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Running Head: DURDEN ET AL.: NEW LEMUR LOUSE

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A New Species of Sucking Louse (Phthiraptera: Anoplura: Polyplacidae) from the Gray Mouse Lemur, *Microcebus murinus*, in Madagascar

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Abstract

Lemurpediculus madagascariensis sp. nov. (Phthiraptera: Anoplura: Polyplacidae) is described from the Gray Mouse lemur, *Microcebus murinus* (J. F. Miller), from Ankarafantsika National Park, Madagascar. Lemurs were trapped using Sherman Live Traps and visually inspected for lice, which were preserved in 90% ethanol. Adults of both sexes and the third instar nymph of the new species are illustrated and distinguished from the four previously known species of *Lemurpediculus: L. verruculosus* (Ward), *L. petterorum* Paulian, *L. claytoni* Durden, Blanco and Seabolt, and *L. robbinsi* Durden, Blanco and Seabolt. It is not known if the new species of louse is a vector of any pathogens or parasites.

Key Words: Phthiraptera, Anoplura, new species, mouse lemur, Madagascar

The mouse and dwarf lemurs of Madagascar (family Cheirogaleidae) are among the smallest primates in the world (Zimmermann and Radespiel 2014, Lehman et al. 2016, Zohdy and Durden 2016). Ectoparasites of cheirogaleid lemurs are inadequately known (Blanco et al. 2013, Zohdy and Durden 2016, Durden et al. 2017) and sucking lice (Phthiraptera: Anoplura) have been described from only three of the more than 30 species of cheirogaleids currently recognized (Hotaling et al. 2016). The three previously described species are *Lemurpediculus verruculosus* (Ward), an ectoparasite of the eastern mouse lemur, *Microcebus rufus* É. Geoffroy, *Lemurpediculus claytoni* Durden, Blanco and Seabolt, an ectoparasite of Sibree's dwarf lemur, *Cheirogaleus sibreei* Forsyth Major, and *Lemurpediculus robbinsi*, which parasitizes Crossley's dwarf lemur, *Cheirogaleus crossleyi* A. Grandidier (Durden et al. 2017). Another congeneric species, *Lemurpediculus petterorum* Paulian, parasitizes a different species of lemur which was stated to probably be *Lepilemur mustelinus* I. Geoffroy by Paulian (1958). Sucking lice are often host-specific (Durden and Musser 1994), and because few lemur species have been sampled for ectoparasites, there are undoubtedly additional undescribed species of *Lemurpediculus* associated with other species of cheirogaleids. Durden et al. (2017) amended the description of the genus *Lemurpediculus* to accommodate new developments in the systematics of Anoplura since the genus was erected by Paulian (1958). In this paper, we describe a new species of *Lemurpediculus* from the gray mouse lemur from Ankarafantsika National Park in northwestern Madagascar.

Materials and Methods

As part of a study on lemur health and communication, mouse lemurs were trapped in Ankarafantsika National Park, Madagascar in Jardin Botanique A using Sherman Live Traps (H.

B. Sherman Traps, Inc., Tallahassee, FL) baited with banana between May and November in 2010 and 2011 (dry season). The study was conducted in the dry season because that is when the lemurs most readily enter traps for the banana. Lemurs were removed from the traps, manually restrained, and inspected for parasites by parting the fur down to the skin. Ectoparasites were collected primarily from the face, ears, legs, back and tail and stored in 90% ethanol in individually labeled vials. All lemurs were released at their capture site following collection of data and ectoparasites. This study was approved by Madagascar National Parks (N101/11/MEF/SG/DGF/DCB.SAP/SCB, N102/11/MEF/SG/DGF/DCB.SA/SCB) and the Arizona State University Institutional Animal Care and Use Committee (Protocol: 10-1077R).

