Morphology of preimaginal stages of *Cryptonevra flavitarsis* (Diptera: Chloropidae) – an inquiline in galls formed by *Lipara* flies on common reed

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Grochowska, M. 2008: Morphology of preimaginal stages of *Cryptonevra flavitarsis* (Diptera: Chloropidae) – an inquiline in galls formed by *Lipara* flies on common reed. — Entomol. Fennica 19: 18–24.

The paper presents a complete description of the morphology of the preimaginal stages of *Cryptonevra flavitarsis* (Meigen, 1830), an inquiline in galls of *Lipara* flies on common reed (*Phragmites australis*). All stages are described for the first time. In descriptions of the larval stages, particular attention is given to the diagnostic characters: facial mask, cephaloskeleton, anterior and posterior spiracles, and locomotory structures. The paper forms a basis for future descriptions of the yet unknown life cycle of this fly.

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Received 4 August 2006, accepted 20 April 2007

1. Introduction

The genus *Cryptonevra* Lioy, 1864, contains 12 species (Nartshuk 1984). Four of them: *C. flavitarsis* (Meigen, 1830), *C. diadema* (Meigen, 1830), *C. consimilis* Collin, 1932 and *C. nigritarsis* (Duda, 1933), are inquilines in galls formed by *Lipara* and *Platycephala* flies on *Phragmites australis* (Cav.) Trin. (Kramer 1917, Collin 1932, Duda 1933, Hennig 1935, 1943, Blair 1932, 1944, Erdös 1957, Wendt 1968, Nartshuk 1969, 1972, 1996, Waitzbauer 1969, Waitzbauer *et al.* 1972, Beschovski 1984, De Bruyn 1985, Ferrar 1987). *C. flavitarsis* is the most common of these four (Pokorný & Skuhravý 1981). Larvae of *C. flavitarsis* develop among leaves in the apical part of a gall or in the larval chamber next to larvae of *Lipara* and *Platycephala*. *C. flavitarsis* is also found among normal and decaying leaves in the apical parts of reed stems not inhabited by other insect species and inside stems of *Triticum repens* (Giraud 1863, Duda 1933, Hennig 1943, Nartshuk 1962). The overwintering stage is the pupa. Adults can be seen flying in reed beds from late April to August (Blair 1932, 1944, Erdös 1957, Wendt 1968, Nartshuk 1969, Waitzbauer 1969, Waitzbauer *et al.* 1972, Pokorný & Skuhravý 1981). *C. flavitarsis* is distributed from Great Britain in the west to the Angara subregion in Western Siberia and Mongolia in the east. The northern limit of its range reaches as far as Finland, while the southern limit extends from northern Africa (Morocco) to Armenia, Azerbaijan, to China (Beschovski 1984, Nartshuk 1984).

The developmental stages of *C. flavitarsis* comprise the egg, three larval stages, the pupa and the imago. Of these, the imago has been well described (Meigen 1830). Habitus drawings of the puparium are also available (Waitzbauer 1969). Data on the other stages are lacking.

The aim of the present study was to describe the morphology of all preimaginal stages of *C.*

flavitarsis, thus paving the way to describing the species' life-cycle and its impact on common reed stems.

2. Materials and methods

The materials included all stages of *C. flavitarsis* obtained from the apical part of common reed stems. The stems were collected in reed beds in and around Lublin (central-eastern Poland) in the years 2002–05. Fresh stems were randomly collected in May and June, and stems bearing visible galls in the apical part without inflorescences were collected outside of this period. The stems were subsequently sectioned in the laboratory under a stereoscopic microscope. The sectioning of galls yielded all larval stages and pupae. Eggs were obtained from the upper surface of leaf blades and from sectioned abdomens of females. First-instar larvae were reared from eggs collected in the field or laid by females in the laboratory. Imagines were obtained by rearing larvae and pupae in Petri dishes lined with filter paper.

Overall, the study material comprised: 30 eggs, 15 first-instar larvae, 10 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.

Imagines were determined according to the key by Nartshuk *et al.* (1970) and compared to the first description of the species (Meigen 1830). The descriptions of larvae follow the terminology of Courtney *et al.* (2000). Parts of the larval body not defined in Courtney *et al.* (2000) are named according to Nye (1958) and Nartshuk (1987).

