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Larval morphology of the antlion *Neuroleon microstenus* (McLachlan, 1898) (Neuroptera, Myrmeleontidae), with notes on larval biology

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Abstract

Larval stages of the Mediterranean antlion species *Neuroleon microstenus* (McLachlan) are described and illustrated. Larvae do not build pitfall traps. They pursue prey by digging in sand backwards and waiting a prey. Second and third instar larvae move backwards or forwards on sand surface, whilst first instar larvae only forwards. Characteristic for the larvae of *N. microstenus*—like for other non-pit-builders—are prominent eye tubercles and sparse mandibular bristles. On the abdominal tip two bulges occur, each with four digging bristles. On the dorsal side of the head of second and third instar larvae black pigmentation occur forming "V" mark. Campaniform sensilla, sensilla coeloconica and sensilla basiconica are recognized for the first time in antlion larvae.

Key words: Larvae, rearing, sensilla coeloconica, sensilla basiconica, campaniform sensilla

Introduction

Antlions (Neuroptera: Myrmeleontidae) with about 2000 described species represent the largest family of lacewings. Myrmeleontidae occur on all continents and most large islands of the world, further emphasizing their success which is attributed largely to their colonization of sand habitats (Mansell 1996). The genus *Neuroleon* Navás, 1909 includes about 120 valid species and is confined to Africa, southern Europe and large parts of Asia (Hölzel 1986; Aspöck *et al.* 2001). It is assumed that larvae of all *Neuroleon* species live in sand without constructing pits (Gepp & Hölzel 1989). In Europe there are only 8 species of *Neuroleon*, and knowledge of their ecology and distribution—as well as for non-European *Neuroleon* species—is poor; usually only single specimens have been collected in European countries. The exception in this respect is France, where Steffan (1971, 1975) studied five species of the genus in detail. In his paper Steffan (1975) erroneously describes larvae of *Neuroleon distichus* (Navás, 1903) under the name *Neuroleon microstenus*. *Neuroleon microstenus* (McLachlan, 1898) is a polycentric Mediterranean species (Aspöck *et al.* 2001). Adults of *N. microstenus* can easily be distinguished from other *Neuroleon* species following key-characters given by Aspöck *et al.* (1980), principally by a small spot on fore wings, and the abdomen of male being much longer than the wings. Till now, the morphology of *Neuroleon microstenus* larvae has not yet been described comprehensively. Previously, the first instar larva was depicted by Gepp (1974) without detailed description. Due to the fact that only scarce information is available concerning the species, we present larval morphology and biology of this species originating on the basis of specimens collected in Istria, Croatia.

Material and methods

Antlion larvae were collected from surroundings of Rovinj, Istria, Croatia (N 45° 3.9', E 13° 39.5'). Single specimens of first instar larvae were collected by hand in September 2007 and second instar larvae in June 2005 and June 2006. Second instar larvae of *N. microstenus* collected in June 2005 and June 2006 occurred

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syntopically with second instar larvae of *Distoleon tetragrammicus* (Fabricius, 1798). The larvae of both species were found in sand or under small stones in a grassland with sporadic bushes of *Spartium junceum* L. Coarser sand (dominant sand particle size 0.2–1 mm) was reddish brown and classified as terra fusca. In September 2007 five first instar larvae were collected in sand among grass roots. All five individuals were close together and in the same substrate embiopterans were recorded. In the northwestern parts of the Balkans adults occur in places similar to larval habitats which are devoid of trees and rich in scrubs and bushes (Devetak & Devetak 2004; Devetak 2007).

