

Morphology of preimaginal stages of *Lipara rufitarsis* Loew 1858 (Diptera: Chloropidae), a parasite of the common reed (*Phragmites australis* (Cav.) Trin.)

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Abstract. All preimaginal stages of *Lipara rufitarsis* Loew are described and illustrated in 39 drawings. The facial mask, cephaloskeleton, spiracles and locomotory structures are considered. This is the first description of first- and second-instar larva. Descriptions of third-instar larva, egg and puparium are expanded. The paper forms a basis for a complete description of the life history of this fly.

Résumé. La morphologie des stades préimaginaux de *Lipara rufitarsis* Loew 1858 (Diptera : Chloropidae), parasite du roseau commun (*Phragmites australis* (Cav.) Trin.). Tous les stades préimaginaux de *Lipara rufitarsis* Loew sont décrits et présentés par 39 illustrations. Les éléments suivants sont caractérisés : le masque facial, le squelette céphalo-pharyngien, les trachées antérieures et postérieures et l'appareil locomoteur. Le premier et le second stades larvaires sont décrits pour la première fois. La description de la larve au troisième stade, de l'oeuf et de la puppe sont complétées. Cette recherche constitue une base fondamentale à la connaissance du cycle vital complet de cette mouche.

Keywords: *Lipara rufitarsis*, morphology, *Phragmites australis*.

Eleven species of *Lipara* Meigen are known from the Holarctic (Nartshuk 1996; Tewksbury et al. 2002). *L. lucens* Meigen, *L. pullitarsis* Doskočil & Chvála, *L. rufitarsis* Loew and *L. similis* Schiner, occur in Poland (Karl 1935; Durska 1970; Siedlar 1991; Nartshuk 2002).

All developmental stages of *L. lucens* are known at present (Ruppolt 1957; Waitzbauer 1969), and only the egg, third-instar larva and puparium of *L. pullitarsis*, *L. rufitarsis* and *L. similis* (Chvála et al. 1974).

This paper describes the morphology of the previously unknown first- and second-instar larva of *L. rufitarsis* 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 preimaginal developmental stages of *L. rufitarsis*.

L. rufitarsis forms galls in the apical part of common reed stems (Ruppolt 1957; Pokorný 1971, 1981; Chvála et al. 1974; Skuhřavý 1978). It is a monophagous and univoltine fly which lays eggs on the surface of common reed stems (*Phragmites australis* (Cav.) Trin. All larval stages and pupae develop inside the galls to leave the stem only as adults, which can be found in reedbeds from May to July (Chvála et al. 1974; Skuhřavý 1978).

Europe, Russia, Israel, Mongolia, Japan and North America (Nartshuk 1984, Tewksbury et al. 2002).

Material and Methods

All developmental stages of *L. rufitarsis* collected from the apical part of common reed stems were studied. Stems of common reed were collected in reedbeds in and around the town of Lublin in mid-eastern Poland in the years 1977–79 and 2001–04. 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: 35 eggs; 18 first-instar larvae, 15 exuviae of first-instar larvae; 11 second-instar larvae, 12 exuviae of second-instar larvae; 18 third-instar larvae; 15 pupae; and 19 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. rufitarsis* females. Second-instar larvae of *L. rufitarsis* were identified in a three-stage process. First, all larvae were isolated from stems collected in May and June. The second stage consisted in isolating only larvae of *Lipara*. Finally, only second-instar larvae of *L. rufitarsis* were isolated. The typing of second-instar larvae of *L. rufitarsis*

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Accepté le 28 septembre 2006

