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DESCRIPTION OF THE PUPARIUM AND REDESCRIPTION OF THE THIRD-INSTAR LARVA OF *BRACHYOPA PANZERI* (DIPTERA, SYRPHIDAE) WITH NEW DATA ON ITS BIOLOGY

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Description of the Puparium and Redescription of the Third-Instar Larva of *Brachyopa panzeri* (Diptera, Syrphidae) with New Data on its Biology. Shparyk, V. Yu., Zamoroka, A. M. — In this study, we report the rearing of *Brachyopa panzeri* Goffe, 1945 from larva and elucidate its life circle with a duration of the pupal stage for the first time. We describe the puparium of *B. panzeri* of which the morphology was unknown prior to the current study. The study of the larva of *B. panzeri* showed discrepancies between our observations and its original description. These allow us to redescribe the larva, explaining the uncertainties that have existed in the literature since the description of the larva. We clarify larval morphology and propose new characters for identification with the map of its chaetotaxy. Morphological features of the larva and pupa are illustrated using both light- and electron microscopy. Additionally, we reared the endoparasitoid wasp *Tetrastichus brachyopae* Graham, 1991 (Hymenoptera: Eulophidae) from *B. panzeri* for the first time. Moreover, *T. brachyopae* is recorded from Ukraine for the first time.

Key words: Syrphidae, immature morphology, larvae, puparium, Tetrastichus, Brachyopa, Ukraine.

Introduction

Brachyopa Meigen, 1822 is a widespread Holarctic genus which includes at least 33 species (Pérez-Bañón et al., 2016). Currently, three major hotspots of *Brachyopa* diversity have been identified. These include Europe, the Far East and western North America (Pérez-Bañón et al., 2016). The species diversity of *Brachyopa* in Europe had been insufficiently studied until a series of revisions which gradually increased the number of known species (Thompson, 1980; Pérez-Bañón et al., 2016). Twenty-two species are known in Europe so far (Speight, 2020). Thompson & Rotheray (1998) also included *Hammerschmidtia* Schummel, 1834 in the genus *Brachyopa* due to very similar morphological features of their imago and larva. An on-going study of the hoverfly fauna of the Ukraine has found eight species of *Brachyopa* and one species of *Hammerschmidtia* (Prokhorov et al., 2018, 2020).

Brachyopa contains small to medium-sized (5–10 mm) hoverflies with an unusual brown body colour that makes them look like fly species of Scathophagidae, Anthomyiidae, Muscidae, Dryomyzidae and Heleomyzidae (Bartsch, 2009). The key morphological features of adult *Brachyopa* include a short, rounded abdomen; wing vein M_1 ending on R_{4+5} in an acute angle; and the basoflagellomere round to oval, often with clearly visible sensory pit. Unlike other genera of Syrphidae, most *Brachyopa* species are rarely observed on flowers. They are often found close to their larval microhabitats, such as sap runs and other decaying material under the bark or inside the cavities of trees (Speight, 2020; van Steenis et al., 2020).

The larvae of *Brachyopa* inhabit tree cavities with accumulations of wet rotting sap, under tree bark with decaying accumulations (*Brachyopa dorsata* Zetterstedt, 1837, *Brachyopa pilosa* Collin, 1939, *Brachyopa vittata* Zetterstedt, 1843, *Brachyopa minima* Vujić & Pérez-Bañón, 2016) or in sap runs on the bark surface (e. g., *Brachyopa atlantea* Kassebeer, 2000*, Brachyopa bicolor* (Fallén, 1817), *Brachyopa insensilis* Collin, 1939, *Brachyopa scutellaris* Robineau-Desvoidy, 1843) (Krivosheina, 2005; Pérez-Bañón et al., 2016; Speight, 2020; van Steenis et al., 2020). *Brachyopa* larvae develop on wide range of host plants including *Abies* Mill., *Acer* L., *Aesculus* L., *Alnus* Mill., *Betula* L., *Fagus* L., *Fraxinus* L., *Larix* Mill., *Picea* A. Dietr., *Populus* L., *Quercus* L., *Taxus* L., *Tilia* L. and *Ulmus* Distant (Rotheray, 1993; Pérez-Bañón et al., 2016, Speight, 2020). *Brachyopa* larvae are very often associated with larvae of *Hylecoetus flabellicornis* (Schneider, 1791) (Coleoptera: Lymexylidae)*, Gnophomyia lugubris* (Zetterstedt, 1838) (Diptera, Limoniidae) and *Mycetobia pallipes* Meigen, 1818 (Diptera, Anisopodidae) (Krivosheina, 2005, 2019). In addition, the eulophid wasp *Tetrastichus brachyopae* is an exclusive parasitoid of the genus *Brachyopa*, reared from puparia of *B. pilosa B. bicolor and B. insensilis* (van Eck et al., 2016).

