BIODIVERSITAS
Volume 24, Number 6, June 2023
Pages: 3230-3235

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240617

Surface ultrastructure of tick (Acari: Ixodidae) on Moa buffalo from Southwest Maluku District, Indonesia

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Manuscript received: 11 April 2023. Revision accepted: 15 June 2023.

Abstract. *Utami P, Kunda RM. 2023. Surface ultrastructure of tick (Acari: Ixodidae) on Moa buffalo from Southwest Maluku District, Indonesia. Biodiversitas 24: 3230-3235.* Moa buffalo (*Bubalus bubalis* Linnaeus, 1758) is one of the local Indonesian buffalo families in the Southwest Maluku District with very limited distribution and is found only on Moa Island. It exhibits distinct characteristics that distinguish it from clumps of mud buffalo and other local breeds, making it a valuable local livestock resource in Indonesia. Meanwhile, ticks are obligatory hematophagous ectoparasites with direct effects on the host, such as blood and weight loss, and their indirect effects are more associated with their role as vectors of disease-carrying pathogens. This study aimed to identify ticks infesting Moa buffalo by analyzing their surface ultrastructure using a Scanning Electron Microscope (SEM) method. This method enabled the establishment of the taxonomic status to obtain comprehensive data regarding the mapping of tick species in Moa buffalo. The samples were manually collected, and the body of the cattle was divided into four regions to determine the preferred attachment sites of ticks. The head, back, tail, and legs were all carefully examined, and each tick was carefully picked from the surface using tweezers or a pair of thumb forceps. Furthermore, the tick samples were collected from three different parts of the Moa buffalo's body, namely the armpit, inguinal region, and perianal area. Based on observations of morphological characters, the ticks found in Moa buffalo were of two species, namely *Dermacentor auratus* Supino, 1897 and *Haemaphysalis bispinosa* Neumann, 1897. The Dermacentor genus, specifically *D. auratus* exhibited specific morphological characteristics, including a pair of eyes located at the coxae II level, an ornate scutum, capitulum, and feet, as well as a short hypostome with 3:3 tooth arrangement. Members of this genus also had festoons, with the size of coxae increasing from I to IV. On the other hand, *H. bispinosa* was characterized by underdeveloped cornua. The segments of palpal III, for both males and females exhibited posterodorsal and posteroventral protrusions. The posteroventral segment of palpal III formed a wide and blunt triangle in both sexes, while the palpal III section had wide median spurs. These characteristics were keys for the identification of *H. bispinosa* species.

Keywords: Acari, Ixodidae, Moa buffalo, Southwest Maluku District, tick, ultrastructure

INTRODUCTION

Moa buffalo (*Bubalus bubalis* Linnaeus, 1758) is one of the local Indonesian buffalo families in the Southwest Maluku District. According to the Minister of Agriculture Decree No. 2911/Kpts/OT.140/6/2011, it has a very limited distribution and is only found on Moa Island. It exhibits uniformity in both physical form and genetic composition, as well as shows remarkable adaptability to the environmental limitations on Moa Island, which is characterized by short rainfall and prolonged periods of heat in the year. Furthermore, the Moa buffalo has distinct characteristics distinguishing it from clumps of mud buffalo and other local breeds, making it a valuable local livestock resource in Indonesia that needs to be protected and preserved.

Ticks are obligatory hematophagous ectoparasites that infest tropical and subtropical terrestrial and semi-aquatic vertebrates (Karim et al. 2017; Ali et al. 2019). The direct effects of tick infestation on buffalo include blood and weight loss, while the indirect effects are more associated with their roles as vectors of disease-carrying pathogens. Ticks inflict harm on their hosts through a variety of mechanisms, including the transmission of pathogens such as bacteria, viruses, and protozoans (Boulanger et al. 2019). Tick (Ixodidae) is an ectoparasite commonly found in ruminants. Although the frequency of infestations in buffalo is not as high as in cattle (Nithikathkul et al. 2002), this does not imply a lack of impact. Abbasi et al. (2017) reported that among the numerous challenges in ruminant farming, cases resulting from parasitism were considered the foremost inhibiting factor in the development of livestock populations, including buffalo.

