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Short Communication

Ultramorphological characteristics of mature larvae of *Nitidula carnaria* (Schaller 1783) (Coleoptera: Nitidulidae), a beetle species of forensic importance

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ABSTRACT

Beetles of the genus *Nitidula* Fabricius are forensically important, and their adults and larvae have been found associated with human corpses and animal carcasses in many places of the world. The external morphology of the larvae of *Nitidula carnaria* (Schaller 1783) was examined by scanning electron microscopy (SEM) to provide a description enabling identification of this forensically important species. The ultrastructure of the head was examined, antennae, mandibles, epipharynx, maxillary and labial palpi, spiracles, thorax, legs, and abdominal segments (especially segments 9 and 10); the tegument was also emphasised in this examination. Several types of sensilla were observed on the maxillary and labial palpi, including sensilla basiconica, sensilla styloconica, and perhaps a different type of sensilla digitiformia. In abdominal segment 10, a sensilla campaniformia was observed. Two types of plates were noticed in the abdominal tegument. The characteristics described here can be used to identify this species. No other study of the ultrastructure of Nitidulidae larvae is available for comparison. This is the first report of *N. carnaria* in carcasses in Chile.

1. Introduction

Forensic entomology is the study and analysis of insect evidence for legal purposes [1,2]. The primary use of entomology in a forensic context involves the examination of succession patterns and the developmental rates of successive insects and other arthropods to estimate the minimum post-mortem interval (PMI_{min}) [3].

Animal carrion can be colonised by a variable number and diversity of arthropods that belong to several taxa, which provide useful information for forensic investigations. Two orders of insects, Diptera and Coleoptera, are of primary forensic interest due to their activity and frequency on human remains [4]. When dry human skeletal remains are recovered in the later stages of decomposition, Coleoptera represent the main entomological evidence that is used for the determination of the PMI_{min} [5].

In general, beetles are associated with advanced stages of decomposition [6–8] although some are observed during active decay stages [9]. The most common families of Coleoptera that are important in forensic entomology are Silphidae, Staphylinidae, Histeridae, Trogidae, Dermestidae, Cleridae and Nitidulidae.

Nitidulidae (“sap beetles”) is a large family of more than 2000 species [10,11] that are mostly sap-feeders on trees or on the juices of fruits [10,12,13]. The defining characteristics of the family Nitidulidae and its subfamilies as well as new genera were first described by Erichson [14]. The members of this family are pests that feed on a variety of fields and stored products, including trees and small fruits, field and sweet corn, dried fruit, peanuts, honey [15,16] and grain [17]. Their feeding behaviours include mycophagy [18–21], phytophagy [22], saprophagy [23] and necrophagy. They colonise animal remains in the late stages of decomposition

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and are particularly associated with bones and dried carrion [10,24,25] along with species of Dermestidae [26,27]. Nitidulids are well-known as important members of the carrion feeding community [28–30]. Indeed, in the study of Shubeck et al. [30], nitidulids composed over 35% of all of the beetles collected from the carrion. Perris [31] first associated the larvae of Nitidulidae with carcasses when he reported his findings of the larvae of *Nitidula quadripustulata* Fabricius (now called *Nitidula carnaria* Schaller) in the carcass of a hedgehog [31]. *Carpophilus* Stephens, *Omosita* Erichson and *Nitidula* Fabricius have all been reported on carrion and thus may be useful in forensic research [10].

Nitidulidae species have been found on animal carcasses and human cadavers in North America [30,32–38], Europe [39–44], Japan [45], and China [46]. In South America, few studies have mentioned the presence of Nitidulidae on carcasses. However, investigations by Martínez et al. [47] and Wolff et al. [48] in Colombia noted that all nitidulids observed on pigs were present at an advanced stage of decay, occurring 13–51 days after death. A review of forensic cases in Argentina revealed records of *Carpophilus hemipterus* Linnaeus recovered from the medullar cavities of bones [49], nitidulids were collected from pig carcasses near Córdoba [50], and the recent finding and preliminary description of the larva and life cycle of *N. carnaria* Schaller in Bahía Blanca [51]. In addition, *Carpophilus* and three unidentified species of Nitidulidae were collected from a pig carcass during a survey of Coleoptera conducted in southern Brazil [52]. In Chile, there is only a brief mention of the finding of two adults of *Carpophilus* sp. that were collected from a rabbit carcass in Valparaiso [53].

Despite the many references that mention the presence of the family Nitidulidae associated with cadaveric decomposition, including a report of the extraction of human mitochondrial DNA from an *Omosita* sp. larvae recovered from human bone [54], few studies have been conducted to establish the value of nitidulid beetles at the species-level in forensic investigations [55]. The aims of this study were to use scanning electron microscopy (SEM) to describe the morphology of *N. carnaria* larvae collected from pig carcasses to provide information regarding the identification of this forensically important species and to report the presence of this species in carcasses in Chile.

2. Materials and methods

2.1. Experimental area

This investigation was conducted in the spring of 2012 (October). The study location was a semi-urban community of Temuco (Chile) located 3 km from the city centre at 38°42′10″ S, 72°32′59″ W, and 119 m above sea level on the slopes of Ñielol mountain. This general region contains abundant vegetation in the form of Valdivian forests, including arboreal species, such as *Nothofagus* sp., *Ulex europaeus* and *Rubus ulmifolius*. However, the experimental area contained an anthropised prairie with *Ballica* sp. and *Tripholium* sp. The climate in the region is rainy and temperate with a short dry season (cold Mediterranean climate). Every hour, we recorded the temperature of the carcasses and larval masses that developed during the advanced stages of decomposition using a HOBO® U12 4-external channel outdoor Data Logger; the environmental temperature, percentage of humidity, and pluviometry were recorded using a weather station Vantage Pro2 Plus (Davis Instruments) located 50 m from the experimental area.

2.2. Carcasses

Four 15-kg pigs (*Sus scrofa* Linnaeus) were euthanized on site by encephalo-cranial trauma using the method applied in slaughterhouses, without creating skin lesions. The Ethical Commission of the Universidad Católica de Temuco approved this procedure. The carcasses were placed in a left lateral decubitus position and exposed to direct sun in boxes consisting of metallic mesh with dimensions of 100 cm × 80 cm × 60 cm to avoid the intervention of carrion-feeding vertebrates but allowing ready access to the entomofauna. The interval between the boxes containing the carcasses was 20 m. Photographic recording of the carcasses and classification of the stage of decomposition according to the definition of Payne [28] and Anderson and Vanlaerhoven [56] were conducted daily.

2.3. Capture and rearing of adults to obtain N. carnaria larvae

Samples were collected from the carcasses each day throughout the entire decomposition process. Crawling insects (adults and larvae) were captured manually with forceps. At day 22 of the postmortem interval (PMI), the carcasses were in advanced decay; from that day forward, we found *N. carnaria* adults, which were collected and maintained in the laboratory as recommended by Byrd and Castner [55]. The insects were reared in 20-cm × 15-cm × 10-cm plastic containers at 60% mean humidity, 15 °C and a 12:12 light/dark cycle. The food substrate was pork meat with semi-dry subcutaneous fat. Conditions were maintained in a refrigerated Foc225E VELP Thermostat. Breeding resulted in larvae that were processed for SEM as described below.

