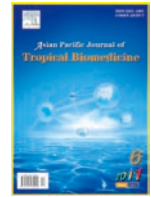




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(11)60095-6 © 2011 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

# Nematode parasite, *Auchenacantha* spp of flying lemur, *Cynocephalus variegatus* (Audebert, 1799) from Indonesia: Morphological study with SEM

Endang Purwaningsih\*

Zoological Division of Research Centre on Biology–LIPI, Widyasatwaloka Building, Jl Raya Bogor Km 46, Cibinong 16911, Indonesia

## ARTICLE INFO

## Article history:

Received 15 April 2011

Received in revised form 27 April 2011

Accepted 28 June 2011

Available online 28 June 2011

## Keywords:

Oxyuridae

*Auchenacantha*

Parasite

Flying lemur

*Cynocephalus variegatus*

Distribution

Indonesia

## ABSTRACT

**Objective:** To observe morphology of *Auchenacantha galeopteri* (*A. galeopteri*), *Auchenacantha spinosa* (*A. spinosa*) and *Auchenacantha parva* (*A. parva*) from Sukabumi and Ujung Kulon, Indonesia using scanning electron microscopy (SEM). **Methods:** Specimens for light microscopy examination were fixed with warm 70% alcohol, cleared and mounted in lactophenol for wet mounting. Drawings were made with the aid of a drawing tube attached to a Nikon compound microscope. Specimens for SEM examination were processed according to Bozzola. Measurements were given in micrometers ( $\mu\text{m}$ ) as the average of findings, followed by the range in parentheses, unless otherwise stated. **Results:** The measurements of *Auchenacantha* spp are same with those of previously described. The striations of male and female *A. galeopteri* are widen towards outside and wavy. The mouth of female with 6 lips, each of them is wider at base than distal end in *A. galeopteri* and rectangular in *A. spinosa*. Both species have dentiform protrusion from inner surface of lips, such structure absent in *A. parva*, but the lips with transverse festoon like pattern at anterior end of each lip. **Conclusions:** Using SEM, the lip and the striation pattern of three species of *Auchenacantha* can be clearly distinguished. Sukabumi and Ujung Kulon are new locality of *A. galeopteri* and *A. spinosa*, and *A. parva* is the new record in Indonesia.

## 1. Introduction

Baylis erected a new genus *Auchenacantha*, the member of family Oxyuridae (Nemataoda: Oxyuroidea) from *Galeopterus variegatus* (*G. variegatus*) (Audebert, 1799) which is the synonym of *Cynocephalus variegatus* (*C. variegatus*) (Audebert, 1799) in Cirebon, West Java, Indonesia, and the genotype is *Auchenacantha galeopteri* (*A. galeopteri*)<sup>[1–3]</sup>. Yamaguti reported that *Auchenacantha* is the synonymy of *Hoeplius*, described from *Cynocephalus volans* from Philippine<sup>[4,5]</sup>. *Auchenacantha* comprises of ten species *i.e.* *Auchenacantha galeopteri* (*A. galeopteri*) (Baylis, 1929), *Auchenacantha spinosa* (*A. spinosa*) (Baylis, 1929), *Auchenacantha magna* (*A. magna*) (Robinson, 1934), *Auchenacantha coronata* (*A. coronata*) (Linstow, 1903), *Auchenacantha parva* (*A. parva*) (Robinson, 1934), *Auchenacantha corollata* (*A. corollata*) (Schneider, 1886), *Auchenacantha boholi* *A. boholi* (Chu, 1931), *Auchenacantha*

*purvisi* (*A. purvisi*) (Robinson, 1934), *Auchenacantha hoeplii* (*A. hoeplii*) (Robinson, 1934), and *Auchenacantha robertrauschi* (*A. robertrauschi*) (Hugot, 1986). All 10 species were reported to be from Dermoptera genus *Cynocephalus* from Malaya, Philippine, and Indonesia<sup>[6]</sup>.