The distribution of ticks and the diseases they transmit are influenced by climate. Infestation of ticks in livestock can cause significant economic losses in the livestock industry. Piroplasmosis (babesiosis and theileriosis) and rickettsiosis (anaplasmosis) are common protozoan diseases caused by ticks in livestock, and have emerged as limiting factors in various countries, causing some health problems within the livestock industry (Abdallah et al. 2017; de la Fuente et al. 2017). Several field studies showed the widespread presence of tick species in buffalo breeds across different countries (Anderson et al. 2013; Abbasi et

al. 2017). Therefore, fundamental studies covering aspects of species identification with surface ultrastructure using a Scanning Electron Microscope (SEM) can help establish taxonomic status to obtain comprehensive data regarding the mapping of tick species in Moa buffalo.

In Indonesia, comprehensive studies of infestation and tick identification in ruminants are rarely reported, and when available, they only provide partial data analysis with minimal novelty. Sahara et al. (2019) successfully identified that the tick infesting cattle in five different provinces of Indonesia were of two genera and three species, namely *Rhipicephalus microplus* Canestrini, 1888, *Haemaphysalis bispinosa* Neumann, 1897 and *Rhipicephalus pilans* Schulze, 1935. The identification of ticks infesting buffalo, particularly Moa buffalo family has not been widely reported in Indonesia. Therefore, this study aimed to contribute to the taxonomic data by providing a complete picture of the ultrastructure.

MATERIALS AND METHODS

Ethical approval

This study received approval from the Animal Ethics Committee at the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia, in compliance with the procedure of using animals in studies.

Study area

This study was conducted through sampling, and ethical approval was not necessary. Tick specimens were collected from a total of 50 buffaloes (20 male and 30 female) at four locations in Moa Island, Southwest Maluku District, Maluku Province, Indonesia (Werwaru, Tounwawan, Klis, and Moain) (Figure 1).

Procedures

Collection of specimens

The collection of ticks was carried out on buffaloes within the age range of 5-10 years. During the collection of field samples, it was observed that each tick organ was in good condition. The head, back, tail, and legs were all carefully examined, and each tick was carefully picked from the surface using tweezers or a pair of thumb forceps. The samples were collected from three different body parts of Moa buffalo, namely the armpit, inguinal region, and perianal area. These body parts were selected based on the level of density and attachment of ticks commonly performed in cows (Sahara et al. 2019), which was confirmed during the initial site survey. Tick sampling was conducted during the first to third weeks of June 2022, both in the morning and evening. The specimens were collected from all stadia (larvae, nymphs, and adults), preserved in 70% ethanol, and sent to the Laboratory of Parasitology, Faculty of Biology, Universitas Jenderal Soedirman, Purwokerto, Indonesia, for initial identification. They were subsequently shipped to the *Laboratorium Penelitian dan Pengujian Terpadu*, Universitas Gadjah Mada (LPPT-UGM), for surface ultrastructure identification using SEM. A total of 220 ticks (110 males, and 59 females of *H. bispinosa* and 51 males of *D. auratus*) were collected during the sampling periods.

Identification of surface ultrastructure using SEM methods

The protocol for analyzing microscopic characters was based on Sahara et al. (2019), and Utami et al. (2021) with some modifications. Specimens were sampled using an ultrasonic device for 10 minutes to effectively clean ticks from any debris remnants of their hosts, and were subsequently observed under a stereo microscope (Olympus-CH20BIM). Surface ultrastructural observation was conducted using a JEOL JSM-651OLA SEM. The observed features included overall appearance, as well as the anterior and posterior parts of the ticks. SEM preparations were performed through LPPT-UGM procedures.

