Morphology and molecular identification of *Eurytrema* spp. worm in Aceh cattle, Indonesia

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Abstract. *Hanafiah M, Helmi TZ, Sutriana A, Bahi M. 2021. Morphology and molecular identification of* Eurytrema *spp. worm in Aceh cattle, Indonesia. Biodiversitas 22: 5654-5661.* The objective of this research was to determine the species of *Eurytrema* spp. in Aceh cattle which were slaughtered in slaughterhouses located in Banda Aceh, Indonesia. Identification of *Eurytrema* spp worm species was performed by Semichon's Carmine staining, SEM and molecular method. The result from Semichon's Carmine staining and SEM method showed that worms coded E2 and E3 were *Eurytrema cladorchis* indicated by clearly visible protruding cirrus while worms coded E1, E4 and E5 were *E. pancreaticum* indicated by the absence of protruding cirrus. Molecular characterization of *Eurytrema* spp. produced fragments of approximately 700 bp in length. The phylogenic tree showed that *Eurytrema* spp. from Aceh cattle consisted of 2 clades: clade 1 was *E. pancreaticum,* while clade 2 was *E. cladorchis.* DNA sequencing result showed that the similarity of worm samples coded 1, 4, and 5 were 98.90% similar to *E. pancreaticum* (KJ767631.1 and KC535543.1)*,* and 96. 33% similar to *E. cladorchis* (MN566134.5 and MN566140.1), whereas the worm samples coded 2 and 3 were 97.53% similar to *E. pancreaticum* (KY490000.1 until KY490004.1)*,* and 97.55 % similar to *E. cladorchis* (MN566135.1 until MN566140.1). This research was able to identify *E. pancreaticum* and *E. cladorchis* in Aceh cattle slaughtered in Banda Aceh slaughter houses.

Keywords: Cattle, *Eurytrema pancreaticum, Eurytrema cladorchis*, identification, molecular

INTRODUCTION

The Aceh cattle breed came from a cross between local cattle (assumed to be a descendant of *Bos sondaicus*) and zebu cattle from India (*Bos indicus*) which occurred hundreds of year ago (Sutarno and Setyawan 2016). At present time, Aceh cattle population has reached an alarmingly low number, so Aceh cattle feedstock needs to be improved by increasing birth rate and lowering death rate percentage (Widyaningrum et al. 2021). The cause of this diminishing number is due to the low growth rate of Aceh cattle, which is much slower than the rate of crossbred cattle (Agus et al. 2018). Other than the slow growth, according to Schwertz et al. (2015) parasitic disease caused by digenetic trematode *Eurytrema* worm, which is a pancreas parasite with low pathogenicity, could be related to the decrease of livestock productive performance and may end in death.

One species of parasitic worm that infect the pancreas and biliary tract of cattle is *Eurytrema* spp. The genus *Eurytrema* appears to be confined to parts of South East Asia, East Asia and Latin America (Mohanta et al. 2015), where annual mortality caused by this pancreatic fluke ranges between 1-3% (Ilha et al. 2005; Okajima et al. 2016). *Eurytrema pancreaticum* infection can be debilitating towards animal health and can cause clinical

and subclinical diseases, as well as causing great economic losses and medical costs and lowering overall productivity (Schwertz et al. 2015), it may be associated with mortality and loss of productive performance in animals due to chronic pancreatitis (Su et al. 2018). The parasites have also been known to impair calf growth, cause progressive weight loss, lower feed conversion, malnutrition, and impair milk production. Eurytrematosis case in cow has been reported in Brazil (Rachid et al. 2011; Quevedo et al. 2013) where clinical and pathological features observed shows chronic and progressive wasting related to interstitial pancreatitis. The prevalence of parasitism in Brazil varied between regions. Lucca et al. (2015) reported in the western region of Santa Catarina State, the prevalence of *Eurytrema* was as high as 69%.

Xu et al. (2015) has researched the expression profile of global MicroRNAs (miRNAs) from adult *E. pancreaticum* utilizing next gen sequencing technology combined with real-time quantitative PCR. Mohanta et al. (2015) was to identify *Eurytrema* flukes both by morphology and molecular properties on the basis of 18-subunit ribosomal RNA (18S rRNA) gene as well as internal transcribed spacer 2 (ITS2) to clarify their phylogenetic status. Figueira et al. (2014) also identified unique morphological characteristics and molecular features in *E. coelomaticum* worms found in cattles in South Brazil. Mohanta et al.

