Light and scanning electron microscopical study on *Antarctophthirus ogmorhini* lice from the Antarctic seal *Leptonychotes weddellii*

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Abstract. Adult lice (*Antarctophthirus ogmorhini*) from the Weddell seal *Leptonychotes weddellii* were investigated by means of light and scanning electron microscopy. We established that this species is characterized by several morphological peculiarities which enable it to survive the low temperatures of the Antarctic sea and the body depression which occurs during the deep (450 m) and long diving periods of the seals. The main peculiarities are: (1) the cuticle of the ventral

and lateral surfaces is much thinner than that of the dorsal surface, (2) the body has numerous stiff spines which are covered by a thick layer of the seal's sebum that closely surrounds the body of the louse, (3) the whole body is covered by a close layer of leaf-like scales which are apparently able to trap air bubbles and thus to provide a small zone of air close to the surface of the cuticle.

Introduction

It has been known for nearly 200 years that blood-sucking lice may occur on the skin - especially along the flippers - of marine mammals in cold or even polar regions (von Olfers *1816*). Comparing the facts known at the time and the material obtained during several German South Polar expeditions, Enderlein described the new genus, *Antarctophthirus*, in 1906 with the type species *Antarctophthirus ogmorhini* (Enderlein *1906*). This taxonomic work, which includes four genera (*Proechinophthirus*, *Echinophthirius*, *Lepidophthirus* and *Antarctophthirus*) within the family Echinophthiriidae (Enderlein *1904*), was still accepted by Durden and Musser (*1994*). These authors added only one other genus, *Latagophthirus*, from river otters. This recent work includes six species in the genus *Antarctophthirus* which are found exclusively either on hosts in the Antarctic region (*A. ogmorhini*, *Antarctophthirus mawsoni*, *Antarctophthirus lobodontis*), in the Arctic or northern zones (*Antarctophthirus callorhini*, *Antarctophthirus trichechi*) or in both northern and southern zones (*Antarctophthirus microchir*). The latter species has been used to argue that both the sea lions (*Arctocephalus* species and otobiids) and the other seals are monophyletic groups, because they are all parasitized by species of the genus *Antarctophthirus*, which apparently coevolved with their hosts (Kim *1972*, *1985*; Raga *1997*). Within the genus, apart from *A. microchir* only *A. ogmorhini* has more than one major host, these being *Hydrurga leptonyx* (leopard seal) and *Leptonychotes weddellii* (Weddell seal). This indicates a rather strict host specificity within this genus. *Echinophthirius horridus*, for example, may be found on all members of the northern phocids (Freund *1928a*, *b*; Hase *1931*).

Although seals around the world are frequently infested with blood-sucking lice - which often produce enormous numbers of progeny - there exist rather few detailed morphological investigations on this group (Scherf *1963*; Murray et al. *1965*; Miller *1971*; Murray *1976*). The present study will contribute to this knowledge by analysing specimens of *A. ogmorhini* obtained from two Weddell seals.

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Materials and methods

Parasites

The parasitic stages and eggs were collected from the hind flippers of two Weddell seals (nos. WE3 and WE7) captured for other purposes during an expedition of the German research vessel "Polarstern" in 1995. Lice and eggs were deep-frozen and then shipped to the Alfred-Wegener-Institut in Bremerhaven (AWI). From there the frozen specimens were transported by car to the Düsseldorf research facilities.

Observation and collection

Prior to the collection of the lice stages, the flippers of the seals were inspected macroscopically and regions with attached lice were photographed using a Nikon F-90 X camera. Pieces of infested skin were then studied using an Olympus stereo microscope SZH 10. Lice were collected by clipping off the hairs to which they were attached. Some of these stages were photographed prior to fixation using the stereo microscope together with an Olympus OM-4 Ti camera.

Light and electron microscopy

For light, scanning and transmission electron microscopy the specimens were routinely fixed in fresh and cold fixative containing 5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.2. After fixation for at least 24 h the specimens were washed in the buffer and separated into two groups:

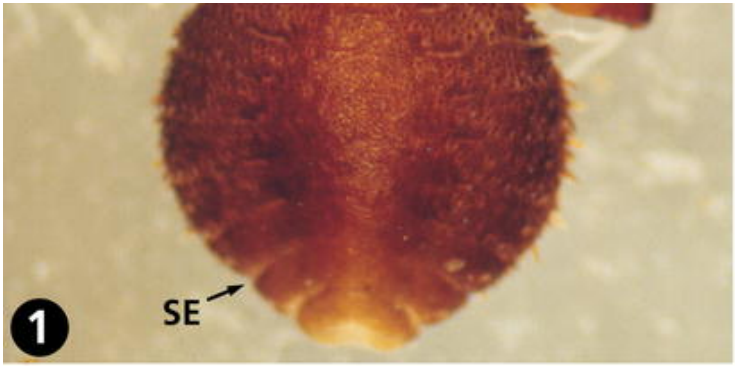
1. Those prepared for scanning electron microscopy. These procedures were done routinely according to the methods established in our laboratory and described elsewhere (Mehlhorn et al. *1983*).
2. Those prepared for light and transmission electron microscopy. This was done using well established routine techniques described elsewhere (Mehlhorn et al. *2001*).

Results

Figures 1, 2, 3, 4 and 5 are light micrographs of the developmental stages of *Antarctophthirus ogmorhini*, and Figs. 6, 7 and 8 are low magnification scanning electron micrographs of the adult stages of this species. Later SEM figures are of a higher magnification (Figs 9, 10, 11, 12, 13), show specific peculiarities (14, 15), or are ultrathin sections

of this species (16, 17). The specimens were found close to the host's skin, attached with their claws to hairs. In principle they showed the features characteristic for the genus *Antarctophthirus*:

1. All stages had a moderately swollen (rounded) hind body (Figs. 1, 2) with visible borders to the segments.
2. Their eyeless, conical head was longer than it was wide (Fig. 8).
3. The antennae of the adults had five segments which appeared marbled in light microscopy by broad, dense annuli (Fig. 5).
4. The forelegs were smaller and more slender than the middle and hind legs and had claws different from those of the other legs (Figs. 2, 5, 8). The claw of the foreleg was needle-like, while that of the other legs was strong and bent (Fig. 8).
5. The quadratic thorax of all stages was narrowly connected to the abdomen (Figs. 1, 6, 7, 8).
6. The pseudopenis of males was v-shaped.
7. Females had patches of genital setae which were arranged convergently (Fig. 10).
8. The dorsal and ventral surfaces of the abdomen were covered with differently shaped scales which apparently trapped bubbles of air around the body (Figs. 9, 11, 13).
9. The dorsal and ventral surfaces were covered with strands of stout spines of different lengths which appeared in different arrangements (Figs. 8, 10, 12).
10. The intersegmental regions of the thorax and the abdomen were insignificantly invaginated compared, for example, to lice from terrestrial mammals (e.g. pigs and humans) (Figs. 2, 9).
11. The outer margin of the abdomen did not form deep invaginations along the border of segments, but was rounded, giving rise to a more or less spherical appearance of the whole abdomen (Figs. 1, 2, 3, 6, 7, 9).
12. The thorax was provided with two pairs of stigmata, while of ten abdominal segments 2-8 also contained two lateral stigmata (Fig. 9).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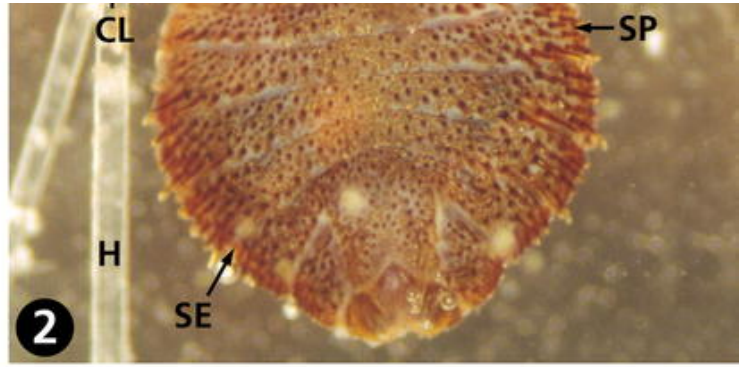
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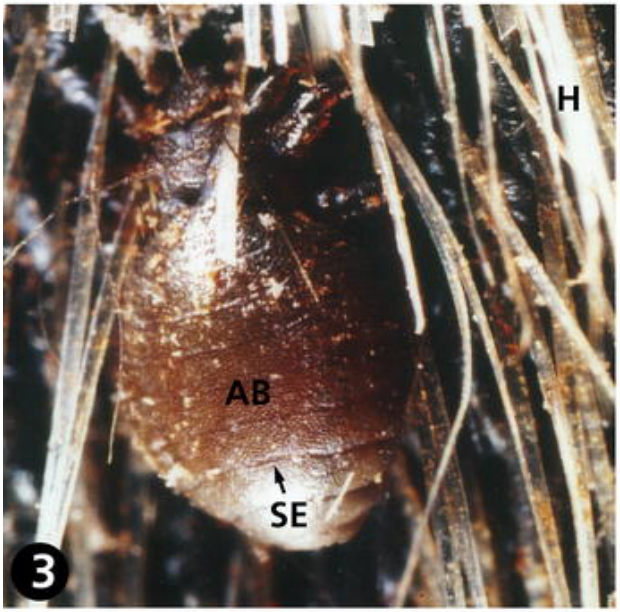
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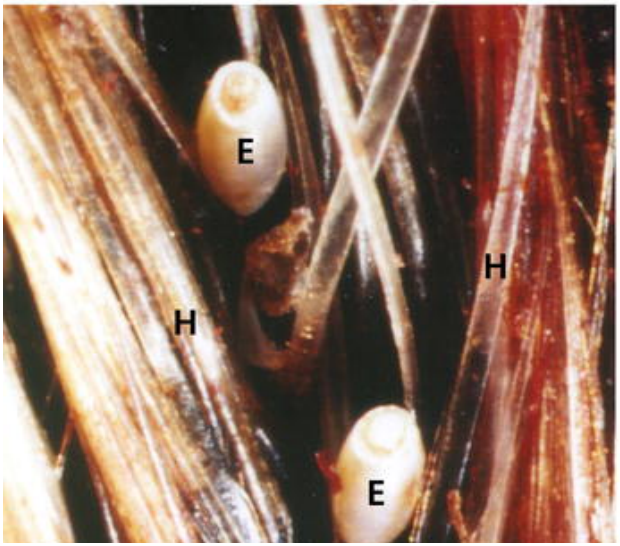
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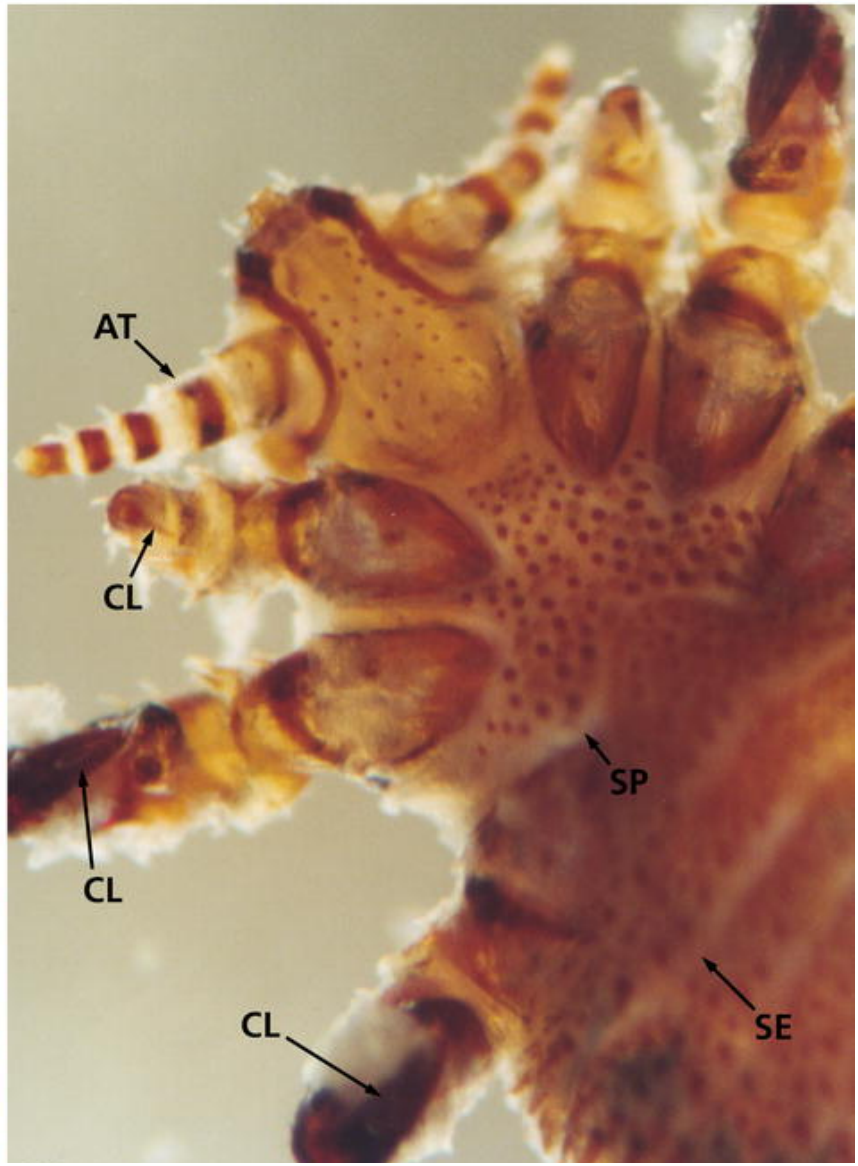
AT
CL
SP
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CL
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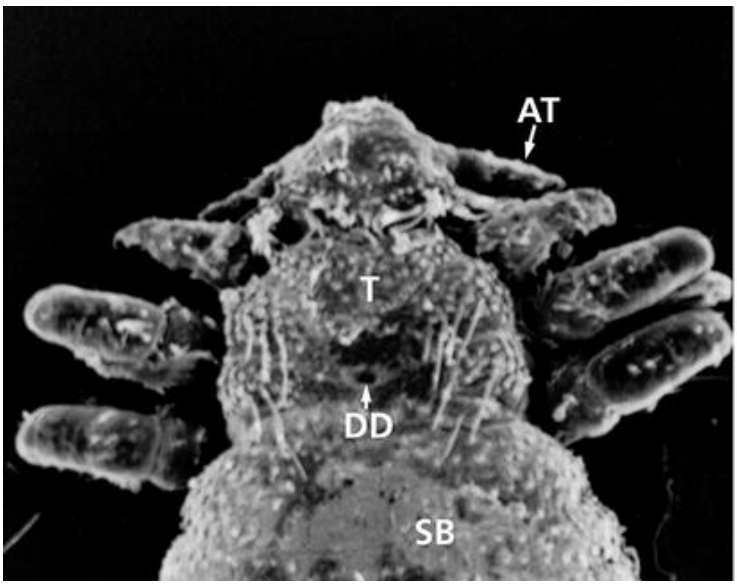
Fig. 1. Color micrograph of a developmental stage of *Antarctophthirus ogmorhini*. Antennae (*AT*), claw (*CL*), dorsal depression (*DD*), segmental border (*SE*). Dorsal view, ×40

