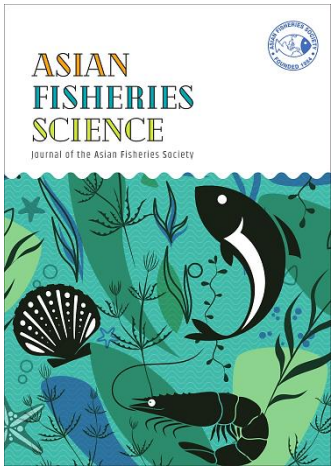

Record of Copepod Parasite (Pennellidae) in Buccal Cavity and Gill Arch of Cultured Groupers, *Epinephelus* spp. in Batam, Indonesia

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Abstract

The crustacean parasites are the most frequently encountered and cause significant economic loss in mariculture. These parasites infect fish fin, skin, gills, and buccal cavity. This study aims to describe copepod parasite in the buccal cavity of cultured groupers, *Epinephelus* spp., from Batam waters using morphological and molecular biology approaches. The tiger grouper, *Epinephelus fuscoguttatus* (Forsskal, 1775), and hybrid grouper, *Epinephelus* sp. showing lethargy and skin darkening were collected from sea cages. The parasite's morphology was observed using light and scanning electron microscopes. The genomic DNA was isolated from the parasites and used as a template for amplification of cytochrome oxidase subunit-1(*Cox1*) gene and followed by sequencing. The fish exhibited red nodules in the mouth cavity, on the lips, and gill arch in varying numbers and size of nodules. The copepodid, chalimus, and adult copepod stages were found from the nodule. Based on the presence of the oral cone, this parasite belonged to Siphonostomatoida order of copepods. Based on the structure of the caudal ramus with four long and four short setae, the first and second pair legs as biramous, and the third pair leg as uniramous, this parasite belonged to Pennellidae family of copepods. Basic local alignment search tool analysis of this *Cox1* showed low homology within 80%, indicating that the DNA sequences of the parasite species were not reported in the GenBank. The unweighted pair group method using arithmetic average phylogenetic trees supported that this parasite belonged to the family Pennellidae. This is the first report on the pennellid parasite infection in the buccal cavity and gill arch of cultured groupers in Batam, Indonesia.

Keywords: cytochrome oxidase subunit-1, red nodule, phylogenic, Siphonostomatoida

Introduction

Aquaculture is the fastest growing food-producing sector in the world and grew on average at 5.3 % per year in the period 2001–2018. The total aquaculture production in 2018 reached 82.1 million tonnes (FAO, 2020). However, intensive aquaculture encounters several problems, such as disease outbreaks and the consequences of introducing pathogens to new hosts or new localities with the transportation of live fish (Guo and Woo, 2009). Especially in tropical regions, the disease progresses more rapidly and results in higher cumulative mortality. The tropical countries especially suffer greater losses in aquaculture due to the disease (Leung and Bates, 2013).

Parasitic infections and associated diseases are becoming more frequent with the intensification of mariculture systems in various parts of the Asia Pacific region (Seng et al., 2006). In the case of net cages, the culture facilities provide an ideal substrate for parasite eggs to entangle and the intensive aggregation of fish facilitates the transmission of parasites among hosts (Yang et al., 2007). In addition, intensive aquaculture conditions, including confinement, overpopulation and stress, enhance transmission of parasites and increase parasitic intensity in cultured fish (Roumbedakis et al., 2013).

Parasitic crustaceans are distributed worldwide in fresh, brackish and marine waters. Parasitic crustaceans showed a great diversity of forms with

marked structural modifications to suit their parasitic mode of life. There are three main groups of parasitic crustaceans affecting commercially important aquaculture species, most of which are external parasites: the Branchiura, Isopoda, and Copepoda (Jithendran et al., 2008).

The Branchiura are obligatory parasites typically found on freshwater fish, with some on marine fish. Generally, these are loosely connected to their hosts and their highly modified cephalic appendages with an advanced attachment system enable them to move around on the host and possess the ability to seek out a new host among the benthic fish, as excellent swimmers (Møller and Olesen, 2012).

Parasitic isopods are ectoparasites that are classified into three major groups: cymothoids, gnathiids and epicaridians. Cymothoids are obligate parasites in immature and adult forms (Rameshkumar and Ravichandran, 2014) are commonly found on marine and freshwater fishes comprise 40 genera and more than 380 species (Smit et al., 2014). They are blood-feeding with several species that settle in the buccal cavity, and others live in the gill chamber or on the body surface, including the fin. This parasite can cause organ and tissue damage (including blood loss) and osmoregulatory problems, serve as vector pathogens and predispose hosts to opportunistic pathogens (Rameshkumar and Ravichandran, 2014).

The family Caligidae parasites, also often referred to as sea lice, are the most commonly reported parasites responsible for disease outbreaks, accounting for approximately 61 % of all, followed by family Ergasiliidae, accounting for 15 % of all (Johnson et al., 2004). The Caligidae, also known as sea lice, is a family of parasitic copepods of fish that consists of 31 genera and 487 species mainly distributed in the genera *Caligus* (250) and *Lepeophtheirus* (121) (Morales-Serna et al., 2016). The Ergasilid copepods damage the gills and cause significant epizootics in cultured and wild populations of fishes, with a total of eight species recorded from this family (Johnson et al., 2004).