Lice were cleared in 10% potassium hydroxide for ~24 h, rinsed in distilled water, transferred to 70% ethanol and then slide mounted in PVA medium (Bioquip Products, Rancho Dominguez, CA). Slide-mounted lice, including specimens of previously described congeneric species (in the Anoplura collections of LAD), were examined at high magnification under phase-contrast using an Olympus BH-2 microscope (Olympus Corporation of the Americas, Center Valley, PA) connected to an Ikegami MTV-3 video camera attachment and monitor (Ikegami Electronics, Neuss, Germany). Drawings of diagnostic morphological features were made from specimens examined at 100x – 400x. Specimen measurements were made using a calibrated graticule fitted into a microscope eyepiece.

Descriptive format for the new species follows Durden et al. (2010) and names and abbreviations of setae and morphological structures follow Kim and Ludwig (1978). Names of setae and certain structures are spelled out in full at first mention (with the abbreviation listed parenthetically) and then abbreviated when subsequently mentioned. The holotype male, allotype female and paratype third instar nymph of the new species are deposited in the U.S. National

Museum of Natural History (NMNH) (Smithsonian Institution), Department of Entomology, Washington DC.

Lemur taxonomy and common names used in this paper follow Groves (2005) and Hotaling et al. (2016).

Results

We trapped a total of 107 *M. murinus.* The entire ectoparasite faunas of these mouse lemurs will be reported in a separate paper.

Lemurpediculus madagascariensis **sp. nov.** (Figs. 1-3)

Male (Fig. 1A,B,C)

Total body length: 1.07–1.13 mm; mean, 1.10 mm (n=5). Head, thorax and abdomen lightly sclerotized.

Head: More heavily sclerotized along anterior dorsal margin and antero-laterally adjacent to first antennal segment; longer than broad with squarish anterior margin. One long Dorsal Principal Head Seta (DPHS), one small Dorsal Accessory Head Seta (DAcHS) anteromedial to DPHS, one Dorsal Posterior Central Head Seta (DPoCHS), 2-3 Dorsal Preantennal Head Setae (DPaHS), two Sutural Head Setae (SHS), three Dorsal Marginal Head Setae (DMHS), 3-4 Apical Head Setae (ApHS) and one Ventral Preantennal Head Seta (VPaHS) on each side. Antennae five-segmented with basal segment slightly wider than long and distinctly broader than second segment; fourth segment slightly extended posterolaterally.

Thorax: Much longer than wide, slightly wider than head. Thoracic sternal plate (Fig. 1B) lightly sclerotized, with narrow anterior extension and broadly curved lateral margins; tiny sclerite bearing two long setae immediately posterior to thoracic sternal plate. Dorsal Principal Thoracic Seta (DPTS) mean length 0.13 mm (range, 0.12-0.14 mm, n=8), with adjacent small Dorsal Mesothoracic Seta (DMsS) on each side; mesothoracic spiracle mean maximum diameter 0.025 mm (range, 0.023-0.027 mm, n=8). Legs with subtriangular coxae; forelegs each terminating in small tibio-tarsal claw; mid and hindlegs subequal in size, each terminating in large, robust tibio-tarsal claw.

Abdomen: Wider than thorax with six annulated spiracles on each side. Paratergal plates, tergites and sternites absent. One row of two long Dorsal Central Abdominal Setae (DCAS) anteriorly, followed by five rows of 4-6 long DCAS and then two rows of two shorter DCAS. Six Dorsal Lateral Abdominal Setae (DLAS) on each side, each adjacent to corresponding spiracle; DLAS 1-5 each with adjacent small seta; DLAS 1 and adjacent small seta both inserted on small ridge. DLAS 6 borne on small sclerite and distinctly longer than other DLAS and extending away from abdomen; five rows of four long Ventral Central Abdominal Setae (VCAS); VCAS in most posterior row slightly shorter than other VCAS. One posterior Ventral Lateral Abdominal Seta (VLAS) on each side adjacent to corresponding DLAS and most posterior spiracle. ~10 tiny to small dorsal setae near posterior apex of abdomen.

