4,5,8}	+	+	–	–
<i>Prosorhynchus</i> sp. 1 (D) ^{8,9}	+	+	+	–
<i>Prosorhynchus</i> sp. 2 (D) ^{8,9}	+	–	+	–
<i>Stephanostomum</i> sp. (D) ⁵	+	–	–	–
<i>Bothriocephalus</i> sp. (C) ^{8,13}	+	–	–	–
<i>Callitetrarhynchus gracilis</i> (C) ⁸	+	–	+	–
<i>Nybelinia indica</i> (C) ^{3,14}	+	+	–	–
<i>Nybelinia</i> sp. (C) ⁰	–	–	–	+
<i>Parotobothrium balli</i> (C) ^{2,3,4,8,14}	+	+	+	–
<i>Tetraphyllidea</i> indet. (<i>Scolex pleuronectis</i>) (C) ^{0,2,3,4,8}	+	+	–	+
<i>Anisakis</i> aff. <i>typica</i> var. <i>indonesiensis</i> (N) ^{0,17}	–	–	+	+
<i>Anisakis</i> sp. (HC-2005) (N) ¹⁵	–	–	+	–
<i>Camallanus carangis</i> (N) ^{2,3,8}	+	+	–	–
<i>Capillaria</i> sp. (N) ^{0,5}	+	–	–	+
<i>Echinocephalus</i> sp. (N) ³	+	+	–	–
<i>Hysterothylacium</i> sp. 1 (N) ^{0,2,3,4,5}	+	+	–	+
<i>Hysterothylacium</i> sp. 2 (N) ⁵	+	–	–	–
<i>Paracuaria adunca</i> (N) ³	+	–	–	–
<i>Philometra</i> cf. <i>lateolabracis</i> (N) ⁰	–	–	–	+
<i>Philometra epinepheli</i> (N) ^{0,16}	+	–	–	+
<i>Philometra ocularis</i> (N) ^{0,3}	+	+	–	+
<i>Philometra</i> sp. 1 (N) ^{2,3}	+	+	–	–
<i>Philometra</i> sp. 2 (N) ^{8,9}	+	–	+	–
<i>Raphidascaris</i> sp. 1 (N) ^{2,3,4,8}	+	+	+	–
<i>Raphidascaris</i> sp. 2 (N) ^{2,3}	+	+	–	–
<i>Spirophilometra</i> sp. (N) ^{3,8}	+	–	–	–

Table 2. (Cont.)

Epinephelid species	<i>Epinephelus coioides</i>	<i>Epinephelus fuscoguttatus</i>	<i>Epinephelus areolatus</i>	<i>Epinephelus ongus</i>
<i>Terranova</i> sp. (N) ^{2,3,4,5}	+	+	–	–
<i>Gorgorhynchoides golvani</i> (A) ⁵	+	–	–	–
<i>Gorgorhynchus</i> sp. (A) ³	+	–	–	–
<i>Neoechinorhynchus</i> sp. (A) ³	–	+	–	–
<i>Rhadinorhynchus</i> sp. (A) ⁵	+	–	–	–
<i>Serrasentis sagittifer</i> (A) ^{3,4,5,8}	+	+	+	–
<i>Southwellina hispida</i> (A) ⁸	+	–	+	–

A, Acanthocephala; C, Cestoda; Cr, Crustacea; D, Digenea; H, Hirudinea; M, Monogenea; N, Nematoda.

Source: ⁰Present study; ¹Diani *et al.* (1999); ²Palm and Rückert (2009); ³Rückert (2006); ⁴Rückert *et al.* (2009b); ⁵Neubert *et al.* (2016); ⁶Bu *et al.* (1999); ⁷Wijayati and Djunaidah (2001); ⁸Kleinertz (2010); ⁹Kleinertz *et al.* (2014a); ¹⁰Koesharyani *et al.* (2000); ¹¹Yuasa *et al.* (1998); ¹²Zafran *et al.* (1998); ¹³Yuniar (2005); ¹⁴Palm (2004); ¹⁵Palm *et al.* (2008); ¹⁶Dewi and Palm (2013); ¹⁷Palm *et al.* (2016).

