

Description of immature stages of cleptoparasitic bees *Epeoloides coecutiens* and *Leiopodus trochantericus* (Hymenoptera: Apidae: Osirini, Protepeolini) with remarks to their unusual biology

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This study presents descriptions of first instars and mature larvae of cleptoparasitic bees *Epeoloides coecutiens* (host *Macropis fulvipes*) and *Leiopodus trochantericus* (host *Diadasina* sp.). Larvae of the genus *Epeoloides* are described for the first time. The larva of *Epeoloides* is similar to other osirine species, but also possesses several characters previously known as autapomorphies of the genus *Leiopodus*. The unusually short stadium of the first instar and killing behaviour of the second instar in *E. coecutiens* are recorded. The first instar of *L. trochantericus* is quiescent for a long time and kills the late second or third instar of the host.

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1. Introduction

Nest parasitism is a life strategy of cleptoparasitic bees that evolved a number of times de novo within the superfamily Apoidea. Recent studies suggest six or eleven independent origins among the bees of the family Apidae (Michener & Greenberg 1980, Rozen 2000, Michel-Salzat *et al.* 2004, Straka & Bogusch 2007). Both opinions are supported by phylogenetic analysis based on adult and larval morphology respectively (Roig-Alsina & Michener 1993, Straka & Bogusch 2007) and by the diversity of the modes of parasitism (Rozen 1991, Rozen *et al.* 2006).

Mature larvae of at least one species of all the totally cleptoparasitic tribes of the family Apidae have already been described, but the number of genera with undescribed morphology is still large (Rozen 2001, Rozen *et al.* 2006). For this reason, Rozen's (2001) taxonomic key to the genera of cleptoparasitic bee larvae is highly incomplete. This key contains all the known genera except the most recently described species of the tribe Osirini (Rozen *et al.* 2006). Thus, members of this tribe are completely missing in the key.

Generalized strategies of egg laying and host offspring killing are known for all of totally cleptoparasitic tribes of the subfamily Apinae and

almost all tribes of the subfamily Nomadinae (Rozen 1991, Rozen *et al.* 2006). This knowledge enables a wide comparison to be made of the various lineages of bees and currently described species of the tribes Osirini and Protepeolini. The most important behavioural questions are: (1) are eggs introduced into open or closed host cells, and (2) does the mother kill the host egg or early instar with her mandible or sting, or do the parasitic larvae kill the host egg or larva? (3) If the latter, which parasitic larval instar(s) does the killing?

Mature larvae of two species of the tribe Osirini (*Protosiris gigas* Melo and *Parepeolus minutus* Roig-Alsina) were described by Rozen *et al.* (2006) with detailed information about the mode of parasitism and first instar morphology for *Protosiris* Roig-Alsina. Both osirines are nest parasites of oil collecting bees of the tribe Tapinotaspidini. *Protosiris* opens finished closed host cells and kills the host egg or larva with its elongate sting. Similar behaviour is known only in the unrelated *Exaerete* Hoffmannsegg (Garófalo & Rozen 2001). Larval instars of *Epeoloides* Giraud were not previously described, but there are some publications directed towards the behaviour of adults (Bogusch 2003, 2005). Bogusch (2005) mentioned an important feature of cocoon spinning in *Epeoloides*, which indicates development of a pronounced labiomaxillary region in its mature larvae.

Mature larvae, first instar larvae and modes of parasitism of two species of Protepeolini [*Leiopodus singularis* (Linsley et Michener) and *L. lacertinus* Smith] have already been described by Rozen *et al.* (1978) and Roig-Alsina and Rozen (1994). Roig-Alsina and Rozen (1994) have also described predefecating larva of *L. abnormis* (Jørgensen), but it seems to be very similar to *L. singularis*. Bees of the tribe Emphorini are the only known hosts of *Leiopodus* Smith. Females enter their opened cell while it is still being provisioned and precisely embed their egg in the cell wall. In *L. singularis* the first instar hatches a long time after the host larva and usually kills it when it is quiescent before molting to the third or fourth instar (Rozen *et al.* 1978, Roig-Alsina & Rozen 1994).

The goal of the current study is to describe mature and first instar larvae as well as to describe

and discuss the modes of parasitism of *Epeoloides coecutiens* (Fabricius) and *Leiopodus trochantericus* Ducke. Larvae of the genus *Epeoloides* have never been described, and parasitic behaviour is known for only one species in the tribe Osirini (Rozen *et al.* 2006). In contrast, larvae of the tribe Protepeolini are well known and the description of those of *L. trochantericus* makes the list of described species almost complete. Five species of the genus *Leiopodus* are the only representatives of the tribe and the larvae of four of them are currently known (Roig-Alsina & Rozen 1994).

2. Material and methods

Larvae of *Epeoloides coecutiens* were collected from 25.VII.2005 to 20.VIII.2005 in Klánovický les, Praha-Klánovice in the Czech Republic, by both authors. The sample contains three dead first instar larvae, two killed by intraspecific siblings, and one died shortly after eclosion in laboratory; two second instar larvae; three undefecated fourth instar larvae; and three mature (post-defecating) larvae.

Larvae of *Leiopodus trochantericus* were collected from 29.I.2006 to 31.I.2006 on the road close to Capitan Solari, Chaco province, in Argentina by the first author. One first instar larva and three mature larvae represent the sample. Other biological observations were performed also on the nesting area on the road close to Mburucuyá, Corrientes province, in Argentina. All specimens are deposited in the collection of the first author.

Biological observations were made on the same localities where the larvae were collected. Construction of the cell and the shape of the provision of *Macropis fulvipes* (Fabricius) were very similar to *M. nuda* (Provancher) (see Rozen & Jacobson 1980). The food mass was formed into compact ovoid loaf and was positioned toward the back of the cell. Egg was placed on the top of the pollen mass. Laboratory rearing of larvae was performed with plastic containers of various shapes in the laboratory at room temperature (20–25°C).