(2015) confirmed the presence of *E. cladorchis* in the biliary tract of cattle (*Bos indicus*) in Bandarban a mountainous district in Bangladesh. Research on the differences of trematode worm parasites *Eurytrema* spp. until now had not been reported for Aceh cattle. Considering the lack of information on species and the morphology of *Eurytrema* spp., this research may aid in better understanding eurytrematiasis in an effort to find efficient strategies to prevent and control infestations of the parasite in cattle from Aceh. In order to better manage cattle in Aceh, more accurate identification of species and morphology of the worms is required.

The purpose of this research is to determine the difference in species of *Eurytrema* spp. worms obtained from Aceh cattle slaughtered in Banda Aceh slaughterhouses by using Semichon's Carmine staining, scanning electron microscopy (SEM) and molecular method.

MATERIALS AND METHODS

Study location and period

A total of 40 pancreas samples were collected from Aceh cattle from Banda Aceh, Indonesia slaughterhouses. The Aceh cattle that were slaughtered were mostly from Aceh Besar district, and partly from Pidie and Aceh Jaya districts. The samples were collected into plastics and labeled. The separation and identification of *Eurytrema* spp. worms were performed in the Laboratory of Parasitology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh. *Eurtyrema* spp. worms were put inside petri dishes, cleaned and later stained using Semichon's Carmine staining. SEM analysis were performed in the Center of Biology Research, LIPI Cibidong, Zoology Division, SEM Laboratory, Cibinong, Indonesia. For DNA extraction, 5 *Eurytrema* spp. were taken from the pancreas. The worms were rinsed in distilled water, fixed in absolute ethanol solution, and then stored at 4ºC. DNA extraction, electrophoresis and amplification of *Eurytrema* spp. worms was performed at Inter University Centre (PAU) Gadjah Mada University. This research was carried out between December 2020 and March 2021.

Semichon's Carmine Staining Method *Eurytrema spp.* was stained using Semichon's Carmine method which referred to Cable (1961). *Eurytrema* worm specimens were put into semichon's carmine staining solution barely covering the specimen thoroughly. The staining solution was previously diluted to an equal volume of 70% alcohol and stored for 1 to a few hours. The over stain color was removed by washing the specimen with 70% alcohol twice. Subsequently, the specimen was dipped in 0.5% to 1% HCL and 70% alcohol until the specimen is pink in color. Then, the specimen was washed twice or three times using 70% alcohol to remove acid and prevent further staining process. Subsequently, dehydration was carried out by dipping the specimens in 80% and 95% alcohol for 1 hour, each solution. Then, the specimen was dipped in another new 95% alcohol or in absolute alcohol if methyl salicylate

was used to clean up the specimen. The next step was cleaning the specimen using neutral creosote, terpinol or methyl salicylate. The specimen was briefly washed in xylol then, attached to a glass object and added entellan, covered with a glass deck carefully to ensure the entellan is distributed thoroughly. The slide was stored for 24 hours to let the entellan dry before observing under a microscope using 40x magnification.

Scanning electron microscope (SEM) method to observe worm micro structure

Eurytrema spp. observations by SEM were performed to explore the small morphological features of *Eurytrema* spp. Specimen preparation was performed in the Center of Biology Research, LIPI Cibinong, Biology Division, SEM Laboratory, Cibinong, according to a particular protocol. Samples were processed at 4ºC in several stages: (i) Cleaning, the sample was soaked in cacodylate buffer (42.6 gr of 0.2 M sodium caccodylate + 1000 mL aquadest with a pH of 8.4) for approximately 2 hours, then agitated in an ultrasonic cleaner for 5 minute; (ii) Presification, the sample was put into a 2.5% glutaraldehyde solution (5 mL glutaraldehyde+caccodylate buffer up to 40 mL) for several hours to 2 days; (iii) Fixation, the sample was soaked in 2% tannic acid (2 g of tannic acid in 100 mL of caccodylate buffer) for 6 hours to several days, then washed with caccodylate buffer for 5 minutes for 4 times; (iv) Dehydration, the sample was soaked 5 minutes in 50% alcohol for 4 times, then immersed in 70% alcohol, 85% alcohol, and 95% alcohol for 20 minutes each (at room temperature), then with absolute alcohol for 10 minutes 2 times; (v) Drying, the sample was submerged for 10 minutes in tert butanol 2 times, then freezed until frozen and transferred to freeze drier/vacuum drier until dry; (vi) Installation, the specimen was fixed on the specimen stub according to the needs and stages; (vii) Coating, the specimen was coated with Au (Aurum) using the ION COATER tool. The microscope used was the JSM-IT200 InTouchScope™ Scanning Electron Microscope. Pictures were taken to observe morphological differences between the available worms using the appropriate magnification (Goldstein et al. 1992).

