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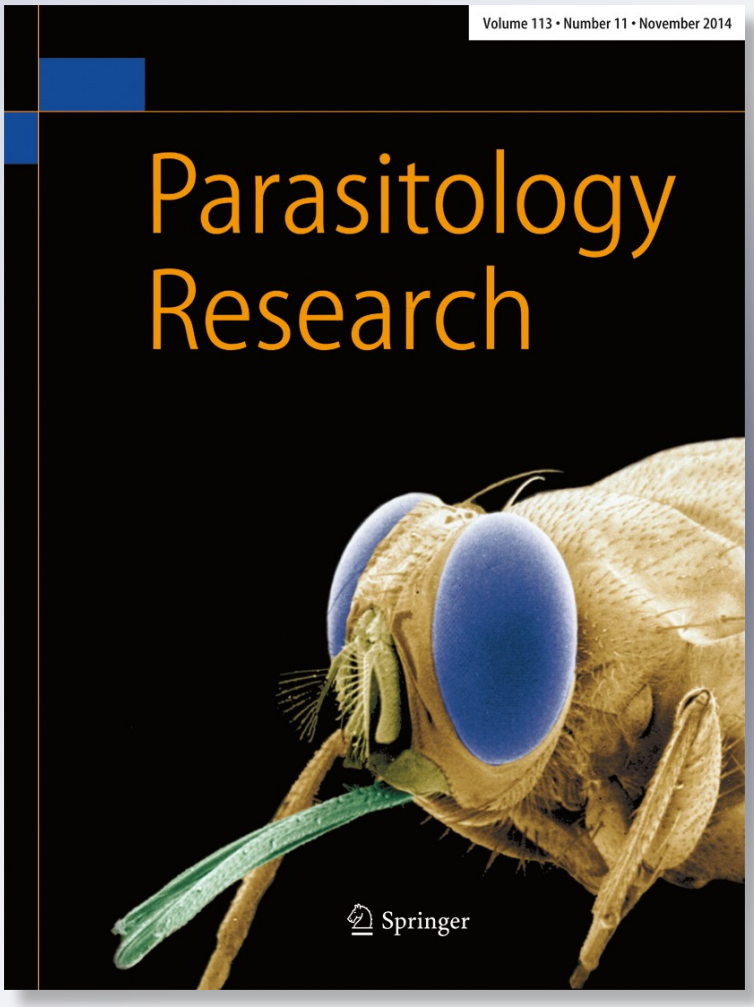

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ORIGINAL PAPER

Antarctophthirus carlinii (Anoplura: Echinophthiriidae), a new species from the Weddell seal *Leptonychotes weddelli*

María Soledad Leonardi · Sebastián Poljak · Pedro Carlini · Juan Galliari · Magalí Bobinac · Mercedes Santos · María E. Márquez · Javier Negrete

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Abstract As a part of an ongoing long-term study on the biology of pack-ice seals in Antarctica, we had the opportunity to collect lice from Weddell seals (*Leptonychotes weddelli*). We did not find the original description of this host-parasite association. *Antarctophthirus ogmorhini* had previously been reported as a parasite for the Weddell seal, but the information is, to a certain extent, confusing. During the development of the present study, we had access to literature concerning the presence of *A. ogmorhini* on this host, which, to our knowledge, was not determined in any of the previous works on this species. We compared lice collected from Weddell seals with *A. ogmorhini* obtained from the type host, the leopard seal (*Hydrurga leptonyx*), and we found that both species can be distinguished. The main differences are the characteristic pattern of chaetotaxy in the dorsal side of the head in lice from Weddell seals, the size and form of the pseudopenis, and the distribution and size of the fringe of setae surrounding the genital opening. Considering the conservative morphology, and ecological and evolutionary features of sucking lice, we proposed that lice from Weddell seals constitute a new species. In the present work, we described and illustrated adults of this new species collected from Weddell seals during the austral summer of 2014 at the Danco Coast, Antarctic Peninsula.

