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Ultrastructural aspects of *Eriogaster catax* and *Eriogaster lanestris* (Lepidoptera: Lasiocampidae)

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Abstract. *Eriogaster catax* (Linnaeus, 1758) and *E. lanestris* (Linnaeus, 1758) are two related species in the family Lasiocampidae with decreasing distribution areas and extremely isolated populations, thus being vulnerable to numerous threats, particularly human impact. Until presently, the biological and ecological studies regarding the species in question are scarce, particularly for *E. catax*, a species protected through Annexes II and IV of the Council Directive 92/43/EEC, Annex II of the Bern Convention and Law no. 49/2011 in Romania. Taking into account the importance of the *E. catax* species and the insufficient information reported on the ecological need of the species, a highly detailed analysis at an ultrastructural level could provide valuable information. The primitive aspect of the wings explained the weak distribution of adults. The different flight period of adults and egg laying season were reflected on the structure, adherence and size of eggs. Also, the structure of the fibers was correlated with the different ways in which *E. catax* and *E. lanestris* use the nest.

Keywords: *Eriogaster catax*, *E. lanestris*, oviposition strategy, ecological niche.

Introduction

Eriogaster catax (Linnaeus, 1758) and *E. lanestris* (Linnaeus, 1758), (Lepidoptera: Lasiocampidae) are two cohabitant species (Sáfián, 2006) which are mainly found in the semi-natural habitats that have a predominant shrubbery vegetation (Kadej *et al.*, 2018).

E.catax is one of the least studied species due to living in very localized areas (Drews and Wachlin, 2003) and it is also considered an endangered species in several European countries. Currently it is protected by law at European level by the *Directive 92/43/EEC*, Annexes II and IV and the *Berne Convention*, Annex II

(Höttinger, 2005), while also being categorized as data deficient (DD) in the IUCN red list of threatened species (Farkač *et al.*, 2005). The species spreads throughout entire Europe, starting from the north of Spain up to the Balkans, and to the south down to the Ural Mountains and Anatolian peninsula (Baillet, 2013; Borges, 2012; Bury, 2015; Freina, 1996; Freina and Witt, 1987; Karsholt and Razowski, 1996; Konvička *et al.*, 2005; Leraut, 2006; Ruf *et al.*, 2003).

E. lanestris has a much larger distribution and can be found in almost all Europe except for the Tundra habitats, the Mediterranean region and in Asia with reports up to north of Caucasus, Kazakhstan, Siberian southern region, Central Yakutia and the Amur basin (Ruf *et al.*, 2003; Ebert, 1994; Freina and Witt, 1987; Dubatolov and Zolotuhin, 1992; Pro Natura, 2005).

In their larval stage, both species feed primarily on the *Prunus spinosa* and *Crataegus monogyna* shrubs. The female moths of both species lay their eggs only once, on the branches of the host plant (Baillet, 2011; Caron, 2009; Höttinger, 2005; Oleksa, 2010). Due to the absence of a “mouth”, the adult moths do not feed in their life span and rely solely on their larval nutrition (Malkiewicz, 2015).

A distinguishing feature of the two species is their highly social behavior during the larval stage, categorized as gregarious (Baillet, 2013; Bury, 2015; Caron, 2009; Chrzanowski *et al.*, 2013; Freina, 1996; Ruf *et al.*, 2003).

In the literature published so far, the growth stages of *E. catax* and *E. lanestris* have been described from a morphological point of view. By using a scanning electron microscopy technique, Fitzgerald (1995) highlights a series of ultrastructural characteristics such as the texture of the silk nest woven by the caterpillars or different types of glands from the genus *Malacosoma*, which is related to the genus *Eriogaster*.

Taking into account the importance of the *E. catax* species and the scarce information reported on the ecological need of the species, a highly detailed morphological analysis at an ultrastructural level could provide valuable information. Due to the fact that *E. catax* and *E. lanestris* share the same habitat and sometimes even the same shrub during their larval development stage, the investigation of both species was considered necessary.

Materials and methods

Electron microscopy analysis

Samples (adults, eggs, larva and nest silk) were prepared for scanning electron microscopy (SEM) analysis using the turbomolecular pumped coater Quorum Q150T ES, from the Integrated Electron Microscopy Laboratory (LIME) of National Institute for Research and Development of Isotopic and Molecular Technologies (INCDTIM), Cluj-Napoca, Romania, and examined at SEM Hitachi SU8230 (LIME-INCDTIM, Cluj-Napoca, Romania).

Statistics

Statistical analyses were conducted using online *t* test calculator.

Results and discussions

Adults

Both species had palmate scales (Fig. 1 A, B, D), fixed on the surface of the wings through a peduncle (Fig. 1 C), and were randomly dispersed on the surface.

The fact that the scales were fixed through a peduncle indicates that they originate from trichomes. Some studies showed the relation between Lepidoptera scales and sensory trichomes (Galant *et al*., 1998; Zhou *et al*., 2009). The scares distribution of the scales is correlated with the underdeveloped flight ability, explaining also the low dissemination of females. Peduncle-like scales and no mouthpieces are primitive characters of *Eriogaster*.

