Surface Ultrastructure of the Eggs of *Malacopsylla grossiventris* and *Phthiropsylla agenoris* (Siphonaptera: Malacopsyllidae)

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Surface Ultrastructure of the Eggs of *Malacopsylla grossiventris* and *Phthiropsylla agenoris* (Siphonaptera: Malacopsyllidae)

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ABSTRACT: The fleas of the Malacopsyllidae are known only by their adults. In this study, we describe the eggs of *Malacopsylla grossiventris* and *Phthiropsylla agenoris* and compare their surface ultrastructure using scanning electron microscopy. Eggs of both flea species are similar in their general appearance, i.e., the presence of disks and absence of lateral aeropyles, but they differ in size, texture of the surface, and number of micropyles and aeropyles. In addition, the eggs of *M. grossiventris* and *P. agenoris* are larger (719–800 μm) than most of the flea eggs known (<600 μm), and the length/width ratio is 2.0, while it varies between 1.6 and 1.8 in other species. As opposed to other large species, malacopsyllids have expandable (telescoping) abdomens that display growth. It is possible that a clutch of eggs has more than 2 eggs. Females of *M. grossiventris* were observed fixed with their mouthparts to the skin of their hosts. Indeed, some of these specimens were observed copulating on the venter of their hosts. These results contribute to the knowledge about the biology of malacopsyllids.

The morphological features of most adult male and female fleas are well documented, whereas their eggs and larval stages are much less well known. The immature stages of 2 monotypic genera of the Malacopsyllidae are no exception. These taxa, *Malacopsylla grossiventris* (Weyenbergh, 1879) and *Phthiropsylla agenoris* (Rothschild, 1904), are both endemic to Argentina and primarily parasitize armadillos (Xenarthra, Dasypodidae). They have also been reported on carnivores (*Lycalopex gymnocercus* Fischer and *Cerdocyon thous* Linnaeus) and some caviid rodents (*Microcavia australis* Geoffroy and d'Orbigny and *Galea musteloides* Meyen) (Autino and Lareschi, 1998; Lareschi et al., 2010). The eggs of *M. grossiventris* and *P. agenoris* have never been described. Their surface ultrastructure is herein described for the first time using scanning electron microscopy (SEM).

Female fleas were collected alive from individuals of the large hairy armadillo (*Chaetophractus villosus* (Desmarest)) from Puerto Lobos (Chubut Province, 42°00′02″S, 65°4′19″W), and the dwarf armadillo (*Zaedyus pichiy* (Desmarest)) captured near Colhué Huapi Lake (Chubut Province, 45°11′S, 68°04′30″W). Flea eggs were obtained from individual female fleas confined to small, clean plastic vials. After eggs were oviposited, respective female fleas were permanently mounted on microscope slides using conventional flea-mounting techniques (Linardi and Guimarães, 2000) and identified as *M. grossiventris* (9 specimens: XNT012-1/9) and *P. agenoris* (7 specimens: XNT005-1/7). Voucher female fleas were deposited in the collection of the División de Entomología, Museo de La Plata, La Plata, Argentina (XNT012-1 and XNT005-1). Five eggs of each species were dehydrated through an ethanol series, critically point dried, coated with a gold-palladium in a Jeol® vacuum metallizer, and examined using a Jeol® JSV 6063 LV scanning electron microscope (SEM) and photographed. Measurements (in μm) were taken using SEM, by means of the digital scale given automatically at different magnifications. In Table I, measurements are expressed as the mean followed by range in parentheses. The terminology used follows Chen and Wang (1993) and Linley et al. (1994).

Posterior and anterior ends of eggs were recognized by the presence of micropyles and aeropyles, respectively. *Malacopsylla grossiventris* eggs (Figs. 1–4) were ovoid, pearly white, with the posterior end more flattened than the anterior end, twice as long as wide (Fig. 1), and with a very porous surface (Fig. 2); micropyles (Fig. 3) and aeropyles (Fig. 4) were grouped at each end of the eggs, respectively; a clear zone existed around each group. No lateral aeropyle fields were observed. Eggs obtained from *P. agenoris* (Figs. 5–8) were also ovoid, pearly white, twice longer than wide, with 1 of the ends more tapered than the other (Fig. 5); the surface was reticulated, forming hexagonal cells (Fig. 6); micropyles (Fig. 7), as well as aeropyles (Fig. 8), were grouped at each end, respectively. Measurements of the main diagnostic characteristics of the eggs of the 2 species are compared in Table I.

Linley et al. (1994) studied the flea eggs of 7 different species (5 families) and reported that their structure was diverse, due to the presence/absence of: (1) reticulation on the surface; (2) aeropyles, or micropyles, or both, grouped or isolated; and (3) lateral aeropyles. Eggs of *M. grossiventris* and *P. agenoris* are similar in their general appearance, as well as in the presence of aeropyles and micropyles grouped, and absence of lateral aeropyles. However, the egg of *P. agenoris* differs from that of *M. grossiventris* due to its larger size and the texture of the surface, as well as to the smaller number of aeropyles and micropyles. Eggs of both species are larger (>703) than most of the flea eggs of most known species (<600), with the exception of *Craneopsylla minerva* Rothschild, 1903 (714). In addition, in malacopsyllid species, the length/width ratio is 2.0, while it varies between 1.6 and 1.8 in the other species (Chen and Wang, 1993; Linley et al., 1994).