All specimens were preserved directly in Pampel's fluid (glacial acetic acid – 4 parts, 5%

formaldehyde – 6 parts, 96% ethyl alcohol – 15 parts, distilled water – 30 parts) according to Švácha and Danilevsky (1987).

Mature larvae, first instar larvae (one of each species) and second instar larva (*Epeoloides* only) were dissected and cleared in a 10% solution of potassium hydroxide (KOH) similarly to Rozen (2001). Head capsules were severed from the rest of the body and cleared in KOH until the soft tissue was removed. Right mandibles were removed from the cleared head capsules and observed separately from dorsal and inner or outer views. Several spiracles were removed from the right side of the body wall and cleared in the same way as the head capsules. Head features, spiracles and all integumental characters were investigated in glycerine preparations, sometimes with cotton wool for better adjusting of the right position. Cleared specimens were stored in glycerine. Mature larvae were described according to McGinley (1981) and some more recent works (Michelette *et al.* 2000, Alves-dos-Santos *et al.* 2002). Descriptions of the first instar larvae are patterned after those in a major work about first instar larvae (Rozen 1991).

Drawings were prepared using a drawing tube (camera lucida) attached to an Olympus BX40 light microscope (from temporary glycerine slides) and an Olympus SZX9 binocular microscope. Any asymmetry shown in the drawing but not mentioned in the respective description is caused by artefacts of preparation. Drawings were scanned using an hp scanjet 5530 photosmart scanner and refined using Adobe Photoshop 7.0. Final figures were treated using CorelDRAW 9.

3. Results

3.1. Postdefecating larva of *Epeoloides coecutiens* (Fig. 1)

Diagnosis. *Epeoloides* is similar to both described Osirini and in some ways resembles members of Protepeolini, Melectini, and Rhathymini. It differs from all other species due to a unique combination of characters and especially due to the morphology of the hypostomal ridge; its posteroventral extension is well visible from a

lateral view (Fig. 1c). This character appears like the feature in Protepeolini, but there the extension of hypostomal ridge is more internalized (see the Discussion). Also the conspicuous body spiculation and distinct setae on the head capsule denote specific features (autapomorphy) of the tribe Protepeolini. However, dorsal body spicules are simple in *Epeoloides*, not transverse and serrate like in *Leiopodus*.

In contrast to Protepeolini, *Epeoloides* larva possesses some other characters of Osirini; short, non-membranous clypeus, upper margin of labrum well below the level of anterior tentorial pits (in frontal view), lower margin without paired tubercles and large spiculate hypopharynx. Several mandibular and labiomaxillary characters of *Epeoloides* are similar to those of *Rhathymus* Lepelletier et Serville, *Melecta* Latreille, and some other Melectini, but differ by the characters mentioned above.

Description. Length (if straight) approximately 12–13 mm (N = 3).

Head: Integument unpigmented except the following areas (see Fig. 1c): mandibular apex and articulations, ventral extension of hypostomal ridge and tentorium in the vicinity of anterior and posterior tentorial pits, apex of labrum, salivary lips, labial and maxillary palpi, faintly pigmented antenna, cardo and stipes; cranium, clypeus, labrum, apices of labium and maxilla with scattered setiform sensillum; apex of labrum, hypopharynx and maxilla with well developed spicules, epipharynx without spiculation.

Head size small compared with body (Fig. 1a); head capsule wider than long in frontal view (Fig. 1b). Tentorium complete; dorsal arm thin and hardly visible; posterior arm well developed, projecting medially, lower in position; anterior tentorial pit high, well separated from mandible. Median longitudinal thickening of head capsule well developed, reaching up to middle of head capsula. Postoccipital ridge very slightly curving forward (almost straight), well defined in all parts by pigmented internal ridge; hypostomal ridge well developed, sinuate, binding to postoccipital ridge at obtuse angle; ventral extension of hypostomal ridge externally visible in lateral view (Fig. 1c), in some specimens is not pigmented, but is recognisable as ventrally salient thickening

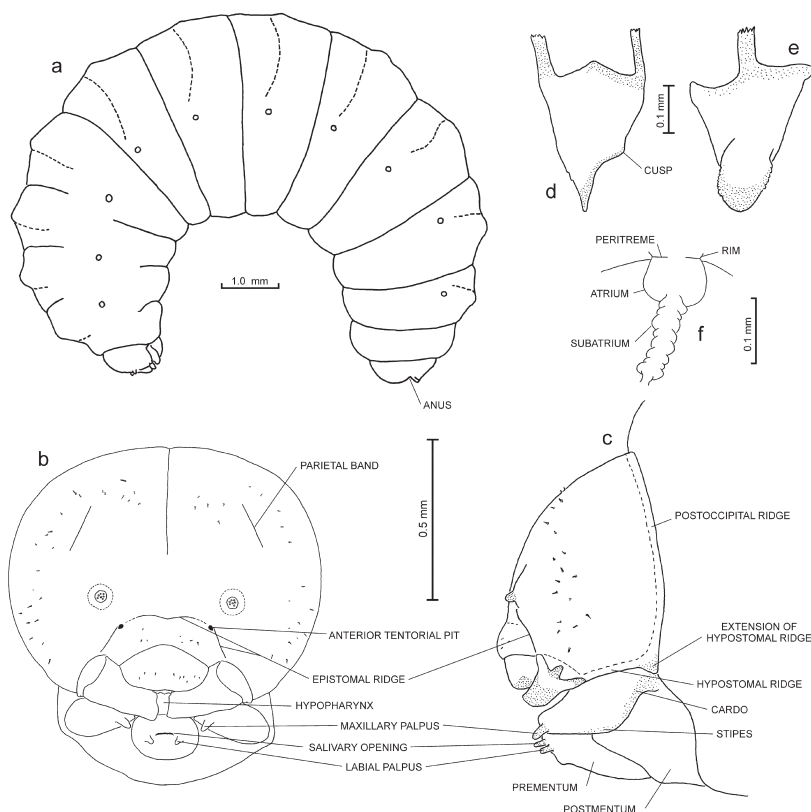


Fig. 1. *Epeoloides coecutiens*, mature larva. – a. Entire larva in lateral view. – b. Head in frontal view. – c. Head in lateral view. – d. Right mandible in dorsal view. – e. Right mandible in inner view. – f. Spiracle in side view.

