PHYLOGENETIC TREE ON LERNAEOSIS IN ARWANA FISH (*Scleropages jardinii*)

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ABSTRACT

Arwana Irian fish is one of the endangered species. Some studies on arwana Irian fish found that Lernaeosis attacked arwana Irian fish. Lernaeosis is one of the diseases that cause the high mortality in juvenile fish. The objectives of this research was to find out the species of *Lernaea* (Copepoda) often attacked arwana Irian fish. *Lernaea* sp. was collected from Papua and Jakarta (Java). They were fixed in the ethanol absolute solution for DNA sequencing in 28S DNA region with primer 28SF (5'-ACA ACT GTG ATG CCC TTA G-3'); 28SR (5' TGG TCC GTG TTT CAA GAC G-3'). It was found five different species of *Lernaea* and one of them was thought as a new species, based on the morphology. However, based on the phylogenetic analysis, they showed three different groups. *Lernaea cyprinacea* G., *L. papuensis*, *L. devastatrix*, and *L. lophiara* were in one group; *L. cyprinacaea* and *L. oryzophila* were in one groups; and the new *Lernaea* sp. was in the different group.

KEYWORDS: ***Scleropages jardinii*, Lernaeosis, Phylogenetic tree**

INTRODUCTION

Lernaeid copepods cause serious deleterious effects on their freshwater fish hosts (Kabata, 1985). One factor which may contribute to the increased infestation level during the drying phase was the reduction in water volume. The economic loss caused by lernaeid ectoparasites has increased due to numerous epizootics occurring among the most important farmed fish in various parts of the world (Tasawar *et al*., 2007). A lernaeid population found only on jaws of *Tilapia* (*Oreochromis* spp.) *Lernaea victoria* (Fryer, 1961) has been identified as *Lernaea cyprinacea*. Other lernaeids of African fish were endemic, *Lernaea barnimiana* may be found on both cyprinid and cichlid hosts (Paperna, 1996). The embedded anchors were surrounded by fibrous granulation tissue, and there was considerable leucocytic response below the dermis. Myofibril degeneration and hemorrhage were noted in most sections of fish (Berry *et al*., 1991). *Lernaea minuta* was found in Javanese carp (*Puntius gonionotus*), which inhibated in Selangor, West Malaysia (Kularatne *et al*., 1994). *Lernaea cyprinacea* was reported from several fish such as goldfish (*Carassius auratus* L.), *Helostoma temmincki*, *Cyprinus carpio*, *Carassius auratus* L. (Shariff *et al*., 1986). Infestations of *Lernaea cyprinacea* was found on four native fish species (*Galaxias occidentalis* Ogilby; *Edelia vittata* Castelnau; *Tandanus bostocki* Whitley) and three introduced fish species (*Carassius auratus* L.; *Gambusia holbrooki* (Girard); *Phalloceros*

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caudimaculatus (Hensel) (Marina *et al*., 2008). The phylogenetic relationships among the Ergasilidae, included *Lernaea cyprinacea* from China, were examined using neighbor-joining, maximum parsimony, maximum likelihood, and Bayesian inference methods based on partial sequences of 18S and 28S ribosomal deoxyribonucleic acid, respectively (Song *et al*., 2008). However, the molecular sequence analysis and phylogenetic relationships among the parasitic *Lernaea* sp. still unclear. Here we would like to provide additional resolution for the interpretation of *Lernaea* sp., in addition to use identification based on morphology. The objective of this paper is to identify *Lernaea* sp. based on molecular analysis and to find phylogenetic relationships among 6 spesies of *Lernaea.*

MATERIALS AND METHODS

A total of 20 of parasitic copepods belonging to Lernaeidae were collected from arwana fish in Papua, Indonesia. Copepods were examined under stereo microscope to identify species. Specimens were fixed in ethanol absolute for molecular study. Primer was used, 28SR(5'–TGG TCC GTG TTT CAA GAC G–3') and 28SF (5'–ACA ACT GTG ATG CCC TTA G–3'); 28SR (5' TGG TCC GTG TTT CAA GAC G–3').

