Omni-Akuatika, 15 (1): 81–92, 2019
ISSN: 1858-3873 print / 2476-9347 online

Research Article

journal homepage: http://ojs.omniakuatika.net

Identification and Prevalence of Parasites in Eel (*Anguilla bicolor*) Captured Along Migration Pathway at Serayu River, Central Java

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Received 9 July 2018; Accepted 19 April 2019; Available online 31 May 2019

ABSTRACT

Eels especially *Anguilla bicolor* has been a major capture species along the migration pathways at Serayu river for both consumption and aquaculture purposes. Yellow eels always exhibited a strong and health when they were caught. However, mass mortalities always found during holding and culture period. Parasites infestation was one of the obstacles of the eels aquaculture. The aims of this study were to observe the health status and parasites infestation of eels along the migration pathway. Three capture stations namely Adipala, Sampang and Purwojati were appointed as sampling sites. Thirty captured eels ranging from 25.48 cm – 28.92 cm were randomly selected at each site during September to December 2018. Ninety eel samples were collected. The samples demonstrated a good health. Results showed that *Trichodina* was the predominant parasite. Further identification revealed that they were belongs to *T. matsu, T. domerguei* and *T. jandarica* with prevalence rate ranged from 40% to 90%. Another protozoan, *Vorticella* was found at low prevalence and intensity namely; 6.7% and 0.2 respectively. Two nematodes, *Anguillicola* and *Spirocamallanus* were found with prevalence rate and intensity 3.3%-6.7%, 0.03 – 0.06 and 13.3%, 0.13 respectively. Molecular identification of nematodes demonstrated that they are closely related to *Anguillicola crassus* (95.40%) and *Spirocamallanus philppinensis* (97.93%). There was no genetically difference between two species of *Anguillicola* from Adipala and Sampang. This study indicated that eel migrate upstream in a good health. The fish was only infested with few parasites in low prevalence and intensity. *Trichodina*, *Vorticella, Anguillicola* and *Spirocamallanus* were found to infest eel during upstream migration.

Keywords: *eels, parasite, prevalence, identification*

1. Introduction

Shortfin eel (*Anguilla bicolor*) as a catadroumous fish has a unique lifecycle. It lives in freshwater, travelling upstream to find water spring then migrate seaward to spawn before they die. Shortfin was split into two subspecies namely *A. bicolor bicolor* and *A. bicolor pacifica* (Jacoby et al., 2014). *A. bicolor pacifica was* found from the western Sulawesi Island to the western Papua Island whilst *A. bicolor bicolor* population was found from the western Sumatera Island to the southern Java Island (Sugeha and Suharti, 2008). Serayu estuary and river in Cilacap District, Central Java is one of the area where eel were migrated upstream and downstream to complete their life cycle (Fahmi et al., 2012). During the migration, mortality was quite significant due to hydropower turbines, pollution, fishing and habitat reduction. In order to prevent over fishing and promote eel aquaculture, Ministry of Marine Affairs and Fisheries enforced a regulation number 18/2009 about the prohibition of exportation of glass eel. After that, exportation of eel should be preceded culture stage until reached allowable size.

The source of seed of eel culture in Indonesia is still relying on natural catch. Therefore, the success of eel aquaculture depends upon the health status of the seeds,

http://dx.doi.org/10.20884/1.oa.2019.15.1.689

and culture management. Research reports on the health status of the eel during upstream migration in Indonesia are still limited. However, parasites were frequently found in cultivation stages; moreover become one of the obstacles in eel production. Abdelmonem et al. (2009) reported that 65 eels (*A. anguilla*) collected from Al Salam cannal, Zagazig markets and Al Manzala Lake were infested by parasites (33.85%). Further identification revealed that the prevalence of each parasites were 10.7% *Anguillicola crassus,* 6.1% *Dactylogyrus*, 7.7% *Pseudodactylogyrus*, 3.07% *Myxidium*, 4.6% *Trichodina*, and 1.5% *Proteocephalus* respectively. Further study conducted by Setyawan et al. (2015) in Banyumas and Cilacap Rivers, Central Java, Indonesia also found four genera of parasites. The parasites were *Procamallanus* sp. and *Anguillicola* sp. from Nematodes, *Dactylogyrus* sp. and *Deropristis* sp. from Platyhelminthes. Parasites are not the only obstacle of the eel cultivation, Steven et al. (2012) reported on their review that at least three viruses detected to cause mortality in European eel farming, namely eel virus European (EVE) from the aquabirnavirus; eel virus European X (EVEX) from the rhabdovirus, and Anguillid herpesvirus 1 (AngHV1) from the alloherpesvirus. This indicated that eel farming would not be able to avoid a risk of disease infection.

Segara Anakan is an estuary of Serayu River. This area is one of eel migration pathway especially *A. bicolor bicolor.* During the rainy season (November – March) abundance of glass eel and elver were migrated to Serayu rivers and its tributary to grow and find spring water. During the process of migration, eel will pass through various changes in the rivers and canal environment. This various environment changes are one of the factors that play an important role in the parasites proliferation. Stressful condition during migration and multiply by poor environmental circumstances resulted in prone to disease. The aims of this study, therefore, were to observe the health status of eels that migrated to Serayu River with special reference to parasites infestation, to calculate their prevalence and predominant species, and furthermore, to identify the parasite using morphological and molecular approach especially for the helminth.

2. Materials and Method

2.1. The Fish Sample

The eels used in this study were catched from Serayu River at three selected stations where eels fishing was occurred in September – Desember 2018. The stations were Adipala sub district (Station 1), Sampang sub district (Station 2) and Purwojati sub district (Station 3) which presented in Figure 1. Eels were captured using traditional fish trap apparatus made from bamboo called "bubu". The bubu was laid on the bottom of the river then left overnight. The following day trapped eels were collected. Thirty eels each station were randomly selected for this study. A total of 90 eels then transported to the Fisheries and Marine laboratory, Jenderal Soedirman University for parasite observation. Sample size was calculated according to modified Martin et al. (1987). Thirty eels as sample size was decided from an assumption that around 40-50 eels caught in each station, the prevalence rate of the parasites was about 5% (Martin et al.,1987).

