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Morphology of surface ultrastructure *of Duthiersia expansa* (Cestoda: Diphyllobothriidea) from water lizards (*Varanus salvator*) from Sidoarjo, Indonesia

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Abstract. *Duthiersia expansa (D.expansa*) is a cestoda of the family Diphyllobothriidae which is often found in the gut of water lizards (*Varanus salvator*). *D. expansa* worm infection is generally chronic and shows no obvious clinical symptoms but can cause weight loss, edema, ulceration of the intestinal mucosa to death. Although there have been no reports of *D. expansa* worm infections in humans, all types of animals have the potential to transmit diseases to humans both directly and indirectly. The present study being the first to describe morphology of surface ultrastructure of *D. expansa* using scanning electron microscopy in sidoarjo, indonesia, managed to compliment and verify the taxonomic valid characteristics of *D.expansa as Asian Duthiersia form.* Samples are collected by making incisions in the duodenum to the ileum. Sample preparation was carried out at the Airlangga University Medical School. The scanning electron microscope (SEM) analysis was carried out at the Central Institute of Life Sciences Universitas Brawijaya. The results of scanning electron microscope examination show that fan-shaped worm scoleks, bothria in the lateral scolex, existence of posterior pores in scolecs, crasspedot-shaped strobila with uterine pore and different forms of microtriches in tegument of the skoleks and strobila

1. Introduction

Water lizards are predators of fish, amphibians, rats, birds, reptiles and large invertebrates such as crustaceans [1,2]. In the wild, water lizards are commonly found on river banks, waterways, swamps, and in bush areas [13]. Based on the habitat and food, water lizards have a high likelihood of being infected by parasites, one of them is intestinal parasite *Duthiersia expansa* [3], although there have been no reports of *Duthiersia expansa* worm infections in humans but all types of animals have the potential to transmit diseases to humans both through direct and indirect contact [4].

Duthiersia expansa is a type of tapeworm (cestoda of the family Diphyllobothriidae) which is often found in intestinal lizards, especially water lizards. According to Raś-Noryńska and Sokół [5], parasitic infections in reptiles are generally chronic and do not show clear clinical symptoms but can cause a lot of harm to water lizards because it can cause a decrease in the quality of the animal itself both in captivity and in the wild [20]. Specific lesions associated with reptile parasites (genus *Duthiersia* spp., *Scyphocephalus* spp., and *Bothridium* spp.) Have been documented previously and are often associated with the occurrence of intestinal mucosal ulceration, bleeding, and edema [6].

The *Scanning Electron Microscope* (SEM) in the Helminth study was used to explain and describe the structures, organs that were considered morphological characteristics [7,8] and had important influences in the taxonomic studies of worm species. Research on the morphological characteristics of *Duthiersia expansa* on water lizards in Indonesia using SEM has never been done before. Difficult reference or literature that discusses thoroughly the parasite makes many researchers who do not understand the *Duthiersia expansa* worm one of which is morphology, so that it becomes less aware and impressed ignored. based on these problems, it is necessary to identify more deeply the morphological characteristics of the *Duthiersia expansa* worm.

2. Materials and methods

Duthiersia expansa specimens adult worms are obtained from the small intestines of water lizards in Sidoarjo, then the worms obtained are put into a petri dish and washed with aquadest until clean. Worm specimens that will be examined using SEM, must be prepared in advance through several stages including the first fixation with 2.5% glutaraldehyde for 3 hours, followed by washing with phosphate buffer pH 7.4 solution three times, the second fixation with osmium tetroxide each for 1 hour. Furthermore, dehydrated in alcohol for 15 minutes, followed by the drying stage / CPD (Critical Point Drying) and coating using a conductive material in the form of carbon, the sample is ready to be examined and photographed by SEM (FEI Quanta FEG 650, Low Vacuum electron microscope). SEM examination used standard SEM Laboratory procedures, Lembaga Sentra Ilmu Hayati (LSIH), Brawijaya University.

3. Result

3.1. Scolex morphology

The *Duthiersa expansa* skoleks are shaped like fan, with two bothria extending laterally. Bothria, the ductile suction tool dangling, consists of two parts namely the dorsal and ventral parts. There are posterior pores in both the skolex as characteristic of Asian duthiersia.

