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The Identification of Laor Worms (Polychaeta) in Marine Areas of Ambon Island, Mollucas Province,Indonesia Based on 16s rRNA Gene Sequence

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Abstract : The identification of Laor worms (Polychaeta)found in marine areas of Ambon Island has been done up till now based on phenotypic characters. The research goal is to find out the species ofthose worms andto determine their kinship relationshipbased on 16S rRNA gene sequences.The data were analyzed using sequencing scanner software (ABI) and multiple alignment was performed using *CLUSTAL W,* the phylogeny tree reconstruction based on nucleotide sequence was conductedusing 5.03 MEGA program. The results of the research show that those Laor worm phenotype differs in body shape, color, and size.The results of the phylogenic analysis based on the 16S rRNA gene sequences and the results of genetic distance and similarity analysis relatedshow that the worm samples classfied into 5 groups, namely group A (samples A2, A4, H1, H2, L3, and L4)identified as*Palola viridis*; group B (samples A5, L1, L2, L6, and L9)identified as*Eunice fucata*; group C (samples A7, H4, H5, L5, and L10)identified as*Eunice*; group D (sample A3)identified as *Lumbrineris magnidentata*; group E (samples H3, A1, A6, L7, and L8)identified as Nereidae. The value of NJ and ML *bootstrap* was 28-100% and 22-100% respectively. The genetic distance range between 0.03851 and 0.22936 with the highest and lowest similarity 96.14897% and 77.06362% respectively. The analysis results based on the 16S rRNA gene sequences of those worms were able to complement the identification phenotypically based on the number of antennas.
Keywords: Ambon Island;Laor Worm;Mollucas;Polychaeta; 16S rRNA gene.

Introduction

Marine worms (Polychaeta) found in marine areas of Mollucasare commonly known as Laor worms. Laor wormsare usually found in *swarming*state, in which they get together in a huge number on the surface of sea water for external mating once a year, either in March or in April. At the time of *swarming,* Laor worms are usually caught by local people as a rich protein food[1].

Based on certain indication it was informed that during *swarming.* Laor worms appearing on the surface of sea water to perform external mating were not just one species[2]. Laor worms of Banda marine areasin Mollucashave been identified as *Lysidice oele* (Eunicidae)[3,4]. Laor worms or Wawo worms, found in the Airlouw village, Ambon island marine areas, were identified as a mixture of 13 species of five (5) different families and mainly consist of *Palola viridis*[5].The Laor worm caught in Latuhalat village marine area of Ambon

Island has been identified Phenotypicallyas *Lysidice oele* whose body consisted of segments, had *chaeta,* and had three antennas on its head[1].

The identification process of Laor worms conducted so far is based on phenotype or morphological character. Phenotype or morphological character used to identify the species is quite limited[6]because it is influenced not only by the genotype or genetic trait, but also by the environment[7]. The weakness of the phenotype marker is that it is time consuming, relatively expensive, and influenced by the environment. Besides, the diversity gained is quite limited and inconsistent[8]. Molecular identification is needed to obtain more accurate informations because the analysis is performed at the DNA level.

Two types of DNA often used as animal molecular markers are nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). Mitochondrial DNA (mtDNA) has been widely used as molecular markers in the phylogenetic studies of animals due to the simple structure of their genomes[9]. There are 13 proteins coding genes,2 rRNA genes (12S and 16S) and 22 tRNA genes found in mitochondria[10]. Those protein coding genes found in mitochondria are *3* subunits of *cytochrome oxidase* genes (CO I-III), 7 subunits of NADH *dehydrogenase* genes (ND 1-6 and ND 4L), 2 subunits of ATPase genes (6 and 6L), as well as *cytochrome* b gene (Cyt b).The 16S rRNA gene is a*non-coding* area as well as the most conserved area of mitochondrial DNAwhich functionsto encode ribosomal RNA that plays a role in the translation process[11,12].

In recent years there have been many effortsto identify Polychaeta using molecular data[13]. The genetic relationships and diversities of palola worms *(Palola,* Eunicidae) of tropical North Pacific and Caribbeahave been analyzed using CO1 and 16S rRNA genes[6]. The phylogenetic position and genetic diversity of Neridae-Polychaeta have been analyzed based on molecular data of 16S rRNA sequences[14]. This study aims to determine the relationship of Laor worm (Polychaeta) based on the sequences of 16S rRNA genes and to determine the species of Laor worm (Polychaeta) inmarine areas of several villages in Ambon Island based on 16S rRNA gene sequences.