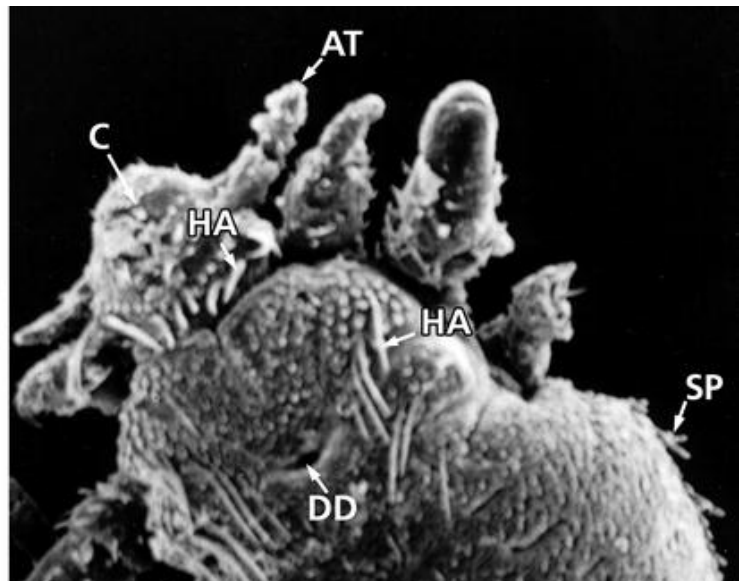
Fig. 2. Color micrograph of a developmental stage of *Antarctophthirus ogmorhini*. Ventral view of an adult which is attached to a seal's hair by means of the claws of the middle and hind legs. Claw (*CL*), hair of Weddell seal (*H*), spines (*SP*), ×40

Fig. 3. Color micrograph of a developmental stage of *Antarctophthirus ogmorhini*. Adult stage hidden in the seal's hairs (*H*). Segmental border (*SE*). ×35

Fig. 4. Color micrograph of a developmental stage of *Antarctophthirus ogmorhini*. Eggs (*E*) attached to the seal's hairs (*H*). ×40

Fig. 5. Color micrograph of a developmental stage of *Antarctophthirus ogmorhini*. Higher magnification of the ventral surface of an adult louse. Note that the antennae (*AT*) appear annulated (dense bands) and that they are composed of five segments. The forelegs and their claws (*CL*) are significantly smaller than the middle and hind legs. ×100