The most complex pattern of microtriches is found in scoleks in both genera of Duthiersia. The apical part of bothria to the anterior scolex is dominated by microtriches in the form of thin capiliformis filitriches and partly leads posteriorly (figure 1). The inner surfaces of bothria are covered by microthriches in the form of gladiate spinitriches. The posterior part of the scoleks is covered with capiliform microtriches mixed with coniform filitriches [9]. The neck has microtriches in the form of gladiate spinitriches interspersed with capiliform filitriches.

3.2. Strobilla

All adult worms examined have the same strobila shape and structure as the others. Strobilla *Duthiersia expansa* in the form of crasspedote consists of immature, mature and gravid proglottids. The entire strobilla is covered with shorter and denser mikrothriches. In immature proglottids, they have a width of 0.7 mm - 1.49 mm and a length of 0.3 - 0.6 mm, horizontal line structures and michrotriches that cover the form of acicular filitriches. In mature proglottids, they have a width of 1.02 mm - 1.11 mm and a length of 0.3 mm - 0.6 mm, vertical line structure, microtriches that cover the form of acicular filitriches and capiliform filitriches. In gravid proglottids, they have a width of 1.2 mm - 1.4 mm and a length of 0.5 mm - 0.9 mm, microtriches that cover the shape of small gladiate filitriches.