Genitalia (Figs 1A,C): Subgenital plate (Fig. 1A) well sclerotized, somewhat urn-shaped with bulging medio-lateral margins and small antero-lateral extensions. Basal apodeme longer than parameres and other genitalic components combined, slightly expanded posteriorly into two paddle-shaped plates on each side; C-shaped anterior endomere with posteriorly converging arms; anteriorly acuminate aedeagal sclerite located inside anterior endomere; posteriorly acuminate central endomere bordered laterally by one broad accessory sclerite on each side;

parameres broad anteriorly and tapering posteriorly to rounded apex; pseudopenis relatively small but extending posteriorly beyond apices of parameres and terminating in acute apex.

Female (Fig. 2A,B,C)

Body length: 1.32-1.50 mm; mean, 1.43 mm (n=6). Head, thorax and abdomen as in male unless indicated otherwise.

Head: Slightly wider than in male.

Thorax: Mesothoracic spiracle mean maximum diameter 0.0275 mm (range, 0.0250-0.0283, n=6).

Abdomen: Dorsally with eight rows of four long DCAS anteriorly followed by one row of six slightly shorter DCAS and one row six small Tergal Abdominal Setae (TeAS) inserted on broad, curved tergite immediately posterior to subgenital plate. One row of one DLAS on each side anteriorly followed by six rows of two DLAS on each side and then one very long DLAS borne on small sclerite posterior to last spiracle. One row of two long VCAS anteriorly followed by five rows of four VCAS. One very long VLAS posteriorly, associated with last DLAS and most posterior spiracle.

Genitalia (Fig. 2C): Subgenital plate broadly rounded anteriorly and extending posteriorly to broad apex, with small, distinct lateral lacuna on each side; each lacuna with four small setae inserted anteriorly; three small setae inserted on each side of subgenital plate near postero-lateral margins. Vulvar fimbriae distinct and extensive collectively forming a V shape; gonopods VIII and IX indistinct and with ~13 setae attached to each gonopod VIII and two slightly larger setae attached to each gonopod IX; gonopod setae collectively forming postero-lateral fan-like patches. Curved subterminal transverse sclerite with small anterior apex situated between gonopods IX. Three small terminal setae ventrally on each side of genital opening.

Third Instar Nymph (Fig. 3)

Body length: 1.00-1.25 mm; mean, 1.11 mm (n=5).

Head: Shape as in male but with slightly more rounded anterior margin. One fairly long DPTS and one adjacent small DAcHS, one DPoCHS, two DMHS, two SHS, 3-4 ApHS and one VPaHS on each side. Antennae approximately as in male.

Thorax: Slightly wider than head, much longer than wide; one long DPTS (mean length, 0.125 mm, range, 0.110-0.129 mm, n=4) adjacent to mesothoracic spiracle (mean maximum diameter, 0.025 mm, range, 0.020-0.028, n=4) on each side. Foreleg coxae subtriangular; mid and hind coxae more irregular; forelegs each terminating in small tibio-tarsal claw; mid and hindlegs subequal in size, each terminating in large, robust tibio-tarsal claw.

Abdomen: Wider than thorax with eight rows of two DCAS and nine rows of VCAS. Eight DLAS on each side; DLAS 2-7 each with accompanying spiracle; DLAS 2 with adjacent small accessory seta, both borne on small protuberance; two additional small setae adjacent to DLAS 2 on each side; one additional small accessory seta on each side adjacent to each of DLAS 3-6; DLAS 7 and 8 both long, extending from postero-lateral abdomen and each associated with one VLAS on each side.

HOLOTYPE ♂ ex *Microcebus murinus* (J. F. Miller) (gray mouse lemur) (male, Animal 25-09), MADAGASCAR: Boeny Region, Ankarafantsika National Park, Jardin Botanique A (46^{0}48’ E, 16^{0}19’ S,), elevation 190 m, 17 October 2010, Coll: Sharon Kessler and Alida I. F. Hasiniaina. Deposited in NMNH (accession barcode, USNMENT00981907).

ALLOTYPE ♀ ex *M. murinus*, same data as Holotype except (male, Animal 82-10) and 13 Nov. 2011. Deposited in NMNH (accession barcode, USNMENT00981908).

