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Description of the larvae of *Nematodes penetrans* (LeConte, 1852)
(Coleoptera: Eucnemidae: Macraulacinae: Nematodini),
with notes on its hypermetamorphic life cycle

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Eucnemid larvae of the Nearctic Region. Part VII: Description of the larvae of *Nematodes penetrans* (LeConte, 1852) (Coleoptera: Eucnemidae: Macraulacinae: Nematodini), with notes on its hypermetamorphic life cycle

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Abstract. Descriptions and illustrations of the triungulin, 5th instar, and prepupal larval forms for *Nematodes penetrans* (LeConte, 1852) (Coleoptera: Eucnemidae: Macraulacinae: Nematodini) are given. The descriptions are based on triungulins collected in a plastic vial as well as 5th instars and prepupal larval stages collected from hard sections of sugar maple (*Acer saccharum* Marshall; Aceraceae) logs and limbs. Adults were reared from those pieces placed in plastic bags. Biological information is provided, based on literature search and personal observations. Comparative studies between larvae of *N. penetrans* and the European species, *Nematodes filum* (Fabricius, 1801) along with other Nearctic larval forms are briefly discussed. This discovery represents the first larval description for the genus in the Nearctic region.

Key Words. False click beetles, morphology, triungulin, 5th instar, prepupal larval stage, Wisconsin

Introduction

Nematodes Berthold, 1827 is largely a tropical genus consisting of 40 extant species, the majority of which occur in the Neotropical region. Twenty-two of those species are distributed in the Neotropical region, mostly from Mexico through South America, particularly the Amazonian basin. Some of those species, *Nematodes biimpressus* Fleutiaux, *Nematodes guadeloupensis* Fleutiaux, *Nematodes puertoricensis* Fisher and *Nematodes simulans* Chevrolat are endemic on some of the Caribbean islands (Cuba, Guadeloupe, and Puerto Rico). Two species, *Nematodes major* Bonvouloir and *Nematodes pubescens* (MacLeay) are distributed along the eastern coastline of the Australian continent. A single species, *Nematodes filum* (Fabricius) occurs in Europe. A single species, *Nematodes* new species occurs on the African continent. Eight species are found in SE Asia and oceanic region, including Japan: *Nematodes beccarii* Fleutiaux, *Nematodes feai* Fleutiaux, *Nematodes incertus* Bonvouloir, *Nematodes lateralis* Otto, *Nematodes sulcatus* Fleutiaux, *Nematodes sumatrensis* Bonvouloir, *Nematodes suturalis* Fleutiaux and *Nematodes watanabei* Hisamatsu. Additionally, Muona (1993) encountered one undescribed species in the region. I encountered four extinct, undescribed species. Two extinct species were discovered from Miocene Dominican Republic amber. The remaining two species were discovered from Eocene Baltic amber and from Madagascar copal. Muona (2000) listed four species of *Nematodes* in the Nearctic region. *Nematodes humpreyi* Muona is a precinctive species in Florida. *Nematodes atropos* (Say) is widespread across south central and southeastern areas of the region. *Nematodes penetrans* (LeConte) is widespread across the northern areas of the region, extending its range as far south as Florida and Texas. *Nematodes collaris* Bonvouloir is very rare in the region; with limited, isolated occurrences in the eastern United States. Muona (2000) had regarded *N. collaris* as an extinct species. However, Otto (2010) identified specimens collected by Rick Buss, Ted MacRae, and Michael Ulyschen belonging to *N. collaris*, which indicates the species is still thriving in its historical range, including several new records for Missouri.

The following account of *N. penetrans* is the seventh in a long-term series of papers (Otto 2012a, 2012b, 2013, 2014, 2015; Otto and Gruber 2016) covering descriptions of larval Eucnemidae with notes on their biology in the Nearctic region.

Materials and Methods

Surrounding pieces of wood determined likely to contain larvae were placed in 3.8-L plastic resealable bags. Collection data were recorded on each bag. These bags were set aside and checked every other day for emergence of adult beetles. Bags were left open for several days to dry out the wood pieces to ensure mold did not grow on the surface. Bags were resealed after the surfaces dried.

