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REDESCRIPTION OF *ANTARCTOPHTHIRUS MICROCHIR* (ANOPLURA: ECHINOPHTHIRIIDAE) FROM THE SOUTH AMERICAN SEA LION, *OTARIA FLAVESCENS*, FROM PATAGONIA, ARGENTINA

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ABSTRACT: *Antarctophthirus microchir* was originally described from *Phocarctos hookeri* on the basis of 1 female and 1 male only. We re-describe adults and describe, for the first time, the 3 nymphal stages from specimens collected from *Otaria flavescens* from Patagonia, using light and scanning electron microscopy. The present material can be distinguished from other *Antarctophthirus* species by the presence of a fringe of setae on the back of the head, only present in *A. trichechi* and *A. callorhini*. However, *A. trichechi* also possess a prominent proboscis with large hooks, and A. *callorhini* presents less abundant and non-uniform abdominal scales in shape and size. Other differential features of *A. microchir* are the pattern of ovoid and uniform scales and longitudinal grooves in the surface of spines. Nymphal stage 1 differs from 2 and 3 mainly by the absence of scales and thorax without ventral spines or hairs. Nymphal stages 2 and 3 may be distinguished by the disposition of the occipital apophyses. *Antarctophthirus microchir* has been reported from 5 sea lion species from both hemispheres. Considering the conservative morphology, and ecological and evolutionary features of sucking lice, we raise the question of whether *A. microchir* from different sea lion hosts may represent a complex of cryptic species.

The Anoplura (Phthiraptera) is composed of lice parasitizing mainly terrestrial mammals, but a few members have been able to adapt to the marine environment. The latter are included in 5 genera within the Echinophthiriidae, which comprises species parasitizing pinnipeds and a river otter (Kim, 1985), i.e., *Proechinophthirus*, *Lepidophthirus*, *Echinophthirius*, *Latagophthirus*, and *Antarctophthirus*. The latter is the most diverse genus, with 6 recognized species (Kim, 1985): *A. ogmorhini*, *A. callorhini*, *A. trichechi*, *A. lobodontis*, *A. mawsoni*, and *A. microchir*. Trouessart and Neumann (1888) described the latter species as *Echinophthirius microchir* from *Phocarctos hookeri*. By current standards, the description was incomplete and was based on just 1 female and 1 male. Later, Enderlein (1906) redescribed the species based on the same material and transferred *Echinophthirius microchir* to *Antarctophthirus*.

As a part of an ongoing project on the biology of the South American sea lion in Patagonia, we had the opportunity to collect lice from pups of this species (see Aznar et al., 2009). Lice were identified as *A. microchir*, following the original description by Trouessart and Neumann (1888) and the key for sucking lice by Ferris (1951). In view of the fragmented and incomplete description of this louse species, the aim of the present study is to re-describe adults and, for the first time, describe the 3 nymphal stages of *A. microchir* from *O. flavescens* from Patagonia.

MATERIALS AND METHODS

Specimens examined

The samples were taken in Punta León rookery (63°03’ S, 47°43’W) during the breeding seasons between 2005 and 2007. Lice were collected from *O. flavescens* pups, which were captured with a noose pole and restrained by 2 people. A third person collected the lice using a fine-tooth comb commonly used for treating human

pediculosis and lice were fixed in 96% ethanol. Combing took approximately 3 min, after which pups were released near their mothers. Twenty males, 20 females, 18 1st instar nymphs (N1), 32 2nd instar nymphs (N2), and 20 3rd instar nymphs (N3) of *A. microchir* ex *O. flavescens*, from Punta León, Chubut Province, Argentina, were examined using light microscopy. Ten males, 10 females, 10 N1, 10 N2, 10 N3, and 2 eggs were examined using scanning electron microscopy.

Our specimens were compared with reference material from the Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand: *A. microchir* from *P. hookeri* (1 male, 1 female) and the New Zealand fur seal, *Arctocephalus forsteri* (1 male, 1 female, 1N1, 1N2); *A. ogmorhini* (1 female) from the Weddell seal, *Leptonychotes weddelli; A. trichechi* (1 male, 1 female) from the walrus, *Odobenus rosmarus*; The Natural History Museum of London: *A. microchir* from the Steller sea lion, *Eumetopias jubatus* (1 male, 1 female), the Californian sea lion, *Zalophus californianus* (1 male, 2 females) and *O. flavescens* from Malvinas (Falkland) Islands (2 males, 3 females); *A. ogmorhini* (4 males, 3 females) from *L. weddelli*; *A. trichechi* (3 males, 3 females) from *O. rosmarus*; *A. lobodontis* (4 males, 4 females) from the crabeater seal, *Lobodon carcinophagus*; *A. callorhini* (1 male, 1 female) from the Northern fur seal, *Callorhinus ursinus;* and the K.C. Emerson Entomology Museum: *A. microchir* from *E. jubatus* (6 males, 14 females) and from *Z. californianus* (3 males, 3 females).

