IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
July 2017 Vol.:7, Issue:1

Identification of Laor Worms (Polychaeta) from the Sea Region of Haria Village of Central Maluku, Indonesia

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

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Submission: 1 July 2017
Accepted: 7 July 2017
Published: 25 July 2017

HUMAN JOURNALS
www.ijsrm.humanjournals.com

Keywords: Central Mollucas, Haria village, Laor worms, phenotype description.

ABSTRACT

This research aims at determining the species of Laor worms from the Haria village of Central Moluccas based on phenotype description and 16S rRNA gene sequences. Based on the phenotype description especially related to the number of antennas, Laor worms found consist of those having two antennas classified as Perinereis genus of the Nereidae family, those having 3 or 5 antennas classified as to Eunice and Lysidice genus of the Eunicidae family, and those having no antennas that cannot be classified into a particular family because its head is not intact. The analysis of phylogeny (NJ and ML approach), genetic distance, and similarity of Laor worms conducted are based on 16S rRNA gene sequences supported by 5.03 MEGA program. The analysis results of the phylogeny, genetic distance, and similarity show that the Laor worms found are classified into 5 groups: group A consisting of sample S1, S3, S7 and S9 is classified into Eunice genus; Group B consisting of samples S2 and S6 is classified into Eunice fucata species; Group C consisting of sample S4 and S5 is classified into Palola viridis species; Group D consisting of sample S10 is classified into Lumbrineris magnidentata species; Group E consisting of sample S8 is classified into Perinereis genus. The value of NJ bootstrap is between 47-100% and the value of ML bootstrap is between 27-100%. Genetic distance ranges from 0.04831 to 0.21774 (4.831% -21.774%) with the highest similarity value of 95.16896% and the lowest similarity value of 78.25638%.

INTRODUCTION

Haria village is one of the villages in Maluku province surrounded by oceans, having coral reefs that act as a physical shield of the shore as a habitat for marine life. Therefore the sea region of Haria village is rich in various kinds of marine life including the marine worms of the Polychaeta class (Yusron, 1987; Mulyadi, 2011; BPS 2015).

The body of Polychaeta has segments as rings having similar shape and size. They have soft, slender, cylindrical and colorful bodies caused by the dye pigment on its body. Polychaeta lives in various habitats such as muddy areas, sandy, rocky, and even can inhabit hard substrates such as corals in large numbers (Yusron, 1985; Nybaken, 1992).

Maluku people have long been familiar with polychaeta, consume it and call it as Laor worm. The Lease people in Central Maluku (Saparua) call Laor worms as Melattono; the people of southeast Maluku (District of Kei Kecil) call it as Lawar, and the people living in Banda call it Oele. Nowadays the Laor term is better known by the Maluku people compared with other terms. Laor is a term used to describe various types of marine worms (Polychaeta) which appear regularly in large quantities at sea level at a specific time. Their appearance regularly in large quantities at sea level is called as swarming. During swarming the Polychaeta undergo external mating (Radjawane, 1982; Monk et al., 1997; Mahulette, 2001).

The swarming of marine worms in Maluku occurs once a year in March or April at a full moon night or a few days after the full moon. Laor worms usually appear for three (3) consecutive nights and are usually taken (collected) by the people of Maluku using traditional seser (a traditional tool like a bucket to collect the laor worms). This event is called as *Timba Laor* (bucketing Laor) in Maluku and it is a significant cultural event in several regions of Indonesia such as Sumba and Lombok (Jekti et al., 1993; Pamungkas, 2009b; Pamungkas, 2015).

When swarming, Laor worms are thought to be not the only one species of worm because the results of identification of Laor worms in Banda sea region (in Maluku) are *Lysidice Oele* (Eunicidae), while the Laor worms or the wawo worms found in sea region of Airlouw village (Ambon Island) are identified as a mixture of 13 species of five (5) different families which mainly consisted of *Palola viridis* (Horst, 1904; 1905; Martens et al., 1995; Pamungkas, 2009a). Phenotypically the body of Laor worms consists of segments, as well as has chaeta, and three antennas on the head. It has small body, and the length of the body is

about 7 cm with the diameter of 1.5 mm. Some Laor worms are larger, thicker and longer than the others. The body has attractive colors such as dark green mixed with gray-white, yellow, red, brown and slightly bluish (Radjawane, 1982).