The immature stages are well known for a dozen species of *Brachyopa* worldwide. At present the larvae for eight of the twenty-two European species have been described (Krivosheina & Mamaev 1967; Rotheray 1991, 1996; Krivosheina, 2005, Pérez-Bañón et al., 2016). These include *B. bicolor, B. insensilis, B. pilosa*, *B. vittata*, *B. scutellaris*, *B. dorsata*, *B. panzeri* Goffe, 1945, and *B. minima*. Pérez-Bañón et al. (2016) discussed the larval morphology of *B. bicolor* and *B. pilosa*, greatly improved by it illustration. Krivosheina (2005) studied the morphology of *Brachyopa* larvae and provided the most complete key for their identification, including six European species. It should be noted, that Krivosheina (2005) has drawn her conclusions on the larva of *Brachyopa* sp. aff. *panzeri* without imago rearing and puparium description. Furthermore, she concluded that the described larva belongs to *Brachyopa* sp. aff. *panzeri* only on the fact of the imago records in the area. Finally, van Steenis et al. (2020) have included Krivosheina's claims to their key of the third-instar larvae of the European species of *Brachyopa* and *Hammerschmidtia*. Finally, van Steenis et al. (2020) included Krivosheina's claims in their key of the third-instar larvae of the European species of *Brachyopa* and *Hammerschmidtia*.

In the current paper we present the results of a study on the biology of *B. panzeri* and the morphology of its preimaginal stages. We provide a description of the puparium of *B. panzeri* for the first time, and re-describe its third-instar larvae with a map of its chaetotaxy.

Material and methods

Abbreviations of institutions:
PUIF — Vasyl Stefanyk Precarpathian National University, entomological collection, Ivano-Frankivsk, Ukraine.
SIZK — Schmalhausen Institute of Zoology, NAS of Ukraine, Kyiv, Ukraine.
KNU — Taras Shevchenko National University of Kyiv, Kyiv, Ukraine.

Studied material. *Brachyopa panzeri*, Ukraine: Khomiakivka, Ivano-Frankivsk Region, 48.866397 N, 24.813515 E, 11 larv. 08.03.2019 (V. Shparyk, A. Zamoroka). 1 ♂, 2 ♀ (ex larvae) 25.03.2019, 2 ♂, 2 ♀ (ex larvae) 28.03.2019, 1♂ (ex larvae) 01.04.2019. Specimens are deposited in PUIF.

Pre-imaginal morphological studies. Eleven third-instar larvae were collected on 08.03.2019 from a crack in a pedunculate oak stump (*Quercus robur* L.), filled with rotten wood. Larvae were carefully removed from the site together with natural substrate and transported to the laboratory. We placed them in a plastic box with small amounts of the decaying wood. After the larvae pupated (13.03.2019) they were placed separately in tubes with cotton lids for air circulation. Further incubation took place in cool and dark conditions at a temperature of 16 °C during two weeks. The first imago emerged from the pupae on 25.03.2019 and the last specimen reared on 01.04.2019. Two larvae did not pupate, one of which was infested with a parasitoid wasp, *T. brachyopae*. Twelve specimens of *T. brachyopae* emerged on 07.04.2019.

One third-stage larva was preserved in ethanol and prepared for further studies. For permanent preservation the larva was slowly boiled in water for about two minutes and immersed in 70 % alcohol.