2.4. Collection of N. carnaria larvae from the carcasses

At day 32 of the PMI, the first larvae of *N. carnaria* were seen. In the following weeks, larvae of different sizes were collected from the carcasses, particularly from areas of the carcasses that had abundant fat and moist skin (Fig. 1A). The larvae were fixed according to the protocol described below. The larvae obtained in the laboratory were compared morphologically with those collected from the carcasses to confirm that both belonged to the same species using a Zeiss Discovery.V12 stereoscopic microscope and later confirmed by SEM.

2.5. Fixation and observation by SEM

The larvae reared in the laboratory (n = 15) and those collected from the carcasses (n = 20) were fixed in 4% paraformaldehyde and 2% glutaraldehyde in phosphate buffer pH 7.4 for 24 h. The larvae were then post-fixed in 1% OsO_4 in a 0.1 M phosphate buffer for 2 h at 4 °C, were subsequently washed three times for 5 min each with a 0.1 M phosphate buffer and were dehydrated using a series of increasing concentrations of ethanol (15–100%). After dehydration with acetone and critical-point drying, the larvae were sputter-coated with gold and visualised using a Leo 420 (Zeiss) scanning electron microscope. The terminology used to describe the larval structures was taken from Carlton and Leschen [57]. Fig. 1B and C shows larvae in different positions before they were prepared for observation using SEM. Additionally we dissected the head to expose the epipharynx and mandibles, which were processed for SEM (n = 4) or mounted on slides (n = 3) for observation with a light microscope Zeiss AxioScope.A1 using differential interference contrast (DIC).

2.6. Abbreviations used in figures

1–9, abdominal segments 1–9; *al*, locomotory appendages/legs; *ant*, antenna; *as*, asperities; *bs*, sensilla basiconica; *cly*, clypeus; *co*, coxa; *fe*, femur; *h*, head; *la*, labrum; *lp*, labial palp, *m*, mesothorax; *md*, mandible; *mn*, mentum; *mo*, mola; *mt*, metathorax; *mx*, maxilla; *mxp*, maxillary palp; *p*, prothorax; *pc*, prostheca; *pm*,

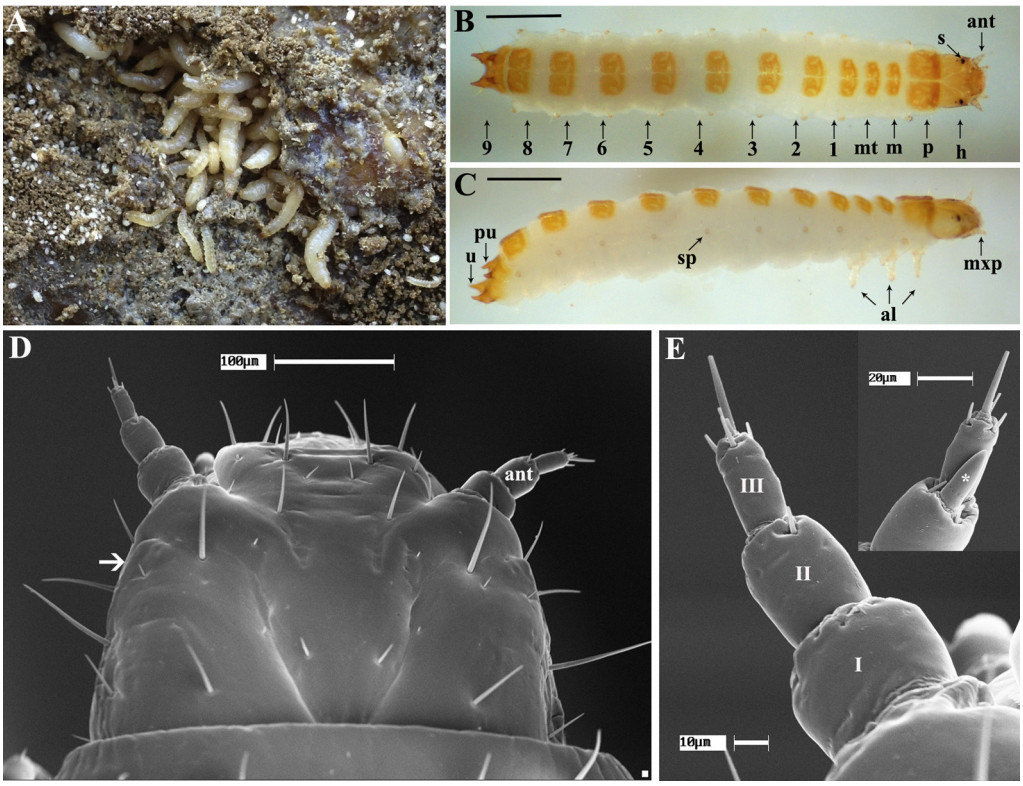

Fig. 1. Site of collection, light microscopic view and SEM of *N. carnaria* larvae. (A) Group of larvae in a pig carcass at the dry stage; (B) Light microscopic photograph of mature larvae, dorsal view. Scale = 500 µm; (C) Light microscopic photograph of mature larvae, lateral view. Scale = 500 µm; (D) SEM of the head-capsule, dorsal view, showing the disposition of the setae and antennae and the position of the ocelli (arrow); (E) Left antenna. Insert: Detail of the second and third segment of antenna, ventral view. Sensorial appendage (white asterisk).

prementum; *pu*, pregomphus; *s*, *stemmata;* *sp*, spiracle; *spa*, abdominal spiracle; *spm*, mesothoracic spiracle; *st1*, sensilla type 1; *st2*, sensilla type 2; *sty*, sensilla styloconica; *ta*, tarsungulus; *ti*, tibia; *tr*, trochanter; *u*, urogomphus.

3. Results

The process of decomposition lasted 40 days from the fresh stage to the dry remains stage. The mean environmental temperature was 15.1 °C (range 5.1–30.8 °C), and the mean carcass/larval mass temperature throughout decomposition was 22.2 °C (range 8.2–48.8 °C). During the experiment 61.4 mm of rainfall was measured, and the mean environmental humidity was 74.5%.

The larvae reared in the laboratory and those collected from the carcasses both exhibited the morphological characteristics described below. The larvae reared in the laboratory at 15 °C that were not processed for SEM their life cycle. The larval period lasted 55 ± 3 days, and the pupal period lasted 12 ± 2 days.

3.1. General description

Mature larvae analysed: 3.5–4.1 mm long and 0.5 mm wide across the abdominal fourth segment. The body is moderately elongate and subcylindrical with all segments nearly equal in diameter, except the head, prothorax and abdominal segments 9 and 10 (Fig. 1B and C).

3.2. Head-capsule (Fig. 1D)

The measurements made by SEM revealed that the head-capsule is 0.25 mm long and 0.4 mm wide. Observed from the dorsal view, the slightly depressed, prognathous head-capsule is somewhat smaller than the prothorax and is covered with numerous setae. Four black stemmata on each side of head were observed (Fig. 1B–D). The setae of the dorsal surface of the head are shown in Fig. 1D. The antenna consists of three antennomeres. The antennal base is membranous and broad. The antennomeres decrease in thickness apically. Antennomeres I and II are subequal in length. Antennomere I is glabrous; antennomere II has three short setae of approximately 7 µm in length and one sensorial appendage that is 23 µm in length. Two setae are lateral to this sensorial appendage, and another one is anterodorsal (Fig. 1E). Antennomere III is the largest antennomere, bearing two apical setae, one of which is approximately 23 µm in length and the other approximately 5 µm in length. Antennomere III also has three subapical setae of approximately 10 µm in length, two of which are lateral and the other dorsal (Fig. 1E, insert).