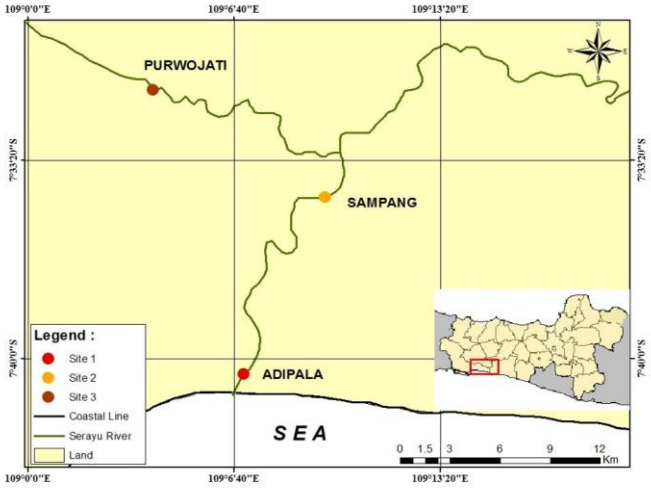

Figure 1. Eel sampling location of this study, station 1 (Adipala), station 2 (Sampang) and station 3 (Purwojati)

2.1. Health status

The health of eel samples was examined by organoleptic and macro parasitic observation.

2.1.1. Organoleptic observation was carried out by looking at the morphology and behavior of the eels. The morphology consisted of body color and abnormalities whilst behavior was observed through active and non active movement. The macro parasites observation was carried out using naked eye to find parasite on the eel skin, fins and mouth.

2.1.2. Observation of parasites was consisted of ectoprasites, endoparasites and followed by calculation of prevalence rate and intensity and predominant species.

Ectoparasites. Ectoparasite infestation was observed through smears method. Eel samples were killed by mean of damaging the brain. Parasites were collected by scrapping each external organ such as gills, fins and skin. Then, mucus was put on glass slides and homogenized with physiological NaCl 0.85% solution. Specifically for morphological characteristic examination of *Trichodina,* the mucus was stained with silver nitrate ($AgNO_3$).
Endoparasites. The eel was dissected using surgical scissors and tweezers so that the intact digestive tract could be removed. The digestive tract was removed and placed on a petri dish. Then the intestine was cut longitudinally and immersed with physiological NaCl solution in a petri dish. The swimbladder was taken out, opened with scissor and put in a petri dish. The parasite was visually examined. The parasite was collected and observed under a microscope. The picture of parasite was obtained using digital camera. Sample of nematode was preserved in 1.5 ml-tube containing 70% ethanol, and stored in freezer.

2.1.3. Prevalence rate, domination and intensity. Calculation of the prevalence rate, domination and intensity were carried out according to formula described by Findyandini et al. (2012); Ramadan et al. (2012), and Fitriyanti et al. (2017).

2.2. Identification of parasites

2.2.1. Ectoparasites

Morphological observation and morphometric measurement were carried out then the result was compared with reference written by Kabata, (1985). Arthur and Lom, (1984) and Bassond and Van As, (1994).

2.2.2. Endoparasites (Nematode)

The nematode was processed for molecular identification. *Anguillicola* sp., was identified based on cytochrome c oxidase subunit I (COX1) gene for the interspecific relationship. The gene was amplified using forward primer LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and reverse primer HCO2198 5'-TAAACTTCAGGGTGACCAAAAAAT-3'. Other nematodes were identified based on nSSU gene. Amplification of the gene was performed using forward primer SSU_F04 5'-GCTTGTCTCAAAGATTAAGCC-3' and reverse primer SSU_R26 5'-CATTCTTGGCAAATGCTTTCG-3' (Laetsch et al., 2012). The amplification product was sent to PT. Genetika Science Indonesia, which send the sample to First BASE Laboratories Sdn Bhd Malaysia for the DNA sequencing. The result was analyzed by BioEdit application. BLAST analysis and multiple sequences alignment analysis (Clustal Omega) were applied.

3. Result and Discussion

3.1. Size and weight.

According to their size, eel samples from three stations were classified as yellow eels. The fish from Adipala had an average length of 28.79 cm with an average body weight of 39.38 g. From Sampang station sample was 26.01 cm of length and 29.50 g of body weight. The sample from Purwojati had average length of 28.29 cm and average body weight of 43.32 g (Table 1).

This result indicated that the yellow eel were migrated upstream. Arai *et al* (2013) reported migration pattern of *A. marmorata* and *A. bicolor pacifica* exhibited three (3) pattern namely (1) typical catadromous life history pattern; (2) constant residence in brackish water; and (3) habitat shifting between sea and brackish waters with no freshwater life. Arai et al. (2013) further stated that as a catadromous fish, they recruited at low latitude then migrated into freshwater habitats of higher productivity for growth then returning to ocean for breeding. Furthermore, a study on otolith Sr:Ca ratio for *Anguilla marmorata* and *A. bicolor pacifica* suggested that these species had flexible migratory behavior. This pattern was also found in tropical eels *A. bicolor bicolor* from Central Java, Indonesia (Chino and Arai, 2010a,b).