All general morphometric parameters (length, width of larva body) were measured at living specimens using a binocular microscope Nikon SMZ-1B with an eyepiece micrometer. Morphometric data are presented in a format of average and one standard deviation. Photographs of immature stages were taken with a trinonocular microscope Leica Z16 APO microscope equipped with a Leica DFC 450 camera and processed by LAS Core software (SIZK). The pictures of anterior and posterior spiracles were taken with a scanning electron microscope Tescan Vega 3 operated at 20 kV.

The terminology on larval morphology and chaetotaxy mapping follows Hartley (1961) and Rotheray et al. (1991, 1993). Reference to a particular sensillum is made thus: MsL1: where Ms (and P, Mt or A1) refers to the name of segment (P — prothorax, Ms — mesothorax, Mt — metathorax, A1 — abdominal segment and it number) and L_1 (or D, V) to L — lateral, D — dorsal and V — ventral position and number of sensilla.

Adult identification. Regarding the adult's identification, we followed Bartsch (2009), Haarto & Kerppola (2007) and Van Veen (2010). To identify *T. brachyopae* we used the characteristics given from the original description following the morphological terminology of Graham (1987, 1991).

Results

Third larval instar of *Brachyopa panzeri*

Overall characters. Brown to pale in colour, brown-grey dorsally, pale ventrally, basal plate dark. Length 7.3 ± 0.7 mm, width 3.1 ± 0.2 mm (n = 8). Body dorsoventrally flattened, rounded anteriorly and tapered posteriorly, with well sclerotized breathing tube. Dorsal and lateral surface covered by sclerotized setae and spicules (fig. 1). Lateral surface of metathorax and abdominal segments with two sensilla, which increase in size in abdominal segments 5–7 and anal segment. The ventral surface vestiture consisting of pale spic-