Descriptions were based on five first instar larvae, five second instar larvae and 12 third instar larvae. Larvae were placed in plastic cups (diameter 10 cm, height 7 cm) or petri dishes (diameter 9 cm) filled with sand brought from the original collecting place. The larvae were kept in laboratory at room temperature (25±1°C). First instar larvae were fed on *Aphis fabae* Scopoli, 1763, second and third instar larvae with ants *Lasius niger* (Linnaeus, 1758) and *Lasius fuliginosus* (Latreille, 1798). Feeding took place every day and responses of the larvae were video recorded. Measurements of larvae were conducted on live and alcohol-preserved specimens using an ocular micrometer. We measured body length (including the mandibles), body width, head length (without mandibles), head width and mandible length. For morphometrics see detailed description in Nicoli Aldini (2007). The three teeth on the inside of the jaw are numbered 1–3, starting from the tooth nearest to the base of the mandible (see Doflein 1916; Lucas & Stange 1981). Morphological observations of larvae were conducted by means of stereo-microscope as well as scanning electron microscope. Body of the larvae is covered with tiny sand particles, so the specimens were cleaned using ultrasound cleaner Sanorex TK 52. Larvae were photographed under a stereoscopic zoom microscope Nikon SMZ800 with a mounted digital camera Nikon DS-Fi1, and processed with NIS-Elements F 3.0 software. Digital images captured at different focal planes were assembled using the application Helicon Focus 4.62 Lite. Larvae used for electron microscopy were fixed in 2 % paraformaldehyde plus 2.5 % glutaraldehyde, dehydrated, critical point dried, sputter-coated with gold and examined with a digital scanning electron microscope Zeiss DSM 950.

Abbreviations: a—antenna; cl—claw; cs—campaniform sensillum; d—dolichaster; db—digging bristle; et—eye tubercle; ga—galea; lp—labial palp; md—mandible; ph—plumose hair; s—scolus; S9—sternite 9; sb—sensilla basiconica; sc—sensillum chaeticum; smt—submedian tooth; sp—spiracle; st—sensilla trichodea; T9—tergite 9; ta—tarsus; To1—tooth 1; To2—tooth 2; To3—tooth 3.

Description

First instar larva of *Neuroleon microstenus*

Body colouration: head black with shiny black jaws, ventral side of the thorax and abdomen whitish straw, dorsal side light brown.

Size: body length (including mandibles) 4–4.5 mm, body width (widest part) 1.5 mm, head length (without mandibles) 0.9–1 mm, head width 0.9–1.2 mm, length of the mandibles 1–1.1 mm.

Head. Anterior margin of the head is weakly sinuate (Figs. 1, 2). The head surface is covered with numerous dolichasters and scarce plumose hairs (Figs. 3, 5). All dolichasters occurring in *N. microstenus* are grooved longitudinally. On the frontal margin of the head, between the mandibles, there are six longer dolichasters arranged in two groups and close to them there is a pair of campaniform sensilla (Fig. 3). Antenna (Fig. 6) about 2.5 times longer than basal width of mandible. The number of antennal segments varies from 15 to 17. On the distal part of the last flagellomere sensilla basiconica are found (Fig. 8). Shiny black mandibles are equipped with three teeth (Fig. 4). Distance between tooth 1 and 3 is longer than greatest mandibular width. Tooth 3 is longer than 2 which is longer than 1. Mandible typically with 2 black longitudinally grooved bristles (sensilla chaetica) between mandibular base and tooth 1, 1 bristle between tooth 1 and 2, and 1 bristle between tooth 2 and 3. No bristles between the third tooth and pointed end of mandible. Bristles on outer margin of mandible extend from its basal region to the level of tooth 3. Mandibular bristles significantly shorter than tooth 3. Mandibles and maxillae bear a great number of contact chemoreceptor organs—sensilla

coeloconica (Figs. 9–11). The sensilla are set on the floor of relatively shallow depressions in the cuticle and are scattered in the whole length of the jaws including mandibular teeth. The labial palps are four-segmented. On the tip of the labial palps sensilla basiconica occur (Fig. 12). Eyes consisting of seven stemmata are borne on prominent eye tubercles (Fig. 6). Six stemmata of each eye are grouped on the dorsal anterior side of the tubercle, the seventh, rudimentary stemma is positioned on its ventral side (Fig. 7). On eye tubercle, several dolichasters are present.

Thorax. Prothorax has fine granular sculpture with longitudinally grooved dolichasters and plumose hairs scattered on its dorsal side (Fig. 13). Between pro- and mesothorax a pair of ungrooved sensilla trichodea occurs. The spiracles of mesothorax are equipped with marginal dent-like structures and are situated laterally on prominent tubercles (Fig. 14). Paired segmental extensions—scoli—equipped with tufts of long, longitudinally grooved bristles (sensilla chaetica), laterally fringe the meso- and metathorax and abdominal segments (Figs. 15–17). The bristles are classified as dolichasters. Wrinkled cuticular surface of thorax and abdomen is rich in bristles and plumose hairs (Fig. 18). Legs are covered with longitudinally grooved bristles (Figs. 19–21). The tip of the tarsus of all legs has two claws. On the tibia of fore leg and on the distal part of the tarsus of hind leg campaniform sensilla are found (Fig. 22). Between the claws two short sensilla trichodea occur (Fig. 23).