Keywords Antarctica · Pack-ice seals · Sucking lice · Taxonomy

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Among the sucking lice (Anoplura), the family Echinophthiriidae is the only family whose species have adapted to survive on pinnipeds (Durden and Musser 1994), a mammalian group that includes fur seals and sea lions (Otariidae), walruses (Odobenidae), and true seals (Phocidae). Currently, the Echinophthiriidae consist of 12 species distributed in five genera: *Latagophthirus*, a monotypic genus from the river otter; *Proechinophthirus* includes two species infesting fur seals and sea lions, whereas species of *Lepidophthirus* (two species); *Echinophthirius* (monotypic) occur in true seals; and *Antarctophthirus*, the most diversified genus of the family.

Antarctophthirus has six recognized species infecting fur seals (*Antarctophthirus callorhini*), sea lions (*Antarctophthirus microchir*), walruses (*Antarctophthirus trichechi*), and Antarctic true seals (*Antarctophthirus lobodontis*, *Antarctophthirus ogmorhini*, and *Antarctophthirus mawsoni*). *Antarctophthirus* is also considered the less host-specific genus of the family, while *A. callorhini*, *A. trichechi*, *A. lobodontis*, and *A. mawsoni* has been described as species-specific; *A. microchir* and *A. ogmorhini* are present in several host species. According to the literature, *A. ogmorhini* is present in the leopard seal, *Hydrurga leptonyx,* and the Weddell seal, *Leptonychotes weddelli*. However, the information is, to a certain extent,

confusing and we did not found the original determination of this species in association with the Weddell seal.

As a part of an ongoing project on the biology of pack-ice seals in the Antarctic Peninsula, we had the opportunity to collect lice from Weddell seals. We compared the lice collected from Weddell seals with those obtained from the type host of *A. ogmorhini*, i.e., the leopard seal. In view of the morphological differences with *A. ogmorhini*, and the ecological and evolutionary history of their hosts, the aim of the present study is to describe a new species of Echinophthiriidae, *Antarctophthirus* n. sp. from the Weddell seal.

Materials and methods

Specimens examined

The samples were taken in the Antarctic Specially Protected Area (ASPA) No. 134 "Punta Cierva", (64° 09′ 23″ S, 60° 57′ 17″ W) in the northern sector of the Danco Coast during the austral summer of 2013/2014. Lice were collected from eight Weddell seals, which were immobilized following the routine procedures (see details in Wheatley et al. 2006). Ten males and 10 females of *Antarctophthirus* n. sp. were examined under light microscope. These specimens were compared with lice from the type host of *A. ogmorhini*, i.e., the leopard seal *H. leptonyx*.

Voucher specimens are deposited at the Parasitology Collection, Centro Nacional Patagónico (Puerto Madryn, Argentina).

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 h. 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, stopping maceration, and avoiding damage by over treatment. The specimens 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 h. A coverslip was placed with some weight upon the lice to flatten them. Lice were finally mounted in Canada balsam.

Figures were made using a drawing tube mounted on a Leica light microscope at×1,000 magnification. Measurements were taken from digital photographs using ImageJ software (Wayne Rasband, NIH, USA). Drawing of the adults is a composite from a series of photographs. All measurements in the text are in millimeters unless otherwise stated and are given as the mean±standard deviation and followed by range, and the number of measurements.

Terminology

Species of Echinopthiriidae are characterized by their modified setae (Kim 1985). Names and abbreviations of setae used in this paper follow those of Kim and Ludwig (1978) and Leonardi et al. (2009): spines are pointed setae, scales are flattened setae, and hairs are the long and thin setae.

Results

Antarctophthirus carlinii n. sp.

Taxonomic summary

Type host: Weddell seal, *L. weddelli* Lesson (Mammalia: Carnivora: Phociidae).

Type locality: Antarctic Specially Protected Area (ASPA) No. 134 "Punta Cierva", Danco Coast, Antarctic Peninsula (64° 09′ 23″ S, 60° 57′ 17″ W). January–February 2014.