3. Description of preimaginal stages

Egg (Fig. 1). 0.90–1.00 mm long, 0.23–0.25 mm wide, mat, white, oblong, slightly rounded at both ends (Fig. 1a). Length to width ratio 4:1. Dorsally flat, ventrally arch-like (Fig.1b). Surface covered with longitudinal and transverse ribs (Fig. 1 c, d) forming squares denser and darker at both ends, more regular and darker at anterior than posterior pole. Chorionic surface covered with microscopic closely-spaced processes making it look like a nap.

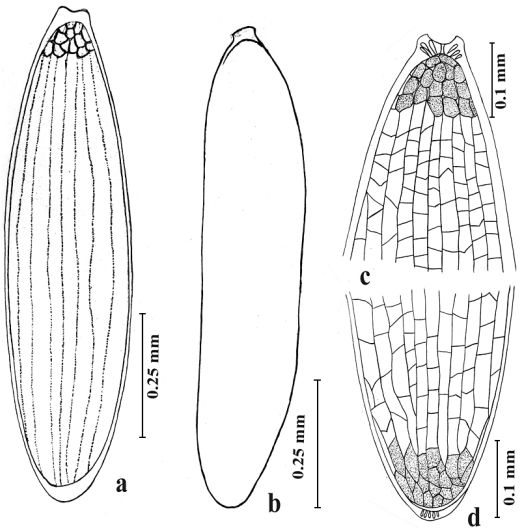

Fig. 1. *C. flavitarsis*, egg. – a. Dorsal view. – b. Lateral view. – c. Anterior section. – d. Posterior section.

First-instar larva (Fig. 2). 1.05–1.30 mm in length, 0.18–0.22 mm at widest diameter. Body white, slender, oval in cross-section (Fig. 2a). Facial mask (Fig. 2b) with a pair of two-segment antenna, maxillary and frontal palpi. Basal segment of antenna ring-like, slightly smaller in diameter than apical segment. Apical segment dome-shaped. Maxillary palpi with numerous sensilla. Frontal palpi with two sensilla over mouthhooks. Oral ridges straight, not branching, arranged symmetrically on both sides of mouth opening (Fig. 2b). Cephaloskeleton (Fig. 2c) of light amber colour, 0.25 mm in length, with 0.06 mm-long mouthhooks. Mouthhooks long, narrow (Fig. 2d). Apical tooth hook-like bending downwards, no oral teeth. Basal tooth poorly distinguishable. Base of mouthhook long and narrow. Additional sclerite shaped like wide plate adjoining external surface of mouthhook at right angle (Fig. 2c, d). Intermediate sclerite closely connected with basal sclerite. Basal sclerite divided into ventral and dorsal cornua. Ventral cornu longer and wider than dorsal cornu. Both cornua poorly sclerotised, mostly membraneous. Dorsal cornu narrowing posteriorly, continuous with dorsal