Abdomen. Sternite 8 bears a pair of small submedian teeth (Fig. 24). Sternite 9 is equipped with stout and pointed fossorial or digging bristles (Fig. 25). On the distal part of the sternite there are two bulges, each of them bearing four digging bristles. On each bulge, the most lateral digging bristle is the longest one and the median is the shortest. All digging bristles are longitudinally grooved. In the proximal part of sternite 9 there is a pair of campaniform sensilla (Fig. 24).

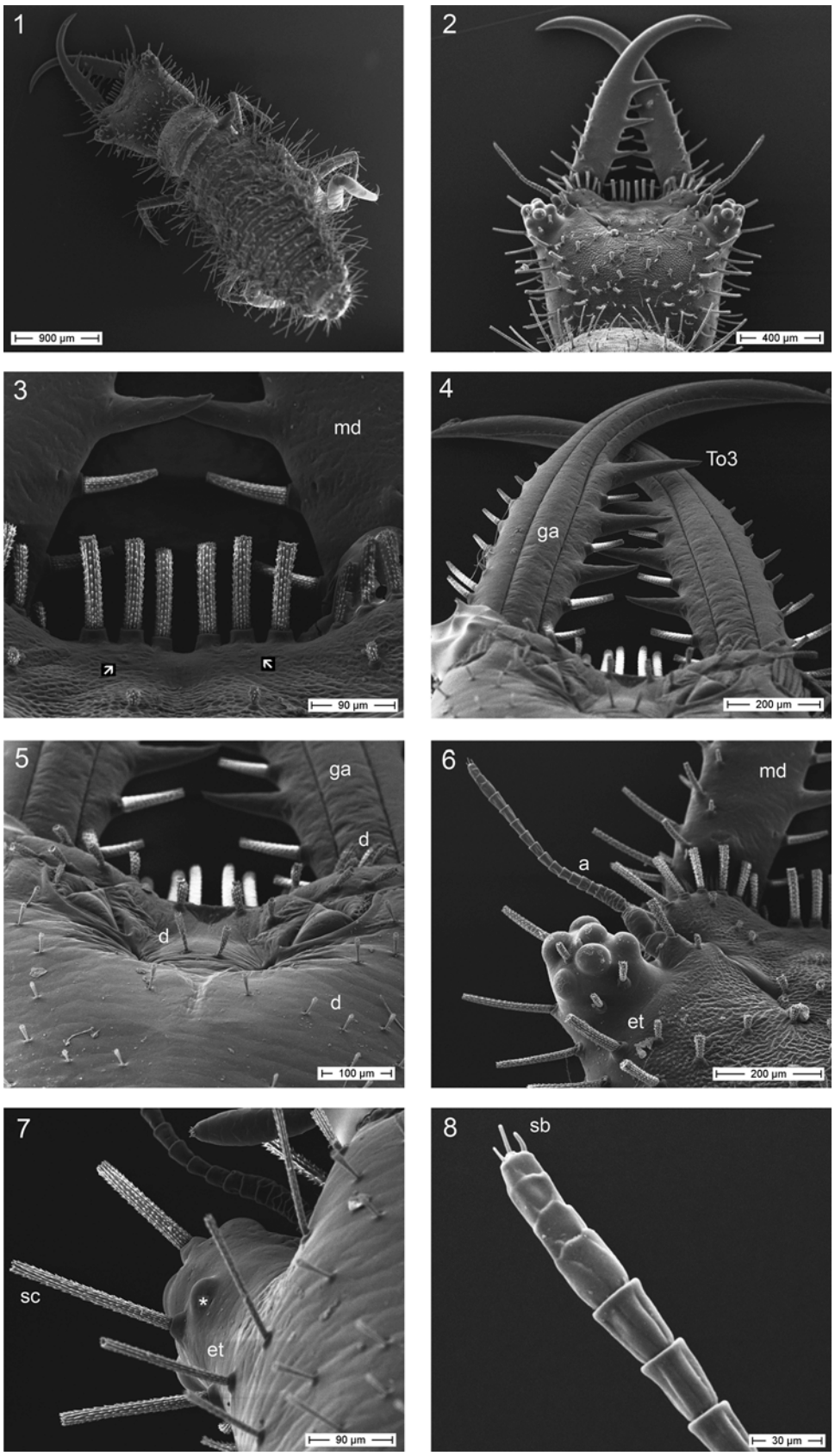
Behaviour: In laboratory conditions, larvae were fed on aphids. When larvae are put on sand surface, they move only forwards, whilst in sandy substrate they move backwards.

