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Research Article

Record on Nematode *Tanqua tiara* Infection on Snakehead Fish *Channa striata* in South Kalimantan Indonesia

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Abstract

Snakehead fish (*Channa striata*) is an important commodity in South Kalimantan Indonesia. The snakehead fish production was increased due to the capture and intensive culture. The disease is one of the obstacles for production that may happened in cultured- and wild-fishes. The aims of this study were to record and to identify parasite which infected on wild snakehead fish from Kandangan Lama, Panyipatan, Tanahlaut, South Kalimantan. The parasite identification was conducted based on the morphology and the molecular characters. The morphology was observed by light microscope and scanning electron microscope. The 18S rRNA of parasite was amplified using designed primers and followed by sequencing. Spherical cysts were found in abdomen cavity and flesh of snakehead fish. The cylindrical worm with needle shape on both tip end with approximately 1 mm length were moving inside of wall cysts. Alignment analysis of 18S rRNA showed the highest homology at 99.83% with *Tanqua tiara*. Phylogenetic tree showed that this worm is located at distance clade with the nematodes that have been reported to infect snakehead fish. The morphology and molecular results verified that and first report the parasite found in snakehead fish in South Kalimantan was *T. tiara* species. This nematode parasite may be served as intermediate host.

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1. Introduction

The snakeheads are members of the freshwater fish family Channidae, elongated, long dorsal fins predatory fish with large mouths, and shiny teeth. These fishes include the species of *Channa argus*, *C. striata*, *C. maculata*, *C. barca*, *Parachanna obscura* (Froese and Pauly, 2022). Snakehead fish (*Channa striata*) or *ikan gabus* or *haruan* in local language is a type of fish that has high economic value and distributed over Sumatra, Java, Kalimantan, Sulawesi, and Papua islands. This species has a distinctive taste, thick and white flesh texture, so the price is quite expensive both in fresh and dried form (salted fish) (Listyanto and Andrianto, 2009). Snakehead fish has the highest price among all freshwater fishes in South Kalimantan and place at the second position as a factor affecting on the inflation rate in South Kalimantan (Huda, 2020). The snakehead fish culture program has been carried out to increase production. The production of snakehead fish in 2012 in the Kalimantan region from pond aquaculture was 420 tons and cage cultivation was 5.895 tons, while capture fisheries production was 18.269 tons (TrobosAqua, 2015). The snakehead fish is further cultured in intensive system and the snakehead fish culture production in 2020 reached 10.988 tons (Huda, 2021).

Intensive aquaculture encounters several problems such as disease outbreaks and 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. Tropical countries especially suffer greater loss in aquaculture due to disease (Leung and Bates, 2013). Disease control in aquaculture also needs to pay attention to diseases infecting wild fish in open waters. Wild fishes may serve as hosts for parasites before infecting commercial species. Egusa and Nakajima (1980) hypothesized that the pomacentrids acted as host of infection for *Kudoa mamiensis* in the carangid. Langdon *et al.* (1992) proposed that clupeoid fish were host of *K. thyrsites* infections found in mahi mahi, *Coryphaena hippurus* L. species. *K. amamiensis* has wide range host on teleost fishes in Australia (Burger *et al.*, 2008).

Crustacean parasite, *Argulus indicus* has been reported to infect striped snakehead (*Channa striata*) in Towuti Lake, South Sulawesi Indonesia with prevalence ranging from 73.3 to 96.7%, mean intensity from 2.18

to 14.43 parasites/fish, while mean abundance ranged from 1.67 to 13.47 parasites/fish (Amriana *et al.*, 2022). *Argulus bengalis* has been reported infecting snakehead *Channa punctatus* from Lalabazar and Ratargul swamp forest of Sylhet in Bangladesh (Das *et al.*, 2018). The anchor worm *Leerne* sp has been identified in *Channa punctatus* at Sylhet Bangladesh (Miah *et al.*, 2013).

Trichodina is the most frequently encountered external parasite in cultured freshwater fishes worldwide. The scraping and movement of these organisms irritate the skin and gill surfaces causing hyperplasia of the epithelium and facilitating secondary infection. Two trichodinid have been found in gills of snakehead fish *Channa punctatus* from the wild and cultured in Sylhet Bangladesh (Miah *et al.*, 2013; Deb *et al.*, 2015). *Trichodina* also has been reported from snakehead fish *C. striata* in South Kalimantan (Novita *et al.*, 2020), in Aceh Besar (Umara *et al.*, 2014) Indonesia. Infection of *Epistylis* sp has been reported in snakehead fish (*Channa striata*) in South Kalimantan Sugiartanti *et al.*, (2020), in Aceh besar (Zaiyana *et al.*, 2022) Indonesia. Other protozoan parasites have been reported to infect snakehead fish such as *Chilodonella* sp *Ichthyobodo* sp. *Actinophrys* sp. (Miah *et al.*, 2013), *Oodinium* sp (Novita *et al.*, 2020) *Tetrahymena* sp. (Zaiyana *et al.*, 2022).

Monogenean *Gyrodactylus* sp. was found from the body surface of snake head fishes *Channa punctatus* in Bangladesh (Miah *et al.*, 2013). This parasite also has been reported to infect snakehead fish (*Channa striata*) in South Kalimantan (Sugiartanti *et al.*, 2020; Novita *et al.*, 2020), in Aceh (Zaiyana *et al.*, 2022) Indonesia. The gill flukes *Dactylogyrus* sp. was found from the gills of the snakehead fish *C. punctatus* in Bangladesh (Miah *et al.*, 2013), in snakehead fish (*Channa striata*) in Aceh (Zaiyana *et al.*, 2022) Indonesia.

