

## A Description of *Lecithocladium angustiovum* (Digenea: Hemiuridae) in Short Mackerel, *Rastrelliger brachysoma* (Scombridae), of Indonesia

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**Abstrak:** *Lecithocladium angustiovum* dikenal pasti daripada perut (87.33%) dan usus (12.67%) ikan makerel Indonesia (*Rastrelliger brachysoma*). Deskripsi termasuk badan yang panjang; min panjang total sebanyak 1018.84 µm; dan eksoma sebanyak 47.52% daripada panjang total. Nisbah penghisap oral dan ventral ialah 1:0.63, dan panjang farinks ialah 97.42 µm. Keputusan jujukan didapati melalui penjujukan gen 18s rDNA untuk segmen DNA sebanyak 354 basepair (bp), dan komposisi min base (%) ialah 17.7 A; 35.7 T; 29.6 G; dan 17.1 C. Pokok filogenetik telah dikonstruksi untuk menunjukkan jarak genetik antara *L. angustiovum* dan jujukan-jujukan daripada *Lecithocladium excisum*, *Dinurus longisinus*, *Plerurus digitatus* dan *Lecithochirium caesionis* yang didapati dari GenBank.

**Kata kunci:** Parasit Helmin, *Lecithocladium angustiovum*, *Rastrelliger brachysoma*, Indonesia

**Abstract:** *Lecithocladium angustiovum* is identified from the stomach (87.33%) and the intestine (12.67%) of Indonesian short mackerel (*Rastrelliger brachysoma*). The description includes an elongated body; a mean total length of 1018.84 µm; and an ecsoma of 47.52% of the total length. The oral and ventral sucker ratio is 1:0.63, and the pharynx length is 97.42 µm. The sequence results were obtained by 18s rDNA gene sequencing of the 354 basepair (bp) DNA segment, and the mean base composition (%) was 17.7 A; 35.7 T; 29.6 G; and 17.1 C. A phylogenetic tree was constructed to demonstrate the genetic distance between *L. angustiovum* and sequences from *Lecithocladium excisum*, *Dinurus longisinus*, *Plerurus digitatus* and *Lecithochirium caesionis* obtained from GenBank.

**Keywords:** Helminth parasitic, *Lecithocladium angustiovum*, *Rastrelliger brachysoma*, Indonesia

## INTRODUCTION

Fish are an important source of animal protein for humans in Indonesia as well as globally. The short mackerel, *Rastrelliger brachysoma*, is the most commercially

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important small pelagic fish because of its low price as a relatively high protein source (Ghazali *et al.* 2012). In addition to its economic value as a human food source, the short mackerel play an important ecological role as host to a range of taxonomically diverse parasites that exhibit a wide variety of life cycle strategies.

Parasitism is a ubiquitous phenomenon in marine environments, and it is probable that all marine fish are infected with parasites (Ruiz 1991) because fish are very vulnerable to helminthic parasitological infections. Some tail or a few species of helminth parasites soften and inhabit the body of fish (Noble & Noble 1982). Parasites are used increasingly as indicators for the differentiation of marine ecology because parasitic fauna might show a distribution parallel to the host distribution (Madhavi & Lakshmi 2011). The prevalence and intensity depend on many factors, such as the parasite species, the feeding habits of the host and the quality of the water the fish inhabit. Additionally, they depend on the presence of intermediate hosts such as copepods (Chandra *et al.* 2011). The effect of parasites on fish has been an attractive theme for two decades (e.g., Barber *et al.* 2000; Lafferty 2008).

Digenean parasites are common and could cause gastro-intestinal tract histopathology in marine fish (Kabata 1985; Chambers *et al.* 2001). The digenean groups of the Hemiuridae family, including the *Lecithocladium* genus, are typically dominant in the digestive tract of marine fishes because the family has a very wide distribution worldwide (Cribb *et al.* 2002). Research on helminthic parasites in Indonesian fish is limited. Indaryanto *et al.* (2014) found four species of helminthic parasites (*Lecithocladium angustiovum*, *Lecitochirium* sp., *Prodistomum orientalis* and *Anisakis typica*) in Indonesian short mackerel (*Rastrelliger brachysoma* and *Rastrelliger kanagurta*) collected from two locations.