Second and third instar larvae

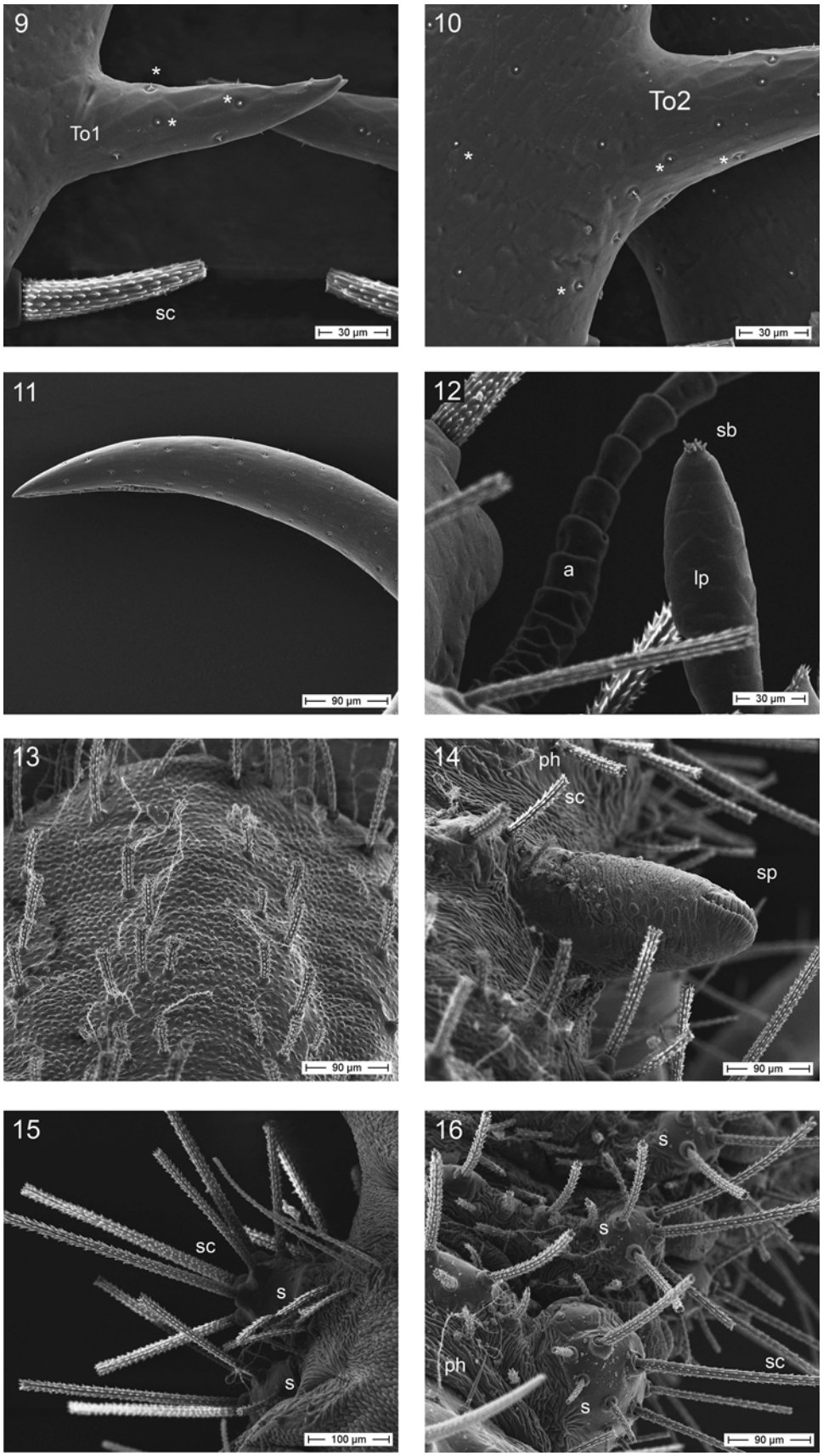
Morphology of second instar is very similar to third instar, but differ in body size.

