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Research paper

First description of the first instar larva of *Sphecapatoclea* and *Sphecapatodes* (Diptera: Sarcophagidae)

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ABSTRACT

The first instar larva is described for the first time for *Sphecapatoclea* Villeneuve and *Sphecapatodes* Villeneuve, two poorly known genera of Palaearctic Miltogramminae. Larval morphology was analysed for one species of each genus using a combination of light microscopy and scanning electron microscopy. The following morphological structures are documented: pseudocephalon, antennal complex, maxillary palpus, facial mask, modifications of thoracic and abdominal segments, anal region, spiracular field, posterior spiracles and details of the cephaloskeleton. Substantial differences between the two genera are noticed in the morphology of labrum and mouthhooks of pseudocephalon, spinulation, sculpture of integument and form of spiracular field. The first instar larva of *Sphecapatoclea* appears to have retained more features of the plesiomorphic ground plan defined for larvae of saprophagous calyptrate flies than the more derived larva of *Sphecapatodes*. Larval morphology points to a systematic position of *Sphecapatoclea* within the tribe Phyllotelini, while *Sphecapatodes* is hypothesized to have a close relationship with the non-phylloteline clade *Metopodia* Brauer & Bergenstamm + *Taxigramma* Macquart.

1. Introduction

Larvae of Palaearctic Sarcophagidae are relatively well studied in comparison to many other groups of brachyceran Diptera (Pape, 1998). This statement is not restricted to the numerous species of flesh flies of medical and veterinary importance in the subfamily Sarcophaginae, but also concerns the biologically diverse Paramacronychiinae and the mostly kleptoparasitic Miltogramminae (Szpila, 2010). However, in case of the last subfamily, available information is almost entirely restricted to genera represented in the European fauna (Szpila, 2010). The few exceptions are the descriptions of larval instars of *Beludzhia* Rohdendorf, *Chorezmomyia* Rohdendorf and *Eremasiomyia* Rohdendorf (Verves, 1988; Szpila and Pape, 2007; Pape and Szpila, 2012). Preimaginal stages of several genera, which seem to be important for phylogenetic and evolutionary reconstructions, still remain unknown. Good examples are the genera *Sphecapatoclea* Villeneuve and *Sphecapatodes* Villeneuve. Both genera, currently represented by 15 and 6 species respectively, are distributed in arid regions of the Palaearctic, with a peak of diversity in the Middle East and Central Asia (Pape, 1996; Zhang et al., 2014). Villeneuve (1909) suggested the two genera to be very closely related, and in the only major phylogenetic reconstruction of Miltogramminae, the genera *Sphecapatoclea* and *Sphecapatodes* were placed close to each other in the tribe Phyllotelini (Verves, 1989). Nothing is known about the breeding habits of their preimaginal stages but recent publications and unpublished data point to the possibility of broad necrophagy in a large subset of Phyllotelini rather than kleptoparasitism in hymenopteran nests, as typical for most other Miltogramminae (Szpila et al., 2010; unpublished).

The main aim of the present study is to provide the first description of the morphology of the first instar larva of *Sphecapatoclea* and *Sphecapatodes*, with a discussion of implications for the morphology-based phylogenetic system of Miltogramminae.

2. Material and methods

Larval material was obtained by keeping wild-caught females under laboratory conditions. Females were collected in a dry stream bed, near the village Daraq, North Khorasan province, Iran (36°57′21″N, 56°10′42″E). To obtain larvae, freshly caught females were kept individually in 3 ml Eppendorf tubes with a finely perforated cover. During the following days, females, if gravid,

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spontaneously and repeatedly larviposited, and the first instar larvae were immediately collected and processed as explained below. All females were subsequently pinned and labeled. Identifications to genus were made using the keys of Rohdendorf (1971), Verves (1994) and Pape (1998). Currently, available identification keys (Rohdendorf, 1971; Zhang et al., 2014) will only provide identifications of males, and molecular libraries have not yet been built based on male specimens, effectively preventing identification from molecular barcoding. Also, from recently collected material (Szpila, unpublished) it is evident that several species new to science will have to be named and described before a stable taxonomy and nomenclature will be attained.

Larvae were killed by soaking in hot water (about 95 °C) to avoid deformation and stored in 70% ethanol. Preserved larvae were slide-mounted in Hoyer's medium for light microscopy. Preparation for SEM involved dehydration through 80, 90 and 99.5% ethanol and critical-point drying in CO_2. The larvae were eventually coated with platinum. SEM images were taken with the use of a JEOL JSM 6335F field emission microscope. Light microscope illustrations were produced from photographs made with the use of a digital NIKON 8400 camera mounted on NIKON ECLIPSE E200 microscope.

Examined females, all larvae, and associated microscope slides have been deposited in Chair of Ecology and Biogeography, Nicolaus Copernicus University, Toruń, Poland (CEB). Larval terminology follows Courtney et al. (2000) with a few modifications proposed by Szpila and Pape (2007) and Szpila (2010).

For testing of potential necrophagous breeding habit, ca. 20 larvae of each genus were transferred to small plastic containers (100 ml). The bottom of each container was covered by a thin layer (2 cm) of humid sand, on the surface of which was placed a small portion of chicken liver (20–30 g). Larvae were observed three times a day.

