The micromorphology of the African buffalo louse *Haematopinus bufali* as observed under the scanning electron microscope

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An extensive literature search revealed that no scanning electron microscopical investigation has, to date, been performed on *Haematopinus bufali*, a sucking louse, host specific to the African buffalo (*Syncerus caffer*).

Live lice were collected from a diseased African buffalo in the Rietvlei Nature Reserve. The specimens were fixed in 70 % ethanol. Graded alcohol was used to achieve dehydration of the lice. The specimens were sonicated. The lice were dried at the critical point of medical wet carbon dioxide, mounted and fixed onto stubs. The lice were then coated with gold and viewed in a Leica Stereoscan 420 SEM at 6–12 kV. Micrographs were recorded as tagged information files (*.tif) and later printed onto Hewlett Packard Premium Glossy Photographic Paper with a Hewlett Packard 1120C Professional Series printer.

The SEM investigation revealed several micro-morphological features not previously described in light microscopical investigations of *H. bufali*. The head was cone shaped and the haustellum was situated on the anterior tip. Setae were positioned laterally to the haustellar opening. Omatidia were absent. The apex of the distal tip of the antenna showed the sensilla basoconica and sensory pegs. The inner face of the last segment revealed a pore, plate and tuft organs. The fourth segment also showed a pore organ. The pro, mesa and metathorax were completely fused. Three pairs of legs with highly modified claws were present. Between the distotibial pad at the base of the tarsal claw, a small structure resembling an empodium was found. Two mesothoracic spiracles were noted. The abdomens of the male and female specimens differed considerably but were closely related. Generally *H. bufali* differs distinctly from related species due to its elongated, slender, dorso-ventrally flattened and elliptically shaped body and the scalloped appearance of the elongated abdomen.

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Introduction

The lice of mammalian and avian species are obligatory ectoparasites, mainly host specific and allocated to one of two groups, according to their feeding techniques. Lice are either anopluric (sucking lice living off the body fluids of the host) or mallophagic (biting lice feeding mainly off the epidermal tissues or feathers of the host) in their feeding strategies.

The superorder Psocodea comprises two orders, one of which is the Phthiraptera, a group with no free living representatives. It comprises obligate ectoparasites found on almost all avians and approximately a quarter of all mammals (Smith 2000; Soler Cruz 1995). Lice are further divided into four groups namely Amblycera, Ischnocera, Rhyncophthirina (a monogeneric group found on elephant and warthog only) and Anoplura. The former three are biting lice (Mallophaga) whilst the latter (Anoplura) are commonly known as sucking lice (Smith, 2000).

Biting and sucking lice are economically important parasites and play an important role in the success of game ranches, reserves and parks all over the world. Heavy lice infestations can lead to lower production yields and lower population numbers in herds of game. Their feeding habits can lead to severe skin irritations of the host and the resultant symptomatic scratching aggravates these conditions even further, thus causing severe dermatitis. High infestations of Anoplura may also cause harmful immune reactions (hypersensitivity and anaphylaxis), skin necrosis, localised haemorrhages, lowered weight gain or weight loss and so forth (USDA 1997). High infestations may lead to anaemia, hypoproteinaemia, secondary infection (Green *et al.* 2001), nutritional deficiencies and reduced vigour as well as secondary bacterial or fungal infections. The spread of lice infestations is promoted by increased population density, as is the case in most infectious situations.

In order to design an effective control progamme in the event of high infestations, it is necessary to correctly identify the ectoparasites infesting the game.

The African buffalo (*Syncerus caffer*) is ranched on many game farms and is also protected in many game reserves in southern Africa. Extensive literature searches revealed that no scanning electron microscopical investigations have, to date, been performed on *Haematopinus bufali* (*Pediculus bufali* De Geer 1778) (Fig. 1), a sucking louse, host specific to the African buffalo (Ledger 1980; Stimie & Van der Merwe, 1968; USDA 1997). The distribution of *H. bufali* coincides with that of its host and includes areas of South Africa, Namibia, Zimbabwe, Zambia, Malawi and Angola (Stimie & Van der Merwe 1968). The Anoplura are small, wingless and often, eyeless insects. The lice are flattened dorso-ventrally and are extremely well adapted to their blood-sucking ectoparasitic lifestyle. The head is conically shaped and adapted for piercing the skin of the host. This enables the dentate proboscis, situated in the trophic pouch (buccal funnel) of the haustellum, to function efficiently in assisting with the blood-sucking feeding mechanism (Kim, Pratt & Stojanovich, 1986). The mouthparts are usually retracted into the head whilst the parasite is at rest and not feeding,. Eversion of the haustellum reveals dentate structures which rotate in an outward direction and in a hook-like fashion. These assist in attaching to the host and facilitates the function of the robust and well developed claws, in their role of attachment to the host.