^a *Didymodictimus* sp. in Rückert (2006); Palm and Rückert (2009); Kleinertz (2010); Kleinertz *et al.* (2014a).

GenBank accession number HF911524.1, Kleinertz *et al.* (2014b)]. The specimen found in this study is similar to *Anisakis* sp. 2 by Palm *et al.* (2008) and *A.* aff. *typica* var. *indonesiensis* Palm *et al.* (2016), which is the most frequent genotype of *A. typica* (*sensu lato*) in Indonesian waters. A nematode which could not be identified to a precise taxonomic level due to its poor condition was found in the stomach, intestine and pyloric caeca (Nematoda indet.). The larval trypanorhynch cestode *Nybelinia* sp. with inverted tentacles was recorded from the pyloric caeca, and larval tetraphyllids named as Tetraphyllidea indet. (*Scolex pleuronectis*), from the intestine of the analysed *E. ongus*. Numeric information on the prevalence, intensity, mean intensity and mean abundance of the recorded endoparasites are given in Table 1.

Parasitological indices

The Shannon index of species diversity for *E. ongus* reached 0.17. If only endoparasites were considered, the Shannon index of species diversity differed distinctly and reached a more than ten times higher value of 1.93. This is the result of the predominating ectoparasite *P. quadratus*, expressed by a Berger–Parker index of dominance of 0.97. If only endoparasites were considered the Berger–Parker index of dominance decreased distinctly to 0.27. A similar pattern was recognised for the Pielou index of evenness with values of 0.06 for the entire parasite fauna and 0.84 for the endoparasite fauna (Table 1).

Comparison of analysed epinephelids

A total of 66 different parasite species, excluding species identified to higher taxonomic levels, represent the parasite fauna of the four considered epinephelid species in Indonesian waters (Table 2). MDS revealed a distinctly different parasite

infection pattern for *E. ongus*, *E. areolatus* and *E. fuscoguttatus* whereas *E. coioides* is not clearly separating from the first three epinephelids (Fig. 2). The ANOSIM significantly demonstrated that the difference between the parasite composition of all four epinephelid species is not distinctive (Global R : 0.30, $P < 0.01$). The pair-by-pair comparisons showed that *E. coioides* is responsible for this finding (*E. coioides* vs *E. areolatus* (R : 0.30, $P < 0.01$), *E. coioides* vs *E. fuscoguttatus* (R : 0.18, $P < 0.01$) and *E. coioides* vs *E. ongus* (R : 0.36, $P < 0.01$)). The remaining three epinephelids *E. ongus*, *E. areolatus* and *E. fuscoguttatus* demonstrated a high separation based on their parasites [*E. ongus* vs *E. areolatus* (R : 0.90, $P < 0.01$), *E. ongus* vs *E. fuscoguttatus* (R : 0.95, $P < 0.01$), *E. areolatus* vs *E. fuscoguttatus* (R : 0.94, $P < 0.01$)]. The SIMPER analysis revealed that *P. quadratus*, *Hysterothylacium* sp. 1 and *P. ocularis* are the main contributors separating *E. ongus* from the remaining three epinephelids. *Allonematobothrium epinepheli*, *Anisakis* aff. *typica* var. *indonesiensis* (Palm *et al.* 2016) and *Hatschekia* sp. contributed most to the separation of *E. areolatus* and *Alcirona* sp., *All. epinepheli* and

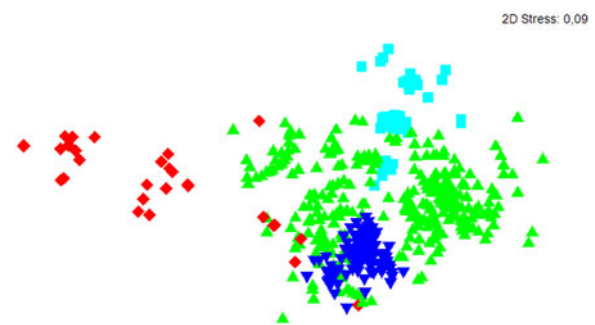


Fig. 2. Multidimensional scaling plot of the parasite fauna from *Epinephelus areolatus* ■, *E. coioides* ▲, *E. fuscoguttatus* ▼ and *E. ongus* ◆ in Indonesian waters based on Bray Curtis similarity

Raphidascaris sp. to the separation of *E. fuscoguttatus*. *Pseudorhabdosynochus lantauensis*, *Prosorhynchus luzonicus* and *Alcirona* sp. are responsible for the minor separation of *E. coioides* from the remaining three epinephelid species.