The identification of Laor worms phenotypically in the sea region of Ambon Island based on the number of antennas by Liline et al. (2016) shows that the Laor worms can be classified into (1) Nereidae family which has two antennas consisting of Perinereis and Hediste genus, and (2) Eunicidae family which has 3 or 5 antennas consisting of Lysidice and Eunice genus. While the Laor worm identification based on the 16S rRNA gene sequences shows that the Laor worms in the sea region of Ambon island are classified into several species such as *Palola viridis*, *Eunice fucata*, *Eunice torquata*, *Eunice gracilicirrata, Eunice antarctica, Lumbrineris magnidentata*, *Perinereis aibuhitensis, Perinereis cultrifera,* and *Hediste atoka*.

To date, Laor worms have been often or always identified based on the phenotype. In fact, detecting species phenotypically is limited because it has some disadvantages. The disadvantages are related to the fact that the phenotype is affected by genotype and environmental factors. Furthermore, this process of identification is time-consuming, expensive, having limited diversity and inconsistent. Due to the weakness of identification phenotypically, molecular identification needs to be done to obtain more accurate data because molecular identification analyzes the DNA (Cahyarini et al., 2004; Schulze, 2006; Zulfahmi, 2013). This research aimed at determining the species of Laor worms found at the Haria village (in Central Maluku) based on phenotype description and 16S rRNA gene sequences.

MATERIALS AND METHODS

The Laor worm samples in the sea region of Haria village, Saparua district in Maluku was collected on March 18, 2014. The samples were collected using traditional net (seser) from the seawater and then rinsed with clean water. After that, phenotype observation of the fresh samples was done by using a stereo microscope brand Olympus SZ-type 51 with a magnification of 100X and documented with a digital camera Samsung 100SSCAM. Laor worms were then put in a solution of 70% alcohol, and phenotype observation was continued by Olympus stereo microscope type SZX 9 with a magnification of 100X.

The DNA of Laor worms was isolated using the CTAB method by Dhakshanamoorthy and Selvaraj (2009) which has been modified for the isolation of Laor worm DNA. Laor worm

DNA samples are processed in PCR using Kapa2G Fast Ready mix to amplify the 16S rRNA supported by forward primer (5'- CGCCTGTTTATCAAAAACAT-3') and reverse primer (5'- CTCCGGTTTGAACTCAGATCA-3') (Palumbi, 1991). The amplification step of DNA PCR of Laor worms was pre denaturation at 94^0C for 5 minutes, denaturation at 94^0C for 30 seconds, annealing at 45^0C for 30 seconds, extension at 72^0 C for 30 seconds, and final extension at 72^0C for 10 minutes. The results of DNA amplification of Laor worms were then sequenced using ABI Prism 373 oxl Genetic Analyzer in First BASE Laboratories Sdn. Bhd. Selangor, Malaysia.

Database of Polychaeta sequences from Genbank (Table 1) was taken to be aligned with the sequences of Laor worms from the sea region of Haria village.

Table 1. Database of Polychaeta and its Accession Numbers retrieved from GenBank

Sample Name	Accession Number
Eunice_antarctica	GQ478137.1
Eunice_fucata	GQ478143.1
Eunice_gracilicirrata	JX559748.1
Eunice_torquata	GQ478145.1
Lumbrineris_inflata	AY838832.1
Lumbrineris_magnidentata	DQ779621.1
Palola_viridis	JN558570.1
Palola_viridis	JN558575.1
Perinereis_aibuhitensis	KF611806.1
Perinereis_cultrifera	KC833495.1

The results of the Laor worms sequencing were analyzed using sequencing software scanner (ABI) and multiple alignments performed using Clustal W with MEGA 5.03 program. Reconstruction of the phylogeny tree was based on the nucleotide sequence with two different approaches, namely Neighbor Joining (NJ) and Maximum Likelihood (ML) using the MEGA 5.03 program Kimura 2-parameter models.