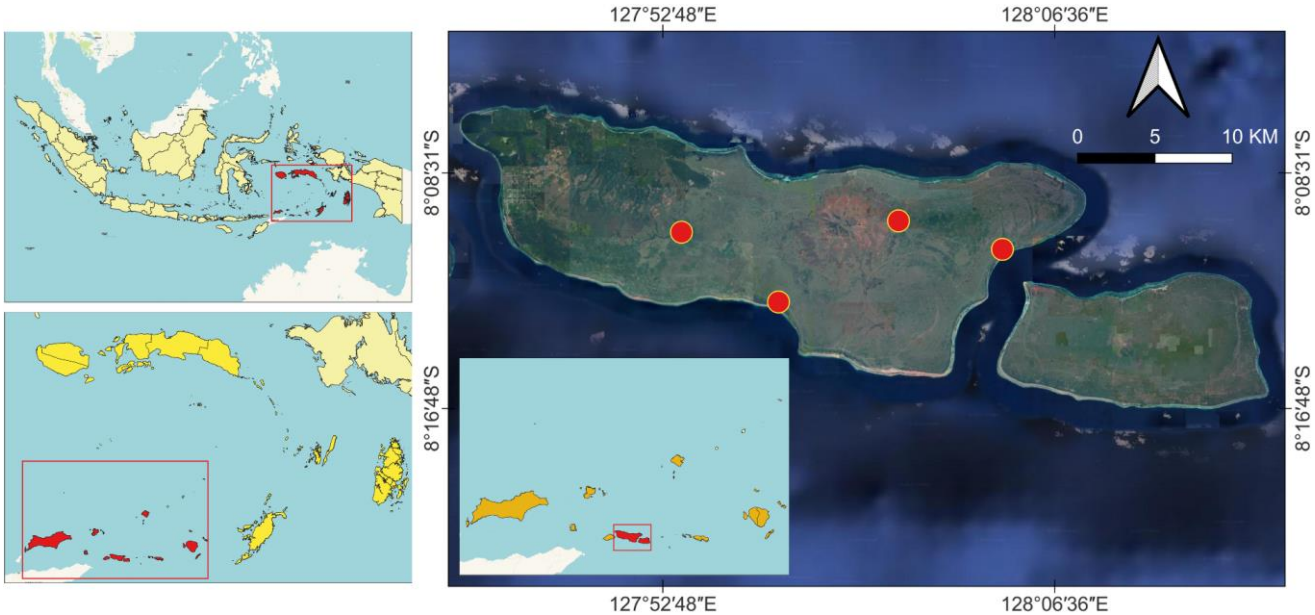

Figure 1. Map of the sampling locations. Tick samples were collected on Moa buffalo from four locations

Data analysis

Tick ultrastructure data were descriptively analyzed based on morphological key characters found in the anterior and posterior parts, and in correlation with the identification keys proposed by Anastos (1950) as well as Tanskull and Inlao (1989).

RESULTS AND DISCUSSION

Morphological characters of Moa buffalo tick

Based on the observation results of morphological characters, tick found in Moa buffalo was of two species, namely *Dermacentor auratus* Supino, 1897 and *Haemaphysalis bispinosa* Neumann, 1897. The *Dermacentor* genus exhibits specific morphological characteristics, including a pair of eyes located at the level of coxae II, an ornate scutum, capitulum, feet, and a short hypostome with a 3:3 tooth arrangement. Members of this genus also have festoons in the posterior region, with the size of coxae increasing from I to IV, which is one of the characteristics of *Dermacentor* spp. (Ajith kumar et al. 2016). The palpal size is longer than the base of the capitulum, and the external and internal spurs on coxae I are visible. *Dermacentor auratus* females have a scutum and easily observable characteristics. When viewed dorsally, the base of the capitulum in *D. auratus* appears rectangular, with a pair of capitulum bases directed medially and protrusions on coxae I. These ticks have short and thick Palpals. The morphological characters of *D. auratus*, based on SEM results are shown in Figures 2A to 2J, respectively.