Body colouration: head brown to reddish brown with black or dark brown pigmentation on dorsal side of the head forming “V” mark (Fig. 27, 28); jaws shiny black; ventral side of the thorax and abdomen light brown, dorsal side brown. Size of second instar larvae: body length (including mandibles) 8–10 mm, body width (widest part) 2.5–3 mm, head length (without mandibles) 1.5–1.6 mm, head width 1.4–1.5 mm, length of the mandibles 1.5–1.7 mm. Size of third instar larvae: body length (including mandibles) 10–13 mm, body width (widest part) 3–3.5 mm, head length (without mandibles) 2–2.2 mm, head width 1.8–2 mm, length of the mandibles 2–2.1 mm. Anterior margin of the head and head surface are covered with numerous bristles (sensilla chaetica); many of them have broken tip or are missing in the whole length. All bristles are grooved longitudinally. Shiny black mandibles are equipped with three teeth. Distance between tooth 1 and 3 is two-times longer than greatest mandibular width. Tooth 3 is longer than 2 which is longer than 1. Mandible is typically equipped with 3–5 short bristles (sensilla chaetica) between mandibular base and tooth 1, 1 bristle between tooth 1 and 2, and 1 bristle between tooth 2 and 3. No bristles are found between the third tooth and pointed end of mandible. The intact bristles occur only in freshly hatched larvae; in older animals most of them are broken and often only a base of the bristle is seen. Bristles on outer margin of mandible are scarce and extend from its basal region to level of tooth 3. Inner jaw bristles are shorter than tooth 1, outer jaw bristles are shorter than tooth 3. Thoracic and abdominal morphology is like in first instar larvae. Sternite 9 is equipped with stout and pointed bristles. On the distal part of sternite 9 there are two bulges, each with four fossorial or digging bristles which are longitudinally grooved (Fig. 26). These digging bristles are longitudinally grooved less clearly than other bristles of the same larva.