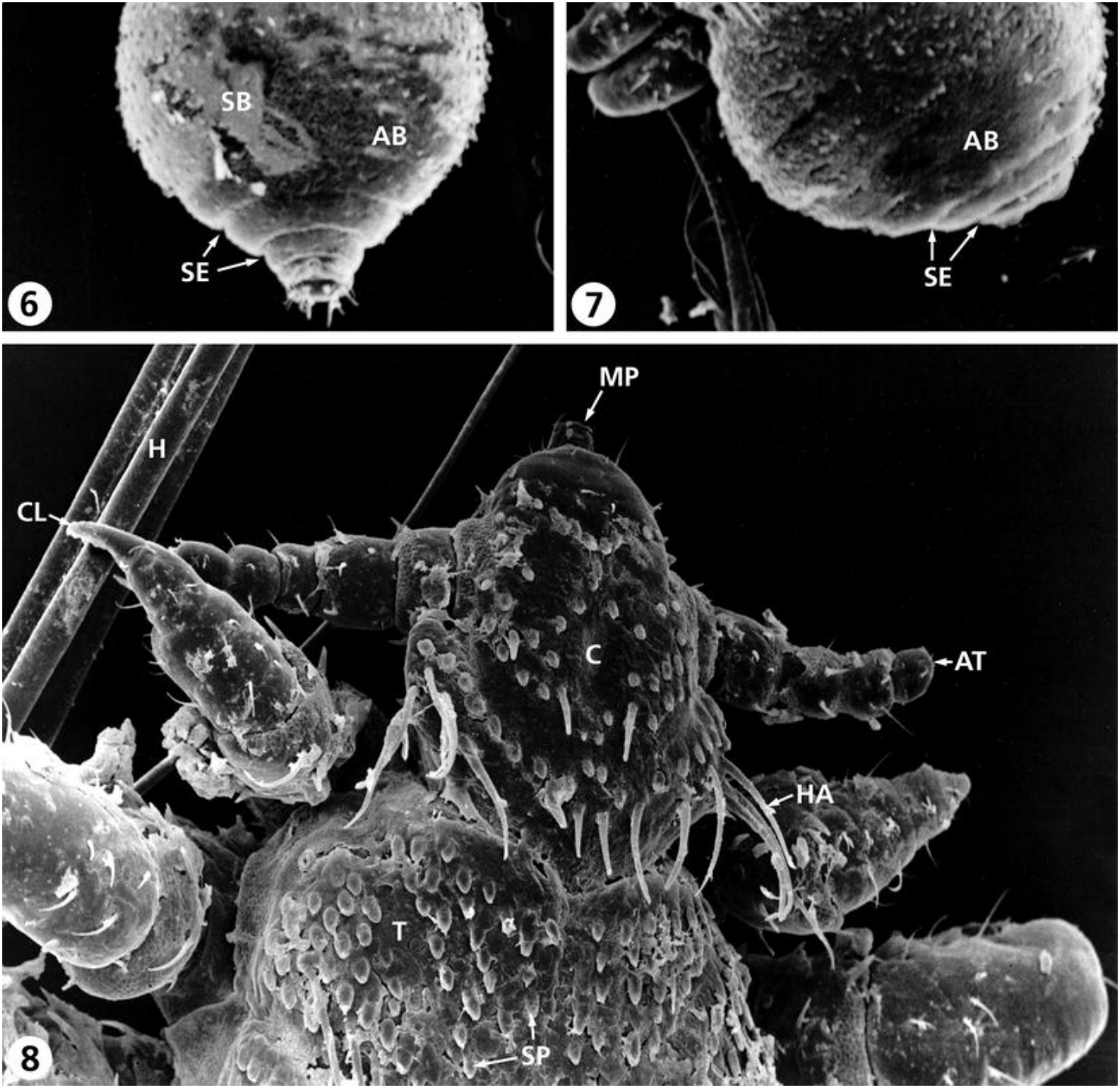
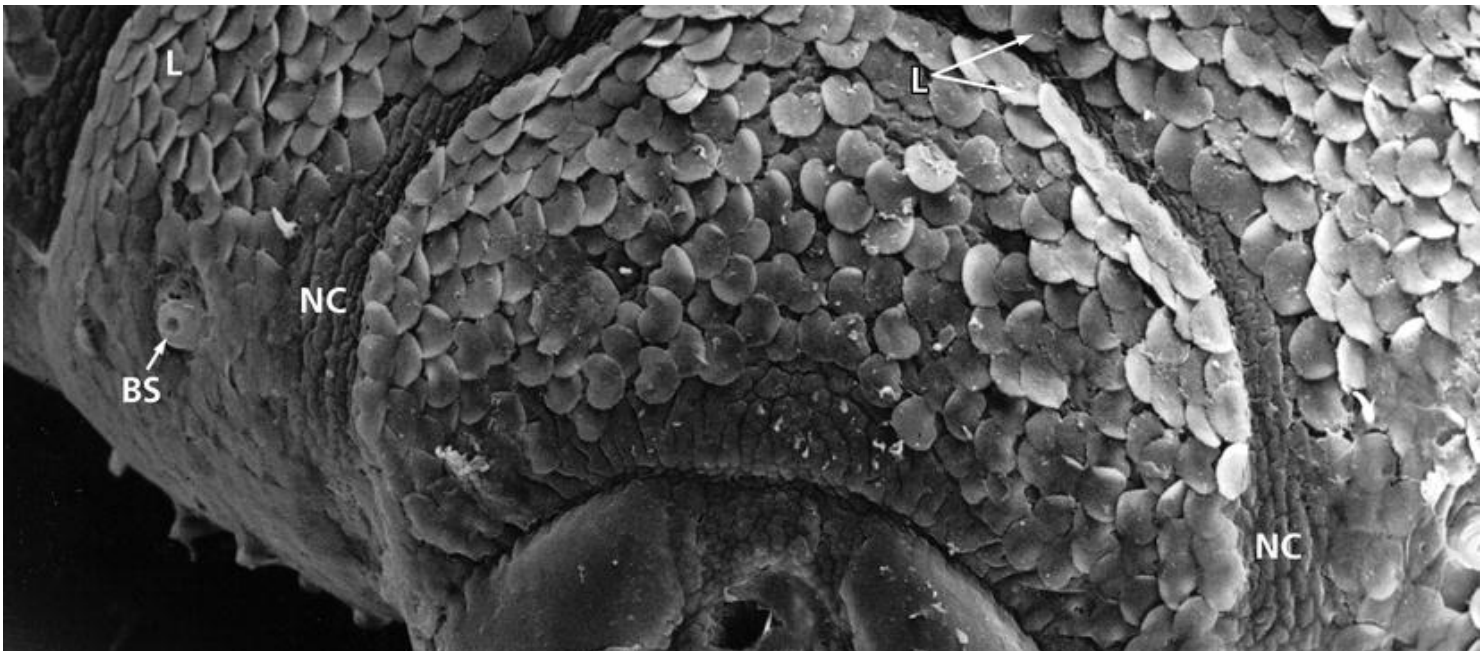


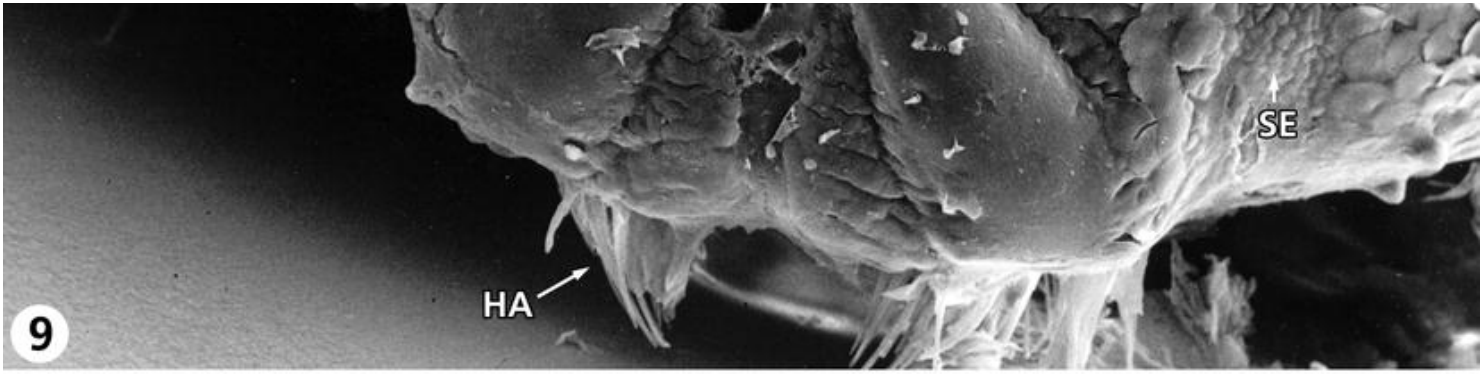
Fig. 6. Scanning electron micrograph of an adult stage of