PARATYPES One nymph (third instar) same data as Holotype except (male, Animal 25-10), 15 October 2010 (accession barcode, USNMENT00981909); 2♂, 2♀, 2 nymphs (third instars) same data as Holotype except different individual lemurs and various dates in 2010 and 2011; deposited in Georgia Southern University Insect Collection (1♂, 1♀) (accession no. L-3813) and Anoplura Collection of L. A. Durden (1♂, 1♀).

ETYMOLOGY: This species is named for the faunistically unique island of Madagascar where both the louse and its host co-occur.

For comparative purposes, the female subgenital plates and associated structures, for the four previously described congeneric species, *L. petterorum*, *L. claytoni*, *L. verruculosus*, and *L. robbinsi*, are illustrated in Fig. 4.

Discussion

Males

Males of *Lemurpediculus* spp. can easily be separated by examination of the genitalia in cleared slide-mounted specimens. In *L. petterorum* males, the parameres are about equal in length to the basal apodeme (shown in Paulian 1958, Fig. 1B), whereas *L. claytoni*, *L. robbinsi*, and *L. verruculosus*, the parameres are much shorter than the basal apodeme. The shape of the parameres and the presence or absence of genitalic endomeres and accessory sclerites can be used to separate these four species. The parameres have slightly concave medio-lateral margins in *L. claytoni* (shown in Durden et al. 2017, Fig. 3B) and distinctly rounded convex medio-lateral margins in both *L. verruculosus* (shown in Durden et al. 2010, Fig. 3) and *L. robbinsi* (shown in

Durden et al. 2017, Fig. 2B). The medial margins of the parameres of *L. robbinsi* have a distinctly rounded bulge (shown in Durden et al. 2017, Fig. 2B) which is absent in *L. verruculosus* (shown in Durden et al. 2010, Fig. 3). Further, the pseudopenis extends well beyond the posterior apices of the parameres in *L. robbinsi* (shown in Durden et al. 2017, Fig. 2B) but just barely beyond the apices in *L. verruculosus* (shown in Durden et al. 2010, Fig. 3). The male genitalia of *L. madagascariensis* sp. nov. (Fig. 1C) have more acute anterior paramere margins than those of the other species in the genus and have additional adjacent small plates that are not present in the other species – a central endomere and a pair of lateral sclerites. Externally, the thoracic sternal plate of *L. petterorum* (shown in Paulian 1958, Fig. 2B) lacks an anterior projection which is clearly present in the other four species.

Females

Females of all five known species of *Lemurpediculus* can easily be separated based on the shape of the subgenital plate which can be observed in either cleared or uncleared specimens. In both *L. petterorum* and *L. madagascariensis* sp. nov., the anterior and posterior portions of the subgenital plate are joined centrally and laterally and have two lateral lacunae in the anterior portion (Figs. 2C, 4A), whereas the anterior and posterior sections of the subgenital plate are not joined laterally in the other three species Fig. 4B-D). The two lacunae in the female subgenital plate of *L. petterorum* are much larger than those in *L. madagascariensis*, collectively making up almost half of the plate size in the former species (Fig. 4A), but less than 10% in the latter species (Fig. 2C). Also, the thoracic sternal plate in the female of *L. petterorum* lacks the anterior extension (shown in Paulian 1958, Fig. 2B) that is present in females of *L. madagascariensis* sp. nov. (Fig. 2B in this paper). In females of *L. verruculosus*, the anterior portion of the subgenital plate is 3-4 times larger than the posterior portion (Fig. 4C). The anterior and posterior sections of the subgenital plate are subequal in size in females of *L. claytoni* (Fig. 4B) whereas the anterior portion is slightly larger than the posterior portion in *L. robbinsi* (4D). Also, the shape of the female subgenital plate is very different between these species (Figs. 2C, 4A-D).

Nymph

The third instar nymph of only one other species of *Lemurpediculus* has been described. This nymphal stage of *L. verruculosus* was described and illustrated by Durden et al. (2010). The third instar nymph is easily separated between these two species because *L. verruculosus* lacks DLAS next to abdominal spiracles 2-5 (see Durden et al. 2010, Fig. 3) whereas *L. madagascariensis* sp. nov. has one long DLAS next to each of these spiracles on each side (Fig. 2C).