Eurytrema spp. observation using PCR

The DNA extraction results were amplified using PCR in the 5.8S and IT2 regions. Polymerase chain reaction (PCR) was performed using a 50 μL mixture that contained 10 μL sample DNA. Reverse primer was JB9 neu (5'-GCT GCA TTC ACA AAC AAC CCG-3') and forward primer was 3SC (5'-CGG TGG ATC ACT CGG CTC G-3') (Mirza and Kurniasih 2002). The PCR mixture contained 0,2 mM of each primer, 100 mM dNTP (Fermentas), 60 mM Tris-HCl (pH 9.0), 15 mM (NH4) 2SO$_4$, 2 mM MgCl2, and 1U Biotaq (Bioline, MA, USA) for each reaction. Amplification was performed using PTC-150 Minicycler thermocycler (MJ Research Inc, MA, USA) with an initial denaturation of 7 minutes on 94ºC, followed by 35 cycles for 1 minute in 95ºC, 1 minute in 60ºC, 1 minute in 72ºC and final incubation for 10 minutes in 72ºC. The amplification product was then electrophorized in 1,1%

agarose gel etydium bromide with 100 bp marker (Biolabs, MA, USA) and a positive control containing *Eurytrema* spp. DNA. A negative control that did not contain DNA material was also used.

Sequencing

PCR product was sequenced by ABI PrismBigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA).

Data analysis

The data was presented in picture and table format and then the accumulated data were analyzed descriptively. Phylogenetic tree and sequence alignments based on the 5.8S and ITS2 gene of the *Eurytrema* spp. were then created by using MEGA version 6 software. Constructed by the neighbor-joining method, based on 1.000 bootstrapped data sets; distance value was calculated by using the Kimura 2 parameter model. *Fasciola hepatica* was used as the out group to provide stability to the generated tree. The sequence identity was generated by using the software Bioedit (Tamura et al. 2013).

RESULTS AND DISCUSSION

Observation on 40 pancreases (Figure 1) from Aceh cattle slaughtered in Banda Aceh slaughterhouses appeared to be infested by trematode *Eurytrema* spp. worms. *Eurytrema* are considered low pathogenic parasites (Ilha et al. 2005; Schwertz et al. 2016), yet are very frequently found at necropsy or at slaughterhouses in Aceh and other parts of West Indonesia.

The results of the examination on pancreas samples of Aceh cattle showed the presence of worms from the trematode class of the genus *Eurytrema* spp. (Figure 1). However, the species of these worms could not be determined due to the morphology of the worms can not be observed specifically. In general, the morphology of *Eurytrema* spp. was reddish in color and slightly oval in shape. This worm has a blunt and rounded anterior end, and has a short tongue-like shape at the posterior end. The body is thick and armed with spines, which often disappear as

they mature. This worm has an oral sucker and a ventral sucker, pharynx, osophagus, testes and genital pore, and cirrus sac. The ovaries are near the midline, behind the testes, and the uterus is at the back of the body. The vitelline glands are follicular and located laterally. The species of Eurytrema worms found in the pancreas was still unknown and thus staining was required.

Morphology observation by worm staining

The results obtained in this research by Semichon's Carmine staining method showed that the worms found in the pancreas (Figure 1) of Aceh cattle were *E. pancreaticum* (Figure 2, E1, E4 and E5) and *E. cladorchis* (Figure 2, E2 and E3). *E. pancreaticum* worms found in this research morphologically were 8-16 mm in length and 5-8 mm in width. The body was flat and tapered. Oral sucker was located at the anterior region of the body while ventral sucker was located around the middle region of the body. Visible reproductive organs were a pair of testicles lobes located opposite of one another above ovarium lobulated with worm eggs situated inside the uterus. Ovarium with lobulated is an identifying characteristic of *E. pancreaticum* (Kumar et al. 2018), uterus, and glandula vitelline. Cirrus sac was located in the middle between oral and ventral sucker, cirrus was present but protruding (Figure 2, E1, Inset).