Table 1. Size of eels caught from 3 station (Mean ± SD)

Collected from	Length (cm)		Weight (g)
	Standard	Total	
Sampang	25.48 ± 6.01	26.01 ± 6.15	29.50 ± 30.45
Adipala	28.34 ± 7.43	28.79 ± 7.56	39.38 ± 36.45
Purwojati	27.83 ± 8.95	28.29 ± 9.15	43.32 ± 56.51

Table 2. Data on the health condition of captured eel samples

Locations	Skin color	Defective organs		Body condition		Eel movement		External Macro parasite
		Yes	No	Normal	Abnormal	Active	Passive	
Adipala	Bright brown, Black	0	30	9	21	30	0	0
Sampang	Brown and Black	0	30	24	6	27	3	0
Purwojati	Brown and Black	0	30	30	0	30	0	0

3.2. Eel health status

The skin colors of captured eels were from bright brown to black. Skin color of eel from Adipala station were bright brown and black whilst from two stations namely Sampang and Purwojati stations were brown and black. Morphologically all ell did not show any macro parasites on their skin, fins and mouth (Table 2). All eel samples showed no abnormalities, moved smoothly. The fins and tail were in perfect condition. However, three samples from Sampang station were lethargic when they were removed from the trap apparatus. In the other study from European eel (*Anguilla anguilla*) from Dutch Rhine river and Ijsselmeer lake, eels were attacked by ectoparasites on the fins and showed haemorrhages. Endoparasites, primary bacterial and virus infection were not found (Haenen et al., 2010). Table 2 indicated that almost all eel samples were in good condition, although the health status examination was partly incomplete.

3.3. Identification and characterization

3.3.1. *Trichodina*

Trichodina Genus belongs to protozoan group in the phylum of Ciliophora. This parasite is a disc-shaped and has organelles in the middle of the disc called denticles. Species identification was based on cell diameter, adhesive disc length, outer membrane width, denticle diameter, denticle shape, length, thorn length and blade length (Kabata, 1985). Microscopic identification of *Trichodina* in this study revealed to three species (Table 3) namely: *Trichodina matsu* (Bassond and Van, 1994), *Trichodina domerguei* (Kabata, 1985) and *Trichodina jandranica* (Arthur and Lom, 1984). *Trichodina domerguei* was collected from eels that caught in Adipala station, *Trichodina matsu* collected from Sampang, while *Trichodina jandranica* was collected from Sampang and Purwojati station.

Arthur and Lom (1984) reported that *T. domerguei* and *T. tenuidens* were able to live in freshwater and marine environment unlike *T. jandranica* that only found in freshwater. *Trichodina jandranica* was species that reported infected cultured eels (*Anguilla japonica*) in Japan (Imai et al., 1991), and cultured *Anguilla anguilla* in Denmark (Madsen et al., 2000; Kristmundsson and Helgason, 2007).

3.3.2. Anguillicola crassus

Molecular identification and characterization for interspesific different of nematode collected from anguillid swimbladder at Adipala and Sampang stations was done using cytochrome c oxidase COX 1. Interspesific alignment with several related species from BLAST identification of gen bank (Table 4) demonstrated that this nematods were 95.20% to 95.40% identical to the DNA sequence of *A. crassus.* The alignment sequence of *A. crassus* from Adipala and Sampang stations showed that there were 100% identical (Figure 2). This means that They were a same species.

This results confirmed that gene sequences analysis using RNA (nSSU) and cytochrome c oxidase COX 1 was able to identify species of nematodes (Fonseca et al., 2010 and Laetch et al., 2012). Camallanidae (*Camalanus* sp. And *Procamallanus* sp.) Nematodes, Anguillicolidae (*Anguillicola* sp.), Pysalopteridae (*Heliconema* sp.) and many other types of nematodes have been identified using the nSSU gene sequence (Cernotikova et al., 2011). *A. crassus* according to Kuwahara et al., 1974 was an exclusive histrotopic nematode that reproduced in eel swimbladder. This parasite could affect the growth and migration process of eels if the prevalence and intensity were high (Nagasawa et al., 1994; Levebvre et al., 2012).

Table 3. Morphometric characteristic of *Trichodina* found in this study

	Trichodina matsu (this study)^{n9}	*Trichodina matsu* (Basson and Van, 1994)	*Trichodina domerguei* (this study)^{n4}	*Trichodina domerguei* (Kabata, 1985)	*Trichodina jandranica* (this study)^{n32}	*Trichodina jandranica* (Arthur and Lom, 1984)
Diameter of (µm)						
Cell	37.5-42.0	35.5-46.5	50.0-55.0	45.0-90.0	27.0-45.0	34.7-51.0
Adhesive	22.5-39.0	26.0-40.0	45.0-51.0	43.0-61.0	16.25-37.0	20.4-30.6
Denticle	15.0-26.0	15.0-22.0	26.0-28.0	28.0-33.0	10.0-18.0	11.2-17.8
Number of						
Denticle	22-24	20-27	25	22-28	15-20	17-22
RP/D	-	6-8	-	8-10	-	5-7
Length of (µm)						
Denticle	9,0-11.0	5.0-7.0	7.0-11.0	11.0-12.0	5.5-10.0	5.1-6.6
Blade	4.0-5.0	3.5-5.0	5.0	3.5-7.0	3.0-6.0	2.6-4.1
Thorn	3.0-3.75	3.0-5.0	3.0-5.0	4.0-5.0	2.0-4.0	1.5-2.6
Width of (µm)						
Border membrane	2.0	3.0-4.0	2.0	3.5-5.0	2-3	3.1-4.1
Central part	2.5-3.0	1.5-2.0	2.5-3.0	3.0	1.0-4.0	1.0-1.5

Legend: RP/D= Radial parts per denticle. ^{nx} = Number identified of *Trichodina*

Table 4. COI gene sequence alignment results of *Anguillicola* samples from Adipala and Sampang with the gene sequences of related species.

