Morphology of preimaginal stages of *Lipara pullitarsis* Doskočil & Chvála, 1971 (Diptera: Chloropidae) – a gall-forming fly in the common reed (*Phragmites australis*)

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All preimaginal stages of *Lipara pullitarsis* are described and illustrated. The facial mask, cephaloskeleton, spiracles and locomotory structures are considered. This is the first description of the first- and second-instar larva. The descriptions of the third-instar larva, egg and puparium are expanded. This paper forms a basis for a complete description of the life history of this fly.

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1. Introduction

The genus *Lipara* consists of 11 species (Nartshuk 1996), five of which ocuur in Europe: *L. lucens* Meigen 1830, *L. similis* Schiner 1854, *L. rufitarsis* Loew 1858, *L. pullitarsis* Doskočil & Chvála 1971, *L. baltica* Karps 1978 (Chvála et al. 1974, Nartshuk 1984).

Lipara pullitarsis was described in 1971 (Doskočil & Chvála 1971). In earlier papers, individuals demonstrating the key features of *L. pullitarsis* were not separated from *L. rufitarsis*. Both species were considered under the common name *L. rufitarsis* (Ruppolt 1957, Wendt 1968, Durska 1970, Doskočil & Chvála 1971). *Lipara pullitarsis* forms galls in the apical part of common reed stems [*Phragmites australis* (Cav.) Trin.] (Doskočil & Chvála 1971, Chvála *et al.* 1974, Skuhravý 1978, Pokorný 1981). It is a monophagous and univoltine fly that lays eggs on the surface of common reed stems. The larvae develop among leaf blades above the growing point. The imagines fly in reed beds from May to July (Chvála *et al.* 1974, Pokorný 1981). All developmental stages of *L. lucens* are known (Ruppolt 1957, Waitzbauer 1969) but for *L. pullitarsis*, *L. rufitarsis* and *L. similis* only the egg, third-instar larva and puparium have been documented (Chvála *et al.* 1974).

This paper describes the morphology of the previously unknown first- and second-instar larva of *L. pullitarsis* and presents previously unknown structural details of the egg, third-instar larva and the puparium. Thus, the paper contains a complete description of the morphology of the preimaginal developmental stages of *L. pullitarsis*.

2. Material and methods

All developmental stages of *L. pullitarsis* collected from the apical part of common reed stems were studied. Stems of common reed were col-

lected in reed beds in and around the town of Lublin in mid-eastern Poland in the years 1977–1979 and 2001–2004. Fresh stems of reed were collected at random in May and June and only those stems with galls visible in the apical part were collected outside of this period. The stems were sectioned in the laboratory using a stereoscopic microscope; this procedure yielded all larval stages and pupae. Additional first-instar larvae were obtained by breeding eggs collected from stems of the common reed in the field or laid by females in the laboratory. Imagines were caught in the field using a sweeping net or developed from larvae and pupae reared in the laboratory. The flies were reared in Petri dishes lined with filter paper.

The study material comprised of 40 eggs, 20 first-instar larvae, 15 exuviae of first-instar larvae, 20 second-instar larvae, 12 exuviae of second-instar larvae, 20 third-instar larvae, 15 pupae, and 20 imagines.

The specimens chosen for detailed study were macerated in 10% KOH for 24 hours at room temperature, washed in distilled water, cleared in chloral hydrate and chloral phenol, and preserved in glycerol. Drawings were based on the microscopic preparations.

First-instar larvae were identified during the rearing of individual eggs obtained from *L. pullitarsis* females. Second-instar larvae of *L. pullitarsis* were identified in a three-stage process. First, all larvae were isolated from stems collected in May and June. At the second stage, only larvae of *Lipara* were isolated, and only second-instar larvae of *L. pullitarsis* were isolated at the third phase. The typing of second-instar larvae of *L. pullitarsis* relied on the presence of the following features: 4–5 lobes on the anterior spiracle (similar to third-instar larvae); numerous, conspicuous, large, amber-coloured spinules on the ventral side of the anterior part of the first thoracic segment. Anterior spiracles with 4–5 lobes appear under the cuticle of typed second-instar larvae just before ecdisis and are also characteristic of the third-instar larvae of these species. Finally, typed larvae were compared to exuviae of second-instar larvae found inside galls near third-instar larvae of *L. pullitarsis*. All the other larvae isolated from stems in May and June will be described in future papers.