DISCUSSION

The information on the parasite fauna of *E. ongus* is very limited. So far, only five species have been recorded (see the 'Introduction'), including the records of *Benedenia fieldsi* (Deveney and Whittington, 2010) and *Pseudorhabdosynochus summanae* (Young, 1969), which must be considered more or less as questionable. *B. fieldsi* was isolated from an aquarium fish and a possible transfer from another fish species in the same fish tank cannot be excluded. *P. summanae* was originally described from *Epinephelus summana* sampled in Australia (Young, 1969), but *E. summana* is endemic to the Red Sea, Gulf of Aden and Socotra Yemen (Heemstra and Randall, 1993; Craig *et al.* 2011). Consequently, Justine (2007) already concluded that *E. summana* cannot be the type-host for this monogenean and declared *E. ongus* or *E. coeruleopunctatus* as potential type-host. Schoelinck and Justine (2011) suggested that *E. ongus* was the original type-host of *P. summanae*, however, not considering Heemstra and Randall (1993) who stated that additional white spotted groupers such as *E. corallicola*, a species which is also known from Australia (Froese and Pauly, 2015), are often confused with *E. summana*. However, Lester and Sewell (1989) reported *Diplectanum summanae* from *E. ongus* sampled at Heron Island, Australia. *D. summanae* is a synonymised name of *P. summanae*. Therefore, we agree with Schoelinck and Justine (2011) which nominated *E. ongus* as type-host of *P. summanae*. Considering the small number of recorded parasites as well as the uncertainty by one of five records, the present study gives a first comprehensive insight into the parasite fauna of *E. ongus*.

Almost all recorded taxa are new host records for *E. ongus*. It is very interesting that only four of 17 taxa represent new locality records, although *E. ongus* was never parasitologically sampled in Indonesia before. Three of these four previously unknown taxa are ectoparasites (*B. hawaiiensis*, *L. epinepheli* and *P. quadratus*) and only one belongs to the endoparasites (*Philometra* cf. *lateolabracis*). Furthermore, the previously recorded endoparasites are known from other epinephelids of Indonesian waters (*E. areolatus*, *E. coioides* and *E. fuscoguttatus*) (Table 2). The only exception is *M. macassarensis*, which was originally found in a lethrinid from Sulawesi by Yamaguti (1952). Thus, the endoparasitic fauna of *E. ongus* was distinctly dominated by generalist parasites, which are already known for epinephelids from Indonesian waters

