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Description of the larva and pupa of *Nasiternella regia* Riedel, 1914 (Diptera: Pediciidae) from Slovakia, with notes on ecology and behaviour

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Abstract: The previously unknown larvae and pupae of *Nasiternella regia* Riedel, 1914 (Diptera, Pediciidae) are described and illustrated from specimens collected in water-filled tree holes in deciduous forests in Slovakia. Brief comments on their ecology and behaviour are provided. Comparisons are made to the larvae of *Nasiternella varinervis* (Zetterstedt, 1851) as described by Krivosheina (2009).

Key words: Pediciidae; *Nasiternella*; larva; pupa; water-filled tree holes

Introduction

Water-filled tree holes (Figs 18, 19), called dendrotelmata, are aquatic microhabitats (e.g., Röhnert 1950; Kitching 1971, 2004) that are unique because of extreme variation in chemical characteristics and other factors (e.g., Walker et al. 1991; Léonard & Juliano 1995). They harbour, among other organisms, more or less specialized immature stages of Diptera and Coleoptera (Schmidl et al. 2008) that use them as breeding sites, and some can breed exclusively in those habitats (Yanoviak & Fincke 2005).

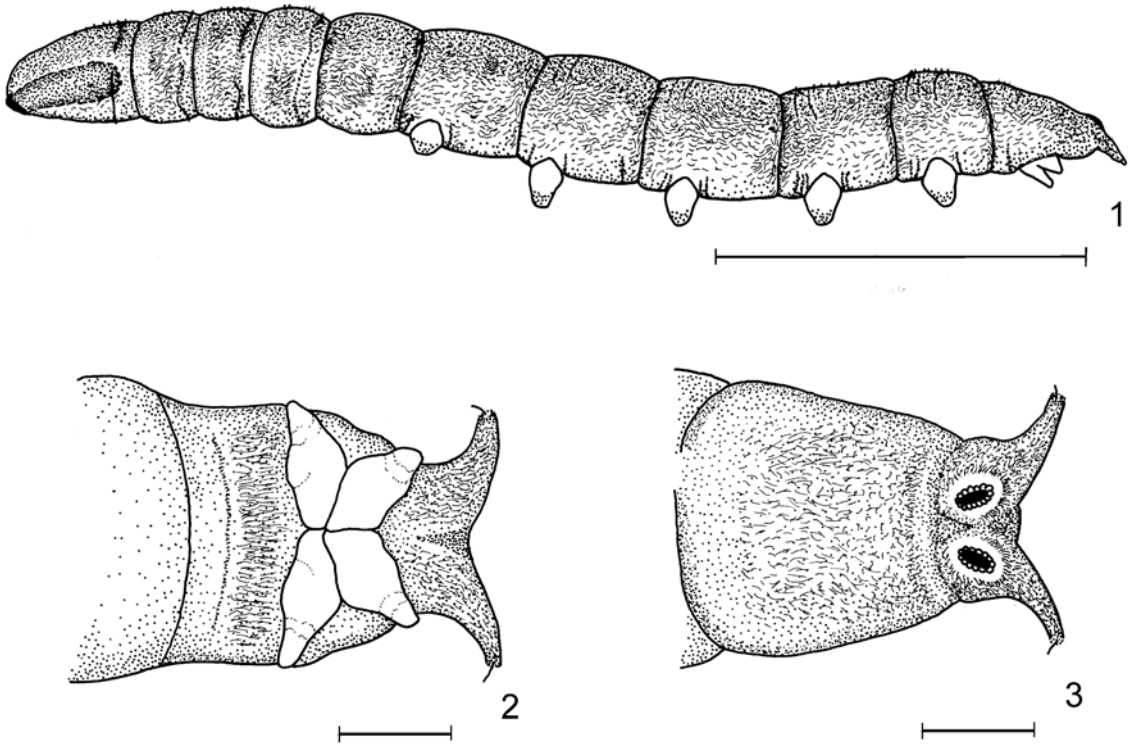
The larvae of the family Pediciidae are distinguished primarily by characteristics of the head capsule which is heavily sclerotized, narrow, rather elongate, with its lateral margins nearly parallel (Lindner 1959). Except for the mycetophagous genus *Ula* Haliday, 1833, the pediciid larvae are predatory, preying on oligochaetes, mites, insect larvae, and other invertebrates. They are predominantly aquatic or semiaquatic, moving to water margins or other drier places for pupation (Oosterbroek & Theowald 1991).