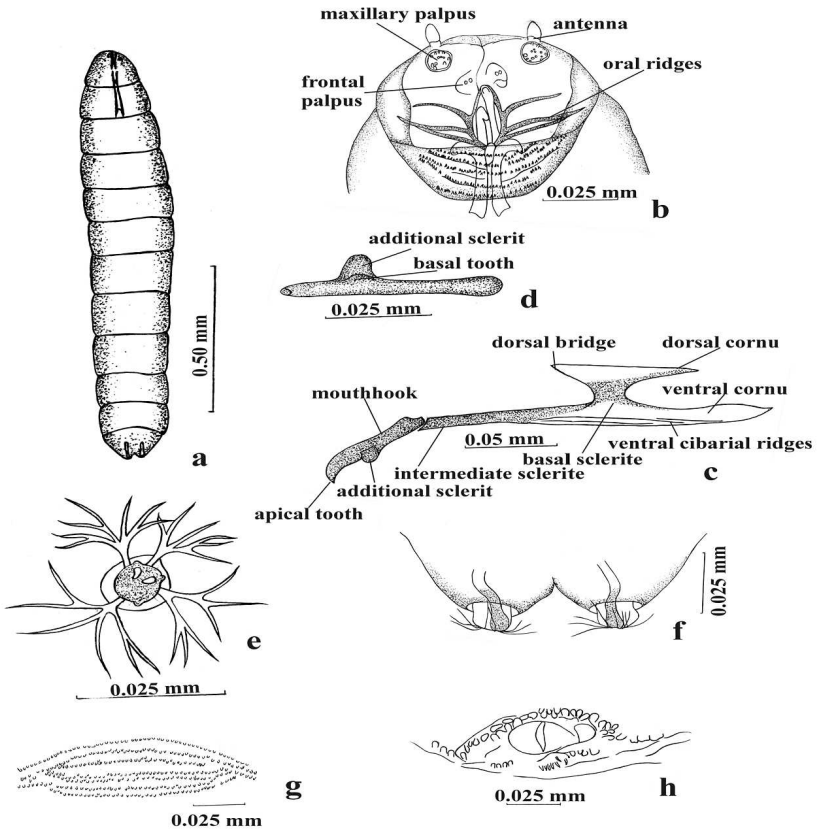

Fig. 2. *C. flavitarsis*, first-stage larva. – a. Ventral view. – b. Facial mask. – c. Cephaloskeleton. – d. Mouthhook ventral view. – e. Posterior spiracle. – f. Posterior segment ventral view. – g. Spinules on ventral intersegmental regions of seventh and eighth abdominal segments. – h. Anal spinules.

bridge anteriorly. Hypopharynx with prominent longitudinal ventral cibarial ridges extending into lumen of cibarial pump. Posterior spiracles on stigmatophores, with two spiracular openings and two pairs of branching interstigmal hairs (Fig. 2e, f). Spiracular trunk long (0.04–0.05 mm) and thin (0.0025 mm). Distance between spiracles 0.03 mm. Spinules present at segment borders ventrally and around anal plate (Fig. 2g, h).

Second-instar larva (Fig. 3). 1.68–3.04 mm long, 0.28–0.55 mm at widest diameter. Body white, long, slender, oval in cross-section, posterior end slightly rounded (Fig. 3a). Facial mask with pair of antennae, maxillary and frontal palpi (Fig. 3b). Basal segment of antenna ring-like, apical segment dome-shaped, narrower at base, located inside basal segment. Maxillary palpi with numerous sensilla. Frontal palpi with two sensilla over mouthhooks. Anterior and prefrontal papillae present. Numerous oral ridges on both sides of mouth opening and over mouthhooks. Cephaloskeleton partly sclerotised, 0.35–0.36 mm in length, including 0.08 mm long mouthhooks. (Fig. 3c). 4–5 oral teeth behind apical tooth (Fig. 3d, e). Basal tooth wide and short, continuous with long and narrow base of mouthhook. Intermediate sclerite long and narrow, connected with basal sclerite (Fig. 3c). Basal sclerite distally divided into ventral and dorsal cornua. Ventral cornu longer and wider than dorsal cornu. Dorsal cornu narrowing posteriorly, continuous with dorsal bridge anteriorly. Rod-shaped parastomal bar projecting anteriorly from basal sclerite. Hypopharynx with prominent longitudinal ventral cibarial ridges extending into lumen of cibarial pump. Anterior spiracle with 5–7 lobes (the number variable between individuals and between left and right spiracles) (Fig. 3f). Posterior spiracle on stigmatophores with three oval spiracular openings (Fig. 3g, h). Spiracular plate quadrilateral with widely rounded corners. Ecdysial scar well visible. Spiracular hair short, branching (Fig. 3g). Spiracular trunk 0.06 mm long, 0.008 mm wide. Distance between posterior