With the description of the new species included in this paper, there are now five recognized species of *Lemurpediculus*. Four of these species, *L. verruculosus, L. robbinsi, L. claytoni,* and *L. madagasarensis* sp. nov., parasitize cheirogaleid lemurs while the host of the fifth species, *L. petterorum* Paulian, was stated by Paulian (1958) to probably be *Lepilemur mustelinus* (weasel sportive lemur) another nocturnal species which belongs to a different lemur family, the Lepilemuridae. All five of these species of lice appear to be host-specific but the host/s of *L. petterorum* requires verification. It would be premature to provide a dichotomous identification key for known *Lemurpediculus* species because we anticipate the collection and description of additional species in this genus in the future, especially considering the highly diverse radiation of mouse lemur species around Madagascar (Yoder et al. 2010, 2016).

With few exceptions, very little is known about the potential for sucking lice of wild mammals to transmit pathogens to their hosts (Durden 2001) and nothing is currently known about any potential vectorial role of lice that parasitize lemurs. However, some pathogens and parasites of lemurs, including certain viruses, bacteria and protozoans could feasibly be transmitted by sucking lice. Future research should address the potential for blood-feeding ectoparasites, including sucking lice, to transmit pathogens to lemurs, particularly in light of the threatened or endangered status of many species of these primates.

Many authors have advocated conserving (or co-conserving) parasites of rare hosts (e.g., Durden and Keirans 1996, Whiteman and Parker 2005, Dunn et al. 2009) and we likewise advocate co-conservation of mouse lemurs and their unique host-specific parasites including their sucking lice.

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Footnotes

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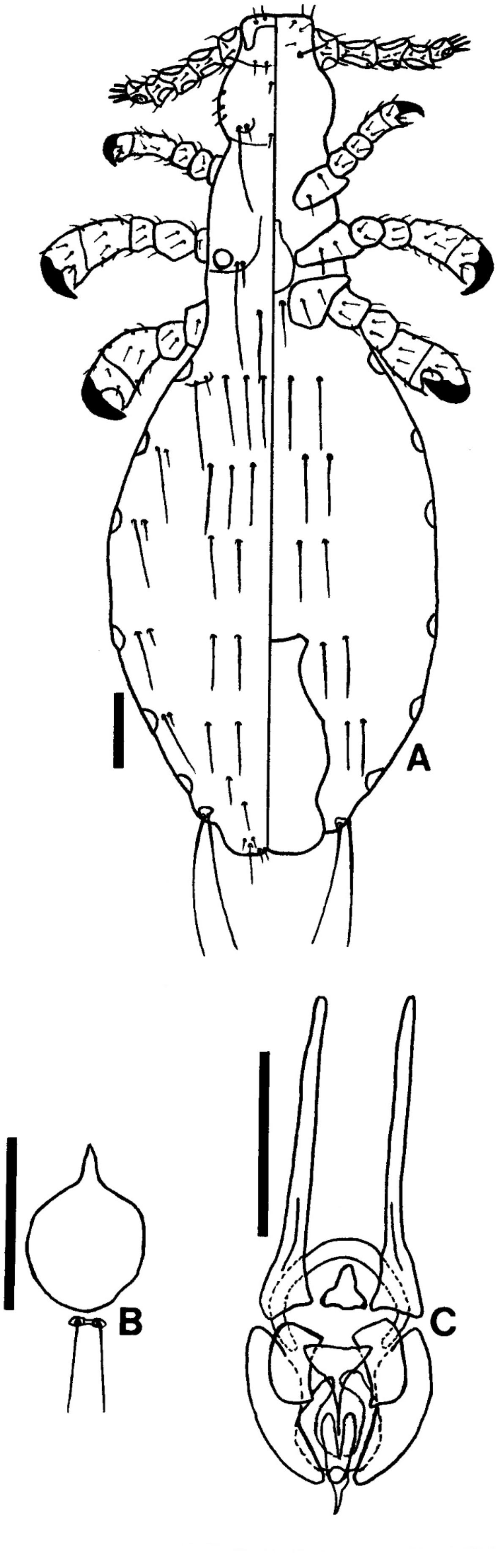
Figure Legends

Fig. 1. *Lemurpediculus madagascariensis* sp. nov., adult male. A: Dorsoventral view. B: Thoracic sternal plate. C. Genitalia. All scale bars, 0.1 mm.