Extracted larvae collected in 2007, 2009 and 2015 were temporarily stored in empty plastic vials and then preserved by initial immersion in a hot water bath for 15-20 minutes. Dimensions were measured using a ruler. Length was measured from the apex of the head capsule to the apex of the ninth abdominal segment. Width was measured across the prothoracic segment. All specimens were subsequently placed in 1 dram, labeled vials filled with 70% ethanol for permanent storage. Some triungulins were preserved directly in 70% ethanol. Larval specimens from this study are deposited in the collection of the Global Eucnemid Research Project (GERP) and in the entomology collection at the University of Wisconsin-Madison (WIRC).

The terminologies of Muona and Teräväinen (2008) as well as Otto (2014) were used in the larval descriptions. Line illustration of the triungulin was completed by free hand through examining some of the specimens under magnification using a dissecting stereoscope. 5th instar and prepupal larval specimens were suspended in Germ-X® hand-sanitizer gel during the imaging process. Images were taken with a JVC KY-F75U digital camera attached to a Leica® Z16 APO dissecting microscope with apochromatic zoom objective and motor focus drive, using a Synchroscopy Auto-Montage® Pro System and software version 5.01.0005. The imaged specimen was rinsed with distilled water to remove the gel, before returning it to the vial. Modifications to 5th instar and prepupal larval stage images were performed using Adobe® Photoshop® Elements 10 program on the computer.

Systematics

Subfamily Macraulacinae Fleutiaux, 1922

Tribe Nematodini Leiler, 1976

Genus *Nematodes* Berthold, 1827

Adult diagnosis. Characters of Nematodini, with mandibles short, ventral secondary tooth present, without expanded lateral surfaces; prothoracic tibiae with one apical spur; male prothoracic tarsomere I with basal sex combs; tarsomere IV originally bilobed; lateral sides of mesothoracic and metathoracic tibiae variable, either with setae and simple spines or with setae and transverse rows of spine combs; hypomeron usually without antennal grooves; prothoracic sternal peg high, either truncated or excavated; median lobe without dorsal basal struts, fused with lateral lobes, distinct, with narrowly and deeply bifurcate apex; bursa divided, simple; spermatheca sclerotized, divided and U-shaped.

Nematodes penetrans (LeConte, 1852)

Larval diagnosis. Larvae of *N. penetrans* can be distinguished from *Isorhipis* Boisduval and Lacordaire and *Melasis* Olivier larvae by the absence of annulets or pseudosegments between each abdominal segment. Presence of mesothoracic sclerome, along with microtrichial patches will further distinguish *N. penetrans* from larvae of *Isorhipis* and *Melasis*. The species can be distinguished from illustrated larvae of *Hylocharis nigricornis* (Say) (Peterson 1951) by its prothoracic scleromes, that being T-shaped in *N. penetrans* and circular in *H. nigricornis*. Furthermore, *N. penetrans* can also be distinguished from *Dirrhagofarsus ernae* Otto, Muona and McClarin by its microtrichial patches on both mesothoracic and metathoracic segments, as well as the absence of microtrichial patch on the ninth abdominal terga. Presence of areole on both the dorsum and tergum of abdominal segment IX will also distinguish *N. penetrans* from *D. ernae*.

Specimens examined. Eight larvae and indeterminate number of triungulins collected at USA: Wisconsin: Oconto County, Rueckert's private property, T29N R17E sec 16, 10 April 2007, 22 April 2007, 9 April 2009, Robert L. Otto, in maple (3 5th instars, 2 prepupal larval stages); Oconto County, Suring, 9–12 June 2007, Robert L. Otto, reared from eggs laid by adults (triungulins); Oconto County, Jeff Otto property, 44°59.075'N, 88°28.725'E, 5 April 2015, leg. Robert L. Otto, in rotten *Acer* limb (3 prepupal larval stages).

Triungulin (Fig. 1)

Description. Length 2.0 mm, width 0.05 mm. Orthosomatic, elateriform (Fig. 1). Body elongate, subcylindrical, sides parallel, translucent white, less sclerotized with head being light brown. Scleromes and microtrichial patches not evident.

Head: Subtriangular, inserted into the prothorax. Lateral projections absent. Dorsal and ventral cephalic discs unmodified. Anterior portion of the head capsule more sclerotized. Mandibles with normal biting structures.