Nasiternella Wahlgren, 1904 is a small pediciid genus comprising only six species worldwide (Oosterbroek 2012). Two of them, *N. regia* Riedel, 1914 and *N. varinervis* (Zetterstedt, 1851), are Palaearctic, and both occur in Europe. *N. varinervis*, also known in the East Palaearctic, was recorded from Norway (Zetterstedt 1851; Mannheims 1967), Slovakia (Starý 1993), and Ukraine (Savchenko 1986). Quite recently, the larva of this species was described by Krivosheina (2009) from specimens collected in the Primorskiy Territory and Kunashir Is., Russia. *N. regia*, only known in Europe and hitherto recorded for Albania, Austria (Lackschewitz 1940), and Romania (Riedel 1914), is reported here from Slovakia for the first time. Until now, the larvae and pupae of this species were unknown. These are described here, and additional information regarding their ecology and behaviour is provided. The adults obtained during this research will be listed, redescribed, and discussed in a forthcoming paper.

Material and methods

Since 2010, the first author has collected unknown pediciid larvae in water-filled tree holes in deciduous forests in Slovakia. These were later identified by the second author as *Nasiternella regia,* based on the adults both reared from the larvae and caught in the field. The tree hole sampling was carried out in the immediate vicinity of the village Diviacka Nová Ves (district Prievidza) at two localities: site 1 (as referred to in Material examined paragraph): an oak forest, (old forest dominated by oak, area approximately 20 ha), northeast of the village, with 8 sampled tree holes, 320 m a.s.l., 48°45′25.1″ N, 18°30′43.5″ E; site 2: a beech forest (beech monoculture, with old trees at margins, area approximately 140 ha), west of the same village, with 2 sampled tree holes in old trees, 340 m a.s.l., 48°44′51.89″ N, 18°28′57.5″ E. All tree holes were to maximum height of 1 m from the ground, with water capacity of 5–30 liters. More than 5 sampled tree holes completely lost water at least once a year. The sampling was conducted in 2010 and 2011, in two-week intervals throughout the year (except for intermittent periods of frost) for site 1, and in spring and autumn for site 2. Eight largest larvae were placed in rearing units (see below), others were preserved in 70% ethanol.

Almost any container with rainwater and a small amount of leaf litter can function as an artificial tree hole.



Figs 1–3. *Nasiternella regia* Riedel, 1914, last-instar larva: 1 – general view, lateral; 2–3 – terminal abdominal segments, ventral (2) and dorsal (3). Scales 10 mm (Fig. 1), 1 mm (Figs 2, 3).

Tree hole analogues with varying degrees of realism can be constructed, for example, from plastic pots (e.g., Yanoviak & Fincke 1992). For rearing the larvae of *N. regia* under laboratory conditions, rearing units were created, each consisting of two containers: a smaller, wide-necked glass bottle (4.5 cm diameter) placed in a larger, plastic bottle crosscut in half (8 cm diameter). The glass was filled with tree hole sediment topped with tree hole water to the brim, and placed in the lower part of the plastic bottle. The space between the containers was then filled with drier tree hole sediment. The larvae of *N. regia* along with their prey were placed in the glass. The unit was closed by putting the upper part of the plastic bottle back on the lower part. For long-term rearing, leaf litter and tree hole sediment must be added periodically as nutrients for prey and additional prey must be supplied. Full-grown larvae leave the glass and pupate in the drier sediment in the space between the containers.

The terminology of morphological features generally follows that of Lindner (1959), Byers (1961), and Oosterbroek & Theowald (1991).