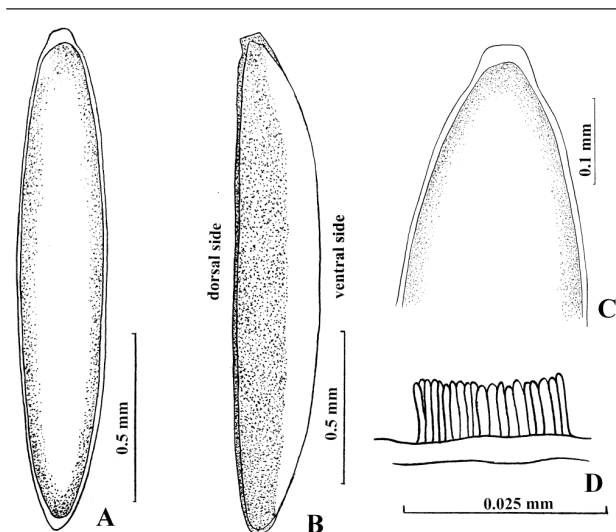


Figure 1
Liparis rufitarsis, egg: A - dorsal view; B - lateral view; C - anterior section; D - processes on chorion surface, lateral view.

relied on the presence of the following features: 6–9 lobes on the anterior spiracle (similar to third-instar larvae); two rows of large spinules on the sides of abdominal segments, rings of small spinules in the anterior boundary on the first thoracic segment. Anterior spiracles with 6–9 lobes appear under the cuticle of typed second-instar larvae just before ecdysis and are also characteristic of 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. rufitarsis*. All the other larvae isolated from stems in May and June shall be described in further papers.

Third-instar larvae and imagines were determined according to the key by Chvála *et al.* (1974). The terminology used in the morphological descriptions follows that of 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).

Results

Egg (Fig. 1), 1.40–1.53 mm long, 0.18–0.20 mm wide, oblong, narrowed at both ends (Fig. 1A). Length-width ratio 8:1. Dorsal side flat, ventral side curving arch-like, (Fig. 1B). Anterior end truncated, posterior end rounded (Fig. 1A). Egg surface smooth (Fig. 1C); dorsal side dark-brown to black, ventral side milky to off-white. Surface of egg finely punctated by the apices of closely spaced processes covering the chorion (approx. 400x power) (Fig. 1D).