Materials and Methods

The samples of Laor wormshad been collected from marine areas of Ambon island especially from marine areas of Latuhalat village Nusaniwe district of Ambon city,Allang Village Leihitu district of Central Maluku,Hutumuri village South Leitimur district of Ambon city. Those samples were collected on March 19, 2014. The samples were collected using traditional tools resembling a net (seser) to collect Laor worms from the sea water, rinsed with clean water and preserved in a 70% alcohol. Samples were then observed using an Olympus stereo microscope type SZX 9, described, and then the results of the observations were identified based onthe book entitled *The polychaete Worms Definitions and Keys to the Orders, Families, and Genera*[15].

DNA of Laor worms was isolated using the modified CTAB method[16]specifiedforLaor worms.The DNA samples of Laor worms especially the 16S rRNA gene were amplified in PCR using *Fast* Kapa2G*Readymix* supported by forward primer 5'-CGCCTGTTTATCAAAAACAT-3' and reverse primer 5'-CTCCGGTTTGAACTCAGATCA-3'[17]. The composition of PCR DNA amplification of Laor worms: Predenaturationprocess was carried out in 94 ^{0}C for 5 minutes, denaturation in 94 ^{0}C for 30 seconds, annealing in 45 ^{0}C for 30 seconds, extension in 72 ^{0}C for 30 seconds, and the final extension in 72 ^{0}C for 10 minutes. The results of PCR DNA amplification of Laor worms were then sent for sequencing in First BASE Laboratories Sdn. Bhd.Selangor, Malaysia, supported by ABI Prism 37 oxl 3 *Genetic Analyzer.*

Polychaeta sequences database is taken from GenBank (Table 1) to be aligned with Laor worm sequences obtained from marine areas of Ambon Island.

Table 1. Database of Polychaeta and accession numbers retrieved from *GenBank*

Sample Name	Sample Name
Eunice_antarctica (GQ478137.1)	Lumbrineris_magnidentata (DQ779621.1)
Eunice_cf._limosa (GQ478135.1)	Palola_viridis (JN558570.1)
Eunice_fucata (GQ478143.1)	Palola_viridis (JN558575.1)
Eunice_gracilicirrata (JX559748.1)	Perinereis_aibuhitensis (KF611806.1)
Eunice_torquata (GQ478145.1)	Perinereis_cultrifera (KC833495.1)
Hediste_atoka (AB703092.1)	Perinereis_nuntia (JX644015.1)
Lumbrineris_inflata (AY838832.1)	

The Results of Laor worm sequencing were analyzed by sequencing scanner software (ABI) and*multiple alignments* were performed using*Clustal W* supported byMEGA5:03 program. The reconstruction of the phylogeny tree based on the nucleotide sequences was carried out in*Neighbor Joining* (NJ) and *Maximum Likelihood* (ML) approaches using the MEGA 5.03 program Kimura 2-parameter model. Genetic dictance and similarity calculation were conducted too using the MEGA 5.03 program Kimura 2-parameter model.

Results and Discussion

Phenotype of Laor Worms

Laor worms from were obtained from three villages namely Allang Village (A), Hutumuri Village (H), and Latuhalat Village (L). Theobservationresults of Laor worm phenotypeare presented atTable 2.

Table 2. Phenotype description of Laor worms

No.	Samples	Body shape/Body color/Body length (cm)
1.	A1	Round, flat, and fat body. Rubberyhead and soft tail. 2 papilla sensors at the head.2 antennas. 8 tentaculars cirri. Parapodia clearly visible.2 eyes. Translucent reddishbrown stripes on theleft and right sides ofthe body. ± 9 cm,139 segments.
2.	A2	Long, smooth, and round body. Parapodia clearly visible. 2 eyes. 3 antennas. Dark green body, translucent head andtail.± 26 cm,182 segments.
3.	A3	Long, smooth, and round body. Parapodia clearly visible. 2 eyes. 3 antennas.Orange body,translucent head andtail.± 8 cm,166 segments.
4.	A4	Long, smooth, and round body. Parapodia clearly visible. 2 eyes. 5 antennas. Brown body,translucent head andtail.± 20 cm,143 segments.
5.	A5	Long, flat, round, and fat body. Parapodia clearly visible.5 antennas.Dark moss greenbody, translucenthead and tail.± 16 cm,109 segments.
6.	A6	Round, flat, and fat body. Rubbery head and soft tail. two sensory papillae at the head. 2 antennas. 8 tentacular cirri. Parapodia clearly visible.2 eyes.Translucent light green, striped brown on leftand right sides of thebody. ± 4 cm,69 segments.
7.	A7	Flat, round, and fat body. Parapodia clearly visible.5 antennas. 5 eyes. Reddish orange body,translucent reddishhead and tail.± 9 cm,114 segments.
8.	H1	Long, smooth, and round body. Parapodia clearly visible. 2 eyes. 3 antennas. Dark green body,translucent head andtail . ± 25 cm,187 segments.
9.	H2	Long, smooth, and round body. Parapodia clearly visible.2 eyes. 5 antennas.Brown body,translucent head andtail . ± 25 cm,74 segments.
10.	H3	Round, flat, and fat body. Rubberyhead and soft tail. Two clamps at the head. Two sensory papilla at the head. 2 antennas. 8 tentacular cirri. Parapodia clearly visible.2 eyes. Translucent reddishbrown stripes on theleft and right sides ofthe body. ± 5 cm,166 segments.