The clypeus has a pair of longer paramedian setae near the anterior margin, a pair of shorter setae near the lateral margin and a pair of short setae in the middle of the clypeus (Fig. 2A). Frontoclypeal protuberances are absent. The labrum is transverse and semi-circular, with a straight anterior margin. Anterior and posterior labral margins each bear a pair of shorter paramedian setae and a pair of longer lateral setae (Fig. 2A). The distance between the posterior pairs of setae is greater than the distance between the anterior setae (Fig. 2A). The dorsal surface of the labrum has a row of 10 decumbent setae of variable sizes, including one pair of short setae, one pair of longer setae and six setae of intermediate length. The epipharynx (Fig. 3A and B) bears a mass of fine asperities, such as thin spines, along diagonal ridges; the ridges convergent on a central ridge to form an inverted Y (Fig. 3A and B). The lateral surface of the epipharynx has thin and abundant setae (Figs. 2B and 3A). The maxilla (Figs. 2C and 4C) has a rounded mala distally bearing a dense brush of thick, variably sized setae. The three-segmented maxillary palpi include a wide basal

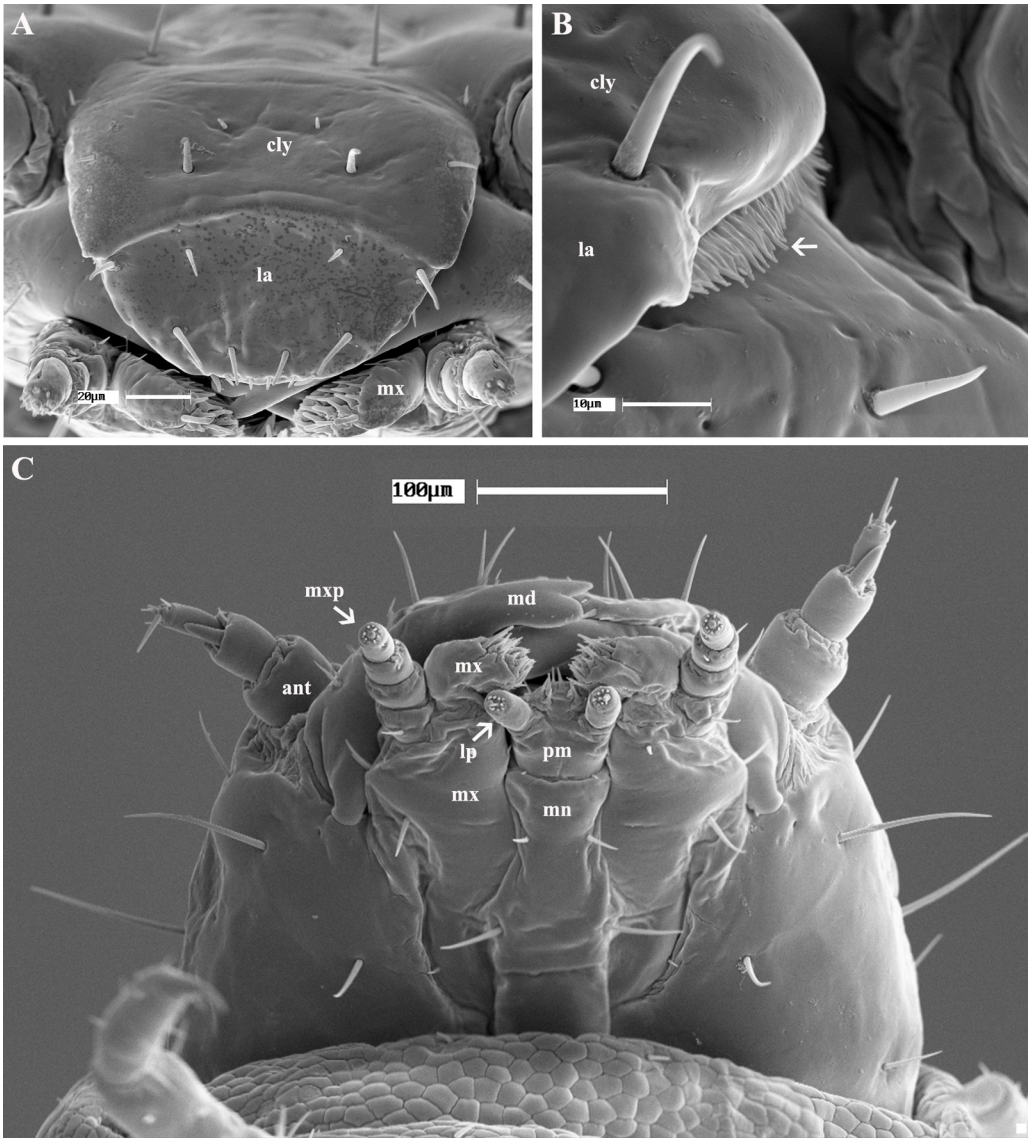

Fig. 2. SEM of the head-capsule of *N. carnaria* larvae. (A) Clypeus and labrum, dorsal view; (B) Clypeus, lateral view, showing setae of the epipharynx (white arrow); (C) Head-capsule, ventral view.

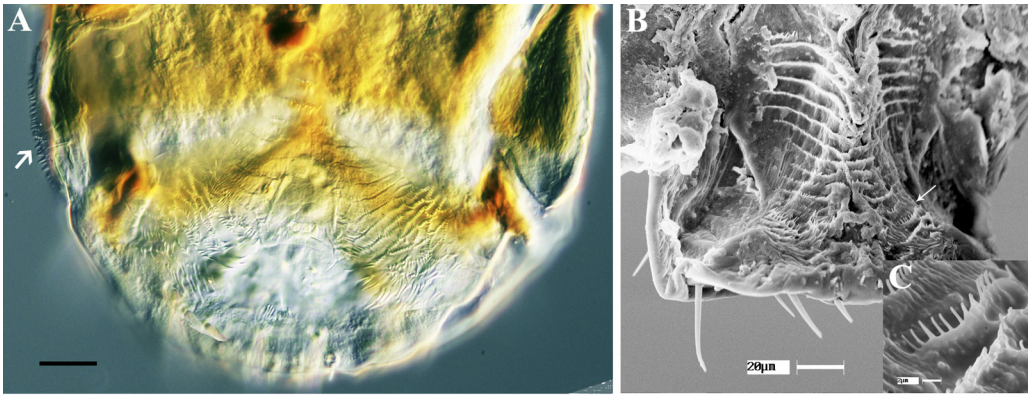

Fig. 3. Light microscopy and SEM of the epipharynx of *N. carnaria* larvae. (A) Light microscopic photograph with DIC of the epipharynx of *N. carnaria* larvae, ventral view. The white arrow shows the lateral setae of the epipharynx. Scale = 20 µm; (B) SEM of the epipharynx, ventral view. The white arrow shows asperities; (C) SEM of the asperities of the epipharynx.