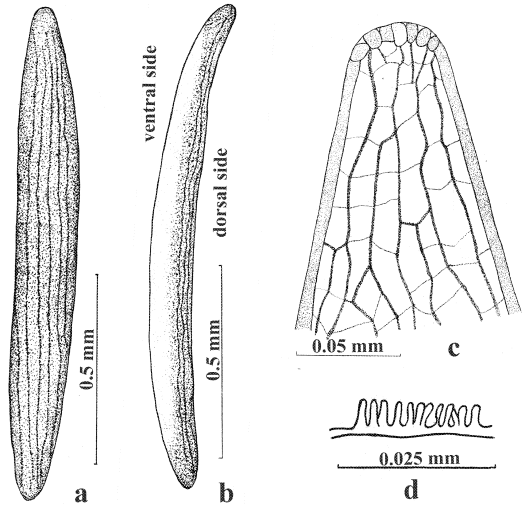

Fig. 1. *Lipara pullitarsis*, egg. – a. Dorsal view. – b. Lateral view. – c. Anterior section. – d. Processes on chorion surface, lateral view.

Third-instar larvae and imagines were determined according to Chvála *et al.* (1974). The terminology used in the morphological descriptions follows Courtney *et al.* (2000). Parts of body of larvae not included in Courtney *et al.* (2000) are named according to Nye (1958) and Nartshuk (1987).

3. Results

Egg (Fig. 1) is 1.08–1.30 mm long, 0.13–0.15 mm wide; length-width ratio 8 : 1. Oblong in shape, narrowed and rounded at both ends (Fig. 1a), curving arch-like ventrally (Fig. 1b). Dorsal side black, ventral side cream-coloured. Broad longitudinal furrows on the dorsal side (Fig. 1a), divided by transverse barriers into numerous polygons (Fig. 1c). Surface of egg finely punctated (approx. 400 power) by the apices of closely spaced rod-like processes covering the egg (Fig. 1d).

First-instar larva (Fig. 2) is 1.33–1.50 mm long, 0.13–0.14 mm in diameter. Body milky-white, slender, cylindrical in cross-section, posterior end with two stigmofores (Fig. 2a). Abdominal segments and third thoracic segment equal in diameter. Facial mask (Fig. 2b) with a pair of two-segment antennae and bright amber-col-