Thorax: Each thoracic segment about 2 to 3 times longer than wide, sides parallel. Pair of small, filiform, 4 segmented legs present on each thoracic segment. Each leg bears one tarsungulis. Seta absent near base of each leg. Scleromes and microtrichial patches not evident.

Abdomen: Segment I about as long as each thoracic segment, but shorter than the remaining abdominal segments. Segments II–VIII at least three times longer than wide, microtrichial patches and areole not evident. Segment IX shorter, bulbous with pair of filamentous setae near caudal end. No indications of circumanal asperities around the anus.

5th Instar (Fig. 2–5)

Description. Length 20.0–25.0 mm, width 0.9 mm (n=3). Orthosomatic, buprestiform (Fig. 2). Body subcylindrical, sides parallel with fused head and prothorax being slightly wider, white with head, prothoracic sclerome patches and caudal end of abdominal segment IX brown. Setae and legs either reduced or absent. Dorsal and ventral microtrichial patches white to slightly darker in color compared to their surrounding areas.

Head (Fig. 3–4): Strongly flattened, subtriangular, prognathous and inserted into prothorax. Dorsal cephalic disc sub-oval, with 2 parallel furrows on either sides, converging towards the posterior side, which forms a small oval ridge and a median carina. Ventral cephalic disc broadly sub-circular, with a single raised carina. Longitudinal furrows absent on ventral cephalic disc. Anterior portion of the head capsule just below the mandibles heavily sclerotized. Remaining head capsule weakly to moderately sclerotized. Each lateral side of head capsule with two projections. First lateral projection small, unsclerotized. Second lateral projection large, heavily sclerotized. Antennae minute, arising between 1st and 2nd lateral projections. Scape not visible. Pedicel elongate. Sensorium and flagellum sub-equal in length. Sensory papillae distinct. Mandibles distinct, resting in the mesal acumination of the head capsule. Each mandible heavily sclerotized, broad, slightly longer than wide, consisting of 2 outwardly projecting teeth. Labial and maxillary palpi extremely small and segmented. Ligula, mala, lacinia and galea not visible. Hypostomal rods absent.

Prothorax: Distinctly wider than all preceding segments. Tergum with pair of T-shaped scleromes. Band of sparse spicules connect T-shaped scleromes with lateral circular microtrichial patch. Sternum with two large scleromes extending from median toward each lateral sides, at which continues to extend at an acute angle down the length of the segment. Pair of small round scleromes present, just laterad of large scleromes. Spiracles absent.

Mesothorax: Three times longer than wide. Both surfaces with medial sclerotized line extending down length of the segment. Oval-shaped microtrichial patch present on both sides of medial sclerotized line. Each patch with series of longitudinal lines of spicules.

Metathorax: Shorter than mesothoracic and abdominal segments. Both surfaces with pair of circular, diverging microtrichial patches near anterolateral sides. Each patch with series of longitudinal lines of spicules. Spiracles absent.

Abdomen: Segments I–VIII three times longer than wide, sub-equal in length. Terga and sterna I–VIII each with oval microtrichial patch and small circular areole near posterior end. Segment IX shorter, dorsally compressed, sparsely punctate and bulbous at caudal end; both surfaces with large circular areole near anterior end; sternum IX (Fig. 5) heavily sclerotized caudad, with complete ring of circumanal asperities. Urogomphi absent on segment IX. Spiracles annular-biforous.

Prepupal Larval Stage (Fig. 6–9)

Description. Length 15.0–18.0 mm, width 1.2 mm (n=5). Orthosomatic, buprestiform (Fig. 6). Body subcylindrical, sides parallel with the fused head and prothorax being slightly wider, cream white with the head and remaining prothoracic sclerome patches brown in color. Setae, legs, microtrichial patches and areole absent.