RESULTS

Phenotype description of Laor worms

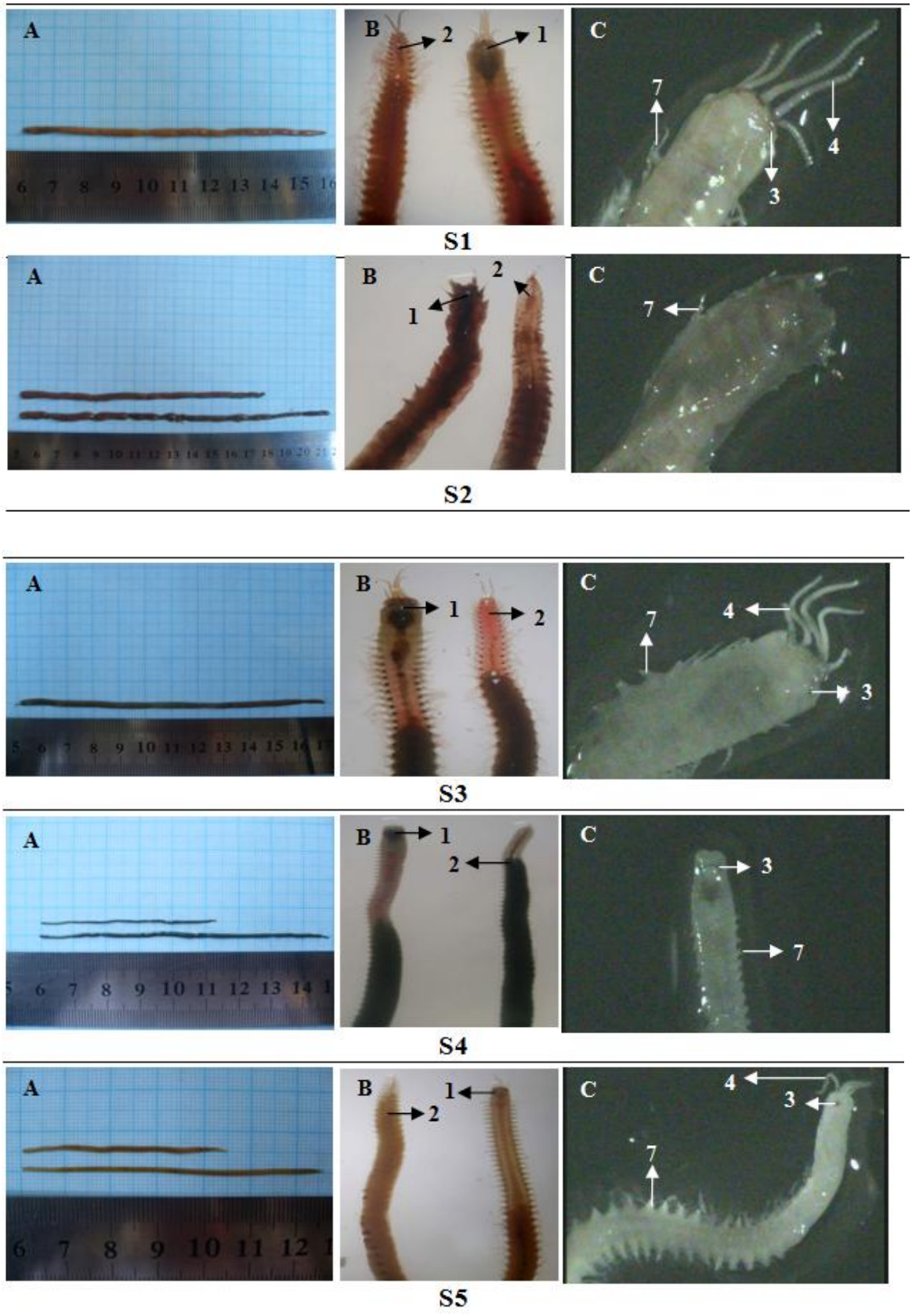
Laor worms are taken from the sea region of Haria village in the Saparua district Central Maluku bounded by petuanan of Tiouw Village in the north, petuanan of Booi village and the area of Banda Sea in the south, petuanan of Paperu village in the east, and the area of Banda sea in the west (Figure 1). Map of the Saparua island is shown in figure 1.

Figure 1: Map of Saparua Island (Central Maluku district)

Source: Provincial Government of Maluku (2015)

Description: Sampling location of Laor worms

The results of phenotype observation of Laor worms in Haria village show that there are variations in the body shape, body color, and body length (Figure 2 and Table 2).