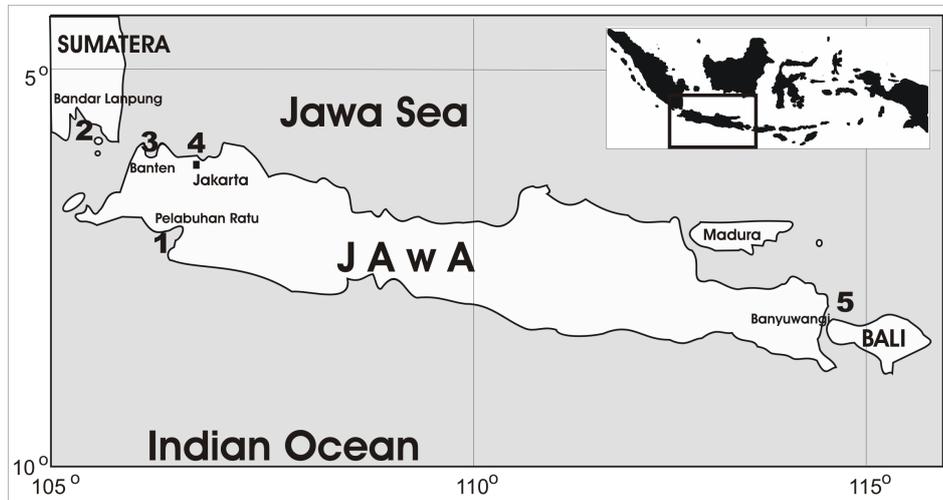
The *Lecithocladium* genera includes at least 83 defined species, and many of them are similar. Information on *L. angustiovum* or the *Lecithocladium* genus is not widely available; the identification of the species has not been clearly defined, which explains the many similar species (Madhavi & Lakshmi 2011). *L. angustiovum* has been reported to frequently infect fish of the Scombridae family (Yamaguti 1953; Bray 1990).

Identification by genetic information and sequence analysis are widely used to describe and determine any type of organism and are considered helpful for classifying organisms into groups for biodiversity study. Blair and Barker (1993) found that 18S rRNA was well suited for the inference of phylogeny among digenean families. In this study, the biological description of *L. angustiovum* (Digenea: Hemiuridae) parasites from Indonesian short mackerel digestive tracts (*R. brachysoma*) is revealed, and the description is supported with genetic information from the sequence analysis technique. Additionally, we present the DNA sequence of *L. angustiovum* and compare it with those of other Hemiuridae family members obtained from GenBank.

## MATERIALS AND METHODS

### Fish Collection and Parasite Identification

The short mackerel (*R. brachysoma*) collections were made in July 2012 from five areas of fish landing sites in Indonesia, *i.e.*, Jawa Island (Pelabuhan Ratu Bay, Banten Bay [Karangantu], Jakarta Bay [Muara Angke] and Banyuwangi Bay) and Sumatera Island (Lampung Bay, Bandar Lampung) (Fig. 1). The fish were caught by local fishermen in surrounding waters near the fish landing sites.



**Figure 1:** Sampling map. The numbers indicate the sites where the Indonesian short mackerel (*R. brachysoma*) were collected: 1) Pelabuhan Ratu Bay; 2) Bandar Lampung Bay; 3) Banten Bay (Karangantu); 4) Jakarta Bay (Muara Angke); 5) Banyuwangi Bay.