Head (Fig. 7–8): Strongly flattened, subtriangular, prognathous and inserted in the prothorax. Dorsal cephalic disc sub-oval-shaped, with 2 parallel furrows on either sides, converging towards the posterior side, which forms a small oval ridge and a median carina. Ventral cephalic disc broadly sub-circular, with a single raised carina. Longitudinal furrows absent on ventral cephalic disc. Anterior portion of the head capsule just below the mandibles heavily sclerotized. Remaining head capsule weakly to moderately sclerotized. Each lateral side of head capsule with two projections. First lateral projection small, unsclerotized. Second lateral projection large, heavily sclerotized. Antennae minute, arising between 1st and 2nd lateral projections. Scape not visible. Pedicel elongate. Sensorium and flagellum sub-equal in length. Sensory papillae distinct. Mandibles distinct, resting in the mesal acumination of the head capsule. Each mandible heavily sclerotized, broad, slightly longer than wide with two outwardly projecting teeth. Labial and maxillary palpi extremely small, segmented. Ligula, mala, lacinia and galea not visible. Hypostomal rods absent.

Prothorax: Distinctly wider than all preceding segments. Tergum with reduced T-shaped scleromes and pair of reduced circular scleromes just laterad of the scleromes. Spicules absent between scleromes. Sternum with pair of reduced scleromes extending from median towards lateral sides, at which continues to extend at an acute angle down the length of the segment. Sternum with reduced, circular scleromes near lateral sides. Spiracles absent.

Mesothorax: As long as abdominal segments I–VIII.

Metathorax: Shorter than mesothoracic and abdominal segments I–VIII. Spiracles absent.

Abdomen: Segments I–VIII sub-equal in length, 2–2 1/2 times longer than wide. Segment IX slightly shorter, parallel and caudally blunt; sternum IX (Fig. 9) with complete ring of sparse, reduced circumanal asperities. Urogomphi absent on segment IX. Spiracles annular-biforous.

Distribution. *Nematodes penetrans* is known from **CANADA:** New Brunswick, Nova Scotia, Ontario, Québec; **USA:** Florida, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maine, Maryland, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Texas, Virginia, West Virginia, and Wisconsin (Muona 2000; Majka 2007; Webster et al. 2012). All specimens used in this study came from Wisconsin.

Biology. Although *N. penetrans* is widespread across eastern North America, the species is uncommon in collections. *Nematodes penetrans* thrives in a variety of forest systems. In Wisconsin, I have collected adults and larvae in northern hardwood swamp, northern mesic forest, northern wet-mesic forest, floodplain forest, southern hardwood swamp, southern mesic forest and oak barrens. Collecting results from examining purple prism traps (Synergy Semiochemicals, British Columbia) from 2009 through 2011 have shown the species are active from mid-June through mid-August in northeastern Wisconsin. Dury (1888) found these beetles running on a dead beech (*Fagus* sp.; Fagaceae). Dury (1904) also found adults emerging from branches of *Acer* sp. (Aceraceae), *Fagus* sp., and *Ulmus* sp. (Ulmaceae) in Ohio. Knull (1947) reared *N. penetrans* from branches of beech. Webster et al. (2012) captured 28

specimens through deployment of Lindgren funnel traps in the canopies of old silver maple (*Acer saccharinum* L.; Aceraceae) forest with green ash (*Fraxinus pennsylvanicus* Marshall; Oleaceae) and an old red oak (*Quercus rubra* L.; Fagaceae) forest systems in New Brunswick during the months of July and August.

On 10 April 2007, a single 5th instar was extracted from a firm section of rotten sugar maple (*Acer saccharum* Marshall; Aceraceae) log. The site (WI: Oconto Co., Rueckert's private property, 5.6 km W of Suring) is identified as a northern mesic forest dominated by sugar maples and American beech (*Fagus grandifolia* Ehrhart; Fagaceae) with white ash (*Fraxinus americana* L.; Oleaceae) and hemlock (*Tsuga canadensis* (L.) Carrière; Pinaceae) as associates. A first subsequent visit to the site took place on 22 April 2007. The visit has yielded a second 5th instar specimen collected from a different hard, seasoned maple log. The site was revisited again on 9 April 2009 from which a third 5th instar and two prepupal larvae were extracted from a firm section of a different rotten sugar maple log. Several prepupal larvae were extracted from a rotten *Acer* limb on my uncle's forested property on 5 April 2015.