No.	Description	Max score	Total score	Query cover	E value	Ident	Accession
1.	Anguillicola crassus_MIK11	867	867	93%	0.0	95.40%	EU376661.1
2.	Anguillicola crassus_MIK08	861	861	93%	0.0	95.22%	EU376659.1
3.	Anguillicola crassus_TUR_5B	857	857	93%	0.0	95.20%	JF805721.1
4.	Anguillicola crassus_JPN_K4B1	857	857	93%	0.0	95.20%	JF805674.1
5.	Anguillicola crassus_CGZ_149a	857	857	93%	0.0	95.20%	JF805658.1

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A       GAGGTATTAAGATTACGATCCATCAACAATATAGTAATAGCGCCTGCTAATACAGGCAAA        60
S       GAGGTATTAAGATTACGATCCATCAACAATATAGTAATAGCGCCTGCTAATACAGGCAAA        60
        ************************************************************

A       GATAATAACAACAAAAAAACAGTTACAAAAACAGACCAAACAAATAACCTCATATGCTCT        120
S       GATAATAACAACAAAAAAACAGTTACAAAAACAGACCAAACAAATAACCTCATATGCTCT        120
        ************************************************************

A       AAAGTAATTGATCTCCTACGAAGATTCTTTGTAGTAGTCATAATATTAATAGCTCCTAGA        180
S       AAAGTAATTGATCTCCTACGAAGATTCTTTGTAGTAGTCATAATATTAATAGCTCCTAGA        180
        ************************************************************

A       ATAGAACTTACACCAGCACAATGAAGACTTAAAATAACAAGATCCACACTTAAACCAGAA        240
S       ATAGAACTTACACCAGCACAATGAAGACTTAAAATAACAAGATCCACACTTAAACCAGAA        240
        ************************************************************

A       TGTCCAATAACACTCAAAGGAGGATAAATAGTCCAACTCGTACCACAACCAGTCCCAACA        300
S       TGTCCAATAACACTCAAAGGAGGATAAATAGTCCAACTCGTACCACAACCAGTCCCAACA        300
        ************************************************************

A       AAAAAGGAATCTAAAATTAAAAATATTGAAACAGGCAATAACCAAAATCTTAAATTATTT        360
S       AAAAAGGAATCTAAAATTAAAAATATTGAAACAGGCAATAACCAAAATCTTAAATTATTT        360
        ************************************************************

A       AAACGAGGAAAACTTATATCAGGTGCTCCTAACATTAAAGGTAAAACTCAATTACCAAAA        420
S       AAACGAGGAAAACTTATATCAGGTGCTCCTAACATTAAAGGTAAAACTCAATTACCAAAA        420
        ************************************************************

A       CCCCCAATTATAGTCGGCATTACTATAAAAAAAATTATAACAATTGCATGAGACGTAATA        480
S       CCCCCAATTATAGTCGGCATTACTATAAAAAAAATTATAACAATTGCATGAGACGTAATA        480
        ************************************************************
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A       ATACAATTATATAATTGCCCGTCACCTAACAAAAGACCCGGTATAGAAAGCTCAAAACGA          540
S       ATACAATTATATAATTGCCCGTCACCTAACAAAAGACCCGGTATAGAAAGCTCAAAACGA          540
        ************************************************************

A       ATTAAAAAAGATAATATACTTCCTACTATCCCCGATCATAAACCAAAAAGAAAATATAAT          600
S       ATTAAAAAAGATAATATACTTCCTACTATCCCCGATCATAAACCAAAAAGAAAATATAAT          600
        ************************************************************

A       ATACCAATATCTTTA-      615
S       ATACCAATATCTTTAT      616
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Figure 2. . A. crassus COI gene sequence alignment between samples from Adipala and Sampang stations

A = *A. crassus* from Adipala; S = *A. crassus* from Sampang.

3.3.3. *Spirocamallanus* sp.

A DNA sequence of nSSU gene from a nematode from the gut of eel from Adipala was aligned with other relevant sequences and presented in figure 3. The gene sequence of nematode was then analyzed using BLAST. It was found that the nematode was closely related to *Spirocamallanus philippinensis* with 97.93% of similarity (Table 5). Multiple alignment of BLAST DNA sequences with related species revealed that Nematode collected from eel gut from Adipala station also closely related to *Procamallanus rebecae* (96.67%), *Procamallanus monotaxis* (95.97%) and two *Spirocamallanus istibenni*, (95.75% and 95.91% respectively).

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Nematoda_A                  GCTCATTACAACAGCCATAATTTACTTGATGTTGACTTTCCCACGTGGATAACTGTGGTA     60
JF934736.1_S.philippinensis GCTCATTACAACAGCCATAATTTACTTGATGTTGACTTTCCCACGTGGATAACTGTGGTA     60
GU170859.1_S.istiblenni     TTTGAGCCATTACGCCATAATTTACTTGATGTTG-ATATTCCACGTGGATAACTGTGGTA     59
DQ442667.1_P.rebecae        GCTCATTACAACAGCCATAATTTACTTGATGTTG-ATTTTCCACGTGGATAACTGTGGTA     59
JF803931.1_P.monotaxis      GCTCATTACAACAGCCATAATTTACTTGATGTTG-ATTTTCCACGTGGATAACTGTGGTA     59
EF180076.1_S.istiblenni     GCTCATTACAACAGCCATAATTTACTTGATGTTG-ATTTTCCACGTGGATAACTGTGGTA     59
                              * *       *********************  * * ********************