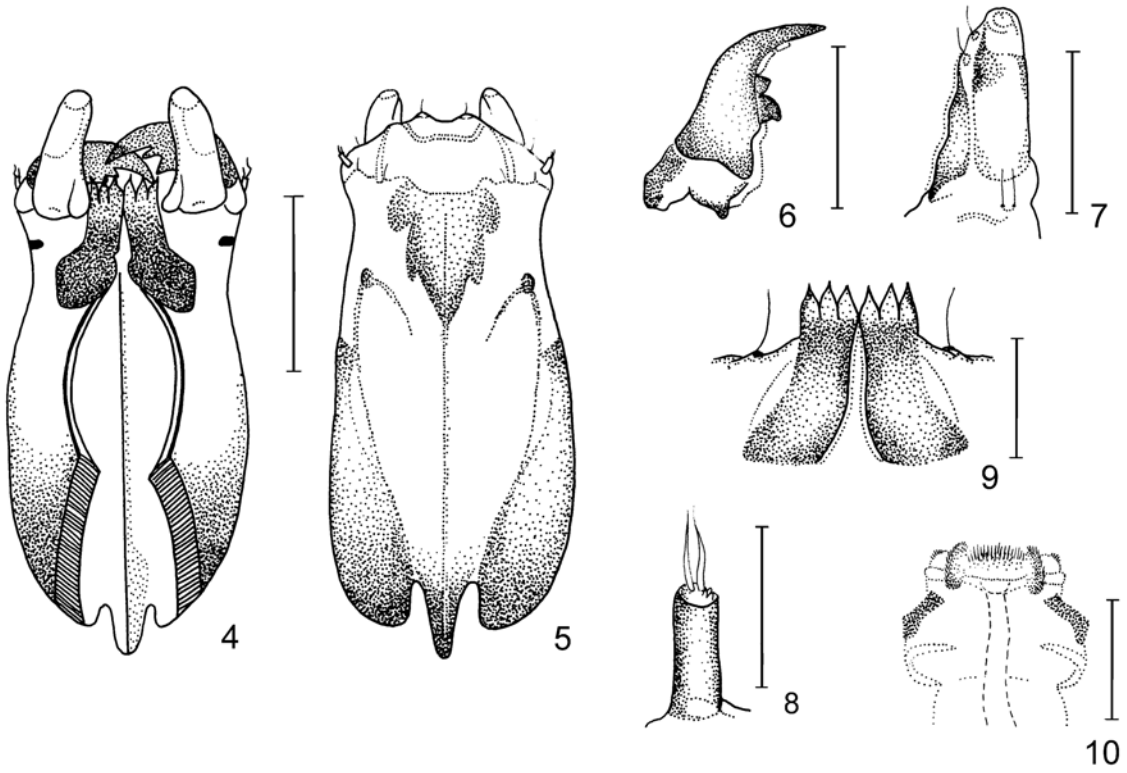
Descriptions

Nasiternella regia Riedel, 1914

Material examined: Slovakia: Diviacka Nová Ves (distr. Prievidza): Site 1, 14.IX.2010, 1 (♀) pupa, 9.X.2010, 1 (♂) exuvia, 19.XI.2010, 1 larva, 17.XII.2010, 1 larva, 14.I.2011, 3 larvae, 26.III.2011, 2 larvae, 9.IV.2011, 1 (?) exuvia, 21.V.2011, 2 larvae, 16.VII.2011, 1 larva, 23.IX.2011, 2 (♂,?) exuviae, 27.IX.2011, 1 (♀) pupa, 5 (2♂♂, 2♀♀,1?) exuviae, 9.X.2011, 1 (♀) exuvia, 23.X.2011, 1 larva, 18.XI.2011, 2 larvae, 2.XII.2011, 1 larvae, 16.XII.2011, 1 larva; site 2, 6.VII.2011, 1 larva, 18.VII.2011, 1 larva (all leg. J. Oboňa); all in coll. J. Oboňa, Faculty of Ecology and Environmental Sciences, Zvolen, Slovakia.