Figure 1. *Eurytema* spp. worms (arrows) in the pancreas of Aceh cattle
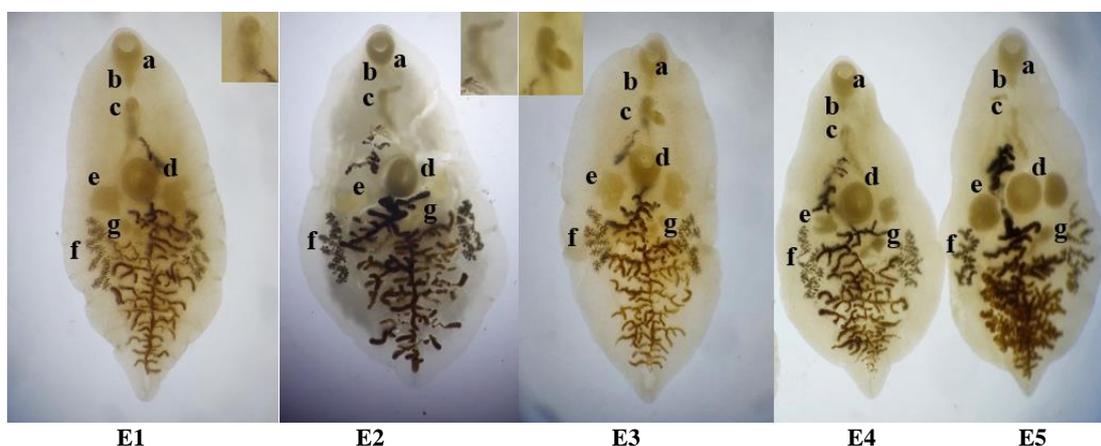
Figure 2. The morphology of genus *Eurytrema* worms. E1, E4 and E5: *E. pancreaticum*, E2 and E3: E. cladorchis. a: oral sucker, b: pharynx; c: cirrus; d: ventral sucker; e: testes; f: vitelline follicles and g: ovarium. Scale bar: 94.5 μm

According to Mohanta et al. (2015), species identification of *Eurytrema* spp. worm has always been room for controversy especially related to the obtained morphologies, making interpretation differs between researchers. In the last several years, significant advancement in microscopic technology has led to new discoveries on the morphology and anatomy of parasitic worms (Faulwetter et al. 2013; Lopes-Torres et al. 2015; Gonçalves et al. 2016; Conn et al. 2018).

According Chai et al. (2013) morphologically *E. pancreaticum* worms have larger oral sucker compared to their ventral sucker, testes with lobes, ovarium with or without lobulated, and grouped vitellaria follicle with clear separation. *Eurytrema cladorchis* worms obtained in this research had morphological features such as protruding digitiform cirrus (Figure 2; E2 and E3. Inset), elongated cirrus sac, branching testicles which were located symmetrically at the posterolateral region towards the acetabulum. Ventral sucker (acetabulum) was larger (on average 322.67 µm) compared to the oval shaped oral sucker (on average 279.07 µm) similiar morphology was also observed previously in research (Figueira et al. 2014; Mohanta et al. 2015) which classifies the worms as the *E. cladorchis* species.

In China, there are at least seven species, including *E. cladorchis, E. coelomaticum, E. fukienensis, E. hydropotes, E. minutum, E. pancreaticum and E. sphaeriorchis*; however, *E. pancreaticum* is considered as predominant species in ruminants (Li et al. 2013). The study of Okajima et al. (2016) explained that eurytrematosis was endemic in Japan particularly in Kagoshima, Okinawa, and Shimane regions. In another study showed that *E. pancreaticum* eggs appeared in fecal samples of five camels in Karamoja sub region of North-eastern Uganda (Nakayima et al. 2017).

Eurytrema cladorchis was discovered living in the pancreatic tract of domestic ruminants. Afterward, the flukes were reported from both wild deer (*Muntiacus muntjak, Hydropotes inermis*) and domestic ruminants in the mountainous area of Fujian, Zhejiang, Jiangxi and Anhui Provinces, China (Cai et al. 2012). Morphological identification of *E. cladorchis* in Nepal has been performed by Mohanta et al. (2015). In this research we compared the morphometry features and morphology of *Eurytrema* worms from Bangladesh with obtained *E. cladorchis*. The result of this study which identifies *Eurytrema cladorchis* in cattle in Indonesia especially in Aceh cattle is the first to be conducted as far as our knowledge. It is the goal of the researchers that this research will add new information on the characteristic of *Eurytrema* worms especially *E. cladorchis*.