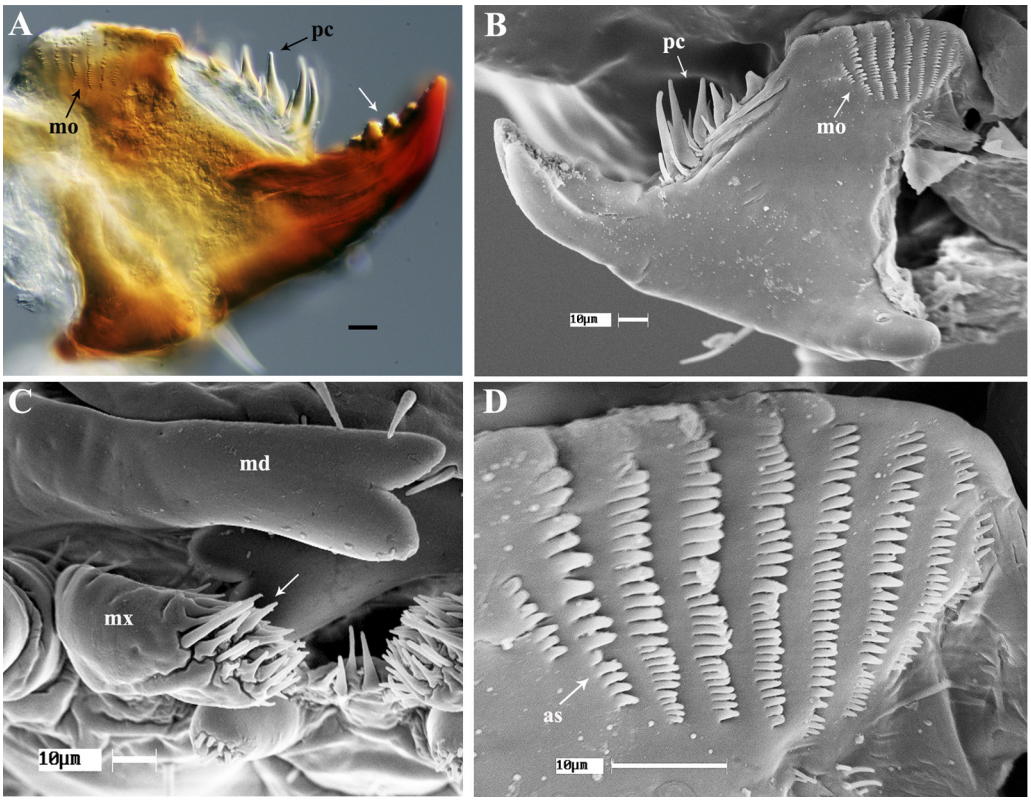

Fig. 4. Light microscopy and SEM of the mandibles of *N. carnaria* larvae. (A) Light microscopic photograph with DIC of the left mandible, ventral view. The white arrow shows the teeth. Scale = 10 µm. (B) SEM of the right mandible, ventral view. (C) SEM of the mandible and maxilla, frontal view. The white arrow shows the brush of setae of the maxilla. (D) SEM of the mola of the right mandible showing the rows of asperities.

palpomere and a thinner terminal palpomere, which is as long as the first and second segments combined (Fig. 2C). The second palpomere bears two large lateral setae, one dorsal and one ventral. The terminal palpomere bears one apical sensilla styloconica and a cluster of ten sensilla basiconica (Fig. 5B). The dorsal surface of the terminal palpomere has a channel or sulcus that extends nearly to the apex (Fig. 5B). The mandibles are symmetrical with bifid apices (Figs. 2C and 4C); the left mandible has five teeth, the largest tooth is located terminally (Fig. 4A); the right mandible has three teeth, the largest tooth is located terminally (Fig. 4B); the prostheca is composed of more than 10 spiny processes of different sizes (Fig. 4A and B); and the mola is composed of approximately nine rows of asperities, the first being the shortest (1/3 of the length of the others rows) (Fig. 4B–D). The labium has a sub-triangular mentum; a rounded prementum bearing large setae along the anterior margin; a small, fully developed palpifer; and a pair of medial labial palpi (Fig. 2C). Each two-segmented labial palp includes four sensilla types on its apical surface: eight sensilla basiconica, one sensilla styloconica, two sensilla (type 1) (one along the medial margin and one along the lateral margin), and one sensillum (type 2) along the medial margin (Fig. 5A).

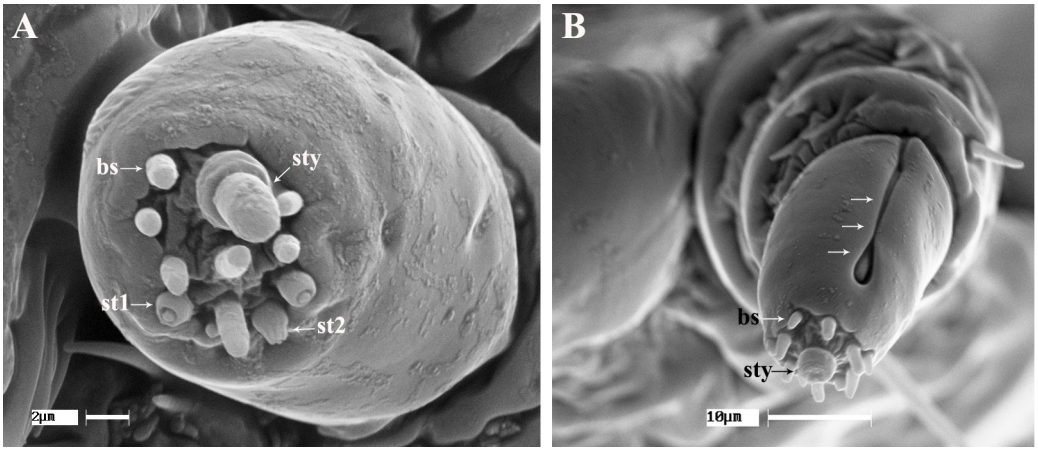

Fig. 5. SEM of the palpi of *N. carnaria* larvae. (A) Labial palp apex, showing the different types of sensilla; (B) Third segment of the maxillary palp, showing the channel crossing the dorsal surface (white arrows) and the sensilla.

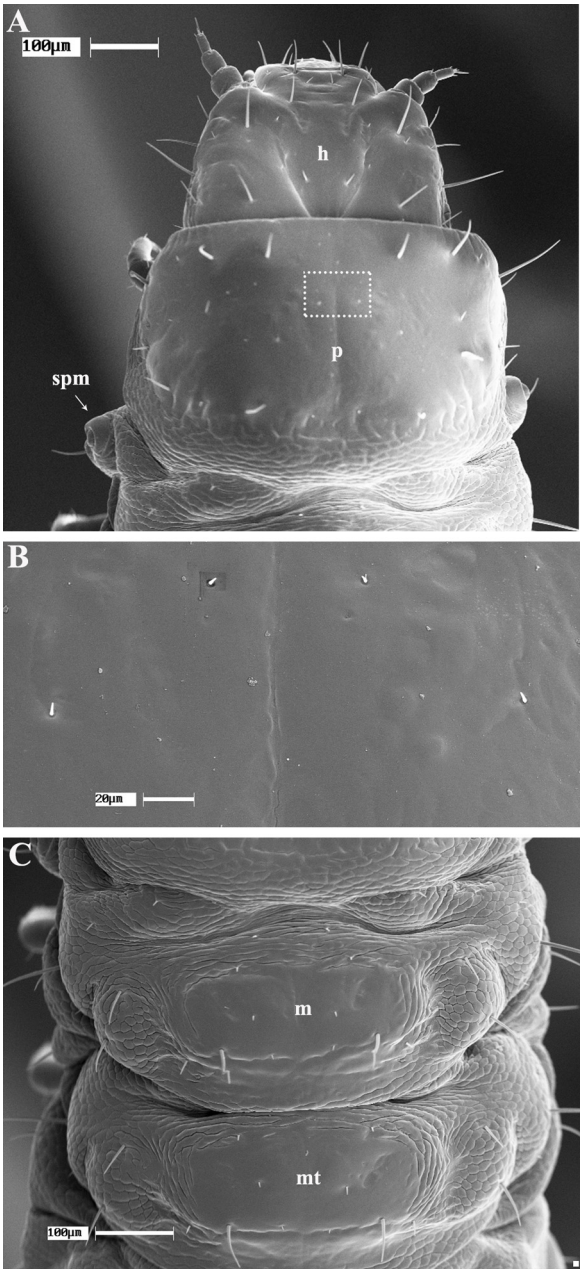

Fig. 6. SEM of thoracic segments of *N. carnaria* larvae. (A) Head-capsule and prothorax, dorsal view. White square magnified in B; (B) Prothoracic surface showing short setae on the pronotum; (C) Meso- and metathorax, dorsal view.