(Palm and Rückert, 2009; Rückert *et al.* 2010; Kleinertz *et al.* 2014a; Kleinertz and Palm, 2015; Neubert *et al.* 2016). The most abundant endoparasitic taxon was the Nematoda, which contributed 67% to all recorded endoparasites of *E. ongus*. This is the main difference to previously studied epinephelids, where the nematodes hold between 25 and 33% of the entire endoparasite records (Palm and Rückert, 2009; Rückert *et al.* 2010; Kleinertz *et al.* 2014a; Kleinertz and Palm, 2015; Neubert *et al.* 2016). Indonesia is the most diverse marine region in the world (Allen, 2008). The fish analysed in this study were obtained from one of the most remote archipelagos in this tropical diversity hotspot. Consequently, the recorded parasite fauna represents the most common parasites of *E. ongus* in an overfished (Yulianto *et al.* 2015), but environmental less affected habitat. It is evident that the parasite richness of *E. ongus* does not reach the species numbers that were recorded for previously investigated *E. coioides* and *E. fuscoguttatus* in Indonesia. However, the records reach a similar level as reported for *E. areolatus* (Table 2). The parasite composition of analysed *E. areolatus*, *E. fuscoguttatus* and *E. ongus* differs significantly, associated with an overarching pattern for *E. coioides* (Fig. 2). This finding as well as the predominance of nematodes is remarkable, because the ecology, behaviour and feeding of these species have been reported as approximately the same (Heemstra and Randall, 1993; Craig *et al.* 2011). However, one striking difference can be found in the maximum size of these four epinephelids. *E. coioides* and *E. fuscoguttatus* reach a total length up to 120 cm, whereas the maximum recorded length of *E. ongus* and *E. areolatus* is about 40 cm (Heemstra and Randall, 1993; Craig *et al.* 2011). The analysed fish specimens in this study ranged between 19.5 and 46.4 cm. Thus, most *E. coioides* and *E. fuscoguttatus* were juveniles (Rückert, 2006; Kleinertz, 2010), whereas all *E. areolatus* and *E. ongus* were adults (for *E. areolatus*, see Kleinertz, 2010). Juvenile *E. coioides* prefer sand, mud and gravel, while juvenile *E. fuscoguttatus* are often found in seagrass areas (Heemstra and Randall, 1993). Adult *E. areolatus* are likewise found over seagrass or on fine sediment bottoms, but in deeper areas (Carpenter and Niem, 1999). *E. ongus* typically occurs in coral reef habitats and on rocky bottoms (Craig *et al.* 2011). Thus, the considered epinephelids prefer different habitats within the analysed size range. This contributes to the recorded different parasite composition (Fig. 2). However, it is remarkable that also generalist parasites, like *Alcirona* sp., *All. epinepheli*, *Hysterothylacium* sp. 1 and *P. ocellaris*, contribute to the differentiation of the analysed fishes. Even if these parasites can use a broad range of hosts, a pattern can be observed, which allows separation of analysed epinephelids (Fig. 2).

Rückert *et al.* (2009b); Rückert *et al.* (2010) and Palm *et al.* (2015) reported the potential risk of parasite transmission between cultured and wild epinephelids. Due to the high number of recorded generalist parasites (see above), this is also the case for *E. ongus*. If one of the recorded parasite species has the potential to increase mortality, decrease fish health or product quality, is a matter of further investigations. This might have relevance in future mariculture development in Indonesia. Parasites infecting several of the four analysed epinephelid species (Fig. 2) contribute most to this potential risk, and can be easily introduced to new localities through the establishment of new mariculture facilities or stocking with non-native species. Karimunjawa is one of the remotest islands of Indonesia. However, mariculture activities are growing in the archipelago (Campbell *et al.* 2010). The present study on *E. ongus* might serve as a reference in terms of: (1) future monitoring programs of these activities (Palm *et al.* 2011) as well as (2) environmental indication based on fish parasites (Kleinertz and Palm, 2015; Neubert *et al.* 2016). We suggest, that *E. ongus* is a suitable species for this purposes due to its increasing contribution to fish landings as well as the decreasing abundance of previously used larger epinephelids like *E. coioides*. Recently, the water quality in Karimunjawa was defined as very good with low, spatial limited inputs of domestic sewage (Sugianti and Mujiyanto, 2014). However, also in Karimunjawa water pollution has increased in recent years and it can be expected that the anthropogenic impact will increase at the same rate as the coastal development increases in Karimunjawa (Campbell *et al.* 2013).

Ectoparasitic flukes have direct life cycles without intermediate hosts (Whittington, 2005). Under polluted conditions increasing infestation rates, high individual numbers and an unequal distribution in favour of ectoparasites were often reported (e.g. Haensly *et al.* 1982; Skinner, 1982; Khan and Kiceniuk, 1988; Marcogliese and Cone, 1996; MacKenzie, 1999; Dzikowski *et al.* 2003). The parasite fauna of *E. ongus* at Karimunjawa was predominated by the diplectanid monogenean *P. quadratus* expressed by a Berger–Parker index of dominance of 0.97 and a Pielou index of evenness of 0.06 (Table 1). The mean intensity of 260.1 is the highest ever recorded for the genus *Pseudorhabdosynochus* from free-living epinephelids in Indonesia (Palm and Rückert, 2009; Rückert *et al.* 2010; Kleinertz *et al.* 2014a; Kleinertz and Palm, 2015; Neubert *et al.* 2016). For a near natural environment, like Karimunjawa, this was previously unknown. This finding might be explained with the age of sampled fish. In contrast to *E. ongus*, the previous studied *E. coioides* and *E. fuscoguttatus* were juveniles (see above). Therefore, *E. ongus* were substantially older in the analysed