Voucher specimens are deposited at the La Plata Museum (Argentina): 2 males, 2 females and 2 of each instar nymphs (1st, 2nd, and 3rd).

Light microscopy

Lice were prepared following the slightly modified protocol of Palma (1978). The specimens were treated with 20% aqueous solution of potassium hydroxide (KOH)

for 24 hr for adults, N2, and N3, and 12 hr for N1 (a longer period damaged the specimens). The KOH macerates the non-chitinous tissues and removes color from the sclerotin, distending the whole body. The KOH was removed and replaced by distilled water for 30 min, and then by a 10% aqueous solution of acetic acid. The acid neutralizes the remaining alkali, stops maceration, and avoids damage by over treatment. Half of the samples were stained with eosin for 12 hr. All the specimens, stained or not, where dehydrated in an ethanol series of 70%, 80%, 90%, and 96%, for 30 min at each concentration. After dehydration, the alcohol was replaced by pure clove oil for 24 hr. A cover slip with some weight was placed upon the lice to flatten them. Lice were finally mounted in Canada balsam.

Scanning electron microscopy (SEM)

Specimens for SEM (10 of each life stage: 5 in dorsal view and 5 in ventral view, and 2 eggs) were dehydrated in an ethanol series, critical point dried in liquid CO_2, mounted on specimen stubs with conductive carbon paint, sputter coated with gold-palladium to a thickness of 25-30 nm in a Bio Rad-Sc 500 coating unit, and examined in a S-4100 scanning electron microscope at 5 kV (Servei Central de Suport a la Investigació Experimental, Universidad de Valencia, Spain). Measurements (in mm): $\overline{X}$ ± S.D., range, n. Abbreviations are explained in Figure 2.

Terminology

Species of Echinopthiriidae are characterized by their modified setae (Kim, 1985). In the literature, we found no uniform terminology regarding the nomenclature of the setae. Most names and abbreviations of setae used in this paper follow those of Kim and Ludwig (1978), slightly modified, i.e., in our abbreviations we have used ‘Sp’ to differentiate our spines from Kim and Ludwig’s setae. However, we used the following criteria: spines are pointed and spiral shaped setae (Figs. 1a, d), scales are

flattened setae (Figs. 1b, e), and hairs (following Mehlhorn et al., 2002), are the long and thin setae (Figs. 1c, f).

REDESCRIPTION

***Antarctophthirus microchir* (Trouessart & Neumann, 1888) Enderlein (1906)**

Syn. *Echinophthirius microchir* Trouessart & Neumann (1888)

Male: Total body length 2.48±0.22, 2.05-2.88, 20. Head lightly longer than wide (length: 0.52±0.05, 0.41-0.60, 20; width: 0.43±0.03, 0.36-0.48, 20); anterior margin heavily sclerotized; maxillary vestige distinct; ventral labrum connected to long apodemes; postantennal angle developed, dorsally with 2 long hairs in both sides; posterolateral angle not developed. Two apical head spines, 4 ventral preantennal head spines (VPreASp), 3-4 ventral posterior marginal head setae modified in long hairs (VPoMHS), 1 supra-antennal head spine, numerous ventral lateral head spines (VLHSp) and ventral anterior marginal spine; 5 sutural head spines (SuHSp), the middle 3 shorter, 4 dorsal marginal head spines (DMHSp), 6 dorsal posterior marginal head setae (DPHS) modified in long hairs forming a fringe. Antennae 5 segments. Basal segment with a short spine. Terminal segment is the longest and with 4 sensoria at apex. Thorax trapezoidal, approximately as long as the head and about twice as wide (width: 0.78±0.05, 0.63-0.88, 20). Thoracic sternal plate covered by scales; 3 spines under each coxa; posterior margin with 2 long hairs. Dorsally, a characteristic inverted Ω pattern of scales; 4 dorsal mesothoracic spines (DMsSp); dorsal metathoracic spines (DMtSp) arranged in 2 rows, the superior with 3 hairs and the inferior with 5 long hairs, marginally 2 spines; 2 dorsal marginal abdominal spines (DMASp) and 2 hairs. Phragmata well developed; occipital apophyses converged at apex delimiting a winkle; mesothoracic phragma continuous across the notum, convergent in a conspicuous dorsal depression. Mesothoracic spiracle membranous and small, but clearly visible; sternal