Sexual dimorphism presents in the genus is characterized by having six lips in the female, and 3 lips in the male, cuticle with transverse striation that is varied in shape, fineness, waviness or spininess. Oesophagus dilated anteriorly with valve and ending in a bulbe with valve. Male has single spicule. In Indonesia, two species of *Auchenacantha*: *A. galeopteri* and *A. spinosa* have been described from *C. variegatus* from Cirebon, West Java. Those two species and *A. parva* have been found from the same host species, *C. variegatus*, but from different locations, Sukabumi, Ujung Kulon and West Java. Their morphology observed using SEM is presented in this study.

## 2. Materials and methods

Nematodes observed in this study were collected from

\*Corresponding author: Endang Purwaningsih, Zoological Division of Research Centre on Biology– LIPI, Widyasatwaloka Building, Jl Raya Bogor Km 46, Cibinong 16911, Indonesia.

E-mail: enpur\_22@yahoo.com.

Foundation Project: Supported by DIPA Project/2010 of Research Centre on Biology–LIPI.

Museum Zoologicum Bogoriensis (MZB), nos MZBNa 318, 477, 478, 479, 480, and 493 were collected from the host *C. variegatus* from Sukabumi and Ujung Kulon, West Java, Indonesia.

Specimens for light microscopy examination were fixed with warm 70% alcohol, cleared and mounted in lactophenol for wet mounting. Drawings were made with the aid of a drawing tube attached to a Nikon compound microscope. Measurements were given in micrometers ( $\mu\text{m}$ ) as the average of findings, followed by the range in parentheses, unless otherwise stated. Specimens for SEM examination were processed according to Bozzola[7]. They were fixed in cacodylate buffer and glutaraldehyde, dehydrated through a graded series of alcohol, and then freeze dried using Labconco Model 79480 (Labconco Co., Kansas City, Missouri) prior to attaching to stubs with double sided cello-tape, coated with gold 400 Å thickness in an Eico I-B2 ion coater and observed with a JSM5310 LV Scanning Electron Microscope.

### 3. Results

#### 3.1. *Auchenacantha galeopteri* Baylis, 1929

In general, *A. galeopteri* is medium in size, cuticle with transverse striation and the first striation is fine beginning from behind the base of lips (Figure 1B, 1C), then widen towards outside and wavy (Figure 1B, 1C), longer interval than posterior region, the wavy striation disappeared at the level of mid oesophagus (Figure 1D), continuing to fine annulation up to posterior extremity, the interval is shorter (Figure 1E). In terms of sexual dimorphism present, the female has mouth circular with 6 lips, bearing two amphid at lateral and four submedian papillae. The tip of each lip curved outwards (Figure 1A, 1B). Male is with three lips, each lip is wider at base than distal end, indented at the middle of distal end (Figure 1F). Lateral alae is extending from behind the base of lip (Figure 1G) to 1/6 of tail length from tip of tail. Its oesophagus is long, slender and dilated anteriorly into a bulb with valvular apparatus, ending with a pyriform bulb, containing valve. Excretory pore is not seen. Nerve ring is found at the anterior region. Male is with single spicule, the cuticle of posterior extremity forms expansion, supported by three slender like processes, one median, two lateral, each of lateral processes with single small branch at the middle of them. A pair of large papilla and small single is around the cloaca. Vulva is at around the oesophageal bulb. Tail of female is tapering.

Male has a total length of 4151 (3600–4580)  $\mu\text{m}$ , width at anterior end is 42 (40–45)  $\mu\text{m}$ , with the maximum width of 209 (195–245)  $\mu\text{m}$ . The first striation is from the base of lips 25  $\mu\text{m}$ , striation intervals are 5–6  $\mu\text{m}$  at anterior region and 1–2  $\mu\text{m}$  at posterior extremity. From base of lips to the beginning of lateral alae 11, posterior end to the tip of alae 317  $\mu\text{m}$ . Anterior end to nerve ring 158 (130–180)  $\mu\text{m}$ , oesophagus

length 959 (905–1050)  $\mu\text{m}$ , oesophageal bulb length 74 (70–75)  $\mu\text{m}$ , width 53 (51–55)  $\mu\text{m}$ . Spicule length 121(112–135)  $\mu\text{m}$ .