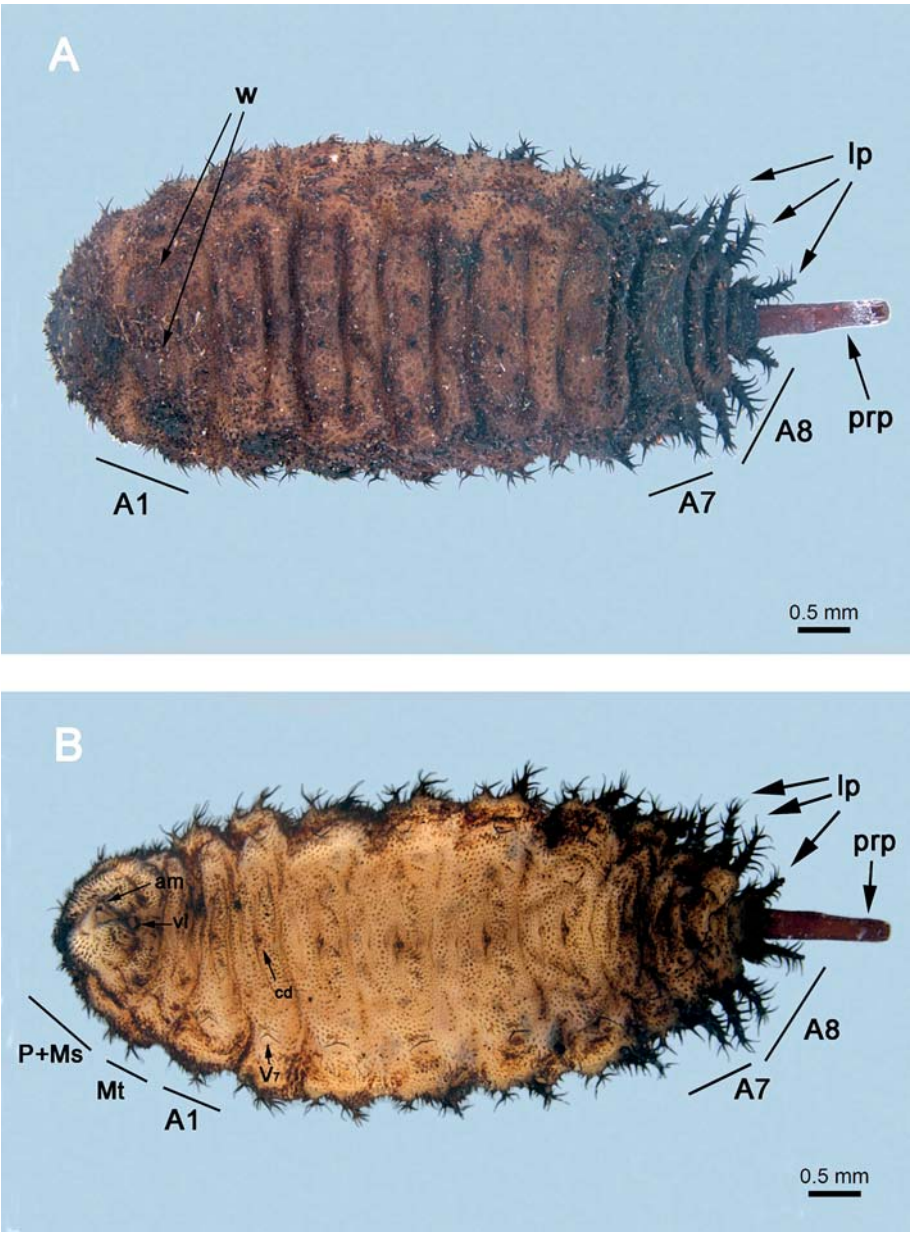

Fig. 1. The dorsal (**A**) and ventral (**B**) views of third-instar larva of *Brachyopa panzeri*. Labels: P — prothorax, Ms — mesothorax, Mt — metathorax, A1, A7, A8 segments of the body (segments A2–6 similar to A1); cd — central dark area, V_7 — ventral sensillum of second abdominal segment; prp — posterior breathing tube; lp — lappets, am — atenno-maxillary organ; vl — ventral lip; w — larval discs.

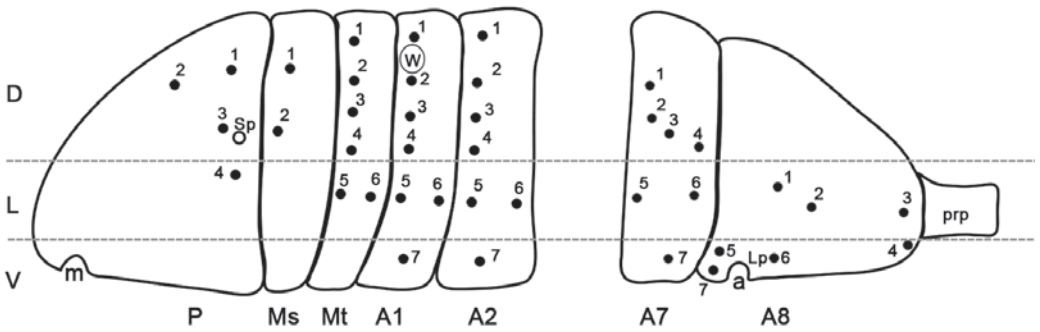

Fig. 2. Chaetotaxy map of the third instar larva of *B. panzeri* showing position and numbering of segmental sensillum (lateral view). Labels: D — dorsal, L — lateral, V — ventral projections; P — rothorax, Ms — mesothorax, Mt — etathorax, A1–A8 — abdominal segments (A3–A6 not shown); prp — posterior breathing tube; Sp — anterior spiracle; W — larval disc; Lp — lappets, m — mouth, a — anal opening.

ules not forming contrasting transverse rows. Ventral part of II–VI abdominal segments (A2–A6) with dark dot-like contrasting areas at the center (fig. 1). Prothorax (P) and mesothorax (Ms) with 6 pairs of sensilla; metathorax (Mt) with 6 pairs of sensilla. Abdominal segments A1–A7 with seven pairs of sensilla, anal segment (A_8) with three pairs of sensilla and four pairs of lappets (fig. 2).

Head and thorax. Mouth-hooks and mandibular lobes internal. Ventral lip forming a well-developed black lunula, contrasting against the background. Lateral lips rounded and covered ventrally by a dense tuft of long thin setae, which are short and thick dorsally and very close together over the dorsal lip. Dorsal lip not clearly visible. Antenno-maxillary organs well developed, located between mouth and dorsal surface of the prothorax. Dorsal surface of prothorax covered by hook-shaped spicules with a sclerotized black tip and a fleshy pale-coloured basal part. The spicules directed radially from the mouth opening. The second pair of sensilla situated near anterior spiracles on the lateral margin of prothorax. Spiracles cylindrical, about 2.5 times longer than wide.