Groupers, *Epinephelus* spp. are of great commercial value and constitute an important commodity in tropical coastal fisheries. The culture of groupers is carried out in tropical and subtropical areas throughout the world but mainly produced from Asia, with three countries responsible for an estimated 92 % of global production: China (65 % of total production), Taiwan Province of China (17 %) and Indonesia (11 %) (Rimmer and Glamuzina, 2017). Several studies have been conducted on the crustacean parasite of grouper in Indonesia. The copepod (*Caligus* sp. and *Lepeophtheirus* sp.) has been found on the skin of tiger grouper (*Epinephelus fuscoguttatus* (Forsskal, 1775)), mangrove grouper (*Epinephelus coioides* (Hamilton, 1882)), humpback grouper (*Cromileptes altivelis* (Valenciennes, 1828)), camouflage grouper (*Epinephelus polyphekadion* (Bleeker, 1849)) in Bali (Slamet et al., 2008). The *Caligus epinepheli* Yamaguti, 1936 and Pennellidae were reported to infect gill filaments on duskytail grouper (*Epinephelus bleekeri* (Vaillant, 1878)) from Segara Anakan waters (Yuniar et al., 2007, Kleinertz and Palm, 2013). There has been no report on crustacean parasite infestation in the buccal cavity of groupers. The present study described the copepod parasite of Pennellidae found in the buccal cavity and gill arch of cultured groupers from the Batam, Riau Islands of Indonesia using morphological and molecular biology approaches.

Materials and Methods

The fish were collected from the sea cage aquaculture facilities at the Batam Mariculture Development Center in Batam, Riau Islands Province of Indonesia. The Batam City area consists of Batam Island and other islands in the Singapore Strait and Malacca Straits. The sea cages were situated at 0.96549° N 104.04590° E (Fig. 1). Approximately 100–500 g of tiger grouper, *E. fuscoguttatus* and hybrid grouper, *Epinephelus* sp. showing lethargy, skin darkening were collected from July to August 2019. The fish handling and study was approved with ethical clearance from the Integrated Research and Testing Laboratory Universitas Gadjah Mada No. 00015/04/LPPT/IV/2020.

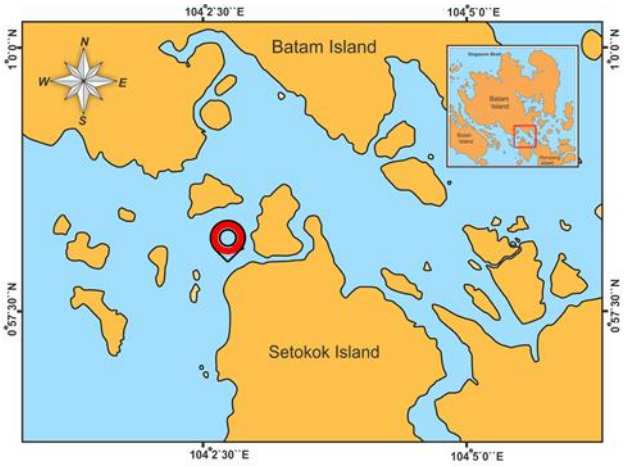

Fig. 1. The sea cage aquaculture facilities at the Batam Mariculture Development Center located between Batam Island and Sekototok Island.

The fish were anesthetised using ice water and observed for clinical signs with naked eyes. The fish were sectioned to collect tissues and parasites for examination and fixation. The parasites were observed in NaCl 0.9 % solution on a glass slide and examined using a microscope. The parasites were also fixed in 96 % ethanol (Merck, Germany) for further morphological examination and molecular analysis. The morphology of several stages of parasites was examined using Olympus Microscope Bx5 (Japan) and Lucida SZ-CTV+ camera.

The adult ethanol fixed parasites were processed for scanning electron microscopy (SEM) analysis. The specimens were processed at 4 °C for cleaning using cacodylate buffer; pre-fixation with 2.5 % of glutaraldehyde; along with fixation with 2 % of tannic acid; following washing with cacodylate buffers and finally dehydrated using a series concentration of ethanol and butanol. The last process was coating the specimens with Au using IB2 Ion Coater (Eiko, Japan). The cephalothorax, legs, and caudal ramus were observed using the JSM IT 200 scanning electron microscope (JEOL, Japan).

The distinct stages of parasites were subjected to DNA extraction following the method described before (Murwantoko et al., 2018). Around 50–100 mg of parasite was homogenized in 400 µL TNES buffer (10 mM Tris-HCl pH 8; 125 mM NaCl; 10 mM EDTA pH 8; 0.5 % SDS; 4M urea). Three µL of RNase (10 mg.mL^{-1}) was added to the mixture followed by incubation at 42 °C for one hour. After incubation, 3 µL of proteinase K (10 mg.mL^{-1}) was added into the mixture and incubated at 42 °C for 2 h. The suspension was extracted using the same volume of phenol: chloroform: isoamyl alcohol (PCIAA). Precipitation of DNA was done using 1 M NaCl and two times the volume of cold absolute ethanol and followed by washing with 70 % ethanol.