Antarctophthirus ogmorhini. Dorsal view. Note that the segmental borders (*SE*) become visible at the abdomen (*AB*). The body is covered by solid spines (*SP*) and by groups of longer hairs (*HA*) at the hind end of the head and at the borders of the thoracic segments. Both the segments of the antennae (*AT*) and of the legs are provided with smooth hairs. Dorsal depression (*DD*), sebum attached to the surface of the louse (*SB*), thorax (*T*). ×120

Fig. 7. Scanning electron micrograph of an adult stage of *Antarctophthirus ogmorhini*. Dorsal view. Note that the segmental borders (*SE*) become visible at the abdomen (*AB*). The body is covered by solid spines (*SP*) and by groups of longer hairs (*HA*) at the hind end of the head and at the borders of the thoracic segments. Both the segments of the antennae (*AT*) and of the legs are provided with smooth hairs. Head (*C*), dorsal depression (*DD*). ×120

Fig. 8. Scanning electron micrograph of an adult stage of *Antarctophthirus ogmorhini*. Dorsal view, higher magnification. Note that the segmental borders (*SE*) become visible at the abdomen (*AB*). The body is covered by solid spines (*SP*) and by groups of longer hairs (*HA*) at the hind end of the head and at the borders of the thoracidal segments. The five segments of the antennae (*AT*) are seen. Both the segments of the antennae (AT) and of the legs are provided with smooth hairs. Claw (*CL*), hair of host (*H*), mouthparts (*MP*), T. ×200





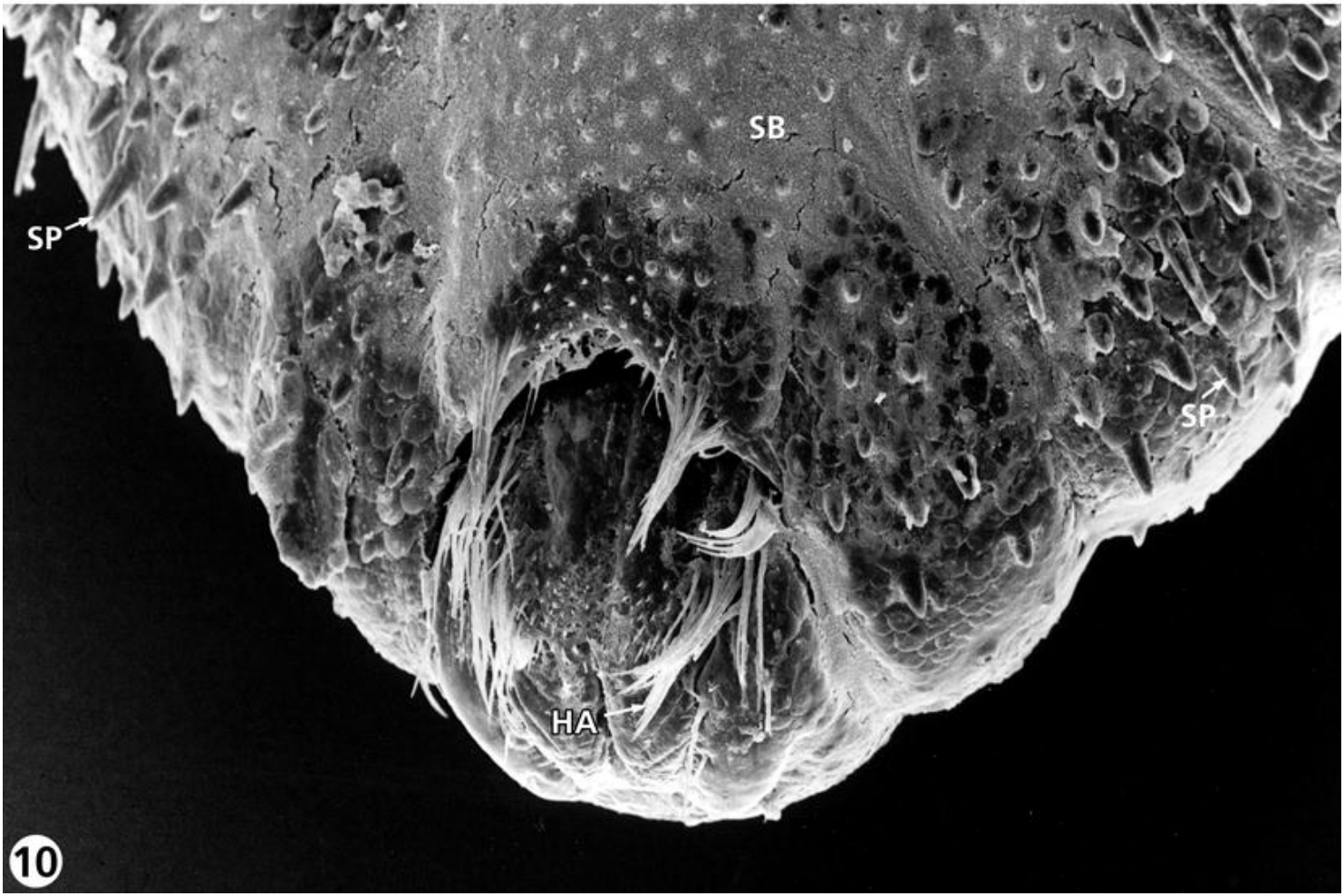


Fig. 9. Scanning electron micrograph (higher magnifications) of the abdomen of an adult *Antarctophthirus ogmorhini*. Dorsal view. Note the occurrence of numerous broad, leaf-like scales on the cuticle

(*L*). However, the border between two segments (*SE*) is not covered by such structures. Opening of tracheal system (*BS*), groups of longer hairs (*HA*), cuticle at segmental borders (*NC*).×320

Fig. 10. Scanning electron micrograph (higher magnifications) of the abdomen of an adult *Antarctophthirus ogmorhini*. Ventral view. Note the thick layer of seals' sebum (*SB*) between the cuticular spines and the occurrence of apparently sensilla-hairs (*HA*). ×320