size range and it appears that the relatively old *E. ongus* accumulated *P. quadratus* over time. In addition, it seems that *P. quadratus* dominated the copepod *Caligus* sp. in quantity of infestation. *Caligus* sp. was found at the same site on the gills with a high prevalence (60.0%), but with a distinctly lower mean intensity of 3.0, although *Caligus* sp. is known to occur with high individual numbers (Neubert *et al.* 2016). Another interpretation of the massive *Pseudorhabdosynochus* infection is that the environmental conditions off Karimunjawa are not as good as reported by Sugianti and Mujiyanto (2014). This would be coherent with the low Shannon index of species diversity. However, if only the endoparasites were considered, the parasite fauna of *E. ongus* must be assessed as highly diverse, as the Shannon index of species diversity reached 1.93. This is the highest value documented for an epinephelid in Indonesian waters (see above). For comparison, the highest recorded Shannon index of species diversity of endoparasites for *E. coioides* reached 1.84, for *E. fuscoguttatus* 1.78 and for *E. areolatus* 1.61, and these values are from unaffected habitats as well (Rückert, 2006; Palm and Rückert, 2009; Rückert *et al.* 2010; Palm *et al.* 2011; Kleinertz *et al.* 2014a; Kleinertz and Palm, 2015; Neubert *et al.* 2016). The Shannon index of species diversity is known to indicate diversity loss of endoparasites in affected environments (Rückert, 2006; Rückert *et al.* 2009a). Referring to the highly diverse endoparasite fauna of *E. ongus*, the environmental conditions in Karimunjawa must be considered as fairly natural. Currently, the marine food web appears to be unspoiled, enabling many endoparasitic species to complete their complex life cycles. The endoparasitic fauna is normally distributed, depicted by a Berger–Parker index of dominance of 0.27 and a Pielou index of evenness of 0.84. However, it is interesting that only a single digenean species was found (see Table 1), although epinephelids appear to harbour rich assemblages of digeneans (Cribb *et al.* 2002). A diverse endoparasite fauna is a hallmark of unpolluted environments (e.g. Lafferty, 1997; MacKenzie, 1999). Consequently, the high number of recorded nematode species indicates that the marine ecosystem off Karimunjawa is healthy and provides the manifold intermediate host fauna, which is needed to fulfil the multiple host life cycles of these parasites. This is underlined by the recorded cestodes, which are indicators of good environmental conditions as well (Palm, 2011). According to Rückert *et al.* (2009a), the documented ecto- to endoparasite ratio of 0.7 classifies Karimunjawa likewise as habitat with natural conditions. However, it should be kept in mind, that on the one hand the massive infection with *Pseudorhabdosynochus* and the low digenean diversity is not characteristic for epinephelids from nearly unaffected habitats such as Karimunjawa

Islands (Kleinertz *et al.* 2014a; Kleinertz and Palm, 2015); and on the other hand that this is the first comprehensive study on the parasites of *E. ongus*. Thus, the findings are restricted to 35 specimens from one locality, which makes further parasitological investigations on this significant fish urgently needed.

ACKNOWLEDGEMENTS

We are thankful for institutional support to the Leibniz Center for Tropical Marine Ecology, GmbH, Germany, and the Bogor Agricultural University (IPB), Indonesia. Special thanks to Dr Am Azbas Taurusman from IPB for his personal initiative, providing laboratory space and organizational support during laboratory work. We would also like to thank the National Park authority for the sampling permit (approval number: 18/BA/BTNKJ-3/2013).

FINANCIAL SUPPORT

Financial support was provided by the German Federal Ministry of Education and Research (grant number 03F0641D) within the framework of the joint Indonesian–German research project SPICE III – MABICO project (Science for the Protection of Indonesian Coastal marine Ecosystems – Impacts of marine pollution on biodiversity and coastal livelihoods).

CONFLICT OF INTEREST

None.

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