Nematoda_A                  ATTCTAGAGCTAATACATGCACCAAAGCTCTGATTCT--CTGACGAGCGCATCTATTAGA     118
JF934736.1_S.philippinensis ATTCTAGAGCTAATACATGCACCAAAGCTCTGATTTT--CTGACGAGCGCATCTATTAGA     118
GU170859.1_S.istiblenni     ATTCTAGAGCTAATACATGCACCAAAGCTCCGATCTC--ATGACGAGCGCATCTATTAGA     117
DQ442667.1_P.rebecae        ATTCTAGAGCTAATACATGCACCAAAGCTCTGATTTTCTTTGACGAGCGCATCTATTAGA     119
JF803931.1_P.monotaxis      ATTCTAGAGCTAATACATGCACCAAAGCTCCGATCTT--ATGACGAGCGCATCTATTAGA     117
EF180076.1_S.istiblenni     ATTCTAGAGCTAATACATGCACCAAAGCTCCGATCTT--ATGACGAGCGCATCTATTAGA     117
                            ****************************** ***      ********************

Nematoda_A                  CCAAAAACCAATCGAGATTATTCGCCTCAAAAACGAATAGCTCGTAAATTTGGTGACTCT     178
JF934736.1_S.philippinensis CCAAAAACCAATCGAGATTATTCGCCTCAAAAACGAATAGCTCGTAAATTTGGTGACTCT     178
GU170859.1_S.istiblenni     CCAAAAACCAATCGAGAATATTCGCCTCAA-AACGAATGCCTCGTAAATTTGGTGACTCT     176
DQ442667.1_P.rebecae        CCAAAAACCAATCGAGAATATTCGCCTCAA-AGCGAATGCCTCGTAAATTTGGTGACTCT     178
JF803931.1_P.monotaxis      CCAAAAACCAATCGAGATTATTCGCCTCAA-AACGAATGCCTCGTAAATTTGGTGACTCT     176
EF180076.1_S.istiblenni     CCAAAAACCAATCGAGAATATTCGCCTCAA-AACGAATGCCTCGTAAATTTGGTGACTCT     176
                            ***************** ************ * *****  ********************

Nematoda_A                  GAATAGCTTAGCTGATCGCATGGTCTCGCACCGGCGACGTATCTATCAAGTGTCTGCCTT     238
JF934736.1_S.philippinensis GAATAGCTTAGCTGATCGCATGGTCTCGCACCGGCGACGTATCTATCAAGTGTCTGCCTT     238
GU170859.1_S.istiblenni     GAATAGCTTAGCTGATCGCATGGTCTCGCACCGGCGACGTATCTATCAAGTGTCTGCCTT     236
DQ442667.1_P.rebecae        GAATAGCTTAGCTGATCGCATGGTCTCGCACCGGCGACGTATCTATCAAGTGTCTGCCTT     238
JF803931.1_P.monotaxis      GAATAGCTTAGCTGATCGCATGGTCTTGCACCGGCGACGTATCTATCAAGTGTCTGCCTT     236
EF180076.1_S.istiblenni     GAATAGCTTAGCCGATCGCATGGTCTCGTACCGGCGACGTATCTATCAAGTGTCTGCCTT     236
                            ************ ************* * ********************************

Nematoda_A                  ATCAACTTTCGATGGTAGTTTATATGCCTACCATGGTTGTAACGGGTAACGGAGAATAAG     298
JF934736.1_S.philippinensis ATCAACTTTCGATGGTAGTTTATATGCCTACCATGGTTGTAACGGGTAACGGAGAATAAG     298
GU170859.1_S.istiblenni     ATCAACTTTCGATGGTAGTTTATATGCCTACCATGGTTGTAACGGGTAACGGAGAATAAG     296
DQ442667.1_P.rebecae        ATCAACTTTCGATGGTAGGTTATGTGCCTACCATGGTTGTAACGGGTAACGGAGAATAAG     298
JF803931.1_P.monotaxis      ATCAACTTTCGATGGTAGTTTATATGCCTACCATGGTTGTAACGGGTAACGGAGAATAAG     296
EF180076.1_S.istiblenni     ATCAACTTTCGATGGTAGTTTATATGCCTACCATGGTTGTAACGGGTAACGGAGAATAAG     296
                            ****************** **** ************************************

Nematoda_A                  GGTTCGACTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGC     358
JF934736.1_S.philippinensis GGTTCGACTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGC     358
GU170859.1_S.istiblenni     GGTTCGACTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGC     356
DQ442667.1_P.rebecae        GGTTCGACTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGC     358
JF803931.1_P.monotaxis      GGTTCGACTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGC     356
EF180076.1_S.istiblenni     GGTTCGACTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCAAGGAAGGCAGCAGGC     356
                            ************************************************************

Nematoda_A                  GCGCAAATTACCCACTCTCAGCACGAGGAGGTAGTGACGAAAAATAACGAGACCGTTCTC     418
JF934736.1_S.philippinensis GCGCAAATTACCCACTCTCAGCACGAGGAGGTAGTGACGAAAAATAACGAGACCGTTCTC     418
GU170859.1_S.istiblenni     GCGCAAATTACCCACTCTCAGCACGAGGAGGTAGTGACGAAAAATAACGAGACCGTTCTC     416
DQ442667.1_P.rebecae        GCGCAAATTACCCACTCTCAGCACGAGGAGGTAGTGACGAAAAATAACGAGACCGTTCTC     418
JF803931.1_P.monotaxis      GCGCAAATTACCCACTCTCAGCACGAGGAGGTAGTGACGAAAAATAACGAGACCGTTCTC     416
EF180076.1_S.istiblenni     GCGCAAATTACCCACTCTCAGCACGAGGAGGTAGTGACGAAAAATAACGAGACCGTTCTC     416
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Nematoda_A                       TTCGAGGCCGGTTATCGGAATGAGTACAACTTAAAGCCGTTAATAAGGATCTATGAGAGG     478
JF934736.1_S.philippinensis      TTCGAGGCCGGTTATCGGAATGAGTACAACTTAAAGCCGTTAATAAGGATCTATGAGAGG     478
GU170859.1_S.istiblenni          TTCGAGGCCGGTTATCGGAATGAGTACAATTTAAAGCCGTTAACGAGGATCTATGAGAGG     476
DQ442667.1_P.rebecae             TTCGAGGCCGGTTATCGGAATGAGTACAACTTAAAGCCGTTAATAAGGATCTATGAGAGG     478
JF803931.1_P.monotaxis           TTCGAGGCCGGTTATCGGAATGAGTACAACTTAAAGCCGTTAACAAGGATCTATGAGAGG     476
EF180076.1_S.istiblenni          TTCGAGGCCGGTTATCGGAATGAGTACAACTTAAAGCCGTTAACAAGGATCTATGAGAGG     476
                                 *************************** *************  ***************