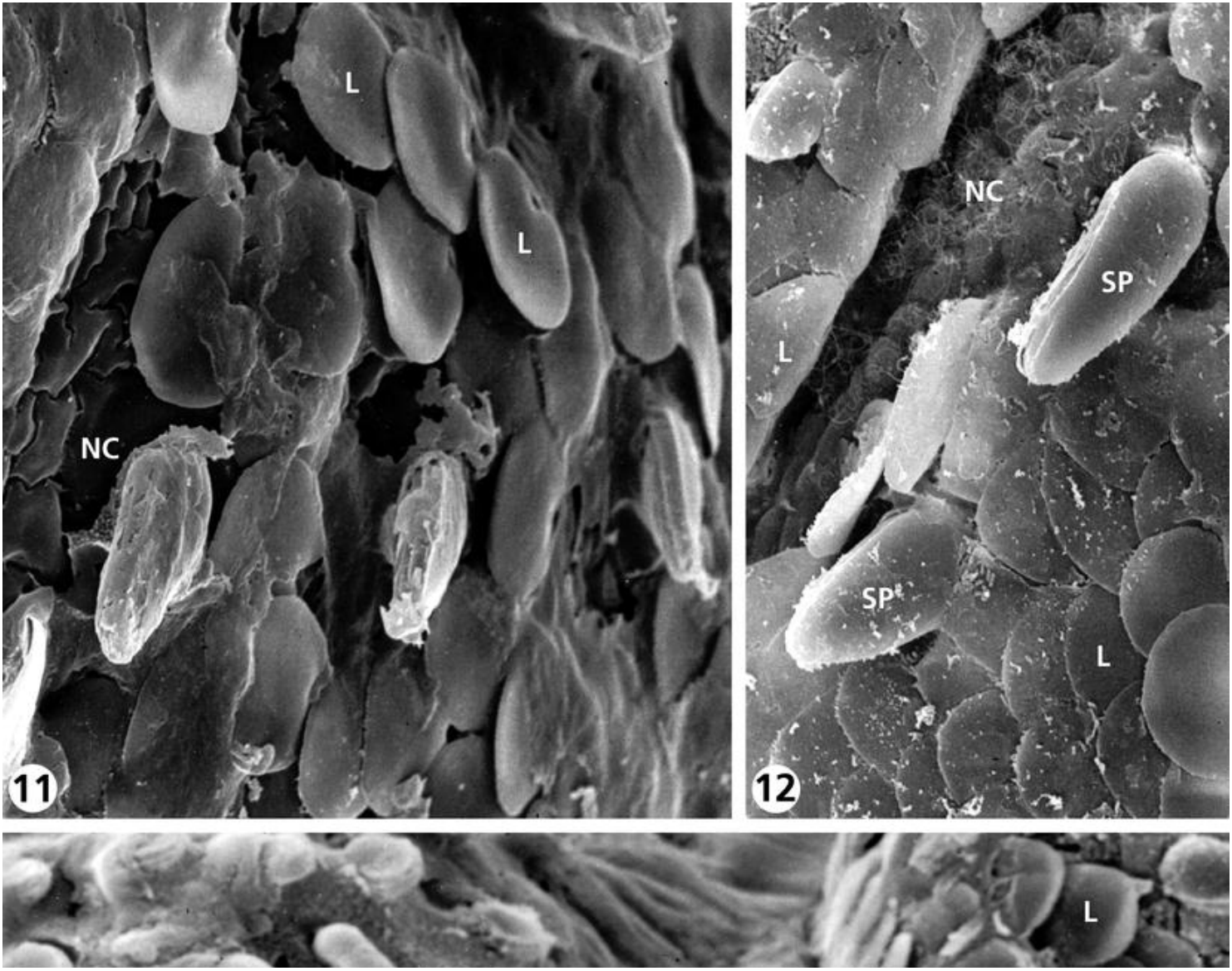


Fig. 11. Scanning electron micrograph of a higher magnification of the body cover of an adult *Antarctophthirus ogmorhini*. Ventral side of abdomen. Note the occurrence of arrow-head like scales (*L*) on the cuticle. Cuticle at segmental borders (*NC*). ×900

Fig. 12. Scanning electron micrograph of a higher magnification of the body cover of an adult *Antarctophthirus ogmorhini*. Dorsal side of abdomen. Note the broad, leaf-shaped scales (*L*). Cuticle at segmental borders (*NC*), spines of the cuticle (*SP*). ×900

Fig. 13. Scanning electron micrograph of a higher magnification of the body cover of an adult *Antarctophthirus ogmorhini*. Dorsal side of thorax. Note the longer hairs and the occurrence of broad, leaf-like scales (*L*). Groups of longer hairs (*HA*), cuticle at segmental borders (*NC*), segmental border (*SE*). ×700