The fish were brought in a cool box for the laboratory work. In the Helminthology Laboratory, (Faculty of Veterinary Science, Bogor Agricultural University), the fish were dissected carefully to observe the digestive tracts and then placed in separate petri dishes containing physiological saline water. The contents of the digestive tract were then observed under a microscope to count and collect *L. angustiovum* parasites. According to Gibson (1996) and Gibson and Bray (1986), the keys to identification of *L. angustiovum* are the presence of ventral sucker organs, an ecsoma, adult parasites found in the gastrointestinal tract or in the body cavity of marine teleosts, a funnel-shaped oral sucker, Scombrid or the *Rastrelliger* genus as the main host, and an oral sucker much larger than the ventral sucker.

We performed morphological measurements on 13 *L. angustiovum* specimens using Leica microscopy applications (LAS EZ V 1.8.0, Leica Microsystems Cambridge Ltd., UK). In addition, data on the mean intensity (level of infection) and prevalence (frequency of infection) were recorded following Bush *et al.* (1997). Three *L. angustiovum* specimens from Pelabuhan Ratu Bay were kept in a sterile 1.5 ml tube that contained 0.5 ml of TNES 8 M urea buffer for the genetic analyses.

### DNA Extraction and Sequence Analysis

The DNA extraction was performed using a standard phenol-chloroform method (Imai et al. 2004). The amplification of 18S rRNA fragments was conducted by PCR using the universal forward primers 5'-ATCCAAGGAAGGCAGCAGGC-3' (18SU467F) and reverse primer 5'-GTGCCCTTCCGTCAATTCCT-3' (18SL1170R) and KapaTAQ™ DNA polymerase (Kapa Biosystems, Cape Town, South Africa). The program used was denaturation (94°C, 2 min), 30 cycles of denaturation (94°C, 30 s), annealing (45°C, 30 s), extension (72°C, 1 min), and a single final extension (72°C, 7 min). The PCR product was separated, electrophoresed on a 1% agarose TreviGel™ 500 (Trevigen, Inc., Gaithersburg, Maryland, USA) and stained with ethidium bromide. The primer and program were designed by Dr. Hideyuki Imai from the University of the Ryukyus, Okinawa, Japan. For DNA sequencing, the sample was sent to Macrogen (Tokyo).

The sequence data were initially aligned using ClustalX2, ver 2.1 (University College Dublin, Belfield, Dublin) (Larkin et al. 2007). A neighbour-joining phylogeny of *L. angustiovum* mitochondria was constructed with Kimura's two-parameter model using the MEGA (version 5) software (Nei & Kumar 2000) and compared with Hemiuridae DNA obtained from GenBank, including *Lecithocladium excisum* AJ287529, *Dinurus longisinus* AJ287501, *Plerurus digitatus* AF029803, and *Lecithochirium caesionis* AJ287528 (Olson et al. 2003).