Last-instar larva (Figs 1–10). Hemicephalic, metapneustic. Body with distinctly separated segments, dark brown dorsally, paler ventrally, densely covered with comparatively long, wavy pubescence appressed to surface, sometimes with velvety or silvery iridescence, depending upon light aspect. Irregular, transverse rows and groups of microscopic, black, suberect spinulae present among the pubescence (Fig. 1). Body length 30–32 mm, body breadth 3.0–3.2 mm (without pseudopods).

Head capsule (Figs 4–10). Length 3.0–3.3 mm, breadth 1.0–1.2 mm. Well-sclerotized, massive, comparatively narrow and elongate (typical for Pediciidae), about three times as long as broad, partially retracted within thorax. Lateral margins of head capsule parallel, heavily sclerotized posteriorly. Frontoclypeal suture shallowly concave. Lateral margins of frontoclypeal apotome with numerous setae and pale rounded sensillae anteriorly. Dorsal suture present, with conspicuous sculpture on both sides. Labrum saddle-shaped, with setae and papillae on its anterior margin. Base of labrum in form of sclerotized plate with cuneiform anterior projection. Antenna (Fig. 8) about three times as long as broad, with two distinct sensory setae at apex, slightly shorter than antennal segment. Mandible (Fig. 6) curved, comparatively long and broad, with long, pointed, apical tooth and two short, roughly triangular teeth along inner margin. Maxilla (Fig. 7) promi-



Figs 4–10. *Nasiternella regia* Riedel, 1914, last-instar larva, head capsule: 4–5 – general view, ventral (4) and dorsal (5); 6 – left mandible, dorsal; 7 – right maxilla; 8 – antenna; 9 – hypostoma, ventral; 10 – prementohypopharyngeal complex, ventral. Scales 1 mm (Figs 4, 5), 0.5 mm (Figs 6, 7, 9, 10), 0.05 mm (Fig. 8).

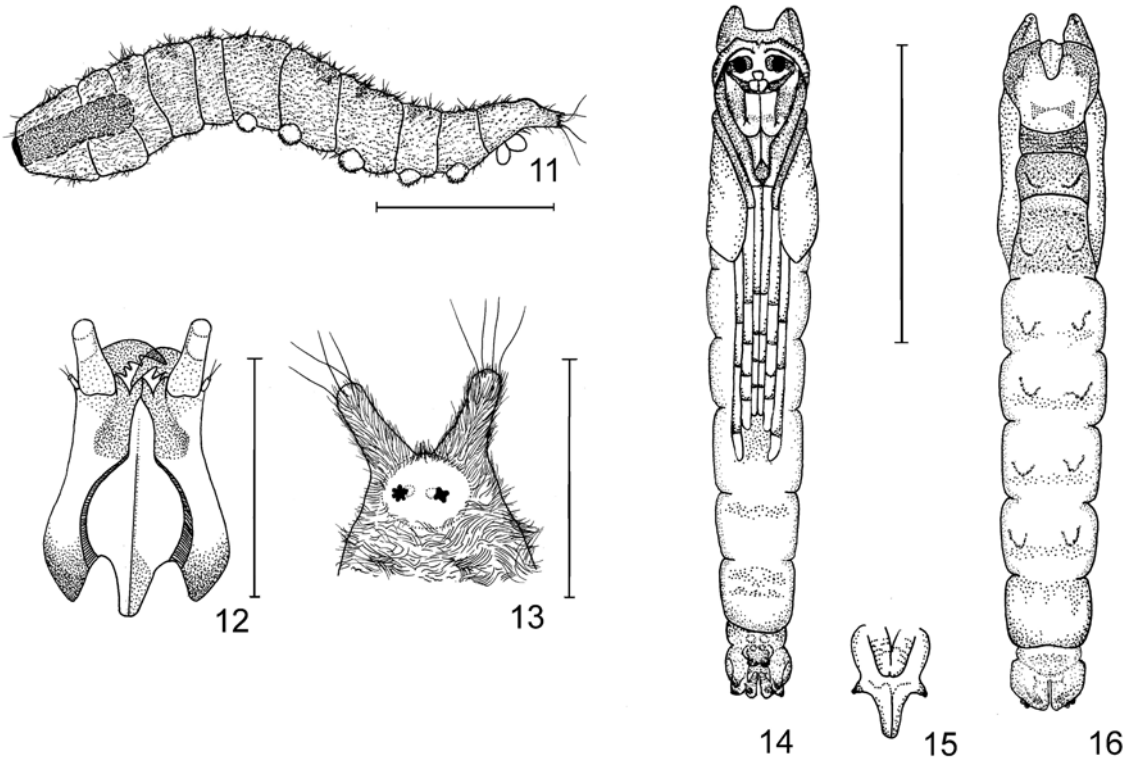
nent, elongate, with outer lobe (palpiger) longer than inner lobe (galea). Hypostoma (Fig. 9) divided into two hypostomal plates, each bearing three narrow, sharp, apical teeth, subequal in size. Prementohypopharyngeal complex (Fig. 10) covered with dense setae, with lateral projection anteriorly on each side.