of posterior part of hypostomal ridge (only in cleared head capsules); junction of hypostomal ridge distinct and pigmented; pleurostomal ridge faintly developed; epistomal ridge well developed, especially its lateral sections. Parietal band represented by weak oblique line above antenna. Antennal prominence moderately weakly developed; disk differentiated from papilla; antennal papilla distinctly projecting, height about one and half basal diameter, with approximately seven sensilla. Short labrum sloping straight downward, bearing numerous conspicuous sensillum, labral apex pigmented; clypeus slightly projecting beyond labrum and frons (Fig. 1c); epipharynx a simple, slightly curved surface beneath labrum.

Mandible (Figs 1d and 1e) moderately robust, short, hardly reaching midline of head; mandibular apex simple, broadly rounded; apical concavity well developed, smooth, scoop-shaped; upper and lower apical edges with distinct teeth (Fig. 1d); outer surface bearing numerous setiform sensilla; cusp slightly distinct, nondentate. Labio-

maxillary region (Figs 1b and 1c) moderately recessed, enlarged only during cocoon spinning. Maxillary apex well separated from labium, bearing elongate palpus apically; galea not evident; cardo and stipes well developed, distinct as pigmented sclerites. Labium divided into prementum and postmentum (Fig. 1c); premental sclerite faintly evident; labial palpus elongated, slightly shorter than maxillary palpus. Salivary lips projecting, sclerotized; slightly narrower than distance between labial palpi. Hypopharynx (Fig. 1b) large, produced dorsally, apical part slightly blobbed; hypopharyngeal groove well developed.

Body: Integument without obvious setae although those of perianal area with scattered, very fine setiform sensillum; integument under high magnification distinctly spiculate, patches of fine, evenly spaced spicules visible on dorsal as well as ventral areas of thorax; integument without any spines or sclerotized tubercles. Body form (Fig. 1a) moderately robust; intersegmental lines moderately defined; dorsal intrasegmental

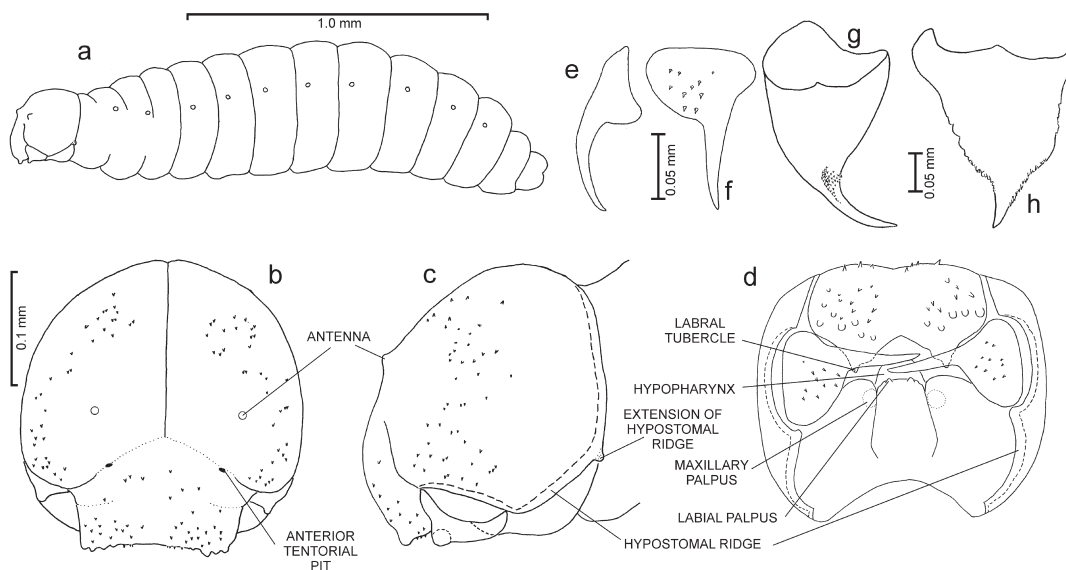


Fig. 2. *Epeoloides coecutiens*, first and second instar. – a. Entire first instar in lateral view. – b. Head, first instar, in frontal view. – c. Head, first instar, in lateral view. – d. Head, first instar, in ventral view. – e. First instar, right mandible in dorsal view. – f. First instar, right mandible in outer view. – g. Second instar, right mandible in dorsal view. – h. Second instar, right mandible in outer view.

lines evident on most body segments; small paired dorsal tubercles well developed on thoracic segments, becoming less evident towards abdominal segment VIII; middorsal and lateral body tubercles missing; abdominal segment IX with venter not produced, so that segment X appears to be attached approximately to middle of IX in lateral view; anus in position slightly dorsal because venter of abdominal segment X is somewhat more produced than dorsum; perianal area with sclerotized transverse line or ill-defined ridge. Spiracles (Figs 1a and 1f) moderately small, subequal in size; atrial wall without ridges; atrium globular, projecting slightly above the body wall; rim well developed; peritreme moderately wide; primary tracheal opening with collar; subatrium short, usually with 8 chambers.

3.2. First instar of *Epeoloides coecutiens* (Fig. 2a–f)

Diagnosis. First instar of *Epeoloides* is simple to recognise due to the combination of following characters: hypognathous head (Fig. 2c), unmodified abdominal segment X (Fig. 2a) and absence

of body setae. It is very similar to the first instar of *Leiopodus* due to the short pad-like maxillary palpus. The large swollen labrum with two apical labral tubercles is directed posteriorly and placed between mandibles and the buccal cavity, which seems to be a unique character of *Epeoloides*.