The amplification of DNA was conducted by polymerase chain reaction (PCR) using a specific primer pair to amplify the region of Cytochrome oxidase subunit-1 (*Cox1*) of mitochondrial DNA (mtDNA). The universal primers for amplification of metazoan *Cox1* are forward primer LCO-1490: 5-GGTCCAA-CAAATCATAAAGATATTGG-3 and reverse primer HCO-2198: 5-TAAACTTCAGGGTGACCAAAAAATCA-3 (Folmer et al., 1994). The PCR mixtures were composed of 2 µL of each oligonucleotide primer, 2 µL DNA template, 20 µL nuclease-free water, and 24 µL PCR mix 2× My Taq Hs Red Mix (Bioline, USA). The amplification reaction was performed using a thermal cycler TM 100 (Biorad, USA) with the following profile, one cycle of denaturation at 95 °C for 120 sec, 37 cycles of denaturation at 95 °C for 30 sec, annealing at 50 °C for 40 sec and extension at 72 °C for 120 sec, following one cycle of final extension at 72 °C for 5 min. PCR products were evaluated by gel electrophoresis on a 2 % agarose gel containing 0.03 % DNA stain (1st Base, Singapore), and compared with molecular size marker od 100 bp DNA ladder (Geneaid, Taiwan).

The direct sequencing of PCR products was performed by a commercial company using BigDye Terminator version 3.1 chemistry (Applied Biosystems, USA) and under an ABI Prism 3100 capillary Genetic Analyser (Applied Biosystems, USA). Obtained sequences were aligned to determine the consensus among those forward and reverse primer sequences. The consensus DNA sequences were analysed using basic local alignment search tool (BLAST) to look for the homology with data on the GenBank through the website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignments for phylogenic analysis of the collected data from GenBank were conducted using the MEGAX software (Kumar et al., 2018). Construction of phylogenetic trees was based on the unweighted pair group method using arithmetic average (UPGMA) and bootstrap analysis was performed with resampling 1000 times.

Results

The samples of tiger grouper and hybrid grouper from sea cages showed clinical symptoms of red nodules in the mouth cavity, lips and gill arch (Figs. 2A, B). The number and size of nodules among the fish varied, indicating the different infection levels in the fish.

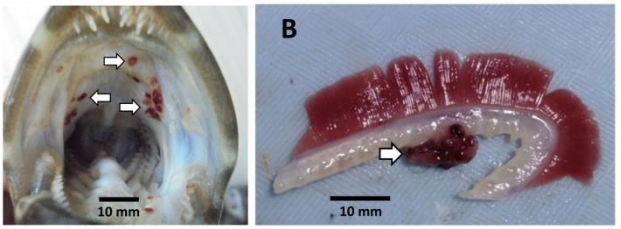

Fig. 2. The mouth cavity (A) and gill arch (B) of hybrid grouper (*Epinephelus* sp.) with the red nodules scattered in the mouth cavity and on the gill arch as indicated by the arrow.

The crustacean parasites were found inside the nodules. The parasite was characterised by paddle-like swimming legs; the body comprises a cephalosome of 6 somites and a postcephalic trunk of 9 somites plus the anal somite, which represents the telson. The cephalosome consists of 5 cephalic somites and the thoracic somite, which bears the maxillipeds. Based on Huys and Boxshall (1991), these parasites belong to subclass copepod. The subclass copepod has a life cycle from the egg, nauplius, copepodid, chalimus, and then become adult male or female (Huys, 2014). In severely infected fishes, several stages of parasites were found from a single nodule as copepodid, chalimus, and adult copepod (Fig. 3A). The copepodid stage was characterised by the presence of a frontal filament organ for attaching the parasite to the host (Fig. 3B). The copepodid develops to chalimus stage before becoming an adult (Fig. 3C).

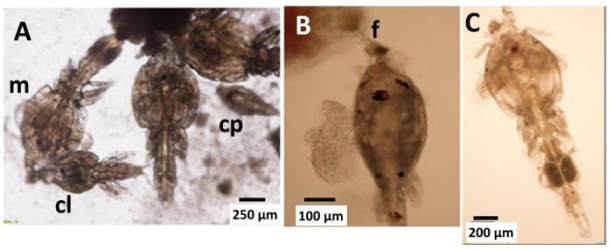

Fig. 3. Various stages of copepodid parasite found in nodules. (A) the mix stages of copepodid (cp), chalimus (cl), adult male (m); (B) copepodid stage with frontal filament (f); (C) adult male parasite.