The prothorax and mesothorax dorsally form a wide plate with multiple sensilla without fleshy papillae. The prothorax dorsally with two transverse rows (anterior and posterior) of sensilla without visible fleshy papillae. The anterior row consists of 2 pairs of sensilla with short setae, approximately the same size. This row of sensilla sits frontward of the anterior respiratory spiracles. The posterior row includes 1 pair of sensilla with 2 long and 2–5 short setae. The lateral sensillum of prothorax (PL_4) sits on a small fleshy papilla (fig. 2). Mesothorax with 2 pairs of dorsal sensilla (MsD_1, MsD_2). Metathorax with 4 pairs of dorsal (MtD_1, MtD_2, MtD_3, MtD_4) and 2 pairs of lateral (MtL_5, MtL_6) sensilla. The area between the sensilla of the pro- and mesothorax covered by blotches darker than the base colour. The border between prothorax and mesothorax is only clearly visible laterally and ventrally. The surface apically and laterally from the sensilla is covered with hook-shaped spicules. Ventral side of pro-, meso- and metathorax without sensilla (fig. 2).

Abdomen. Dorsal part of abdominal segments A1–A6 with 4 pairs of sensilla ($A1D_1$–$A1D_4$ to $A6D_1$–$A6D_4$). Segments A1–A6 dorsally with a distinct transverse groove. The metathoracic an abdominal sensilla D_1–D_4 sit on the anterior margin of Mt, A1–A6. Larval discs (w), which serve to allow protrusion of the anterior pupal spiracles, are situated between $A1D_1$ and $A1D_2$ (fig. 2). Abdominal segments A2-A6 with non-functioning spiracles between D_1 and D_2. Segments A1–A6 posteriorly with 2 transverse rows of black spicules. Dorsal integument in polygonal brown blotches, with clearly visible rows of dark setae. Sensilla $A1D_{1\text{-}4}$–$A6D_{1\text{-}4}$ with 2–3 long and 3–5 short setae and a small papilla. Sensilla $A7D_3$, $A7D_4$ with 2–6 thick lateral setae and a well-developed long papilla.

Sensilla $A1L_{5-6}$–$A7L_{5-6}$ with well-developed basal papilla. Papilla of sensillum L_6 1.5–2 times longer than sensillum L_5. Sensilla $A5L_6$–$A7L_6$ 5–7 large with 6–9 lateral setae (resemble lappets on the anal segment), directed to the posterior margin of the larvae (fig. 1).

The ventral surface of abdominal segments A1–A7 segments bearing 1 pair of sensilla with two apical long setae (fig. 2). Setae bicoloured, with pale base and black apex. Segments A1–A6 ventrally with a distinct transverse groove and black central blotch. Sensilla $A7D_3$ and $A7D_4$ with long, fleshy basal papillae (similar to $A8L_1$, $A8L_2$, $A8L_3$).

Anal segment (A8) about 1.5 times longer than A7, divided in anterior and posterior parts. Anterior part with 2 pairs of long lateral lappets $A8L_1$, $A8L_2$, bearing 8–12 lateral and 7–9 apical setae which vary in size. Posterior part bearing 1 pair of lappets $A8L_3$ with 9–16 apical setae of different size. The lateral surface of A8 with 3 pairs of simple sensilla ($A8V_4$, $A8V_5$, $A8V_7$) and 1 pair of anal lappets ($A8V_6$).

Posterior breathing tube long, at least 6 times as long as wide, dull on the base and lustrous on the apex. A spiracular plate sitting on the apex of breathing tube, with distinct morphological structures (fig. 3) including two central round scars, four pairs of spiracular openings and six pairs of branched inter-spiracular setae. First dorsal pair of spiracular openings C-shaped and approximately 40 μm long; second straight and short (10 μm); lateral third pair slightly S-shaped, and about 35 μm long. Ventral fourth spiracular openings straight, 30–35 μm long (fig. 3).