Thoracic segment 1 longer than broad, two other thoracic segments and abdominal segment 1 much shorter, about half length of thoracic segment 1.

Abdominal segments 3–7 with ventral creeping welts concave in middle, laterally elevated into conspicuous, yellow pseudopod (Fig. 1), with numerous, sclerotized, curved, pale spinulae at apex. Pseudopods on abdominal segment 3 about half as long as others. Pseudopods on abdominal segments 4–7 approximately one third length of body breadth. Spiracular disc (Fig. 3) with pair of oval, slightly elevated spiracles; centre of spiracle almost black, outer part brown. Each spiracle surrounded by pale, glabrous ring. Spiracular lobes two, each slightly more than twice as long as its basal breadth, truncate and bearing short seta at apex. Anal field (Fig. 2) surrounded by four white, fleshy, conical anal papillae, anterior pair slightly longer.

First-instar larva (Figs 11–13). Body transparent, light grey, anterior part slightly darker. Body surface densely covered with pubescence, relatively longer than that of last-instar larva and protruding, with transverse patches of longer hairs giving larva tousled appearance (Fig. 11). Body length 2–3 mm, body breadth 0.1–0.2 mm. Head capsule (Fig. 12) massive, sclerotized anteriorly, differing from that of last-instar larva by mandible bearing three teeth along inner margin and each hypostomal plate with only two apical teeth. Pseudopods on abdominal segments 3–7 shorter relative to those of last-instar larva, subequal in length (Fig. 11), with apices densely covered with numerous curved spinulae. Spiracular disc (Fig. 13) transversely oval, with two small, dark spiracles, spiracular lobes each with four long apical setae. Anal field (Fig. 11) surrounded by four quite clear, bulbous anal papillae, about as long as spiracular lobes.

Pupa (Figs 14–16). Male pupa: body length 22–25 mm, body breadth 3.0–3.5 mm; female pupa: body length 27–29 mm, body breadth 4.0–4.5 mm. Body coloration yellowish brown, some dorsal parts distinctly darker, especially anterior and posterior portions of thorax and abdominal segments 1–2 (Fig. 16). Antennal sheath annulate, curved posterolaterally, with its apex close to mid-tibia laterally. Sheath of maxillary palpus short, directed anterolaterally. Respiratory horn dark, short, and broad, somewhat ear-shaped, with longitudinal suture and minute annulations along entire length. Apex of wing sheath reaching to posterior margin of abdominal segment 2. Leg sheaths with apices in oblique alignment ventrally on abdominal segment 5, with foretarsus shortest, reaching only to anterior margin of abdominal segment 5 (Fig. 14). Abdominal segments 1–7 without any tubercles or spines. Dorsal abdominal segments with U-shaped rows of black punctures on each side as in Fig. 16. Male pair of genital sheaths



Figs 11–16. *Nasiternella regia* Riedel, 1914, first-instar larva: 11 – general view, lateral; 12 – head capsule, ventral; 13 – terminal abdominal segment, dorsal. Pupa: 14, 16 – male pupa, general view, ventral (14) and dorsal (16); 15 – female terminal segments, ventral. Scales 1 mm (Fig. 11), 0.5 mm (Figs 12, 13), 10 mm (Figs 14–16).