Female has a total length of 8652 (7580–9590)  $\mu\text{m}$ , width at anterior end is 85 (70–92)  $\mu\text{m}$ , with the maximum width of 316 (260–470)  $\mu\text{m}$ , from base of lips to first striation 49.8  $\mu\text{m}$ . Striation intervals are 12.5 (9.4–16.2)  $\mu\text{m}$  at anterior region, 8–9  $\mu\text{m}$  at posterior. From base of lips to the beginning of lateral alae 4.09  $\mu\text{m}$ , ended at 214  $\mu\text{m}$  from posterior end. Anterior end to nerve ring reach a length of 268 (220–280)  $\mu\text{m}$ . Oesophagus length 1820 (1560–1900)  $\mu\text{m}$ , oesophageal bulb length, 169 (120–250)  $\mu\text{m}$ , width 141(118–170)  $\mu\text{m}$ . Vulva has a length of 1663 (1540–1790)  $\mu\text{m}$  from anterior end. The tail is long and slender with a length of 1835 (1520–2180)  $\mu\text{m}$ . The eggs is thin shelled, oval 55 (53–9) by 28 (23–31)  $\mu\text{m}$ .

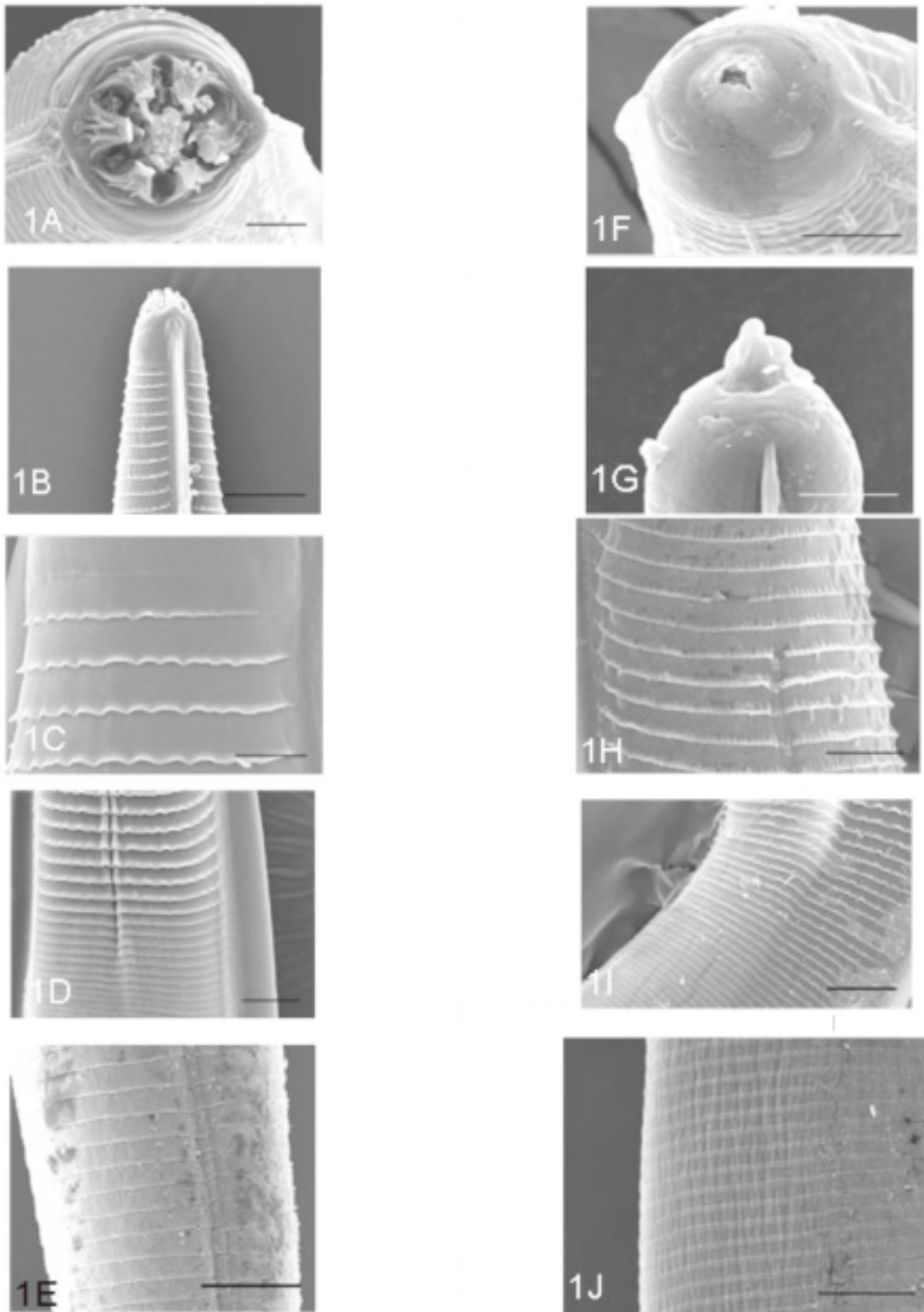
#### 3.2. *Auchenacantha spinosa* Baylis, 1929