11.	H4	Flat, round, and fat body. Parapodia clearly visible. 5 antennas. 2 eyes. Greenish orange body,translucent head andtail . ± 7 cm,97 segments.
12.	H5	Flat, round, and fat body. Parapodia clearly visible. 5 antennas2 eyes. Bright green moss body, translucent headand tail . ± 11 cm,99 segments.
13.	L1	Flat, round, chubby and a little longbody. Parapodia clearly visible.Roundish head, 2 eyes embedded in the head and rather stand out.Bright red meat. ± 28 cm,102 segments.
14.	L2	Flat, round, chubby and little long body. Parapodia clearly visible.Roundish head, 2 eyes embedded in the head and rather stand out. Burgundy. ± 20 cm,118 segments.
15.	L3	Long, smooth, and round body. Parapodia clearly visible. 2 eyes. 5 antennas. Brown body,translucent head andtail. ± 9 cm,163 segments.
16.	L4	Long, smooth, and round body. Parapodia clearly visible. 2 eyes. 3 antennas. Dark green body,translucent head andtail. ± 28 cm,347 segments.
17.	L5	Long, smooth, and round body. Parapodia clearly visible. 2 eyes. 5 antennas. Orange body,translucent head andtail. ± 11 cm,126 segments.
18.	L6	Flat, round, and fat body. Parapodia clearly visible.5 antennas. 2 eyes.Moss bright greenbody, translucent reddishhead and tail. ± 11 cm,111 segments.
19.	L7	Round, flat, and fat body. Rubbery body. Two sensory papillae at the head. 2 antennas. 8 tentacular cirri. Parapodia clearly visible.2 eyes.Bright red meat.± 12 cm,143 segments.
20.	L8	Round, flat, and fat body. Rubberyhead, soft tail. Two sensory papillae at the head. 2 antennas. 8 tentacular cirri. Parapodia clearly visible.4 eyes. Translucent reddishbrown stripes on theleft and right sides ofthe body.± 9 cm,113 segments.
21.	L9	Flat, round, and fat body. Parapodia clearly visible. Roundish head, 2 eyes embedded in the head and rather stand out. 3 antennas.Dark moss greenbody, translucenthead and tail. ± 8 cm,121 segments.
22.	L10	Flat, round, and fat body. Parapodia clearly visible.2 eyes. 5 antennas.Orange body, headand tail somewhattranslucent and orange. ± 21 cm,118 segments.

Based on the descriptions explained at Table 2, it can be concluded that phenotype of those Laor wormsvaries in relation to the shape, color, and size of the body. One of the most important parts of Polychaeta used for identification is the head[18]. The Polychaeta body consisting of presegmental, segmental, and postsegmental parts, with the presegmental parts having the palp, antenna, and prostomium[15]. In this connection, the head of the Laor worm can be used to identify Laor worms phenotypically because it has a number of antennas. Based on the of antenna number, the Laor worms can be classified as those having 2 antennas found in the sample A1, A6, H3, L7 and L8, those having 3 antennas found in sample A2, A3, H1, L4 and L9, those having 5 antennas found in sample A4, A5, A7, H2, H4, H5, L3, L5, L6, and L10, and those having no antenna found in sample L1 and L2. Polychaeta have been identified based on the number of antennas, namely Nereidae having 2 antennas, whereas Eunicidae having 3 antennas or 5 antennas[15]. At the head of the Laor worm *(Lysidice Oele)* there were 3 antennas and having no tiny spines while at the head of Palolo worms *(Eunice viridis)* there were 5 antennas having tiny spines on the antennas[1]. *Lysidice Oele* (*Lysidice* genus) and *Palola viridis* (*Palola* genus) or *Eunice viridis* (*Eunice* genus) were classified into Eunicidae family[15].