H. bufali, has to date, not been described under the scanning electron microscope (SEM) although authors such as Stimie & van der Merwe (1968) recorded diagrammatic sketches drawn from light microscopic observations, with little or no ultra-structural detail. Nevertheless, they briefly described the head, thorax and abdomen of the female and the genitals of both male and female which, in this investigation, aided in the identification of the species. It was hence concluded that the micro-morphology of *H. bufali* was incomplete and scanning electron microscopy was used to add to the existing information available in the literature at present.

Fig. 1. A SEM micrograph of the dorsal view of *Heamatopinus bufali*.

Materials and methods

Live lice were collected from a diseased African buffalo at the Rietvlei Nature Reserve which is situated approximately 20 km to the southeast of Pretoria. The buffalo was heavily infested and at least 30 lice were collected. The lice were found behind the ears, in the proximity of the genitalia and on the inner surfaces of the fore and hind legs. The lice were processed according to the method as described by Turner *et al.* (2002) for the mallophagean type waterbuck louse (*Bovicola hilli*). Reproduceable and consistent results were obtained by following this procedure and it is thus re-documented in order to facilitate the investigations of researchers who wish to obtain similar high quality photo-micrographic results.

The specimens were fixed in 70 % ethanol. Graded alcohol (70 %, 80 %, 96 % and absolute) was used to achieve dehydration of the lice. After dehydration the specimens were sonicated for approximately 30 seconds. Ultrasonic cleaning times may vary according to the toughness of the species and should be experimented with accordingly. The lice were dried at the critical point of medical wet carbon dioxide, after which they were mounted and fixed onto standard SEM pin type aluminium stubs with double-sided tape. The lice were sputter-coated with gold for two minutes at 12 mA under vacuum and in the presence of argon gas. The coating procedure was repeated from different angles to reduce the effects of charging when rotating and tilting the specimen stage (holder). The specimens were viewed in a Leica Stereoscan 420 SEM at 6–12 kV with 3–12 mm working distances.

Although the working distances varied due to the tilting of the specimen stage, good resolution and contrast was obtained by optimally manipulating the beam of electrons. The accelerating voltages varied proportionately to the working distances during tilting. The specimens were tilted from 0–90° and rotated from 0–360° thus covering all planes of the x, y and z axes through the specimens. The tilting and rotation was performed for the sole purpose of viewing the exterior surface of each louse in as many planes as possible. The contrast was set to 14 % whilst the brightness was kept at 32 %. An aperture of 30 µm was placed in the path of the beam to lessen the negating effects of diffraction and interference of the accelerated electrons. Micrographs were recorded as tagged information files (*.tif) and submitted as such.

Results

The lice were identified according to the distinct and pronounced morphological features as recorded by Stimie & van der Merwe (1968) using the light microscope. Their observations included the following:

Female: The head was slightly longer than broad and dorso-ventrally flattened. The fore head and hind head were almost equal in length. The hind head constricts into a 'neck'. The post-antennal angles of the ocular processes were well developed. The thorax was rectangular in shape. The sternal plate was trapezoidal in shape with an anteriorly directed posterior margin. The legs and claws were large and robust. The abdomen was elongated and flatly elliptical in shape. It had a 'scalloped' appearance due to the conical shape and the posterio-lateral angle of projection of the paratergites. The conical shape and angle of projection and the absence of paratergites on segments I and II were crucial observations during identification of the lice. Each tergite had a pair of median and a pair of submarginal sclerotic areas. These were absent on the first and second segments. The submarginal sclerotic areas were heavily sclerotised and formed definite plates. One or two setae were situated caudally to the paratergites. The gonophyses were concave in position and semilunar in shape.

Male: The male was very similar to the female but varied in length. It showed a typical v-shaped pseudopenis. The genitalia were heavily sclerotised. Generally these authors remark that *H. bufali* differs distinctly from related species because of its elongated, slender body, and the scalloped appearance of the abdomen.