Malacopsyllids are large fleas; the engorged females can reach a length of 6.5 mm with an abdominal diameter of 3 mm (Smit, 1987). The large-sized eggs observed for malacopsyllids are in accordance with other large-sized fleas, such as *Sphinctopsylla ares* (Rothschild, 1911) and species of *Hystrichopsylla* Taschenberg, 1880 (Chen and Wang, 1993; Linley et al., 1994; Krasnov, 2008). Species with very large eggs never have more than 2 eggs within the oviduct at any one time (Krasnov, 2008). In contrast to these species, malacopsyllids have expandable (telescoping) abdomens that display growth (Smit, 1987). So, it is possible that the clutch size in numbers of eggs is greater than 2. In addition, Hystrichopsyllidae and *S. ares* differ from malacopsyllids in their more chitinized and structured chorionic surface (Krasnov, 2008). Besides, although laciniae are not heavily serrated, females of *M. grossiventris* were fixed with their mouthparts to the skin of their hosts, like ticks. Indeed, some of these specimens were observed copulating on the ventral region of their hosts. These observations are in accordance with Weyenbergh (1879). The present results provide useful information regarding the heretofore poorly known biology of these 2 species.

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TABLE I. Comparison of dimension and characteristics of the eggs of *Malacopsylla grossiventris* and *Phthiropsylla agenoris*. Measurements (in micrometers) are given by the mean followed by range in parentheses.

Characteristics	*Malacopsylla grossiventris*	*Phthiropsylla agenoris*
Length	719 (703–740)	804 (773–849)
Width	364 (351–374)	393 (384–399)
Number of aeropyles	68–88	40–60
Diameter of aeropyles	1.8 (1.6–2)	1.6 (1.4–1.9)
Number of micropyles	188–212	120–130
Diameter of micropyles	1.7 (1.5–2)	2.2 (1.9–2.7)

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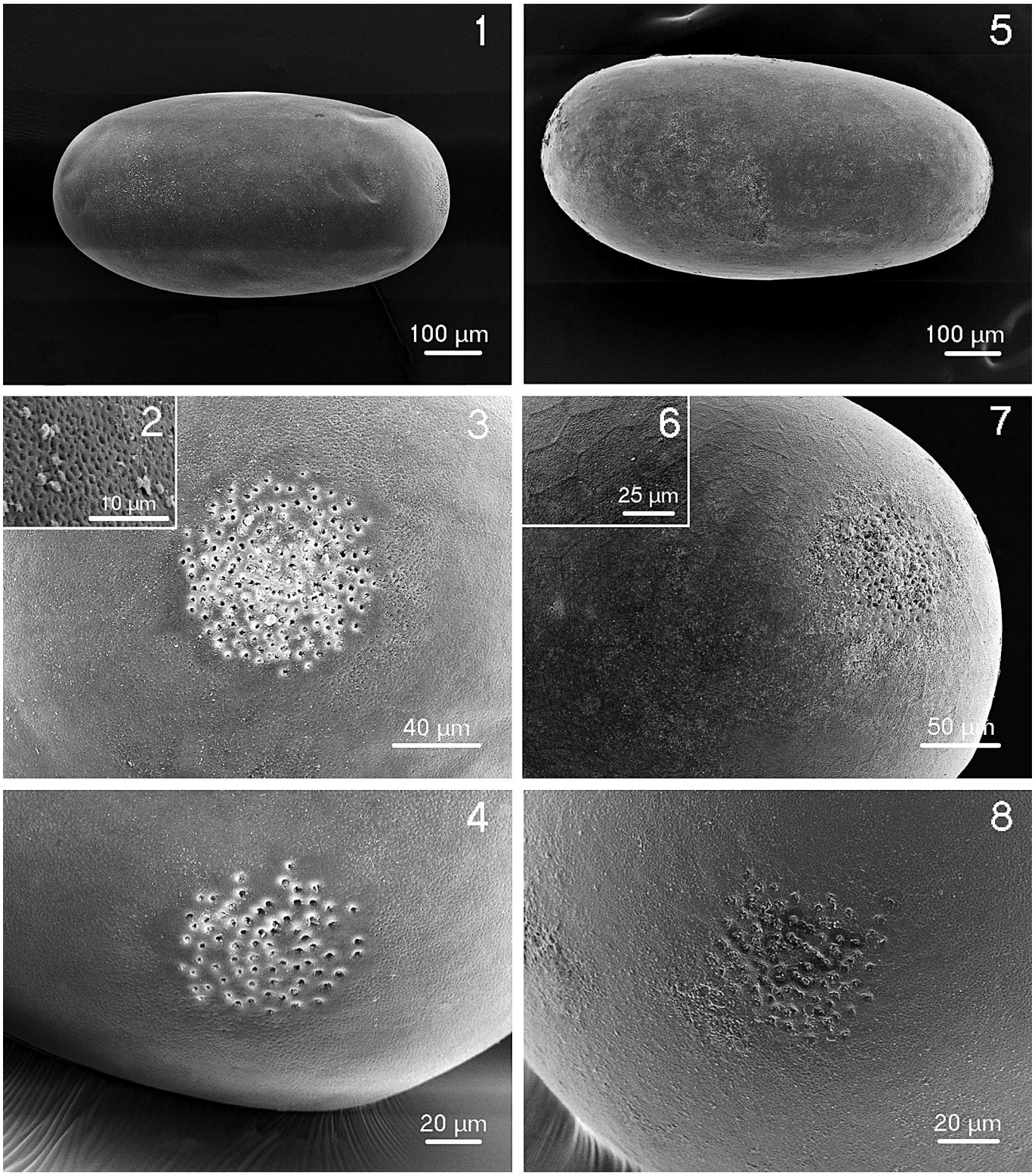

FIGURES 1–4. Egg of *Malacopsylla grossiventris.* (**1**) General. (**2**) Detail of surface showing porous chorion. (**3**) Micropyles. (**4**) Aeropyles.
FIGURES 5–8. Egg of *Phthiropsylla agenoris.* (**5**) General. (**6**) Detail of surface showing chorion with reticulation. (**7**) Micropyles. (**8**) Aeropyles.

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