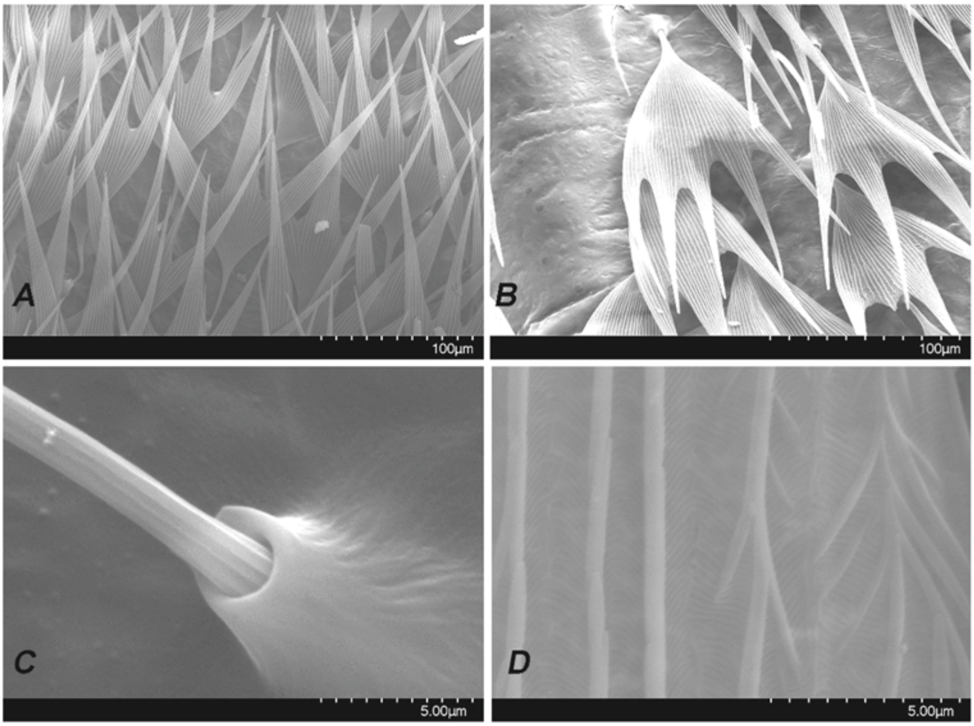

Figure 1. A. Palmate scales of *E. catax*; B. Palmate scales of *E. lanestris*; C. The insertion point of a peduncle to the scale; D. The surface of a scale in detail.

The antennae of both sexes were consisted of three segments: scape, pedicel and a long (Ma *et al.*, 2017; Mark *et al.*, 2017) bipectinate flagellum (Fig. 2 A). The bipectinate flagellum is composed of approximately same number of flagellomeres in males and females. Each flagellomere bears two lateral branches (Fig. 2 C, 4 A). The females of both species had shorter and thicker branches than the males. There are also differences in shape and thickness between the flagellomere branches of the two species i.e., those of *E. lanestris* were significantly thickened towards the base giving them a pear-like shape (Fig. 2 C, D).

Branches of flagellomeres in males of the two species increased progressively in length from the proximal end to the middle, and then decreased towards the apex. Branch length was sexually dimorphic, with males possessing significantly longer branches throughout.

The dorsal surface of the flagellum (antennal spindle) and the entire scape and pedicel were covered in overlapping lamellar scales (Fig. 2 B) (Mark *et al.*, 2017; Yuvaraj *et al.*, 2018).

Based on the external morphology of the sensilla observed under SEM, many sensilla with role in olfaction which plays a critical role for insects were identified (Yuvaraj *et al.*, 2016).

Sensilla trichodea were predominantly distributed on the ventral side of the lateral branches (Fig. 3A, B; 4A) (Fernandes *et al.*, 2017; Li *et al.*, 2018; Mark *et al.*, 2017). In males they were grouped in clusters of 5–6 sensilla (Fig. 4 B, C, D). Sensilla trichodea are the most abundant sensory receptors on the antennae of both sexes and species (Fig. 3 A, B and 4 A).

On the dorsal side of the lateral branches (Fig. 2 C, D; 4 A) we identified 8-9 (n=100) sensilla chaetica (Fig. 3 E, F and 4 E, F) (Mark *et al.*, 2017) at antennae of both species.

On the apex of of the lateral branches of both sexes we identified 1-3 sensilla basiconica (Fig. 3 C, D) (Mark *et al.*, 2017).

Eggs

For *E. catax* the eggs had an elongated, cylindrical form, nicely organized in rows and very well welded together and to the substrate. On the apex, the eggs had the micropyllar region formed like a small dent, with numerous pores (Fig. 5 A, C, E).

E. lanestris had less organized eggs, with weak connections in between and to the surface. The eggs were elongated and with a wider apex compared with the basal part. The micropyllar region is slightly curved (Fig. 5 B, D, F). Because the eggs are not so close to each other, the pores developed on the lateral of the egg, and not on top, like *E. catax* (Fig. 6 A).

Both species hatch by penetrating the micropyllar region and the missing part indicated that the caterpillars consumed this region which contributed to their nutrition (Fig. 6 B).