The prevalence of *E. pancreaticum* was detected in Aceh cattle with a prevalence rate of 0.4% (Hanafiah et al. 2019). Lucca et al. (2015) mentions that the prevalence of *E. pancreaticum* in cattle may occur, in extreme cases up to 100%, while (Okajima et al. 2016) reported that the prevalence of eurytrematiasis was less than 1% in animal slaughterhouses in Japan.

Scanning electron microscope (SEM)

SEM observation on obtained *Eurytrema* spp. displayed micro non-protruding cirrus on *Eurytrema pancreaticum* (EP), while clearly protruding cirrus is visible on *Eurytrema cladorchis* worms (EC) (Figure 3).

This result is supported by research conducted by Chai et al. (2013) which stated that morphologically, *E. pancreaticum* did not have a protruding cirrus (Figure 3. a), while *E. cladorchis* has a protruding cirrus (Figure 3. b). clearly protruding cirrus is visible on *E. cladorchis* worms (EC) (Figure 3) Mohanta et al. (2015).
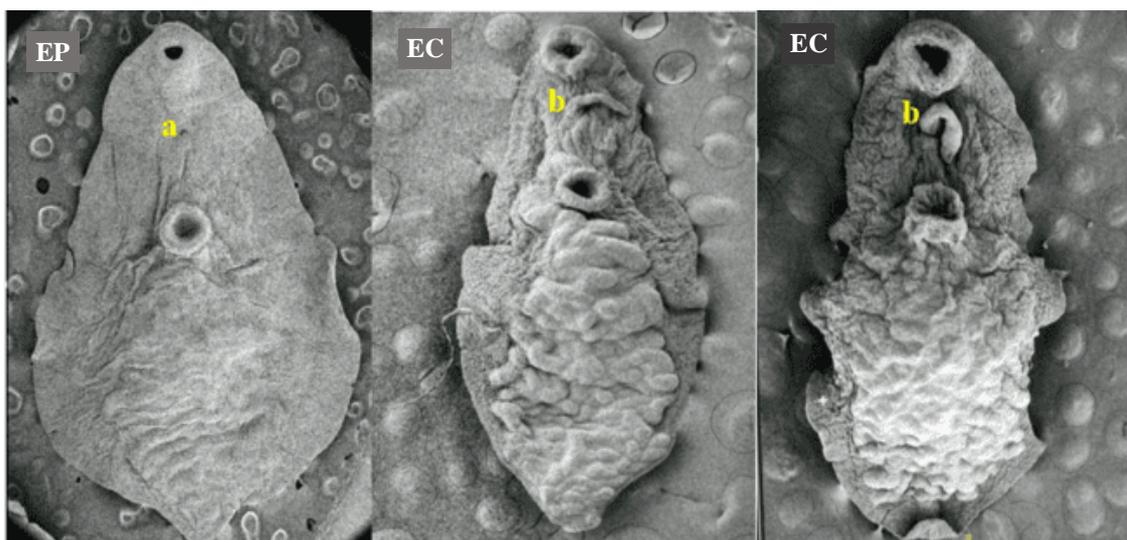
Figure 3. The morphology of genus *Eurytrema* spp. worm. EP: *Eurytrema pancreaticum*, EC: *Eurytrema cladorchis*, a: Cirrus does not protrude, b: Cirrus visibly protrudes. Scale bar: 500 µm

Repetitive R529 sequence amplification

Amplification results of DNA from blood, pancreas, and *Eurytrema* spp. samples were obtained by using forward primer 3SC and reverse primer JB9 neu with different master mix, in which mix PCR Go Tag specifically attached to sample DNA copy of D1, E1, E2 as well as J1 dan J2 producing 700 bp in length, while D2 did not show any band. Using master mix PCR Bio Line, E1, E2 and J1 samples produced 700 bp in length, while D1, D2 the band is amplified in an unspecified region with 5.8 S and IT2 primers (Figure 4). Meanwhile, worm samples E1, E2, E3, E4 and E5 produced 700 bp in length (Figure 5).