The gastro-intestinal helminth parasites have been studied from various fishes for many purposes including identification of threat of zoonotic transmission to consumers' issues. The cestode *Senga* sp. has been found in *Channa orientalis*, while cestode *Bothriocephalus* sp. was found in *C. striata* in Nepal (Shrestha *et al.*, 2019). Cestode *Senga ophioccephalina* was reported on *Channa punctatus* in Bangladesh (Das *et al.*, 2018). Cestode (*Senga* sp.) was also found in *Channa punctatus* in Dhaka Bangladesh (Sultana and Salam, 2015) and also in *Channa punctatus*, and *Channa striatus* in Uttar Pradesh, India (Gautam *et al.*, 2018).

The Ecanthocephalan *Pallissentis* sp was found in the digestive tract of snakehead fish (*C. striata*) from Indra Makmur, East Aceh (Tanjung *et al.*, 2019), in *C. Striata* and *C. punctatus* from Uttar Pradesh, India (Gautam *et al.*, 2018), in *C. striatus* from Nepal (Shrestha *et al.*, 2019) in *C. punctatus* from Bangladesh (Sultana and Salam, 2015). The parasitic acanthocephalan *Leptorhynchoides* sp. has been reported to infest the intestine of *C. striatus* from Nepal (Shrestha *et al.*, 2019). *Neoechinorhynchus tylosuri* has been found in *C. punctatus* from Bangladesh (Sultana and Salam, 2015).

A disease case has been reported in Kandangan Lama Village, Panyipatan District, Tanahlaut Regency, South Kalimantan. Residents found worms in the flesh of snakehead fish (Wahid 2018). As far as authors understanding, this is the first report on the infection of *Tanqua tiara* on snakehead fish. The objective of this study is to identify parasite from the wild snakehead fish *C. striata* in Kandangan Lama based on the morphology and nucleotide sequences of 18S rRNA.

2. Material and Methods

2.1 Sampling

Snakehead fish (*C. striata*) at 600 – 1300 g of weight were obtained from the fisherman in Kandangan Lama Village located at S 4.04767, E 114.68412. The fish was necropsied and the presence of parasite was observed in abdomen cavity and flesh. The parasites were collected for morphological observation.

2.2 Morphological Observation

The parasites were placed in water on a glass slide and observed using a microscope. The parasites were also fixed in 70 % ethanol for further morphological examination and for molecular analysis in laboratory. The scanning electron microscopy (SEM) analysis was performed from ethanol fixed parasites. The parasite specimens were processed for cleaning at 4°C with cacodylate buffer; pre-fixation with 2.5 % of glutaraldehyde; then fixation with 2 % of tannic acid; following washing with cacodylate buffers and finally dehydrated using a series concentration of ethanol and butanol. The specimens were coated with Au using IB2 Ion Coater (Eiko, Japan). The parasite morphology was observed using the JSM IT 200 scanning electrons microscope (JEOL, Japan).

2.3 DNA Extraction

The ethanol fixed parasites were subjected to DNA extraction following method as described before (Murwantoko *et al.*, 2018). Approximately 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 and incubated for one hour at 42°C. After incubation, three µL of proteinase K (10 mg.ml⁻¹) was added into the mixture and incubated at 42°C for 2 hours. 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.

2.4 DNA Amplification

The universal primer was designed to amplify 18S SSU rRNA from various taxa of fish parasites. Multiple Alignment was performed from nucleotide sequences of 18S rRNA SSU ribosome of several parasites which were obtained from GenBank at NCBI. The primers were designed to recognize the conserved region of sequences among those parasites. The designed primers are Univ_Prst_F: 5 ‘ACGGCTACCACATCCAAGGAA 3’ as forward primer, and Univ_Prst_R1: 5 ‘TGTACAAAGGGCAGGGACGTA3’ as reverse primer.

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 2x My Taq Hs Red Mix (Bioline, US). The DNA amplification was performed using a thermal cycler TM 100 (Biorad, US) 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 % Floresafe DNA stain (1st Base, Singapore), and compared with molecular size marker of 100 bp DNA ladder (Geneaid, Taiwan).

2.5 Sequencing and Analysis

The direct sequencing of PCR products was performed by a commercial company using BigDye Terminator version 3.1 chemistry (Applied Biosystems, US) and under an ABI Prism 3100 capillary Genetic