The SEM investigation showed several micro-morphological features not previously described in *H. bufali*. The following specializations and anatomical features were revealed:

Head: The general light microscopic model of the head as described by Stimie & Van der Merwe (1968) was correct but incomplete

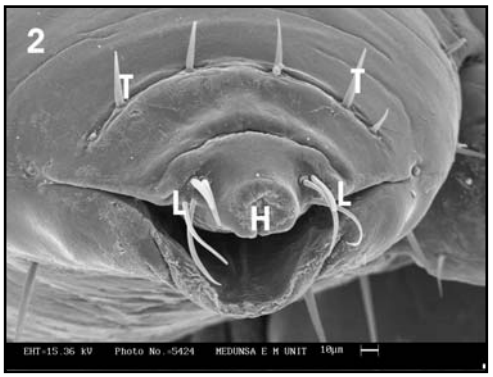

Fig. 2. The haustellum (H), two pairs of laterally (L) situated setae and six short transverse (T) setae.

Fig. 3. The labrum (L) was folded in a membranous fashion.

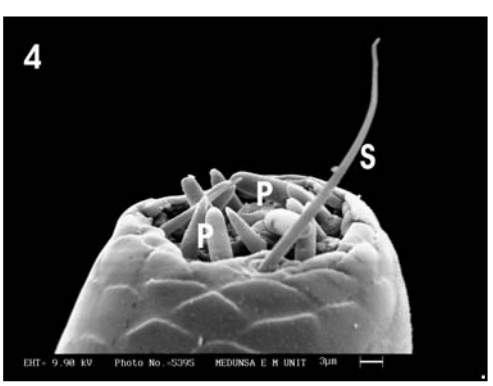

Fig. 4. The sensilla basoconica comprised 12 sensory pegs (P) and a long seta (S).

when viewed under the SEM. This is due to the higher resolution of the SEM when compared to that of a light microscope. The head was conically shaped and the haustellum was situated on the anterior tip (Fig. 2). Dentate processes as seen in the trophic pouch of *H. phacochoeri*, the warthog louse (Green *et al.* 2000), were not noted due to the orientation of the specimen in the microscope. Two pairs of setae were positioned laterally to the haustellar opening, naturally on the left and right side. The labrum was folded in a membranous fashion dorsally (Fig. 3). Omatidia were absent although prominent ocular processes were noted.

The five segments of each of the antennae of the male seemed thicker and more robust than those of the female but this could not be verified by measurement. The apex of the distal tip of the male antenna showed that the sensilla basoconica comprised 12 sensory pegs and a long seta at the rim of the apex (Fig. 4). At higher magnifications, the surfaces of the pegs seemed smooth, with no evidence of pores or other structures on their round and blunt tips. The inner face of the fifth segment revealed a complex of one pore organ (sensilla coeloconica) and two adjacent plate organs (Fig. 5). A pit organ (tuft organs) was observed within the sensilla coeloconica (Fig. 6). The fourth segment showed another pore organ but with no adjacent plate organs. No tuft organ protruded from this pore organ.

Thorax: The pro, mesa and metathorax were completely fused and the thorax was characterised dorsally by two notal pits on the prothorax and a central notal pit on the mesothorax (Fig. 7). Two spinous processes originated from the metathorax and extended toward the posterior. Three pairs of legs with highly modified claws were present. The three pairs of legs and claws were rather similar in size. Each leg had a large claw which gradually acuminated towards the most distal end. The legs showed the usual five segments namely the coxa, trochanter, femur, tibia and tarsus (Fig. 8). The tibia and tarsus were highly modified to form powerful grasping organs for attachment to the

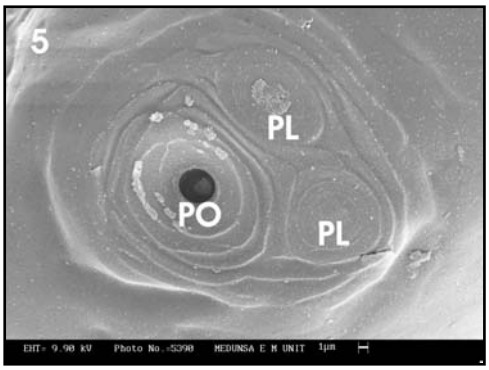

Fig. 5. The sensilla coeloconica showing the pore organ (PO) and two adjacent plate organs (PL).