In General, *A. spinosa* is medium in size, cuticle with transverse striation, the first striation is without spine and the second bearing eight large spine, arranged in circular, followed the striation (Figure 2A), then the spines become smaller in size but more in number (Figure 2B, 2C, 2D), the spine disappears at the level of vulva, continuing with fine striation to posterior region (Figure 2E). The mouth is circular with 6 lips bearing two amphid at lateral and 4 submedian papillae in the female, the tip of each lip curved outwards (Figure 2A, 2B), while male were not found. Lateral alae present, extending from the front of vulva to near the tip of tail. The oesophagus is short, slender, ending in a bulb, containing valve. The nerve ring is at anterior region, excretory pore is not seen, female tail is short, tapering with vulva behind the posterior end of oesophagus.

Female: Total length 5058 (4420–6040)  $\mu\text{m}$ , width at head 86 (60–90)  $\mu\text{m}$ , maximum width 321 (230–410)  $\mu\text{m}$ , from base of lips to first striation 18.1  $\mu\text{m}$ , striation interval 27–35  $\mu\text{m}$  at anterior region, 4  $\mu\text{m}$  at posterior region. From base of lips to the beginning of lateral alae 11  $\mu\text{m}$ , ended at 110  $\mu\text{m}$  from tip of tail. Anterior end to nerve ring 188 (150–250)  $\mu\text{m}$ , excretory pore not seen. Oesophagus length 427 (430–530)  $\mu\text{m}$ , esophagus bulb length 88 (73–94)  $\mu\text{m}$ , width 72 (60–81)  $\mu\text{m}$ , vulva 1004 (860–1300)  $\mu\text{m}$  from anterior end, tail length 776 (770–880)  $\mu\text{m}$ , eggs thin shelled, banana shaped, 64 (56–63) by 19 (18–28)  $\mu\text{m}$ .

#### 3.3. *Auchenacantha parva* Robinson, 1934

In general, *A. parva* is medium in size, cuticle with fine transverse striation along the body, beginning from the behind lips (Figure 2G, 2H, 2I, 2J). The female's mouth has six lips bearing two amphid at lateral and 4 submedian papillae. Each lip has transverse festoon like pattern at the anterior end (Figure 2F). Lateral alae extends from behind lips to near the tip of tail. The oesophagus is short, ending in a pyriform bulb, with valve, nerve ring at anterior region and the excretory pore is not seen. The tail of female is long, slender with vulva behind the posterior end of esophagus.