Thus, the samples of those Laor worm can be classified into 3 groups. The first group is Nereidae family having2 antennas namely samplesof A1, A6, H3, L7 and L8, may consist of Perinereis and Hediste genus. The second group is Eunicidae family having 3 antennas namely samples of A2, A3, H1, L4 and L9 or having 5 antennas namely samples of A4, A5, A7, H2, H4, H5, L3, L5, L6, and L10 which may consist of *Lysidice* and *Eunice* genus. The third group is Laor worms which do not have any antennas namely samples of L1 and L2. The third group cannot be grouped into a certain family because the head of the samples identified might be damaged, so the antennas could not be observed. Phenotypical Identification often has some difficulties because the Laor worms found are often damaged. The Laor wormsare very soft and mushy, and the way of catching the samples using traditional tools (seser), so it can only catch the Laor wormsin sea

levels, often swayed away by the waves or currents of sea water[2]. Therefore, the molecular identification needs to be done to support the phenotypical identification result.

25 samples of Laor worms in marine areas of Ambon Island have been identified phenotypically based on the epitoke (body parts of the worms containing sex cells), namely the epitoke schizogamy (stolon) and the epitoke epigamy[19]. Those samples of Laor worm identified based on epitoke schizogamy consist of *Eunicidae* family including *Palola* sp.,*Eunicidae* sp. 1, and *Eunicidae* sp. 2. Furthermore, on the other hand, Laor worm samples identified based on epitoke epigamy consist of five families namely Eunicidae family that including *Eunice* sp. 1, *Eunice* sp. 2, *Eunice* sp. 3, *Eunice* sp. 4, *Eunice* sp. 5, *Eunice* sp. 6, and *Lysidice oele;* Euphrosinidae family that including *Euphrosine* sp.; Lumbrineridae family including *Lumbrineris* sp. 1, *Lumbrineris* sp. 2, and *Lumbrineris* sp. 3; Nereidae family including*Ceratonereis singular australis, Composetia marmorata, Neanthes cf. Gisserana, Neanthes masalacensis, Neanthes unifasciata, Nereis* sp., *Perinereis helleri, Perinereis nigropunctata,* and *Solomonereis marauensis;* and Scalibregmatidae family including *Hyboscolex verrucosa* and *Scalibregmatidae* sp. The results of the identification indicated that there were a few Laor worm groups that could not be identified to the species level because of the lack of taxonomic information on Polychaeta in Indonesia.

Identification based on 16S rRNA gene sequences

The identification of laor worms using 16S rRNA gene was carried out based on the results of phylogenetic analysis, genetic distance, and similarity value (similarity) of Laor worms. The length of 16 S rRNA genessuccessfully amplified from 22 samples of Laor worms ranged from 465 until 520 bp. The results of *multiple alignment* were then used to reconstruct the phylogeny tree using two different approaches, namely*Neighbor Joining* (NJ) (Figure 1) and *Maximum Likelihood* (ML) (Figure 2). The results of genetic distance and similarity calculationof Laor worms using the MEGA5.03 program Kimura 2-parameter models are presented in Table 3.