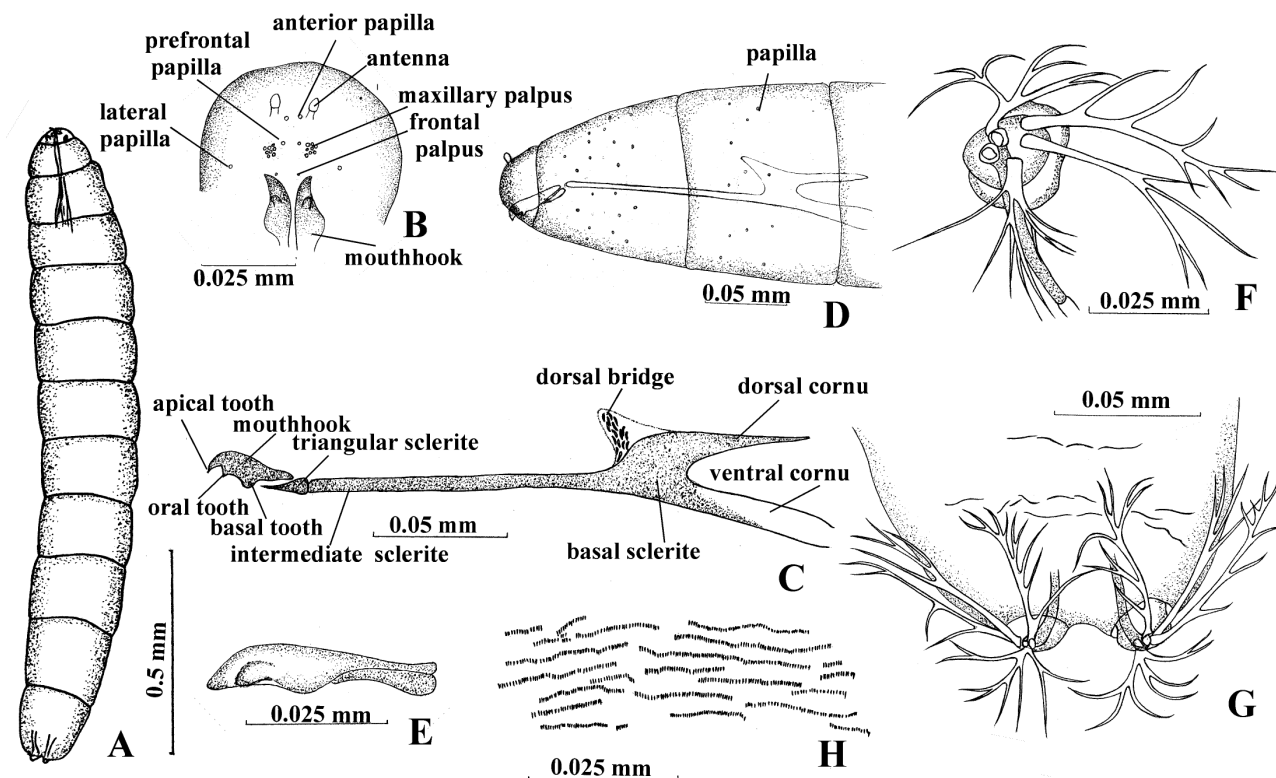


Figure 2
Liparis rufitarsis, first-instar larva: A - ventral view; B - facial mask; C - cephaloskeleton; D - anterior segments - lateral view; E - mouthhook, ventro-lateral view; F - posterior spiracle; G - posterior segment, ventral view; H - spinules on anterior part of second thoracic segment.

First-instar larvae (Fig. 2), 1.33–1.48 mm long, 0.20–0.30 mm maximum diameter. Body milky-white, slender, cylindrical in cross-section, posterior end slightly curved (Fig. 2A). Facial mask (Fig. 2B) with a pair of two-segment antennae and amber-coloured maxillary palpi. Basal segment of antenna annular, slightly shorter in diameter than apical segment, which is dome-shaped. Maxillary palpus with 7–8 sensillia. 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) reaches as far as third thoracic segment (Fig. 2D), 0.25–0.27 mm long, including 0.03 mm long mouthhooks. One oral tooth behind apical tooth (Fig. 2C, E). Basal tooth massive, in the middle of the mouthhook, continuing with posteriorly narrow base of mouthhook. Triangular sclerite ventrally below mouthhook.

Intermediate sclerite closely connected with basal sclerite. Basal sclerite is divided into ventral and dorsal cornua. Ventral cornu longer than dorsal cornu. Dorsal cornu fully sclerotised, ventral cornu only sclerotised at base, with membranous end. Dorsal cornu tapering posteriorly, anterior part continuous with dorsal bridge. Posterior spiracle on stigmofores, with two large spiracular openings and two pairs of branching spiracular hairs (Fig. 2F, G). A pair of longer hairs adjoining lateral surfaces of last segment of the larva, with shorter hairs adjoining ventral and dorsal surfaces (Fig. 2G). Spiracular trunk long (0.035 mm) and narrow (0.0025 mm). Broad, inconspicuous band of spinules at anterior borders of all segments (Fig. 2H). Papillae in all segments, more numerous in first thoracic segment and last abdominal segment than in other segments.