Fig. 17. *Nasiternella regia* Riedel, 1914, pupal cocoon (detritus removed).

short, strongly curved ventrally at apex, or even recurved, each sheath with three darkly pigmented lobes (Fig. 14). Female pair of genital sheaths elongate, slightly curved dorsally, each sheath with darkly pigmented tubercle laterally (Fig. 15).

Biology. The larvae of *N. regia* were collected in water-filled tree holes in oak (Figs 18–19) (site 1) and beech (site 2) trees. Due to their considerable size they probably represent one of top predators for such habitats. In the laboratory, they showed sensitivity to light, being active at twilight and by night and hiding during the day. They stayed attached to the water surface with their spiracular discs exposed to the air for breathing, while preying beneath the surface. Young instars often swam on the water surface. Full-grown larvae sometimes clung with their pseudopods to various objects just below the water surface. When preying, the larvae could almost double their lengths by extending their bodies and protruding their heads. They mainly fed on the larvae of flies (such as Culicidae) and beetles (Scirtidae) and even attacked drowning terrestrial invertebrates. They can survive in saturated tree hole sediment without free water. Only a single larva could be kept in each rearing unit, because, if there were two, the smaller one would either escape into the interspace where it died, or it died in the water. In a natural tree hole, however, three or more exuviae could be found. Larvae ready for pupation constructed a silken tube (Fig. 17), 35–40 mm in length and 3.5–4.8 mm in breadth, covered with small fragments of tree hole sediment. The construction of the cocoon and pupation took about one week. Pupae were motile, protruding their heads (usually only respiratory horns) from the pupal tubes, probably for breathing. They were very shy, quickly hiding in cocoons as a respond to any nearby movement or change of light. Duration of the pupal stage was about two weeks. Adults were observed in nature from late September to early October. First-instar larvae were found in mid-October. Thus, most probably, there is one generation per year.

Figs 18, 19. Oak water-filled tree holes in which five and one larvae, respectively, of *N. regia* were collected in 2011.

Of the eight larvae reared (see Material and methods) three yielded pupae (2 ♂♂, 1 ♀) and two developed to adults (2 ♀♀). Three larvae died. The two females emerged on 26.X.2011, i.e., about two weeks later than flies in nature.

Remarks. The larva of *N. regia* seems to be most closely related to that of *N. varinervis*, but no other larvae of *Nasiternella* are known. Based on the description of the larva of *N. varinervis* by Krivosheina (2009), which is presumably the last-instar larva, the larvae of the two species can be differentiated as follows: A full-grown larva of *N. regia* is about twice as large as that of *N. varinervis*, corresponding to the size of the adults. The mandible of *N. regia* has only one group of teeth along the inner margin (Fig. 6), whereas there is another group of teeth in *N. varinervis* (cf. Krivosheina 2009, Fig. 14), and the maxilla is distinctly more slender and longer in *N. regia* (cf. Fig. 7 and Krivosheina 2009, Fig. 15). The creeping welts are elevated laterally into conspicuous pseudopods in *N. regia*. There was no mention of such pseudopods in the description of *N. varinervis*. It can be assumed, therefore, that the pseudopods, if developed in *N. varinervis*, are relatively much shorter than those of *N. regia* (cf. Fig. 1 and Krivosheina 2009, Fig. 1). In *N. regia*, the spiracular disc has oval rings around comparatively large spiracles, with moderately long spiracular lobes (Fig. 3), whereas in *N. varinervis*, based on the figures by Krivosheina (2009, Figs 3, 4), the spiracular disc is transversely oval, the spiracular lobes seem to be shorter and the spiracles relatively smaller.

The two species also differ from each other ecologically. *N. varinervis* seems to occur in cool and humid places such as mountains, being associated with rotting wood of (mostly) fir trees as a saproxylic predator. *N. regia* prefers warm and drier places at lower altitudes being confined to deciduous (predominantly oak) trees that contain water-filled tree holes. As a saproxylic species that appears to be a specialized inhabitant of tree holes and seldom if ever breeds elsewhere, *N. regia* can be termed a dendrolimnetobiont (Röhnert 1950; Kitching 2004).

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