Following conditions of cycles were 94°C for 5 minutes, followed by 30 cycles of 94°C for 30 second, 54°C for 30 second and 72°C for 1 minute with a final extension at 72°C for 5 minutes (Song *et al*., 2008). Six specimens of *Lernaea* were then purified and sequenced in the 28S region.

Results of DNA sequencing were sorted using Clustal W, continued with limited editing manually (Thompson *et al*., 1994). The distances between pairs were corrected with Kimura two-parameter model using MEGA 4 (Kumar *et al*., 2004). All sequences were aligned with Clustal W. Maximum parsimony (MP) and Neighbor joining (NJ) methods using 1,000 bootstrap re-sampling were used to get the phylogenetic tree.

RESULTS AND DISCUSSIONS

The sequense results showed that there were several gene variation between *Lernaea lophiara* (L1), *L. papuensis* (L10), and *L. devastatrix* (L11) in one group; *Lernaea cyprinacaea* (L2) dan *L. oryzophilla* (L3) were in one group; and *Lernaea* sp. (L4) was in one group (Figure 1). The sequences result of *Lernaea* in Indonesia has not been reported yet.

From a total of 16 sequences at 28S rDNA region where 430 is variable from 754 of parsimony, including gaps. And 289 is the Converse of 754 including the gap (Table 1).

Phylogenetic tree using maximum parsimony (MP) analysis found three groups among

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L 1 :  AGCACTGA –CCGCCAGCTTTTGAAAGGGTTGCGCGGAATGTAGTGTTTG      50
L 2 :  AGCACTGAAC- GCCAGCTTTTGAAAGGGTTGCGCGGAATGTAGTGTTTG
L 3 :  AGCACTGAAC- GCCAGCTTTTGAAAGGGTTGCGCGGAATGTAGTGTTTG
L 4 :  AGCACTGAACCGCCAGCTTTTGAAAGGGTTGCGCGGAATGTAGTGTTTG
L10:  AGCACTGA - CCGCCAGCTTTTGAAAGGGTTGCGCGGAATGTAGTGTTTG
L11:  AGCCTGGA - CCGCCAGCTTTTGAAAGGGTTGCGCGGAATGTAGTGTTTG

L 1 :  GGAGAGCCTTCTCATGATGCGCGGTGCAAAATCTGTCTAAGTCCACCTTG      100
L 2 :  GGAGAGCCTTCTCATGATGCGCGGTGCAAAATCTGTCTAAGTCCACCTTG
L 3 :  GGAGAGCCTTCTCATGATGCGCGGTGCAAAATCTGTCTAAGTCCACCTTG
L 4 :  GGAGAGCCTTCTCATGATGCGCGGTGCAAAATCTGTCTAAGTCCACCTTG
L10:  GGAGAGCCTTCTCATGATGCGCGGTGCAAAATCTGTCTAAGTCCACCTTG
L11 :  GGAGAGCCTTCTCATGATGCGCGGTGCAAAATCTGTCTAAGTCCACCTTG

L 1 :  ACTGGGGCCACTACCCATAGAGGGTGATAGGCCCGTAAGACAGTCTGCGT      150
L 2 :  ACTGGGGCCACTACCCATAGAGGGTGATAGGCCCGTAAGACAGTCTGCGT
L 3 :  ACTGGGGCCACTACCCATAGAGGGTGATAGGCCCGTAAGACAGTCTGCGT
L 4 :  ACTGGGGCCACTACCCATAGAGGGTGATAGGCCCGTAAGACAGTCTGCGT
L 10:  ACTGGGGCCACTACCCATAGAGGGTGATAGGCCCGTAAGACAGTCTGCGT
L 11:  ACTGGGGCCACTACCCATAGAGGGTGATAGGCCCGTAAGACAGTCTGCGT
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Figure 1. Sequencing result of *Lernaea* species from arwana Irian fish

Figure 1. (Continued)