A
B
2
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S5

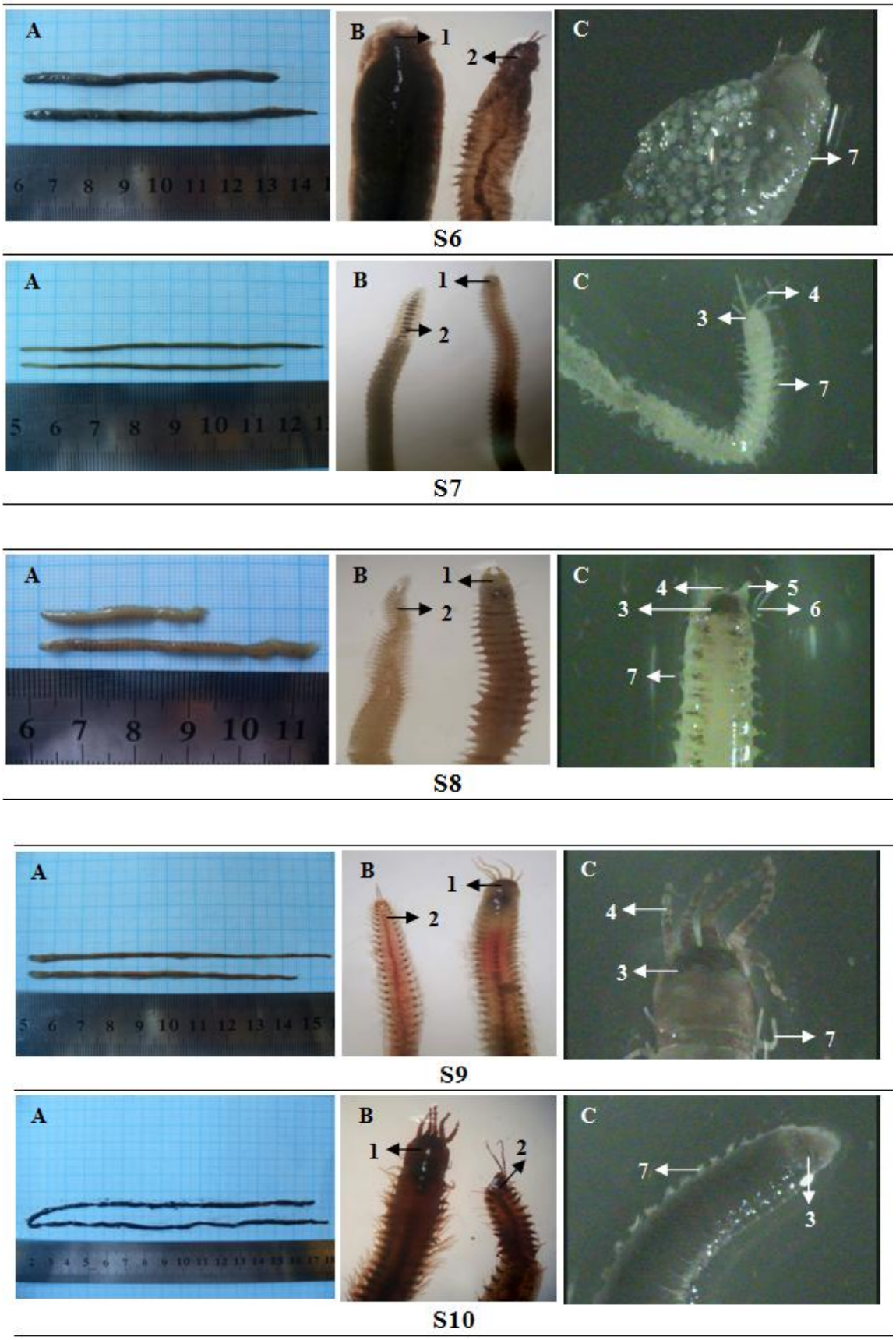

Figure 2. Phenotype of Laor worms from Haria Village

(A) Observations of fresh Laor worms with digital camera brand Samsung 100SSCAM,

(B) Observation of fresh Laor worms with Olympus stereo microscope types

SZ 51 (magnification 100X)

(C) Observations of preserved Laor worms with Olympus stereo microscope SZX type 9 (magnification 100X).

Source: Personal documents (2014)

Description:

1. Head

2. Tail

3. Eye

4. Antenna

5. Sensory papillae

6. Tentacular Cirri

7. parapodia

Table 2. Phenotype Description of Laor Worms

Sr. No.	Code of Worm Laor	Body shape	Body color	Body length (cm) and Total Segment
23.	S1	• Flat round fat • Parapodia evident • 5 antennas • 2 eyes	reddish orange body, translucent reddish head, and tail	± 10 cm 115 segments
24.	S2	• Flat round fat and little long	Blackish red	± 17 cm 93 segments