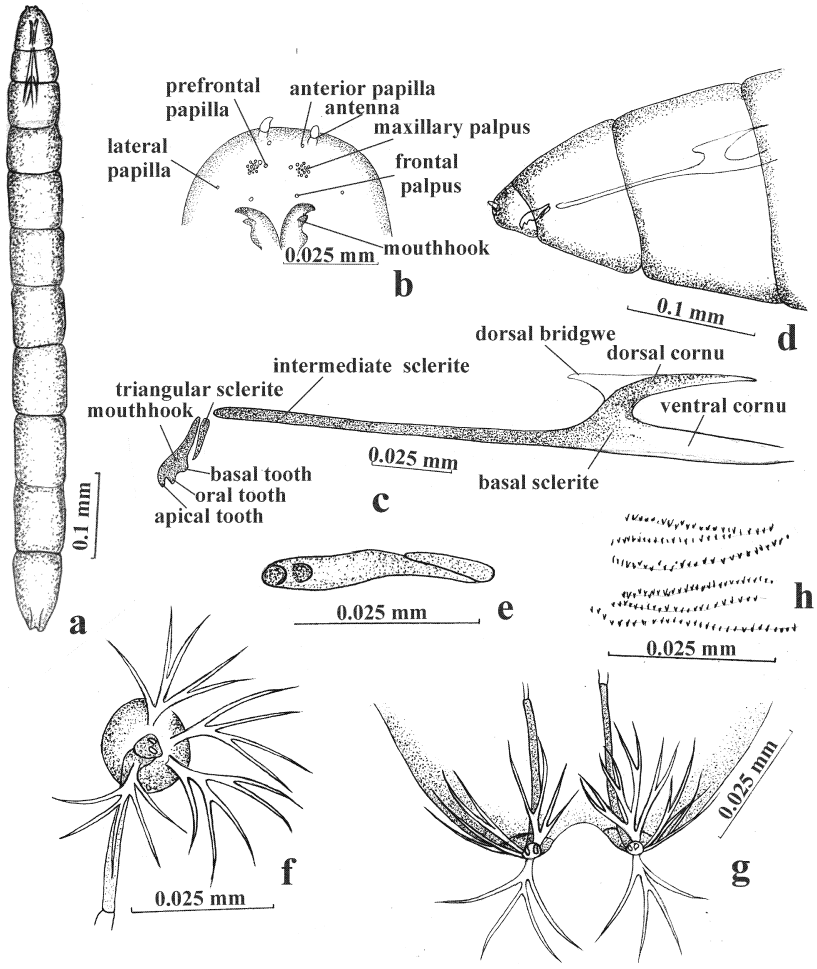

Fig. 2. *L. pullitarsis*, first-stage larva. – a. Ventral view. – b. Facial mask. – c. Cephaloskeleton. – d. Anterior segments, lateral view. – e. Mouthhook, ventral view. – f. Posterior spiracle. – g. Posterior segment, ventral view. – h. Spinules on anterior part of second thoracic segment.

oured maxillary palpi. Basal segment of antenna annular, slightly shorter in diameter than apical segment, which is dome-shaped. Maxillary palpus with 7–8 sensilla. A pair of frontal palpi over the mouthhooks, each containing 1 sensillum. Anterior, prefrontal and lateral papillae present (Fig. 2b). Bright amber-coloured cephaloskeleton (Fig. 2c) 0.22 mm long, including 0.03 mm long mouthhooks, reaches as far as the beginning of third thoracic segment (Fig. 2c–d). One oral tooth behind apical tooth (Fig. 2c, e). Basal tooth massive, in the middle of the mouthhook, continuous with posteriorly narrow base of mouthhook. Triangular sclerite situated ventrally below mouthhook. Intermediate sclerite closely connected with basal sclerite. Basal sclerite divided into ventral and dorsal cornua. Ventral cornu longer than dorsal cornu. Dorsal cornu fully sclerotised, ventral cornu only sclerotised at the base, with membraneous end. Dorsal cornu narrowing posteriorly, anterior part continuous with membraneous dorsal bridge. Posterior spiracle with two large spiracular openings and branching spiracular hairs (Fig. 2f–g). Hairs not variable in length. Spiracular trunk long (0.025 mm) and narrow (0.003 mm). Broad, inconspicuous bands (Fig. 2h) of spinules at anterior borders of all segments. Papillae not present.

Second-instar larva (Fig. 3) is 2.72–4.96 mm long, 0.40–0.48 mm at widest diameter. Body milky, long, slender, cylindrical in cross-section, posterior end slightly curved (Fig. 3a). Facial mask with a pair of two-segment antennae and maxillary palpi (Fig. 3b). Basal segment of antenna annular, somewhat smaller in diameter than apical segment. Apical segment dome-shaped.