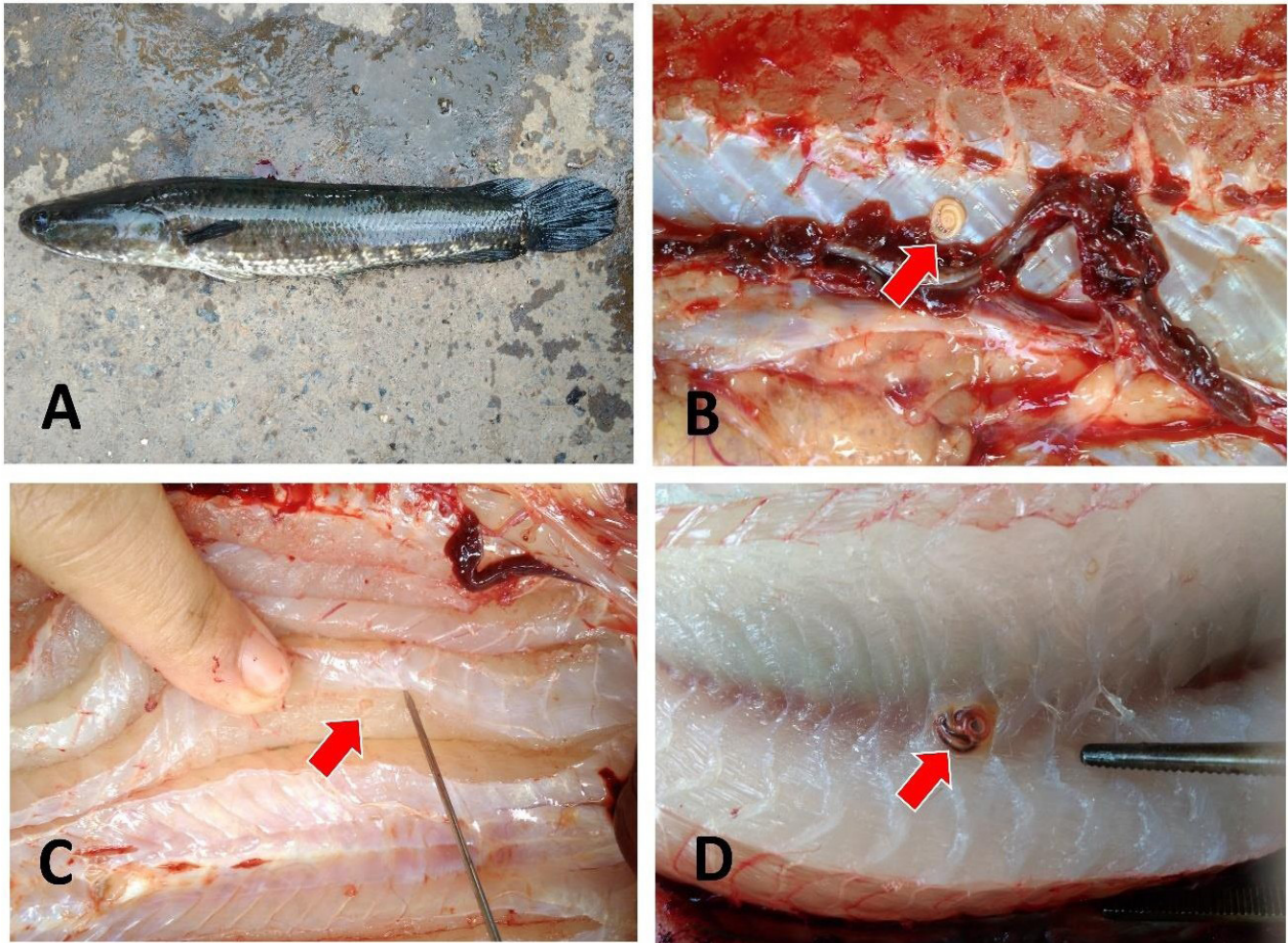


Figure 1. The snakehead fishes (*Channa striata*) from Kandangan lama showed normal color and shape (A). A spherical cyst with coiled structure inside was found in abdomen cavity (B). Fish flesh was translucent white in color and spherical white cysts with approximately 2 mm in diameter was found, as indicated by arrow (C). A larger red dark cyst was also found in the flesh as indicated by arrow (D).

Analyzer (Applied Biosystems, US). Obtained sequences were aligned to determine the consensus among those reverse and forward primer sequences. The consensus DNA sequences were analyzed using basic local alignment search tool (BLAST) to determine the homology with data on the GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Multiple alignments for phylogenetic analysis of the collected data from GenBank was conducted using the MEGAX software (Kumar *et al.*, 2018). Phylogenetic tree was constructed on the neighbor-joining method Saitou and Nei (1987).

3. Results and Discussion

The snakehead fishes (*Channa striata*) which have been caught by fisherman showed normality both in behavior, color and shape (Figure 1A). The internal organ such as gill, liver, stomach, intestine, heart, and spleen

were in normal condition. Spherical cyst with coiled structure was found in the abdominal cavity of fish (Figure 1B). Fish flesh was translucent white in color. Incision on flesh showed several spherical white cysts with approximately 2 mm in diameter (Figure 1C). A larger red dark cyst was also found in the flesh (Figure 1D).

The cylindrical worm was found moving inside of wall of cysts. When the cyst wall was ruptured, the whitish cylindrical worm with needle shape on both end shows (Figure 2A). The length of this worm was approximately 1 mm in length (Figure 2B). The larger worm with color of dark red has size more than 5 mm in length (Figure 2C).

Microscopic observation showed that the worm was cylindrical in shape with round anterior part as

a mouth (Figure 3A). From the mouth, an esophagus was present and occupy around twenty percent of total length. The clear border between esophagus and intestine was observed (Figure 3B). This nematode was characterized by homogeneous ornamentation of the cuticle with annules, with mouth in anterior (Figure 4A), and a point end of tail at posterior end (Figure 4B). The shape and body structure indicated that this animal belonged to nematode (Bogale *et al.*, 2020).