The result of amplification of Polymerase chain reaction (PCR) on 5.8S and ITS2 site in this research showed that the DNA length of the *Eurytrema* spp. found was the same with research conducted by Mirza and Kurniasih (2002) which was 700 bp in samples from Makassar, Yogyakarta, and Aceh, while using ITS2, 15 samples of *Eurytrema* spp. worms on agarose gel 1% which produced 1500 bp. Different results were also obtained in research by Mohanta et al. (2015) who used 8S rRNA on *Eurytrema* spp. worms which showed 1784 bp DNA, while the ITS2 was 229 bp in length.

The results of nucleotide BLAST (*Basic Alignment Search Tool*) which are shown in NCBI showed the homology between *Eurytrema* spp. worms in this research (sequenced product) with the sequence of several worms in GenBank. For worm samples E1, E4, and E5 they were 98.90% homologous with *E. pancreaticum,* and 96. 33% homologous with *E. cladorchis*. Worm samples E2 and E3 were 97.53% homologous with *E. pancreaticum,* and 97. 55 % homologous with *E. cladorchis.*

Estimated evolution differences with the *E. cladorchis* genotype from Bangladesh according to research by Cai et al. (2012) stated that the difference is low in values between 0,000 (identical) up to 0,002 (similarities 99.8%) for 18S rRNA and for ITS2 was 0,000. In 18S region rRNA, the estimated divergent evolution between *E. cladorchis* from Bangladesh and *E. pancreaticum* (DQ401034) from China showed similarity of 99.4-99.6%, while using ITS2 *E. cladorchis* and *E. pancreaticum* from Bangladesh has a similarity 96.2%. Genetic identity of *E. cladorchis* from Bangladesh with *E. cladorchis* and *E. fukienensis* from China respectively were 98.1-98.3 and 98.4-98.6% respectively.

Table 1 showed the genetic distance of *Eurytrema pancreaticum* Aceh isolate obtained from Aceh cattle (isolate number 4101133_1, 4101139_4, 4101141_5) with other *Eurytrema pancreaticum* isolates. *Eurytrema pancreaticum* Aceh isolate had genetic distance of 0. 018 and 0.055 when compared to *Eurytrema_pancreaticum* isolate 5.8S_ribosomal_RNA_gene (KJ767631.1) and *Eurytrema_cladorchis_*isolate_LS09_5.8S_ribosomal_RNA_gene (MN566140.1), respectively. Whereas *E. cladorchis* Aceh isolate (isolate number 4101135_2 dan 4101137_3) had genetic distance of 0.032 as compared to isolate of *Eurytrema_pancreaticum_*5.8S ribosomal_RNA gene (KJ767631.1) and *Eurytrema_cladorchis_*isolate

LS09 5.8S_ribosomal_RNA_gene (MN566140.1), and had 0.023 genetic distance compared with *E. pancreaticum* Aceh isolate (isolate number 4101133_1, 4101139_4, 4101141_5).

Phylogenetic analysis result

Sequence results obtained were later used to construct a phylogenetic tree which was then compared to determine the difference level between isolates. *Eurytrema* spp. sequences from Aceh cattle slaughtered in Banda Aceh slaughterhouses were then compared to sequence data from 10 different strain/isolates from the GenBank. The sequence organizing and phylogenetic tree were performed by MEGA X.0 software (Figure 6).
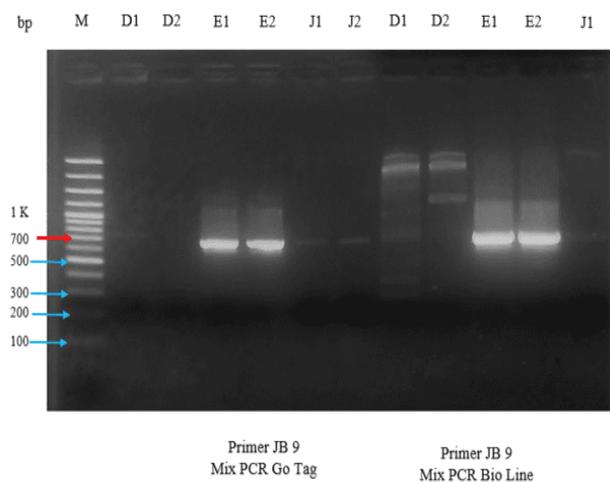