## RESULTS AND DISCUSSION

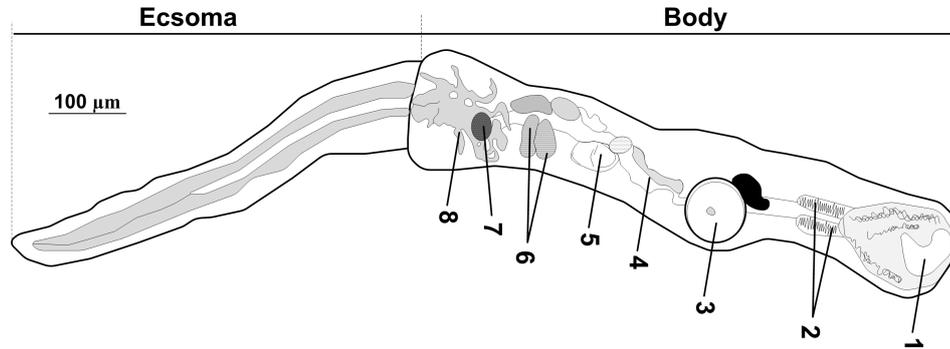
### Descriptions

Body elongate, mean total length of 1018.84±145.30 µm (range 775.57 to 1401.58 µm) and mean maximum width of 118.54±16.67 µm (a range of 89.73 to 150.71 µm) at the middle of the body or slightly posteriorly. Mean body length was 534.70±61.81 µm (range 442.80 to 668.78 µm), and mean ecsoma of 484.14±89.09 µm (range 328.13 to 732.80 µm). Ecsoma tapered posteriorly, usually extended, short and blunt. Body length was 52.48% of total length, and ecsoma was 47.52% of total length. Cuticular denticulations present on the entire surface of the body proper. The lateral cuticular folds curve backward in a longitudinal direction at the ventral posterior end of the body proper, so that this small midventral area was covered with smooth cuticle, similar to the ecsoma.

Oral sucker terminal, cup-shaped, with a pair of distinct submedian incisions ventrally. Oral sucker is much larger than the ventral sucker. The dimensions of oral sucker were 93.06 × 75.82 µm (range from 65.76 × 51.26 to 122.33 × 111.99 µm) and of ventral sucker were 59.00 × 58.09 µm (range from 42.20 × 48.08 to 72.69 × 66.48 µm), with an oral and ventral sucker ratio 1:0.63 (range from 1:0.52 to 1:0.75); distance from anterior to ventral sucker is 224.34 µm (range from 169.45 to 294.58 µm) or at 22% of total length. Size of body width in this ventral sucker area is 113.12±27.17 µm (range from 93.00 to 131.03 µm) or ventral sucker size is 52.16% of body width.

The pharynx is cylindrical, 97.42±13.18 µm (range from 68.15 to 133.52 µm) in size. Oesophagus is very short and frequently turned dorsally. Uterus extends into the ecsoma more than half of its length, coiled between the posterior

testis and ovary, running in a dorsal or dextral direction, and occasionally in a sinistral direction. Excretory pore terminal; arms uniting the dorsal and posterior end of the oral sucker. The anatomies of the fresh specimens and the preserved specimens of *L. angustiovum* are presented in Figure 2.



**Figure 2:** Anatomy of *L. angustiovum* collected from Indonesia short mackerel fish (*R. brachysoma*).

Note: 1) oral sucker; 2) pharynx; 3) ventral sucker; 4) prostate cell; 5) vesicula seminalis; 6) testis; 7) ovary; 8) vitellarium

#### Level of Infection

The helminth parasites of *R. brachysoma* are dominated by *L. angustiovum*, with 1365 individuals (96.47%) of a total of 1415 individual parasites. Among the 160 examined fish, 124 fish (77.50%) were infected by the parasite, and the intensity of the infection ranged from 1 to 69 parasites per fish (see Table 1).

**Table 1:** Abundance, intensity and prevalence of infection with *L. angustiovum* digenean parasites in Indonesian short mackerel (*R. brachysoma*).

Fish landing site	Number of collected fish	Number of infected fish	Total abundance (intestine – stomach)	Mean intensity (min – max)	Prevalence (%)
Pelabuhan Ratu Bay	36	28	92 (5–87)	2.99 (1–7)	77.78
Lampung Bay	40	33	252 (24–228)	7.54 (2–26)	82.50
Banten Bay	40	35	666 (109–557)	19.03 (1–69)	87.50
Jakarta Bay	26	19	275 (27–248)	14.47 (2–65)	73.08
Banyuwangi	18	9	80 (8–72)	8.89 (3–15)	50.00

Additionally, *L. angustiovum* infected five perciform families in the Indo-West Pacific, with Carangidae (47%) and Scombridae (44%) being the most frequently reported families. The Indian mackerel, *Rastrelliger kanagurta*, had a