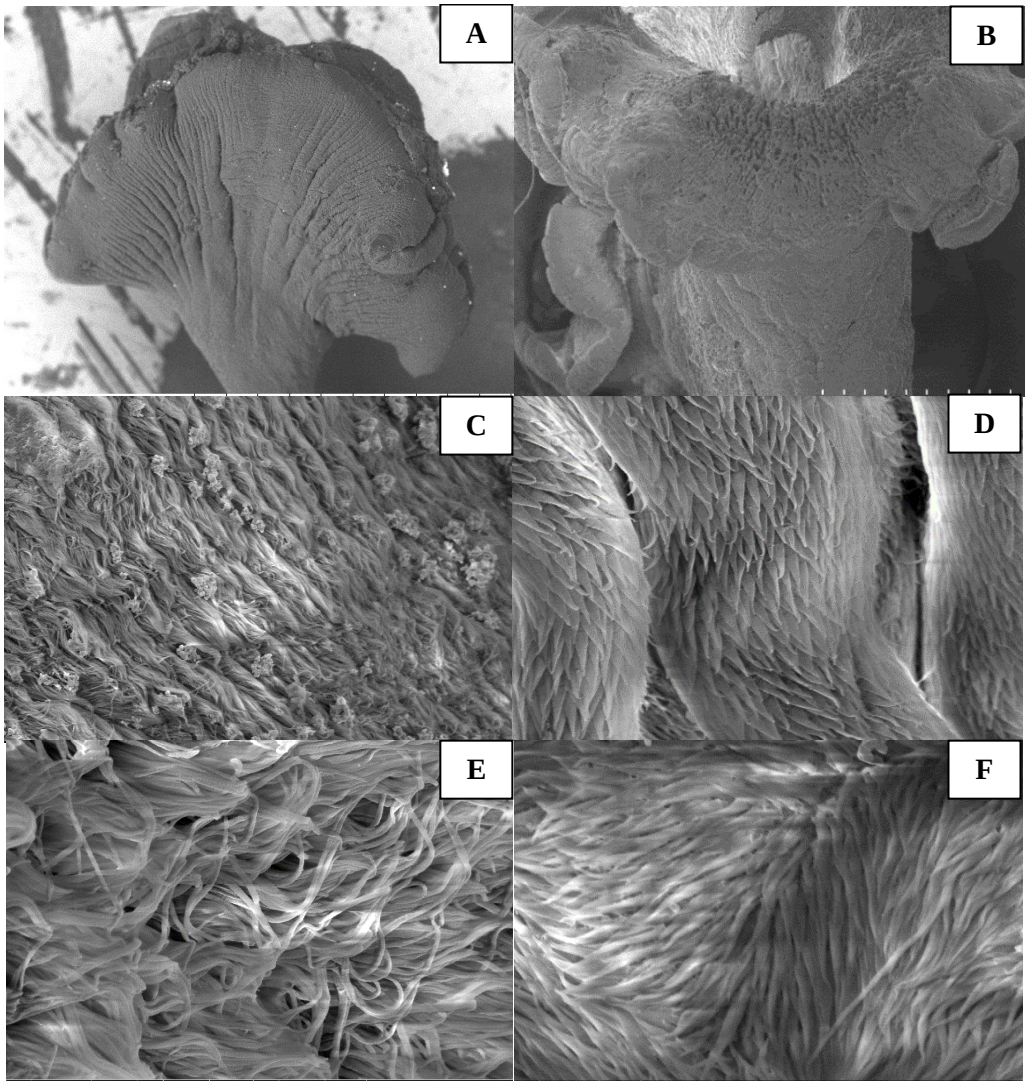

Figure 1 A-F. Scanning electron microscope of *Duthiersia expansa*. **A**, Scolex (Sc) like a fan shaped with two bothria (B) (Scale bar: 1 mm). **B**, Lateral view of scolex; Higher magnification of posterior region of bothria with pores (P) (Scale bar : 500 µm). **C**, Thin capilliform filitriches on the apical (Ap) region of bothria to the anterior scolex (Scale bar : 10 µm). **D**, Gladiate spinitriches covered on the inner surface (IS) of bothria. **E**, capilliform microtriches mixed with coniform filitriches on the posterior region of scolex. **F**, Gladiate spinitriches interspersed with capilliform filitriches on the neck (N) (Scale bare D-F : 3 µm).

4. Discussion

This specimen was identified as the genus Duthiersia based on the following genetic characteristics given by Bray *et al* [10]. *Duthiersia expansa* (*D.expansa*) is a cestoda of the family Diphyllobothriidae found in mammals, birds and reptiles (including monitor lizards and snakes). Cestodes in reptiles are usually characterized by the presence of bothria and are divided into three genera: Bothridium, Duthiersia, and Scyphocephalus [9]. Adult *D.expansa* worms generally have a flat, dorsoventral body, elongated like a ribbon, do not have a digestive tract or vascular canal and are usually divided into segments called proglottids which when mature contain male and female reproductive organs. Adult

worm body length reaches 8.60 -9.10 mm and width 0.58 - 3.94 mm. All definitive hosts infected with only one adult worm except one host infected with 4 different worms [11-13]. This specimen has similar morphological and measurement characteristics from the Asian Duthiersia form that was described earlier by Perrier [14], Baer [15], and Woodland [16]. It has been previously reported from Varanus salvator in the Philippines, and Malaysia [5,17]. This is also reinforced by Woodland's statement in 1939 that all Asian form variants belong to one species, namely *Duthiersia expansa.* Research on Asiatic Duthiersia spp.