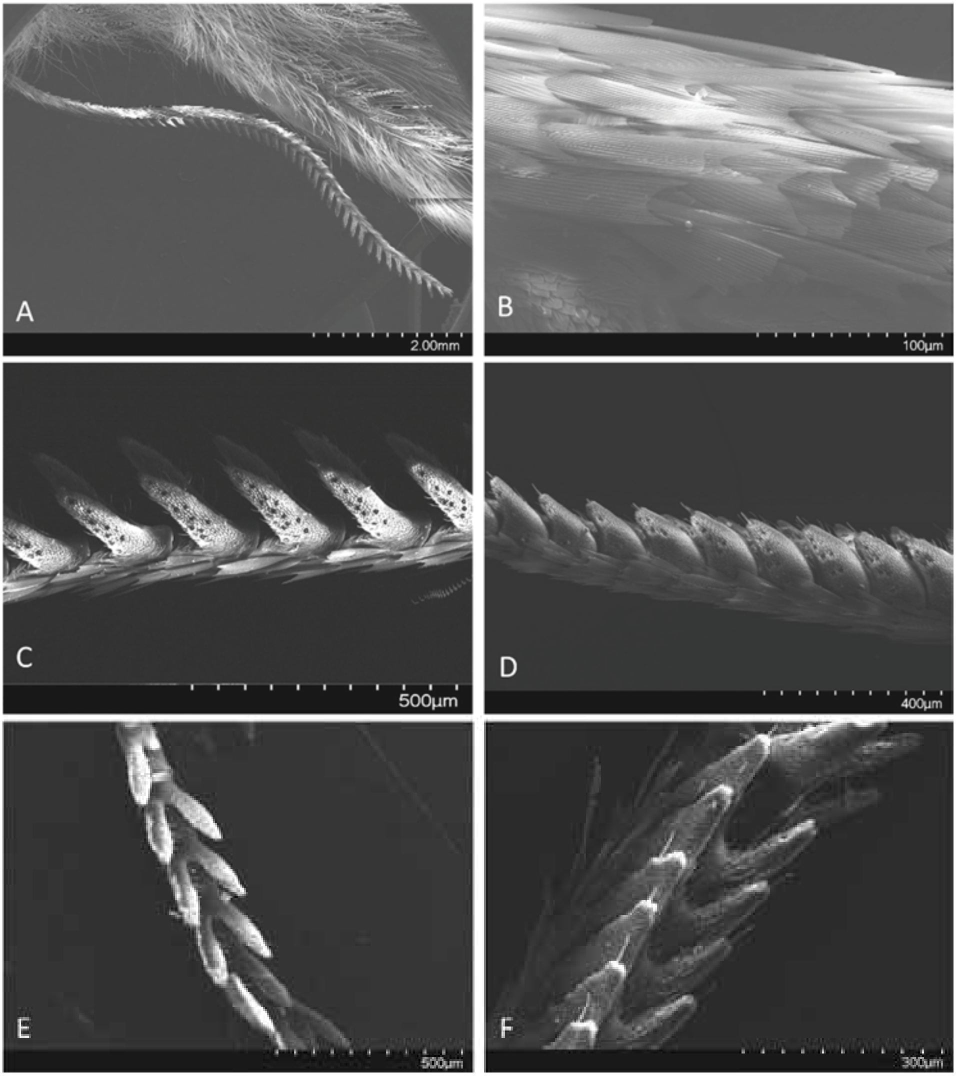

Figure 2. SEM micrographs showing the external morphology of the antennae of *E. catax* and *E. lanestris:* A. The bipectinate antennae of the *E. catax* female; B. The dorsal surface of the flagellum is covered with overlapping lamellar scales at both species; C. Female antenna of *E. catax* - lateral view. D. Female antenna of *E. lanestris* - lateral view; E. Female antenna of *E. catax* - ventral view. F. Female antenna of *E. lanestris* - ventral view;

