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Developmental Changes in Salivary Glands of Nymphs and Adults of the Spinose Ear Tick *Otobius megnini*

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ABSTRACT: *Otobius megnini* has an autogenous 1 host life cycle, where larvae and nymphs stay attached inside the ear canal for long periods, but the adult tick is free living and can lay several egg batches without feeding. In order to obtain information about anatomical structures involved in this particular life cycle, nymphs and adults of *O. megnini* were dissected and salivary gland images were obtained in situ with the use of scanning electronic microscopy. Measurements of salivary alveoli were obtained with the use of ImageJ 1.40g software. In the nymphs, the Type I alveoli are relatively small (mean diameter: 19.6 μm) compared with those of the adults (mean: 43.4 μm) and other soft ticks in the literature. Type II alveoli in nymphs are similar (mean: 82.6 μm) to previously described alveoli in adult soft ticks. In contrast, the adults of *O. megnini* Type II alveoli are smaller (mean: 36.8 μm) and have a wrinkled surface. These findings provide more evidence that Type I alveoli take part in absorption of moisture during the free-living tick stages.

Otobius megnini (Dugés, 1883) is an argasid tick with a worldwide distribution; cattle, sheep, goats, South American camelids, and horses are the principal hosts. Human infestation is also quite frequent (Keirans and Pound, 2003). The tick has been incriminated in pathogen transmission. Although it is generally considered as being adapted to arid and semiarid environments, it has also been reported from humid areas in several countries. Larvae and nymphs stay attached inside the ear canal for extended periods of time, whereas the adult tick is free living and can lay several egg batches without feeding (Nava et al., 2008).

Salivary glands (SGs) are the major route by which pathogenic organisms and toxins access vertebrate hosts (Kaufman, 1989). In argasid ticks, the SGs are composed of both Type I and Type II alveoli (Roshdy, 1972; Coons and Roshdy, 1981). Type II alveoli are composed of granular cells that have a storage and secretory function for proteins involved in the regulation of blood feeding at the tick–host interface (Mans et al., 2004). Type I alveoli are thought to be responsible for secreting hygroscopic substances involved in the uptake of atmospheric moisture (Bowman et al., 2008). Active absorption of water vapor from the surrounding atmosphere compensates for water losses and, together with integumental waterproofing, assures maintenance of body water above a critical equilibrium humidity in nonparasitic tick stages (Knülle and Rudolph, 1982). Studies on the ultrastructure of *O. megnini* SGs (Stricker, 1993) have shown that Type I alveoli (namely, Type A acini) are like those of other ticks in adults, but are relatively small in nymphs. It was also shown that Type II alveoli (namely Type B acini) are granular and comprise the bulk of the glands in the nymphs, but are shrunken and degenerated in adults. In the particular biological cycle of *O. megnini*, different stages are exposed to different environmental conditions. Although the nymphs can spend as many as 200 days attached in a protected and relatively stable environment like the ear channel, adults can survive for several months in an external environment under more variable conditions.

To increase our understanding of the anatomical features involved in water balance processes, dissections of the SGs of 2 adults and 2 nymphs of *O. megnini* were performed. The live ticks were chilled at 4 C, fixed on a melted paraffin layer in a Petri dish, and covered with phosphate-buffered saline (PBS). The entire dorsal cuticle was removed with the aid of a stereomicroscope. To facilitate the observation of the SGs, some of the organs and tissues were also removed. The ticks were washed with PBS and fixed for 24 hr in modified Karnovsky medium (2 parts 10% formaldehyde, 1 part 25% glutaraldehyde, 2.5 parts PBS, and 4.5 parts distilled water). Karnovsky medium was subsequently removed by rinsing in PBS. At this point, ticks were removed from the paraffin layer and dehydrated in a succession of 10%, 30%, 50%, and 70% ethanol. Each solution was changed 3 times, every 25 min. Finally, they were placed in absolute ethanol for 60 min. In each step of the process, the tissue/solution ratio was maintained at 1:10. After that, the samples were dehydrated by critical point drying and coated with sublimated gold. Scanning micrographs were taken at the Electron Microscope Service of the Museo de Ciencias Naturales de La Plata, Universidad Nacional de La Plata. The major diameters of 20 alveoli of each type were measured in nymphs and adult ticks with the use of the free software ImageJ 1.40g. A *t*-test was performed to compare measurements of alveoli of different *O. megnini* stages. The sizes of other argasid SGs described in the literature were also compared.