Nematoda_A                       GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCTCAAAGTGTATATCGTCATTGCT     538
JF934736.1_S.philippinensis      GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCTCAAAGTGTATATCGTCATTGCT     538
GU170859.1_S.istiblenni          GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCTCAAAGTGTATATCGTCATTGCT     536
DQ442667.1_P.rebecae             GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCTCAAAGTGTATATCGTCATTGCT     538
JF803931.1_P.monotaxis           GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCTCAAAGTGTATATCGTCATTGCT     536
EF180076.1_S.istiblenni          GCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCTCAAAGTGTATATCGTCATTGCT     536
                                 ************************************************************

Nematoda_A                       GCGGTTAAAAAGCTCGTAGTTGGATTTAAGCGCGATGACTCGGTCCGTCCATGGGACGCG     598
JF934736.1_S.philippinensis      GCGGTTAAAAAGCTCGTAGTTGGATTTAAGCTCGATGATTCGGTCCATCCATGGGATGTG     598
GU170859.1_S.istiblenni          GCGGTTAAAAAGCTCGTAGTTGGATTTAAGCTCGATGACTCGGTCCATCCATAGGATGAG     596
DQ442667.1_P.rebecae             GCGGTTAAAAAGCTCGTAGTCGGATTTAAGCTCGATGACTCGGTCCATCCATGGGATGAG     598
JF803931.1_P.monotaxis           GCGGTTAAAAAGCTCGTAGTTGGATTTAAGCTCGATGACCTGGTCCATCCATAGGATGAG     596
EF180076.1_S.istiblenni          GCGGTTAAAAAGCTCGTAGTTGGATTTAAGTTCGATGACTCGGTCCATCCATAGGATGAG     596
                                 ******************* *********  ******   ***** ***** *** * *

Nematoda_A                       AACTGAGCTCATCGGCTAGTCA-CCGCTGGTTTTGTCTAGTTGGCTTTAAACGGTCGCCT     657
JF934736.1_S.philippinensis      AACTGAACTCATGAGCTCTTAT-TCGCCGGTTTTGTTTAGGTGGCTTTAAACGGTCGCCT     657
GU170859.1_S.istiblenni          AACTGAGCTCATCGGCTA-TCATCGGCTGGTTTTGTCTAGGTGGCTTTAAACGGTTGCCT     655
DQ442667.1_P.rebecae             AACTGAGCTCATCGGCTAATCATCGGCTGGTTTTGTTTAGGTGGCTTTAAACGGTCGCCT     658
JF803931.1_P.monotaxis           AACTGGGCTCATCGGCTAT-CACCAGCTGGTTTTGCCTAGGTGGCTTTAACCGGTCGCCT     655
EF180076.1_S.istiblenni          AACTGAGCTCATCGGCTAATCATCAGTTGGTTTTGTTTAGGTGGCTTTAAACGGTCGCCT     656
                                 *****  *****  ***        *  *******  *** ********* **** ****

Nematoda_A                       AGACTGGCTAGCAAGTTTACTTTGAAAAAATTAGAGTGCTCAACGCGGGCTTAATGCCTG     717
JF934736.1_S.philippinensis      AGACTGGCTAACAAGTTTACTTTGAAAAAATTAGAGTGCTCAACGCGGGCTTAATGCCTG     717
GU170859.1_S.istiblenni          AGGCTGGCTAGCAAGTTTACTTTGAAAAAATTAGAGTGCTCAACGCGGGCTTAATGCCTG     715
DQ442667.1_P.rebecae             AGACTGGCTAGCAAGTTTACTTTGAAAAAATTAGAGTGCTCAACGCGGGCTTAATGCCTG     718
JF803931.1_P.monotaxis           AGGCTGGCTAGCAAGTTTACTTTGAAAAAATTAGAGTGCTCAACGCGGGCTTAATGCCTG     715
EF180076.1_S.istiblenni          AGGCTGGCTAACAAGTTTACTTTGAAAAAATTAGAGTGCTCAACGCGGGCTTAATGCCTG     716
                                 ** ******* ************************************************

Nematoda_A                       AATAGTCGTGTATGGAATAATGAAATAGGATCTTGGTTCTATTTTGTTGGTTTCCTGAAC     777
JF934736.1_S.philippinensis      AATAGTCGTGTATGGAATAATGAAATAGGATCTTGGTTCTATTTTGTTGGTTTCCTGAAC     777
GU170859.1_S.istiblenni          AATAGTCGTGCATGGAATAATGGAATAGGATTTCGGTTCTATTTTGTTGGTTTTTTTAAC     775
DQ442667.1_P.rebecae             AATAGTCGTGCATGGAATAATGGAATAGGATCTCGGTTCTATTTTGTTGGTTTTTCTAAC     778
JF803931.1_P.monotaxis           AATAGTCGTGCATGGAATAATGGAATAGGATTTCGGTTCTATTTTGTTGGTTTTTTTAAC     775
EF180076.1_S.istiblenni          AATAGTCGTGCATGGAATAATGGAATAGGATTTCGGTTCTATTTTGTTGGTTTTTTTAAC     776
                                 ********** *********** ******** * ******************    ***