Description. Length: 1.7–1.8 mm (N = 3).

Head: Shape (Fig. 2c) hypognathous, parietals globose, extending upwards and backwards; in frontal view (Fig. 2b) approximately as wide as long; foramen widely opened, almost as wide as the head capsule; head not pigmented except the posterior tentorial arm and apex of mandibles. Head capsule sensilla short. Anterior tentorial pit conspicuous; posterior tentorial pit darkly pigmented; extension of hypostomal ridge extending posteroventrally. Postoccipital and hypostomal ridges well developed; pleurostomal ridge faintly developed, extending medially as lateral section of epistomal ridge; median section of epistomal ridge faintly developed; pale median ecdysial line weakly developed; posterior margin of head capsule forms obtuse angle with hypostomal ridge (if tentorial pit is excluded). Parietal band not evident. Antennal prominence weakly developed, fused with parietal; disk not differentiated from

papilla; antennal papilla slightly projecting, with numerous non-setiform sensilla. Labrum broad and perhaps large (clypeal margin not completely obvious), strongly curving towards buccal cavity, with transverse irregular rows of tubercles on each side (projections of sensillary basement) which give illusion of labral apex (Figs 2b and 2c); these sensillary tubercles are asymmetrically distributed, each bearing one very small apical papillate sensillum; possible true apical tubercles (Fig. 2d) widely separated from each other, projecting posteriorly between mandibles and buccal cavity.

Mandible (Figs 2e and 2f) broad at base, apically attenuate, sharply pointed, curved into the buccal cavity when closed, inner edge smooth, with ten setiform sensilla on outer surface. Labio-maxillary region (Fig. 2d) unsclerotized; maxilla well separated from labium, bearing numerous small spicules; maxillary palpus flat and short, slightly pad-like, born ventrally, bearing several small sensilla; maxillary lobe small; labium covered with short, posteriorly directed spicules; apically positioned labial palpus distinctly projecting, conical, short. Hypopharynx (Fig. 2d) large, densely spiculate; hypopharyngeal groove well distinct; salivary opening hardly visible, well separated from hypopharyngeal groove.

Body: Form (Fig. 2a) moderately elongate, straight, without distinct tubercles; abdominal segment X rounded, without apicolateral lobes (pygopods), but with distinct vertical ridge in dorsal lobe dividing it into two swellings. Integument without any conspicuous setae; all body segments with distinct band of spicules directed posteriorly; segment X with rounded spicules dorsally and without spicules ventrally; dorsal spiculation sparser and shorter than ventral spiculation. All spiracles present, normal in position, subequal in size. Anus positioned apically.

3.3. Biology of *Epeoloides coecutiens* (Figs 2g–h)

We did all observations in a small area with three nesting aggregations with one to nine nests of host bees (depending on time and activity). *Macropis fulvipes* (Fabricius) was recorded as the only host of *Epeoloides*, even though *M. euro-*

paea Warncke was also present in the locality (but less common).

Behaviour of adults and egg deposition: Flight activity occurs from approximately 9:00 a.m. to 7:00 p.m. (Central European Time). Females searched for the host nests only in warm weather (23–37°C) and usually only in the sunny parts of the nesting area. When the temperature fell (20–25°C) the bees flew strictly in shafts of sunlight or they were inactive. Only during the warmest days were they able to fly in the shade for a longer time. The parasitic female was usually able to find all active host nests at the aggregation, even if the host nests were several meters apart. Females returned to the nest up to ten times during the day and checked the cell condition (opened, provisioned, or closed). Some females entered the nest to check the cell condition immediately after finding it.

This behaviour often resulted in conflict if the host female was inside. Parasitic females flew away immediately after the first contact with the hosts. A real conflict situation happened when the host female returned from foraging trip and the *Epeoloides* female had been just inside. The bees fought for several minutes and then the parasite escaped. In another type of visiting behaviour, the parasitic female waits five to twenty centimetres from the entrance on grass or other elevated points (they usually used our nest markers), until the host female returns from foraging and subsequently leaves the nest. As soon as the host female flies away, the cuckoo bee enters the nest. Two females together were often observed in such behaviour in front of a nest.

If the host nest was inappropriate for egg laying, the female stayed inside several seconds to five minutes. When the nest was appropriate, the cleptoparasitic female stayed one hour or longer inside. Sometimes such a female was seen in the entrance taking the soil from tumulus when finishing the cell closure. Also the closing of the nest by two females was twice observed. One was more inside near the cell and the second closed the middle part of the tunnel with soil. *Epeoloides* female was usually closing the cell as well as the large part of tunnel, when enough soil was available from the tumulus.

Successful parasitization of the nest was artificially initiated five times. Elimination of host fe-

male during good weather, when this host female remained more than one hour at the entrance and did not forage, always caused subsequent successful parasitisation. Freshly closed host cell (*M. europaea*) was not accepted by the female of the cleptoparasite, but such a situation was observed only once. In no other case we were certain if the cell was really freshly closed and subsequently visited by the cleptoparasite.

25.VII. – one nest excavated thirty minutes after the entry by *Epeoloides* female: Two females were found inside, one was burrowing and one was closing the nest. One fully provisioned (or nearly so) cell was found with one *Epeoloides* egg. This egg was laid between provision and closure, adhering to the bottom right side of the cell wall. Host egg was not found, probably missing.

28.VII. – one nest excavated after departure of *Epeoloides* female: One provisioned cell was found with one *Epeoloides* egg. This egg had almost the same position as in the previous cell. Host egg was not found, probably missing.

18.VIII. – one nest excavated after *Epeoloides* female leaves: One provisioned cell was found with one *Epeoloides* egg. Normal position of the egg was disrupted, but it was not on the provision. Host egg was not found, probably missing.