		• Parapodia evident • Roundish head		
25.	S3	• Flat round fat • Parapodia evident • 5 antennas • 2 eyes	Bright green moss body, reddish translucent head, and tail	± 11 cm 106 segments
26.	S4	• Long smooth round • Parapodia evident • 3 antenas • 2 eyes	Dark green body, translucent head, and tail	± 9 cm 157 segments
27.	S5	• Long smooth round • Parapodia evident • 5 antennas • 2 eyes	Orange body, translucent head, and tail	± 7 cm 154 segments
28.	S6	• Flat round fat • Parapodia evident • Roundish head • Transparent and small round circles visible in the body	Dark moss green bodies, translucent reddish heads, and tails	± 9 cm 76 segments
29.	S7	• Long smooth round • Parapodia evident • 5 antennas • 2 eyes	Light moss green bodies, translucent heads, and tails	± 8 cm 123 segments
30.	S8	• Round flat fat, • chewy head, soft tail, • Two sensory papillae at the head • 2 antennas • 4 eyes • 8 tentacular cirri	Translucent reddish brown stripes on the left and right sides of the body	± 6 cm 56 segments
31.	S9	• Parapodia clearly visible • Flat round fat	Bright red meat	± 11 cm

		• Parapodia evident • 5 antennas • 2 eyes • Flat round fat	body, translucent reddish head, and tail	36 segments
32.	S10	• Parapodia evident • 5 antennas • 2 eyes	Purple body, translucent reddish head, and tail	± 31 cm 128 segments

The results of the identification of Laor worms phenotypically from the Haria village based on the number of antennas are divided into the Laor worms having two antennas (sample S8), having three antennas (sample S4), having five antennas (samples of S1, S3, S5, S7, S9, and S10), and those having no antennas or the antennas are not visible (samples of S2 and S6).

Identification of Laor Worms based on 16S rRNA Gene Sequences

The identification of Laor worms based on 16S rRNA gene was carried out based on the results of phylogeny analysis, genetic distance, and similarity value of Laor worms. The length of 16 S rRNA gene successfully amplified from 10 samples of Laor worm ranged between 476-520 bp. The results of the sequencing of Laor worm sample were then aligned with the Polychaeta database of 16S rRNA gene from Genbank (multiple alignments). The results of multiple alignments were used to reconstruct the phylogeny tree with different approaches, namely Neighbor Joining or NJ (Figure 3) and Maximum Likelihood or ML (Figure 4).