126 plate not developed. Fore legs characteristic of genus, small and weak; middle and hind
127 legs very large and strong, very similar in shape and size. Tarsus and tibia merged in a
128 tibiotarsal segment; tibiotarsus with distinct basal lobe and strong claw, with 3 holdfast
129 pads. Abdomen large, oval and pointed (width: 1.26±0.12, 1.08-1.45, 20); without
130 distinctive tergites or sternites; paratergal plates not developed; 6 spiracles present on
131 each side. Ventral central abdominal setae (VCAS), dorsal central abdominal setae
132 (DCAS), dorsal lateral abdominal setae (DLAS) and ventral lateral abdominal setae
133 (VLAS) modified in scales, covering entire abdomen. DCA scales of sternite 1 are
134 lanceolate and very distinctive. Six rows of VLA spines. Dorsal marginal abdominal
135 setae (DMAS) and dorsal lateral abdominal setae (DLAS) modified in numerous shrew
136 setae. Five to 6 apical hairs. Scales ovoid, pointed with irregular serration at apex and
137 vary in size (Figs. 1b, e). Spines pointed, spiral-shaped, vary in size but not in shape
138 (Fig 1a, d). Basal plate (Fig. 4b) relatively long, short parameres; very long V-shaped
139 pseudopenis, the arms of which articulate with bases of parameres.
140 *Female (Fig. 3b):* Total body length 2.78±0.34, 2.01-3.53, 20. Head (width:
141 0.44±0.04, 0.37-0.50, 20; length: 0.55±0.04, 0.46-0.61, 20), thorax (width: 0.92±0.08,
142 0.81-1.06, 20), legs and abdomen as in male, except for genitalia and associated
143 characters; abdomen more rounded (width: 1.64±0.30, 1.19-2.42, 20) and without
144 lanceolate scales. Without distinct genital plate, gonopods and spermatheca; with a
145 fringe of setae surrounding the genital opening.
146 *Egg (Fig. 6):* (0.93±0.018, 0.90–0.95, 8) Smooth, white, with operculum distinctly
147 raised, tapering to a blunt apex.
148 *Nymph 1 (Fig. 3c):* Total body length 0.98±0.10, 0.79-1.21, 18. *Head:* About as
149 long as wide (width: 0.27±0.04, 0.21-0.38, 18; length: 0.30±0.05, 0.21-0.39, 18);
150 anterior margin rounded; labroclypeal area heavily sclerotized; haustellum with well

developed denticles; postantennal angle developed; oral spines present; ventrally without spines or scales; dorsally 3 SuH spines, 1 DPoMH hair, 2 DPreA spines; fringe not developed. Antenna with 4 segments; basal segment wide; terminal segment longest; sensoria developed; 1 spine in basal segment and setae pattern as in adult. Occipital apophyses not developed. Thorax (width: 0.40±0.06, 0.28-0.55, 18) with weakly developed phragmata (Fig. 7a); without scales; 1 DMs spine and 1 DMs hair; 1 DMt hair. Leg as in adult; spines of coxas developed; coxal plate not highly developed; claws weakly sclerotized; pads present as in adult. Abdomen (width: 0.45±0.07, 0.30-0.59, 18) Short, oval; tergites, sternites or paratergites not distinctive. Six rows of DCAS: rows 1, 2 and 3 with 3 setae; 4, 5 and 6 with 1 seta. Six rows of 3 short DLA spines. Seven rows of VCAS: row 1 with 1 short hair and 1 spine; rows 2 to 5 with 2 hairs and 2 spines; rows 6 and 7 with 2 hairs; rows 2 to 5 with 1 VLA spine in each row.

Nymph 2 (Fig. 3d): Total body length 1.51±0.18, 1.09-1.79, 32. Features not mentioned here as in N1. Pattern of spines and scales as described in adult, unless mentioned otherwise. Hairs shorter and scales less dense than in adults. Head about as long as wide (width: 0.35±0.04, 0.25-0.45, 32; length: 0.40±0.04, 0.31-0.47, 32); occipital apophyses short and not convergent (Fig. 7b). Antennae with 4 segments; like N1 but terminal segment beginning to differentiate. Thorax width 0.65±0.09, 0.49-0.82, 32. Dorsally with pro-, meso-, and metathoracic phragmata well developed. Thoracic sternal plate with fewer scales than in adults. Abdomen width 0.83±0.14, 0.58-1.03, 32. Oval, scales developed, setae pattern as in adults, but less dense.