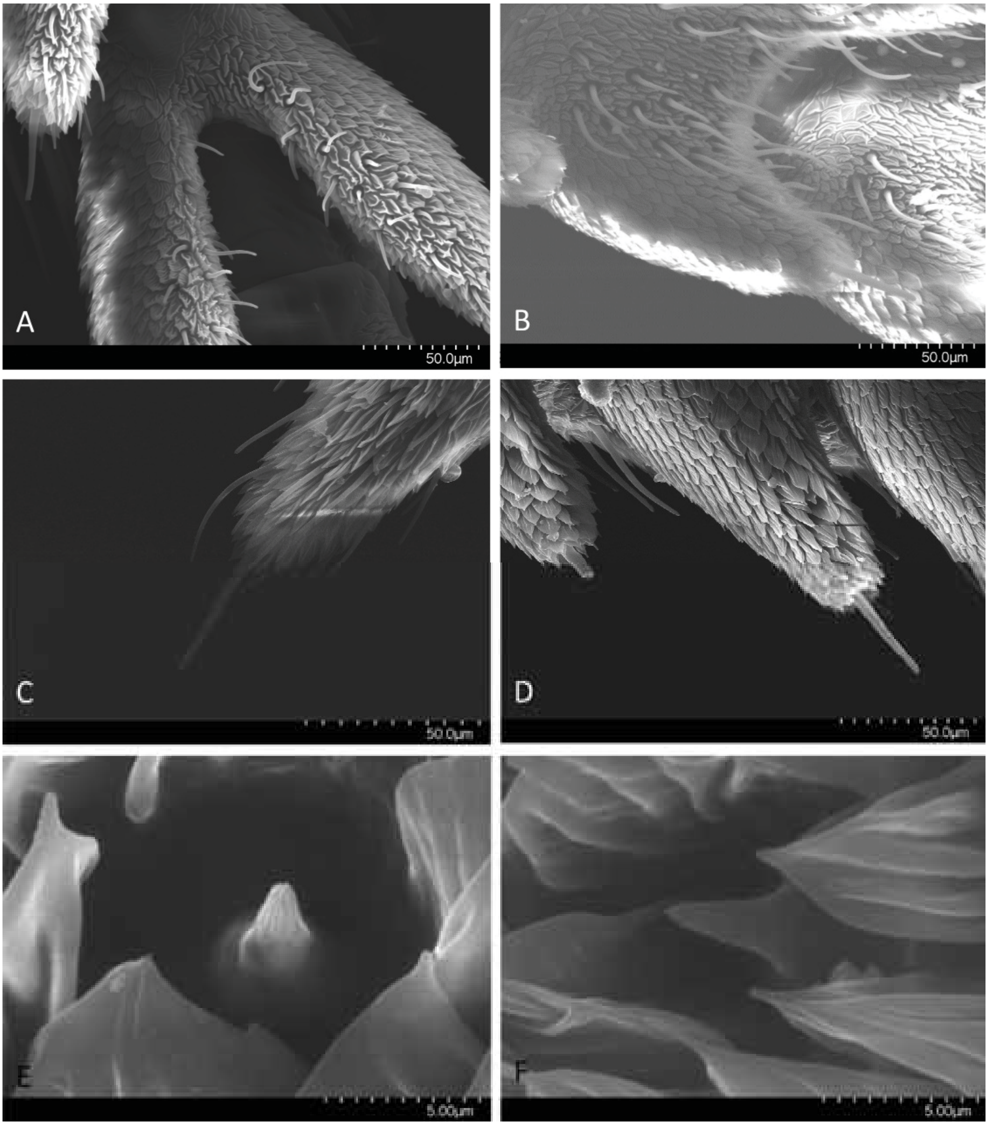

Figure 3. The external morphology of the sensilla observed using SEM on the female antennae of *E. catax* (A., C., E.) and *E. lanestris* (B., D., F.). A., B. Sensilla trichodea on the ventral side of the lateral branches; C., D. Sensilla basiconica on the apex of the lateral branches; E., F. Sensilla chaetica on the dorsal side of the lateral branches.