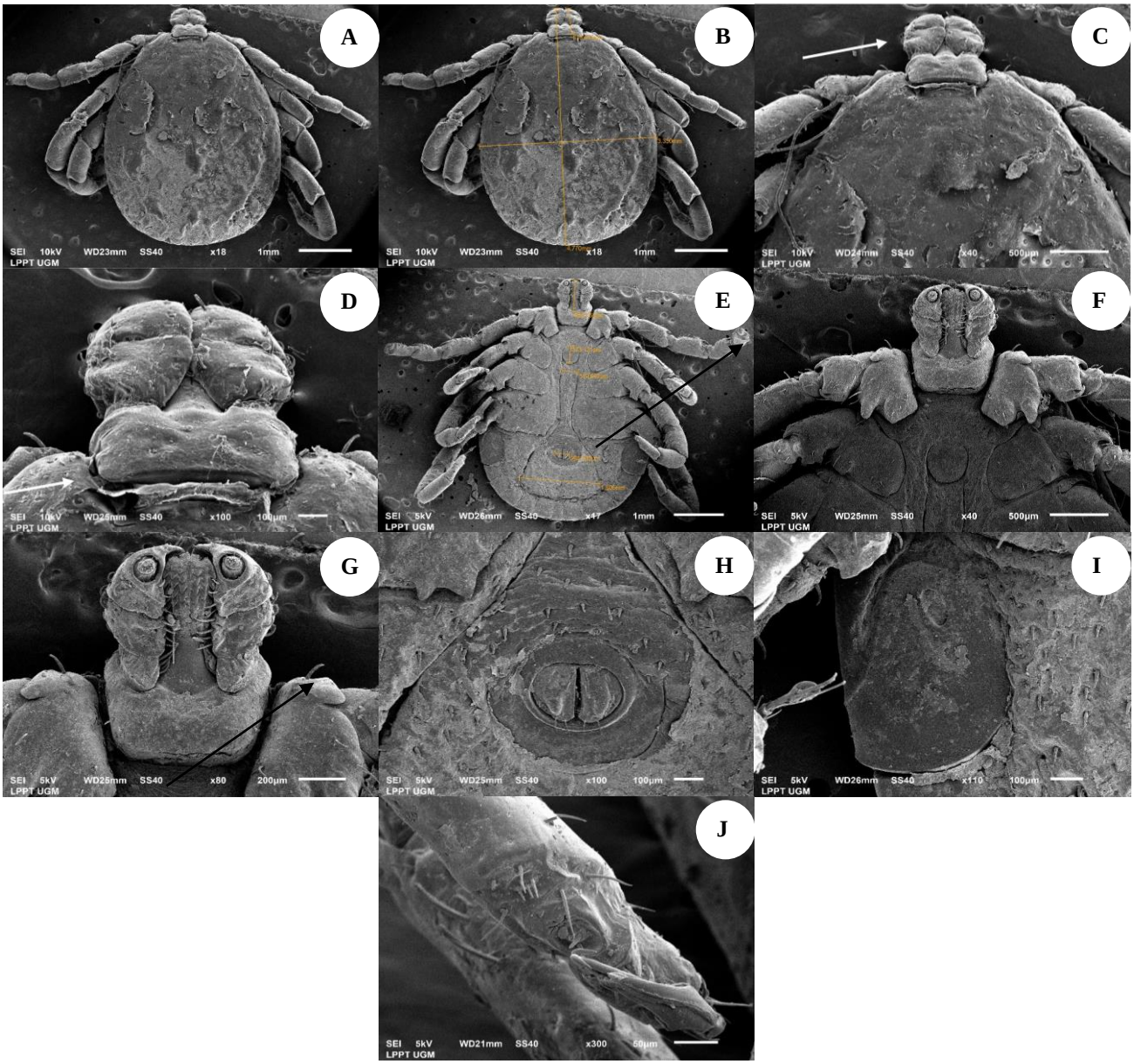

Figure 2. Morphological characters of *Dermacentor auratus* based on SEM results. A. Dorsal plate, B. Dorsal plate by size, C. Capitulum plate, D. Basis of capitulum, E. Ventral plate, F. Hypostome, G. Palpals, H. Anus, I. Spiracle plate, J. Haller's organ

The morphological characters of *Haemaphysalis* genera members found in Moa buffalo were identified using the classification provided by Anastos (1950). *Haemaphysalis bispinosa* is a species of tick found in Indonesia. The morphological characteristics of *Haemaphysalis* members, specifically this species, include a long and pointed tarsal section. The pulvillus part is long and narrow, but slightly shorter than the paws. The length of the capitulum ranges from 0.54 mm, and the base of the female capitulum is much wider than that of the male, with a large shaft area. Furthermore, this species has a short hypostome of a 4:4 tooth arrangement, with a structure of approximately eight teeth per file, as well as a short and blunt cornua shape. The morphological characters of *H. bispinosa* are presented in Figures 3 and 4, illustrating the males (Figures 3A to 3J) and females (Figures 4A to 4F), respectively.