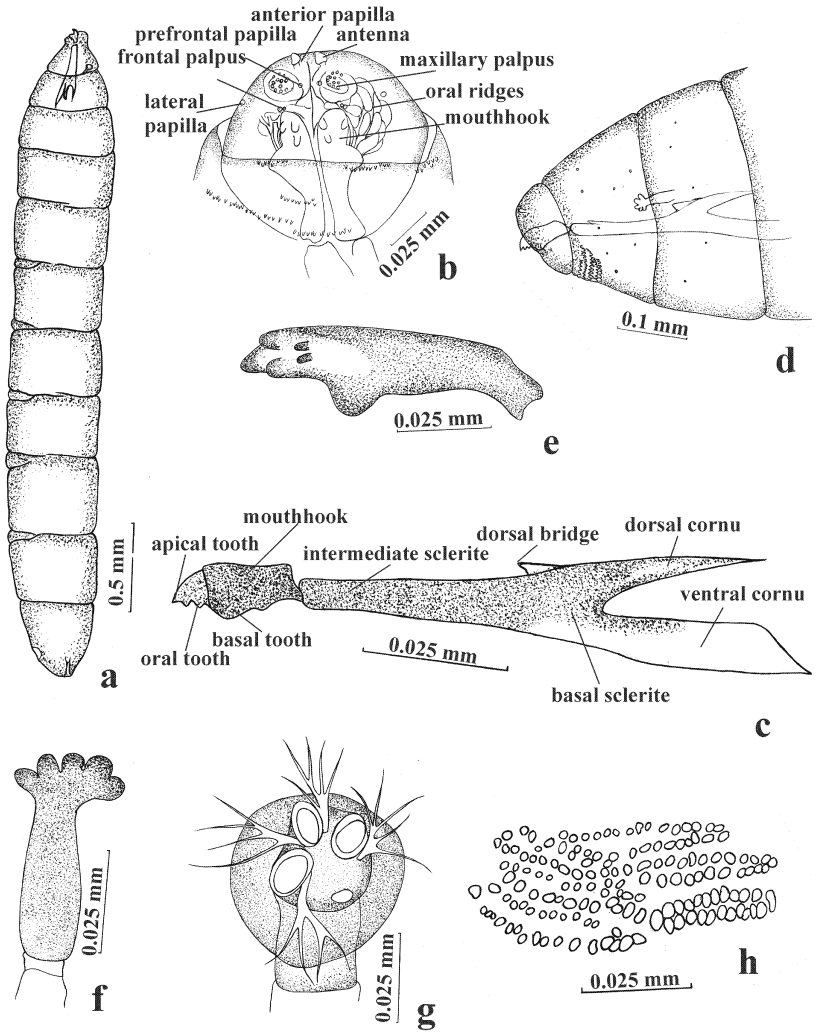

Fig. 3. *L. pullitarsis* second – stage larva. – a. Lateral view. – b. Facial mask. – c. Cephaloskeleton. – d. Anterior segments, lateral view. – e. Mouthhook, ventrolateral view. – f. Anterior spiracle. – g. Posterior spiracle. – h. Spinules on ventral, anterior part of first thoracic segment.

Maxillary palpus with 11–12 sensilla, 3 of which are situated dorso-laterally, apart from the others. A pair of frontal palpi over mouthhooks, each with 2 sensilla. Anterior, lateral and prefrontal papillae present. Oral ridges initially straight, then branch, forming cells around mouth opening. Cephaloskeleton (Fig. 3c) reaches as far as the beginning of third thoracic segment (Fig. 3d), is 0.45 mm long, including 0.08 mm long mouth hooks. Mouthhook black except markedly brighter, brown anterior part with teeth. Two pairs of large oral teeth behind apical tooth (Fig. 3e). Basal tooth in the middle of mouthhook, massive, continuous with rather broad mouthhook base. Base of mouthhook with small dorsal process. Intermediate sclerite narrow, closely connected with basal sclerite. Basal sclerite divided into ventral and dorsal cornua. Ventral cornu longer and broader than dorsal cornu. Both cornua fused anteriorly and markedly sclerotised in that part, with membraneous ends. Dorsal cornu narrows posteriorly, and is continuous with dorsal bridge anteriorly. Anterior spiracle (Fig. 3f) with 4–5 lobes (the number variable in some larvae). Spiracular trunk long (0.05 mm) and narrow (0.01 mm). Posterior spiracle without stigmofores with three oval spiracular openings (Fig. 3g). Spiracular plate diameter 0.03 mm. Spiracular hair short, branching (Fig. 3g). Spiracular trunk short (0.06 mm), broad (0.03 mm). Small papillae on all segments, more numerous on first thoracic segment and last abdomi-