Puparium of *Brachyopa panzeri*

Overall description. Puparium elongated-ovate, flattened dorsoventrally, expanded anteriorly and narrowed posteriorly. Cuticle dull, reddish to dark brown, covered with dried remnants of fleshy projections bearing setae. Setae dark coloured, the same length as in the larvae. Posterior breathing tube sclerotized, dull and dark coloured at the base, lus-

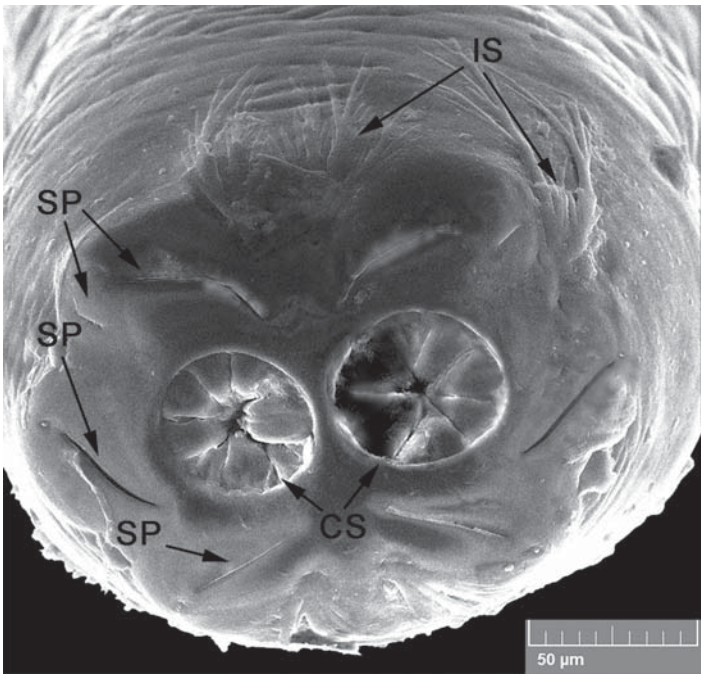

Fig. 3. Scanning electron microphotograph of larval spiracular plate on the apex of breathing tube of *Brachyopa panzeri* (SEM). Labels: CS — central scars; IS — interspiracular branched setae; SP — spiracular opening.

trous and light brown on the apex, translucent (fig. 4). Length including prp: 5.1 ± 0.8 mm; width: 3.1 ± 0.3 mm; height: 2.5 ± 0.3 mm (n = 8).

Pupal spiracles. Anterior pupal spiracles (fig. 4, A: arp) cylindrical (0.62 ± 0.02 mm long, 0.19 ± 0.03 mm wide, n = 8), slightly narrowed toward apex and slightly curved backward. Cuticle dull, reddish to light brown, densely tuberculate. Distinct tubercles 3–6 μm in diameter, arranged in subspiral rows (fig. 4, B). Large tubercles arranged in subradial longitudinal rows of 4–7 pieces. Spiracular openings slit-like,