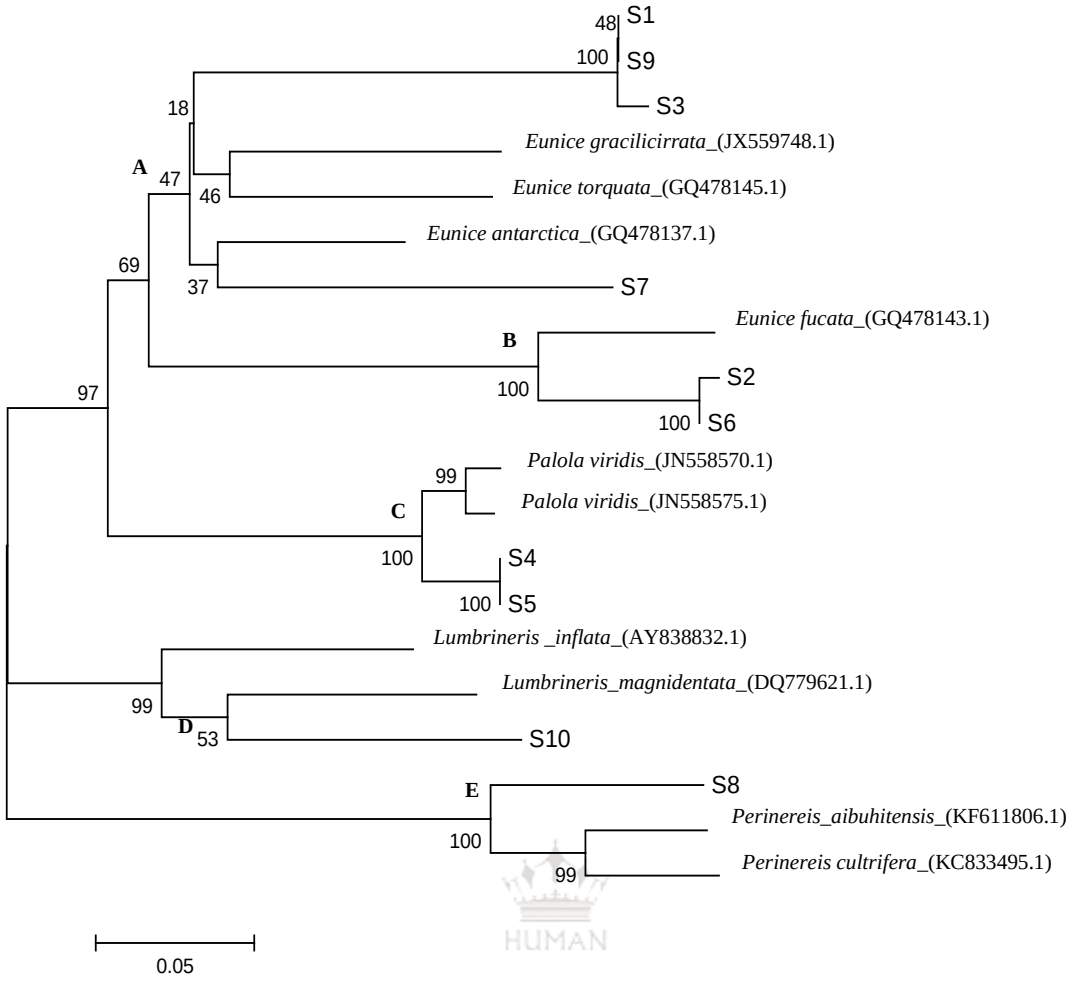

Figure 3. Phylogeny tree of nucleotide sequence based on Neighbor Joining approach (NJ) with Kimura 2-parameter models, bootstrap 1000 replications.

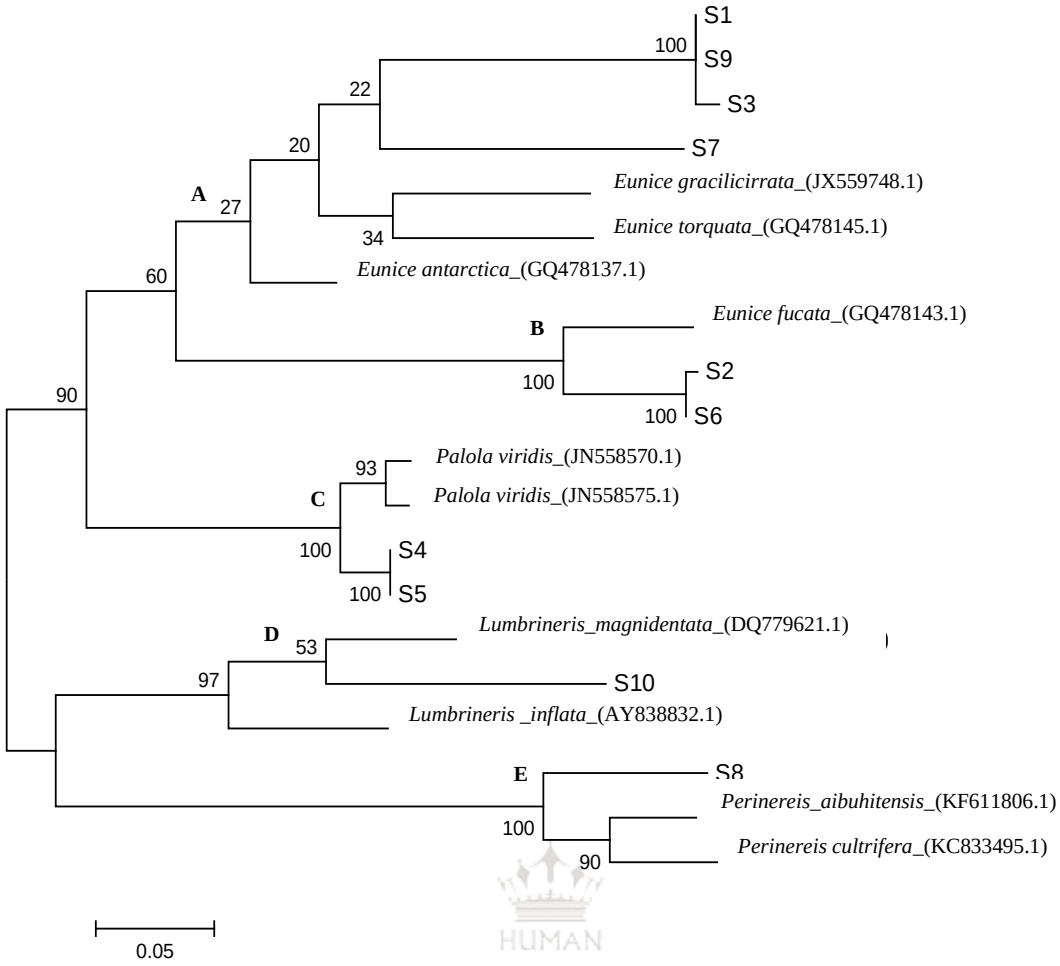

Figure 4. Phylogeny tree of nucleotide sequence based on Maximum Likelihood approach (ML) with Kimura 2-parameter models, bootstrap 1000 replications.