The copepodid length measured 0.72 mm (Figs. 4a, b). The body consisted of the cephalothorax, somite, genital segment and urosom. The cephalothorax part was bigger than the posterior body part. At this stage, the parasite had frontal filament, antennae organ, and maxilliped was starting to develop. Its legs were formed and equipped with setae. Pinnate setae were also growing on caudal ramus.

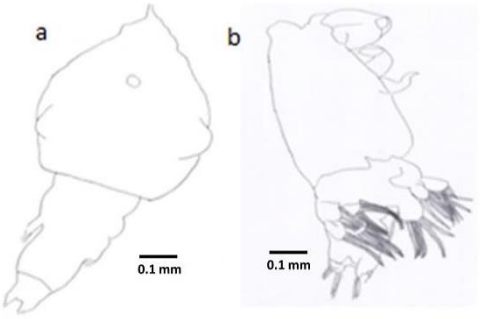

Fig. 4. Copepodid stage. (a) dorsal view showed the body parts divided into cephalothorax, somite, genital segment and urosom; (b) ventro-lateral view showed complementary organs starting to develop.

The chalimus stage was characterised by the presence of digestion system (Fig. 3). It measured 1.4 mm in length, with the length of the caudal ramus at 0.4 mm. The organ of the frontal filament had disappeared; and flattened dorsoventrally. At this stage, the body consisted of cephalothorax, including the first pedigerous somite and four posterior body segments and the antennules (Fig. 5a).

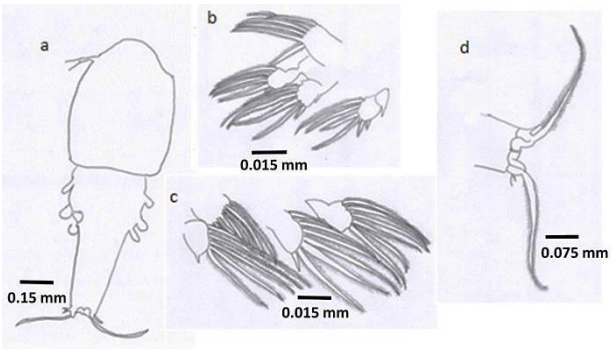

Fig. 5. Chalimus stage of the copepod parasite. a: whole body of chalimus; b: right legs; c: left legs; d: caudal ramus.

The adult copepod had nauplius eyes and an oral cone. The oral cone was located on the ventral area and formed a ring-like shape (Fig. 6c). Based on the presence of the oral cone, this parasite belonged to Siphonostomatoida order of copepods (Huys and Boxshall, 1991). It requires more than one host to complete the life cycle (Boxshall and Halsey, 2004). In this study, only male copepods were found on hybrid groupers and tiger groupers, based on the morphological characterisation by Izawa (2019).

The adult male parasite had cyclopoid shape and its length was 2.67 mm. The body consisted of cephalothorax including pediger, posterior body with pediger 2–4, genital area, and two abdominal somites. The antennules' length was 0.35 mm and equipped with several setae. The size of the maxilliped was 0.75 mm was claw-shaped with a pointy tip. The antennae's length was 0.3 mm and was hook-shaped and jagged. Caudal ramus had 4 length setae around 0.31 mm and had 4 short setae, biramous leg on the first and second pair legs, and uniramous on the third pair leg (Fig. 6). The surface structure of this parasite was observed using a SEM as seen in Figure 7. There was a pair of antennules on the ventral cephalon, a pair of antennae, a pair of nauplius eyes, an oral cone, and a pair of maxilliped (Figs. 7a, b). The maxilliped was crescent moon-like in shape (Fig. 7c). The body segment was seen on the dorsal area, with the first pediger located on the cephalothorax, and the second pediger located on somite 1 (Fig. 7d). The first leg consisted of several coxa, two bases, and setae (Fig. 7e). The first pair leg biramous on exopod basis has a spin and four setae, while on the second endopod basis and six setae (Fig. 7e).

The PCR amplification of cytochrome subunit1 of this parasite was performed using DNA genomes from the adult and chalimus stages as templates. The agarose electrophoresis of the PCR products showed the specific band with the size around 750 bp. The read nucleotide sequences of PCR products from chalimus and adult genome were 693 nt in size. The DNA sequences have been deposited in the GenBank with accession numbers MW590277 and MW590278, respectively.

The BLAST analysis of the sequenced DNA showed a low homology of 80 % with the data from the GenBank. The sequence of this parasite has a homology of 80.47 %, 80.51 %, 79.84 %, and 79.41 % with *Lernaeenicus ramosus* Kirtisinghe, 1956 (LC317014), *Acartiella sinensis* Shen & Lee, 1963 (KF977238), *Speleoithona bermudensis* Rocha C.E.F. & Illife, 1993 (MF077893), *Mesocyclops pehpeiensis* Hu, 1943 (MK159096), respectively. These results indicated no record of this related parasite species in the GenBank.