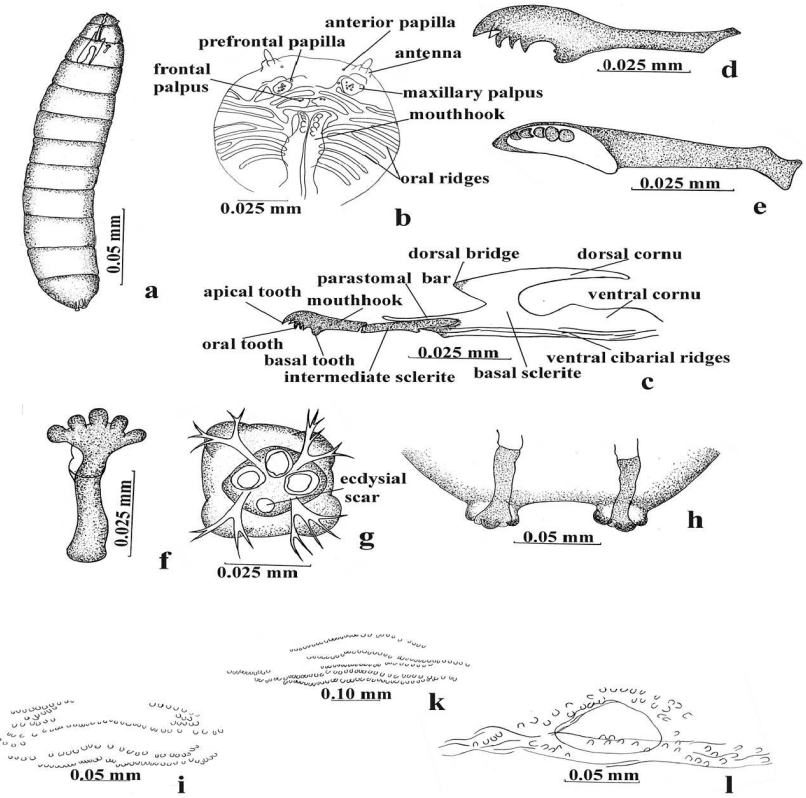

Fig. 3. *C. flavitarsis* second-stage larva.
– a. Lateral view.
– b. Facial mask.
– c. Cephaloskeleton.
– d. Mouthhook, lateral view. – e. Mouthhook, ventro-lateral view.
– f. Anterior spiracle.
– g. Posterior spiracle.
– h. Posterior segment, ventral view. – i. Spinules on ventral intersegmental regions of seventh and eighth abdominal segments.
– k. Spinules on ventral intersegmental regions of second and third abdominal segments.
– l. Anal spinules.

spiracles 0.06 mm. Small spinules present at segment borders ventrally and around anal opening (Fig. 3 i, k, l).

Third-instar larva (Fig. 4). 4.00–5.12 mm long, 0.64–1.12 mm at widest diameter. Body milky white, long, slender, oval in cross-section, posterior end slightly rounded (Fig. 4a). Facial mask with pair of two-segment antennae and maxillary palpi (Fig. 4b). Basal segment of antenna ring-like, apical segment dome-shaped, narrowed at the base, located inside basal segment. Maxillary palpus with numerous sensilla (Fig. 4b). Pair of frontal palpi over mouthhooks, each with 2 large sensilla. Anterior and prefrontal papilla present. Numerous oral ridges on both sides of mouth opening and over mouthhooks. Cephaloskeleton partly sclerotised, 0.61–0.68 mm in length, including 0.07–0.08 mm long mouthhooks (Fig. 4c). Numerous, very small oral teeth behind apical tooth along lateral surfaces of mouthhook. Oral teeth reaching as far as basal tooth (Fig. 4d). Basal tooth massive, in middle of mouthhook, continuous with broad base of mouthhook. Intermediate sclerite elongated, trianuglar, connected with basal sclerite. Basal sclerite distally divided into dorsal and ventral cornua. Ventral cornu longer and wider than dorsal cornu. Dorsal cornu narrowing posteriorly, continuous with dorsal bridge anteriorly. Rod-shaped parastomal bar projecting anteriorly from basal sclerite. Hypopharynx with prominent longitudinal ventral cibarial ridges extending into lumen of cibarial pump. Anterior spiracle with 4–9 lobes (the number variable between individuals and between left and right spiracles) (Fig. 4e). Posterior spiracle on stigmatophores with three oval spiracular openings (Fig. 4f). Ecdysial scar well visible. Spiracular plate quadrilateral with widely rounded corners. Spiracular hair branching (Fig. 4f). Spiracular trunk 0.08 mm long and 0.05 mm wide. Distance between spiracles 0.05 mm (Fig. 4g). Large, plate-like spinules in closely-spaced rows on lateral surfaces along anterior border of first thoracic segment (Fig. 4h), much smaller and less numerous spinules on ventral surface of same segment (Fig. 4i). Small spi-