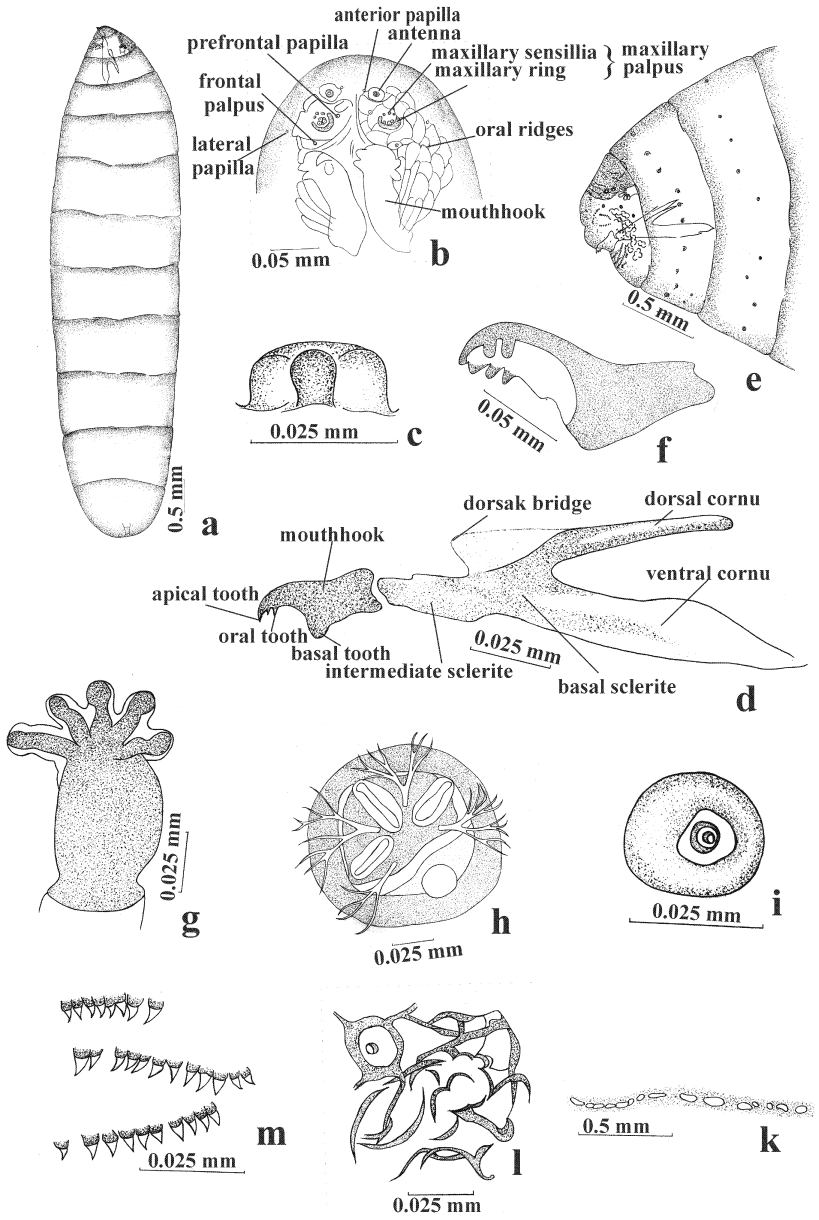

Fig. 4. *L. pullitarsis*, third-stage larva. – a. Lateral view. – b. Facial mask. – c. antenna. – d. Cephaloskeleton. – e. Anterior segments, lateral view. – f. Mouthhook, ventro-lateral view. g. Anterior spiracle. – h. Posteror spiracle. – i. Papilla, dorsal view. – k. Plate-like cuticular thickenings on intersegmental regions of seventh and eighth abdominal segments. – l. Cuticular ornament on first thoracic segment. – m. Spinules on ventral, anterior part of first thoracic segment.

nal segment. Numerous, conspicuous, large amber-coloured spinules on ventral side of anterior part of first thoracic segment (Fig. 3d, h). Small spinules at anterior border of anal opening.

Third-instar larva (Fig. 4) is 5.84–8.60 mm long, 2.20–2.60 mm at widest diameter. Body milky, long, cylindrical, flattened ventrally, slightly curved at posterior end (Fig. 4a). First thoracic segment sclerotized on dorsal side. Facial mask with a pair of two-segment antennae and maxillary palpi (Fig. 4b). Apical segment of antenna inside basal segment (Fig. 4c). Maxillary palpus with 9–10 sensilla surrounded by sclerotized half-ring (Fig. 4b), 3 of the sensilla separated from others, between the free ends of the half-ring. A pair of frontal palpi over mouthhooks, each with 1 large sensillum. Anterior, prefrontal and lateral papillae present (Fig. 4b). Oral ridge shallow, lobes on either side with 4 or 5 ridges radiating laterally from the mouth opening,

ridges branching anterolaterally in a reticulate manner. Cephaloskeleton (Fig. 4d) dark-brown to black; 0.66–0.75 mm long, including 0.16–0.18 mm long mouthhooks, reaching as far as the beginning of third thoracic segment (Fig. 4e). Mouthhooks black. Oral teeth behind apical tooth (Fig. 4f) always in two rows. Number of teeth varies among individuals: 2 : 1; 2 : 2; 3 : 1; 3 : 2; 4 : 3. Basal tooth in the middle of mouthhook, massive, continuous with mouthhook base. Base of mouthhook long, with a posterior wedge and a broad dorsal process. Intermediate sclerite broad and short, closely connected with basal sclerite. Basal sclerite divides into dorsal and ventral cornua. Ventral cornu longer and broader than dorsal cornu. Both cornua fused and sclerotized at the front, with membraneous ends. Dorsal cornu narrows posteriorly, anteriorly continuous with dorsal bridge. Anterior spiracle with 4–5 lobes (Fig. 4g), the number variable in some larva. Spiracular trunk 0.07 mm long and 0.06 mm broad. Posterior spiracle without stigmofores, with three oval spiracular openings (Fig. 4h). Spiracular plate 0.10 mm in diameter, peritreme weakly sclerotised. Spiracular hair delicate, branching (Fig. 4h). Spiracular trunk 0.07–0.08 mm long and 0.09–0.10 mm broad. Distance between spiracles 0.06 mm. Locomotory structures variable (Fig. 4i–m). Papillae (Fig. 4i) on all segments, most numerous on first two thoracic segments and last abdominal segment. Delicate plate-like cuticular thickenings at all segment borders (Fig. 4k). Characteristic ornament at anterior border of first thoracic segment (Fig. 4l), with small spinules on ventral side (Fig. 4m).