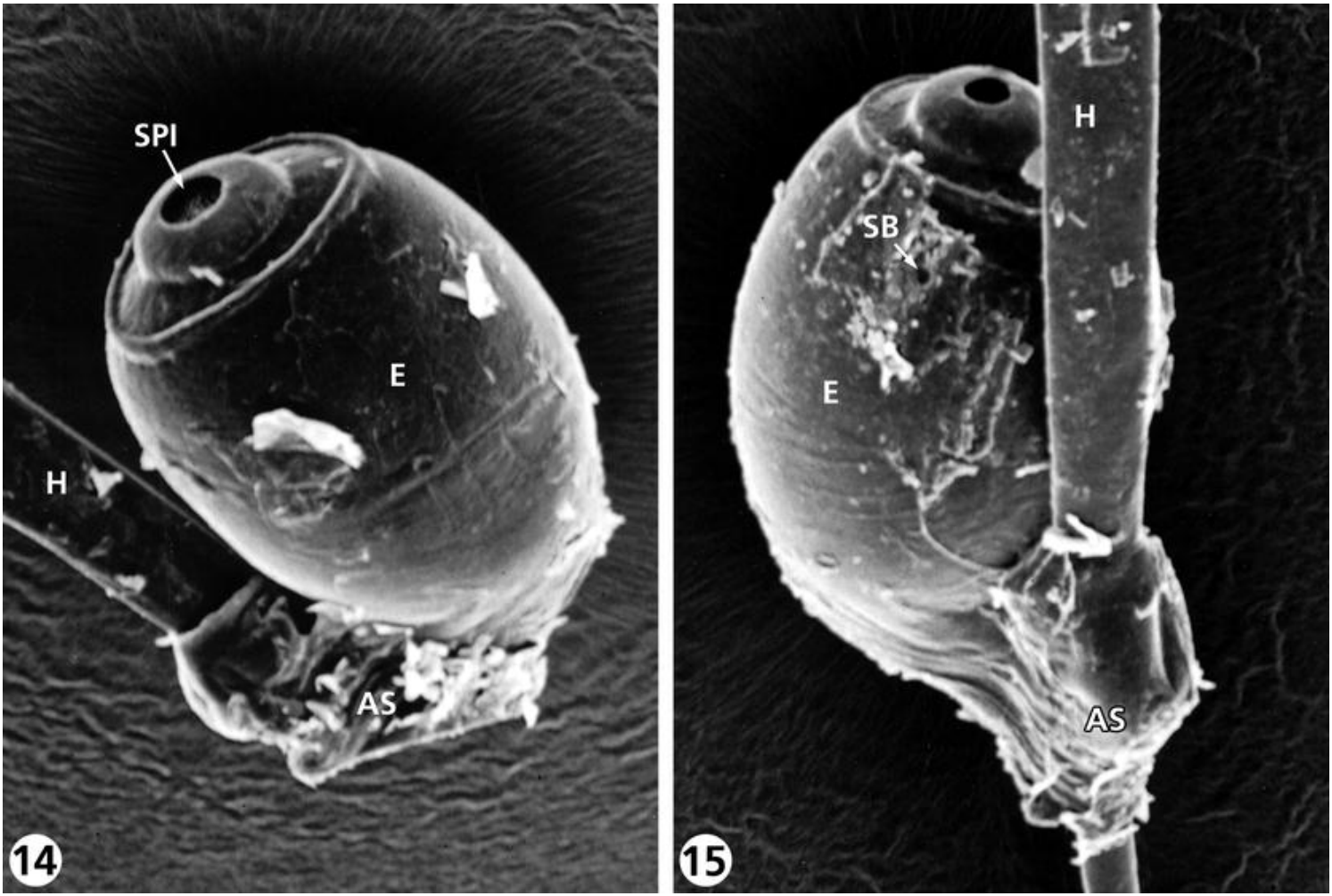


Fig. 14. Scanning electron micrograph of an *A. ogmorhini* egg attached (*AS*) to the hairs of the Weddell seal. Egg (*E*), hair (*H*) of host, spiracle (*SPI*). ×200

Fig. 15. Scanning electron micrograph of an *A. ogmorhini* egg attached to the hairs of the Weddell seal. Attachment substance of eggs (*AS*), egg (*E*), hair (*H*), sebum attached to louse surface (*SB*). ×200

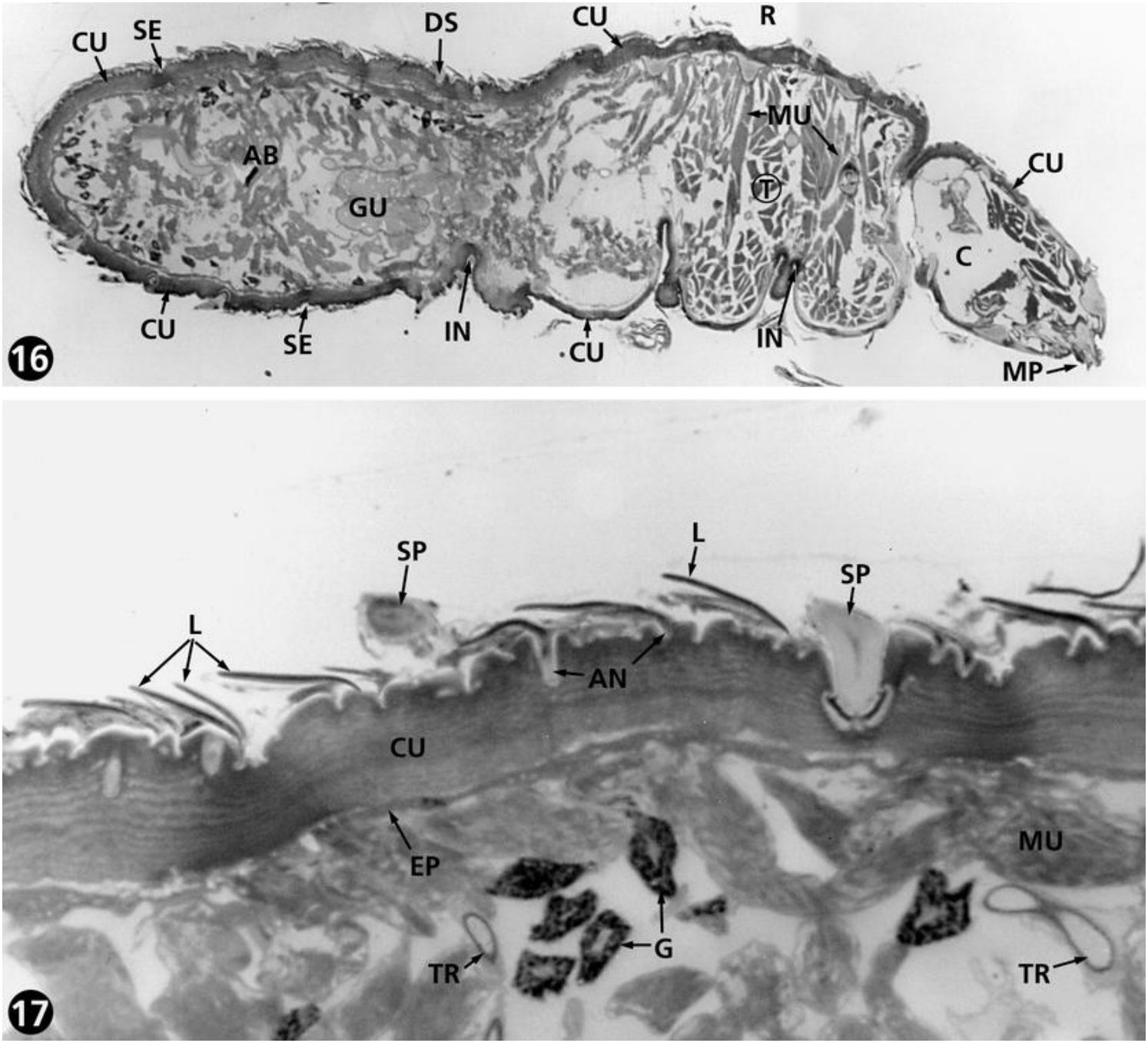


Fig. 16. Light micrograph of a semithin section through adult *Antarctophthirus ogmorhini*. Longitudinal section: the abdomen is not cut through its full length. Note that the cuticle at the dorsal side (*DS*)

is considerably thicker than that of the ventral side (especially in the region of the thorax). Head (*C*), cuticle (*CU*), gut (*GU*), inter-segmental membranes (*IN*), muscles (*MU*), mouthparts (*MP*), segmental border (*SE*), thorax (*T*). ×80

Fig. 17. Light micrograph of a semithin section through adult *Antarctophthirus ogmorhini*. Magnification of the dorsal cuticle with its characteristic protrusions. Epidermis (*E*), tips of glands (*G*), leaf-like scales (*L*), muscles (*MU*), cuticular spine (*SP*), trachea (*TR*). ×380

Besides these general features, *A. ogmorhini* had some more specific characteristics which were found mostly in adult males and females. The males were considerably smaller than the females, which reached, at the maximum, 2.2 mm in length (the mean length was around 2 mm). Both females and males appeared light to dark brown depending on the amount of ingested blood (Figs. 1, 2, 3). The cuticle of the whole body showed some peculiarities which can be seen in the scanning electron micrographs and in light microscopical sections:

1. The diameter of the cuticle was rather thick compared to sucking lice from terrestrial hosts and reached about 1/6-1/10 of the whole diameter in the abdominal portion of the body (Figs. 16, 17).
2. In general, it was at least double the width on the dorsum compared to the ventral part of the body (Figs. 16, 17).
3. The cuticle of the head was thinner than in other parts of the body (Fig. 16).
4. At the segmental borders of the abdomen, the cuticle was smooth and the plates were connected by intersegmental membranes (Figs. 16, 17).
5. On the inner side of each of the three thoracic segments, a thick ridge was formed which was used as an anchor-point for the muscle strands. These ridges were also visible from outside (Fig. 1) and ran to a central point in the metathorax, where a depression was seen when viewed from above (Figs. 1, 6, 7).
6. The thorax and abdomen were closely covered by small scales which were arranged in a tile-like manner (Figs. 9, 11, 12, 13). While they appeared like the leafs of a European lime tree (Fig. 9) on the dorsal surface, they had an arrowhead shape on the ventral surface (Figs. 11, 12). In both cases, however, there was some air-filled space before the solid layers of the cuticle were reached (Figs. 16, 17).
7. The segmental plates of the thorax and the abdomen, as well as the head, were spotted with regular rows of short, arrowhead-like, solid spines which were formed by the cuticle (Figs. 1, 2, 8, 10, 17). These

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spines, which were directed obliquely to the posterior end of the louse, were shorter and broader in the head region and along the mid-thorax and abdomen, while they were longer and more pointed on the lateral sides of the body (Fig. 10). These spines were apparently used to envelope the louse in a thick layer of the seal's sebum and thus provided another means of protection against low temperatures (Figs. 6, 10).

8. The dorsal hind border of the head as well as the dorsal surface of the thoracic segments had symmetrically arranged, long, cuticular hairs (Figs. 6, 7, 8, 13). These were found in a semicircular arrangement on the head, but occurred only at the margin of the thorax (mostly in groups of four; Figs. 6, 7).
9. At the posterior end of the lice - especially around the genital openings - smooth hairs were found. These were considerably shorter than the thoracic hairs but longer and smoother than the body spines (Fig. 10).
10. Similar, rather short hairs were seen along the five segments of the relatively thick antennae, which appeared striated due to alternating dense and white bands when seen by light microscopy. Other fine hairs were found on the segments of the legs (Fig. 8).

Only a few of the smaller males were seen These had a rather similar outer body shape (Fig. 6); however the abdomen appeared less rounded than in the females (Fig. 7).

The females glued their eggs onto the hair of the seal with the operculum pointing towards the tip of the shaft. The glue of *A. ogmorhini* was so tenacious that it could not be dissolved without destroying the hair, although it covered only one fourth of the egg. The eggs reached a length of about 0.5 mm and were ovoid (Figs. 4, 14, 15). Macroscopically they appeared white (Fig. 4) due to air penetration. The egg's operculum had a single opening consisting of a spherical stigma situated at the tip of a concentric protrusion (Figs. 14, 15).

There was no sign that the body of the lice which were studied by transmission electron microscopy contained the larvae of worms (e. g. lung worms, filariae) which have been described from some seals (Fig. 16).

Discussion

Antarctophthirus ogmorhini is a blood-sucking louse of the family Echinophthiriidae, order Anoplura, which parasitizes two different hosts: the Weddell seal *Leptonychotes weddellii* and the leopard seal *Hydrurga leptonyx*. Most phases of the life cycle of *A. ogmorhini* were described by Murray et al. (*1965*) and Murray

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(1967). Details of the morphology of *A. ogmorhini* as well as related species of the same family, the members of which are only found on marine carnivores of the suborder Pinnipedia, were summarized by Scherf (1963), Miller (1971) and Hinton (1976). Comparing these published results with those of the present study, it can be seen that the different species and genera of the family Echinophthiriidae are characterized by peculiar morphological features:

1. All genera except *Echinophthirius* have forelegs which are smaller than the middle and hind ones. A similar phenomenon is seen in the human crab louse *Phthirius pubis* (Martini 1946 ; Mehlhorn 2001), while human head and body lice (genus *Pediculus*) have legs all of the same size and shape.
2. All species have a characteristic body shape. While human body lice and head lice have, for example, a rather slender abdomen with marginal striations at the segmental borders, the hind body - especially in species of the genus *Antarctophthirus* - appears more ovoid to spherical with rounded margins, although the segmental borders are visible even at low magnification.
3. The outer surface of lice of the family Echinophthiriidae is absolutely unique and apparently represents an adaptation to the cold temperatures in the biotopes of their hosts.

    a. The body surface is covered with regular rows of stout spines of a species-specific length. Those of *A. ogmorhini* are medium-sized in comparison to *Echinophthirius horridus*, *Lepidophthirus macrorhini* or *A. trichechi* (Murray 1976; Scherf 1963). The spines on the ventral surface of the body and on the outer body margin are considerably larger and thicker than those on the mid-body and thorax. The main function of these spines became evident in our scanning electron micrographs. They are apparently used to fix a thick layer of the seal's sebum to the body surface. This sebum layer would offer protection against low water temperatures. The contact of the host's body surface with the spines of the lice probably induces an increased production of sebum.
    b. In addition to these stiff body spines there are longer hairs seen on the dorsal surface of the louse's body. These thoracic hairs - probably representing sensillae (setae) - are species specific. Thus, *A. ogmorhini* has groups of four while *A. trichechi* (Scherf 1963) has only two on each side. The function of these longer hairs, however, is unknown.
4. Another prominent characteristic of the surface of the Antarctic and Arctic lice (except for *Echinophthirius*) is the presence of numerous small scales which cover the abdomen and thus are produced by the rather thick cuticle. These scales, which may offer some air-filled space,

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are postulated to function in the same way as the plastron found in other insects, and apparently trap bubbles of air when the louse (together with its host) is immersed in water. These structures would therefore increase the oxygen uptake of the lice via the body surface, when direct contact via the stigmata is impossible (Hinton *1976*; Murray *1976*).

5. The fact that the dorsal cuticle of specimens of the family Echinophthiriidae is considerably thicker (reaching up to 1/6 of the whole diameter) than that of the ventral cuticle may also be explained as an adaptation to the cold environment, since the dorsal surface interfaces directly with the cold water, while the ventral surface, with the thinner cuticle, is attached to the warmer surface of the seal's body.

Thus, considering items 3-5, the body surface of these lice from cold waters has three peculiarities that do not occur in lice from temperate climates, and guarantee that they can maintain a suitable body temperature. In addition the surface scales, which apparently trap air bubbles, may help the lice to survive the rather long (30 min) and deep (up to 450 m) diving periods of the seal (Plötz et al. *2001*).

The present study, which deals with lice of only two seals species, did not provide evidence of superparasitism with worms - especially with filariae (e.g. *Dipetalonema*) or metastrongylids. Much more material must be considered before this question can be addressed.

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