Construction of phylogenetic trees of UPGMA using cytochrom oxydase subunit 1 (*Cox1*) sequences from crustaceans parasite fishes showed that this parasite is located at the branch together with *Lernaeenicus raditus* (Lesueur, 1824), *Lernaeenicus ramosus* Kirtisinghe, 1956, *Lernaeenicus sprattae* (Sowerby, 1806), *Lernaeocera branchialis* (Linnaeus, 1767), *Peniculus fistula* von Nordmann, 1832, *Metapeniculus antofagastensis* Castro-Romero & Baeza-Kuroki, 1985, *Penella* spp. The *Caligus* and *Lepeophtheirus* were located at different branches. The *Ergasilus* and Cymothoa are located at a distance branch (Fig. 8). The outcome supports the morphological result that this parasite belongs to the Pennellidae family as the

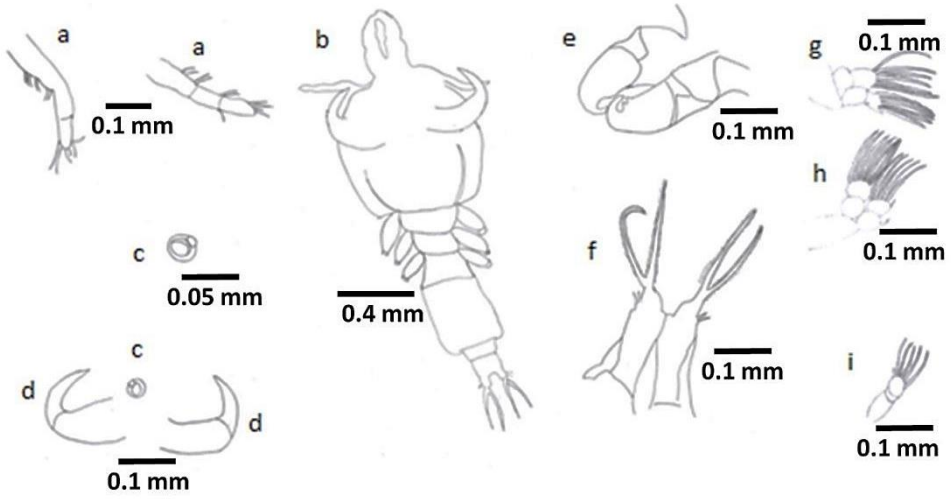

Fig. 6. Adult stage of the copepod parasite. (a) antennules; (b) whole body of adult male dorsal; (c) oral cone; (d) maxilliped; (e) antennae; (f) caudal ramus; (g) first leg; (h) second leg; (i) third leg.

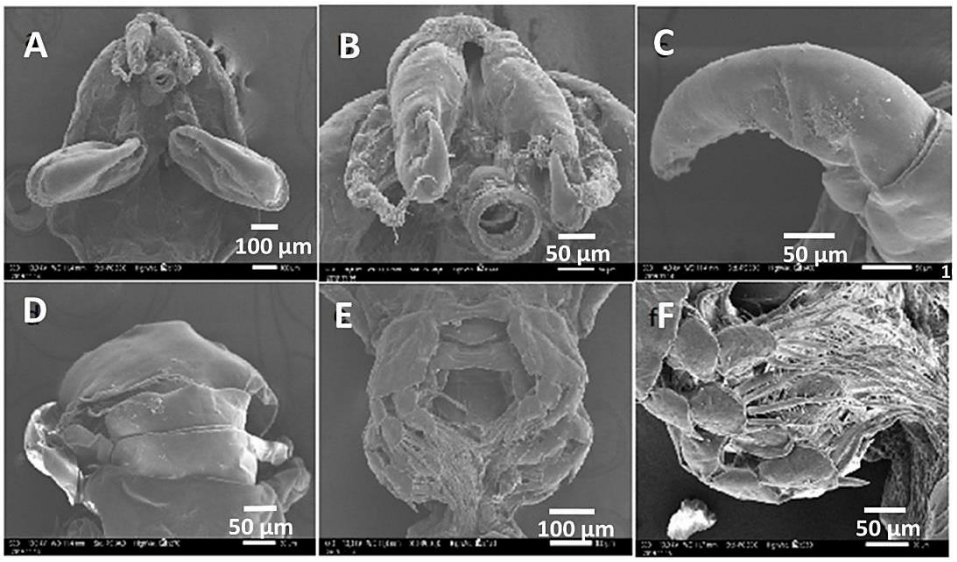

Fig. 7. Scanning electron micrograph of adult stage of the copepod parasite. (a) cephalon ventral; (b) oral cone, antenna, and antennules; (c) maxilliped; (d) somite; (e, f) first leg biramus.