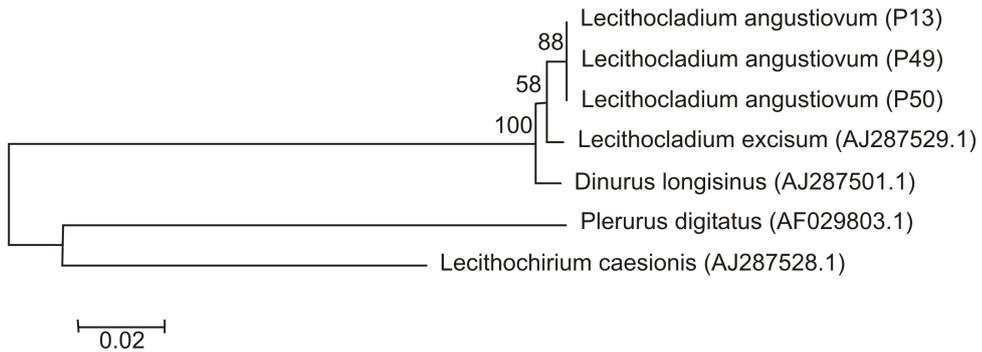
large proportion (37%) of the recorded parasites (Bray 1990). A fish parasitism study in India showed that *L. angustiovum* in *R. kanagurta* collected from the Visakhapatnam coast occurred with a prevalence of 88.5% (Madhavi & Lakshmi 2011). In Ghana, this parasitic species was found in different hosts, including *Upeneus prayensis* (Mullidae), *Trachinotus glaucus* and *Trachinotus gorensis* (Fischthal & Thomas 1971).

### Site in Host

This parasite is frequently found in the stomach and intestine (Yamaguti 1953; Fischthal & Thomas 1971; Bray 1990). In this study, *L. angustiovum* parasites were found in the stomach, with a 87.33% occurrence, and in the intestine, with a 12.67% occurrence; this finding was similar to earlier results. A study on the distribution of another digenean species, *Genarchopsis dasus* (the Hemiuridae family), in *Channa punctatus* from the Mymensingh District in Bangladesh (72.8%) reported that the parasites were found in 6.6% of the anterior part, 9.0% of the middle part and 11.6% of the posterior part of the intestine (Chandra et al. 2011). Digeneans might cause little or no overt pathology in the gastro-intestinal tract of fish (Kabata 1985) because digenean are typically small (typically 1–2 mm long), mobile (they do not create permanent feeding scars) and do not feed deeply on host tissues (e.g., few ingest blood) (Chambers et al. 2001).

### Genetic Information

Identification of genetic information is a classical taxonomy method for revealing genetic variations. In this study, the sequence results were obtained by DNA sequencing of 354 basepairs (bp) of the DNA segment, and the mean base composition (%) was 17.7 A, 35.7 T, 29.6 G and 17.1 C. The neighbour-joining phylogeny position is presented in Figure 3.



**Figure 3:** Phylogenetic tree (Neighbour Joining [NJ] with a Kimura 2 parameter model) of the *L. angustiovum* compared with other members of the Hemiuridae family obtained from GenBank.

In the *Lecithocladium* genera, 32 among 83 defined species were recorded in marine fish of India. Most of these species are not well defined, and many of them might not be valid species (Madhavi & Lakshmi 2011). Gibson and

Bray (1986) revised poorly described species of the Indian Ocean and reduced these species to six species (Table 2).