3. Results

The general habitus of the first instar of *Sphecapatoclea* sp. and *Sphecapatodes* sp. follows the general pattern for the Calyptrata, with the body being divided into a bilobed pseudocephalon (pc) equipped with the antennal and maxillary sensory organs, three thoracic segments (t1–t3), seven abdominal segments (a1–a7), and the anal division (ad) carrying the posterior spiracles (sp).

3.1. Sphecapatoclea *sp. (Figs. 1A–I, 2A–H and 5A–C)*

3.1.1. *Material examined*

19 larvae, larviposited by female labeled: "Iran, North Khorasan, Daraq 013, 1096m, N36 57 21.1 E56 10 42.5, 10 V 2014, Iran Milt6, leg. Iran Expedition".

3.1.2. *Pseudocephalon*

Antennal complex (an) large, antennal dome and oval, with rounded tip, antennal basal ring (abr) high (Fig. 1A–E); maxillary palpus (mp) shaped as a flat disc clearly distinguished from surrounding cuticle, first sensillum basiconicum (sb1) long with slightly swollen tip and shifted away from central cluster of sensilla toward medio-dorsal border of palpus, additional sensilla (ns1–2) large and both situated at level of adjacent surface of palpus dorsally to central cluster of sensilla (Fig. 1F); ventral organ (vo) on flat, fleshy lobe (Fig. 1A, C, D and G), edge of sensillar pit with a few conical protuberances (Fig. 1G and H); pseudocephalon laterally with broad, flat lobe ('cheek organ') (Fig. 1A, C and D); oral ridges (or) well developed (Fig. 1G); dorsal surface of pseudocephalon directly behind antennal complex with small cuticular warts, more posteriorly with longitudinal cuticular ridges (Fig. 1B).

3.1.3. *Cephaloskeleton*

Labrum (lb) straight but with anterior part bent down perpendicularly, tip pointed (Fig. 5A); mouthhook (mh) slightly curved, basal part with lateral arm (Fig. 5A), tip of mouthhook with row of 4–5 pointed teeth placed at an angle of about 30 ° to median plane of mouthhook (Fig. 1G); intermediate sclerite (is) slightly below parastomal bars (pb) in lateral view (Fig. 5A), in ventral view intermediate sclerite longer than wide (Fig. 5B); parastomal bars long (Fig. 5A and B); vertical plate (vp) slightly narrower than ventral cornu (vc) and broader than dorsal cornu (Fig. 5A); dorsal bridge absent.

3.1.4. *Thoracic segments*

Anterior spinose bands (asb) with from 3–4 (dorsal surfaces) to 11–12 (ventral surface of first segment) rows of spines, spines arranged separately from each other (Fig. 1A–D); lateral surface of first thoracic segment with aperture of anterior spiracle (as) (Fig. 1D); remaining area of thoracic segments with densely set cuticular ridges (Fig. 1A–D); Kelin's organ with short sensilla (Fig. 1I).

3.1.5. *Abdominal segments*

Anterior spinose bands on abdominal segments (a1–a7) with from 2–3 to 9–10 rows of spines, all bands complete (Fig. 5C); posterior spinose band (psb) on segments a1–a4 incomplete without spines on lateral and dorsal surfaces, complete on segments a5–a7; spines small and arranged separately from each other (Fig. 2A, B, D and E), spines on ventral and dorsal surfaces of segments similar (Fig. 2B and E); lateral creeping welts (lcw) developed and covered by spines; all abdominal segments with densely set cuticular ridges on entire surface (Figs. 2A, D, G and 5C).

3.1.6. *Anal division*

Anterior spinose band on anal division (ad) incomplete, without spines on dorsal surface (Fig. 2G); surface of anal division except for spiracular field with cuticular ridges (Fig. 2C and G); papillae (p1–p7) around spiracular field clearly visible as flat protuberances with an apical sensillum (Fig. 2F and G); spiracular field ringed by hair-like spines (Fig. 2F, G and H); posterior spiracles (sp) with four small peristigmatic tufts each with a few (1–3) branches; anal papillae rounded (Fig. 2C and G); anal tuft (at) with numerous spines (Fig. 2F and G).

3.1.7. *Remarks on breeding*

All first instar larvae deposited on chicken liver started to feed on this breeding medium. However, after moulting to the second instar, all larvae eventually died without reaching further developmental stages.

3.2. Sphecapatodes *sp. (Figs. 3A–J, 4A–I and 5D–F)*

3.2.1. *Material examined*

15 larvae, larviposited by female labeled: "Iran, North Khorasan, Daraq 013, 1096m, N36 57 21.1 E56 10 42.5, 10 V 2014, Iran Milt 3, leg. Iran Expedition".

3.2.2. *Pseudocephalon*

Antennal complex (an) large (Fig. 3A, B, D and E), antennal dome (and) parallel and robust with rounded tip (Fig. 3E), basal ring (abr) high (Fig. 3E); maxillary palpus shaped as a flat disc clearly distinguished from surrounding surface of pseudocephalon, first sensillum basiconicum long with swollen, spherical tip (Fig. 3F), first sensillum basiconicum shifted from central cluster of sensilla to dorsal periphery of palpus, second additional sensillum (ns2) shifted to a level below the adjacent surface of maxillary palpus (Fig. 3F); ventral organ on flat, fleshy lobe (Fig. 3B and G); oral