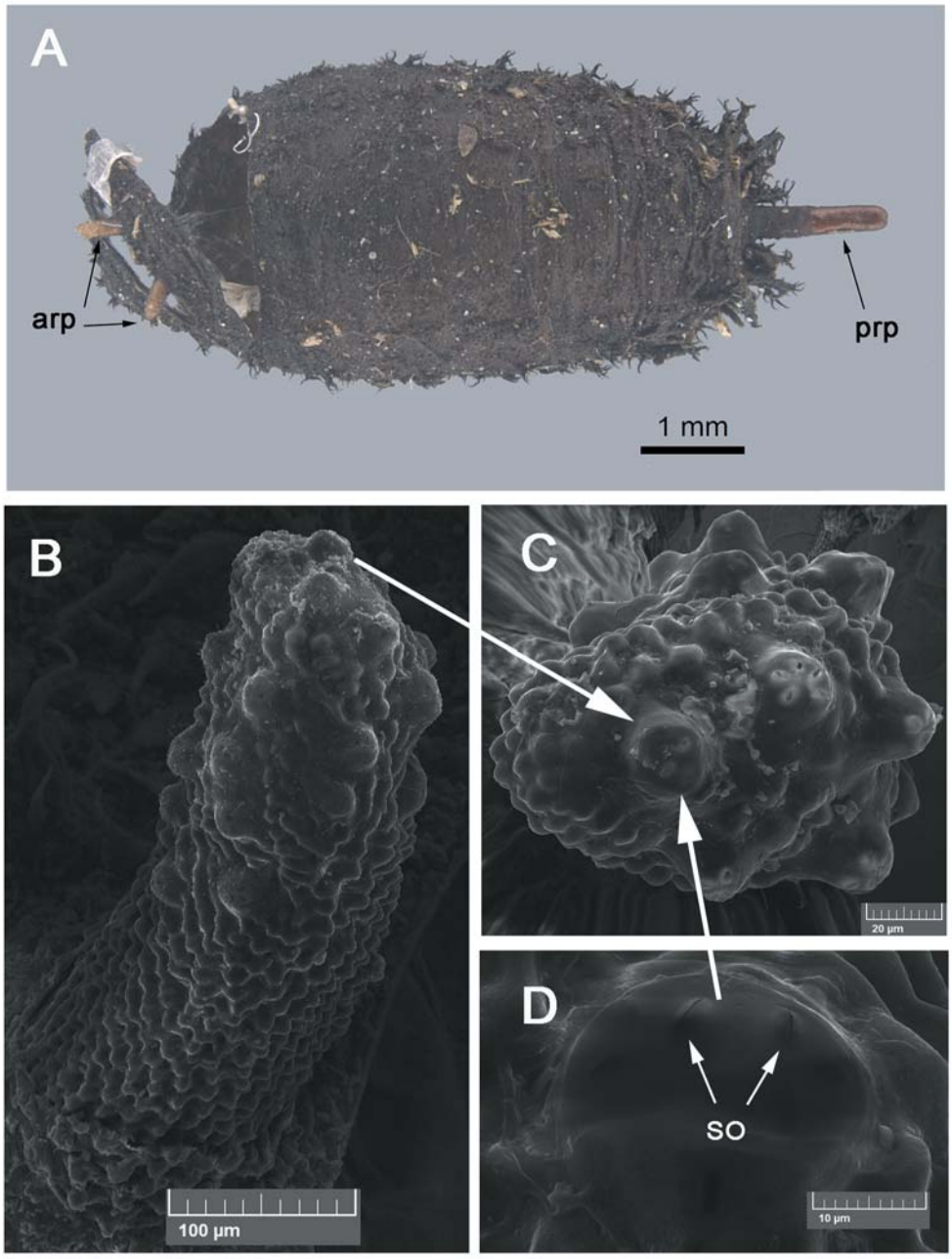

Fig. 4. Morphology of *Brachyopa panzeri* puparium. A — opened pupa in dorsal view; B — upal spiracle, lateral view; C — pupal spiracle, apical view; D — spiracular openings of pupal spiracles (SO).

3–7 grouped radially (sometime chaotically) on the apex of large tubercles (fig. 4, C). These slit-likes openings are located on oval prominences, which are longitudinally divided into two parts by slits (fig. 4, D: SO). Both spirals are based on the middle of the upper part of the operculum, separated by a distance of approximately the length of the spiracle.

Discussion

Our results fill significant gaps in the life cycle of *B. panzeri*, especially in the biology of the third-instar larva and pupal development. We reared adults *B. panzeri* for the first time, and estimated the duration of pupal development at 12–17 days. Our findings also shed light on morphological features of the preimaginal stages of *B. panzeri*. We described its puparium for the first time and redescribed the third-instar larva due to new data missed by previous authors (e. g., Krivosheina, 2005).

Krivosheina (2005) described larva of *B. panzeri* collected in the Eastern Carpathian Mountains (Ukraine). However, she did not support the description by rearing of the adult. She suggested that the described larva might be *B. panzeri* because (in her opinion) only two species of *Brachyopa* (i. e., *B. vittata, B. panzeri*) were recorded within the mentioned territory. This claim is repeated in Speight (2020) and van Steenis et al. (2020). However, we now know at least 6 species of *Brachyopa* occur in the Eastern Carpathian Mountains, including *B. dorsata*, *B. panzeri*, *B. pilosa*, *B. plena*, *B. testacea* and *B. vittata* (Prokhorov et al., 2018, 2020; Mielczarek et al., 2019). The immature stages of *B. plena* and *B. testacea* still remain unknown.