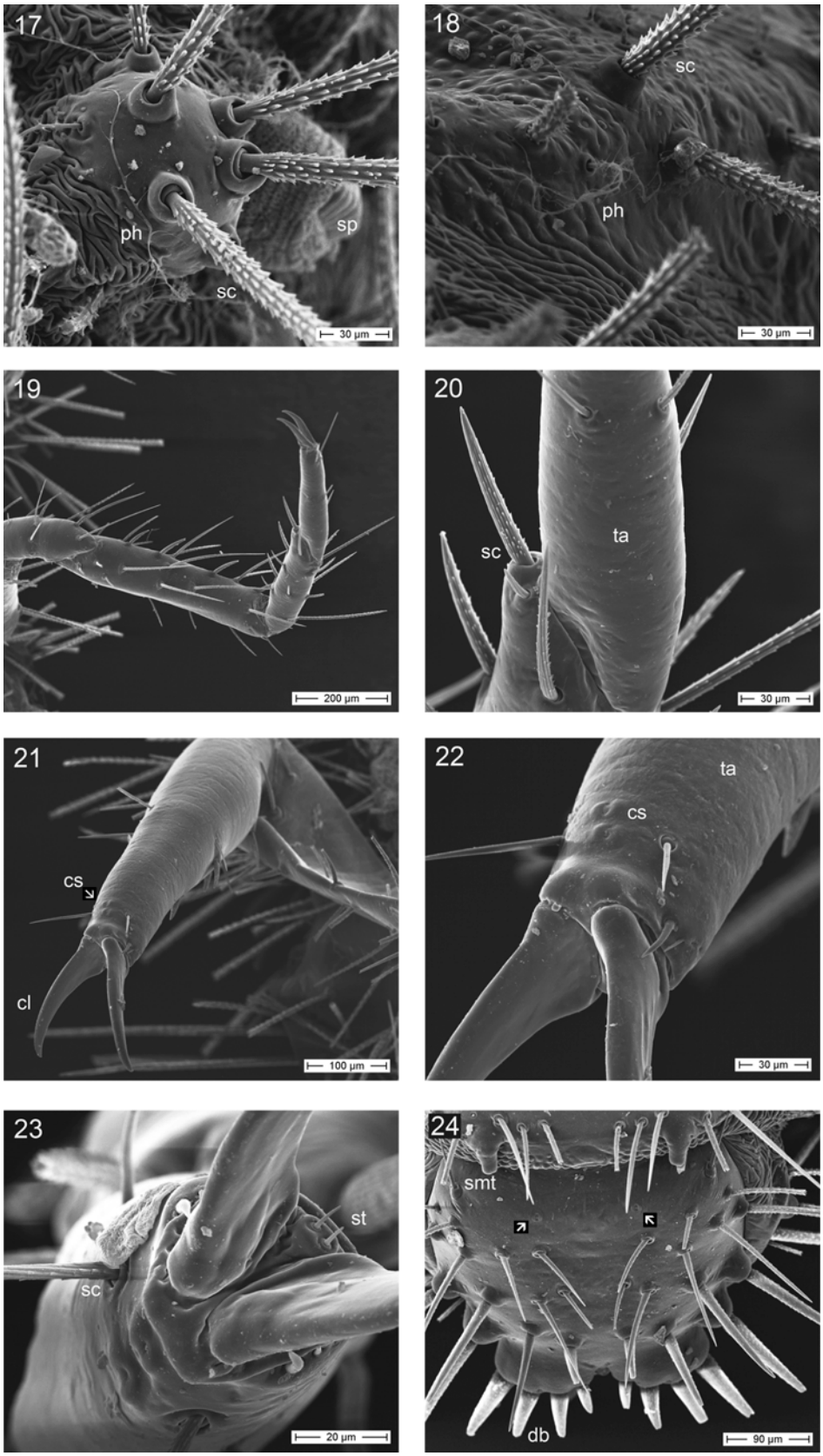
Behaviour of second and third instar larvae: On sand surface larvae move backwards or forwards. They pursue prey by digging slowly backwards in sand and then whipping the head backwards to grasp prey when it is beneath the larvae. Larvae never ran forwards after prey.



FIGURES 1–8. SEM micrographs of first instar larva of *N. microstenus*. 1. First instar larva, dorsal view. 2. Head, dorsal view. 3. Frontal margin of the head with six dolichasters, dorsal view. 4. Head with jaws, ventral view. 5. Frontal margin of the head, ventral view. 6. Eye tubercle with six stemmata, dorsal view. 7. Ventral surface of the eye tubercle with seventh stemma (marked with an asterisk). 8. Right antenna with sensilla basiconica on the last flagellomere.



FIGURES 9–16. SEM micrographs of first instar larva of *N. microstenus* (continued). 9. and 10. Sensilla coeloconica (see asterisks) on the first and second mandibular tooth. 11. Sensilla coeloconica on the tip of the right mandible. 12. Labial palp with sensilla basiconica. 13. Prothorax, dorsal view. 14. Mesothoracic spiracle on a prominent tubercle. 15. Ventral view of two mesothoracic scoli. 16. Scoli on the abdominal segments 6–8.



FIGURES 17–24. SEM micrographs of first instar larva of *N. microstenus* (continued). 17. Scolus on the fourth abdominal segment. 18. Sensilla chaetica and plumose hairs on the right side of prothorax. 19. Fore leg covered with bristles. 20. Tarsus of fore leg. 21. Hind leg with paired claws. 22. Two campaniform sensilla and paired claws of hind leg. 23. Two sensilla trichodea between the claws of hind leg. 24. Digging bristles and campaniform sensilla (see arrows) on the sternite 9.