Nematoda_A                       TAAGATAATGGTTAAGAGGGACAGACGGGGGCATTCGTATCGCTACGTGAGAGGTGAAAT     837
JF934736.1_S.philippinensis      TAAGATAATGGTTAAGAGGGACAGACGGGGGCATTCGTATCGCTACGTGAGAGGTGAAAT     837
GU170859.1_S.istiblenni          CGAAATAATGGTTAAGAGGGACAGACGGGGGCATTCGTATCGCTACGTGAGAGGTGAAAT     835
DQ442667.1_P.rebecae             TGAGATAATGGTTAAGAGGGACAGACGGGGGCATTCGTATCGCTACGTGAGAGGTGAAAT     838
JF803931.1_P.monotaxis           CGAAATAATGGTTAAGAGGGACAGACGGGGGCATTCGTATCGCTACGTGAGAGGTGAAAT     835
EF180076.1_S.istiblenni          CGAAATAATGGTTAAGAGGGACAGACGGGGGCATTCGTATCGCTACGTGAGAGGTGAAAT     836
                                   * ************************************************************

Nematoda_A                       TCTTGGACCGTAGCGAGACGCCCGACTGCG  867
JF934736.1_S.philippinensis      TCTTGGACCGTAGCGAGACGCCCGACTGCG  867
GU170859.1_S.istiblenni          TCTGGAC-CGTAGCGAGACGCCCGACTGCG  864
DQ442667.1_P.rebecae             TCTTGGACCGTAGCGAGACGCCCGACTGCG  868
JF803931.1_P.monotaxis           TCTTGGACCGTAGCGAGACGCCCGACTGCG  865
EF180076.1_S.istiblenni          TCTTGGACCGTAGCGAGACGCCCGACTGCG  866
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Figure 3. Multiple sequences alignment result of nSSU gene sequences of a Nematode sample from Adipala (Nematoda A) and other related species.

Table 5. Analysis result using Basic local alignment search tool (BLAST) of nSSU gene sequence of a nematode sample from Adipala.

No.	Description	Max score	Total score	Query cover	E value	Ident	Accession
1.	*Spirocamallanus philippinensis*	1506	1506	100%	0.0	97.93%	JF934736.1
2.	*Procamallanus rebecae*	1443	1443	99%	0.0	96.67%	DQ442667.1
3.	*Procamallanus monotaxis*	1408	1408	99%	0.0	95.97%	JF803931.1
4.	*Spirocamallanus istibenni*	1399	1399	100%	0.0	95.75%	EF180076.1
5.	*Spirocamallanus istibenni*	1380	1380	98%	0.0	95.91%	GU170859.1

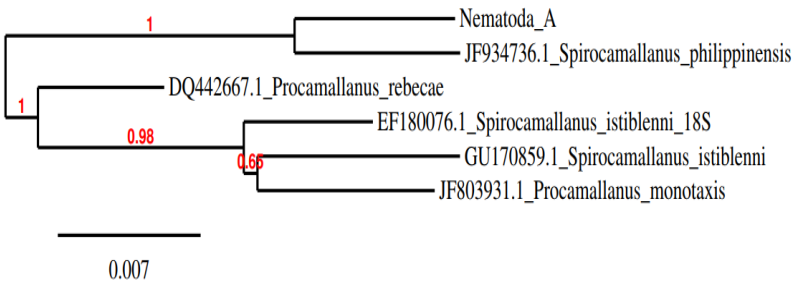

Figure 4. Phylogenic tree of Nematoda A from Adipala with other related nematodes based on nSSU gene sequence

BLAST analysis results showed that the closest species to the nematodes obtained in the gut of eel based on the nSSU gene sequence was *Spirocamallanus philippinensis* JF934736.I (97.93%; table 5). The phylogenic tree showed that the nematodes obtained in this study was in one cluster with *Spirocamallanus philippinensis* (figure 4). Moravec *et al* (2013) stated that *Spirocamallanus anguillae* was found in Indonesian eels shortfin at India in the first time.

3.4. Prevalence, domination and intensity of parasite

Ectoparasites found in this study were *Trichodina* and *Vorticella* (Table 6 and Figure 5). These ectoparasites were very common and frequently found in the environment that organic materials were quite high (Axelrod, 1989). Jabal et al. (2015) further stated that *Trichodina* sp. was a cosmopolite organism that able to survive at any waters. The total numbers of *Trichodina* in 3 stations were 231, 2,242, and 1,764 respectively (Table 6). *Vorticella* was only found in Sampang station at low numbers. This indicated that organic matters in both Adipala and Purwojati stations during sampling was relatively low. Tumbol et al (2011) in their study found 3,159 trichodinids and 1 (one) *Vorticella* on 50 cultured eels from North Sulawesi. They found that trichodinids were the predominant ectoparasites found in gills and skin. Anisah et al (2016) reported that trichodinid was also predominant ectoparasites in gouramy juveniles (*Osphronemus gouramy*). Meanwhile, protozoan parasites found in Anguillid that caught in Central Sulawesi were *Myxidium* sp., *Myxobolus* sp., *Henneguya* sp., *Ceratomyxa* sp., *Chilodonella* sp., *Balanidium* sp. and *Glugea* sp. (Jabal et al., 2015). *Pseudodactylogyrus bini*, *Pseudodactylogyrus anguillae*, *Pseudodactylogyrus microchis* and *Pseudodactylogyrus* sp. are found in *Anguilla japonica* which cultured in China (Guangzheng et al., 2015). *Gyrodactylus anguillae* also found in the cultured of *Anguilla Anguilla* (Elgendy et al*.,* 2016).