The results of the phylogeny tree reconstruction of Laor worms in the Haria village based on the NJ approach (Figure 3) and ML approach (Figure 4) show the same tree topology (having different bootstrap values); Laor worms cluster into five groups.

Based on the phylogeny tree reconstructed with NJ approach Laor worms cluster into group A consist of sample S1, S3, S7 and S9 clumped into Eunice genus with a bootstrap value of 47%. Laor worms clustered into group B consist of sample S2 and S6 clumped into *Eunice fucata* species with a bootstrap value of 100%. Laor worms clustered into group C consist of sample S4 and S5 clumped into *Palola viridis* species with a bootstrap value of 100%. Laor worms clustered into group D consist of sample S10 identified as *Lumbrineris magnidentata* species with the bootstrap value of 53%, and those clustered into group E consist of sample S8 identified as the Perinereis genus with the bootstrap value of 100%.

Related to ML approach there are also five clusters of Laor worms. Laor worms clustered into group A consist of sample S1, S3, S7, and S9 clumped into the Eunice genus with a bootstrap value of 27%. Group B consist of sample S2 and S6 clumped into *Eunice fucata* species with a bootstrap value of 100%. Group C consist of sample S4 and S5 clumped into *Palola viridis* species with a bootstrap value of 100%. Group D consists of sample S10 classified as *Lumbrineris magnidentata* species with a bootstrap value of 53%. Group E consist of sample S8 clumped into the Perinereis genus with a bootstrap value of 100%.

The calculation results of genetic distance and similarity of Laor worms using the MEGA 5.03 program Kimura 2-parameter model are shown in Table 3.

Table 3. Genetic Distance and Similarity of Laor Worms

Sr. No.	Samples	Genetic distance	Similarity	Species	Group
1.	S1	0.20393	79.60661	*Eunice antarctica*	A
2.	S3	0.21774	78.25638	*Eunice antarctica*	
3.	S7	0.18330	81.6697	*Eunice antarctica*	
4.	S9	0.20393	79.60661	*Eunice antarctica*	
5.	S2	0.11174	88.82575	*Eunice fucata*	B
6.	S6	0.10601	89.39868	*Eunice fucata*	
7.	S4	0.04831	95.16896	*Palola viridis*	C
8.	S5	0.04831	95.16896	*Palola viridis*	
9.	S8	0.13132	86.8681	*Perinereis aibuhitensis*	D
10.	S10	0.17103	82.89727	*Lumbrineris magnidentata*	E

Note: the determination of the name of the species obtained from Genbank was based on the

The value of Similarity and Genetic Distance.

The calculation results of genetic distance and similarity of 10 samples of Laor worms from Haria village obtain genetic distances ranging from 0.04831 to 0.21774 (4.831% -21.774%) with the highest similarity score is 95.16896% and the lowest similarity is 78.25638%. Based on the calculation of genetic distance and similarity values the Laor worm samples can be classified into 5 groups. Genetic distance and similarity do not reach 100% but it can illustrate the similarity of the Laor worm samples closer to particular genus or species from

GenBank. Group A consisting of sample S1, S3, S7, and S9 is identified as *Eunice antarctica* species. Group B consisting of sample S2 and S6 is identified as *Eunice fucata* species. Group C consisting of sample S4 and S5 is identified as *Palola viridis* species. Group D consisting of sample S8 is identified as *Perinereis aibuhitensis* species, group E consisting of sample S10 is identified as *Lumbrineris magnidentata* species.

DISCUSSION

The phenotype description of Laor worm of the Haria village is based on the number of antennas. The Laor worms are classified into 3 groups: Laor worms having two antennas are identified as Perinereis genus of Nereidae family; those having 3 or 5 antennas are identified as Lysidice genus and Eunice (Palola) genus of Eunicidae family; those having no antennas (or the antennas are not visible) cannot be classified even into a particular family.