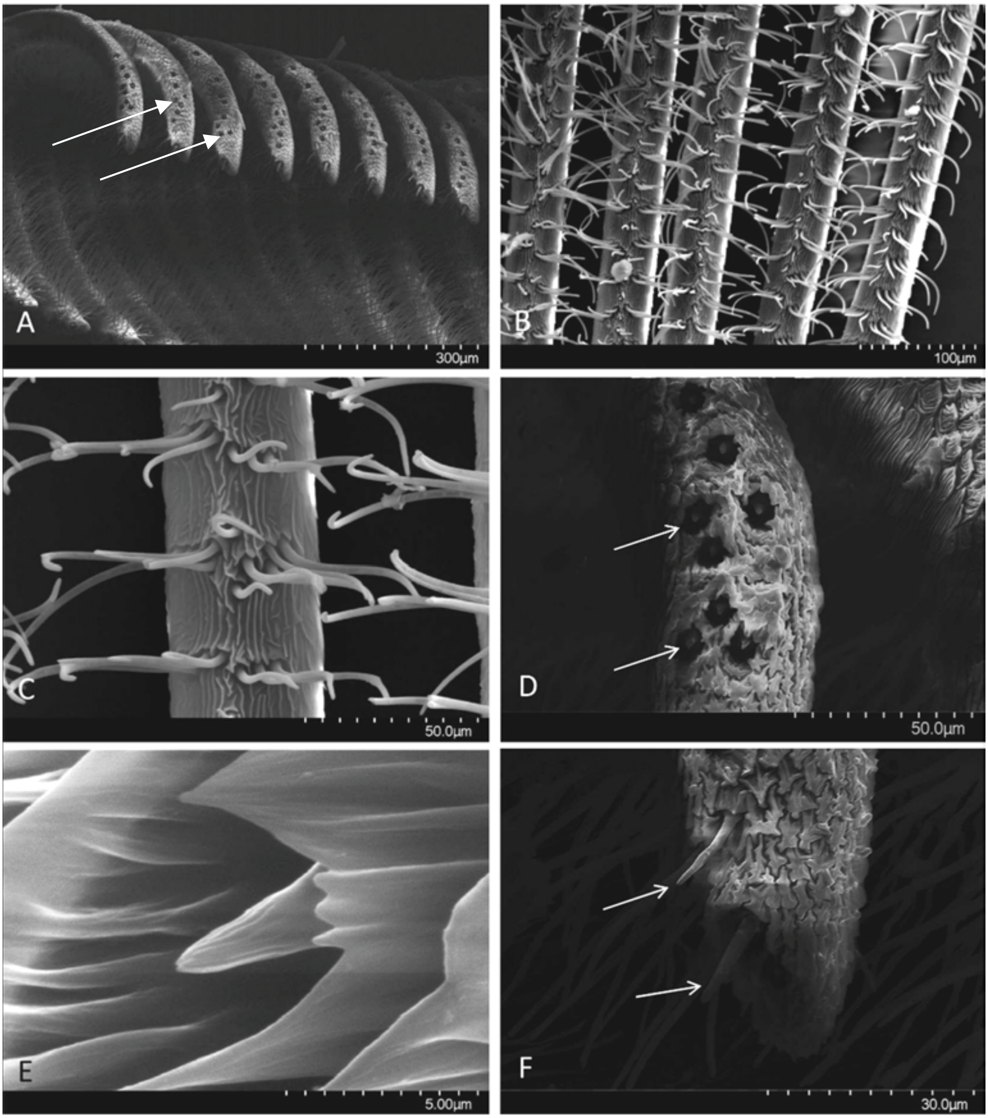

Figure 4. The external morphology of the antennae and the sensilla observed on SEM on the male's antennae of *E. catax*. A. Male antenna of *E. catax* - ventral view. Arrows indicate the location of the chaetica sensilla. B., C. Sensilla trichodea on the ventral side of the lateral branches of both species; They were grouped in clusters of 5–6 sensilla; E. Sensilla chaetica on the dorsal side of the lateral branches. F. Sensilla basiconica on the apex of of the lateral branches.

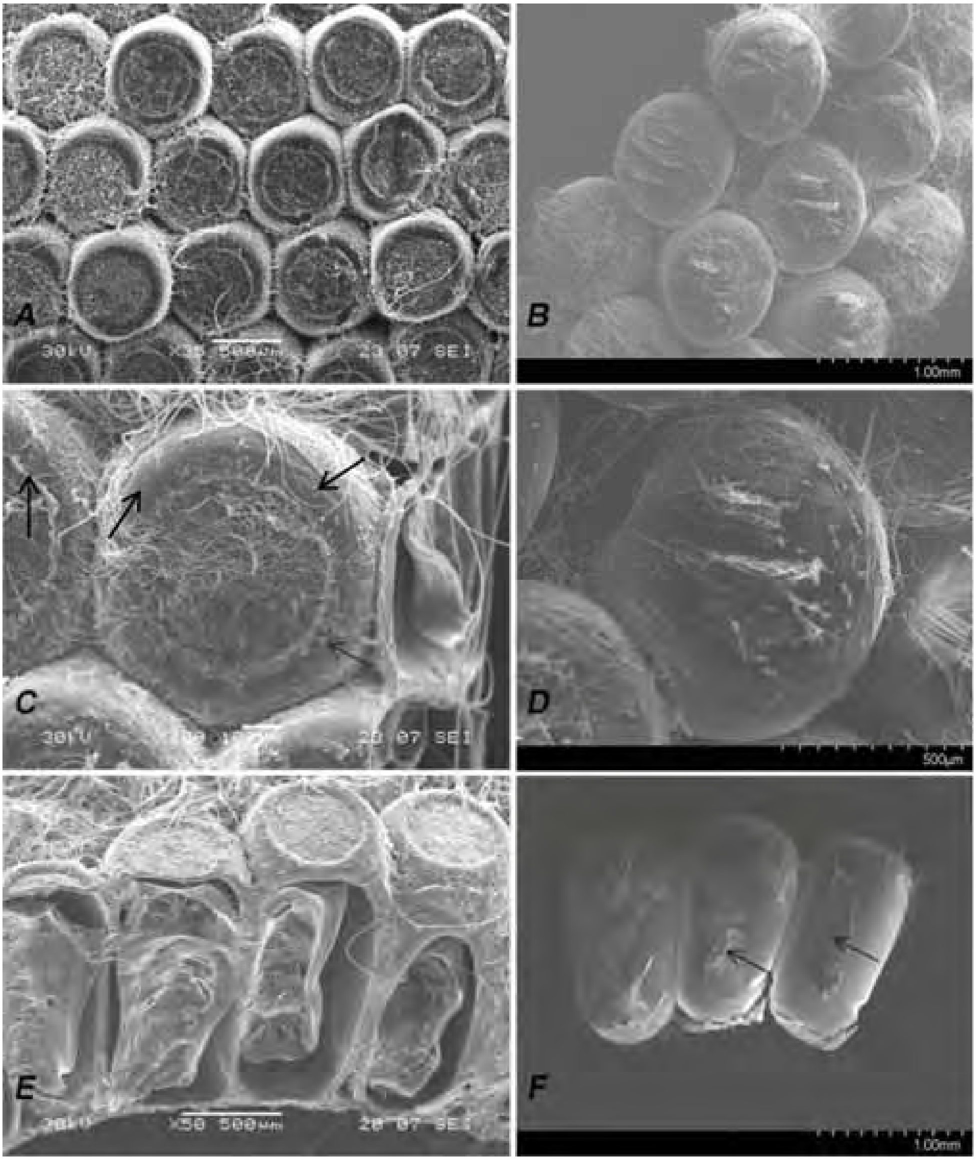

Figure 5. A. Eggs of *E. catax* arranged in ordered.rows The eggs are tightly bound together and adheres very well to the substrate; B. Eggs of *Eriogaster lanestris* - the rows are more slightly ordered and the adhesion to the substrate is weak; C. Around the micropillary region of the *E. catax* egg, numerous pores indicated by arrows can be observed; D. The micropillary region of the E. *lanestris* egg is slightly bulged like a dome. E. Lateral view of *E. catax* eggs; F. Lateral view of *E. lanestris* eggs. Arrows indicate the contact area with adjacent eggs.