Nymph 3 (Fig. 3e): Total body length 1.87±0.14, 1.68-2.29, 22. Features similar to adults, unless mentioned otherwise. Hairs shorter than in adults. Head width 0.42±0.05, 0.34-0.61, 22; length: 0.47±0.04, 0.42-0.54, 22. Occipital apophyses further prolonged and connected at apex, wrinkle not developed. Antennae with 4 segments. Occipital

apophyses of thorax converge at apex (Fig. 7c); width 0.78±0.05, 0.66-0.85, 22. Abdomen width 1.07±0.07, 0.66-0.85, 22. Scales and spines denser than in nymph 2.

Taxonomic summary

Type host: *Phocarctos hookeri* (Gray, 1844).

Locality: Auckland Island, New Zealand (50°30'S; 166°17'E).

Remarks

The redescribed louse can be distinguished from other species of the genus by the presence of the fringe of setae on the back of the head. *Antarctophthirus trichechi* has a proboscis unusually prominent, bearing large hooks; *A. microchir* and *A. callorhini* clearly differ in the distribution of abdominal scales, being more abundant and uniform in shape and size in *A. microchir*. Other useful characters to differentiate *A. microchir* from other *Antarctophthirus* species are the pattern of ovoid and uniform scales and longitudinal grooves in the surface of spines.

To confirm our identification we tried to examine the holotype, but it was not located in any public or private louse collections. Therefore, the holotype might never have been deposited. Our specimens fit the descriptions of *A. microchir* from Trouessart and Neumann (1888) and Ferris (1951). We also compared our specimens with the only available material from the type host, *P. hookeri* (1 male and 1 female from the Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand) and no meaningful differences were detected and, therefore, we assigned our specimens to this species.

N1 are distinguishable from other nymphal stages by having shorter occipital apophyses, shorter thoracic phragmata and by the absence of scales and thorax without ventral spines or hairs. N2 and N3 may be distinguished by their occipital apophyses, which are parallel in N2 and converging in N3 at the apex (Fig. 7).

DISCUSSION

The South American sea lion, *O. flavescens*, had previously been reported as a host for *A. microchir,* but the information was, to a certain extent, confusing. According to Kim et al. (1975) and Lauckner (1985), *A. microchir* was reported by Ferris (1951) from *O. flavescens*. However, the latter referred to *Otaria hookeri* (syn. *Phocarctos hookeri*), not to *O. flavescens*. We think that the synonymy of *Otaria* with *Phocarctos* (indicated in Ferris' [1951] monographs) may have led to confusion in considering *O. flavescens* as a host for *A. microchir*. During the development of the present study, we had access to literature concerning the presence of *A. microchir* on this host, which, to our knowledge, was not reported in any of the previous works on this species. Hamilton (1939) recorded several specimens of *Antarctophthirus* from the Malvinas (Falklands) Islands, which were sent to the British Museum for identification. However, the specific identification was not confirmed due to the absence of material from the type host. Later, Carrara (1952) reported *A. microchir* from the same host species. The specimens were identified at the Museo de La Plata (Argentina). One of the authors (MSL) could not find voucher specimens at the collection of the Museo de La Plata. Recently, *A. microchir* was identified from *O. flavescens* in Chile (Crovetto et al., 2008).

Members of the Phthiraptera (chewing and sucking lice) generally show a high level of host specificity, with over 70% of the species recorded from a single host species (Smith, 2007). A well-known example is the chewing lice-pocket gopher association. Usually, each species of louse is restricted to a single gopher species (Hafner et al., 1994) because the life cycle of the chewing lice occurs entirely in the fur of the host. Moreover, pocket gophers are asocial mammals, with limited dispersal capabilities, and the different species rarely interact (Hafner et al., 1994; Light and Hafner, 2007). In addition, these parasite-host life styles have resulted in a high degree

of codivergence and cospeciation between chewing lice and their hosts (e.g., Hafner and Nadler, 1988; Page et al., 1995). Sucking lice (Anoplura) are also obligate and permanent parasites of mammals, living in host fur. The Anoplura have evolved closely with their mammalian hosts for a long time and, as a consequence, sucking lice show a high level of host specificity, i.e., more than 60% of sucking louse species are associated with one host species (Kim, 1985).