### Figures 1.

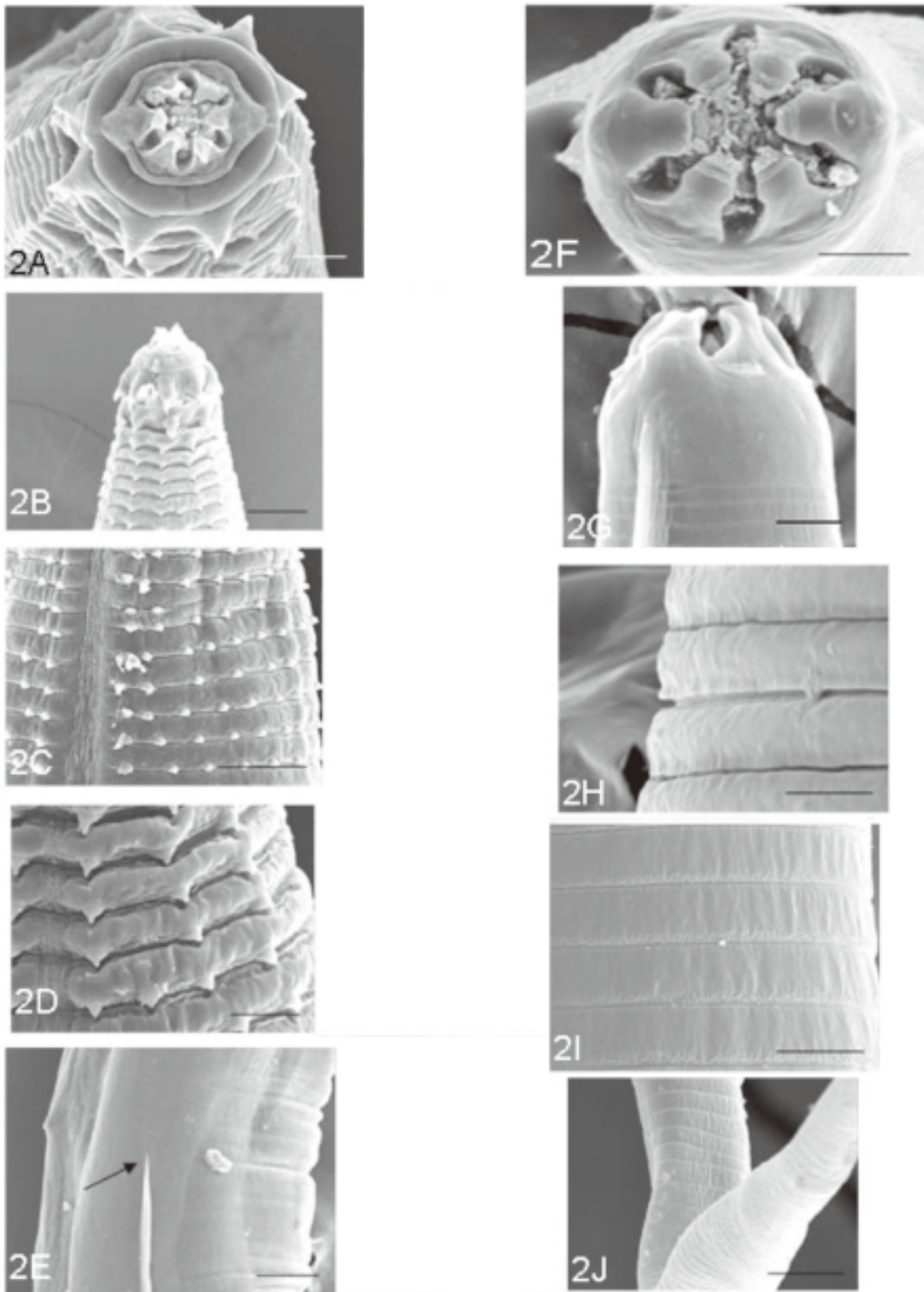
*A. galeopteri*, female.

1A: Mouth, enface view; 1B: Anterior end, lateral view; 1C: The beginning of wavy annulation, ventral view; 1D: The end of wavy and the beginning of fine annulation, ventral view; 1E: Annulation at the tail, ventral view;

*A. galeopteri*, male.