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L 1 :  GTTGTGCTGGCTTTTTCCCTAGAGTCGAGTTGCTTGGGAGTGCAGCTCAAA      200
L 2 :  GTTGTGCTGGCTTTTTCCCTAGAGTCGAGTTGCTTGGGAGTGCAGCTCAAA
L 3 :  GTTGTGCTGGCTTTTTCCCTAGAGTCGAGTTGCTTGGGAGTGCAGCTCAAA
L 4 :  GTTGTGCTGGCTTTTTCCCTAGAGTCGAGTTGCTTGGGAGTGCAGCTCAAA
L10:  GTTGTGCTGGCTTTTTCCCTAGAGTCGAGTTGCTTGGGAGTGCAGCTCAAA
L11:  GTTGTGCTGGCTTTTTCCCTAGAGTCGAGTTGCTTGGGAGTGCAGCTCAAA

L 1 :  GTGCGTGGTAAACTCCACGTAAGGCTAAATATCACCCCGAGACCGATAGC      250
L 2 :  GTGCGTGGTAAACTCCACGTAAGGCTAAATATCACCCCGAGACCGATAGC
L 3 :  GTGCGTGGTAAACTCCACGTAAGGCTAAATATCACCCCGAGACCGATAGC
L 4 :  GTGCGTGGTAAACTCCACGTAAGGCTAAATATCACCCCGAGACCGATAGC
L10:  GTGCGTGGTAAACTCCACGTAAGGCTAAATATCACCCCGAGACCGATAGC
L11:  GTGCGTGGTAAACTCCACGTAAGGCTAAATATCACCCCGAGACCGATAGC

L 1 :  GAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAGTTCA      300
L 2 :  GAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAGTTCA
L 4 :  GAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAGTTCA
L10:  GAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAGTTCA
L11:  GAACAAGTACCGTGAGGGAAAGTTGAAAAGAACTTTGAAGAGAGAGTTCA

L 1 :  ATAGTACGTGAAACTGTGTAGCGGTAAACAGAGGGGCTCTCGAAGTCCAG      350
L 2 :  ATAGTACGTGAAACTGTGTAGCGGTAAACAGAGGGGCTCTCGAAGTCCAG
L 3 :  ATAGTACGTGAAACTGTGTAGCGGTAAACAGAGGGGCTCTCGAAGTCCAG
L 4 :  ATAGTACGTGAAACTGTGTAGCGGTAAACAGAGGGGCTCTCGAAGTCCAG
L10:  ATAGTACGTGAAACTGTGTAGCGGTAAACAGAGGGGCTCTCGAAGTCCAG
L11:  ATAGTACGTGAAACTGTGTAGCGGTAAACAGAGGGGCTCTCGAAGTCCAG

L 1 :  GCTGGAGATTCAGGTTGCCAGATGGCTAGTTTGGCTGGTGCGAAGATCTG      400
L 2 :  GCTGGAGATTCAGGTTGCCAGATGGCTAGTTTGGCTGGTGCGAAGATCTG
L 3 :  GCTGGAGATTCAGGTTGCCAGATGGCTAGTTTGGCTGGTGCGAAGATCTG
L 4 :  GCTGGAGATTCAGGTTGCCAGATGGCTAGTTTGGCTGGTGCGAAGATCTG
L10 :  GCTGGAGATTCAGGTTGCCAGATGGCTAGTTTGGCTGGTGCGAAGATCTG
L11 :  GCTGGAGATTCAGGTTGCCAGATGGCTAGTTTGGCTGGTGCGAAGATCTG

L 1 :  CATAGGACTTTGTGCCTGGTCAAATGTTGTTGGCTGGATGGCGATTGCAC      450
L 2 :  CATAGGACTTTGTGCCTGGTCAAATGTTGTTGGCTGGATGGCGATTGCAC
L 3 :  CATAGGACTTTGTGCCTGGTCAAATGTTGTTGGCTGGATGGCGATTGCAC
L 4 :  CATAGGACTTTGTGCCTGGTCAAATGTTGTTGGCTGGATGGCGATTGCAC
L10 :  CATAGGACTTTGTGCCTGGTCAAATGTTGTTGGCTGGATGGCGATTGCAC
L11 :  CATAGGACTTTGTGCCTGGTCAAATGTTGTTGGCTGGATGGCGATTGCAC