Our findings are different from Krivosheina's (2005) description of *B. panzeri* larva. She claimed that two apical setae on the lateral sensilla (A7–A8) are pale and recognized this as the diagnostic feature for *B. panzeri* larva. In fact, these setae are dark-coloured in our larvae. The colour of the setae might be an artefact, since Krivosheina described larvae from material stored for more than 40 years. Thus, it is not appropriate to use them as diagnostic features. We also found that the number of these setae is highly variable among the studied larvae (n = 8), varying from 2 to 4. This trait was missed by Krivosheina, although she examined 6 specimens of *Brachyopa* larvae. Instead, she stated there was to a constant number (2) of these setae.

It should be noted that Krivosheina (2005) used her own terminology, which is poorly consistent with the conventional one developed by Rotheray from Hartley (Hartley, 1961; Rotheray et al., 1991, 1993, 1996; Pérez-Bañón et al, 2016). For instance, she used the term "papilla" instead of both "sensilla" and "appet", and "appendage" instead of "seta". In addition, she did not separate abdominal segments 7–8, using instead the term "terminal plate". These cause big confusions in determining the position of the certain cuticular structures. We should clarify that the term "chaetotaxy" is used for the larval morphology of Syrphidae in a fairly broad sense (Hartley, 1961). Contrary to many other Diptera, hoverfly setose vestiture are represented by aggregations of sensilla, each with one or many setae around it. The eighth and sometimes the seventh abdominal segments are differently modified in many species, with laterally up to three pairs of fleshy protuberances or lappets at the end of the body (Hartley, 1961). The lappets of *Brachyopa* larva are homologous to sensilla on the other abdominal segments and morphologically very similar. Therefore, it remains unclear how to characterize these cuticular growths properly.

We also found certain differences between our findings and characteristics used in the key for identifying *Brachyopa* larva proposed in van Steenis et al. (2020). Van Steenis et al. (2020) generally follow Krivosheina's (2005) description using the following diagnostic characteristics: "Lateral papillae of posterior segment unequally sized, 5th and 6th as long as wide, 7th about 1.5 times longer than wide; 3rd and 4th pair of papillae short…". Our results showed that the lateral lappets ($A8L_1$, $A8L_2$,

Fig. 5. Female of *Tetrastichus brachyopae* reared from puparium of *Brachyopa panzeri* for the first time.

$A8L_3$) are approximately the same size (fig. 1), 3–4 times longer than wide. Only the lateral papillae of the seventh posterior segment are of different sizes. Thus, the mentioned characters in Krivosheina (2005) and van Steenis et al. (2020) are unsuitable for distinguishing the larvae of *B. panzeri* from *B. dorsata*. As a consequence, the diagnostic characters of the larvae of the *B. panzeri* and *B. dorsata* species group should be reconsidered, and subsequent descriptions and re-descriptions should be made using detailed mapping of the cuticular formations.

Additionally, we reveal a relationship of *B. panzeri* with parasitoid wasps. We reared 12 specimens of *Tetrastichus brachyopae* Graham, 1991 (fig. 5) from the puparium of *B. panzeri* for the first time. Moreover, our finding of *T. brachyopae* is the first record for Ukraine. We observed wasps emerging from the apical part of the puparium through a single opening, about 0.4 mm wide. All emerged specimens (n = 12) of *T. brachyopae* were females. The puparium cavity was not examined for dead eggs or specimens.

Tetrastichus brachyopae is a gregarious koinobiont endoparasitoid of *Brachyopa* species, with a clutch size varying from 7–18 specimens with a strong female bias (van Eck et al. 2016). To date, four species of *Brachyopa* are confirmed hosts for *T. brachyopae*. These include *B. pilosa* (Noyes 2016, Vidal, 1997), *B. bicolor* and *B. insensilis* (van Eck et al., 2016) and *B. panzeri* (this publication). *Tetrastichus brachyopae* is widespread within Europe (Hansson and Schmid, 2020) including Czech Republic (Graham, 1991), The Netherlands (van Eck et al., 2016), Sweden and Switzerland (Hedqvist, 2003), Georgia (Kostjukov and Japoshvili, 2016) and Ukraine (this publication).

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