In this study primer pair were designed to amplify 18S rRNA of mtDNA for fish parasites. The specific band at approximately 1300 bp was appeared after amplification with Univ_Prst_F / Univ_Prst_R primers using this nematode genome as a template. This PCR product was used for DNA sequencing and 1161 nucleotides could be read. This DNA sequences have been stored in the Genbank with accession number ON254915.

The BLAST analysis of the sequenced DNA showed the highest homology at 99.83% with *Tanqua tiara* (accession JF934728.1), followed by *Tanqua* sp (OL830843.1) at 98.62%. The homology with other species were less than 97% i.e with *Linstowinema* sp (JF934727.1) at 96.99%, *Spiroxys japonica* (AB818381.1) at 95.66%, *Spiroxys hanzaki* (AB818383.1) at 95.35%, and at less than 95% for *Anguillicola crassus* (DQ118535.1), *Hysterothylacium* sp (MF072698.1), *Baylisascaris ailuri* (JN256991.1), *Toxocara vitulorum* (EF180078.1) (Table 1). Only one nucleotide was different between this nematode from South Kalimantan with the *Tanqua tiara* (accession JF934728.1) from Northern Territory Australia. This result indicated that the parasite found in snakehead fish in South Kalimantan was *Tanqua tiara* species. Phylogenic analysis showed that the nematodes were distributed into four clades and the *Tanqua tiara* was placed at same clade with *Linstowinema* sp (Figure 5).

3.1 Discussion

Snakehead fish (*Channa striata*) is an important fish commodity in South Kalimantan and can cause inflation when the supply is not enough. The production of snakehead fish in Kalimantan in 2012 from pond and cage cultivation was 6,310 tons (TrobosAqua, 2015) and in 2020 could reach 10,988 tons (Huda, 2021). The increasing production indicated the intensification

of snakehead fish culture that have been conducted. However, intensive aquaculture encounters several problems such as environmental issues, disease (Guo and Woo, 2009). Mitigation of the diseases is important on the controlling disease program. The fish diseases have occurred in wild fishes or cultured fish. In this study we investigated the parasite disease contracted by the wild snakehead fish from Kandangan Lama Village, Panyipatan District, Tanahlaut Regency, South Kalimantan.

Several parasites have been reported to infect snakehead fishes. Infection on snakehead fishes by crustacean parasite *Argulus* spp has been reported in Bangladesh (Das *et al.*, 2018), in Indonesia (Amriana *et al.*, 2022), and crustacean anchor worm *Lernea* sp have been reported in Bangladesh (Miah *et al.*, 2013). The protozoan *Trichodina* has been reported to be found in snakehead fish *C. striata* (Umara *et al.*, 2014; Novita *et al.*, 2020) and *Channa punctatus* (Miah *et al.*, 2013; Deb *et al.*, 2015). Monogenean *Gyrodactylus* sp. was found on the body surface of snakehead fishes (Miah *et al.*, 2013; Sugiartanti *et al.*, 2020; Novita *et al.*, 2020; Zaiyana *et al.*, 2022). The gill flukes *Dactylogyrus* sp. was found on the gills of the snakehead fish (Miah *et al.*, 2013; Zaiyana *et al.*, 2022).

The gastro-intestinal helminth parasites have been studied from fishes for many purposes including the issue of threat of zoonotic transmission to consumers. The cestode *Senga* sp. has been found in *Channa orientalis*, while cestode *Bothriocephalus* sp. was found in *C. striata* in Nepal (Shrestha *et al.*, 2019). Cestode *Senga ophiocephalina* was reported on *Channa punctatus* in Bangladesh (Das *et al.*, 2018). Cestode (*Senga* sp.) was also found in *Channa punctatus* in Dhaka Bangladesh (Sultana and Salam, 2015), in *Channa punctatus*, and *Channa striatus* in India (Gautam *et al.*, 2018). The acanthocephalan *Pallissentis* sp was found in the digestive tract of snakehead fish (*C. striata*) from Indonesia (Tanjung *et al.*, 2019), in *C. striata* and *C. punctatus* from India (Gautam *et al.*, 2018), in *C. striatus* from Nepal (Shrestha *et al.*, 2019), and in *C. punctatus* from Bangladesh (Sultana and Salam, 2015). The parasitic acanthocephalan *Leptorhynchoides* sp. has been reported to be found in the intestine of *C. striata* from Nepal (Shrestha *et al.*, 2019). *Neoechinorhynchus tylosuri* has been found in *C. punctatus* from Bangladesh (Sultana and Salam, 2015).



Figure 2. Small cysts and nematodes in slide with a large nematode in right (A). The nematodes was approximately 10 mm in length (B), and the larger nematode with color of dark red was more than 50 mm in length (C).