Conclusive evidence regarding the evolutionary patterns of echinophthiriids in pinnipeds is not available. However, specificity to their hosts and their particular morphological traits suggest a coevolutionary process beginning when the ancestors of pinnipeds entered the ocean (Kim et al., 1975). Host specificity of echinophthiriids ranges from 100%, involving 1 or 2 host genera (*Lepidophthirus*, *Proechinophthirus*, and *Latagophthirus*), to echinophthiriids, such as *Echinophthirus* spp. and *Antarctophthirus*, which infect species in 5 and 9 host genera, respectively (Kim et al., 1975). The 2 latter genera include the polytypic species *E. horridus*, which infests 7 Phocinae species and *A. microchir*, which infects 5 Otariinae species. In the case of *A. microchir*, it is striking that the same louse species has been reported from 5 sea lion species from both hemispheres (Australia, New Zealand, and North and South America). Fahrenholz (1939) noted morphological differences, regarding the shape of abdomen and thorax margins and scales from the 6th tergite, when comparing the illustrations of *A. microchir* by Ferris (1934) and Enderlein (1906) and, consequently, he erected a new subspecies (*A. m. californianus* from *Z. californianus*). Ferris (1951) refuted this subspecies, arguing that the discrepancies were probably due to different slide-mounting of the specimens.

There are 2 approaches that have influenced the development of Phthiraptera taxonomy (especially for bird lice) at the species level (Mey, 1998). One approach

considers that morphologically identical lice of different hosts are different species (based on the host specificity criterion). The other approach emphasizes the morphological criterion as the clue to differentiate species (Page et al., 2004). Within this context, it is difficult to establish host specificity in lice. In addition, the taxonomy may be problematic because morphological characters of lice are often conservative (Page et al., 2004). Several authors have suggested that only multivariate analysis can detect significant variations between related lice from different hosts (Ramli et al., 2000). However, morphologically identical species may be genetically different. In fact, several examples of cryptic species in lice have been reported (Page et al., 2004). In the case of *A. microchir*, the second approach would include the criteria followed so far to support its occurrence in different host species and geographical areas. However, the question raised here is whether *A. microchir* from different sea lion hosts represents a complex of cryptic species. Until molecular data become available, this question is far from resolved.

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FIGURE 1. Types of modified setae. (**a**) Scanning electron micrograph (SEM) of spine (scale bar= 10 μm); (**b**) SEM of scale (scale bar= 10 μm); (**c**) SEM of hairs and spines

(scale bar= 50 μm); (**d**) Line drawings (LD) of spine (scale bar= 50 μm); (**e**) LD of scale (scale bar= 50 μm); (**f**) LD of hairs (scale bar= 50 μm).

FIGURE 2. Chaetotaxy of *Antarctophthirus microchir*. Terminology follows Kim & Ludwig 1978. *Head:* 1- APHSp, apical head spine; 2- OrS, oral setae; 3- VPreASp, ventral preantennal spine; 4- VPHSp, ventral principal head spine; 5- VPoMHS, ventral posterior marginal head setae; 6- SuHSp, sutural head spine; 7- DMHSp, dorsal marginal head spine; 8- DPoMHS, dorsal posterior marginal head setae; 9- DPreASp, dorsal preantennal spine; 10- MAHSp, marginal anterior head spine. *Thorax:* 11- DPtSp, dorsal principal thoracic spine; 12- DMsSp, dorsal mesothorax spine; 13- DMtSp, dorsal metathorax spine; 14- DPTSp, dorsal principal thoracic spine; 15- DMASp, dorsal marginal abdominal spine. *Abdomen:* 16- VCAS, ventral central abdominal setae; 17- VLAS, ventral lateral abdominal setae; 18- VMAS, ventral marginal abdominal setae; 19- DCAS, ventral central abdominal setae; 20- DLAS, dorsal lateral abdominal setae; 21- DMAS, dorsal marginal abdominal setae; 22- AAS apical abdominal setae.

FIGURE 3. Ligth microscope micrograph of *Antarctophthirus microchir*. (**a**) Male; (**b**) female; (**c**) Nymph 1; (**d**) Nymph 2; (**e**) Nymph 3. (Scale bar= 500 μm).

FIGURE 4. Line drawings of *Antarctophthirus microchir*. (**a**) Female, dorsoventral view (scale bar= 1 mm); (**b**) pseudopenis (scale bar= 250 μm).

FIGURE 5. Thoracic dorsal scales showing an inverted Ω pattern (scale bar=100 μm).

FIGURE 6. Egg (scale bar= 250 μm).

FIGURE 7. Thorax showing development of phragmata. (**a**) N1 (scale bar= 500 μm); (**b**) N2 (scale bar= 500 μm); (**c**) N3 (scale bar= 500 μm).