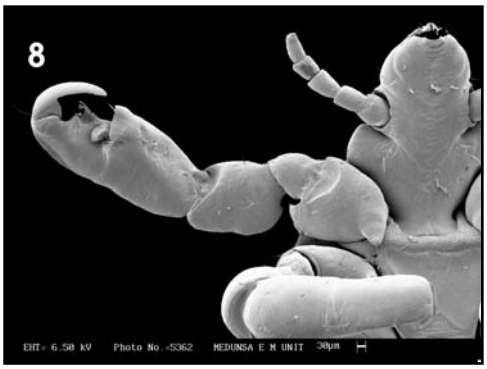

Fig. 8. Each leg had a large claw which gradually acuminated towards the most distal end.

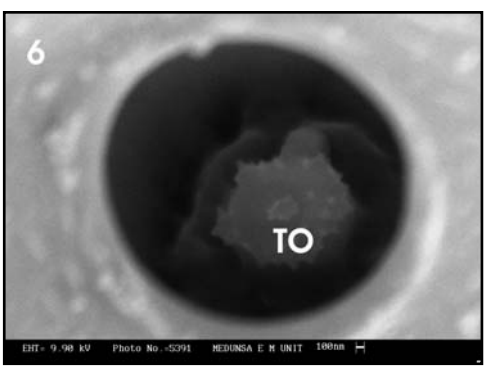

Fig. 6. The enlarged pit organ (tuft organ)(TO) within the sensilla coeloconica.

host (Fig. 9). This grasping was further aided by a distotibial thumb, as well as a distotibial pad-like structure. This seemingly acts as a shock absorbing pad. Between the distotibial pad at the base of the tarsal claw, a small structure was found. This structure strongly resembles the spiked empodium found on the claws of *Pseudolynchia canariensis*, a common ectoparasite of pigeons and wild birds (Green & Turner 2001) and as far as could be established, it has not been recorded in *H. bufali* until now (Fig. 10). Two mesothoracic spiracles were noted (Fig. 11), each situated laterally on the left and right

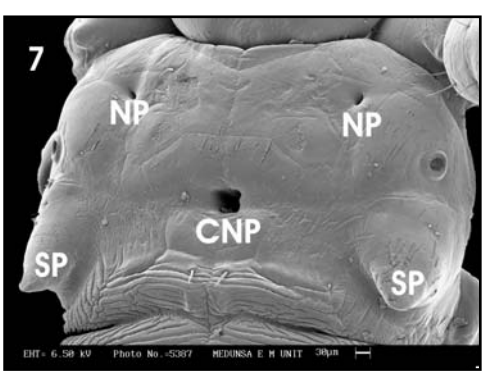

Fig. 7. A dorsal view of the fused pro, mesa and metathorax showing two notal pits (NP) on the prothorax and a central notal pit (CNP) on the mesothorax. Two spinous processes (SP) protruded towards the rear.

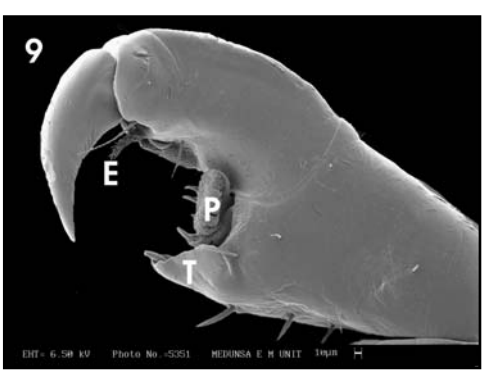

Fig. 9. The claw with the distiotibial thumb (T), distiotibial pad (P) and empodium (E).

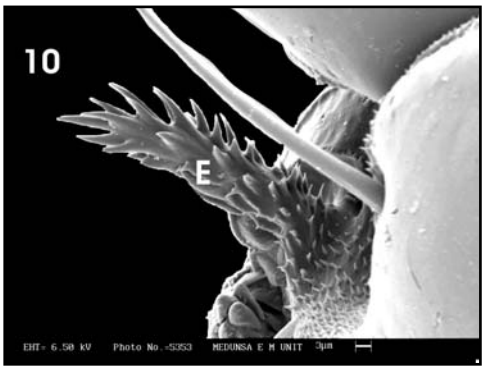

Fig. 10. An enlarged view of the empodium (E)

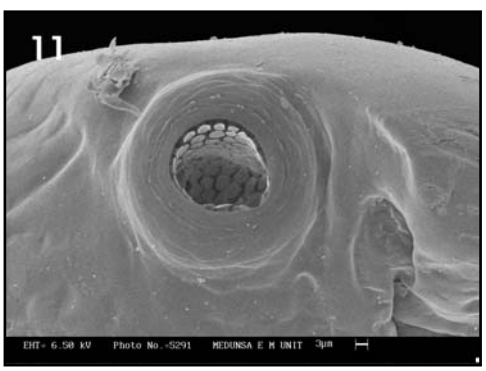

Fig. 11. A mesothoracic spiracle.