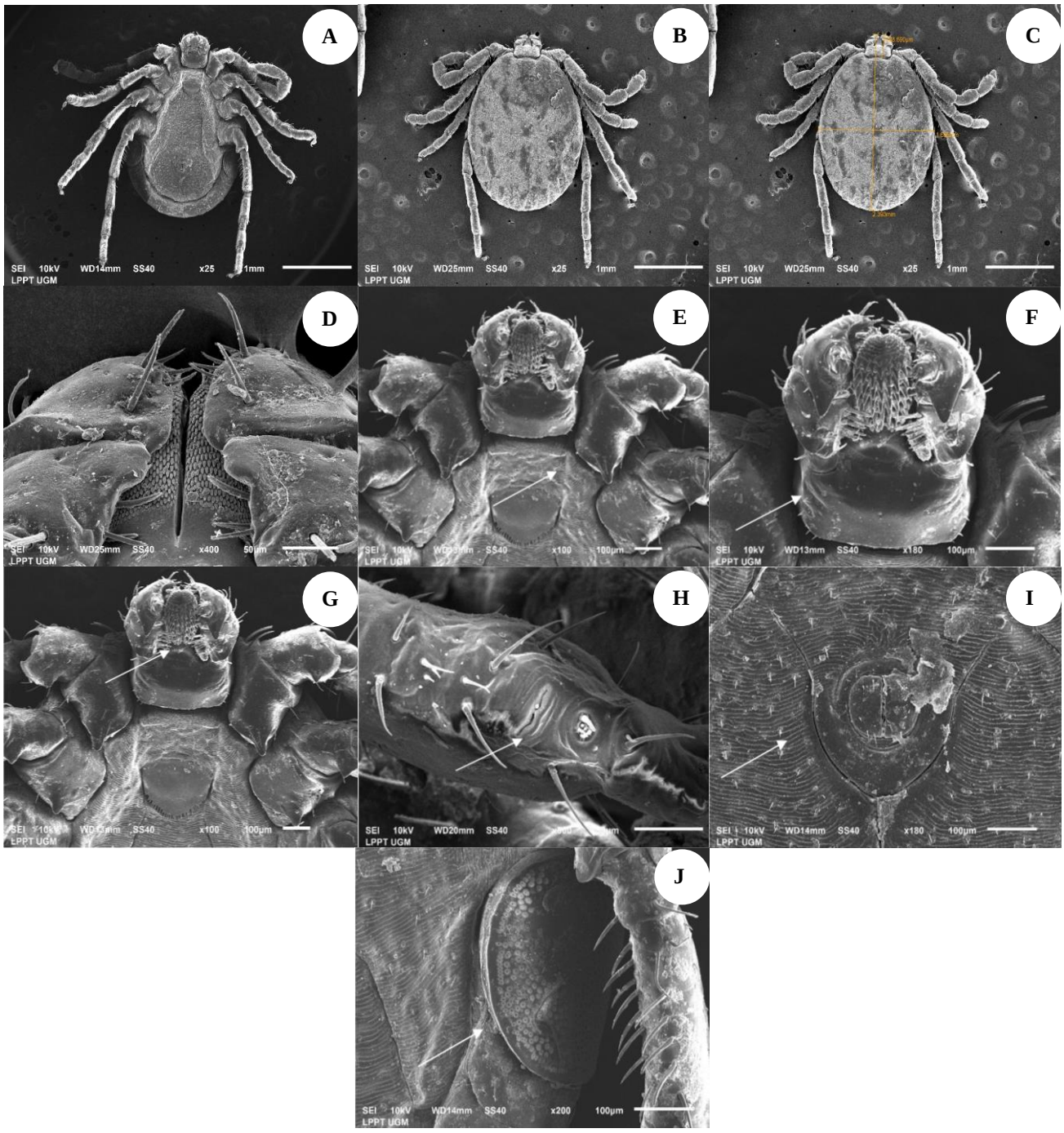

Figure 3. Morphological characters of male *Haemaphysalis bispinosa* based on SEM results. A. Ventral plate of male, B. Dorsal plate of male, C. Dorsal plate of male by size, D. Capitulum and Hypostoma, E. Coxae of male, F. Basis capitulum of male, G. Palpals of male, H. Haller's organ of male, I. Anus of male, J. Spiracle