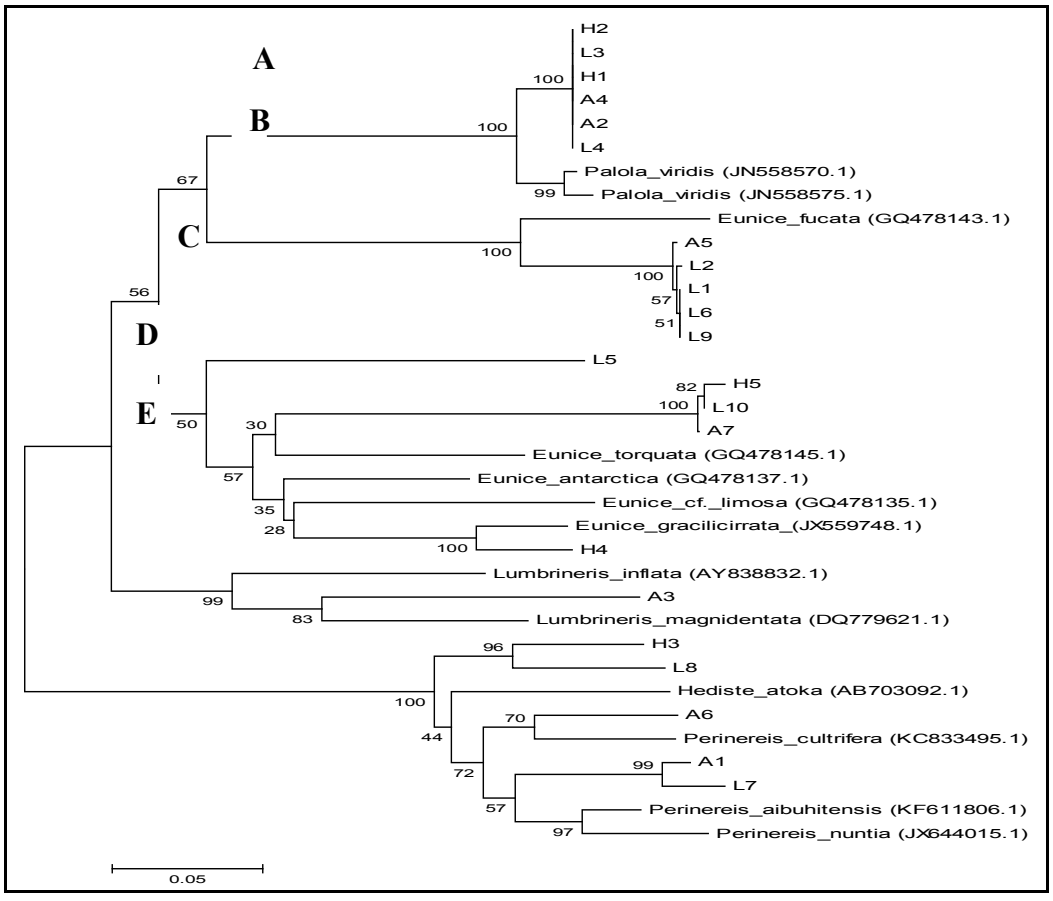

Figure 1. Phylogeny tree of laor wormsbased on 16S rRNA gene sequences according to*Neighbor Joining* (NJ) approach(*bootstrap* 1000 replications)

Table 3. Genetic Distance and Similarity of Laor Worms

No.	samples	Genetic distance	similarity	Species	Group
1.	A2	0,03851	96.14897	*Palola viridis*	A
2.	A4	0,03851	96.14897	*Palola viridis*	
3.	H1	0,03851	96.14897	*Palola viridis*	
4.	H2	0,03851	96.14897	*Palola viridis*	
5.	L3	0,03851	96.14897	*Palola viridis*	
6.	L4	0,03851	96.14897	*Palola viridis*	
7.	A5	0,11620	88,37970	*Eunice fucata*	B
8.	L1	0,11313	88,68650	*Eunice fucata*	
9.	L2	0,11636	88,36392	*Eunice fucata*	
10.	L6	0,11313	88,68650	*Eunice fucata*	
11.	L9	0,11313	88,68650	*Eunice fucata*	
12.	A7	0,22234	77,76618	*Eunice torquata*	C
13.	H4	0,06210	93,79001	*Eunice gracilicirrata*	
14.	H5	0,22936	77,06362	*Eunice Antarctica*	
15.	L5	0,20177	79,82255	*Eunice Antarctica*	
16.	L10	0,22200	77,80039	*Eunice Antarctica*	
17.	A3	0,17423	82,57723	*Lumbrineris magnidentata*	D
18.	A1	0,10993	89,00702	*Perinereis aibuhitensis*	E
19.	A6	0,09455	90,54519	*Perinereis cultrifera*	
20.	H3	0,14445	85,55492	*Perinereis aibuhitensis*	
21.	L7	0,10689	89,31143	*Perinereis aibuhitensis*	
22.	L8	0,12805	87,19476	*Perinereis aibuhitensis*	