Development cycle and larval behaviour: In two nests, where the time of egg deposition was known, the time period of larval cycle were recorded. Both larvae were dug out together after 22 and 27 days respectively and both larvae were still feeding. They were reared in the laboratory and the older one spun a cocoon 33 days after parasitisation of the nest. The second one fell from the provision and after one day without provision began spinning of the cocoon as well. Its larval development took 28 days.

The nest with the egg from 28.VII. was kept in the laboratory until the first instar emerged. The first instar hatched overnight in an interval of between 24 and 34 hours when the checks were made. Similar observation was made in the field. A nest that we excavated during the evening on 1st August was provisioned during 28th July and probably finished (and parasitised) the next day (29.VII.). One early second instar feeding on top of the provision was found inside. On 5.VIII., exactly 72 hours after the parasitisation, a third nest

was excavated. Three cells were found inside. One, freshly parasitised, contained two eclosed egg chorions adhered to the bottom right of the cell wall, two fresh dead (probably killed) first instars close to the eggs and two second instar larvae on the top of the provision. One of these second instar larvae was decapitated and being eaten by the other second instar. These killing larvae had sharp mandibles almost like the first instar (Figs 2g and 2h). There were no vestiges connected with the presence of host larva.

Cocoon fibre of *Epeoloides* is ochraceous and thus very slightly differentiable from the dark brown host cocoon.

3.4. Postdefecating larva of *Leiopodus trochantericus* (Fig. 3)

Diagnosis. Larvae of three other species of the genus *Leiopodus* have already been studied. *Leiopodus trochantericus* differs from them in the following characters: maxillary spiculation absent, mandible attenuate apically and its apical concavity weakly developed, body integument apparently nonsetose, body spiculation distinctly shorter than in other species, spiracular atrial wall smooth and subatrial length short.

Leiopodus trochantericus is a typical member of the tribe Protepeolini and in the key to mature larvae of cleptoparasitic bees (Rozen 2001) goes to the genus *Leiopodus* (couplet 11).

Description. Length (if straight) approximately 12–13 mm (N = 3).

Head: Integument unpigmented except for following areas: mandibular apex and articulations, internal head ridges, posterior extension of the hypostomal ridge, two vertical lines mesad of antennae, salivary lips and stipes; labial and maxillary palpi and antennae faintly pigmented; cranium, labrum and apex of labium with scattered setiform sensillum; spiculation not developed.

Head size small compared to the body (Fig. 3a); head capsule very wide, distinctly wider than long in frontal view (Fig. 3b). Tentorium complete; dorsal and anterior arms thin and hardly visible; posterior bridge very thin, lateral part of bridge is more probably posteroventral and internalized extension of hypostomal ridge (see Dis-

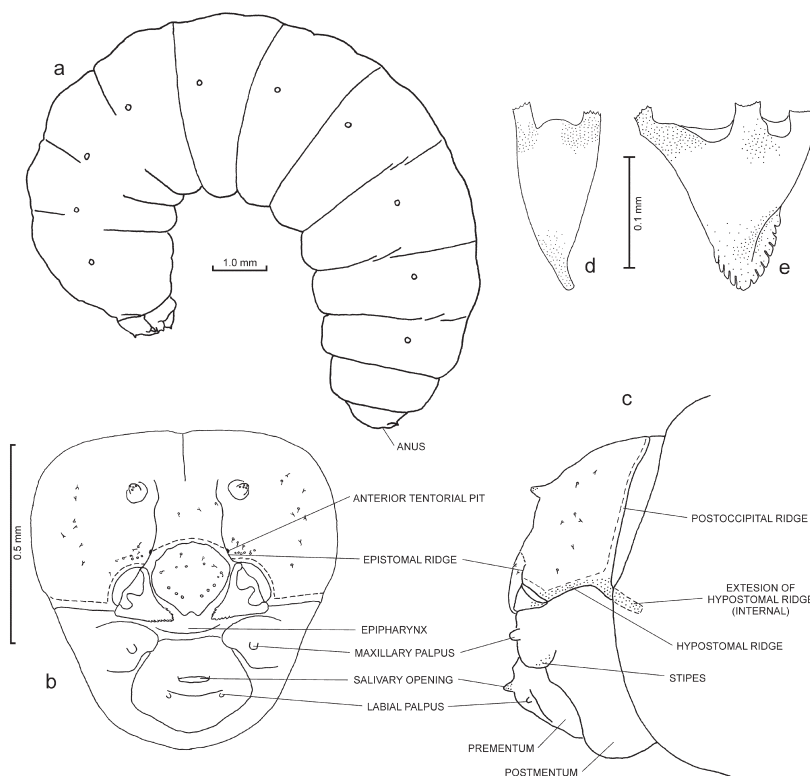


Fig. 3. *Leiopodus trochantericus*, mature larva. – a. Entire larva in lateral view. – b. Head in frontal view. – c. Head in lateral view. – d. Right mandible in dorsal view. – e. Right mandible in inner view.

cussion and Fig. 5b); anterior tentorial pit low, much closer to mandible than to antenna (Fig. 3b). Median longitudinal thickening of head capsule well developed, reaching middle of the head capsule. Postoccipital ridge straight, well defined in all parts; hypostomal ridge well developed, sinuate, binding to postoccipital ridge at obtuse angle; long posteroventral extension of hypostomal ridge visible in lateral view including external and internal parts (see description of tentorium and figs 3c and 5b); pleurostomal ridge well developed; epistomal ridge short, well developed only laterally. Parietal band not distinct. Antennal prominence moderately weakly developed; disk differentiated from the papilla; antennal papilla distinctly projecting, height about one and half basal diameter, with approximately six sensilla. Labrum large, rounded, but not projecting as seen in lateral view (Fig. 3c), bearing numerous conspicuous sensilla; labral apex without tubercles, slightly emarginated ventromedially; clypeus extremely short laterally, fusing with frons above, but the frontoclypeal morphology is not fully understood, short membra-

nous area between frons and labrum developed; frons with two heavily sclerotized vertical ridges mesad of antennae of uncertain origin; epipharynx forms separate large swollen area beneath the labrum clearly visible in frontal view (Fig. 3b).