Second-instar larva (Fig. 3), length 3.76–4.80 mm, 0.65–

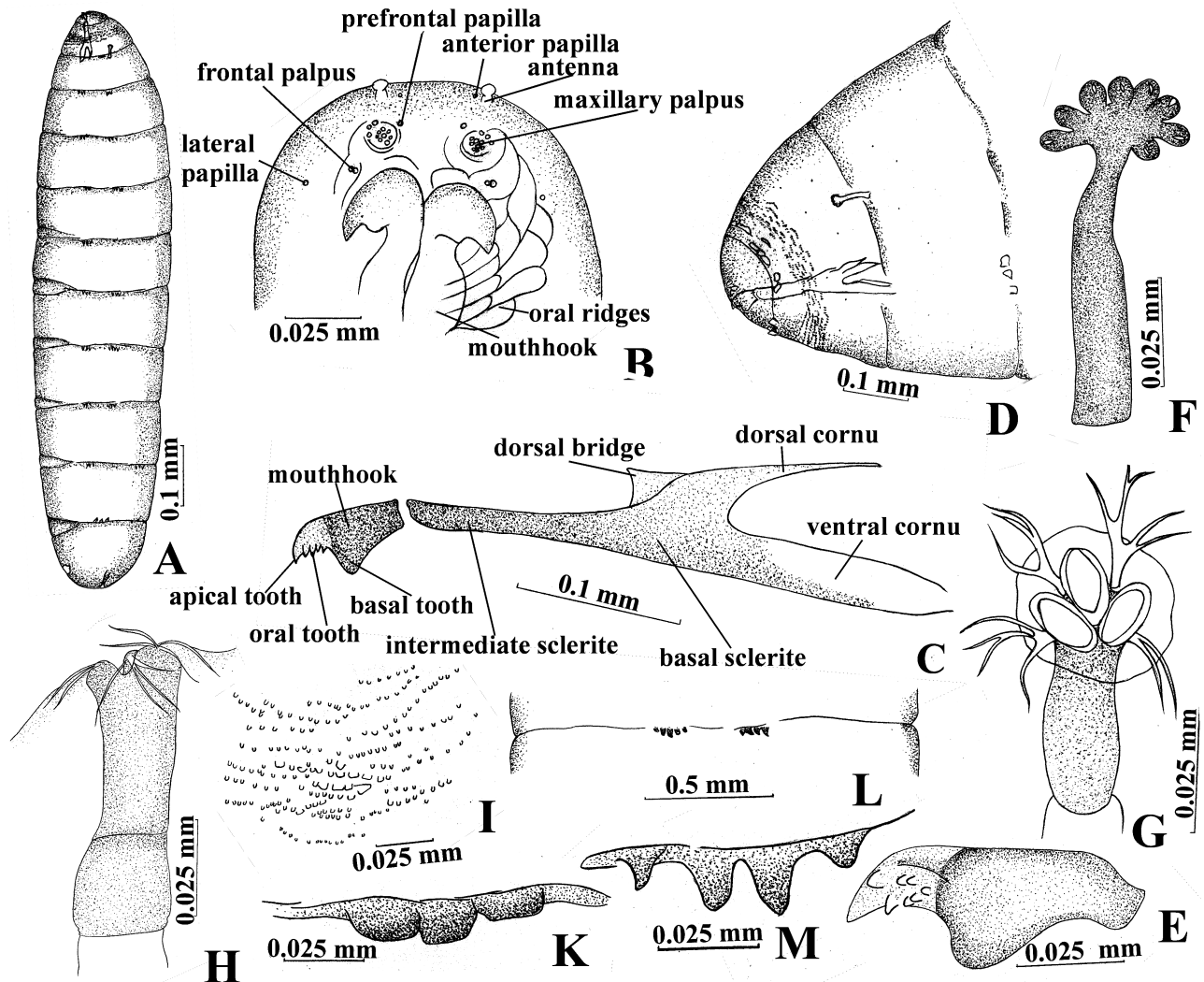


Figure 3

Liparis rufitarsis second-instar larva: A - lateral view; B - facial mask; C - cephaloskeleton; D - anterior segments, lateral view; E - mouthhook, ventro-lateral view; F - anterior spiracle; G, H - posterior spiracle; I - spinules on ventral anterior part of first thoracic segment; K - plate-like cuticular thickenings on intersegmental regions of pseudocephalon and first thoracic segment; L-M - spinules on lateral intersegmental regions of fifth and sixth abdominal segments.