3.3. Thorax

0.4 mm long and 0.55 mm wide. The prothorax is longer than the meso- or metathorax but is not as wide. The pronotum occupies most of the dorsum (Fig. 6A) and is clothed with thick, large, short setae (Fig. 6A and B). The meso- and metanota are subequal in size and structure, are less sclerotised than the pronotum but bear setae similar to those of the prothorax (Fig. 6C). The legs are well developed, with five well-differentiated segments, each terminating in a claw-like tarsungulus that bears two broad setae (Fig. 7, insert). The thoracic and abdominal spiracles are subequal in size and elevated above the body surface, occurring on the mesothorax and on abdominal segments 1–8. All of the spiracles have one seta near the spiracular vent (Fig. 8A and B).

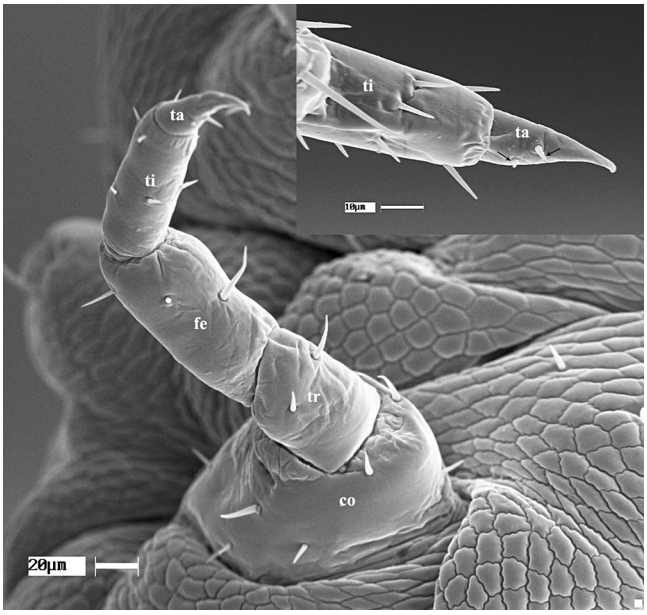

Fig. 7. SEM of left metathoracic leg of *N. carnaria* larva, anteroventral view. Insert: Detail of the tibia and tarsungulus, and tarsungular setae (black arrows), ventral view.

3.4. Abdomen

The abdomen is 10-segmented. Segment 9 bears both pregomphi and urogomphi (Fig. 9A). The pregomphi curve dorsally, and each bears a terminal macroseta. The urogomphi, which are larger than the pregomphi, curve dorsally and decrease in size apically and bear four ventrolateral macrosetae originating at the base of a stout tubercle and two apical short setae (Fig. 9A). Segment 10 has one pair of medial setae, eight large setae surrounding the segment and two short basal lateral setae (Fig. 9B). Two sensilla campaniformia are present at the anterior margin of segment 10 (Fig. 9B, insert).

Abdominal venter is comprised of two different types of cuticular plates: smooth plates (Fig. 9C) lining the ventral thinning (do not appear to contact the ground) (Fig. 1C) and scale-like plates with four to nine flat spine-like projections posteriorly (Fig. 9D) (appear to contact the ground).

4. Discussion

The present study is the first to characterise the ultrastructure of *N. carnaria* larvae using SEM, and this is the first report of this species in carcasses in Chile.

The study was performed in spring in southern Chile, and we observed the presence of *N. carnaria* in areas of the carcasses that had abundant fat and moist skin. In a separate experiment performed with pig carcasses during the summer at the same experimental site, we did not find *N. carnaria* in any of the carcasses at any stage of decomposition [58]. The main differences between these studies were the weather conditions and, therefore, the duration of the decomposition process. In the present study, the environment was moist and the process of decomposition lasted for 40 days, whereas in the summer study, the environment was dry (mean humidity = 58%; rain = 3.7 mm) and decomposition was more rapid (only 11 days) [58]. Byrd and Castner [55] observed that these beetles prefer a moist environment, conditions that only occur in spring in this region of Chile. In contrast to our findings, a species of the family Nitidulidae was found in both summer and spring in a study performed in Córdoba (Argentina) [50]. In that

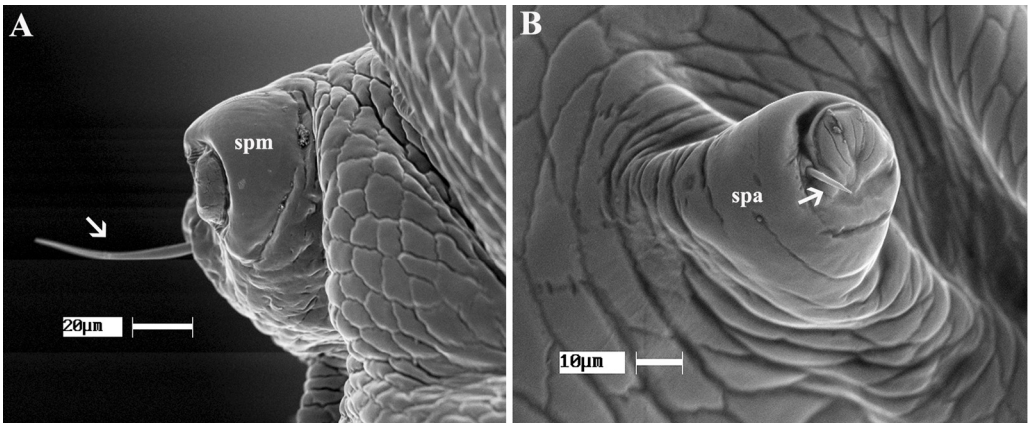

Fig. 8. SEM of the spiracles of *N. carnaria* larvae. (A) Left mesothoracic spiracle showing setae (white arrow), dorsal view; (B) Left spiracle of abdominal segment 5 showing setae (white arrow), anteroventral view.