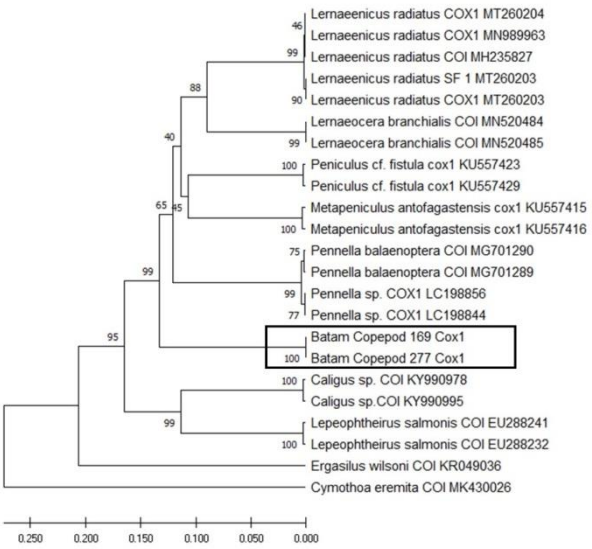

Fig. 8. Phylogenic tree of fish copepod parasites analysis using UPGMA methods with copepod from Batam indicated by box.

genera *Lernaeenicus*, *Lernaeocera*, *Peniculus*, *Metapeniculus* and *Penella* are in the family of Pennellidae.

Discussion

The morphology of the oral cone on the ventral area and formation of a ring-like shape suggested that the parasite in this study belong to Siphonostomatoida order of copepods. The morphology analysis indicates that the parasite belongs to Pennellidae family as Kabata (1979) stated that Pennellidae family has maxilliped developed into a prehensile appendage. They are characterised by attachment organs (frontal filament), that belong to the family Pennellidae (Yuniar et al., 2007). The frontal filament of pennellids appears to differ from that of caligids in the bifurcate (Brooker et al., 2012), whereas in caligids, the filament consists of a single strand (Gonzalez-Alanis et al., 2001). In pennellids, the antenna consists of two segments, whereas in caligids the endopod is fused to form a single segment (Brooker et al., 2012). The location of *Cox1* sequences of the parasite in one clade with the genera of *Lernaeenicus*, *Lernaeocera*, *Peniculus*, *Metapeniculus* and *Penella* supported that the parasite in this study belonged to Pennellidae family. The phylogenic tree of UPGMA showed this parasite made a distinct clade separate from other genera under Pennellidae family (Fig. 8).

The Pennellidae are unique among parasitic copepods of fishes in having two host life-cycles but also consist single host life-cycle (Boxshall and Halsey, 2004). Most members of the Pennellidae are mesoparasitic as the thorax and abdomen become deeply embedded in the host's tissues, with the egg sacs hanging outside (Raja et al., 2014). While attached to the final host, females exhibit gigantism due to the massive expansion in the length and girth of the genital complex. In this study, we could not find the female parasite on fish and animals surrounding the aquaculture facilities. This result indicates the possibility that this parasite has several hosts, and the final hosts for females may not be the tiger grouper nor hybrid grouper.

The morphology of female pennelleid is essential for the classification of the parasite. However, because the female parasite could not be found, it was not possible to determine the genus or species. The cephalothorax shape, somite, number of legs, caudal ramus, and maxilliped of this parasite were similar with the characteristics of *Lernaeenicus ramosus* Kirtisinghe, 1956 by Izawa (2019). However, the male adult's length around 2-3 mm, was larger than *L. ramosus* with size of only 0.81-0.84 mm excluding caudal ramus. The first legs of the two are biramous but *L. ramosus* on exopod has 5 setae and endopod 7 setae, while the parasite found on exopod has 4 setae and endopod has 6 setae. This parasite has a long shape antenna when it is stretched and it has jagged hook-like shape (Fig. 6e), which differs from the illustration of *L. ramosus's* antenna as a short one (Izawa, 2019).

Several crustacean parasites from genera of *Ergasilus*, *Caligus* and *Alella* were reported to infect the gills. The member of Ergasilidae as *Ergasilus labracis* Kroyer, 1863, *Ergasilus lobus* Lin & Ho, 1998, *Ergasilus lizae* Kroyer, 1863, *Diergasilus kasaharai* Do, 1981 infect gill lamellae of several fish species causing severe gill changes such as hyperplasia, inflammation, necrosis, high levels of mucous, and formation of vacuoles. The infection of *Alella macrotrachelus* (Brian, 1906) on black sea bream caused hyperplasia of the gill lamella. The infection of *Caligus spinosus* Yamaguti, 1939 on farmed Japanese amberjack, *Seriola quinqueradiata* Temminck & Schlegel, 1845, occurs mainly on the gill arches and rakers (Johnson et al., 2004). In the present study, the Pennellidae parasite infected the gill arches forming the red nodules.

Pennellidae parasites have been reported to infect the gills of fish. *Lernaeocera branchialis* infect the gills of several fishes and the Atlantic cod, *Gadus morhua* Linnaeus, 1758, as definitive hosts (Brooker et al., 2007). *Lernaeenicus radiatus* that has long been known with metamorphosed females infecting the muscle (Hogans, 2018), was newly identified based on its larval development to an adult and sexual reproduction in the gills of black sea bass, *Centropristis striata* (Linnaeus, 1758) (Lovy and Friend, 2020). With limited evidence, Yuniar et al. (2007) reported infection of larval pennellid on the gill filaments and gill rakers of *Epinephelus coioides* (Hamilton 1822). Based on the result, we can conclude that a pennellid parasite infecting the gills of groupers showed distinct morphology and *Cox1* sequences with *Lernaeocera branchialis* or *Lernaeenicus radiatus* (Fig. 8).