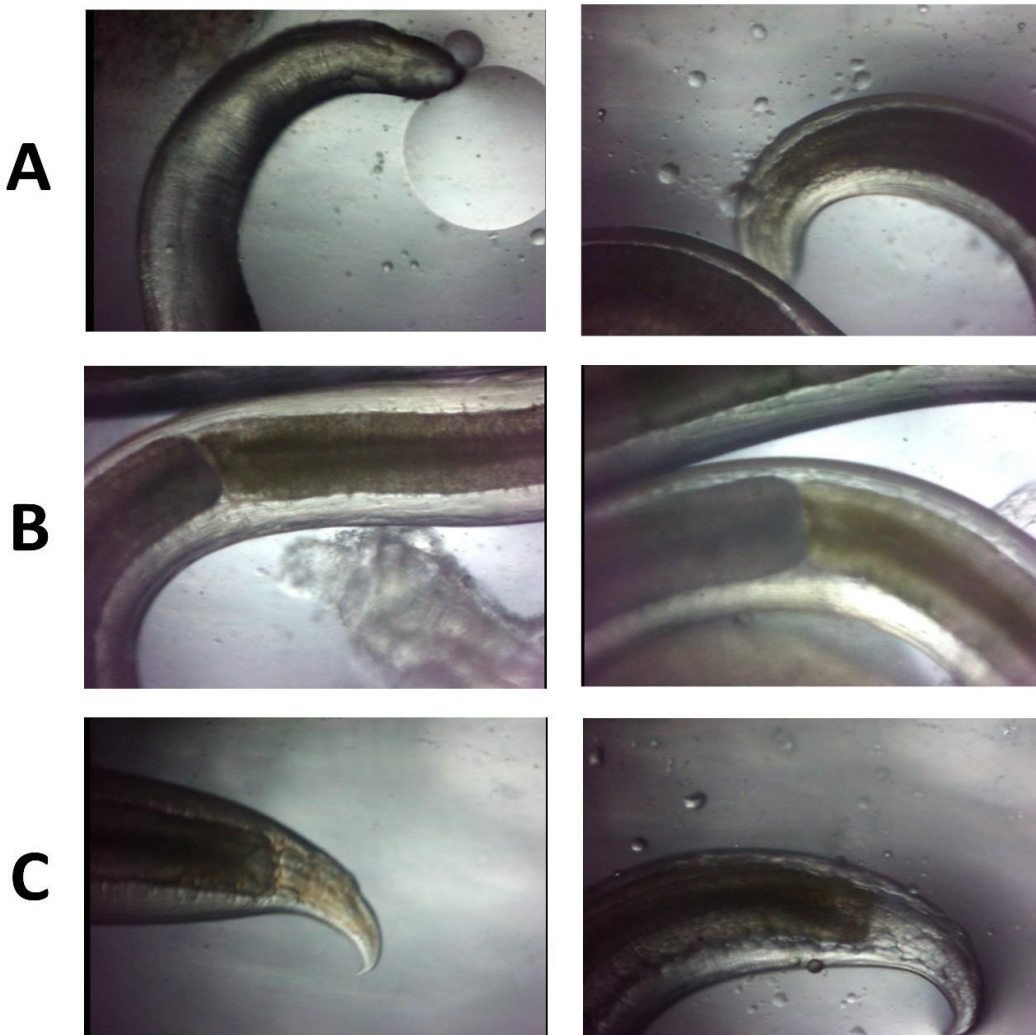


Figure 3. Light assisted microscopic observation of the nematodes. Nematode has cylindrical shape, with round anterior part as a mouth (A). The clear border between esophagus and intestine was observed (B). Posterior end showed a sharp end (C)