The paired SGs comprise 2 strings spread from the internal face of the spiracular plate to the capitular foramen (Fig. 1A, B). The alveoli surround a main duct. Although the Type I alveoli are aggregated on the medial ventral face of the anterior third of the organ, the rest of the gland includes Type II alveoli and is completely covered by a sheath (Fig. 1C). This general structure of the SGs of nymphs and adults of *O. megnini* is similar to those of other argasid tick species (Roshdy, 1972; Mans and Neitz, 2004) and to the anatomical description performed by Stricker (1993). As shown in Table I, there is a significant difference in size among alveoli of the same type in nymphs and adults. SGs of adult argasids have been measured in *Argas persicus* (Roshdy, 1972) and in *Ornithodoros papillieps* (Balashov, 1972). In these studies, the diameters of Type I alveoli were 30–40 μm and 40–60 μm, respectively, whereas Type II alveoli diameters were 70–100 μm and 70–150 μm, respectively. In both ticks, the size ratios of Type II and Type I alveoli was 2.2–2.4:1. In *O. megnini* nymphs, the size of Type II alveoli is similar to those of other ticks, but Type I alveoli are smaller. The size ratio in this stage is 4.2:1 (Fig. 1C, D). Although the size of Type I alveoli of adults is similar to adults of other ticks, this is not true for Type II alveoli, which are smaller than Type I alveoli of the same stage, and the size ratio (0.84:1) is inverted. The wrinkled surface of the former alveoli type is also different from the smooth surface shown by the other alveoli (Fig. 1E, F).

Quantitative information about immature stages in soft ticks for comparative purposes is scarce in the literature, so we do not know the normal size of the SG alveoli in nymphs. Till (1961) described that in ixodid ticks, the differences in the size of the SGs among stages are due to an increase in the number of alveoli, because the size of the alveoli does not vary significantly between stages, being of the same size in unfed larvae, nymphs, and adults. In the present study, nymph Type II and adult Type I alveoli are of the same size of those of other species described in the literature (Balashov, 1972; Roshdy, 1972). The differences observed between the Type I alveoli in the different tick stages provide more evidence that these structures take part in uptake of moisture during the free-living stages, as was hypothesized by Rudolph and Knülle (1978). Because larvae of *O. megnini* molt on the host, most nymph development is in the ear canal, where temperature and humidity conditions are relatively constant. Thus, the function of these alveoli is limited, probably due to the absence of external stimuli. When the last nymphal stage emerges from the ear canal and drops to the ground to molt into the adult stage, it is exposed to external environmental conditions. At this point, passive resistance to water loss is associated with the waxy lipids of the epicuticle (Knülle and Rudolph, 1982).

Adult ticks carry out their reproductive activity under external environmental conditions, without feeding. In order to survive, they must be able to take water from the atmosphere. During this life stage, Type I alveoli are well developed, but Type II alveoli are not; they are visibly shrunken when compared to those in the nymphs and hidden by the sheath that covers them.