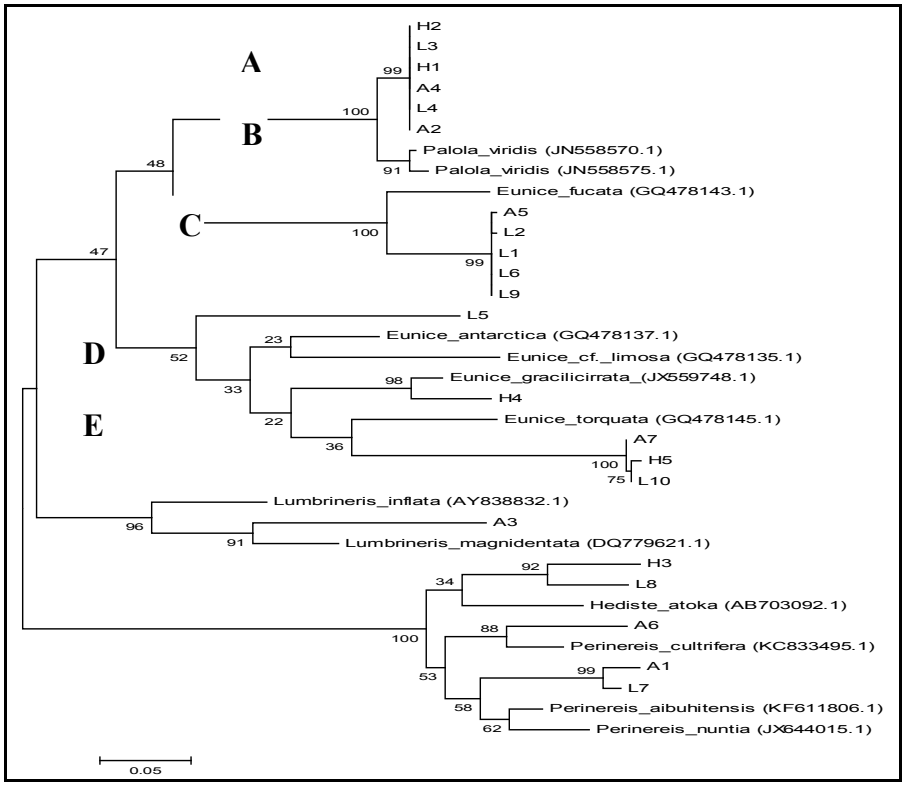

Figure 2. Phylogenytree of laor worms based on 16S rRNA gene sequences according to *Maximum Likelihood* (ML) approach(*bootstrap* 1000 replications)

The reconstruction of the phylogeny tree of Laor worms based on the nucleotide sequence of 16S rRNA gene using the *Neighbor Joining* (NJ) and *Maximum Likelihood* (ML) approaches shows the same topology tree but having different *bootstrap* values (Figure 1 and Figure 2). The results of phylogenetic analysis of 16S rRNA gene sequences using NJ and ML approaches showthat the samples of Laor Wormsare grouped into 5 groups (A, B, C, D, and E).

Related to NJ approach group A of Laor worms consists of samples A2, A4, H1, H2, L3, and L4grouped to*Palola viridis* having a bootstrap value of 100%. Group B consists of the samples A5, L1, L2, L6, and L9 grouped to*Eunice fucata* havinga bootstrap value of 100%. Group C consists of samples A7, H4, H5, L5 and L10 grouped to the *Eunice* having bootstrap value of 50%. Group D consists of sample A3 grouped to *Lumbrineris magnidentata* havinga bootstrap value of 99%. Group E consists of a samples of H3, A1, A6, L7 and L8 grouped to Nereidae consisting of *Hediste* and *Perinereis*havinga bootstrap value of 100%.

Related to ML approachgroup A of Laor worms is grouped to *Palola viridis* having a bootstrap value of 100%. Group B isgrouped to *Eunice fucata* havinga bootstrap value of 100%.Group C is grouped to the *Eunice* having a bootstrap value of 52%. Group D isgroupedto*Lumbrineris magnidentata* havinga bootstrap value of 96%, and Group E is grouped to Nereidaeconsisting of *Hediste* and *Perinereis*having a bootstrap value of 100%. Based on a phylogenetic analysis conducted related to Neridae-Polychaeta, it was foundthat the bootstrap valuewas 1-100%[14]. *Bootstrap* value below 50% cannotbe categorized that the results of topology are proper to be used to determine the species[20].

The calculation results of genetic distance as well as similarityof the 22 Laor worm samplesfind out that genetic distance ranged from 0,03851 - 0,22936 with the highest similarity valueof 96.14897% and the lowest similarity valueof 77.06362%. Based on the calculation of genetic distance and similarity values, Laor worm samples can be classified into 5 groups. Genetic distance and similarity values uncovered in this researchare are below 100% but those values can illustrate the closeness of Laor worm samples to particular genus or species documented in the GenBank. Referring to the GenBank datathe group A samples are grouped to P*alola Viridis*,the group B samplesare grouped to*Eunice fucata*, and the group C samplesare grouped to*Eunice*. Furthermore,the group D sample is grouped to*Lumbrineris magnidentata*, and the group E samples are grouped to*Nereidae*belonged to *Hediste* and *Perinereis*. The lowest and the highest genetic distance of Laor worm samples are 0,03851 or 3,851% and 0,22936 or 22,936%respectively. Species having >3% genetic distance were classified into different species[21].