Mandible (Figs 3d and 3e) robust, very short, attenuate towards the apex; mandibular apex simple, acute; apical concavity poorly developed, smooth, not scoop-shaped; upper and lower apical edges with large teeth (Fig. 3d); outer surface bearing several setiform sensillum; cusp not evident. Labiomaxillary region (Figs 3b and 3c) large in relation to the head capsule; maxilla moderately fused to labium at base; maxillary apex produced medially; palpus large, positioned apically; galea not evident; cardo reduced, stipes distinct as pigmented sclerite. Labium divided into prementum and postmentum (Fig. 3c); premental sclerite faintly evident; labial palpus small, much smaller than maxillary palpus, considerably lower than salivary opening. Salivary lips large, projecting, sclerotized; much narrower than distance between labial palpi. Hypopharyngeal area

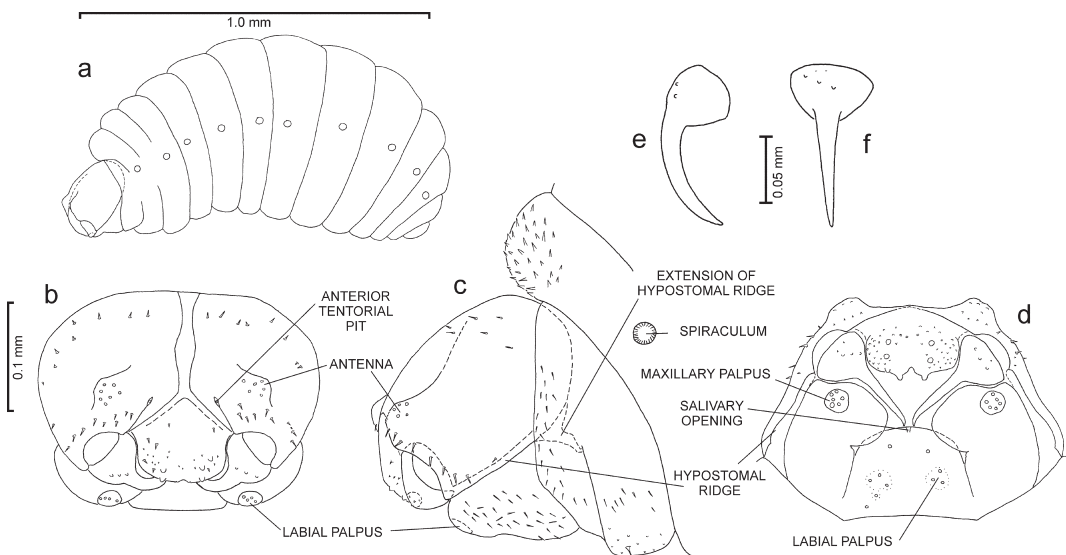


Fig. 4. *Leiopodus trochantericus*, first instar. – a. Entire larva in lateral view. – b. Head in frontal view. – c. Head in lateral view. – d. Head in ventral view. – e. Right mandible in dorsal view. – f. Right mandible in outer view.

not differentiated from dorsal surface of labium; hypopharyngeal groove absent.

Body: Integument with obvious pigmented setae on abdominal segment X; lateral part of majority of segments with a few very short setiform sensilla visible only under high magnification, body not conspicuously setose; integument distinctly spiculate, patches of fine, often transverse spicules visible on dorsal as well as ventral areas of thorax; integument without any spines or sclerotized tubercles. Body form (Fig. 3a) moderately robust; intersegmental lines not defined, not divided into cephalic and caudal annulets; small paired dorsal tubercles poorly developed on thoracic segments; small middorsal body tubercles distinct between thoracic and anterior abdominal segments; lateral body tubercles missing; abdominal segment IX with non-produced venter, but segment X attached dorsally to IX in lateral view; anus slightly dorsal in position because venter of abdominal segment X is somewhat more produced than dorsum; perianal area with distinct sclerotized transverse ridge. Spiracles moderately small, subequal in size, born on small tubercles; atrium globular, projecting distinctly above the body wall; atrial wall without any ridges or spines; rim poorly developed; peritreme moderately narrow; primary tracheal opening with a collar; subatrium short.

3.5. First instar of *Leiopodus trochantericus* (Fig. 4)

Diagnosis. The first instar of *L. trochantericus* is similar to both described first instars of the genus *Leiopodus*. This species looks like the first instar of *L. singularis* with a more circular head, narrower labrum and flat, padlike maxillary palpus but it differs because of a wider head (but not so wide as in *L. lacertinus*) and sparsely spiculate mandibles. Also the body shape, head capsule (lateral view), mandible and antennal prominence differ from both described species.

Description. Length: 1.1 mm (N = 1).

Head: Shape (Fig. 4c) more or less hypognathous, parietals globose, extending upwards and backwards; in frontal view (Fig. 4b) wider than long; foramen magnum very large; mandibles and head capsule darkly pigmented except the frontoclypeal area and labrum; labiomaxillary region entirely membranous and unpigmented. Head capsule sensilla long, setiform. Anterior tentorial pit conspicuous; posterior tentorial pit internalized. Hypostomal ridge strongly curving upward to the postoccipital ridge, both with fluent conversion from one to another; extension of hypostomal ridge very large, internalized and directed posteroventrally, darkly pigmented; pleurostomal ridge well developed, extending

medially as the lateral section of the epistomal ridge; median section of the epistomal ridge not developed; pale median ecdysial line present. Parietal band indistinct. Antenna located on large lateral swelling; antennal papilla and disk not distinct, fused with parietal; swelling bears six or seven large nonsetiform sensilla. Labrum large, moderately wide, strongly curving towards buccal cavity, with seven large sensillary tubercles (Fig. 4d); these sensillary tubercles distributed into two lines, each bearing one very small apical papillate sensillum; apical tubercular pair directed towards the buccal cavity; area between these tubercles distinctly emarginated.