Figure 4. Sample DNA amplification result on agarose gel 1%. M: marker, D1 and D2: Aceh cattle blood, E1 and E2: *Eurytrema* spp. worm, J1 and J2: Aceh cattle pancreas
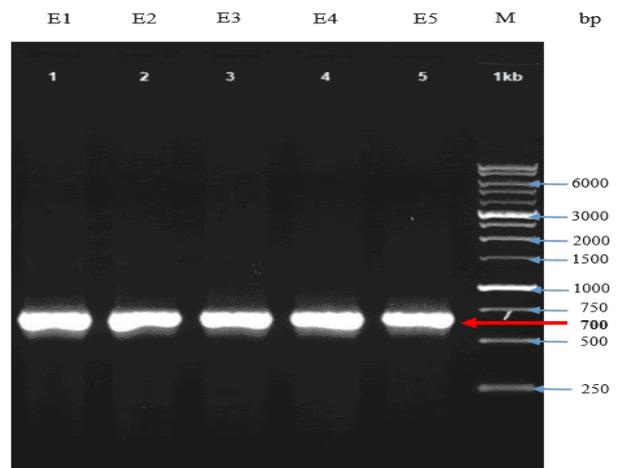
Figure 5. Sample DNA amplification on gel agarose 1%. M: marker, E1, E2, E3, E4 and E5: *Eurytrema* spp. worms

Table 1. Data of genetic distance between *Eurytrema* isolate from Aceh with data from GenBank

Code/number of isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. KJ767631.1															
2. 4101133_1	0,018														
3. 4101139_4	0,018	0,000													
4. 4101141_5	0,018	0,000	0,000												
5. 4101135_2	0,032	0,023	0,023	0,023											
6. 4101137_3	0,032	0,023	0,023	0,023	0,000										
7. MN566140.1	0,055	0,046	0,046	0,046	0,032	0,032									
8. LC006031.1	0,051	0,041	0,041	0,041	0,027	0,027	0.004								
9. EF547132.1	0,240	0,246	0,246	0,246	0,240	0,240	0,247	0,246							
10. KF734795.1	0,221	0,234	0,234	0,234	0,227	0,227	0,234	0,234	0,023						
11. KU563717.1	0,221	0,227	0,227	0,227	0,221	0,221	0,215	0,215	0,080	0,075					
12. DO379986.2	0,234	0,240	0,240	0,240	0,227	0,227	0,234	0,234	0,027	0,023	0,070				
13. MN831475.1	0,234	0,240	0,240	0,240	0,227	0,227	0,234	0,234	0,027	0,023	0,070	0,000			
14. KC535543.1	0,000	0,018	0,018	0,018	0,032	0,032	0,055	0,051	0,240	0,221	0,221	0,234	0,234		
15. MW620063.1	0,523	0,522	0,522	0,522	0,522	0,522	0,552	0,551	0,515	0,549	0,563	0,517	0,517	0,523	
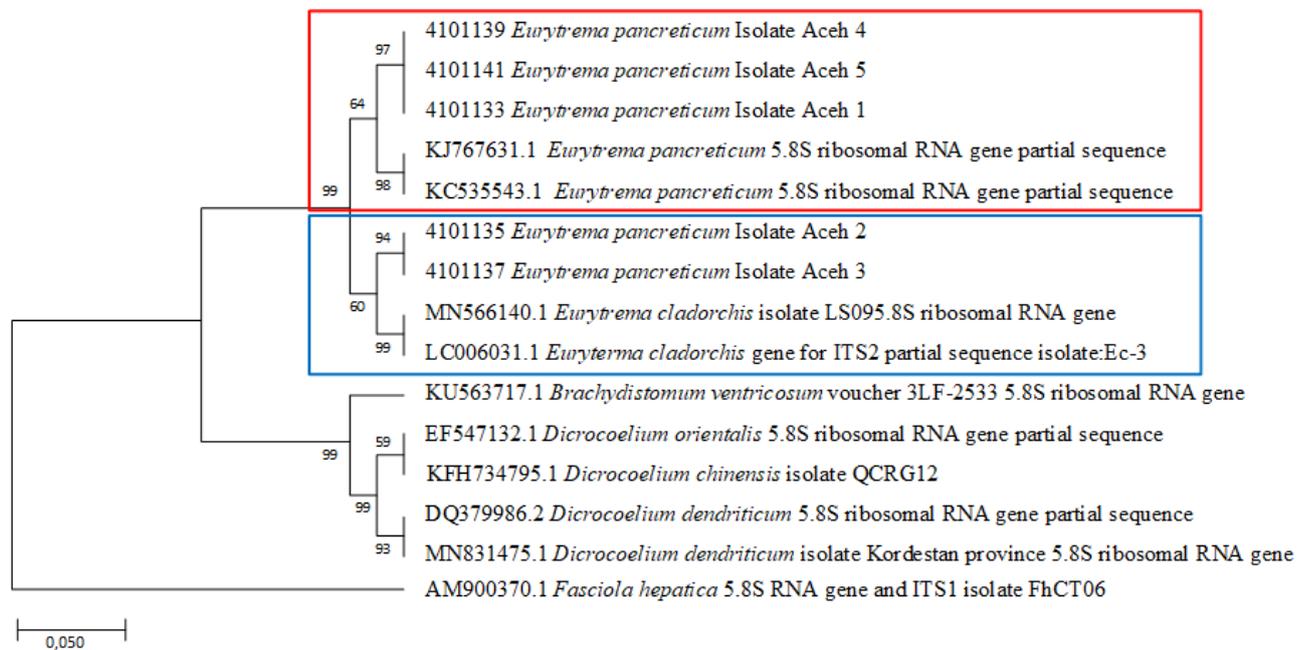
Figure 6. Neighbour Joining by 5,8S and ITS2 (700 bp) sequence of *E. pancreaticum* and *E. cladorchis* from Indonesia and other sequences related to Dicrocoelidae family. *Fasciola hepatica* was used as outgroup. Bootstrap value higher than 50% is shown in tree