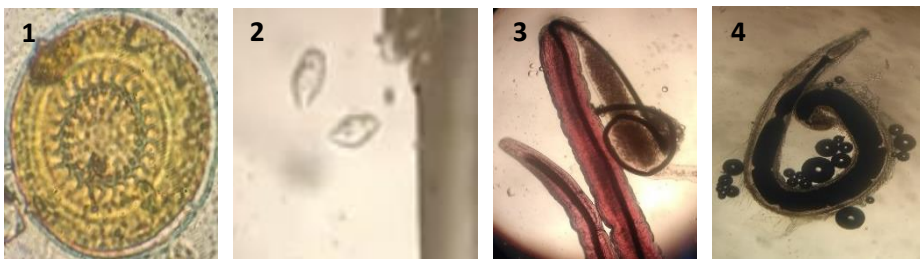

Figure 5. Morphology of Trichodina, Vorticella, Spirocamallus and Anguillicola collected in this study

Note: Picture 1. *Trichodina* sp., with AgNO3 400x, Picture 2. *Vorticella* sp. with 100x, Picture 3 and Picture 5. Endoparasites with 40x

The prevalence rate of trichodiniasis was 40.0%, 66.67% and 90.0% at Adipala, Sampang and Purwojati stations respectively. *Vorticella* was only found in the Sampang station with prevalence rate as low as 6.67%. This results was in contrast with Ali et al. (2009) that the tricodinid prevalence was only 4.6%. Furthermore, Madsen et al. (2000) reported that trichodinid was found in Danish cultured eels at the prevalence rate of 66%. From this study, it was clear that *Trichodina* was the predominant species at three station namely 97.8%, 99.6% and 100% respectively. The intensity was ranged from 7.7 to 58.8 trichodinids/eel and tended to increase along the upstream pathway.

Endoparasites *Anguillicola* found in Sampang and Adipala stations whilst *Spirocamallus* only found in Adipala station (Table 6 and Figure 5). Both endoparasites *Anguillicola* and *Spirocamallanus* were found in Swimbladder and intestine at low numbers namely 5 and 2 parasites with prevalence rate 3.3% - 6.7% and 13.3% respectively (Table 7). The intensity of *Anguiillicola sp was* 0.4 and 0.08 parasite/eel, and *Spirocamallus sp* was 1.6 parasite/eel (Table 7). This result was in line with previous study by Setyawan et al. (2015) in Cilacap that the prevalence rate and intensity of *Anguillicola* sp. were 5.56% and 2 parasites/ind respectively. These results were relatively low compared to Japanese eels which have a prevalence rate of 33%-58% (Han et al., 2008) and European eels in Turkey from 4 rivers at 9.52%-50.00% (Koyucu et al., 2017).

Table 6. The number of infected eels and parasites

Type of parasites	The location of parasites	The total number of parasites			The number of infected eels		
		Adipala	Sampang	Purwojati	Adipala	Sampang	Purwojati
Ectoparasites							
Trichodina sp.	Skin, Gills	231	2,242	1,764	12	20	27
Vorticella sp.	Skin	0	6	0	0	2	0
Endoparasites							
Anguillicola sp.	Swimbladder	1	2	0	1	2	0
Spirocamallanus sp.	Intestine	4	0	0	4	0	0
Total parasites		**236**	**2,250**	**1,764**			

Table 7. The prevalence, domination and intensity of parasites in this study

Type of parasites	The location of parasites	Prevalence rate (%)			Intensity (parasite ind/eels number)			Domination (%)		
		Adp	Smg	Prt	Adp	Smg	Prt	Adp	Smp	Prt
Ectoparasites										
Trichodina sp.	Skin, Gills	40	66.7	90	7.7	74.7	58.8	97.8	99.6	100
Vorticella sp.	Skin	0	6.7	0	0	0.2	0	0	0.2	0
Endoparasites										
Anguillicola sp.	Swimbleader	3.3	6.7	0	0.03	0.06	0	0.4	0.08	0
Spirocamallanus sp.	Intestine	13.3	0	0	0.13	0	0	1.6	0	0

Ali et al. (2009) reported that *A. crassus* was found in Al Salam channel, Egypt at prevalence rate 10.7%. Whilst Tumbol et al. (2011) found endoparasites level of incidence for *Capillaria* sp 24%, *Oxyurida* sp 6%, and *Acanthocephalus* sp 2%. Moravec et al. (2013) found nematode *Heliconema ahiri* and *Procamallanus anguillae* in Indonesian *Anguilla bicolor*. Other study conducted by Moravec & Scholz (2015) in European eel (*Anguilla anguilla*) from 3 rives located in Czech Republic found a total of 35 species of macroparasites which were dominated by Nematodes and Acanthocephala groups at varying intensity and prevalence. This study indicated that endoparasite helmints were common threat for eels during migration.

4. Conclusions

Eel migrated upstream along the pathways at Adipala, Sampang and Purwajati

stations were infected by both ectoparasites and endoparasites. Ectoparasite *Trichodina* sp. was the predominant species with prevalence rate 40.07% and intensity 90.0 parasite/ind. *Vorticella* sp. was found at low prevalence rate and intensity namely; 6.7% and 0.2. respectively. Morphometric identification and characterization of Trichodina revealed that 3 species were found namely *Trichodina matsu, T. domerguei,* and *T. jandranica*.

The endoparasite nematodes obtained were *Anguillicola* and *Spirocamallanus* with prevalence rate and intensity 3.3%-6.7%, 0.03 – 0.06 and 13.3%, 0.13, respectively. Molecular identification of nematodes demonstrated that they were closely related to *Anguillicola crassus* with similarity 95.40% and *Spirocamallanus philppinensis* with similarity 97.93%. There were no genetically differences between two species *Anguillicola crassus* from Adipala and Sampang. From this study it can be seen that eel migrated upstream in a good health. *Trichodina*, *Vorticella, Anguillicola* and *Spirocamallanus* found infested eels during upstream migration.

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