Mandible (Figs 4e and 4f) broad at base, apically attenuate, sharply pointed, curved into buccal cavity when closed, inner edge smooth, with three large and a few small spicules on outer surface. Labiomaxillary region (Figs 4c and 4d) not sclerotized; maxilla distinct from labium, bearing long, but very thin setiform posteriorly directed spicules; large, flat and padlike maxillary palpus located ventrally, bearing six distinct sensilla; maxillary lobe large, slightly pronounced over the labium; labium covered with short, posteriorly directed spicules; labial palpus may be represented by large short sensilla distributed over lower surface of labium, with several concentrated on the bare area (Fig. 4d). Hypopharyngeal area reduced; salivary opening distinct, directed into buccal cavity.

Body: Form (Fig. 4a) moderately robust, slightly curved, with small lateral tubercles below spiracles; abdominal segment X hardly visible, probably small, rounded, without distinct apicolateral lobes (pygopods). Integument with conspicuous setae spread over lateral tubercles; all body segments with distinct band of spicules directed posteriorly; spicules long on thoracic segments I and II; thoracic segment III and abdominal segments I–VIII with rather short and sometimes indistinct spicules; spicules of segment IX and X long and distinct, directed posteriorly; dorsal spiculation longer than the ventral spiculation. All spiracles present, normal in position, subequal in size. Anus not evident.

3.6. Biological observations of *Leiopodus trochantericus*

Several females were observed in the nesting area of *Diadasina* (*Diadasina*) sp. in Chaco prov. as well as in Corrientes prov. Females usually entered the nest soon after the host left it. They were often found sitting on the ground near the active nest and waiting until the host female left. From nine excavated cells, five cells of *Diadasina* were parasitised. A first instar larva was found half inside a decapitated host second (or possibly third) instar. All mature larvae were found in cocoons. The cocoon is ochraceous, superficially hardly differentiable from the host cocoon.

4. Discussion

In the key to the larvae of cleptoparasitic bees *Epeoloides* goes next to *Rhathymus* (couplet 17) (Rozen 2001), but other Osirini were described later so they are all missing from this key. Recently described Osirini *Protosiris* and *Parepeolus*, (Rozen *et al.* 2006) go to the couplet 17 as well as *Epeoloides*. The partial similarity of *Epeoloides* to *Leiopodus* is not important for the keying, because they differ in many other characters that are more easily visible. Their common features, like conspicuous setae on head capsule, distinct body spiculation, and externally visible extension of hypostomal ridge, may be phylogenetically important, because all of them were previously considered as unique for *Leiopodus* (Roig-Alsina & Rozen 1994). On the other hand, body spiculation basically differs between these two genera. Especially the dorsal spicules of *Leiopodus* are distinctly serrate and transverse, which is a unique shape of spicules among Long-Tongued bees (Megachilidae and Apidae). In addition, the extension of the hypostomal ridge is similar only externally (compare figs 1c and 3c). The internalized part of the hypostomal ridge of *Leiopodus* is extremely large and posteroventrally moved, unlike any other bee species (compare figs 5a and 5b). Roig-Alsina and Rozen (1994) discuss this unusual character and consider that the posterior arm of the tentorium is the same as the posterior extension of the hypostomal ridge. We are convinced that this is right and we

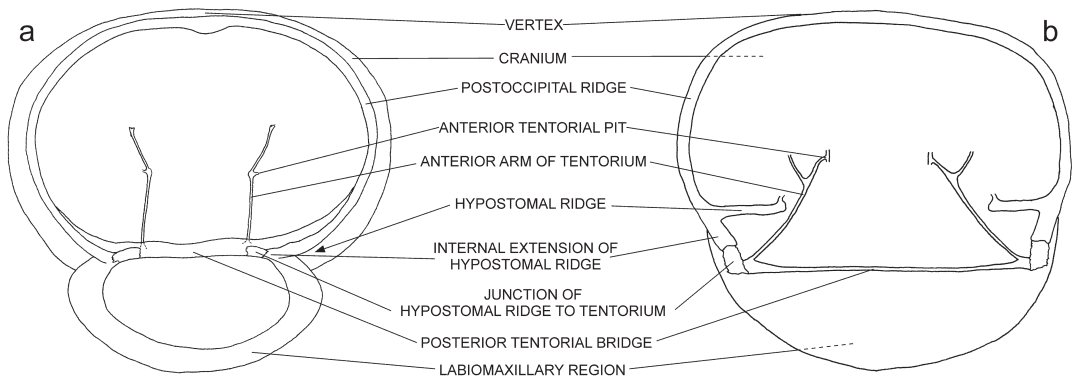


Fig. 5. Foramen magnum of mature larvae, distal view. – a. *Epeoloides coecutiens*, position of hidden hypostomal ridge indicated by arrow. – b. *Leiopodus trochantericus*.

use the general term “extension of hypostomal ridge”, which could be internal, external, and variously directed (ventrally, posteriorly, etc.). The distinctive, darkly pigmented junction of the hypostomal ridge to the tentorium, and in *Epeoloides* also to the labiomaxillary region supports this statement. Homology is also indicated by branching of the anterior tentorial arm to the posterior tentorial bridge close to the junction (Figs 5a and 5b).