The phylogenetic tree construction result (Figure 6) showed that *Eurytrema* spp. from Aceh consisted of 2 clades, where clade 1 consists of 2 sub clades in which each consisted of *E.pancreaticum* from Aceh cattle in Indonesia and *E. pancreaticum* (KJ767631.1), *E. pancreaticum* (KC535543.1) from Bangladesh while clade 2 consisted of 2 sub clades as well which consisted of *E.cladorchis* form Aceh Indonesia, *E. cladorchis* (LC006031.1) isolate from Bangladesh and *E. cladorchis* (MN566140.1) from Liangshan, China.

Cai et al. (2012) stated that phylogenetic analysis of *E. cladorchis* 18S and ITS2 genotypes from Bangladesh formed a monophyletic clade in phylogram. Phylogram constructed from 18S rRNA has 2 clades, where clade 1 consists of *E. coelomaticum*, *E. cladorchis* and *E. fukienensis* from China while clade 2 consists of *E. cladorchis* from Bangladesh whereas *D. dendriticum* is different from genus *Eurytrema*. In phylogram obtained from ITS2 sequences, *E. pancreaticum* is a relative from clade *E. cladorchis* while genus *Dicrocoelium* members form a different clade.

Clades formed by *E. cladorchis* from Bangladesh is vastly different from *E. cladorchis* and *E. fukienensis* reported by Cai et al. (2012) in China. The estimated evolutionary divergent value showed that the genetic distance between *E. cladorchis* from Bangladesh and *E. cladorchis* reported from China was 1.7-1.9%. This showed that *E. cladorchis* in Bangladesh and in China are genetically quite different. *E. pancreaticum* Aceh isolates from this research when compared to *E. pancreaticum* from

Bangladesh, India, has an evolutionary divergence value of 1.8%, while the *E. cladorchis* Aceh isolate and *E. cladorchis* from Liangshan, China, showed the genetic distance of 3.2%. This result showed that *E. pancreatium* from Aceh cattle when compared with those from Bangladesh are genetically quite different, while the *E. cladorchis* from Aceh cattle when compared with China and Bangladesh are genetically vastly different.

In conclusion, this research was able to identify *E. pancreaticum* and *E. cladorchis* in Aceh cattle obtained from Banda Aceh slaughterhouses. *E. cladorchis* indicated by clearly visible protruding cirrus while worms *E. pancreaticum* indicated by the absence of protruding cirrus. The DNA amplification of *Eurytrema* spp. worm was 700 bp in length. The phylogenetic tree showed that *Eurytrema* spp. from Aceh cattle formed 2 clades, clade 1 consisted of *Eurytrema pancreaticum,* while clade 2 consisted of *Eurytrema cladorchis.*

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