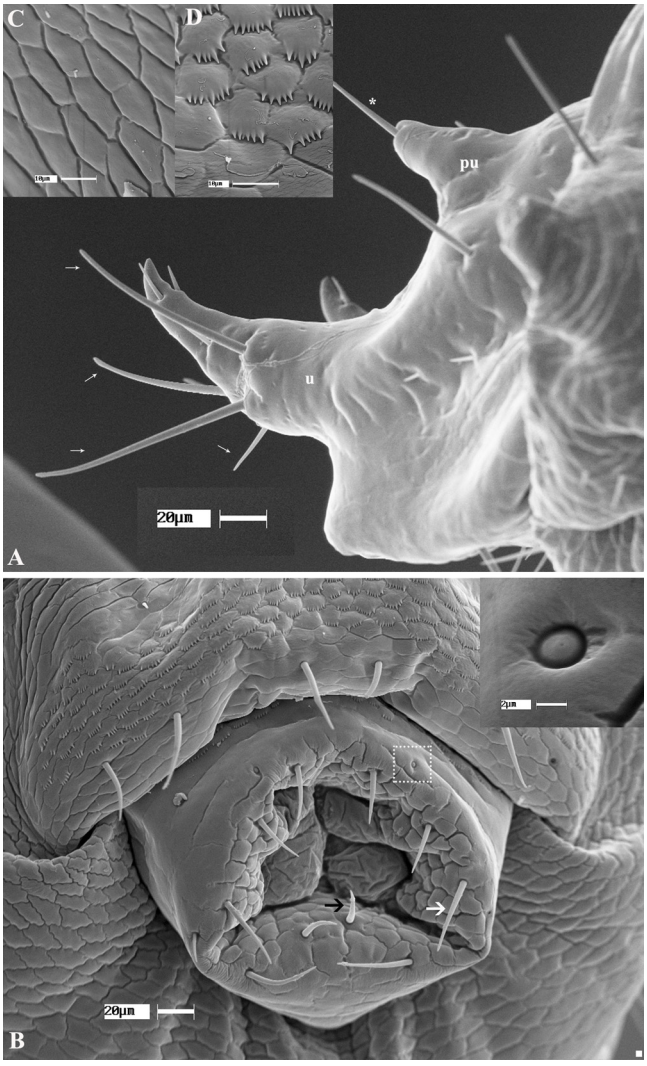

Fig. 9. SEM of abdominal segments 9 and 10 of *N. carnaria* larvae. (A) Abdominal segment 9 showing the pregomphus and urogomphus, lateral view. Terminal macrosetae of the pregomphus (white asterisk). Ventrolateral and ventromedial macrosetae of the urogomphus (white arrows); (B) Abdominal segment 10 showing lateral setae (white arrow) and internal setae (black arrow), ventral view. White square magnified in upper right insert. Insert: Detail of sensilla campaniformia; (C) Integument type I (smooth) of the ventral surface of abdominal segments; (D) Integument type II (scale-like) on the ventral surface of the abdominal segments.

region of Argentina, both seasons are warm and wet, unlike Temuco (Chile), where such climatic conditions are only characteristic of the spring. The strong seasonality exhibited by *N. carnaria* in this region of Chile suggests this species as forensic indicator of the season in that a person (or animal) died. However other successional studies need to be performed in Chile during other seasons in different regions (i.e., to determine the presence or absence of this species during the different seasons in other regions). Moreover, Matuszewski et al. [44] indicated that *Nitidula* and other Nitidulidae can serve as indicators of the relocation of bodies from rural open areas to rural forest habitats in Europe [44]. Our observations and those of Matuszewski et al. [44] show that this species has a strong potential as an indicator in forensic entomology due to either its seasonality, observed in this study, or its geographical preference, described by Matuszewski et al. [44], although more studies are needed to strengthen both ideas.

The life cycle of *N. carnaria* was described by Zanetti et al. [51]. These authors reported that for larvae reared at 25 °C at a 54% mean humidity, the larval period lasted for 38.3 ± 1.7 days, and the pupal period lasted for 8.7 ± 0.7 days. In our study, the larvae were reared at 15 °C at a 60% mean humidity, and the larval period lasted for 55 ± 3 days, and the pupal period lasted for 12 ± 2 days. These differences in the development rates are mainly due to the environmental temperature used in both experiments. Temperature is one of the most important environmental factors that regulates the development rates in insects, as has been observed in other beetles, such *Dermestes maculatus* DeGeer [59]. The results obtained in both experiments contribute to the knowledge of the life cycle of *N. carnaria*.

The general description of the larval morphology of *N. carnaria* is consistent with the description of other larval nitidulids by Böving and Rozen [60], Peterson's [61] and Hayashi [45]. The measurements of the total length and some structures of larvae were consistent with the measurements of *N. quadripustulata* reported by Perris [31] and recently by Zanetti et al. [51]. However, there are slight differences between the measurements described here and those reported by these authors, especially on the measurements of other smaller structures of the larvae, such as the head. These differences are most likely due to the technique used to measure the larvae in different studies: light microscopy versus SEM. This latter technique is much more accurate, although the dehydration that the specimens undergo in this technique may cause the smaller measurements that are registered for the structures.

Several authors have described the main features of the larvae of many species of the Nitidulidae family [45,51,60,61]. In the present report we provide new ultramorphological details of the *N. carnaria* larvae.

Giglio et al. [62] suggested that the number and location of the sensory structures vary among larvae of different species of some beetles. These authors compared the labial and maxillary palpi of 22 species of Carabidae beetle larvae and demonstrated the presence of four types of sensilla: sensilla digitiformia, sensilla campaniformia, sensilla basiconica and sensilla chaetica. Using transmission electron microscopy (TEM), the authors established that sensilla campaniformia and sensilla chaetica are mechanoreceptors, whereas sensilla basiconica are chemoreceptors. Sensilla digitiformia can be both mechanoreceptors and chemoreceptors and may be implicated in hygroreception. In all of the *N. carnaria* larvae that we observed, the presence, number, and distribution of at least two types of sensilla on the labial and maxillary palpi: sensilla basiconica and sensilla styloconica were observed; however, two other types of sensilla were also discovered. The fine structure and function of the channel on the dorsal surface of the third segment of the maxillary palpi is not yet known. It is likely that the channel represents a type of sensilla digitiformia, although ultrastructural analysis using TEM is required to confirm this hypothesis and to determine its function. Chaetotaxy is another important characteristic used in taxonomic descriptions and in phylogenetic systematics [63]. Indeed, the number and disposition of the setae of *N. carnaria* larvae were different from those of other nitidulid larvae [45,57]. Although some authors question the utility of larval chaetotaxy, we believe it may prove useful for identification purposes.

On the ventral surface of abdominal segment 10, one pair of sensilla campaniformia was discovered. In general, sensilla campaniformia are considered to be mechanoreceptors [64,65] and have been found on the antennae of several insects, e.g., ground beetles [66] and Australian spittlebugs [67]. The two types of cuticular plates observed on the abdominal segments may have different functions. One type of plate is located in the areas of the abdomen that appear to contact the ground and is characterised by caudally oriented projections. A similar structure is known from the larvae of other necrophagous insects, e.g., the maggots of the fly family Calliphoridae, where they are involved in the locomotion and traction of larvae [68]. It is likely that they serve a similar function in *N. carnaria* larvae.

It is possible that morphological criteria based on differences in the number and location of the sensory structures and setae can be used to characterise different taxa of Nitidulidae, but it is necessary to study and describe the larvae of other species of Nitidulidae with SEM. The results presented here contribute to the improved identification of *N. carnaria* larvae using SEM. Our observations on *N. carnaria* larvae indicated that the presternum of prothoracic segment is much shorter than the eusternum. Hayashi [45] also observed the same characteristic for *O. colon* Linnaeus larvae, but he described that the presternum was large in *C. hemipterus* larvae was subequal to the eusternum in length. Another distinct characteristic of *C. hemipterus* larvae is the lack of paired sclerites in the meso- and metathoracic terga; *O. colon* larvae have mesothoracic to the 8th abdominal terga with a pair of dorsoparamedian sclerites on each [45]. We also observed this finding for *N. carnaria* larvae. These species can be distinguished because these structures are obscure in *O. colon* larvae but are orange in *N. carnaria* larvae. Furthermore, the body length of the first species is approximately 5–6 mm, and the head capsule is approximately 0.56 mm in breadth [45]; those of *N. carnaria* larvae are approximately 3.5–4.1 mm, and approximately 0.4 mm in breadth, respectively, as measured by SEM.