1.05 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. 2B). Basal segment of antenna annular, somewhat smaller in diameter than apical segment. Apical segment dome-shaped. Maxillary palpus with 11–12 sensillia, 3 of which are situated dorso-laterally, apart from the others. A pair of frontal palpi over mouthhooks, each with 2 sensillia. Anterior, lateral and prefrontal papillae present. Oral ridges initially straight, then branch and fuse together, forming cells around mouth opening. Cephaloskeleton (Fig. 3C) reaches as far as the beginning of second thoracic segment (Fig. 3D), 0.41 mm long, including 0.07 mm long mouthhooks. Mouthhooks

black except markedly brighter, brown anterior part with teeth. Behind apical tooth is a pair of large oral teeth with five smaller ones (Fig. 3E), the last, and smallest, of which is found in the middle part of the mouthhook, and the other four, bigger ones form two pairs on either side of the mouthhook. Basal tooth in the middle of mouthhook, massive, continuous with mouthhook base which tapers posteriorly (Fig. 3C). Base of mouthhook with a 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 membranous ends. Dorsal cornu narrows posteriorly, and continuous with dorsal

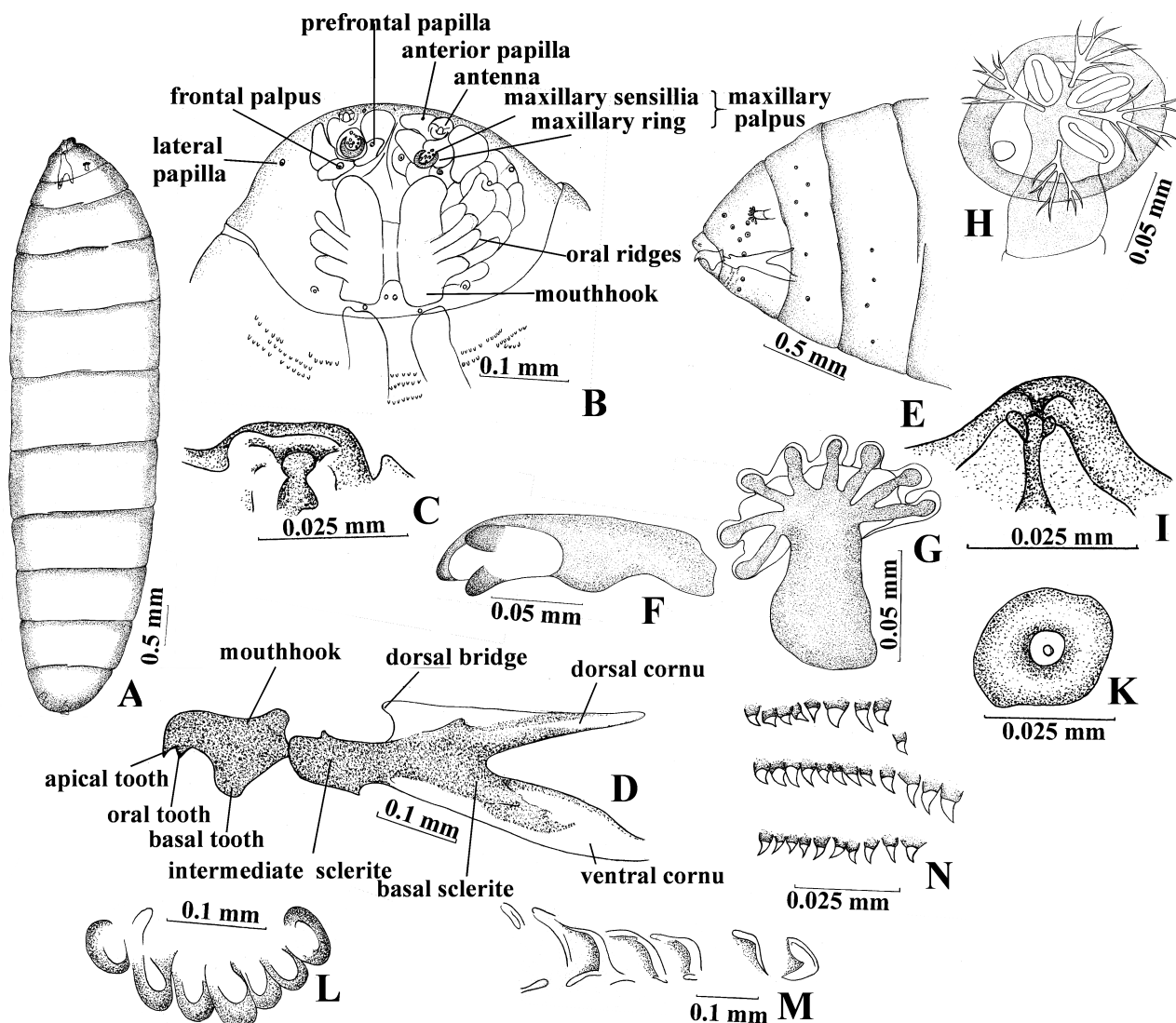


Figure 4