1F: Mouth, enface view; 1G: Anterior end, lateral view; 1H: Annulation at anterior region, ventral view; 1I: The end of wavy and the beginning of fine annulation, ventral view; 1J: Annulation at posterior end, lateral view.

Scale bars: Figure 1 A, C, F, G, H = 20  $\mu$  m; Figure 1 B = 100  $\mu$  m; Figure 1 D = 50  $\mu$  m; Figure 1 I, E = 10  $\mu$  m.



### Figures 2.

*A. spinosa*, female.

2A: Mouth, nface view; 2B: Anterior end, lateral view; 2C: Annulation at anterior region, ventral view; 2D: Annulation with smaller spine, ventral view; 2E: The annulation without spine and the beginning lateral alae ventral (arrow), ventral view;

*A. parva*, female.

2F: Mouth, nface view; 2G: Anterior end, lateral view; 2H: Annulation at anterior region, ventral view; 2I: Annulation at mid body, ventral view; 2J: Annulation at the tail, ventral view.

Scale bars: Figure 2 A, D, F, G = 20  $\mu$  m; Figure 2 B, C, E = 50  $\mu$  m; Figure 2 H, I, J = 10  $\mu$  m.

Male not found.

Female has a total length of 4454 (3970–4890)  $\mu\text{m}$  and the width at head is 65 (60–70)  $\mu\text{m}$ . The maximum width is 353 (310–410)  $\mu\text{m}$ . The length from base of lips to the first striation is 34.4  $\mu\text{m}$  with striations interval 5–7  $\mu\text{m}$  at the anterior end, 2–2.5  $\mu\text{m}$  at the posterior end. From base of lips to the beginning of lateral alae the length is 13.9  $\mu\text{m}$ , ended at 135  $\mu\text{m}$  from tip of tail. The length from anterior end to nerve ring is 195 (180–210)  $\mu\text{m}$  and the excretory pore is not seen. The oesophagus has a length of 970 (960–980)  $\mu\text{m}$ , oesophageal bulb reach a length of 174 (170–180)  $\mu\text{m}$  and width 153 (140–178)  $\mu\text{m}$ , vulva from anterior end 1147 (993–1300)  $\mu\text{m}$ . The tail length is 920 (670–1385)  $\mu\text{m}$ . The eggs oval 44 (43–45) by 21 (20–26)  $\mu\text{m}$ .

#### 4. Discussion

The measurements of *A. galeopteri*, *A. spinosa* and *A. parva* in the present study are still within the measurement range of those previously described. Some characters of *A. galeopteri* in the previous description by Baylis<sup>[1]</sup> and Hugot<sup>[6]</sup>, are different from that of the present study using scanning electron microscope (SEM). The head of *A. spinosa* is similar with that of *A. galeopteri*<sup>[1]</sup>, but, different lip shapes. The lips of *A. galeopteri* are wider at base than distal end (trapezoid-shaped) and it is rectangular in *A. spinosa*. Dentiform protrusion appears from the inner surface of each lips, it seems identical with line cuticle thickening in the inner surface of the lips<sup>[1]</sup>. The striations of *A. galeopteri* are spiny<sup>[1]</sup>, those are wavy in this study. Hugot also presented the figures of wavy striation of *A. galeopteri*<sup>[6]</sup>. The lips of *A. parva* are similar with that in *A. robertraushci* (Hugot, 1986), in having transverse festoon like pattern at anterior end of the lips, but, *A. parva* have circular mouth not hexagonal as in *A. robertraushci*.

The males found were believed as *A. galeopteri*, because the striations are wavy at anterior and fine at posterior region, same with that of the female of *A. galeopteri*. Baylis also found the male of *A. galeopteri* with the same characters with the male in present study, except the striations pattern. The sexual dimorphism in *Auchenacantha* is one of the difficulties in determining the male species, because in one individual host usually more than one species of *Auchenacantha* are found. Hugot found four different kinds of male (*Auchenacantha* sp1–sp 4), but there is no matching of females with males<sup>[6]</sup>. The males in the present study is close to *Auchenacantha* sp 2, in having the similar lips, spicule and tail shape and the pattern of striation.