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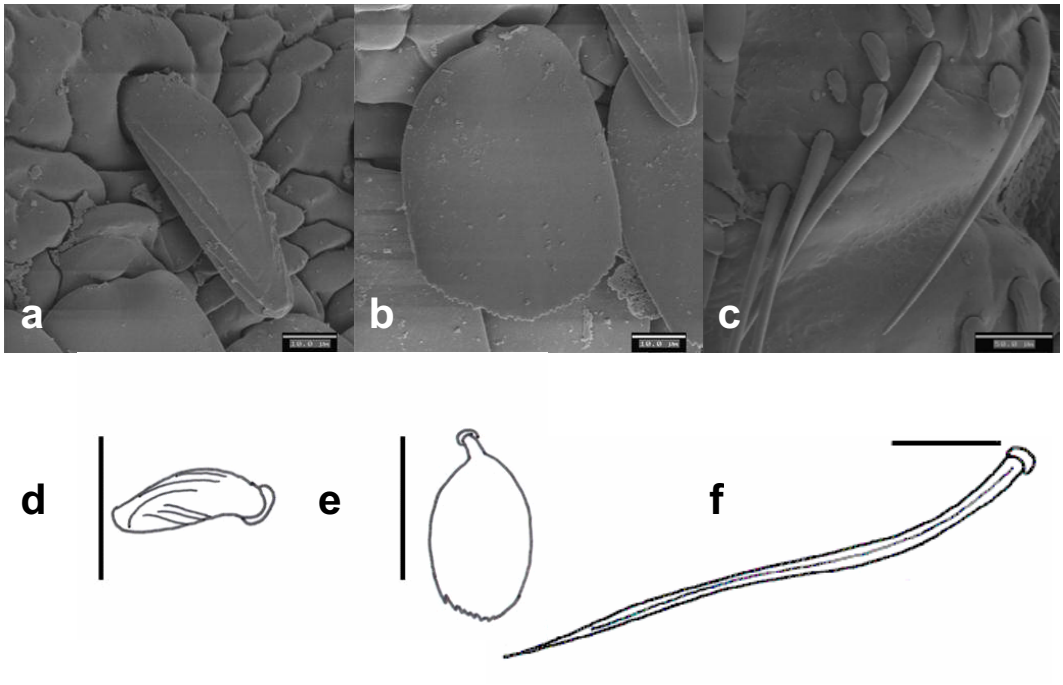
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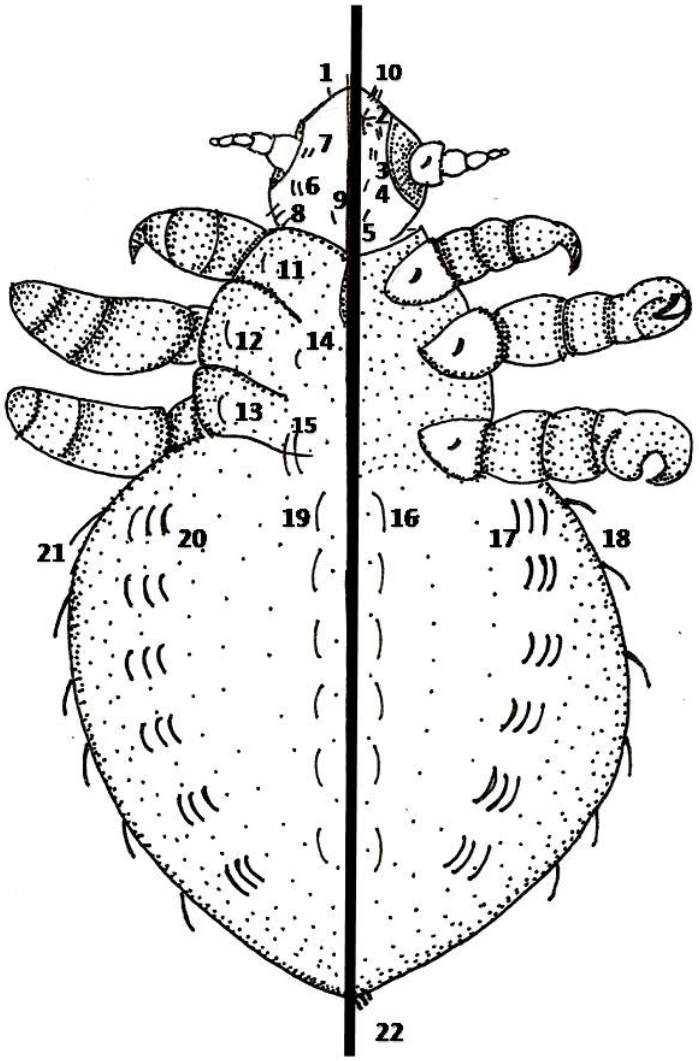