Based on the results of phylogeny analysis, the Laor worms from the Haria village are classified into 5 groups, namely Laor worms grouping into Eunice genus (Eunicidae family), *Eunice fucata* species (Eunicidae family), *Palola viridis* species (Eunicidae family), grouping into *Lumbrineris magnidentata* species (Lumbrineridae family), and Perinereis genus (Nereidae family). The analysis results of genetic distance and similarity of Laor worms in Haria village show that the Laor worms consist of 5 species, namely *Eunice antarctica* (Eunicidae family), *Eunice fucata* (Eunicidae family), *Palola viridis* (Eunicidae family), *Perinereis aibuhitensis* (Nereidae family), and *Lumbrineris magnidentata* (Lumbrineridae family).

Identification results of Laor worms from Haria village related to the phylogeny analysis, genetic distance and similarity based on 16S rRNA gene sequences show that those Laor worms consist of three family, namely Nereidae family, Eunicidea family, and Lumbrineridae family. These results are not entirely consistent with the phenotype based identification related to the number of antennas, in which the Laor worms only consist of 2 families, namely Nereidae family and Eunicidae family.

The analysis results of phylogeny, genetic distance, and similarity based on 16S rRNA gene sequences found that sample S4 having 3 antennas and samples of S1, S3, S5, S7, and S9 having 5 antennas are identified as Eunice genus and *Palola viridis* species of Eunicidae family. The results of the analysis using 16S rRNA gene only found Eunice genus or Palola genus. These results are in line with the phenotype based identification result of Laor worms,

that those having three or five antennas are identified as Eunice genus or Palola genus of Eunicidae family. On the other hand, Lysidice genus was not found. This condition might be caused by the fact that Lysidice genus *(Lysidice Oele* species) has not been registered in *Genbank* (Liline, *et al* 2016).

Sample S8 having two antennas, based on 16S rRNA gene sequences, was identified as Perinereis genus of Nereidae family. This result is in line with the phenotype based identification result of Laor worms that those having two antennas are identified as Perinereis genus of Nereidae family. Pamungkas (2009a) have identified Laor worm of Latuhalat village phenotypically based on the shape of the intact body as Nereis sp species of Nereidae family. The phenotype observation shows that the Nereis sp. has special features on the head such as having two antennas, 2 pieces of palp, 4 pieces of relatively large eyes and four pairs of tentacular cirri located on the left and right of the head.

Sample S10 having 5 antennas, based on 16S rRNA gene sequences, was identified as *Lumbrineris magnidentata* species of Lumbrineridae family. This result is different from the phenotype based identification result of Laor worms that those having 5 antennas are identified as Lysidice genus and Eunice genus or Palola genus of Eunicidae family. Sample S2 and S6 which do not have an antenna or that the antenna does not appear phenotypically, based on 16S rRNA gene sequences, are identified as *Eunice fucata* species of Eunicidae family. Fauchald (1977) classified Polychaeta based on the number of antennas, consisting of Polychaeta having 2 antennas classified as Nereidae family, while those having 3 or 5 antennas classified as Eunicidae family. Identification of Laor worms done molecularly based on partial 16S rRNA gene sequences can complete the phenotype-based identification particularly with regard to the number of antennas (Liline et al., 2016).

CONCLUSION

1. Phenotype based identification results of Laor worms in the Haria village related to the number of antennas consist of Laor worms having 2 antennas (sample S8), are classified into Perinereis genus of Nereidae family, those having 3 antennas (sample S4) and those having 5 antennas (samples of S1, S3, S5, S7, S9 and S10) are classified into Eunice and Lysidice genus respectively of Eunicidae family, those having no antenna (samples of S2 and S6) cannot be classified into a certain family, because the head is possibly not intact.

2. The results of the phylogeny analysis, genetic distance, and similarity of Laor worms of the Haria village based on 16S rRNA gene sequences show that sample S8 having two antennas are closer to *Perinereis aibuhitensis* species of Nereidae family. Sample S4 having 3 antennas and sample S1, S3, S5, S7, and S9, S10 having 5 antennas are closer to *Palola viridis* species and *Eunice antarctica* species of Eunicidae family. Sample S10 having 5 antennas, is closer to *Lumbrineris magnidentata* species of Lumbrineridae family. Sample S2 and S6 having no an antenna which is possible because the head was not intact are closer to, particularly *Eunice fucata* species of Eunicidae family.

3. The phylogeny tree of Laor worms reconstructed with NJ approach has bootstrap value between 47-100% and the phylogeny trees reconstructed with the ML approach has bootstrap value between 27-100%. The calculation result of genetic distance ranges from 0.04831 to 0.21774 (4.831% -21.774%) with the highest similarity score is 95.16896% and the lowest similarity is 78.25638%.

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