Family Oxyuridae were commonly found in mammals than other groups, such as rodent and chimpanzee<sup>[8–11]</sup>, but the ten species of *Auchenacantha* were found only on an archaic and small mammal group. They have strongly differentiated character existing in their hosts of complex parasitic fauna so *Auchenacantha* can be considered as highly specialized parasites. According to Hugot this specialization and the

complex distribution of the morphological forms permit the hypothesis of a long association of the hosts and parasites during the Tertiary<sup>[6]</sup>. Sukabumi and Ujung Kulon are the new locality for *A. galeopteri*, *A. spinosa* and *A. parva*. In Indonesia *C. variegatus* distributed in Kalimantan, Sumatra and Java but in Java only found in West Java<sup>[3,12]</sup>.

#### Conflict of interest statement

We declare that we have no conflict of interest.

#### Acknowledgements

I wish to thank the team of Mammals Survey who provided me with the specimens for this study from Ujung Kulon and to Mr. and Mrs. Mushashino who donated the specimens from Sukabumi. The author is also greatfull to Dr. Hideo Hasegawa for providing me with the literature which help to improve this paper. This Research was financially supported by DIPA Project/2010 of Research Centre on Biology–LIPI.

#### References

- [1] Baylis HA. Some new parasitic nematodes and cestodes from Java. *Parasitol* 1929; **21**(3): 256–265.
- [2] Peter AJ, Quentin JC. Keys to genera of Oxyuroidea. Anderson RC, Chabaud AG, Sheilla W, editors. In: *CIH keys to the nematode parasites of Vertybrates*. England: CAB International; 1976, p. 174–203.
- [3] Boeadi, Steinmetz. IUCN red list of threatened species version 2010. [On line] Available from: www. iucnredlist.org. downloaded 2–2–2011.
- [4] Yamaguti S. *Systema helminthum. Vol. 3: Nematode parasites of vertebrates*. London: Interscience Publisher; 1961.
- [5] Chu HJ. Nematode from flying lemurs in the Philippine island and from birds in China. *J Parasitol* 1931; **17**(3): 155–160.
- [6] Hugot JP. Sur le genre *Auchenacantha* (Oxyuridae: Nematoda) parasites de Dermopteres. Etude de la morphologie et de la distribution des formes. *Syst Parasitol* 1986; **8**(4): 243–266.
- [7] Bozzola JJ, Russel LD. *Elektron microscopy: Principle and techniques for biologist*. Boston: Jones and Bartillet; 1992.
- [8] Anderson RC. *Nematode parasites of vertebrates: Their development and trasmission*. 2nd ed. Wallingford: CABI Publishing; 2000.
- [9] Hideo H, Ikeda Y, Fujisaki A, Moscovice LR, Petzelkova KJ, Kaur T, et al. Morphology of chimpanzee pinworm, *Enterobius (Enterobius) Anthropophiteci* (Gedoelst, 1916) (Nematoda: Oxyuridae), collected from chimpanzees, *Pan troglodytes*, on Robondo Islan, Tanzania. *J Parasitol* 2005; **91**(6):1314–1317.
- [10] Robles MR, Graciela TN. A new species of *Syphacia* (Nematoda: Oxyuridae) from *Oligoryzomys nigripes* (Rodentia: Cricetidae) in Argentina. *Parasitol Res* 2007; **101**(4): 1069–1075.
- [11] Weave HJ, Smiles LR. Three new species of *Syphacia* (*Syphacia*) (Oxyurida: Oxyuridae) from Queensland, Australia, and a key to the species present in the Australian bioregion. *Comp Parasitology* 2010; **77**(1): 9–19.
- [12] Suyanto A, Yoneda M, Maryanto I, Maharadatunkamsi, Sugardjito J. *Checklist of the mammals of Indonesia*. Bogor: LIPI– JICA– PHKA; 2002.