The Comparison of the Results between the Phenotype basedIdentification and the 16S rRNA Gen Sequence based Identification

According to the results of the phenotype identification based on the number of antennas, the Laor worms were classified into 3 groups: Laor worms having 2 antennas identified as Nereidae family, Laor worms having 3 antennas or 5 antennas identified as Eunicidae family, and Laor worms having no antenna. The results of the tree phylogeny analysis, genetic distance, and similarity analysis based on the 16S rRNA gene sequences show that the Laor worms grouped into several species, such as: *Palola viridis, Eunica fucata, Eunice torquata, Eunice gracilicirrata, Eunice antarctica, Lumbrineris magnidentata, Perinereis aibuhitensis, Perinereis cultrifera, and Hediste atoke.* The results of the analysis of phylogeny, genetic distance, and similarity explain that the Laor wormshaving no antenna, namely sample L1 and L2, are grouped into *Eunice fucata*species.

Based on the results of the analysis of phylogeny, genetic distance calculation, and similarity analysis (the result of 16S rRNA gene sequence analysis), it is found that the Laor wormsgrouped as samples A1, A6, H3, L7, L8 having2 antennas tend to be closer to Nereidae consisting of *Perinereis aibuhitensis, Perinereis cultrifera, and Hediste atoke.* The results of this analysis are similar with the phenotype identification consisting of *Perinereis* and *Hediste.* On the other hand samples A2, H1 L4, L9 having 3 antennas, and the samples A4, H2, L3, A5, L6, A7, H4 , H5, L5, L10 having 5 antennas tend to be closer to *Palola viridis, Eunice torquata, Eunice gracilicirrata, Eunice antarctica* (based on 16S rRNA gene sequences)are included in Eunicidae. The results of molecular analysis show that only *Eunice* or *Palola* are matched with the phenotype identification. On the other hand *Lysidice* (*Lysidice oele)* cannot be traced to the molekuler identification because the species as well as the genus has not yet been listed in *GenBank.* Sample A3 having3 antennas, which iscloser to the Lumbrineris including *Lumbrineris magnidentata* (based on 16S rRNA gene sequences),

is apparently different from the phenotype identification results grouping the sample to Eunicidae. Samples L1 and L2 having no antennas,which cannot be identified phenotipically are closer to Eunicidae particularly *Eunice fucata* (based on 16S rRNA gene sequences). Based on the explanation above, it can be stated that the molecular identification of the Laor worms can complement the phenotype identification particularly relating to the number of antennas.

Conclusion

1. The Phenotype of Laor worms inhabiting marine areas of Ambon island waters vary from body shape, body color, and body size. Based on the number of antenna, there are 4 group of Laor worms: thosehaving 2 antennas (samples A1, A6, H3, L7 and L8),those having 3antennas (samples A2, A3, H1, L4 and L9), those having 5antennas(samples A4, A5, A7, H2, H4, H5, L3, L5, L6, and L10) and those having no antenna (samples L1 and L2).
2. The results of phylogenetic analysis based on the 16S rRNA gene sequences (NJ and ML approaches), the distance calculationand genetic similarity analysis of Laor worm samples show that those worm are grouped into 5 groups: group A (A2, A4, H1, H2, L3 and L4)identifiedas *Palola viridis,* group B (A5, L1, L2, L6, and L9)identifiedas *Eunice fucata,* group C (A7, H4, H5, L5 and L10)identified as*Eunice,* group D (A3)identified as *Lumbrineris magnidentata,* and group E (H3, A1, A6, L7 and L8)identified as Nereidae . Phylogenetic analysis using NJ approach get the *bootstrap* values of 28-100%, and ML approach get the *bootstrap* values of 22-100%. The results of genetic distance calculation of Laor worm samples range from 0.03851-0.22936 with the highest similarity score 96.14897% and the lowest similarity score 77.06362%.
3. The results of the Laor worm identification based on the phylogeny analysis, genetic distance calculation and similarity analysis complement the phenotype based identification particularly relating to the number of antennas. The first group having2 antennas, namely samples A1, A6, H3, L7, L8 tend to be closer to the Nereidae consisting of *Perinereis aibuhitensis, Perinereis cultrifera, and Hediste atoke;* the second group having 3 antennas, namely samples A2, H1 L4, L9, as well as those having 5 antennas namely samples A4, H2, L3, A5, L6, A7, H4, H5, L5, L10, tend to be closer to the Eunicidae consisiting of *Palola viridis, Eunice torquata, Eunice gracilicirrata, Eunice antarctica* included in Eunicidae; sample A3 having3 antennas, which is closer to Lumbrineridae including *Lumbrinerismagnidentata* is apparently different from the phenotype identification result grouping the sample to Eunicidae; the third group consisting the samples having no antennas (sample L1 and L2) are closer to Eunicidae *(Eunice fucata).*