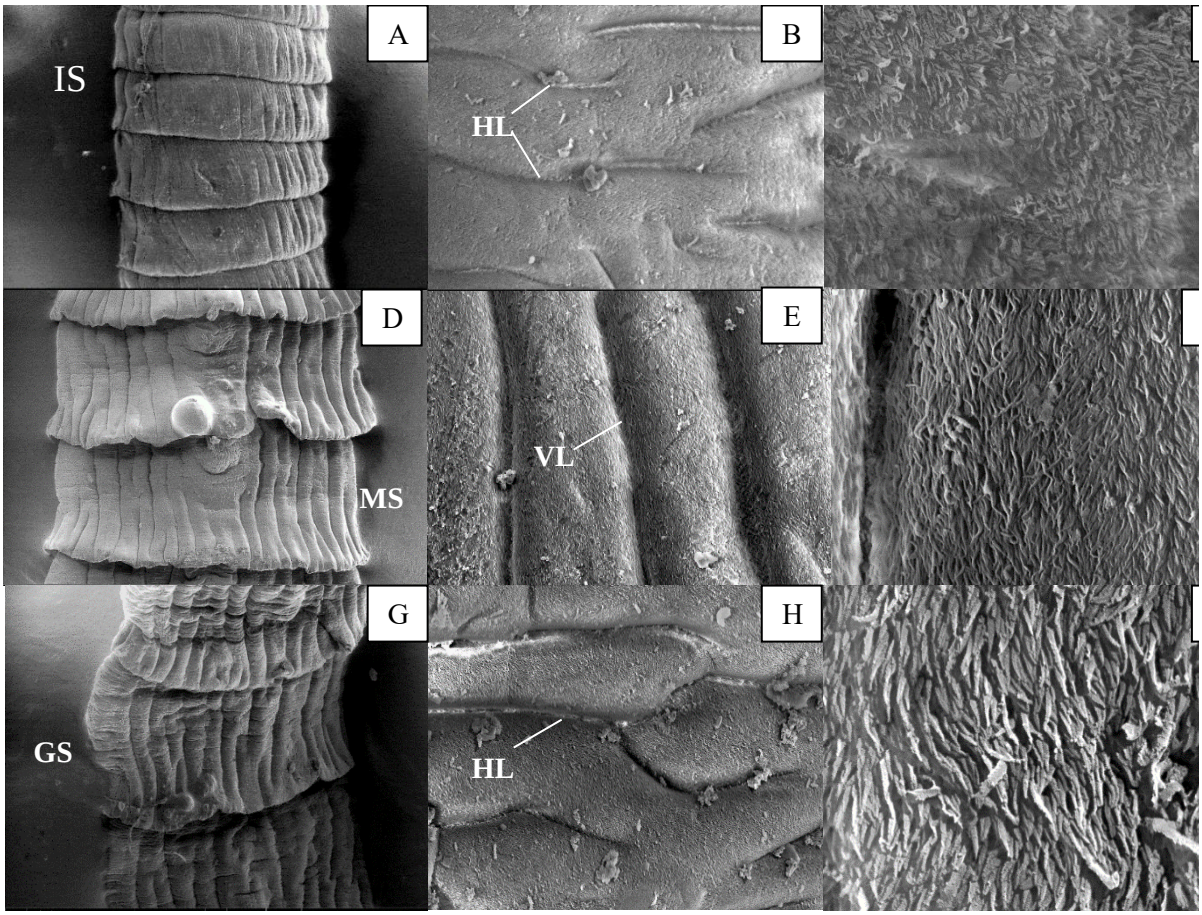

Figure 2. A – I. Scanning electron microscope of *Duthiersia expansa* proglottid. **A**, Immature segment (IS) (Scale bar : 500 µm). **B**, Horizontal line (HL) structures on the immature segment (Scale bar : 10 µm). **C**, Acicular filitriches on the immature segment (Scale bar : 3 µm). **D**, Mature segment (MS) (Scale bar : 500 µm). **E**, Vertical line (VL) structures on the mature segment (Scale bar : 10 µm). **F**, Acicular filitriches and capiliform filitriches on the mature segment (Scale bar : 3 µm). **G**, Gravid segment (GS) (Scale bar : 500 µm). **H**, Horizontal line structures on the gravid segment (Scale bar : 500 µm). **I**, Small gladiate filitriches on the gravid segment (Scale bar : 1 µm).

Based on SEM results, it can show the structure and morphology (scolex, pores, and microvilli) which vary in size, shape, and distribution throughout the worm's body. The morphology of the surface scolex ultrastructure is considered important to know phylogenetically and can represent a significant taxonomic character [18]. In this study, *D. expansa* scolex have a wide fan-like shape with non-curved bothria. This is in accordance with the statement of Woodland [16] that Scolex *D. expansa* has a varying shape depending on the maturity of the worm. In young specimens, the scolex resembles a funnel with

the edges of bothria which are not too curved. In adult specimens, the scolex is triangular or fan shaped with bothria curved edges that are very curved to slightly hang (with a length of 1.12-2.46 mm and width 1.19-3.14 mm). In the posterior scolex there are pores or holes [11]. Microtriches are small villi that cover the entire surface of the worm's body. Microtriches has several functions including absorption of nutrients, excretion, movement and external protection [15]. The shape of microtriches is found to vary between worm species, at each stage of the life cycle and body parts (especially on the skoleks), using SEM. This research has never been done before in Indonesia. In other countries, previous studies have been disclosed about the form of microtriches in *D. expansa* from Vietnam [9] and in other species of *D.fimbriata* from Africa [13].

Based on its width, Chervy [19] recognize two types of microtriches: filitriches (≤ 200 nm base width) and spinitriches (> 200 nm base width). Filitriches are distinguished by their authors by length, including: papilliform (width ≤ 2 times the length), acicular (2-6 times longer), and capilliform (> 6 times longer). Spinitriches forms are much more varied, 25 spinithrix forms; where 13 shapes have a width that greatly exceeds thickness and 12 other shapes between width and thickness are almost the same. Filitriches are a type of microtriches that are most often found in all cestode groups and are considered as prototypes [20]. Based on the results of our SEM almost in common with the research of Yoneva *et al* [9] where there are differences in morphology, especially from microtriches scolex *D. expansa*. Scolices of the species studied contain various types of microtriches such as acicular and capilliform filitriches, and coniform and gladiate spinitriches, while those in strobila, contain microtriches in the form of acicular filitriches, capiliform and small gladiate spinitrices. Previous studies of ultrastructures in the diphillobothriide family comparing plerocercoid stages and adult Spirometra spp. worms, showed that scoleks and strobili were also covered by capiliform filitriches [21]. In another family cestoda, microtriches on strobilla were dominated by acicular filitriches on *Caryophyllaeus brachycollis* (Cestoda: Caryophyllidea) [22,23].