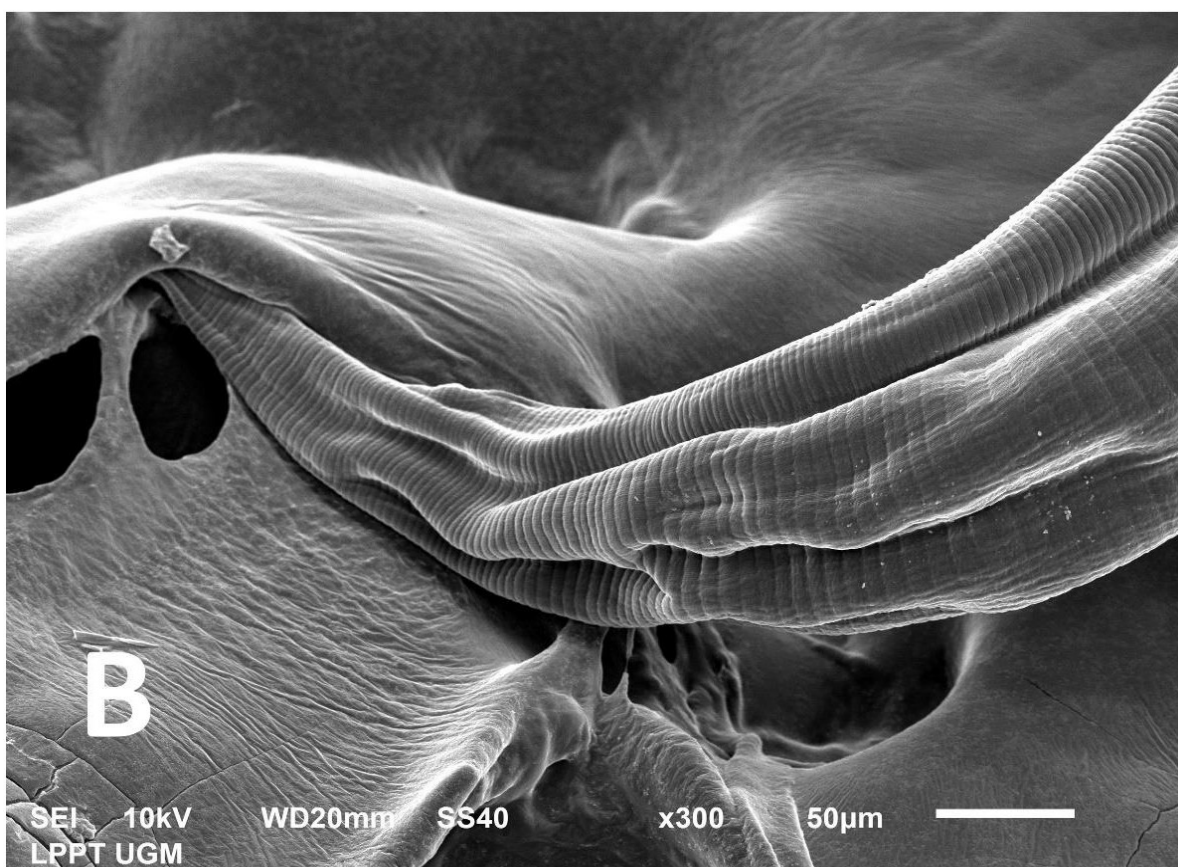
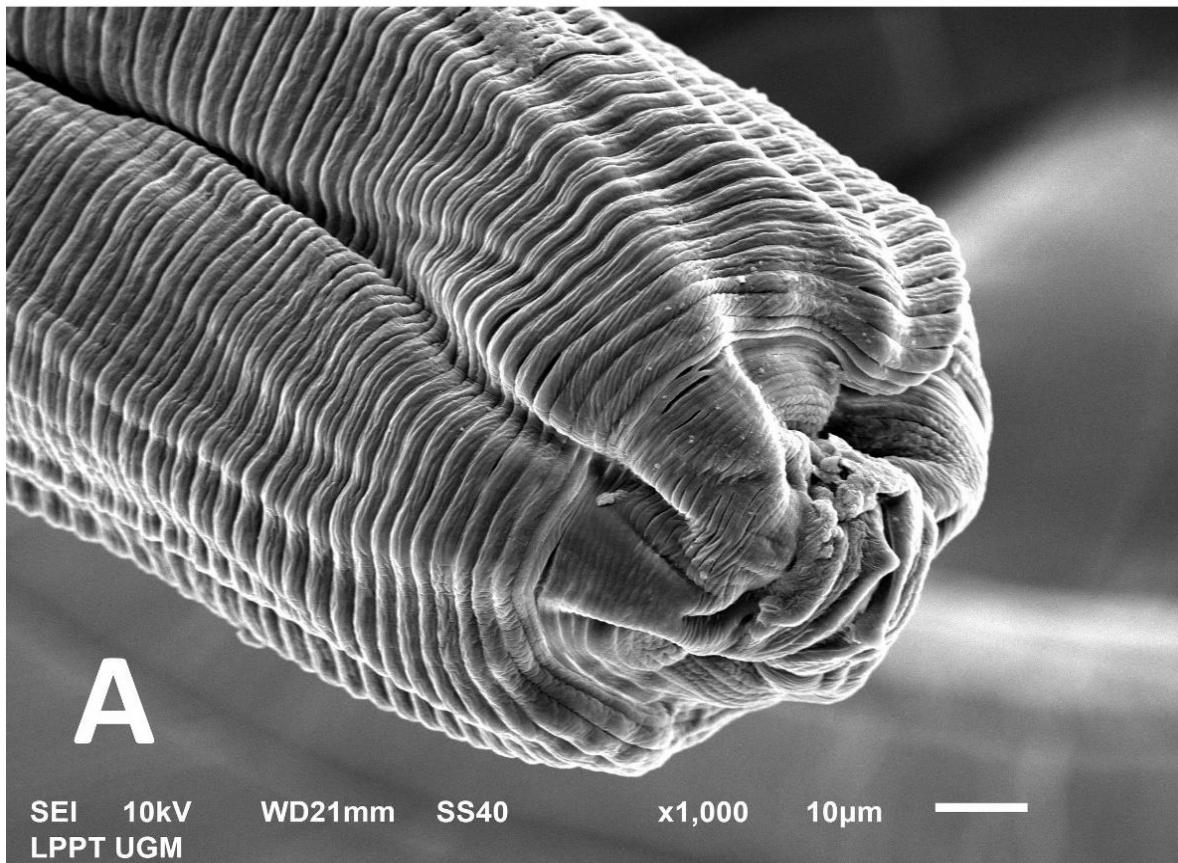


Figure 4. Scanning electron microscope observation of the nematodes showing the homogeneous ornamentation of the cuticle with annules, with mouth in anterior (A), and a point end of tail at posterior end (B)

Table 1. Alignment analysis on nucleotide of 18S rRNA from the nematode from Kandangan Lama with the data on the GenBank with indicated accession numbers