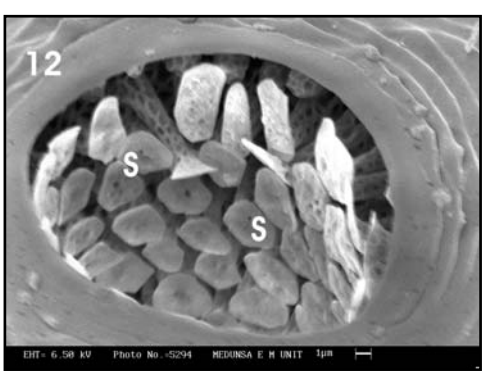

Fig. 12. The spiracular lumen lined with pedunculated irregular pentagonally shaped scales (S).

side of the thorax. The spiracles were well developed. The spiracular lumen was lined with irregular pentagonal shaped scales (Fig. 12). Some micrographs suggest that being pedunculated, these scales, besides filtering the air during discontinuous respiration, could purposely restrict entrance to the lumen of the spiracle in order to prevent dust particles and other debris from entering. Thoracically there seemed to be no micromorphological differences in the male and female subjects. However, a single distinguishing feature which could suggest sexual dimorphism and which could be of value in future investigations, was the presence of a anterio-dorsal ridge resembling a thoracic collar in the male specimens. This collar was absent in the females.

Abdomen: The abdomens of the male and female specimens differed considerably but were closely related (Fig. 13) (Fig. 14). As recorded by Stimie & Van der Merwe (1968), the abdomen was elongated, dorsoventrally flattened and elliptical in shape. The acute posterio-lateral angles of projection and the conical shape of the paratergites led to the scalloped ridge of the outer edge of the abdomen. No paratergites were observed on segments I and II and this was a crucial observation in the identification of the species. A single slender marginal or postspiracular seta was situated midway between each tergite and paratergite. Caudally situated and almost on the apical tip of each of the paratergites a spiracular opening was observed (Fig. 15). The spiracles were also lumenally lined with the irregular pentagonal shaped and pedunculated scales closely resembled that of the thoracic spiracle. A dorsal view of the male and female abdomen showed that in the female, the scalloping was more irregular and projected radially as opposed to the caudal direction of projection in the male. The genitalia of the female were concave, semi-lunar protrudences, whilst a row of marginal setae lined a pair of flap-like claspers (Fig. 16). Two small tufts of setae were situated to the anterior base of the claspers (Fig. 17). The anal opening was situated posterio-dorsally and just above the sexual orifice (Fig. 18). The

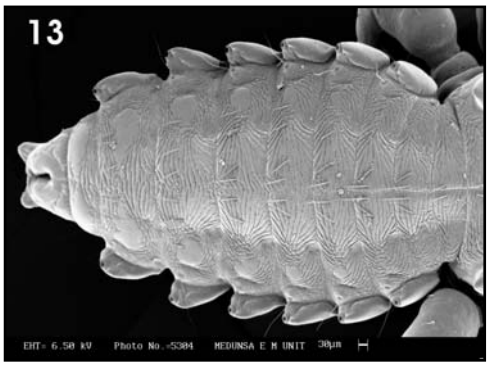

Fig. 13. Dorsal view of the male abdomen.

male showed the typical v-shaped pseudopenis with the tip of the aedegus (penis) protruding (Fig. 19).

Generally *H. bufali* distinctly differs from related species due to its elongated, slender, dorso-ventrally flattened, elliptically shaped body and scalloped appearance of the abdomen.