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References

[1] E.P. Catts, M.L. Goff, Forensic entomology in criminal investigation, Annu. Rev. Entomol. 37 (1992) 253–272.
[2] J. Amendt, C.P. Campobasso, E. Gaudry, C. Reiter, H.N. LeBlanc, M.J.R. Hall, Best practice in forensic entomology – standards and guidelines, Int. J. Legal Med. 121 (2007) 90–104.
[3] G.D. de Jong, W.W. Hoback, Effect of investigator disturbance in experimental forensic entomology: succession and community composition, Med. Vet. Entomol. 20 (2006) 248–258.
[4] C.P. Campobasso, G. Di Vella, F. Introna, Factors affecting decomposition and Diptera colonization, Forensic Sci. Int. 120 (2001) 18–27.
[5] P. Kulshrestha, D.K. Satpathy, Use of beetles in forensic entomology, Forensic Sci. Int. 120 (2001) 15–17.
[6] J.A. Payne, E.W. King, Coleoptera associated with pig carrion, Entomol. Monthly Mag. 105 (1970) 224–232.
[7] L.A. Olaya-Másmela, Entomofauna sucesional en el cadáver de un cánido en condiciones de campo en la Universidad del Valle (Cali-Colombia), Cuad. Med. Forense 23 (2001) 5–14.
[8] A.M. García-Rojo, L. Honorato, La Entomología Forense y la práctica policial en España: estimación del intervalo post-mortem en un cadáver hallado en el interior de una arqueta en la comunidad de Madrid, Ciencia Forense, Rev. Aragon. Med. Legal 8 (2006) 57–62.
[9] A.M. García-Rojo, Estudio de la sucesión de insectos en cadáveres en Alcalá De Henares (Comunidad Autónoma de Madrid) utilizando cerdos domésticos como modelos animales, Bol. SEA 34 (2004) 263–269.
[10] K.G.V. Smith, Manual of Forensic Entomology, British Museum, Natural History, London, 1986.
[11] A.G. Kirejtshuk, Nitidulidae (Coleoptera) of the Himalayas and Northern Indochina, Part 1: Subfamily Epuraeinae. Theses Zoologicae, vol. 28, Koeltz Scientific Books, Koenigstein, 1998.
[12] C.T. Parsons, A revision of the nearctic nitidulidae (Coleoptera), Bull. Museum Comp. Zool. 92 (1943) 121–278.
[13] R.A. Crowson, The Biology of the Coleoptera, Academic Press, London, 1981.
[14] W.F. Erichson, Versuch einer systematischen Eintheilung der Nitidularian, Z. Entomol. 4 (1843) 225–361.
[15] H.E. Hinton, A Monograph of the Beetles Associated with Stored Products, vol. I, British Museum of Natural History, London, 1945.
[16] P.F. Dowd, T.C. Nelsen, Seasonal variation of sap beetles (Coleoptera: Nitidulidae) populations in Central Illinois Cornfield-Oak Woodland habitat and potential influence of weather patterns, Popul. Ecol. 23 (1994) 1215–1223.
[17] J.H. Durrant, Insects associated with grain, Rep. Grain Pests Comm. Roy. Soc. 9 (1921) 33–52.
[18] J.F. Lawrence, Nitidulidae (Cucujoidea) (including Brachypteridae, Cateretidae, Cybocephalidae, Smicripidae): sap beetles, dried fruit beetles, in: F.W. Stehr (Ed.), Immature Insects, vol. 2, Kendall Hunt Pub. Co, Dubuque, Iowa, 1991, pp. 456–460.
[19] W.A. Connell, Nitidulidae of Delaware, Del. Agric. Exp. Sta. Bull. 318 (1956) 1–67.
[20] W.A. Connell, Bibliography of *Carpophilus humeralis* (Fab.) in support of a revision of the genus *Carpophilus* Stephens, Bull. Entomol. Soc. Am. 27 (1981) 263–266.
[21] A.R. Cline, R.A.B. Leschen, Coleoptera associated with the oyster mushroom, *Pleurotus ostreatus* Fries, in America north of Mexico, Southwest Natl. 4 (2005) 409–420.
[22] R.A. Crowson, The biology of the Coleoptera, Academic Press, London, 1981.
[23] R.S. Anderson, J.S. Ashe, Leaf litter inhabiting beetles as surrogates for establishing priorities for conservation of selected montane cloud forests in Honduras, Central America (Coleoptera: Staphylinidae, Curculionidae), Biodivers. Conserv. 9 (2000) 617–653.
[24] M.H. Hatch, The beetles of the Pacific Northwest. Part III: Pselphidae and Diversicornia I, Univ. Wash. Publ. Biol. 16 (1961) 1–503.
[25] D.E. Gennard, Forensic Entomology: An Introduction, University of Lincoln/British Library, UK/England, 2007.
[26] M.I. Arnaldos, M.D. García, E. Romera, J.J. Presa, A. Luna, Estimation of postmortem interval in real cases based on experimentally obtained entomological evidence, Forensic Sci. Int. 149 (2005) 57–65.
[27] S. Özdemir, O. Sert, Determination of Coleoptera fauna on carcasses in Ankara province, Turkey, Forensic Sci. Int. 183 (2009) 24–32.
[28] J.A. Payne, A summer study of the baby pig *Sus scrofa* Linnaeus, Ecology 46 (1965) 592–602.
[29] A. Peterson, Larvae of Insects: An Introduction to Nearctic Species Part II, Edward Brothers, Inc., Ann Arbor, Michigan, 1979.
[30] P.P. Shubeck, N.M. Downie, R.L. Wenzel, S.B. Peck, Species composition and seasonal abundance of carrion beetles in an oak-beech forest in the great swamp national wildlife refuge (N.J.), Entomol. News 92 (1981) 7–16.
[31] E. Perris, Larves des Coléoptères, Deyrolle, Paris, 1877.
[32] G.B. Vogt, Occurrence and records of Nitidulidae, Coleopt. Bull. 4 (1950) 81–91.
[33] W.C. Rodríguez, W.M. Bass, Insect activity and its relationship to decay rates of human cadavers in East Tennessee, J. Forensic Sci. 28 (1983) 423–432.
[34] R.N. Williams, J.L. Blackmer, D.S. Richmond, M.S. Ellis, Nitidulidae (Coleoptera) diversity in three natural preserves in Portage County, Ohio, Ohio J. Sci. 92 (1992) 82–87.