L 1 :  TTCTCTGGCCTAGCAATGGGCGCGACGAGCCACTGAGAGCGAATCAAGTG      500
L 2 :  TTCTCTGGCCTAGCAATGGGCGCGACGAGCCACTGAGAGCGAATCAAGTG
L 3 :  TTCTCTGGCCTAGCAATGGGCGCGACGAGCCACTGAGAGCGAATCAAGTG
L 4 :  TTCTCTGGCCTAGCAATGGGCGCGACGAGCCACTGAAAGCGAATCAAGTG
L10 :  TTCTCTGGCCTAGCAATGGGCGCGACGAGCCACTGAGAGCGAATCAAGTG
L11 :  TTCTCTGGCCTAGCAATGGGCGCGACGAGCCACTGAGAGCGAATCAAGTG

L 1 :  CGTGGGTGAAGTTTGCTTCAACAGTCTTATGGCTGGTGTTGTGAGCCCCG      550
L 2 :  CGTGGGTGAAGTTTGCTTCAACAGTCTTATGGCTGGTGTTGTGAGCCCCG
L 3 :  CGTGGGTGAAGTTTGCTTCAACAGTCTTATGGCTGGTGTTGTGAGCCCCG
L 4 :  CGTGGGTGAAGTTTGCTTCAACAGTCTTATGGCTGGGGTTGGGAGCCCCC
L10 :  CGTGGGTGAAGTTTGCTTCAACAGTCTTATGGCTGGTGTTGTGAGCCCCG
L11 :  CGTGGGTGAAGTTTGCTTCAACAGTCTTATGGCTGGTGTTGTGAGCCCCG
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Figure 1. (Continued)

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L 1 : CGTTTTCTGGCTTCGATTTCGGTGGTCTTATGTATGGAGATAGGACAGAC      600
L 2 : CGTTTTCTGGCTTCGATTTCGGTGGTCTTATGTATGGAGATAGGACAGAC
L 3 : CGTTTTCTGGCTTCGATTTCGGTGGTCTTATGTATGGAGATAGGACAGAC
L 4 : CGTTTTCTGGCTTCAATTTCGGGGGTCCTTAAAAAATGAGATAGGGACAC
L10 : CGTTTTCTGGCTTCGATTTCGGTGGTCTTATGTATGGAGATAGGACAGAC
L11 : CGTTTTCTGGCTTCGATTTCGGTGGTCTTATGTATGGAGATAGGACAGAC

L 1 : TCGTTTATAGCGAGTGCCGCTTTTGTGGCACTGTCTTTGTCCGACATCTG      650
L 2 : TCGTTTATAGCGAGTGCCGCTTTTGTGGCACTGTCTTTGTCCGACATCTG
L 3 : TCGTTTATAGCGAGTGCCGCTTTTGTGGCACTGTCTTTGTCCGACATCTG
L 4 : CACTCCAAAATAGCGGAGTGCCGGCCTTTGTTGGCCCTGTTCTTTTGTGC
L10 : TCGTTTATAGCGAGTGCCGCTTTTGTGGCACTGTCTTTGTCCGACATCTG
L11 : TCGTTTATAGCGAGTGCCGCTTTTGTGGCACTGTCTTTGTCCGACATCTG

L 1 : TCGCGAGTAGGTCGGTGGCCTCTCTGACCCGTCTTG      754
L 2 : TCGCGAGTAGGTCGGTGGCCTCTCTGACCCGTCTTG
L 3 : TCGCGAGTACGTCGGTGGCCTCTCTGACCCGTCTTG
L 4 : GTTGGCCCCTGGTTCTTTGTGGCGGGACATACGGG
L10 : TCGCGAGTAGGTCGGTGGCCTCTCTGACCCGTCTTG
L11 : TCGCGAGTAGGTCGGTGGCCTCTCTGACCCGTCTTG
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Table 1. The distance between bases of sequence 6 species in the 28S region of *Lernaea*