Accession	Description	Scientific name	Homology	Reference
JF934728.1	<i>Tanqua tiara</i> 18S ribosomal RNA gene, partial sequence	<i>Tanqua tiara</i>	99.83%	Laetsch <i>et al.</i> (2012)
OL830843.1	<i>Tanqua</i> sp. MAW-2021 isolate 678-12 small subunit ribosomal RNA gene, partial sequence	<i>Tanqua</i> sp	98.62%	Unpublished
JF934727.1	<i>Linstowinema</i> sp. SAN-2011 18S ribosomal RNA gene, partial sequence	<i>Linstowinema</i> sp.	96.99%	Laetsch <i>et al.</i> (2012)
AB818381.1	<i>Spiroxys japonica</i> gene for 18S ribosomal RNA, partial sequence, isolate: Kanto6	<i>Spiroxys japonica</i>	95.66%	Hasegawa <i>et al.</i> (2013)
AB818383.1	<i>Spiroxys hanzaki</i> gene for 18S ribosomal RNA, partial sequence, isolate: Hyogo1	<i>Spiroxys hanzaki</i>	95.35%	Hasegawa <i>et al.</i> (2013)
DQ118535.1	<i>Anguillicola crassus</i> small subunit ribosomal RNA gene, partial sequence	<i>Anguillicola crassus</i>	94.49%	Unpublished
MF072698.1	<i>Hysterothylacium</i> sp. Aa1 18S ribosomal RNA gene, partial sequence	<i>Hysterothylacium</i> sp.	94.23%	Li <i>et al.</i> (2018)
JN256991.1	<i>Baylisascaris ailuri</i> isolate Afl 18S ribosomal RNA gene, partial sequence	<i>Baylisascaris ailuri</i>	94.23%	Li <i>et al.</i> (2012)
EF180078.1	<i>Toxocara vitulorum</i> 18S small subunit ribosomal RNA gene, partial sequence	<i>Toxocara vitulorum</i>	94.23%	Nadler <i>et al.</i> (2007)

The nematode parasites have been studied from several snakehead fishes in Asia and Africa. The *Procamallanus* sp., *Camallanus* sp. were identified in the intestine of *Channa punctatus* (Miah *et al.*, 2013; Gautam *et al.*, 2018; Das *et al.*, 2018), in *C. Striata* (Gautam *et al.*, 2018; Tanjung *et al.*, 2019). The nematodes *Neocamallanus* sp. and *Paracamallanus cyathopharynx* (Esther *et al.*, 2015), *Procamallanus* sp and *Contracaecum* sp (Adegbehingbe and Umezurike, 2018) was found in African snakehead fish *Parachanna obscura*. In this study we record a nematode from *C. Striata* in South Kalimantan. In the previous studies, the nematode was identified from the intestine, but in this study the nematode was identified from muscle.

Tanqua tiara is a long-shaped nematode and can be distinguished between the male and female. Investigation by Agustin *et al.* (2017), *T. tiara* which infected *Varanus salvator* from East Java showed the male animal has total length of 9.4-32.0 mm with body width of 0.26-1.77 mm, while the female animal has total length of 6.8-22.0 mm with body width of 0.14-2.33 mm. Male *T. tiara* which infected *V. salvator* and *V. magna* was reported to have total length of 13.8 – 28.6 mm with body width of 0.39-0.77 mm, while the female has total length of 13.0-25.7 mm with body width of 0.26-0.42 mm (Purwaningsih *et al.*, 2020). Sou (2020) summarized that the male has total length of 13.50–39.00 mm with body width of 0.19–1.10 mm, while the

female has total length of 20.00–45.46 mm with body width of 0.52–1.51 mm. In this study, the length of most worms were approximately 10 mm (Figure 2A). This indicated that the length is in the range of *T. tiara* species. This study also found a worm with larger size, with color of dark red and more than 50 mm in length (Figure 2C). This larger worm may be the female worm.

Nematodes are one of among the most diverse but least studied organisms. Morphological approach has proved insufficient to the study of nematode identification and diversity. DNA and protein-based methods have been developed to supplement or circumvent the limitations associated with morphology-based classification. Many forms of DNA-based methods have been employed for the identification of nematodes which can be broadly categorized into nucleotide sequence- and fingerprint-based methods. Sequence-based methods may involve analyses of nucleotide sequence information from specific segment(s) of the mitochondrial DNA (mtDNA), nuclear DNA, or the whole genome. The rRNA and mitochondrial cytochrome oxidase sub unit I (COX1) genes of MtDNA are preferred by most studies (Bogale *et al.*, 2020). In this study we employed the study based on the nucleotide sequence method 18S rRNA as a target. Bogale *et al.* (2020) proposed to use the 18s rRNA for study of nematodes. Floyd *et al.* (2002) used sequence information from the 18S (small subunit; SSU) to group soil nematodes into molecular operational taxonomic units (MOTUs). The universal primers were designed to amplify 18s rRNA region of various fish parasites. The primers pair which was designed in this study can amplify 18s rRNA region from this parasite from snakehead fish as indicated by the presence of specific band at approximately 1300 bp.

The PCR product of 18S rRNA was used for DNA sequencing and the 1161 nucleotides could be read. The BLAST analysis of the sequenced DNA showed the highest homology at 99.83% with the *Tanqua tiara* (accession JF934728.1) and with *Tanqua sp* (OL830843.1) at 98.62% and the homology with other species were less than 97% (Table 1). The homology of the nematode in this study with other nematodes which had been reported to infect the snakehead fish (*Camallanus*, *Procamallanus* and *Neocamallanus*) were so low it did not appear in BLAST analysis. Phylogenetic tree showed that *Camallanus* and *Procamallanus* were located on different and distant clade (Figure 5). These results of homology analysis and phylogenetic tree confirmed that the nematode in this study was *Tanqua tiara* (v. Linstow, 1879) Blanchard, 1904 (Nematoda: Gnathostomatoidea).