This investigation was performed in order to assist entomologists, veterinarians and biologists in the identification of this species epidemiological studies, control as well as for the purpose of comparative studies. The study does, however, not purport to be taxo-

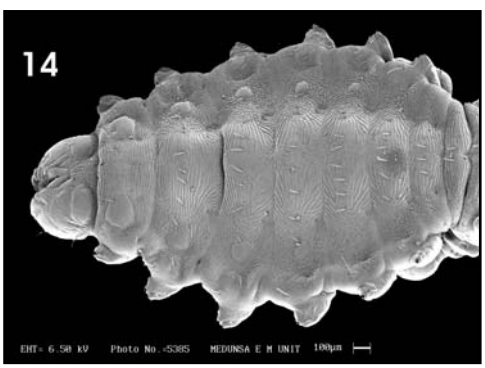

Fig. 14. Dorsal view of the female abdomen.

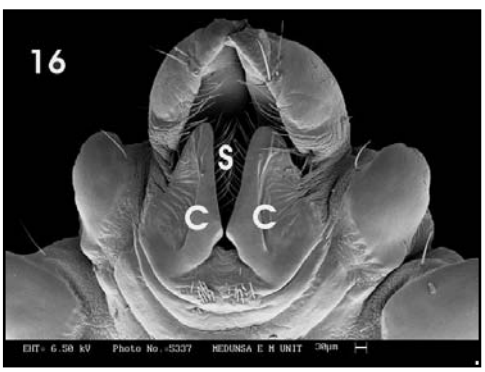

Fig. 16. The flap-like claspers (C) of the female were lined with setae (S).

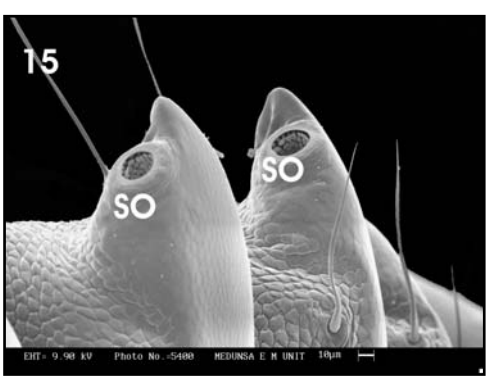

Fig. 15. The apical tip of each of each paratergite revealed a spiracular opening (SO).

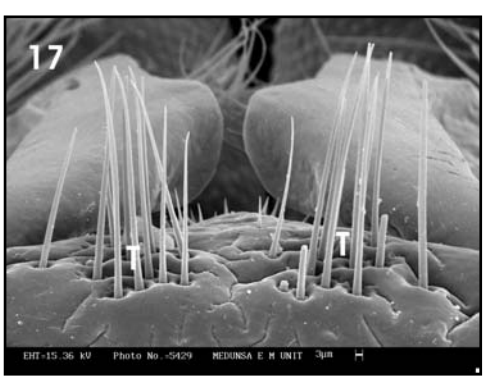

Fig. 17. Two small tufts (T) of setae were situated to the anterior base of the claspers.

nomic in nature but rather to serve as a guide to further studies on *H. bufali*.

Acknowledgements

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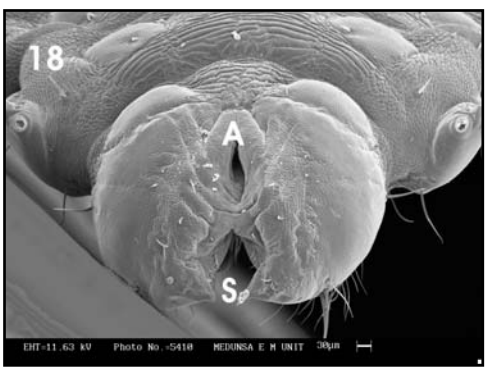

Fig. 18. The anal opening (A) above the sexual orifice (S).

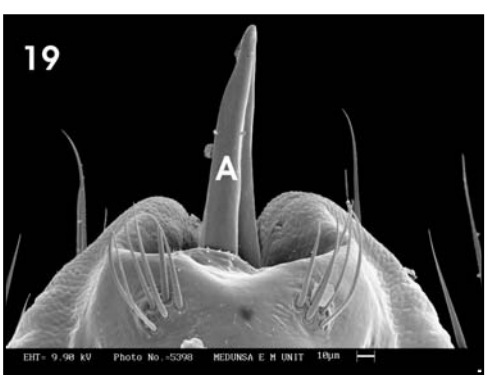

Fig. 19. The male showed the typical v-shaped pseudopenis (A).

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