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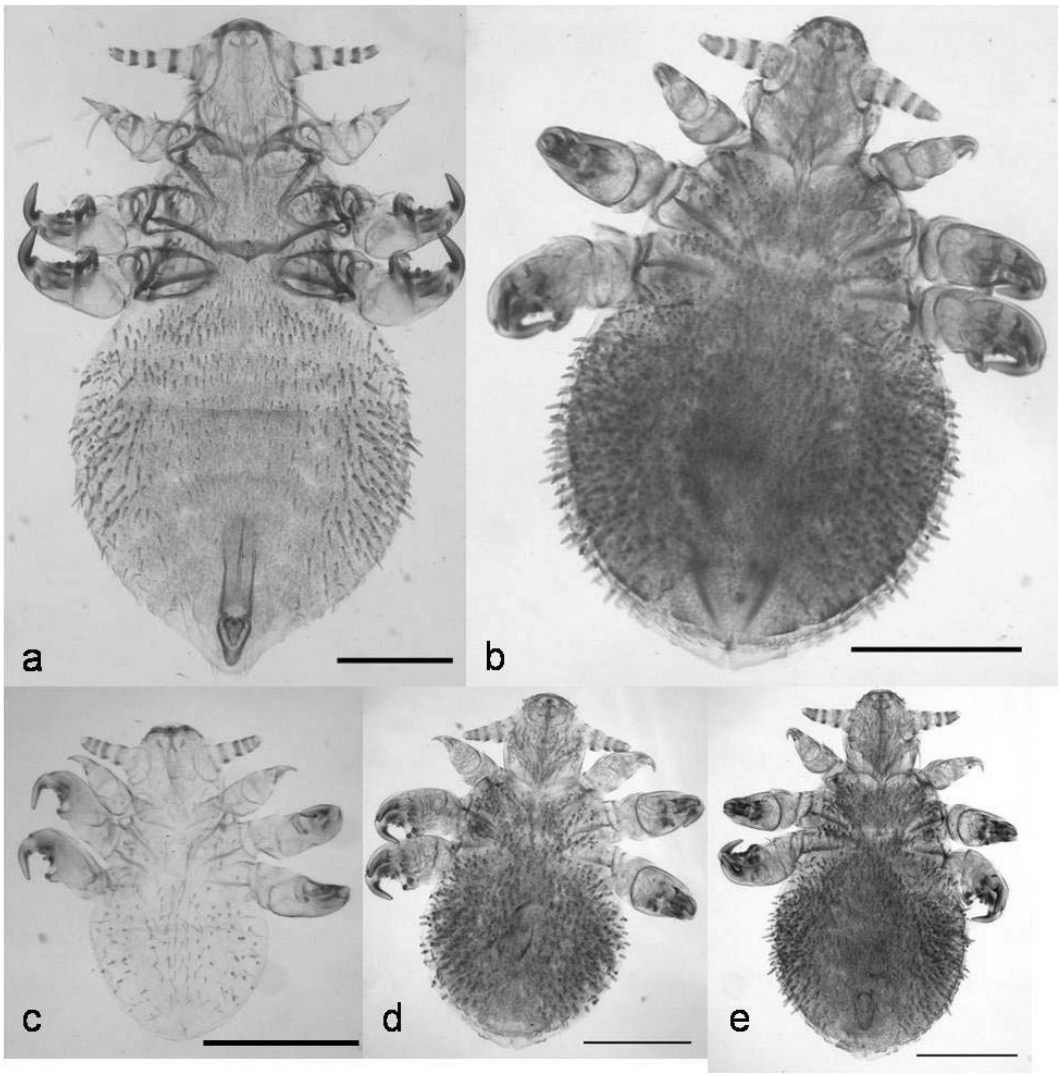
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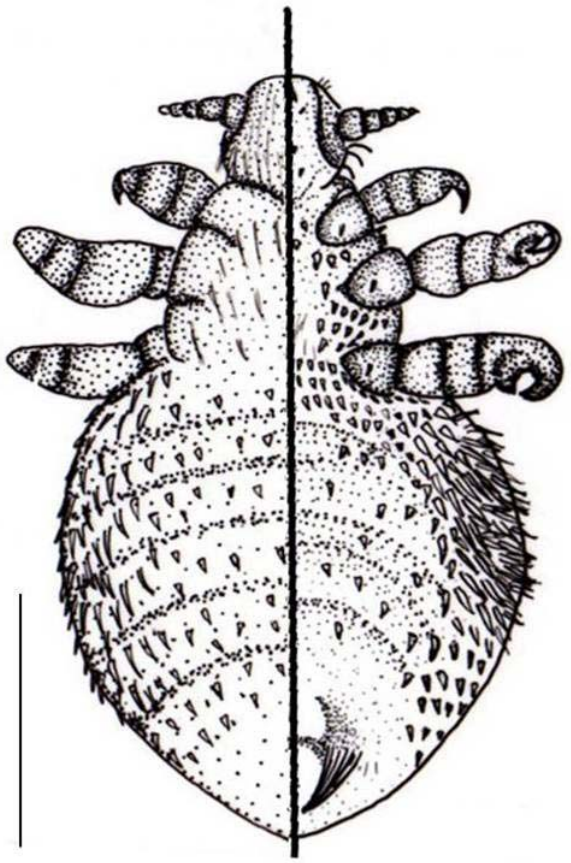