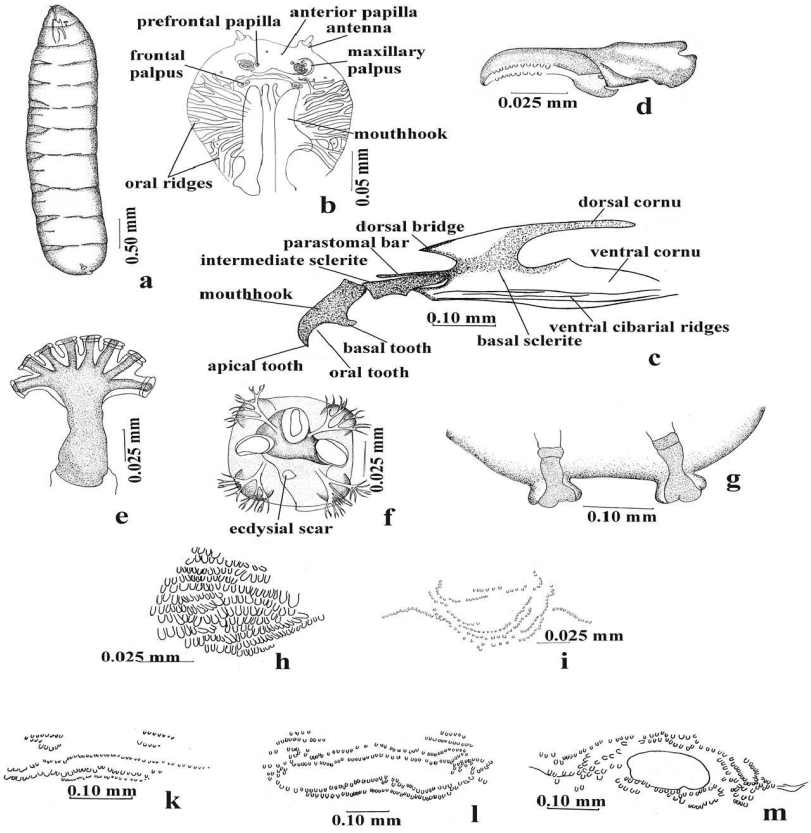

Fig. 4. *C. flavitarsis*, third-stage larva. – a. Lateral view. – b. Facial mask. – c. Cephaloskeleton. – d. Mouthhook, ventrolateral view. – e. Anterior spiracle. – f. Posteror spiracle. – g. Posterior segment, ventral view. – h. Spinules on lateral anterior part of first thoracic segment. – i. Spinules on ventral anterior part of first thoracic segment. – k. Spinules on ventral intersegmental regions of second and third abdominal segments. – l. Spinules on ventral intersegmental regions of seventh and eighth abdominal segments. – m. Anal spinules.

nules at segment borders ventrally and around anal opening (Fig. 4k, l, m).

Puparium (Fig.5). 2.76–4.44 mm long, 0.66–1.14 mm at widest diameter, oblong, straw-coloured to brown. Narrowed anteriorly, flattened dorsoventrally, posterior end broad, slightly rounded (Fig. 5a). Anterior spiracles present on both sides of anterior border of puparium (Fig. 5b, a). Posterior spiracles strongly sclerotised, on stigmatophores (Fig. 5c, d). Numerous folds of cuticle in anterior part dorsally and all along ventral surface (Fig. 5a, d).

4. Discussion

Common reed is among the plants supporting the richest phytophage faunas in the Palaearctic (Emeljanov 1967), with about 200 consumer species belonging to seven orders of Insecta and one family of Acarina. The order of Diptera is represented by eight families, the most numerous of which are the Chloropidae with 26 species (Nartshuk 1996). Accordingly, common reed has been considered a model plant for studies of phytophage-plant relationships (Skuhravý 1981, Nartshuk 1996). Such relationships are important for the functioning of many ecosystems. Gaining a comprehensive understanding of the phytophage–plant relationship is not possible without understanding the morphology and biology of the phytophages.