**Table 2:** Regional distribution area, main host and size of genera *Lecithocladium* according Gibson and Bray (1986).

Group species	Regional distribution	Main host	Body length (mm)	Sucker size
<i>L. angustiovum</i> Syn: <i>L. scombri</i> <i>L. bulbolabrum</i> <i>L. unibulbolabrum</i>	Indo-Malaysian	Scombrid genus <i>Rastrelliger</i>	±4	Oral sucker much larger than the ventral sucker
<i>L. excisum</i>	Mediterranean, Northeast Atlantic	<i>Scomber scombrus</i>	±3–8	Same size sucker or slightly larger oral sucker
<i>L. apolecti</i> Syn: <i>L. excisiforme</i> <i>L. psenopsis</i> <i>L. hexavitellarii</i> <i>L. anteporus</i> <i>L. tetravitellarii</i> <i>L. microductus</i> <i>L. arabiana</i> <i>L. microcaudum</i> <i>L. stomatei</i>	Indo-Malaysian	<i>Stromateids</i> , <i>Stromateus</i> and <i>Formioniger</i>	±14	Oral sucker much larger than the ventral sucker
<i>L. parviovum</i> Syn: <i>Cleftocolleta magnum</i> <i>L. karalense</i> <i>L. indicum</i>	Indo-Malaysian	<i>Rastrelliger</i> , <i>Carangids</i> and <i>Stromateids</i>	±12	Oral sucker much larger than the ventral sucker
<i>L. glandulum</i> Syn: <i>L. carultum</i> <i>L. triacantha</i> <i>L. thapari</i>	Indo-Malaysian	<i>Sciaenids</i>	±6	Same size sucker or slightly larger ventral sucker
<i>L. harpodontis</i> Syn: <i>L. Brevicaudum</i> <i>L. ilishae</i> <i>L. chauhani</i>	Indo-Malaysian	<i>Hilsa</i>	±6	Same size sucker or slightly larger ventral sucker
<i>L. karachii</i> Syn: <i>L. pakistanensis</i>	Indo-Malaysian	<i>Carangids</i>	±5	A ventral sucker much larger than the oral sucker

**Table 3:** Comparison of some morphological characteristics of *L. angustiovum* and *L. excisum*.

	<i>L. excisum</i> <sup>a</sup>			<i>L. angustiovum</i>		
	Mediterranean	Black Sea	NE Atlantic	Macassar Indonesia <sup>b</sup>	Indian Ocean <sup>c</sup>	Pelabuhan Ratu Indonesia <sup>d</sup>
Total length (mm)	2.92–4.85	5.00–8.00	3.5–7.30	–	–	0.75–1.40
Body (mm)	–	–	0.6–1.20	2.50–4.00	1.30	0.44–0.66
Ecsoma (mm)	0.64–1.03	–	0.15–4.00	1.30–2.10	1.36	0.32–0.73
Oral sucker (mm)	0.14–0.48	0.33–0.67	0.35–0.65	–	185–240	0.07–0.09
Ventral sucker (mm)	0.23–0.35	0.33–0.56	0.3–0.50	–	145–160	0.05
Sucker ratio	–	–	1:0.72–0.95	–	1:0.86	1:0.63

Note: a) Gibson and Bray (1986); b) Yamaguti (1953); c) Bray (1990); d) present study

The species most similar to *L. angustiovum* is *L. excisum*. The differences between *L. angustiovum* and *L. excisum* are the distribution area, main host, body size and position in the neighbour-joining phylogeny (Tables 2 and 3, and Fig. 3). These data show that *L. angustiovum* and *L. excisum* are two distinctly different species. Additional convincing records of *L. angustiovum* are reported by Yamaguti (1953), Bray (1990), Arthur & Lumanlan (1997), Liu *et al.* (2010), and Madhavi & Lakshmi (2011); however, most of them were collected from *R. kanagurta* as the host, and Yamaguti (1953) and Bray (1990) gave descriptions of the morphological measurements of *L. angustiovum*. No measurements are detailed enough to permit a dependable identification. The morphological examination of *L. angustiovum* in this study demonstrated that some characteristics (the length of the body, an ecsoma, oral sucker and ventral sucker) of *L. angustiovum* in Pelabuhan Ratu Bay were much smaller than those of *L. excisum* and *L. angustiovum* collected from other areas (Table 2).

## ACKNOWLEDGEMENT

This study was a collaborative research program and partly funded by Bogor Agricultural University and University of the Ryukyus, conducted as part of the international graduate program for the Asia-Pacific region. We are grateful to Dr. Makoto Tsuchiya (Program Leader and Professor of Science, University of the Ryukyus).

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