The *Cymothoa* sp. (Isopoda) was attached to the urface, in the buccal or branchial cavity of fish (Jithendran et al., 2008). Several copepods have been reported to infect the buccal cavity. *Eobrachiella elegans f. Seriolae* Yamaguti & Yamasu, 1960 was found on the walls of the oral cavity of cultured Japanese amberjack, *Seriola quinqueradiata* Temminck & Schlegel, 1845. An unidentified species of Caligidae or Pennellidae was reported on the walls of the buccal cavity of tiger puffer, *Takifugu rubripes* (Temminck & Schlegel, 1850) (Johnson et al., 2004), which was similar to the findings in the present study where the Pennellidae parasites were found in the buccal cavity of fish.

The BLAST analysis of the *Cox1* sequenced parasite showed a low homology of 80 % of data from the GenBank, indicating that this related parasite species is not recorded in the GenBank. The molecular analysis is important for taxonomy studies. The DNA barcodes have been applied as a highly effective identification system for the analysed marine

crustaceans and represent an important milestone for modern biodiversity assessment studies using barcode sequences (Raupach et al., 2015). Future studies on taxonomy, life cycle, ecology, and molecular studies are still required to present a complete understanding of the important taxon (Smit et al., 2014).

Conclusion

Batam is an important site for mariculture in Indonesia, producing several marine fishes such as groupers, *Epinephelus* sp, barramundi, *Lates calcarifer*, pompano, *Trachinotus* sp. and ornamental fishes. Since Batam is at the northern border of Indonesia, it has the advantage to export live fish to east Asian countries. The groupers are of great commercial value among the cultured marine fish and constitute an important commodity. However, grouper culture encounters several problems, such as disease outbreaks. The tiger grouper, *Epinephelus fuscoguttatus* and hybrid grouper, *Epinephelus* sp. from sea cage of Batam showing lethargy and skin darkening with varying numbers of red nodules in the mouth cavity, lips, and gill arch were infected with copepods of Pennellidae family. Even though no significant reports on fish mortality were reported, the copepod infection may result in secondary disease by bacteria or viruses. The control strategy of this parasite requires information on the life cycle and host range. The study on taxonomy and presence of this parasite on other cultured and wild marine fishes should be studied.

Conflict of interest: The authors declare that they have no conflict of interest.

References

Boxshall, G.A., Halsey, S.H. 2004. An introduction to copepod diversity. The Ray Society. London. 966 pp.

Brooker, A.J., Bron, J.E., Shinn, A.P. 2012. Description of the free-swimming juvenile stages of *Lernaeocera branchialis* (Pennellidae), using traditional light and confocal microscopy methods. Aquatic Biology 14:153–163. https://doi.org/10.3354/ab00388

Brooker, A.J., Shinn, A.P., Bron, J.E. 2007. A review of the biology of the parasitic copepod *Lernaeocera branchialis* (L., 1767) (Copepoda: Pennellidae). Advances in Parasitology 65:297–341. https://doi.org/10.1016/S0065-308X(07)65005-2

FAO. 2020. The state of world fisheries and aquaculture. FAO, Rome. 244 pp.

Folmer, O., Black, M., Hoen, W., Lutz, R., Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit-1 from diverse metozoan invertebrates. Molecular Marine Biology and Biotechnology 3:294–299.

Gonzalez-Alanis, P., Wright, G.M., Johnson, S.C., Burka, J.F. 2001. Frontal filament morphogenesis in the salmon louse *Lepeophtheirus salmonis*. Journal of Parasitology 87:561–574. https://doi.org/10.1645/0022-3395(2001)087[0561:FFMITS]2.0.CO;2

Guo, F.C., Woo, P.T.K. 2009. Selected parasitosis in cultured and wild fish. Veterinary Parasitology 163:207–216. https://doi.org/10.1016/j.vetpar.2009.06.016

Hogans, W.E. 2018. Functional morphology and structural variability in *Lernaeenicus* (Copepoda: Pennellidae) parasitic on teleost fishes from the northwest Atlantic Ocean. Comparative Parasitology 85:13–26. https://doi.org/10.1654/1525-2647-85.1.13

Huys, R. 2014. Copepoda. In: Atlas of crustacean larvae. Martin JW, Olesen, J, Hoeg, JT (Eds.), Johns Hopkins University Press, Baltimore, pp. 144–151.

Huys, R., Boxshall, G.A. 1991. Copepod evolution. The Ray Society. London. 468 pp.

Izawa, K. 2019. Redescription of *Lernaeenicus ramosus* Kirtisinghe, 1956 (Copepoda, Siphonostomatoida, Pennellidae), with description of its Male and the postnaupliar developmental stages. Crustaceana 92:119–128. https://doi.org/10.1163/15685403-00003852

Jithendran, K.P., Natarajan, M., Azad, I.S. 2008. Crustacean parasites and their management in brackishwater finfish culture. Aquaculture Asia Magazine 13(3): 47–50.