Liparis rufitarsis, third-instar 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 - posterior spiracle; I - papilla, lateral view; K - papilla, dorsal view; L - plate-like cuticular thickenings on lateral intersegmental regions of second and third abdominal segments; M - plate-like cuticular thickenings on intersegmental regions of second and third abdominal segments; N - spinules on ventral anterior part of first thoracic segment.

bridge anteriorly. Anterior spiracle (Fig. 3F) with 8–9 lobes (the number variable in some larvae). Spiracular trunk long (0.06 mm) and narrow (0.01 mm). Posterior spiracle without stigmofores, with three oval spiracular openings (Fig. 3G). Spiracular plate diameter 0.04 mm, with poorly visible external border. Spiracular hair short, branching (Fig. 3G). Spiracular trunk long (0.08 mm), diameter at base 0.03 mm, above base 0.02 mm (Fig. 3H). Locomotory structures variable. Small papillae on all segments, more numerous in first thoracic segment and last abdominal segment. A ring of small spinules at anterior border of first thoracic segment (Fig. 3I, D). Plate-like cuticular thickenings at the border of pseudocephalon and first thoracic segment (Fig. 3K, D). Two rows of large spinules along lateral surfaces of abdominal segments (Fig. 3A). Spinules at segment borders form two groupings (Fig. 3L), with 3–4 spinules in each (Fig. 3M). Distance between groupings of spinules at the border of sixth and seventh segments, and seventh and eighth segments longer than in other segments. Short groupings of plate-like cuticular thickenings on ventral surface in the middle part of last two abdominal segments. Small spinules before anal opening.