Four adults emerged on 13 May 2007. All were placed in a vial with a piece of wood to observe their behavior and to attempt breeding these beetles. Adults were observed to live up to three weeks under laboratory conditions. These beetles will snap into the air when placed on their back. Eucnemidae, like several families within Elateroidea utilizes a clicking mechanism involving a well-developed prosternal spine and the cavity of the mesothoracic sternum, activated through contraction of a strong muscle in the pronotum to create a strong pressure. As observed with other eucnemid species, *N. penetrans* will quiver their extended antennae. No theory has been explained with regards to the uniqueness of that behavior present in Eucnemidae in relation with other families within the superfamily Elateroidea. Female beetles were observed ovipositing eggs on wood surfaces and sides of the vial. Triungulins hatched from 9–11 June 2007 when they were observed crawling on surfaces of the vial and wood pieces, two weeks after eggs were laid by reared adults.

Nematodes penetrans selects hard, firm wood to lay eggs either on the trunk or on large lateral branches. Larvae tunneling in softer wood or near the base of a dead tree were not observed during any collecting trips in northeastern Wisconsin. Eggs hatched approximately two weeks later. Free-living triungulins were observed crawling using ventral side of the ninth abdominal segment and hair-like legs to keep them firmly attached on surfaces, in search of a suitable opening. Once triungulins burrow into a suitable crevice or opening, their body contracts through peristalsis, allowing the immature beetle to enter the crevice.

Larvae spent the rest of their time burrowing between the fibrous layers in the sapwood. Van Horn (1909) observed that the related *N. atropos* do not construct galleries inside the wood. My observation is somewhat similar to Van Horn's, except I did find faint indications of galleries in the sapwood when collecting larvae of *N. penetrans*. Larvae were observed burrowing through several layers in the sapwood. It was difficult to obtain an undamaged specimen, when extracting larvae in hard, seasoned wood. Otto (2012a, 2012b) found several larval eucnemid species utilizing the same log. *Nematodes penetrans* were found in an isolated part of the log consisting of hard, firm sapwood. Larvae of *Rhagomicrus bonvouloiri* (Horn) and *Deltometopus amoenicornis* (Say) were present in same log consisting of softer, white rotten, moist sapwood.

Mature 5th instars transition into a prepupal larval stage. I observed prepupal larval forms have reduced prothoracic T-shaped scleromes. Areoles and microtrichial patches are absent on both surfaces of each abdominal segments. Segments contracted and became shorter. Prepupal larval forms are darker in coloration. They assume a U-shaped position inside the pupal chamber, about an inch beneath the surface. Pupation requires approximately 14–21 days before eclosion. Development may take at least one to two years to complete.

Discussion

Based on larval illustrations and descriptions provided by Leiler (1976), Mamaev (1976), and Burakowski (1991) as well as specimens received from Tamás Németh, *N. penetrans* is best separated from the European *N. filum* by the shape and size of microtrichial patches on each thoracic and abdominal segment, as well as size of areole on the ninth abdominal terga. Microtrichial patches for *N. filum*

are smaller and more rounded. *Nematodes penetrans* have larger, oval shaped microtrichial patches. Areoles are larger and more prominent on all nine abdominal segments for *N. penetrans*. *Nematodes filum* dorsally has smaller areoles on the eighth and ninth abdominal segments and ventrally from the seventh to the ninth abdominal segments. *Nematodes penetrans* have dorsal mesothoracic microtrichial patches present on both sides of the medial sclerotized line with a pair of offset circular patches on the metathoracic segment. *Nematodes filum* also has dorsal mesothoracic microtrichial patches on both sides of the medial sclerotized line with a pair of kidney-shaped microtrichial patch on the metathoracic segment. Relative lengths between these two species are significant. 5th instar larvae of *N. filum* are shorter, 15 mm long, whereas *N. penetrans* are longer, 20–25 mm long.