The first instar of *Epeoloides* seems to be more similar to *Leiopodus* than to *Protosiris* according to Rozen’s table of derived first instar characters (Rozen 1991, Rozen *et al.* 2006). *Epeoloides* shares four possible synapomorphies with *Leiopodus*: parietals extending upwards and backwards; angle of posterior margin of head to the hypostomal groove is broadly curved; antenna fused with head capsule; and maxillary palpus short, large and pad-like. Only two derived characters are similar to the only known osirine first instar (*Protosiris*): the egg is free in cell; and the antenna is fused with head capsule. It is not substantial evidence of the relationship between *Leiopodus* and *Epeoloides*, but this conclusion is similar to the result of the cladistic analysis of larval characters (Straka & Bogusch 2007) *Leiopodus* and *Epeoloides* may be more related than *Epeoloides* to *Protosiris* or other osirine bees. The mentioned morphological characters should not be behaviourally dependent adaptations, because the gap between the behaviour of *Leiopodus* and *Epeoloides* is very large (see discussion below).

Information about the most important behavioural features of *Epeoloides coecutiens* has still not been favourably resolved. Several observations reflect the unusual behavioural strategy of this cleptoparasitic bee. Females, when laying eggs, stay inside the appropriate host nest for a very long time. Construction of the closure is clearly a major part of this time, but the remaining behaviour is unknown. The situation found in excavated nests indicates two almost equally probable modes of behaviour. First, they are laying their eggs in unfinished cells with enough provisioning. The second possible strategy is the female cleptoparasite opening freshly closed cells and consuming host eggs. Thus, host eggs were not found in any opened host cells. Both possible egg depositions are unique among bees of the family Apidae. Consuming of host eggs is known in cleptoparasitic bees of the families Halictidae and Megachilidae (Michener 2000) where two or more eggs do not occur in a host cell together. Halictid parasitic bees probably destroy the eggs of other conspecifics as well, which is probably not true in the case of *Epeoloides*. In one excavated nest we found four larvae per cell. In this point of view, the first propounded suggestion is slightly more likely than the second. Observed behaviour could be a halfway strategy between the opening of finished closed cells and entering unfinished host cells without finished provisioning. Until now, these were thought to be the only two possibilities.

Other unusual features of *Epeoloides* are represented by the very brief duration of the first

instar followed by the molting to the second and the killing behaviour of the second instar. This behaviour is probably connected with the missing host egg or young larva. Selective pressure on the first instar depends more on intraspecific conflict than on the elimination of the host. The early developed second instar possesses mandibles almost two times larger than those of the first instar and thus the survival probability of the first instars should be low in unequal combat. Killing behaviour of second instars is quite rare in bees and is often caused by the fact that first instars are inactive and remain surrounded by the egg chorion (Torchio 1989, Garófalo & Rozen 2001). In megachilid bees there are some known species that are hospicial in higher instars (Torchio 1989, Rozen & Özbek 2004). *Epeoloides* first instar possess typical killing morphology, thus the killing behaviour of the first instar is not out of the question.

The short-term duration of the first instar *Epeoloides* is in high contrast with the long-term first instar of *Leiopodus singularis* and *L. trochantericus*. The *L. singularis* egg incubation period is equalled the duration of the host's cell provisioning (often partly) plus egg incubation period plus the duration of at least the first two larval stadia and usually the length of the third stadium as well (Rozen *et al.* 1978). On the other hand, *L. lacertinus* does not display such an extreme form of behaviour (Roig-Alsina & Rozen 1994). The only found first instar of *L. trochantericus* fed on second or third instar of *Diadasina*, so that its hospicial behaviour could be similar or the same as in *L. singularis*, with late eclosion and killing the quiescent host larva closely before molting to the third or higher instar.

Both described species of larvae differ much from each other both in morphology and behaviour. They may be different because they have different life strategies, and, due to selective pressure, they changed a lot from the common plesiomorphic growth plan. This is surely right especially for mature larva of *Leiopodus*, which is superficially in the least degree dissimilar to any other bee larvae. After all, both tribes (Osirini and Protepeolini) may be closely related as the cladistic analysis of mature larvae suggests (Straka & Bogusch 2007). Even though, they

might have had a common cleptoparasitic ancestor, the question arose – what might be the reason for such a differentiation? We are convinced that it is caused by host antistrategy against its nest parasite. There are several host behavioural features that are almost surely connected with these coevolutionary effects. First, egg-laying underneath the provision is interesting and unique behaviour of emphorine bees (subtribe Emphorina only) (Michener 2000). It may represent an adaptive answer to the behaviour of their nest parasite, which was destroying their eggs inside. Such behaviour is currently known in *Protosiris*, which destroys host eggs with its sting. It was also observed that no first instar was alive in the cell of *Protosiris*, even if the cell was repeatedly parasitised (Rozen *et al.* 2006). In such cases the defending behaviour, like emphorine bees have, should be successful. Laying the egg beneath the provision clearly evolved in a common ancestor of the subtribe Emphorina, because all genera possess this character. We must think of an historical nest parasite of emphorine bees and a possibly ancestor of current *Leiopodus*, which according to cladistic analysis, probably arose from some osirine bee similar in parasitic behaviour to *Protosiris*.

Analogously, the *Macropis* female staying at the entrance (and probably guarding it) during warm days may be the most important event that affects the *Epeoloides* female in its mode of parasitism. The cuckoo bee was clearly unsuccessful when it tried to attack the guarded nest. We found one nest with five cells and all of them contained cocoons of *Macropis*, even if several *Epeoloides* females frequently visited this nest. When a *Macropis* female left its nest during the most risky time period (it also means if a female was artificially eliminated) the nest was always successfully parasitised. This leads to the conclusion that this host behaviour is a very important strategy against *Epeoloides*. *Epeoloides* must elude this antistrategy by changing its mode of parasitism. The parasitic female could return to the nest later (1) after closing of the cell, or earlier, (2) before the host female begins to guard the nest. If it comes to the nest later (1), the available cell would be closed or closed and parasitised by the female that came first (2). *Epeoloides* has adaptively shortened the course of the first instar and

for this reason, the brood of the second visitor (1) of the nest cannot be successful in intraspecific conflict.

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