Site in host: Mostly in the hind flippers

Type material: Holotype (male CNP-Par 63); alotype (female CNP-Par 64) paratypes (CNP-Par 65). Parasitology Collection, Centro Nacional Patagónico- CENPAT/CONICET. Puerto Madryn, Argentina.,

Collectors: MS Leonardi, S Poljak, P Carlini, J Galliari, M Bobinac, J Negrete.

Prevalence: 75 % (in six out of eight seals analyzed).

Intensity: 7.67.

Etymology: This species is named after our dear collage and friend Doctor Alejandro Carlini, who led the program of Marine Mammals of the Argentinean Antarctic Institute from 1995 until he suddenly passed away in December of 2010.

Description

Male (Fig. 1a) Total body length 2.29±0.23, 1.77–2.49, 10. *Head:* Almost as long as wide (length 0.51±0.05, 0.41–0.58, 10; width 0.49±0.05, 0.36–0.48, 10); anterior margin heavily sclerotized; maxillary vestige very distinct; ventral labrum connected to long apodemes; postantennal angle developed, posterolateral angle not developed. The proboscis is prominent, formed by two pieces with three hooks each one; around it, three short and thin apical hairs in each side. Along the external border, seven ventral principal head spines followed by two column of short spines. Three to four ventral posterior marginal spines of different sizes. Dorsally, this species present a very conspicuous pattern of chaetotaxy (Fig. 2). Below, the sclerotized anterior margin four dorsal anterior head

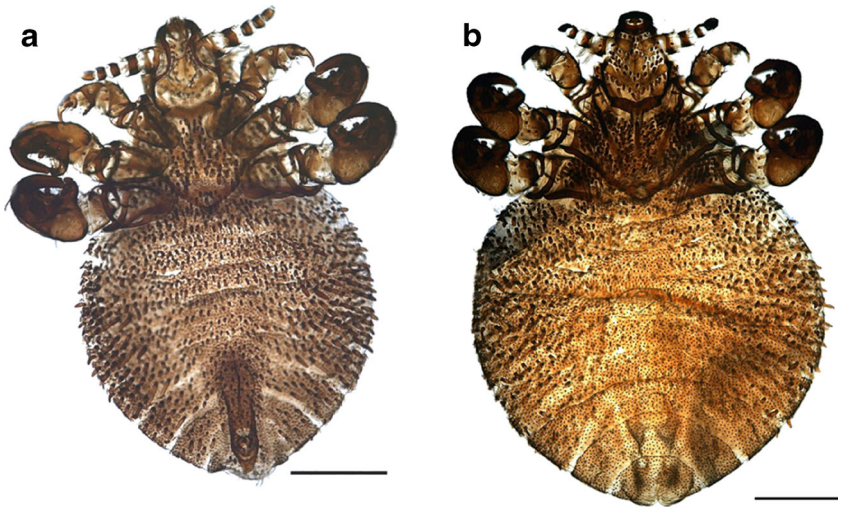

Fig. 1 Light microscope micrograph of *Antarctophthirus carlinii* n. sp. **a** Male; **b** female (*scale bar*=500 μm)

spines. The suprantennal central head spines form a triangle on the basis of which converge a line of four dorsal preantennal spines. Three short and one longer suprantennal head spines and the same pattern of suprantennal central head spines. Five sutural head spines, the central one a little bit shorter than the other. The dorsal and central anterior head spines form a continuum of seven spines of the same shape and size. Four dorsal posterior marginal very long hairs and two dorsal posterior principal long hairs around the posterior border of the head. *Antennae:* (Fig. 3) Typical of the genus. Five segments. The basal segment is the biggest, and possesses a short spine. The sclerotized margin of each segment has six short hairs. The terminal segment with sensoria at apex. *Thorax:* (Fig. 4) Trapezoidal, posteriorly much wider, as long as the head and twice as wide (length 0.51±0.07, 0.34–0.51, 10; width 0.78±0.05, 0.63–0.88, 20). Occipital apophyses are well developed and parallel; above them, a very sclerotized and notorious plate that covers the posterior margin of the head. Thoracic sternal plate covered by scales and spines; three spines under each coxa; anterior superior margin with one strong spine. Dorsally, pleural apophysis is very sclerotized, the space between them is covered by scales and spines. Very conspicuous notal pit. Mesothoracic spiracles very notorious. Four dorsal principal thoracic long hairs and three metathoracic dorsal long hairs, shorter than the principal ones. *Legs:* Fore legs characteristic of genus; small and weak; the middle and hind legs are very large and strong, very similar in shape and size. *Abdomen:* Very large, rounded, nearly as long as wide (length 1.33±0.13, 1.06–1.44, 10; width 1.43±0.12, 1.21–1.53, 10); without distinctive tergites or sternites; paratergal plates are not developed; six spiracles present. Ventral central abdominal setae, dorsal central abdominal setae, dorsal lateral abdominal setae, and ventral lateral abdominal setae are mainly modified in scales, covering entire abdomen. Dorsal marginal abdominal setae and