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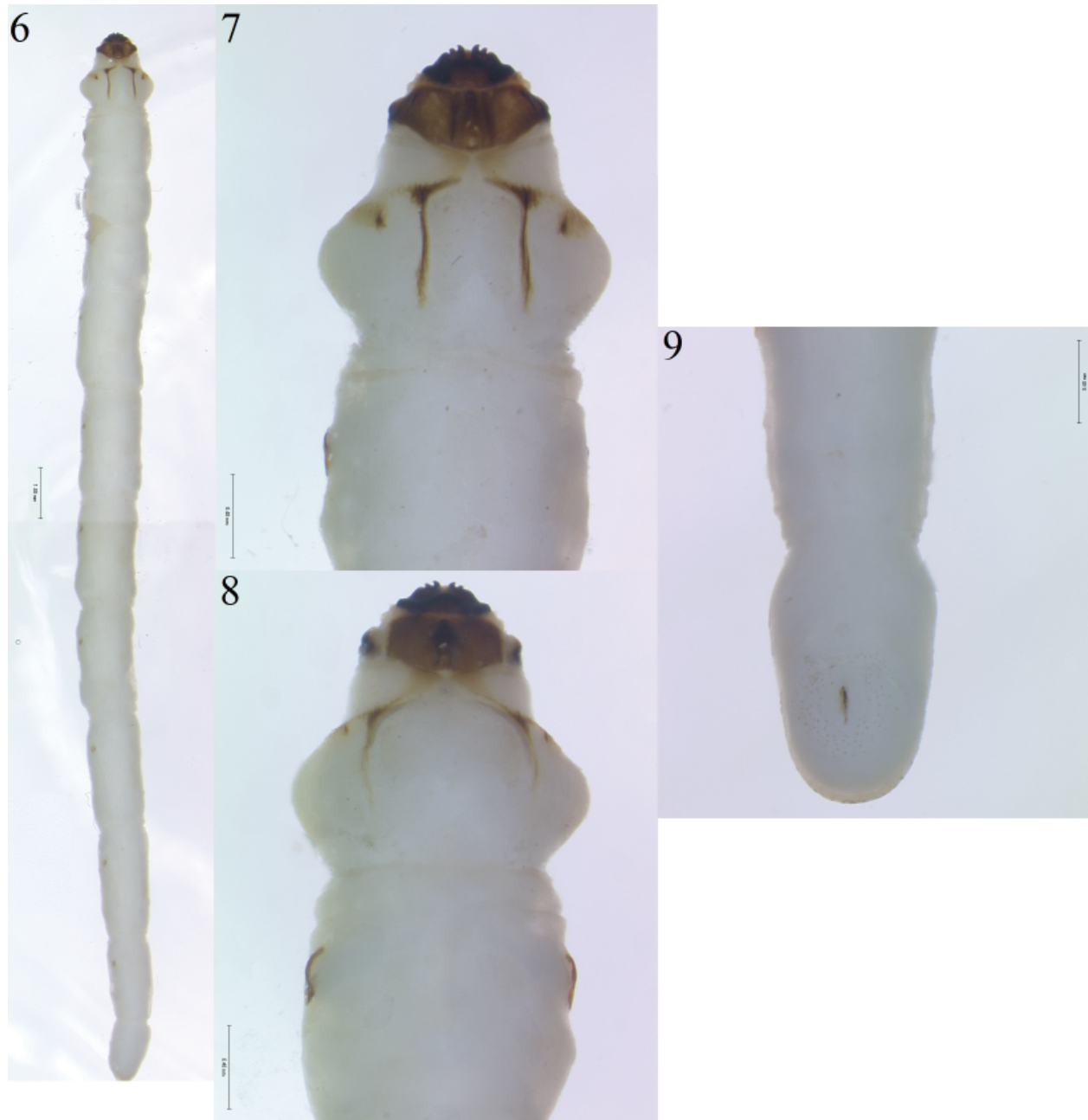
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Figures 1–5. *Nematodes penetrans*, triungulin and 5th instar. **1)** Triungulin, dorsal view. **2)** Dorsal habitus. Scale line = 1.0 mm. **3)** Head and thoracic regions, dorsal view. **4)** Head and thoracic regions, ventral view. **5)** Abdominal segments VII–IX, ventral view. Scale line = 0.4 mm.



Figures 6–9. *Nematodes penetrans*, prepupal stage. **6)** Dorsal habitus. Scale line = 1.0 mm. **7)** Head and thoracic regions, dorsal view; **8)** Head and thoracic regions, ventral view; **9)** Abdominal segments VII–IX, ventral view. Scale line = 0.4 mm.