Third-instar larva (Fig. 4) 8.00–9.00 mm long, 1.70–2.50 mm at widest diameter. Body milky, long, cylindrical, flattened ventrally, slightly curved at posterior end (Fig. 4A). First thoracic segment slightly sclerotized. 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 sensillia (Fig. 4B) surrounded by sclerotized half-ring, with 3 of the sensillia somewhat bigger separated from others, between the free ends of half-ring. A pair of frontal palpi over mouthhooks, each with 1 sensillum. Anterior, prefrontal and lateral papillae present (Fig. 4B). Oral ridges shallow, lobes on either side with 4 or 5 ridges radiating laterally from the mouth opening and branching anterolaterally in a reticulate manner. Cephaloskeleton (Fig. 4D) dark-brown to black, 0.60 mm long, including 0.15 mm long mouthhooks. Mouthhooks black. Two large oral teeth behind apical tooth (Fig. 4F). Basal tooth in the middle of mouthhook, massive, continuous with mouthhook base. Base of mouthhook short, with a large 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. Dorsal cornu narrows posteriorly, anteriorly continuous with membranous dorsal bridge. Both cornua fused and sclerotized at the front, with membranous ends. Anterior spiracle with 6–9 lobes (Fig. 4G), the number variable in some larvae. Spiracular trunk 0.08 mm long and 0.04 mm broad. Posterior spiracle without stigmofores, with three oval spiracular openings (Fig. 4H). Spiracular plate 0.1 mm in diameter, peritreme weakly sclerotized. Spiracular hair delicate, branching (Fig. 4H). Spiracular trunk 0.06 mm long and 0.07 mm broad. Distance between spiracles 0.13 mm. Locomotory structures variable (Figs 4I–N). Papillae (Fig. 4I, K) in all segments. Most numerous in first two thoracic segments (Fig. 4E) and last abdominal segment. Groupings of plate-like cuticular thickenings poorly visible along lateral surfaces of larva, at the border between abdominal segments (Fig. 4L). Plate-like cuticular thickenings of slightly different shape (Fig. 4M) form rings at borders of all segments. Small spinules at anterior ventral border of first thoracic segment (Fig. 4N) and near anal opening.

Puparium (Fig. 5) 6.0–7.3 mm long, 1.5–2.1 mm at widest diameter (♂ 6.0–6.4 mm long, 1.5–2.0 mm in diameter; ♀ 7.0–7.3 mm long, 1.8–2.1 mm in diameter), oblong, narrow at both

ends (Fig. 5A), ventrally flat, dorsally convex, straw-coloured to light brown, thoracic segments darker than abdominal segments. First thoracic segment and last abdominal segment brown. Flat anterior spiracles with 6–9 lobes at posterior border of first