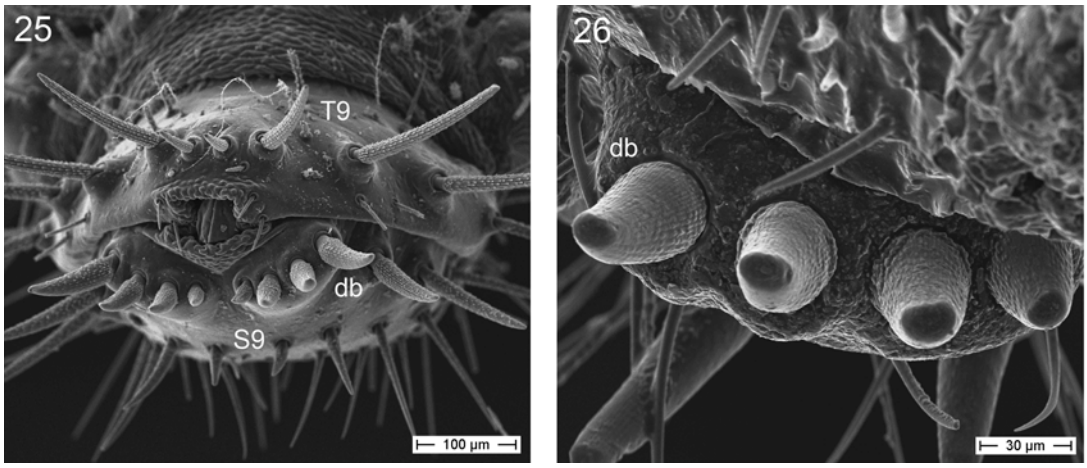


FIGURE 25. Tip of the abdomen with digging bristles of first instar larva.
FIGURE 26. Bulge with four digging bristles of third instar larva.

FIGURE 27. Third third instar larva of *N. microstenus*.
FIGURE 28. Head of third instar larva, dorsal view.

Remarks on development

In the laboratory rearing, second instar larvae moulted into third instar larvae at the end of June, rarely in July or August. Third instar larvae form ball-like cocoons made of silk and covered with sand grains glued to silk. The diameter of cocoon is 7–9 mm. Pupation lasts 28–30 days. Adults emerged in July and August, rarely in June. The pupa only partly emerged from the cocoon before the adult appeared. Shortly after completion of the adult form, a single pellet of larval excrement (meconium) was deposited. This is elongate, measuring 3.5–4 mm in length and 1 mm in diameter, hard and shining.

Discussion

Although a number of species of myrmeleontid larvae are mentioned in the literature, only a small proportion is adequately described (reviews: Gepp 1984; Stange & Miller 1990). More papers describing larval morphology and biology deal with pit-building antlion species than non-pit-builders (Gepp 1984). Larvae of *N. microstenus* have prominent eye tubercles and sparse short bristles on the jaws. Prominent eye tubercles and sparse mandibular bristles are both characteristic for non-pit-builders, like *Distoleon* (Satar *et al.* 2006) and other *Neuroleon* species (Steffan 1975). On the abdomen apex of *N. microstenus* the two groups of digging bristles are arranged in the same way as in *Neuroleon ochreatus* (Navás, 1904) (Steffan 1975). Four digging bristles on a bulge are also found in *Distoleon* (Satar *et al.* 2006) but their length differs from bristle length in *Neuroleon* where they are not of equal size. Black pigmentation on the dorsal side of the head forming "V" mark in *N. microstenus* is similar to the pigmentation pattern in a closely related species, *Neuroleon nemausiensis* (Borkhausen, 1791) (Steffan 1975). Three types of sensory receptors—campaniform sensilla, sensilla coeloconica and sensilla basiconica—are recognized for the first time in antlion larvae. Campaniform sensilla are recorded on the head, legs and abdomen. It is proposed that two campaniform sensilla on the 9th abdominal sternite play a role in digging into sandy substrate because these mechanoreceptors are known to detect stretch forces in cuticle (McIver 1985; Chapman 1998; Römer 2003). Sensilla coeloconica are present on the mandibles and maxillae of *Neuroelon microstenus*, and sensilla basiconica were found on the tips of the antennae and labial palps. These receptors play role in detection of chemical substances important in feeding and sensing animal's environment (Zacharuk 1985; Galizia 2008; Glendinning 2008). Koch (1983) recorded chemoreceptor sensilla on the mandibles of *Euroleon* and Satar *et al.* (2006) mentioned antennal sensilla as "three finger shaped lobes" in *Distoleon*. Chemoreceptors of *Neuroleon* are present on maxillae and both surfaces of mandible, even on mandibular teeth. These receptors play a role during catching behaviour when prey is grasped and fixed with mandibles and tip of mandibles and teeth penetrate a prey. It is suggested that antlions estimate taste of prey with these chemoreceptors.

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