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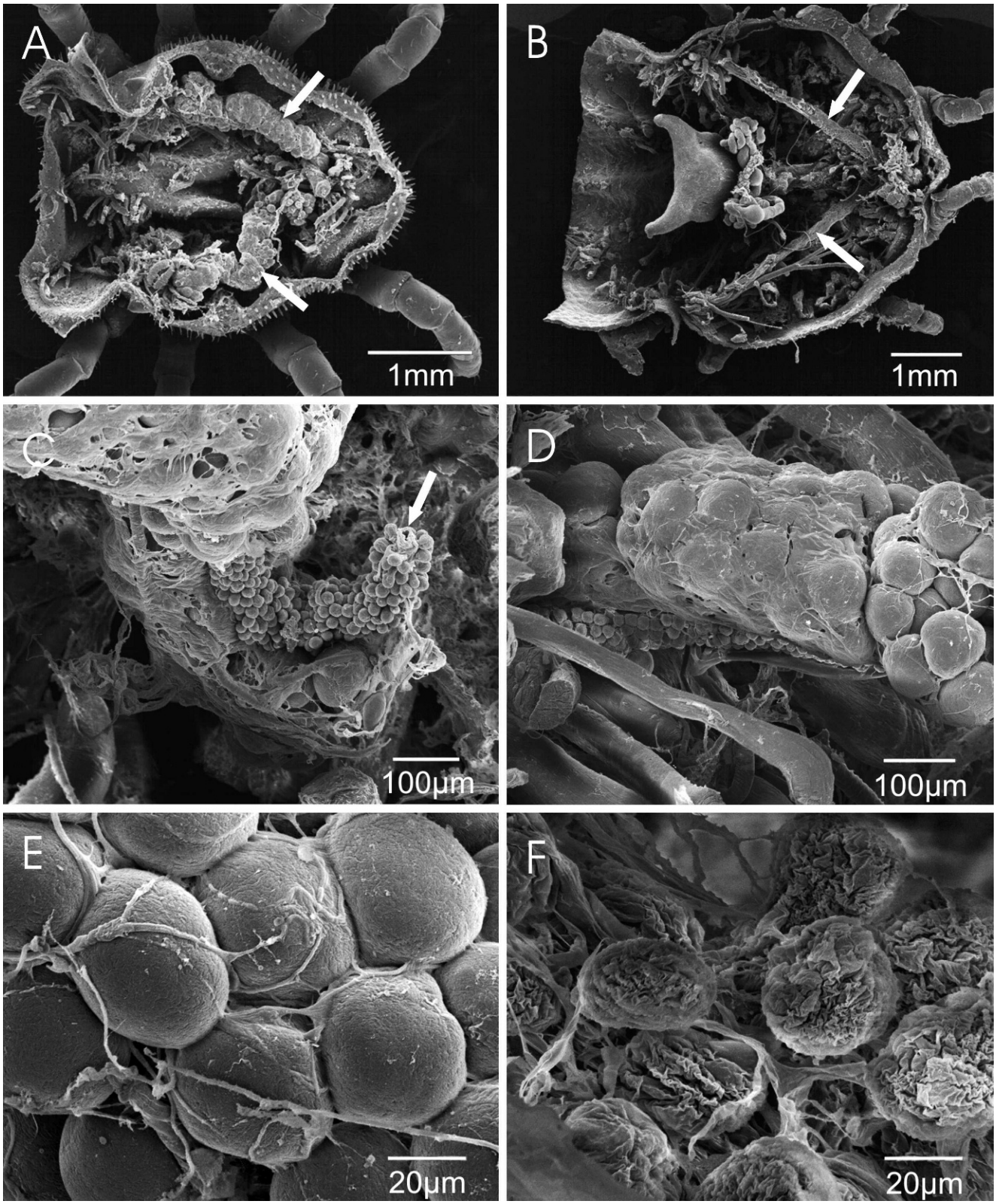

FIGURE 1. *Otobius megnini* (**A**) nymph and (**B**) adult. Arrows point to salivary glands. (**C**) Type I alveoli of nymph. Arrow points to main salivary duct. (**D**) Type II alveoli of nymph. The sheath was partially removed. (**E**) Type I alveoli of adults. (**F**) Type II alveoli of adults.

TABLE I. Means ± standard deviations (SDs) and ranges of the size (μm) measured on the major diameter of Type I and Type II alveoli of 2 nymphs and 2 adults of *Otobius megnini*. Twenty alveoli of each type were measured in each specimen. *t* indicates *t*-test for difference between means of alveoli of the same type but different stage ($P < 0.0001$).

	Mean	SD	Range	*t*
Nymph Type-I alveoli	19.61	1.92	14.7–21.7	
Adult Type-I alveoli	43.37	3.69	37.1–48.7	7.52
Nymph Type-II alveoli	82.60	5.53	72.5–91.4	
Adult Type-II alveoli	36.75	3.53	32.5–42.9	5.83

The biology of this tick remains unclear in several respects. For example, it would be good to undertake the same type of analysis, including the larvae, and to complete it with cellular biology and ultrastructure studies to assess the functional characteristics of both alveoli types. In this scenario, *O. megnini* would seem to be a suitable natural model for analyzing SG physiology, especially with respect to Type I alveoli.

The presence of a sheath surrounding the entire SG of argasids is described by Coons and Alberti (1999) and Bowman and Sauer (2004). However, we found it covering only Type II alveoli in nymphs and adults. Mans and Neitz (2004) provide scanning electron micrographs of *Ornithodoros savignyi* with an encasing sheath around the entire SG. A sheath is not always included in many descriptive publications of SG in soft ticks, or in other comprehensive works of tick anatomy. It is, therefore, unclear if the sheath is a common feature for all argasids, or if it is shared just by some genera. Visualization of this structure may be difficult with just stereomicroscopy. The methods used here could help to clarify this issue.

Finally, *O. megnini* nymphs have a unique spinose cuticle, not shared by other stages or other tick species (Sonenshine, 1991). Although not the aim of the present study, the micrographs revealed the internal face of the cuticlar spines, but no associated muscles or nerves were recognized. We suggest that a function for these spines could be to increase earwax production by irritation, and also help in keeping this wax smeared over the nymph epicuticle once it leaves the host. Further work is necessary to confirm this hypothesis.

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