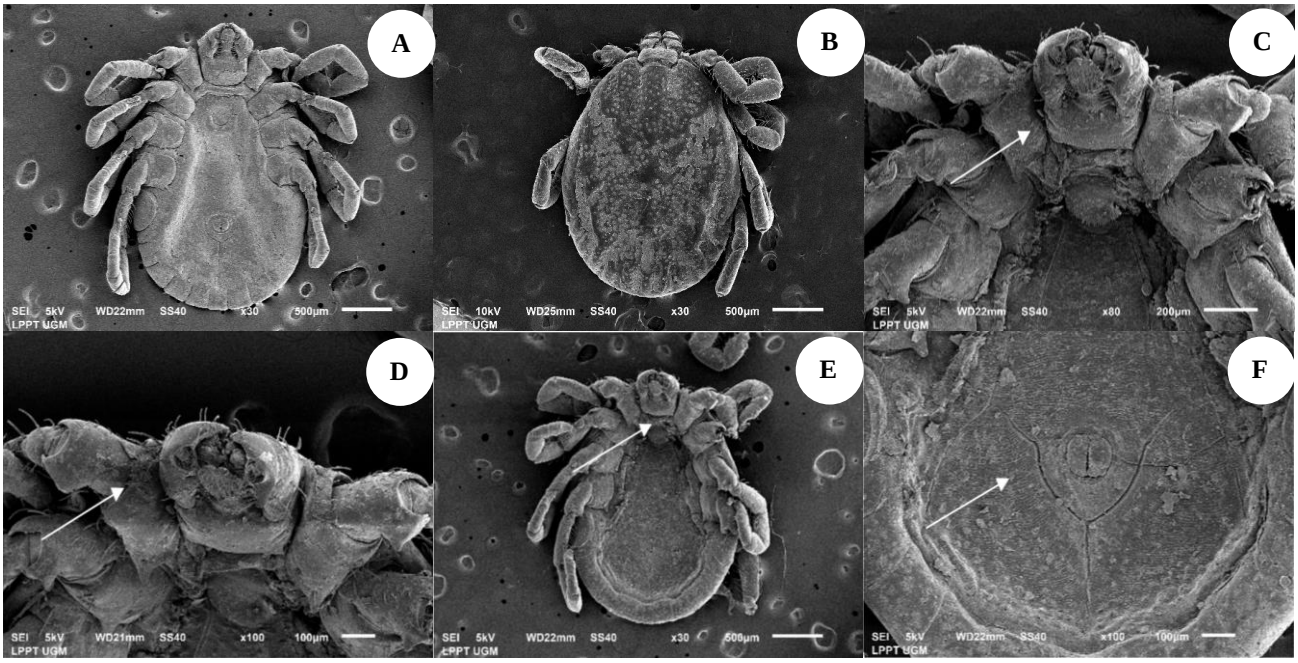

Figure 4. Morphological characters of female *Haemaphysalis bispinosa* based on SEM results. A. Ventral plate of female, B. Dorsal plate of female, C. Basis capitulum of female, D. The part of Palpals III, E. Coxae of female, F. Anus of female

Discussion

Dermacentor is one of the globally important genera of ticks, although its species diversity is relatively limited compared to members of the genera *Ixodes* and *Amblyomma* (Guglielmone et al. 2014). Petney et al. (2019) stated that the ecology and diversity of this species in Southeast Asia were less known. *Dermacentor auratus* is a hard-bodied tick of the genera *Dermacentor*, and has a wide geographical distribution throughout almost all of Southeast Asia, namely Indonesia, and Vietnam to India, Sri Lanka (Kwak 2018; Petney et al. 2019). The length from the capitulum to the middle festoon is 7 mm, and the maximum breadth at its mid length is 5 mm. Adult ticks have highly ornate scutum, capitulum, and legs, with a pair of eyes at the second level of coxae. It is also characterized by a brown base color marking on the dorsal scutum. In Indonesia, Kadarsan and Suwito (1993) noted the occurrence of *D. auratus* in the host *Sus barbatus* Müller, 1838 from Long Alango, Kayan Mentarang (East Kalimantan), based on morphological studies and a very limited number of individuals. The presence of *D. auratus* along the Malay Peninsula and Sumatra strongly indicated that this species entered Indonesia during the last ice age when it was still connected by land (Kwak et al. 2021).