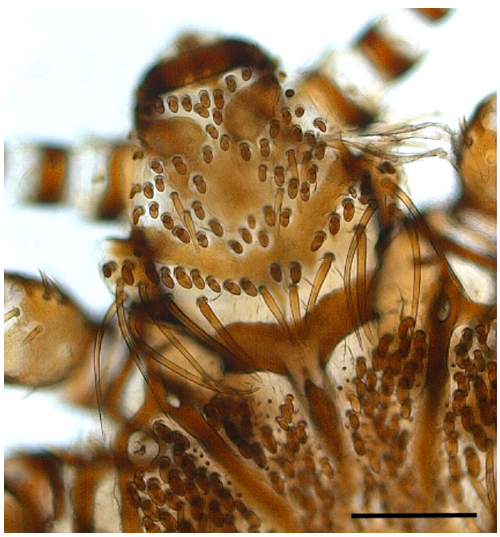

Fig. 2 Characteristic pattern of chaetotaxy on the dorsal side of head. **a** Light microscope micrograph; **b** line drawing (*scale bar*=200 μm)

Fig. 3 Detailed of the antenna of *Antarctophthirus carlinii* n. sp. by light microscope photograph (*scale bar*=100 μm)

Fig. 4 Light microscope micrograph of the thorax of *Antarctophthirus carlinii* n. sp. (*scale bar*=200 μm)

dorsal lateral abdominal setae modified in numerous spines. The fifth, sixth, and seventh abdominal segments with three long spines in addition to the short one present in all the abdominal segments. The size and density of spines decrease for the margin to the central of the abdomen. Ten to 12 apical short hairs. Scales ovoid, uniform in shape and size. Spines pointed, spiral-shaped, vary in size but not in shape. *Genitalia.* (Fig. 5) Basal plate relatively long, wider in its insertion, short parameres; elongated U-shaped pseudopenis, slightly less wide in its extreme; the arms of the pseudopenis articulate with bases of parameres.

Female (Fig. 1b) Total body length: 2.77±0.54, 1.93–3.79, 10. Head (length 0.53±0.09, 0.42–0.69, 10; width 0.50±0.09, 0.40–0.68, 10), thorax (length 0.52±0.09, 0.41–0.69, 10; width 1.22±0.19, 1.00–1.58, 10), legs and abdomen as in male, except for genitalia and associated characters; abdomen more rounded (length 1.80±0.38, 1.23–2.53, 10; width: 1.83 ±0.39, 1.37–2.55, 10). *Genitalia* (Fig. 6)*:* Without distinct genital plate, gonopods, and spermatheca; with a fringe of setae surrounding the genital opening from the tenth abdominal segment to the apex.

Discussion

According to the available information, *A. ogmorhini* had been reported as a parasite for the Weddell seal, *L. weddelli*. However, the information is to a certain extent, confusing. The original determination of this host-parasite association was not found. Therefore, we analyzed the literature concerning the presence of *A. ogmorhini* in this host.