References

1. Radjawane T.R., Laor: Cacing Laut Khas Perairan Maluku (Laor: Typical Sea Worms of Maluku), Youth Competition of Scientific Research, Jakarta, Ministry of Education and Culture of the Republic of Indonesia, Jakarta, 1982, 1- 29.
2. Pamungkas J., Pengamatan Jenis Cacing Laor (Annelida, Polychaeta) di Perairan Desa Latuhalat Pulau Ambon, dan Aspek Reproduksinya (Observation on Laor type worms (annelids, Polychaeta) in Marine Area of Latuhalat Village of Ambon Island, and reproductive aspects), J. Triton, 2009,5(2): 1-10.
3. Horst R.,Wawo and Palolo Worms, J. Nature, 1904, 69: 582. [In English]. DOI: 10.1038 / 069582a0
4. Horst R.,Over de "Wawo" von Rumphius (Lysidice Oele n.sp.), Rumphius Gedenkbook Kolon MusHaarlem, 1905, 105-108. [In Dutch].
5. MartensJ.M, Heuer U, Hartmann-ö Schr der G, Mas-senschw ä rmen des S ü DSEE-Palolowurms (Palola viridis Gray) und wie weiterer PolychaetenLysidice Oele Horst and Lumbrineris natans n. sp. auf Ambon (Molukken; Rodel), Mitt. Hamb., Zool. Mus. Inst, 1995, 92: 7-34. [In German].
6. Schulze A., The phylogeny and Genetic Diversity of Palolo Worm (Palola, Eunicidae) from The Tropical North Pacific and the Caribbean, J. Biol. Bull, 2006, 210: 25-37.
7. Cahyarini R.D, Yunus A., and Purwanto E.,Identifikasi keragaman genetik beberapa varietas lokal kedelai di Jawa berdasarkan analisis isozim (Identification of the genetic diversity of local varieties of soybean in Java based on isozyme analysis). J. Agrosains, 2004, 6 (2): 79-83.

8. Zulfahmi, Penanda DNA untuk Analisis Genetik Tanaman (DNA Markers for Plant Genetic Analysis). J. Agroteknologi,2013, 3 (2): 41-51.
9. AviseJ.C., phylogeography: The History and Formation of Species, Cambridge (Massachusetts): Harvard University Press. 2004, 447.
10. Melnick D.J. and Hoelzer G.A., What is good for the mtDNA in the study of primate evolution? J. Evol. Anthropol, 1993, 2 (1): 2-10.
11. Case R.J., Boucher Y., Dahlloff I., Holmstrom C., Doolittle W.F.,andKjelleberg S., Use of 16S rRNA and rpoB Genes as Molecular Markers for Microbial Ecology Studies, J. Applied and Environmental Microbiology, 2007, 73: 278-288.
12. Parker P.G, Snow A.A., Schug M.D., Booton G.C.,andFuerst P.A., What Molecules Can Tell Us About Populations: Choosing and Using A Molecular Marker, J. Ecology, 1998, 79 (2): 361-382.
13. Bleidorn C., Vogt L., and Bartolomaeus T., A contribution to polychaete phylogeny using 18S r DNA sequence data, Evolutionary Systematics Zoological J. Res., 2003, 41: 186-195.
14. SatheeshkumarP. and Jagadeesan L., Phylogenetic Position and Genetic Diversity of Neridae-Polychaeta Based on Molecular Data from 16S rRNA Sequences, J. of Scientific Research, 2010, 6 (6): 550-555.
15. Fauchald K., The Polychaete Worms Definitions and Keys to The Orders, Families, and Genera. Los Angles: The Allan Hancock Foundation Universityof Southtern California, 1977, 1-188.
16. Dhakshanamoorthy D. and Radhakrishnan S., Extraction of Genomic DNA From Jatropha sp. Using Modified CTAB Method, Rom. J. Biol. Plant Biol., 2009, 54 (2): 117-125.
17. Palumbi S.R., Martin A.P., Romano S.L., McMillan W.O.,SticeL., Grabowski G.,The Simple Fool's Guide to PCR, Dept. Of Zoology, University of Hawaii, Honolulu, 1991, 46.
18. Day J.H., A Monograph on the Polychaeta of Southern Africa, Part I: Errantia. British Museum (Natural Hisstory) Publications, 1967,1-458.
19. Pamungkas J., Species richness and macronutrient content of Wawo worms (Polychaeta, Annelida) from Ambonese waters, Maluku, Indonesia, J. Biodiversity Data, 2015, 3: e4251. doi: 10.3897 / bdj.3.e4251
20. Gregory T.R., Understanding Evolutionary Trees, Evo Edu Outreach, 2008, (1): 121-137.
21. Hebert P.D.N., Cywinska A., Ball S.L., and deWaard J.R., Biological Identifications through DNA Barcodes, The Royal, 2003, 270: 313-321.

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