The present paper is a rare detailed account of the morphology of one of many species inhabiting stems of common reed. Most available data pertain to with the morphology of *Lipara* flies (Waitzbauer 1969, Chvála *et al.* 1974, Grochowska 2006a–c, 2007), with few papers describing the genus *Cryptonevra* (Hennig 1943, Waitzbauer 1969). Known descriptions include the third-instar larva and pupa of *C. nigritarsis* (Hennig 1943, Waitzbauer 1969) and a drawing of the pupa of *C. flavitarsis* (Waitzbauer 1969). The third-instar larva of *C. flavitarsis* differs from the

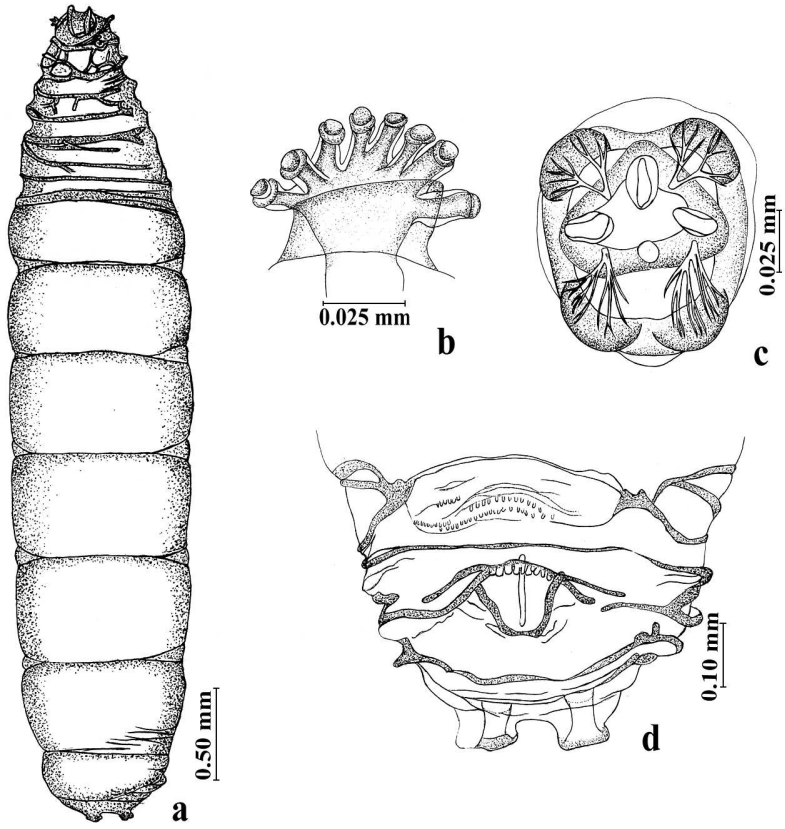

Fig. 5. *C. flavitarsis*, puparium. – a. Dorsal view. – b. Anterior spiracle. – c. Posterior spiracle. – d. Posterior segment, ventral view.

larvae of *C. nigritarsis* described by Hennig (1943) in several characters. The former is larger, with the pseudocephalon not divided into lobes, two-segment antennae and mouthhooks with conspicuous, closely spaced, small teeth. The corresponding larva of *C. nigritarsis* has a mouth-hook without teeth and three-segment antennae. The structure of spiracles is also different. The anterior spiracles of the *C. flavitarsis* larva have 4–9 lobes, and the spiracular plate of posterior spiracles is quadrilateral, while the larva of *C. nigritarsis* has anterior spiracles with 4–5 lobes and the spiracular plate of posterior spiracles is oval. The puparium of *C. flavitarsis*, previously known only from drawings (Waitzbauer 1969), is described for the first time in this paper and illustrated with original drawings. In his description of the puparium of *C. nigritarsis*, Hennig (1943) merely stated that the puparium bore characters of the third-instar larva. As a result, his description can hardly be compared to the above description of the puparium of *C. flavitarsis*.

Larvae of *C. flavitarsis* are typical saprophages. They are found in galls of *Lipara* and *Platycephala* flies together with larvae of *C. diadema*, *C. nigritarsis* and *Incertella zuercheri*. There have also been very rare records of these larvae found among decaying leaves of the apical part of stems not inhabited by other species of insects.

Acknowledgements. I would like to thank Dr Lech Lechowski for his suggestions and comments on this paper.

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