[	1	2	3	4	5	6	7	8]
[1]		[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.0]
[2]	1.0		[0.0]	[0.0]	[0.0]	[0.0]	[0.0]	[0.1]
[3]	1.0	1.0		[0.0]	[0.0]	[0.0]	[0.0]	[0.1]
[4]	1.0	1.0	1.0		[0.0]	[0.0]	[0.0]	[0.1]
[5]	0.1	0.1	0.4	0.1		[0.0]	[0.0]	[0.0]
[6]	1.0	1.0	1.0	1.0	0.4		[0.0]	[0.1]
[7]	1.0	1.0	1.0	1.0	0.1	1.0		[0.1]
[8]	0.3	0.3	0.4	0.3	0.4	0.3	0.3	

Note:
[1] *#Lernaea_cyprinaceae_G*; [2] *#Lernaea_lophiara*; [3] *#Lernaea_cyprinacea*; [4] *#Lernaea_devastatrix*
[5] *#Lernaea*_sp.; [6] *#Lernaea_oryzophila*; [7] *#Lernaea_papuensis*; [8] *#Dactilogyrus*

Lernaea (Figure 2). First group were *Lernaea cyprinacea* G., *L. papuensis*, *L. devastatrix*, and *L. lophiara*. Second group were *Lernaea cyprinacea* and *L. oryzophilla*, *Lernaea* sp. was in the third group. However, phylogenetic tree using neighbor joining (NJ) analysis found two groups among *Lernaea* (Figure 3). First groups were including *Lernaea cyprinacea* G., *L. cyprinacea*, *L. papuensis*, *L. devastatrix*, *L. lophiara*, and *L. oryzophila*; and second group was including *Lernaea* sp.

All species *Lernaea* were sequence on 28SrDNA region based on study by Song *et al.* (2008) who found relationships among species in genus Ergasilidae (copepods) with *Lernaea cyprinacea* as an outgroup. The average percentage of codon G + C was 51.6%. *Learnea cyprinacea* G., *Lernaea lophiara*, and *Lernaea papuensis* had the same percentage of codon G + C (51.7%). The percentage of G + C codon for *Lernaea cyprinacea* is 51.6%. *Lernaea devastatrix*, *Lernaea* sp., and *Lernaea oryzophila* are 51.8%, 51.3%, and 51.5%, respectively. There is no avalailable data sequence on *Lernaea* sp., except *Lernaea cyprinacea* in GenBank, to compare the result.

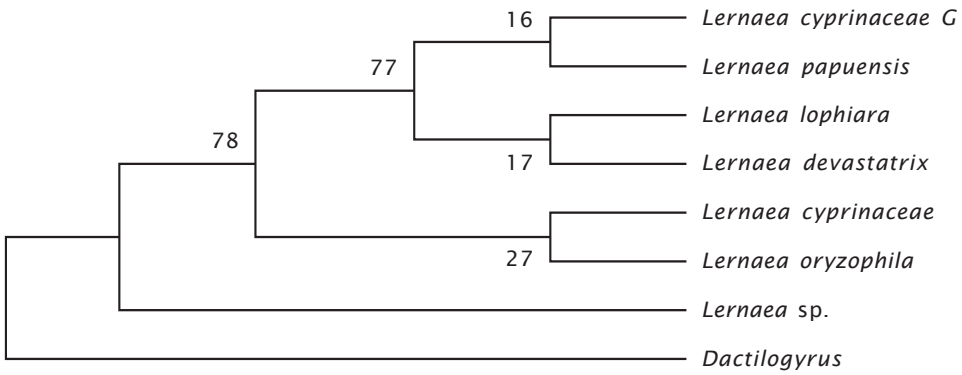

Figure 2. Phylogenetic tree from *Lernaea* sp. analyzed with maximum parsimony from sequence 6 species of *Lernaea* and *L. cyprinacea* GenBank with *outgroups Dactilogyrus* in 28S region

Based on maximum parsimony (Figure 2) there are 3 clade among species of *Lernaea*. Clade I consists of *Lernaea cyprinacea* G., *L. papuensis*, *L. Devastatrix*, and *L. lophiara*. Clade II consist of *Lernaea cyprinacea*, and *L. oryzophilla*. *Lernaea* sp. is the only member of Clade III. Phylogenic relationship between clade I and clade II is closer than clade III. The closeness similarity among the species of *Lernea* is probably due to proximity of the historical relationship between Indonesia archipelago and Asian and Australian continents. *Lernaea cyprinacea* G., and *L. papuensis*; *L. devastatrix*, and *L. lophiara*; and *L. cyprinacea* and *L. oryzophilla* can be considered as sister taxon (sister). It means that they all share a common anchestor. *Lernaea* sp. can be regarded as the begining of other species of *Lernaea* because it appears on the earliest in the phylogenetic tree. *Lernaea cyprinacea* G. and *L. papuensis* are the last descendant of *Lernaea* sp.