5. Conclusion

Based on the results of ultrastructural morphology of the scolex, pores, and microtriches of worms that were examined using SEM, the characteristics of the specimens studied strongly lead to *Duthiersia expansa* and are considered to be Asian forms of Duthiersia.

This parasite species is important to study, because most Indonesian people now use water lizards for consumption. In addition, there is still a lack of studies that review more deeply about internal parasites in wild reptiles. Although this parasite infection does not show the potential for zoonoses, every wildlife has the potential to transmit the disease directly or indirectly, and still cause harm to the health of its animals.

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Nomor : 2934/UN3.1.6/KP/2022

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Jabatan : Lektor Kepala

Telah melaksanakan penelitian dengan judul sebagai berikut :

No.	Judul Karya Ilmiah	Tahun pelaksanaan Penelitian
1.	Morphological Detection of The Intestinal Parasite Blastocystis sp. In Fresh and Cultured Feces of Pet Sugar Glider (Petaurus breviceps) (Mammalia: Petauridae) In Surabaya, Indonesia.	2018
2.	Identification of Active Compounds of Ethanol Extract of Citrus amblycarpa leaves by Analysis of Thin-layer Chromatography and Gas Chromatography-Mass Spectrometry as Bioinsecticide Candidates for Mosquitoes	2020
3.	Histopathological studies on *Leucocytozoon Caulleryi* infection on broiler in endemic area of Indonesia	2020
4.	Potential Extract Ethanol Citrus Amblycarpa as a Bioinsecticide Against Aedes Aegypti Larvae	2021
5.	Protein Profile of Sporozoite of Leucocytozoon sp. from Culicoides sp.	2010
6.	Deteksi Cryptosporidium canis pada Anjing di Kota Surabaya	2020
7.	Eksplorasi Protein Antigenik *Leucocytozoon caulleryi* sebagai Kit Diagnostik Leucocytozoonosis pada Ayam Broiler	2013

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8.	Uji reaktivitas protein 30 kDA bakteri *Aeromonas hydrophila* yang diisolasi dari ikan air tawar dengan teknik indirect ELISA.	2016
9.	Penambahan Sari Air Laut (Nigarin) Dalam Pengencer Skim Kuning Telur Terhadap Viabilitas Dan Motilitas Spermatozoa Sapi Limousin Post Thawing	2018
10.	The Effectiveness of Ethanol Extract of Red Betel Leaf (Piper crocatum) Againts Mortality of Boophilus microplus Larvae In Vitro	2020
11.	Prevalence of Ectoparasites in Bean Goats on the Sub-District of Prambon, District of Nganjuk	2020
12.	Repellent Effectiveness of Permot Leaf Ethanol Extract (Passiflora Foetida Linn.) against Aedes Aegypti Adult Mosquitoes	2021
13.	Detection of Goat Digestive Tract Protozoa Through Feces Examination in Kwanyar Sub-District, Bangkalan District	2021
14.	Identification and Prevalence of Digestive Tract Endoparasites of Goats in Ujungpangkah, Gresik District	2021
15.	Morphology of surface ultrastructure of Duthiersia expansa(Cestoda Diphyllobothriidea) from water lizards (Varamus salvator) from Sidoarjo, Indonesia	2014
16.	Antigenic Protein of *Leucocytozoon caulleryi* schizont Inducing Cellular Immune Resonse: TLR-2 and CD4 as Marker	2017

Adapun penelitian tersebut tidak perlu dilakukan ***Uji Etical Clearence*** karena tidak menggunakan hewan coba.

Demikian surat kerangan ini kami buat untuk dapat dipergunakan sebagai persyaratan pengusulan Jabatan Fungsional **Guru Besar**

Surabaya, 8 Agustus 2022

Dekan,

Prof. Dr. Mirni Lamid, drh., MP
NIP 196201161992032001