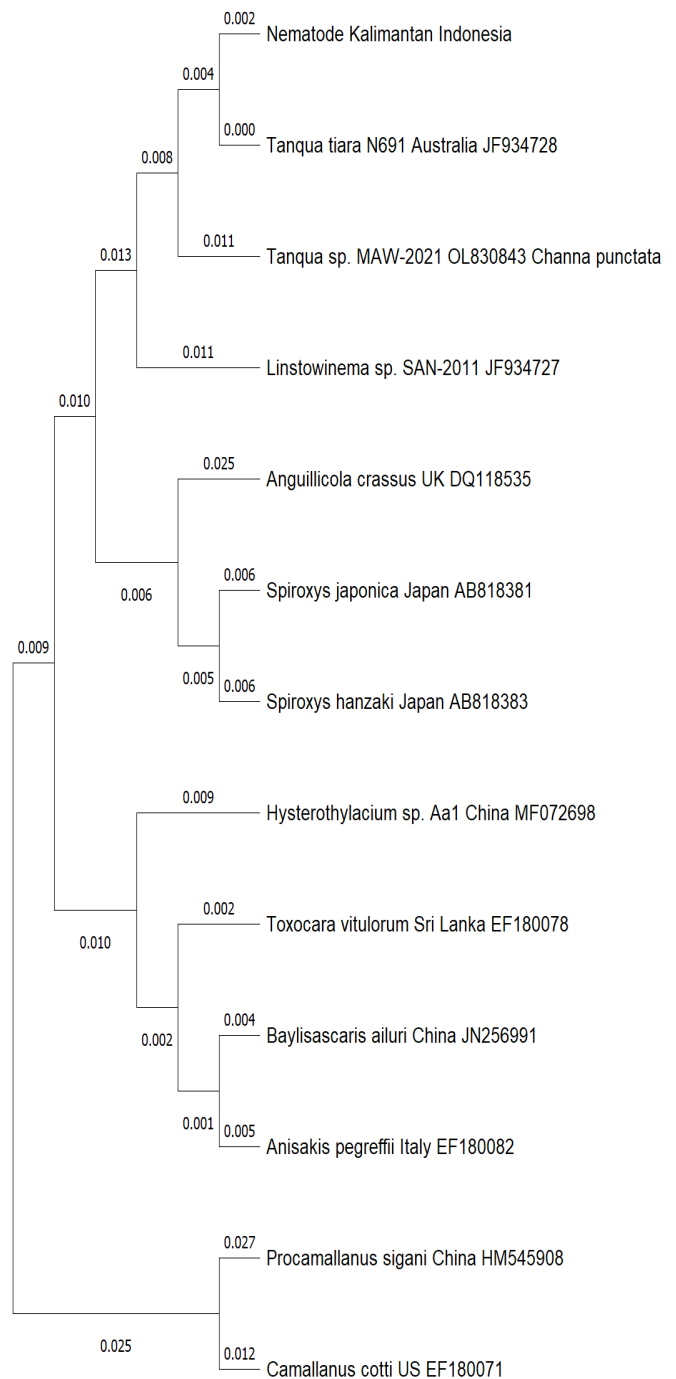


Figure 5. Phylogenetic tree based on nucleotide sequences of 18S rRNA from the nematode from Kandangan Lama with the data on the Genbank with indicated accession number was constructed on the neighbor-joining method

T. tiara has been reported to infect the Asian water monitor *Varanus salvator* (Agustin *et al.*, 2017), *V. magna* (Purwaningsih *et al.*, 2020), Yellow-spotted Monitor (*Varanus panoptes*) (Barton and Jones, 2018), and Nile monitor lizard (*Varanus niloticus*) (Gibbons and Keymer, 2005). *T. tiara* also has been found as a parasite

in non-venomous snakes *Lycodon laoensis* in Thailand (Chaiyabutr and Chanhome, 2002). In this study we proved data of the new host of *T. tiara* as the snakehead fish (*Channa striata*). *T. tiara* is found in the stomach of the monitors (Agustin *et al.*, 2017; Purwaningsih *et al.*, 2020) and cause death of many *V. Salvator* in Hainan China. Different with those reports, in this study the nematode is found in the flesh of snakehead fish, and does not change behavior and performance.

Nematodes have three main life-cycle stages: eggs, larvae and adults. Adult worms infect definitive hosts (those animals in which sexual development of the worm occurs) whereas larval stages may also be free-living or parasitize on intermediate hosts or invertebrate vectors. In this study, the infected snakehead fish shows normal performance. This normal performance can be considered that the snakehead fish might have served as intermediate host. While Asian water monitor served as definitive host. Therefore, the pathology and pathogenicity of this nematode on fish is a challenge to be explored in the future.

The eggs hatch, producing the first larval stage (L1) that then undergoes several metamorphoses (molts) to L2, L3. The L3 is the infective stage which when it's usually ingested by the host animal such as shrimp or fish in order for the life cycle to continue. The snakehead fish is a carnivore animal which may indicate that it was infected directly from release L3 or by consuming infected shrimp or fish. Furthermore, the life cycle of this nematode is important to be studied in the future.

4. Conclusion

Snakehead fish (*Channa striata*) is a freshwater fish that has high economic value in South Kalimantan. The disease has been found in this species in the form of spherical cysts in the abdominal cavity and flesh of the fish. The cylindrical worm with needle shape on both tip end with approximately 1 mm length were moving inside of wall cysts. The analysis on 18S rRNA showed this parasite is the nematode *Tanqua tiara*. The pathology, pathogenicity, and life cycle of this nematode are suggested to be studied in the future.

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Author's Contribution

Mw; developed the methods, analyzed the molecular data, earned fund for laboratory activities, and finalized manuscript. JH; conducted field experiment, analyzed clinical signs, handled the samples, earned fund for field activities, and improved the manuscript. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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