Puparium (Fig. 5) is 6.0–7.9 mm long, 1.5–2.0 mm in diameter (male 6.0–6.8 mm long, 1.5–2.0 mm in diameter; female 7.0–7.9 mm long, 1.8–2.0 mm in diameter), oblong, narrow at both ends (Fig. 5a), ventrally flat, dorsally convex, straw-coloured to brown. First thoracic segment and last abdominal segment brown to black. Flat anterior spiracles with 4–5 lobes at posterior border of first thoracic segment. Posterior spiracles in the central part of eighth abdominal segment. Plate-like cuticular thickenings at segment borders. Papillae on all segments; well visible on thoracic segments and eighth abdominal segment, delicate on other abdominal segments. Folds of cuticle all over outer surface of puparium, more numerous ventrally (Fig. 5b).

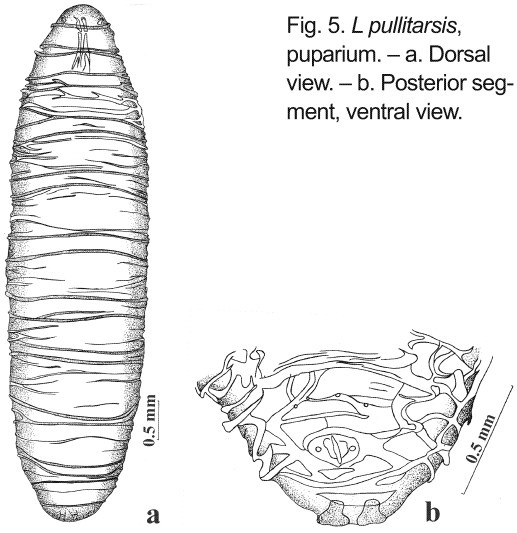

Fig. 5. *L pullitarsis*, puparium. – a. Dorsal view. – b. Posterior segment, ventral view.

4. Discussion

A third-instar larva of *Lipara* with 5 lobes on anterior spiracles was determined as *L. rufitarsis* by Ruppolt (1957), who also included larvae with 6–8 lobes into the same species. In his paper, Ruppolt also took notice of morphological and biological differences between these larvae, suggesting that they might actually represent two different species. Seventeen years later Ruppolt's doubts were confirmed by Chvala *et al.* (1974), who concluded that larvae with 4–5 lobes on anterior spiracles are larvae of *L. pullitarsis*, while larvae with 6–9 lobes belong to *L. rufitarsis*. Chvala *et al.* (1974) established the following features as relevant for determination of third-instar larvae of *Lipara*: number of lobes on anterior spiracles, sclerotization, cuticular striation and the pattern of papillae on the thoracic segments. While the above features certainly allow for determination of larvae of *L. pullitarsis*, they do not fully characterise this stage of development. The present paper supplements previous descriptions of third-instar larvae of *L. pullitarsis* with descriptions of the facial mask, cephaloskeleton, posterior spiracles and locomotory structures, and contains the first descriptions of the morphology of first- and second-instar larvae.

A detailed analysis of the morphology of all larval stages of *L. pullitarsis* has revealed differences between individual stages in the structure of antennae, maxillary palpi, frontal palpi, cephaloskeleton, spiracles, and in the type and position of locomotory structures.

The first-instar larva of *L. pullitarsis* has an oblong body. The diameters of abdominal segments and the third thoracic segment are the same. Spiracular hair on posterior spiracles are of equal length and branch in a bush-like manner.

The second-instar larva, like the third-instar larva, has 4–5 lobes on anterior spiracles and numerous, conspicuous, large, amber-coloured spinules on the ventral, anterior part of the first thoracic segment.

Previous descriptions of the egg (Chvala *et al.* 1974) are now complemented by a characterisation of the microsculpture of the chorion, and those of the puparium, with additional details regarding the size and microsculpture.

A knowledge of the morphology of all stages of *L. pullitarsis* is helpful in identifying this species at different stages of its natural history and provides a basis for a comprehensive description of the life-cycle of this fly (Chvala *et al.* 1974, Pokorny 1981).

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