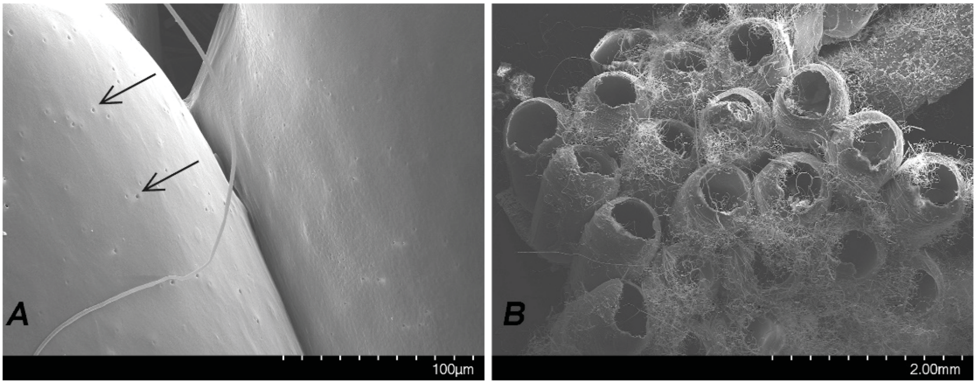

Figure 6. A. Respiratory pores on the egg surface of *E. lanestris*;
B. Hatch orifices of *E. lanestris* caterpillars.

E. lanestris has smaller eggs compared to *E. catax* and this could be explained by the different time periods when the eggs are laid. *E. catax* lays its eggs in September-October (Bury, 2015; Chrzanowski et al., 2013; Freina, 1996), and *E. lanestris*, during March-April (Ruf *et al.*, 2003).

Compact and well adhered eggs on the surface of the leaves, along with a small number of large eggs are adaptations of *E. catax* to overcome unfavorable conditions during winter period.

Larva

The polypod caterpillar of *E. catax* had five stemmata (the visual organs of the larva) on each side of the cephalic capsule. Also, numerous pores were observed on the cephalic capsule most probably act like atmospheric pressure sensors (Fig. 7 A, B, C).

Both species had protective tubular trichomes on the surface of the body and on the cephalic capsule. To evaluate the differences, trichomes' width on the cephalic capsule were measured and very significant differences were observed ($p<0.05$). *E. catax* had an average width of trichomes of 8.96 µm ± 0.2 µm (N=30, ± SEM) and for *E. lanestris* the trichomes measured 4.51 µm ± 0.25 µm (N=30, ± SEM) (Fig. 4 D, E, F).

On the sides of the body, eight pairs of spiracles were observed. Spiracles contain numerous atrial structures that close or open the airway. The atrial structures of the spiracles are finger-shaped and they have many cilia (Fig. 8. A, B).

On the ventral side the larva had three pairs of thoracic legs and four pairs of prolegs provided with numerous crochets (Fig. 9).

E. catax hatch in early spring, along with bud break of *Prunus spp.*and *Crataegus spp.* (Baillet, 2013; Caron, 2009; Höttinger, 2005).

Access to food is still limited during this period and cold weather in the morning and at night lead to high consumption of energy for thermoregulation. *E. lanestris* hatches 3 to 4 weeks later (Konvička *et al*., 2006), when both *Prunus* spp. and *Crataegus* spp. are blooming, the temperatures slightly increased and food sources are plenty.