[35] T.W. Adair, B.C. Kondratieff, The occurrence of *Nitidula flavomaculata* (Coleoptera: Nitidulidae) on a human corpse, Entomol. News 107 (1996) 233–236.
[36] E.J. Watson, C.E. Carlton, Insect succession and decomposition of wildlife carcasses during fall and winter in Louisiana, J. Med. Entomol. 42 (2005) 193–203.
[37] M.B. Price, D.K. Young, An annotated checklist of Wisconsin sap and short-winged flower beetles (Coleoptera: Nitidulidae, Kataretidae), Insecta Mundi 20 (2006) 68–84.
[38] B.J. Sharanowski, E.G. Walker, G.S. Anderson, Insect succession and decomposition patterns on shaded and sunlit carrion in Saskatchewan in three different seasons, Forensic Sci. Int. 179 (2008) 219–240.
[39] M. Castillo-Miralbés, Artrópodos presentes en carroña de cerdos en la Comarca de la Litera (Huesca), Bol. SEA 28 (2001) 133–140.
[40] P. Kočárek, Decomposition and coleoptera succession on exposed carrion of small mammal in Opava, the Czech Republic, Eur. J. Soil Biol. 39 (2003) 31–45.
[41] S. Matuszewski, D. Bajerlein, S. Konwerski, K. Szpila, Insect succession and carrion decomposition in selected forests of Central Europe. Part 2: Composition and residency patterns of carrion fauna, Forensic Sci. Int. 195 (2010) 42–51.
[42] M.I. Saloña, M.L. Moraza, M. Cales-Tolrá, V. Iraola, Ñ.P. Bahillo, T. Yélamos, R. Outerelo, R. Alcaraz, Searching the soil: forensic importance of edaphic fauna after the removal of a corpse, J. Forensic Sci. 55 (2010) 1652–1655.
[43] E. Anton, S. Niederegger, R.G. Beutel, Beetles and flies collected on pig carrion in a experimental setting in Thuringia and their forensic implications, Med. Vet. Entomol. 25 (2011) 353–364.
[44] S. Matuszewski, M. Szafalowicz, M. Jarmusz, Insects colonising carcasses in open and forest habitats of Central Europe: search for indicators of corpse relocation, Forensic Sci. Int. 231 (2013) 234–239.
[45] N. Hayashi, A contribution to the knowledge of the larvae of Nitidulidae occurring in Japan (Coleoptera: Cucujoidea), Insecta Matsumurana 14 (1978) 1–97.
[46] J. Wang, Z. Li, Y. Chen, Q. Chen, X. Yin, The succession and development of insects on pig carcasses and their significances in estimating PMI in south China, Forensic Sci. Int. 179 (2008) 11–18.
[47] E. Martínez, P. Duque, M. Wolff, Succession pattern of carrion-feeding insects in Páramo, Colombia, Forensic Sci. Int. 166 (2007) 182–189.
[48] M. Wolff, A. Uribe, P. Ortiz, A. Duque, A preliminary study of forensic entomology in Medellín, Colombia, Forensic Sci. Int. 120 (2001) 53–59.
[49] A. Oliva, Insects of forensic significance in Argentina, Forensic Sci. Int. 120 (2001) 145–154.
[50] M. Battan-Horenstein, A.X. Linhares, Seasonal composition and temporal succession of necrophagous and predator beetles on pig carrion in central Argentina, Med. Vet. Entomol. 25 (2011) 395–401.
[51] N.I. Zanetti, E.C. Visciarelli, N.D. Centeno, Preliminary data on larval morphology and life cycle of *Nitidula carnaria* (Coleoptera: Nitidulidae), a species of forensic interest, Rev. Soc. Entomol. Argent. 72 (2013) 195–198.
[52] K.M. Mise, L.M. Almeida, M.O. Moura, Levantamento da fauna de Coleoptera que habita a carcaca de *Sus scrofa* L., em Curitiba, Parana, Rev. Bras. Entomol. 51 (2007) 358–368.
[53] F. Saíz, E. Tosti-Croce, M.S. Leiva, Estudio de los cambios de la mesofauna asociada a la descomposición de cadáveres de conejo en clima mediterraneo, An. Mus. Hist. Nat. Valparaiso 20 (1989) 41–74.
[54] J.A. DiZinno, W.D. Lord, M.B. Collins-Morton, M.R. Wilson, M.L. Goff, Mitochondrial DNA sequencing of beetle larvae (Nitidulidae: *Omosita*) recovered from human bone, J. Forensic Sci. 47 (2002) 1–3.
[55] J.H. Byrd, J.L. Castner, Forensic Entomology: The Utility of Arthropods in Legal Investigations, CRC Press, Boca Raton, FL, 2001.
[56] G.S. Anderson, S.L. Vanlaerhoven, Initial studies on insect succession on carrion in Southwestern British Columbia, J. Forensic Sci. 42 (1996) 617–625.
[57] C. Carlton, R.A.B. Leschen, Description of *Soronia* complex (Coleoptera: Nitidulidae: Nitidulinae) larvae of New Zealand with comments on life history and taxonomy, N. Z. Entomol. 30 (2007) 41–51.
[58] A. Ortloff, P. Peña, M. Riquelme, Preliminary study of the succession pattern of necrobiont insects, colonising species and larvae on pig carcasses in Temuco (Chile) for forensic applications, Forensic Sci. Int. 222 (2012) e36–e41.
[59] M.S. Richardson, M.L. Goff, Effects of temperature and intraspecific interaction on the development of *Dermestes maculatus* (Coleoptera: Dermestidae), J. Med. Entomol. 38 (2001) 347–351.
[60] A.G. Böving, J.G. Rozen, Anatomical and systematic study of the mature larvae of the Nitidulidae (Coleoptera), Entomol. Med. 31 (1962) 265–299.
[61] A. Peterson, Larvae of Insects: An Introduction to Nearctic Species, Part II, Edward Brothers, Inc., Ann Arbor, Michigan, 1979.
[62] A. Giglio, E.A. Ferrero, E. Perrotta, S. Tripepi, T. Zetto, Ultrastructure and comparative morphology of mouth-part sensilla in ground beetle larvae (Insecta, Coleoptera, Carabidae), Zool. Anz. 242 (2003) 277–292.
[63] A.Y. Solodovnikov, Larval chaetotaxy of Coleoptera (Insecta) as a tool for evolutionary research and systematics: less confusion, more clarity, J. Zool. Syst. Evol. Res. 45 (2007) 120–127.
[64] L. Ågren, Flagellar sensilla of some colletidae (Hymenoptera: Apoidea), Int. J. Insect Morphol. Embryol. 6 (1977) 137–146.
[65] R.Y. Zacharuk, Antennae and sensilla, in: G.A. Kerkut, L.I. Gilbert (Eds.), Comprehensive Insect Physiology Biochemistry and Pharmacology, Sensory, Pergamon, Oxford, UK, 1985, pp. 1–69.
[66] A. Must, E. Merivee, M. Mänd, A. Luik, M. Heidemaa, Electrophysiological responses of the antennal campaniform sensilla to rapid changes of temperature in the ground beetles *Pterostichus oblongopunctatus* and *Poecilus cupreus* (Tribe Pterostichini) with different ecological preferences, Physiol. Entomol. 31 (2006) 278–285.
[67] A.P. Liang, M.J. Fletcher, Morphology of the antennal sensilla in four Australian spittlebug species (Hemiptera: Cercopidae) with implications for phylogeny, Aust. J. Entomol. 41 (2002) 39–44.
[68] N. Ubero-Pascal, R. López-Esclapez, M.D. García, M.I. Arnaldos, Morphology of preimaginal stages of *Calliphora vicina* Robineau-Desvoidy, 1830 (Diptera, Calliphoridae): a comparative study, Forensic Sci. Int. 219 (2012) 228–243.