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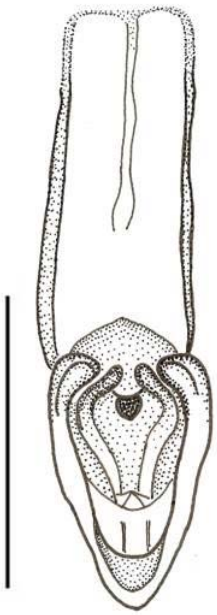

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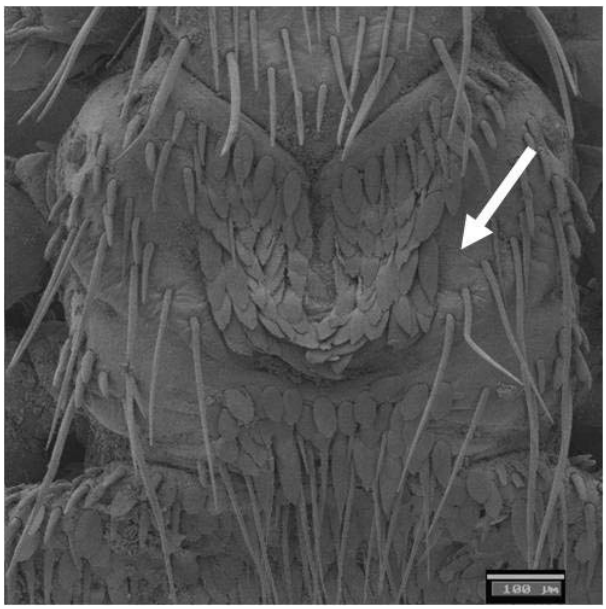
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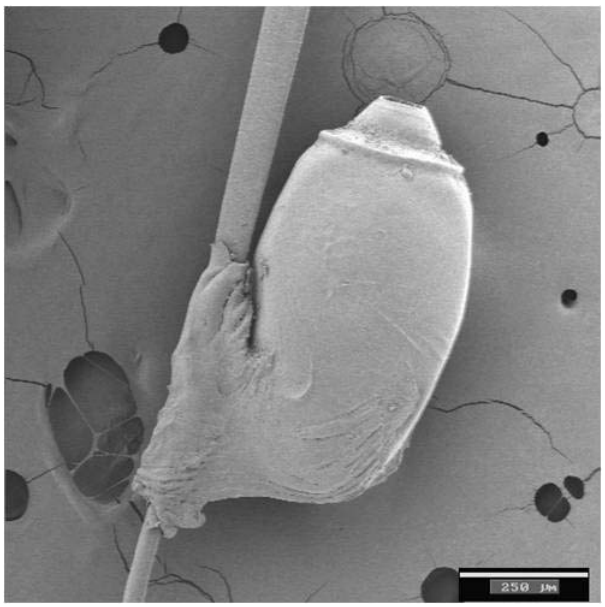
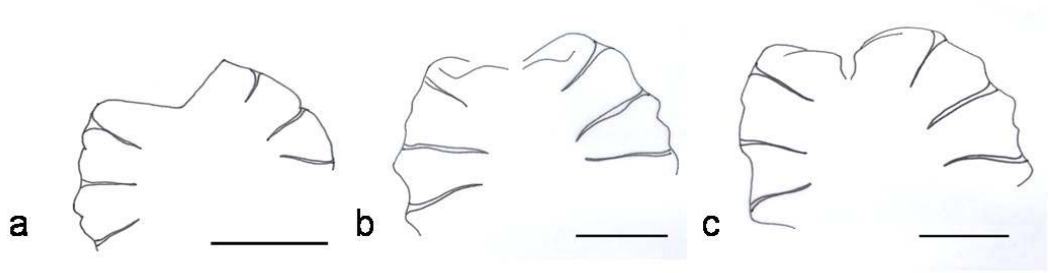

250 µm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