Fig. 2. *Lemurpediculus madagascariensis* sp. nov., adult female. A: Dorsoventral view. B: Thoracic sternal plate. C. Genitalia. All scale bars, 0.1 mm.

Fig. 3. *Lemurpediculus madagascariensis* sp. nov., third instar nymph: Dorsoventral view. Scale bar, 0.1 mm.

Fig. 4. Female subgenital plates, associated setae, and vulvar fimbriae of the four previously described species of *Lemurpediculus*. A: *L. petterorum* Paulian ex (probably) *Lepilemur mustelinus*, Ambatolampy District, Madagascar. B. *L. claytoni* Durden, Blanco and Seabolt ex *Cheirogaleus sibreei*, Tsinjoarivo, Amabatolampy District, Madagascar. C: *L. verruculosus* (Ward) ex *Microcebus rufus*, Ranomafana National Park, Madagascar. D: *L. robbinsi* Durden, Blanco and Seabolt ex *Cheirogaleus crossleyi*, Tsinjoarivo, Amabatolampy District, Madagascar. Scale bar = 0.05 mm.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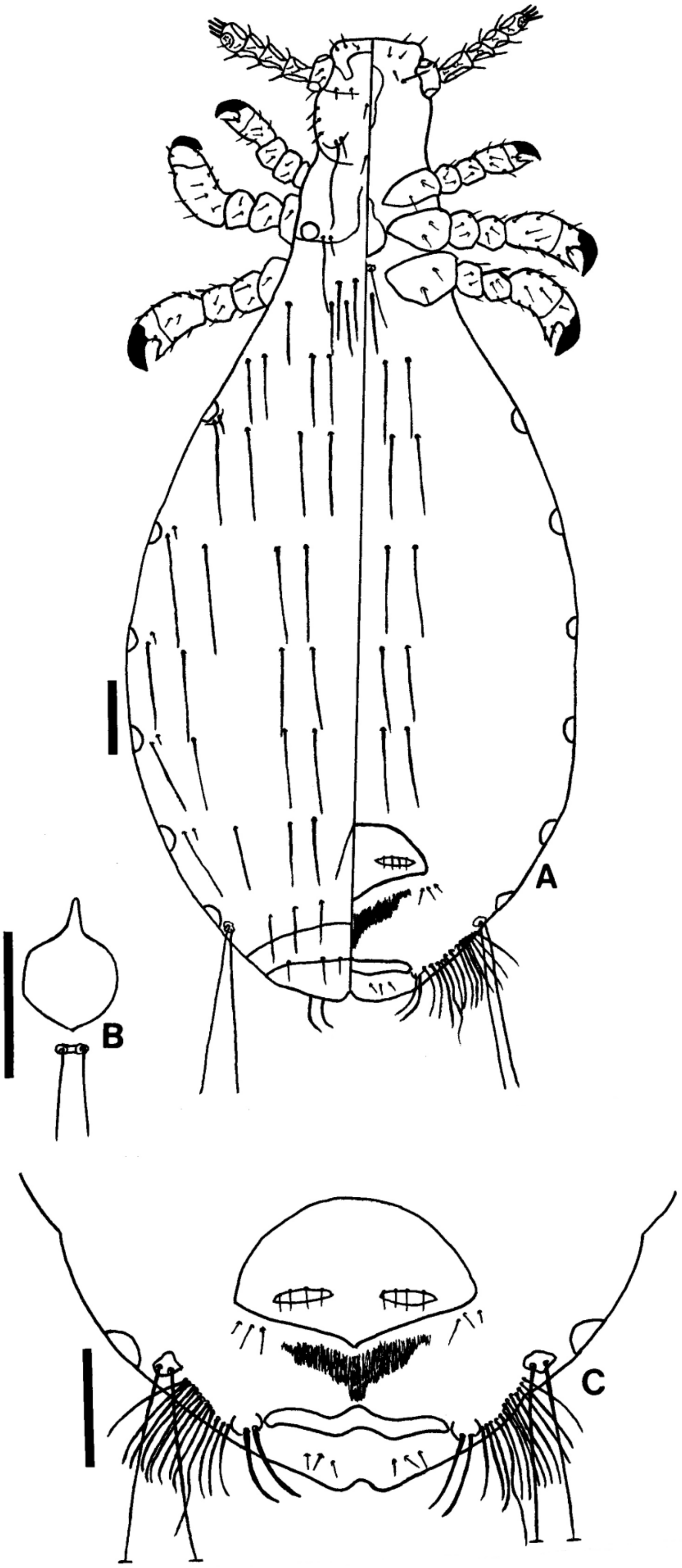
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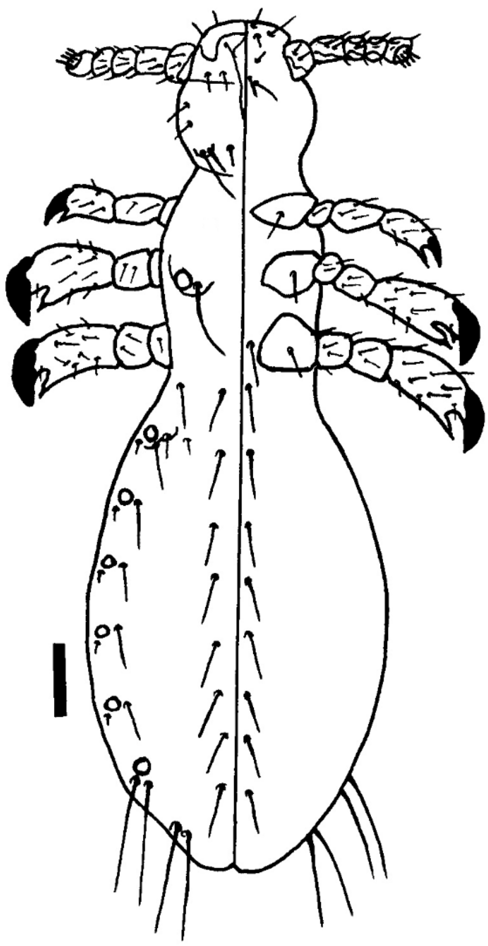