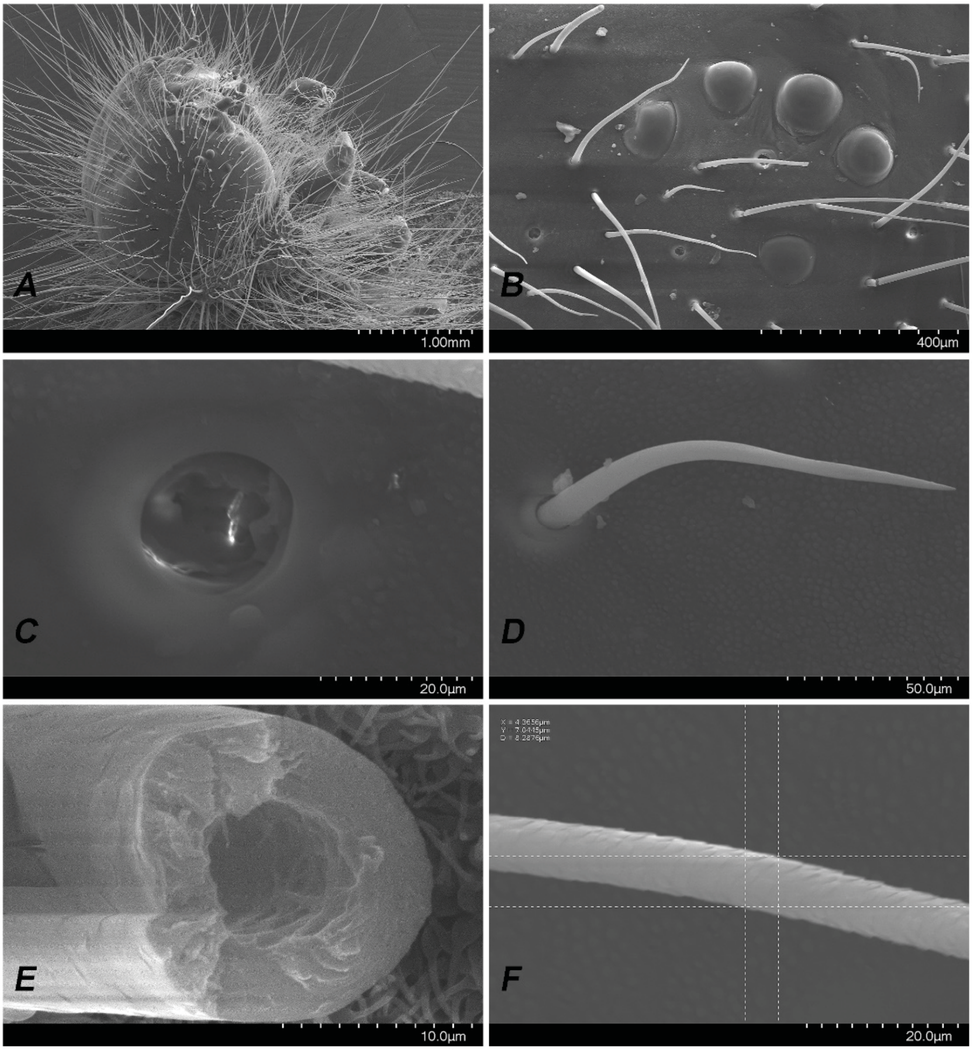

Figure 7. A. Cephalic capsule of the caterpillar; B. Five stemmata - the visual organs of the larva; C. Cephalic capsule of the caterpillar has many pores - atmospheric pressure sensors; D. Protective trichomes present on the body and on the cephalic capsule; E. Protective trichome - cross section; F. *E. catax* average width of trichomes - 8.96 µm.

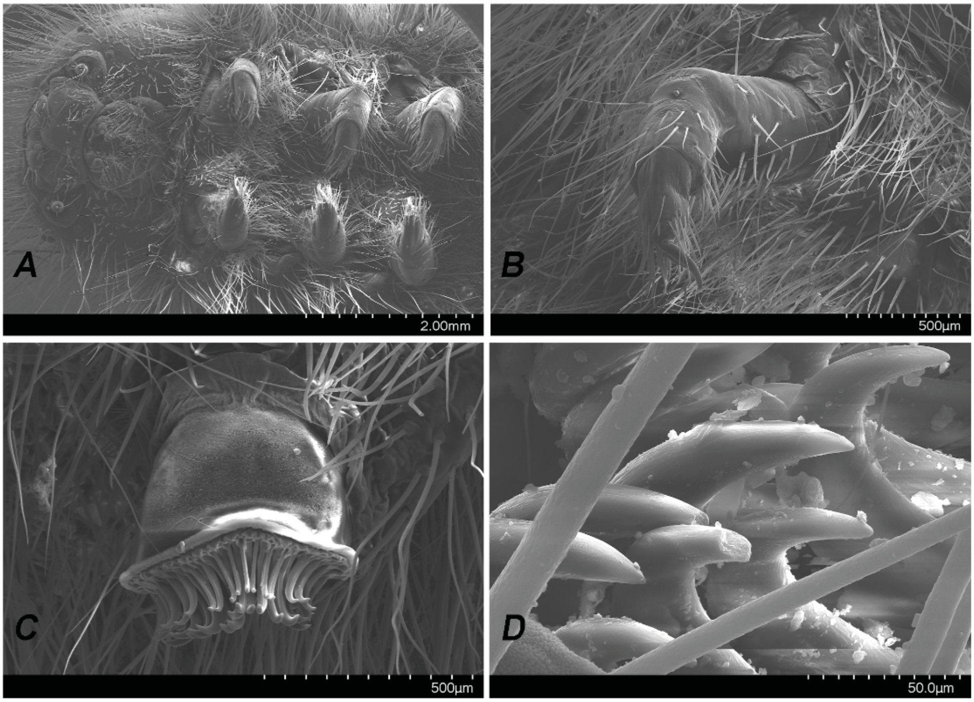

Figure 8. Two different types of caterpillars legs. A., B. Three pairs of articulated abdominal legs C., D. Proleg provided with numerous crochets.

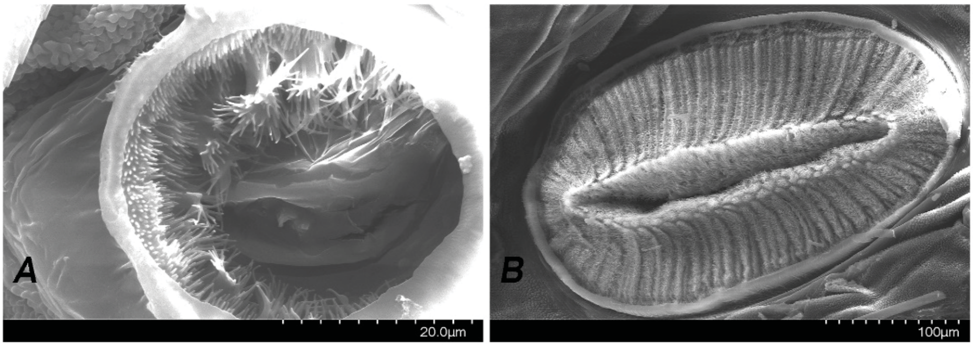

Figure 9. Spiracle with atrial structure. A. Caterpillar in first instar *L1*; B. Caterpillar in last instar *L5*.