Johnson, S.C., Treasurer, J.W., Bravo, S., Nagasawa, K., Kabata, Z. 2004. A review of the impact of parasitic copepods on marine aquaculture. Zoological Studies 43: 229–243.

Kabata, Z. 1979. Parasitic Copepoda of British fishes. Ray Society, London. 468 pp.

Kleinertz, S., Palm, H.W. 2013. Parasites of the grouper fish *Epinephelus coioides* (Serranidae) as potential environmental indicators in Indonesian coastal ecosystems. Journal of Helminthology 89:86–99. https://doi.org/10.1017/s0022149x1300062x

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35:1547–1549. https://doi.org/10.1093/molbev/msy096

Leung, T.L.F., Bates, A.E. 2013. More rapid and severe disease outbreaks for aquaculture at the tropics: implications for food security. Journal of Applied Ecology 50:215–222. https://doi.org/10.1111/1365-2644.12017

Lovy, J., Friend, S.E. 2020. Black sea bass are a host in the developmental cycle of *Lernaeenicus radiatus* (Copepoda: Pennellidae): Insights into parasite morphology, gill pathology and genetics. Parasitology 147:478–490. https://doi.org/10.1017/S0031182019001781

Møller, O.S., Olesen, J. 2012. First description of larval stage 1 from a non-African fish parasite *Dolops* (Branchiura). Journal of Crustacean Biology 32:231–238 https://doi.org/10.1163/193724011X615541

Morales-Serna, F.N., Rosa Maria Medina-Guerrero, R.M., Fajer-Avila, E.J. 2016. Sea lice (Copepoda: Caligidae) parasitic on fishes reported from the Neotropical region. Neotropical Biodiversity 2:141–150. https://doi.org/10.1080/23766808.2016.1236313

Murwantoko, Negoro, S.L.C., Isnansetyo, A., Zafran. 2018. Identification of marine leech and assessment of its prevalence and intensity on cultured hybrid groupers (*Epinephelus* sp.). Biodiversitas 19:1798–1804. https://doi.org/10.13057/biodiv/d190529

Raja, K., Maran, B.A.V., Gopalakrishnan, A., Saravanakumar, A., Vijayakumar, R., Sinduja, K. 2014. Infestation of *Lernaeenicus seeri* (Copepoda: Pennellidae) and *Hirudinella ventricosa* (Digenea: Hirudinellidae) on Wahoo *Acanthocybium solandri* collected from Parangipettai, southeast coast of India. Tropical Biomedicine 31:477–486.

Rameshkumar, G., Ravichandran, S. 2014. Problems caused by isopod parasites in commercial fishes. Journal of Parasitic Disease 38:138–141. https://doi.org/10.1007/s12639-012-0210-4

Raupach M.J., Barco A., Steinke D., Beermann J., Laakmann S., Mohrbeck I., et al., 2015. The application of DNA barcodes for the identification of marine crustaceans from the North Sea and

adjacent regions. PLoS ONE 10:e0139421. https://doi.org/10.1371/journal.pone.0139421

Rimmer, M.A., Glamuzina, B. 2017. A review of grouper (Family Serranidae: Subfamily Epinephelinae) aquaculture from a sustainability science perspective. Reviews in Aquaculture 11:58–87. https://doi.org/10.1111/raq.12226

Roumbedakis, K., Marchiori, N.C., Paseto, A., Gonçalves, E.L.T., Luque, J.L., Cepeda, P.B., Sanches, E.G., Martins, M.L. 2013. Parasite fauna of wild and cultured dusky-grouper *Epinephelus marginatus* (Lowe, 1834) from Ubatuba, Southeastern Brazil. Brazilian Journal of Biology 73:871–878. https://doi.org/10.1590/S1519-69842013000400025

Seng, L.T., Tan. Z, Enright, W.J. 2006. Important parasitic diseases in cultured marine fish in the Asia-Pacific region. Aquaculture Asia Pacific 2(1):14–16.

Slamet, B., Agus, T., Setiadharma, T., Giri, N.A., Suwirya, K. 2008. Inventarisasi dan pengendalian penyakit parasit pada induk ikan laut di bak pemeliharaan. Jurnal Perikanan 10:276–281. https://doi.org/10.22146/jfs.8906 (in Indonesian).

Smit, N.J., Bruce N.L., Hadfield K.A. 2014. Global diversity of fish parasitic isopod crustaceans of the family Cymothoidae. International Journal for Parasitology Parasites and Wildlife 3:188–197. https://doi.org/10.1016/j.ijppaw.2014.03.004

Yang, T.B., Chen, A.P., Chen, W., Li, A.X., Yan, Y.Y. 2007. Parasitic diseases of cultured marine finfishes and their surveillance in China. Parassitologia 49:193–199.

Yuniar, A.T., Palm, H.W., Walter, T. 2007. Crustacean fish parasites from Segara Anakan Lagoon, Java, Indonesia. Parasitology Research 100:1193–1204. https://doi.org/10.1007/s00436-006-0391-9