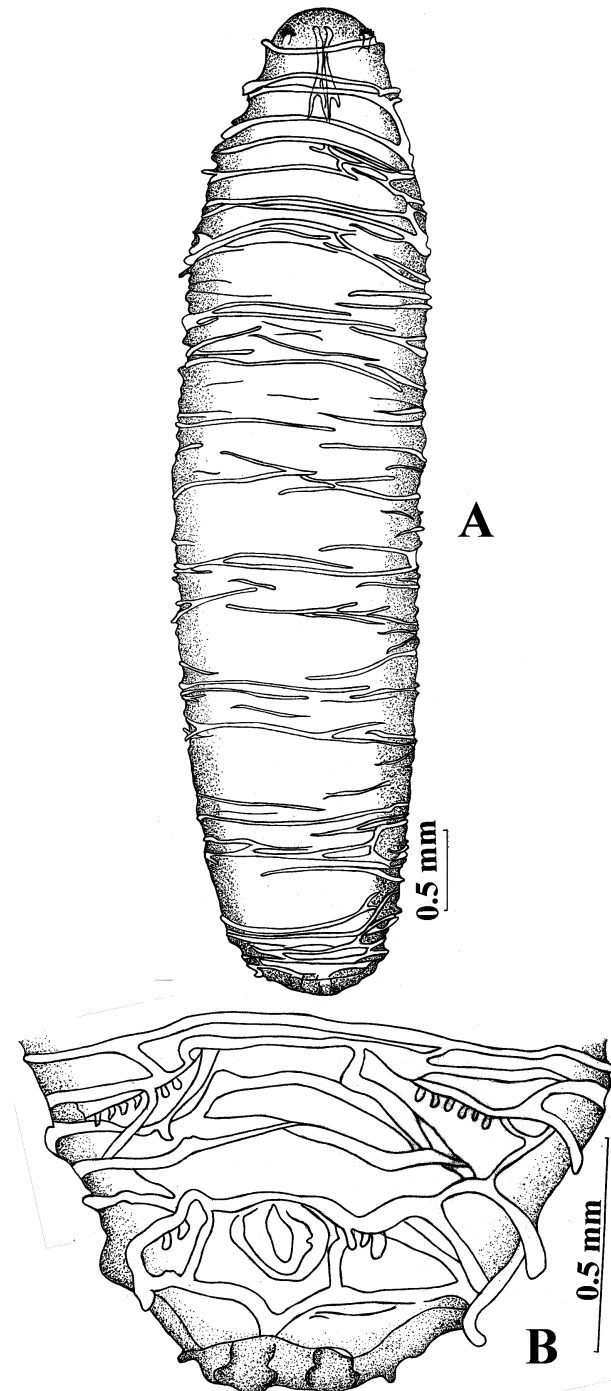


Figure 5
Liparis rufitarsis, puparium: A - dorsal view; B - posterior segment, ventral view

thoracic segment. Posterior spiracles in the central part of eighth abdominal segment. Plate-like cuticular thickenings at segment borders and on either side of anal opening (Fig. 5B). Papillae on all segments; slightly larger on thoracic segments and eighth abdominal segment than on other abdominal segments. Folds of cuticle all over outer surface of puparium, more numerous ventrally (Fig. 5A, B).

Discussion

A third-instar larva of *Lipara rufitarsis* was first described by Vimmer (1925), under the name of *L. lucens*. This can be concluded from his description and drawing of the anterior spiracle with the 7 lobes characteristic of *L. rufitarsis*. The other features listed in the description are not relevant for the identification of this species. Chvala *et al.* (1974) developed a key for the identification of third-instar larvae of European *Lipara* species. The following features were regarded as relevant for distinguishing species: sclerotisation of the first and last segment of the larva and the number of lobes on anterior spiracles. The description of the larva of *L. rufitarsis* is confined to these structures. The present paper contains the first-ever description of first- and second-instar larva of *L. rufitarsis*. Particular attention is paid to the facial mask, cephaloskeleton, anterior and posterior spiracles, and locomotory structures. The same structures are described in the third-instar larva, which led to the conclusion that the larval stages of *L. rufitarsis* can be distinguished between themselves by the structure of antennae, maxillary palpi, frontal palpi, oral ridges, the structure of the cephaloskeleton, anterior and posterior spiracles, and the type and placement of locomotory structures.

The description of the structure of the surface of the egg of *L. rufitarsis* is in contradiction to existing data. Chvala *et al.* (1974) reported the presence of oblong, poorly visible furrows there. Observations by the present author show that the egg of *L. rufitarsis* has a smooth surface. Higher magnification demonstrates uniform punctation, corresponding to the apices of closely spaced rod-like processes.

Chvala's *et al.* (1974) description of the puparium is now supplemented with features of cuticular sculpture. Plate-like cuticular thickenings are better visible on the surface of the puparium than in the third-instar larva.

Pokorný (1981) attributes the lack of descriptions of the younger stages of dipteran larvae of the genus *Lipara* in papers written to date to difficulties in finding such specimens in the field. In the case of *L. rufitarsis* these difficulties are due to a number of causes. First, all *Lipara* species are found in galls in the apical part of common reed stems growing in the same area, at the same time and represent the same developmental stage. Second, young larvae of *Lipara* are often accompanied by insect larvae of the genera *Cryptonevra*, *Tropidoscinis*, *Calamoncosis* and

Anthomyza. Third, at the time when young larvae of *L. rufitarsis* appear (May, June), the identification of stems poses considerable difficulty or is often impossible.

Dipterological papers have notably been devoting too little attention to the younger larval stages. The number of Chloropidae species for which descriptions of the younger larval stages are available is not precisely known. Nartshuk (1987) reports that these descriptions are very scarce and only refer to representatives of seven genera compared to the total of 170 genera and 2325 species in the world inventory of this family (Nartshuk 1987).

Acknowledgments. I like to thank Dr Lech Lechowski for his suggestions and commentaries to this work. A special thanks is due to Prof. Przemysław Trojan for his kind appraisal and constructive comments of my work.

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