Nest silk

E. catax had curvy-like fibers, welded together in different spots, and *E. lanestris* had straight fibers which did not form agglomerations, and with calcium oxalate crystals for increased strength. The width of the fibers was measured and highly significant differences were observed ($p<0.001$). *E. catax* had 0.89 µm ± 0.04 µm thick fibers, and *E. lanestris* had 1.78 µm ± 0.07 µm (N = 100, ±SEM), making it double in width (Fig. 10).

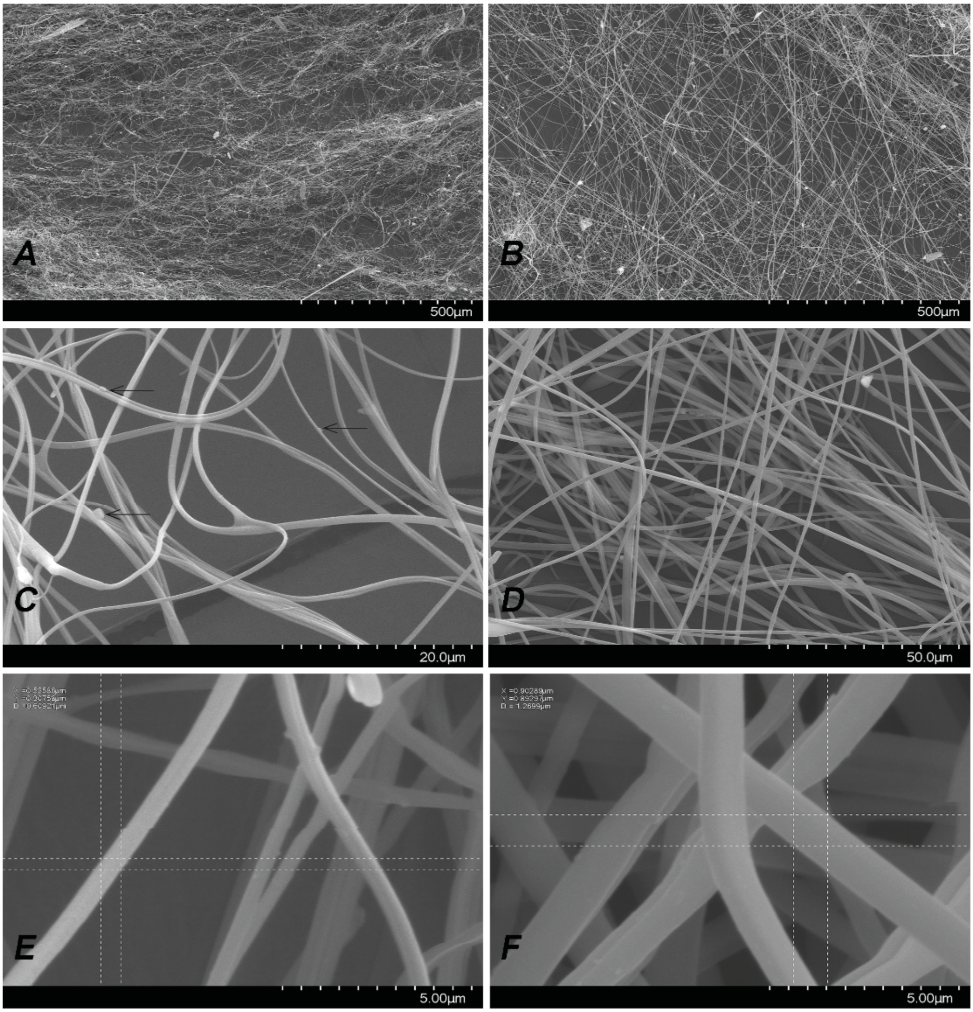

Figure 10. The ultrastructural aspects of *E. catax* (A, C, E) and *E. lanestris* (B, D, F) silk

The small width of the fibers of *E. catax* is compensated by their capacity to weld and form a felt-like structure, which confers rigidity to the nest. For *E. lanestris* the time required for the fibers to stiffen is shorter compared to *E. catax.* This explains why for *E. catax* the fibers adhere to each other.

The nest made by *E. catax* is used as a platform to rest on, gain warmth and shelter. The nest grows until the larva reach L3 stage, but they do not have enough space to stay inside it, compared to *E. lanestris*, where the larva can get inside the nest.

The nest serves as an activity center and plays an important role in the thermoregulation of the colony (Fitzgerald and Costa, 1999; Costa, 1997).

Conclusions

SEM analysis revealed precious information about the ecology and biology of the species. The primitive aspect of the wings explained the weak spatial distribution of the adults. The different flight period of adults and egg laying season were reflected in the structure, adherence and size of eggs. Also, the structure of the fibers was influenced by the ways in which *E. catax* and *E. lanestris* use the nest.

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