However, there are only two clade among species *Lernaea* based on neighbor joining tree (NJ) method (Figure 3). Clade I consists of *L. cyprinacea GenBank*, *L. cyprinacea*, *L. oryzophila*, *L. lophiara*, *L. devastarix*, and *L. papuensis*. Clade II is *Lernaea* sp. From the obtained result we concluded that the species *Lernaea* is monophyletic. Ho (1998) stated that Lernaeidae, Ozmanidae, and Ascidicolida are monophyletic group, which are derived from a common ancestor.

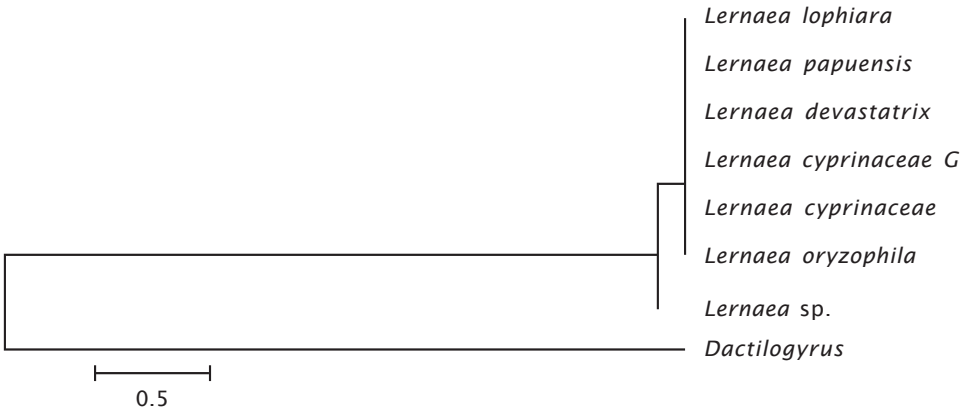

Figure 3. Phylogenetic tree from *Lernaea* sp. analyzed with neighbor joining from sequence 6 species of *Lernaea* and *L. cyprinacea* GenBank with *outgroups Dactilogyrus* in 28S region

The evolution of *Lernaea* mainly associated with the development and modification of the holdfast organ. The final form of the holdfast organ is determined by the consistency of fish tissues by which a parasitic animal attaches itself to its host. So many variations found in holdfast's shape. Strong influence of environment on morphology of *Lernaea* expressed by Poddubnaja (1974) *in* Kabata (1985) who found *L. cyprinacea*, *L. ctenopharyngdonis*, *L. quadrinucifera* adult females origates from the same individual eggs. So far, there is no studies on genetic molecular of *Lernaea* sp. This study perhaps is the iniatial study of molecular *Lernaea*.

CONCLUSION

Phylogenetic tree from maximum parsimony (MP) and neighbor joining (NJ) based on sequence in 28S rDNA do not show differences among species *Lernaea*. There are 3 variation groups of *Lernaea* from arwana Irian fish in Indonesia based on maximum parsimony. *Lernaea papuensis*, *L. devastatrix*, and *L. lophiara* are in one group; *L. cyprinacea* and *L. oryzophilla* are in one group; and *Lernaea* sp is in different group. However, based on neighbour joining there are 2 groups variation of *Lernaea* from Arwana Irian, ie. *Lernaea papuensis*, *L. devastatrix*, *L. lophiara*, *L. cyprinacea*, and *L. oryzophilla* are in one group; and *Lernaea* sp. is in different group. This indicates that phylogenetic reconstructions should be in other region, such as in the ITS region. This result is also suggested that *Lernaea* was monophyletic.

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