The *Haemaphysalis* genus exhibits distinct morphological characteristics, including an oval-shaped body, brown color, lack of ornate features and eyes, as well as the presence of festoons. The length and width of the capitulum are almost the same, with a rectangular base. The second segment of the palps is slightly wider with a short hypostome. In all life stadia, there is a posterodorsal margin on palpal segment 3 with distinct spurs, and a posteroventral margin with broadly triangular spurs. The spurs on Coxa I are long and tapered, while those on Coxae II-IV are short. The species *H. bispinosa* has a short and blunt cornua. Kwak and Ng (2022) described the cornua of males as blunt and medium in size. According to Brahma et al. (2013), *H. bispinosa* exhibits an underdeveloped cornua. The segments of palpal III in both male and female, have posterodorsal and posteroventral protrusions. The posteroventral segment of palpal III forms a wide and blunt triangle in both sexes, and the palpal III section has wide median spurs. These characteristics are keys to the identification of *H. bispinosa* species (Kwak and Ng 2022). Spur formation can be observed in Coxae 1 through IV, with both males and females exhibiting similar spur patterns in Coxae 1-IV. Female *H. bispinosa* has a longer and wider scutum, while the cornua of males are shorter compared to those of females. According to Sahara et al. (2019), *H. bispinosa* is a tick species with three mammalian hosts in Asia and Australia. Its distribution in Australia is limited to coastal areas of New South Wales and southeastern Queensland, with high infestations reported in sheep and cattle.

Haemaphysalis bispinosa is known to have distribution areas in India, Sri Lanka, Nepal, Pakistan, Burma, China, Japan, Australia, New Zealand, Thailand, Malaysia, and Vietnam (Geevarghese and Mishra 2011). However, Chen et al. (2015) showed *H. bispinosa* was not found in China, which was contradictory to Geevarghese and Mishra (2011). It was later discovered that *H. bispinosa* found in China was species of *H. longicornis* Neumann, 1901. Anastos (1950) stated that *H. bispinosa* was distributed in Sumatra, Java, Sumbawa, Flores and Bali regions of Indonesia, but none had been reported in Maluku. Although Moa Island region is administratively included in Maluku Province, it ecologically falls within the biogeography of Nusa Tenggara, Indonesia. *Haemaphysalis bispinosa* has a

wide range of hosts, including sheep, goats, wolves, ferrets, mules, antelopes, buffaloes, cows, cats, dogs, donkeys, horses, ponies, rabbits, mice, chitals, tigers, birds, wild mammals, and rodents (Geevarghese and Mishra 2011). In this case, Moa buffalo in Southwest Maluku District is only infested by two species of ticks, namely *H. bispinosa* and *D. auratus*. Reports in Indonesia showed instances of *H. bispinosa* infesting chickens, goats, and even cats (Sahara et al. 2019). Infestations of *H. bispinosa* had been observed in cattle raised both within and outside pen areas of Yogyakarta Province, Indonesia. In various regions of Bangladesh, goats are more infested than cows, pigs, or buffaloes (Islam et al. 2006; Ghosh et al. 2007). In addition, Gosh et al. (2007) reported that *H. bispinosa* mostly parasitized goats (74%), cattle (12.0%), and buffaloes (10.8%). It is also worth noting that each developmental stage of *H. bispinosa* exhibits a different host, a pattern commonly found among all other three-host tick species, regardless of their genera or species.

ACKNOWLEDGEMENTS

The authors are grateful to the authorities of the agriculture office of Southwest Maluku District, Laboratory of Parasitology, Faculty of Biology UNSOED, Purwokerto, and LPPT-UGM Yogyakarta for the assistance in the permit process for field sample collection and the identification of ultrastructure surfaces. The authors are also grateful to Albertus Sairudy for helping with the collection of tick samples on Moa Island. This study received funding support or grants from the Universitas Terbuka, Indonesia, through the Institute for Research and Community Service in 2022. The authors declare no conflict of interest in this study.

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