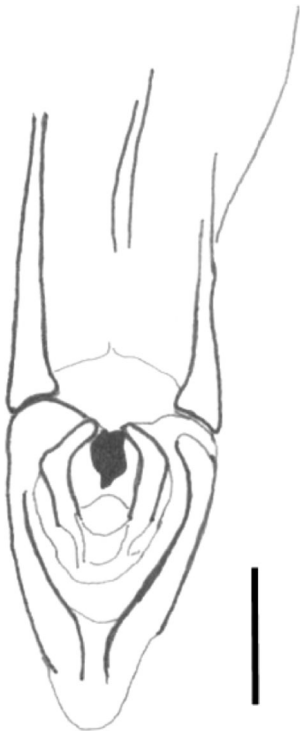

Fig. 5 Line drawing of a male genitalia of *Antarctophthirus carlinii* n. sp. (*scale bar*=100 μm)

A. ogmorhini was described by Enderlein (1906) from specimens collected in the leopard seal, *H. leptonyx*. Harrison (1937), as part of the research performed by the Australasian Antarctic Expedition from 1911 to 1914, collected lice from Weddell seals. He recognized a very distinctive pattern of chaetotaxy but the lack of adult specimens prevented to describe a new species. Some years later, Clay (1940) analyzed material from the British Graham Land Expedition of 1934–1937. She analyzed 31 females, 4 males and 1 nymph from young Weddell seals and determined that probably the specimens were conspecific of *A. ogmorhini* (Clay 1940). However, the material was in a very poor condition and she established that the determination was impossible (Clay 1940).

Ferris (1951), in his detailed monograph of sucking lice, made no mention about the occurrence of *A. ogmorhini* in the

Fig. 6 Line drawing of a female genitalia of *Antarctophthirus carlinii* n. sp. (*scale bar*=200 μm)

Weddell seal. Murray et al. (1965) conducted a series of experiments with lice from Weddell seals. They mentioned the species as *A. ogmorhini* but along the text the authors did not perform a taxonomic determination of the species. Durden and Musser (1994) in their checklist of association between sucking lice and their mammal hosts referred to Ferris (1951) and determined Weddell seal as host to *A. ogmorhini*. Finally, Mehlhorn et al. (2002) based in this publication determined *A. ogmorhini* as the species present in the Weddell seal.

According with our bibliographic research, we could determine that the association between *A. ogmorhini* and the Weddell seal (WS hereafter) should be consequence of a confusion or misinterpretation. Moreover, it seems likely that since Enderlein (1906) in the last a hundred years, nobody has had access to lice from leopard seals. During the 2013/2014 summer season, in the Antarctic Specially Protected Area (ASPA) No. 134, Danco Coast, Antarctic Peninsula, we had the opportunity to sample leopard seals (LS hereafter) and lice (*A. ogmorhini*) were collected on two specimens (Leonardi et al. in preparation). Comparing the lice obtained on LS with those from WS, we found that the morphological differences were conclusive.

More precisely, we conclude that the unique pattern of chaetotaxy in the dorsal side of the head, the shape and size of the male genitalia, and the pattern of setae surrounding the female genital opening are enough evidence to describe *A. carlinii* n. sp. as a new species. Furthermore, differences in behavior, ecology, and evolutionary history between WS and LS broadly support the fact that different lice species infected them. For instance, WS are mainly associated with coastal regions of Antarctica and usually congregate in "pupping colonies" during breeding seasons (Siniff et al. 1977). Meanwhile, LS are solitary animals that inhabit the pack ice of Antarctica although they usually migrate northwards and were registered in continental cost of America, Africa, and Australia (Rogers 2009).

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).

According to Kim and Ludwig (1978), 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). The morphological, behavioral, and physiological features of echinophthiriids are the evidence of a long evolutionary process, which begins when the ancestors of pinnipeds entered the ocean (Kim et al. 1975). The determination of this new species opens the discussion about the evolutionary history of echinophthiriids and invites to look more closely the association of *A. microchir* and the sea lions. The combination of molecular and morphological analysis of all the species of the genus will finally resolve the question about the specificity of *Antarctophthirus* lice.

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