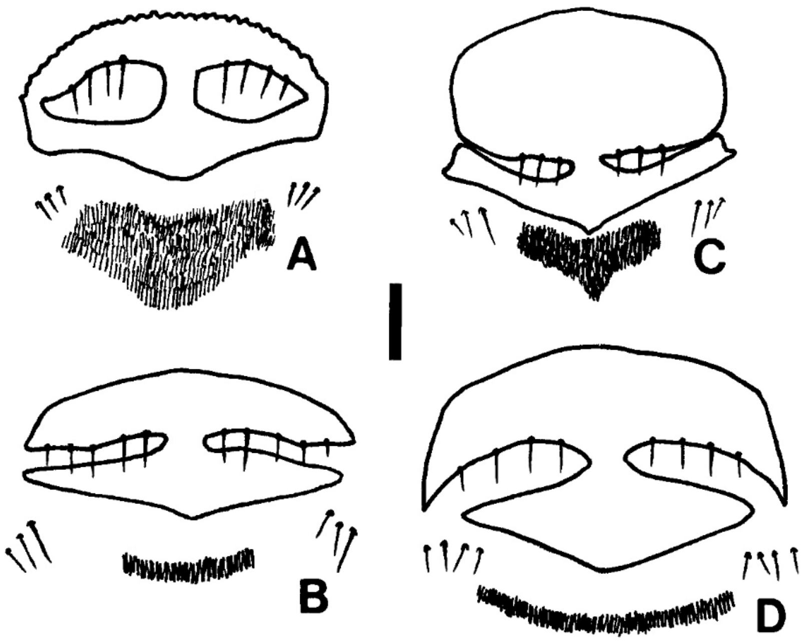
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