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Larva, pupa and DNA barcodes of the Neotropical geometrid moth *Glena mielkei* (Lepidoptera: Geometridae: Ennominae: Boarmiini)

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ABSTRACT

Glena mielkei Vargas, 2010 (Lepidoptera: Geometridae: Ennominae: Boarmiini) is a Neotropical geometrid moth native to the Atacama Desert of northern Chile whose larvae are folivorous on the shrub *Trixis cacalioides* (Asteraecae). The last instar and pupa are described and illustrated, and DNA barcode sequences are provided for the first time for *G. mielkei*. Descriptions are made based on larvae collected in the type locality. Comparisons with the available descriptions of congeneric species suggest that the chaetotaxy of the SV group of the abdominal segment and the morphology of the cremaster could be useful tools to species identification based on last instar and pupa, respectively. A search in BOLD (Barcode of Life Data System) showed that the only DNA barcode haplotype found in the two specimens sequenced was closest to *Physocleora* Warren, 1897 than *Glena* Hulst, 1896. These results coincide with the morphological peculiarities of the genitalia highlighted in the original description of *G. mielkei*, suggesting that a definitive assessment of the generic status of this geometrid moth deserves further integrative studies.

Introduction

Species of the family Geometridae tend to exhibit a close relationship with the vegetation (Scoble, 1995; Brehm and Fiedler, 2005; Brehm et al., 2005; Bolte, 1990). Records of the Geometridae fauna in Chile show more than 450 species for the central and southern areas of the country, with most of the described species represented by the subfamilies Ennominae and Larentiinae (Parra, 1995). Records for the north area of Chile, however, remain scarce, with only few species having been described (Vargas, 2010).

Glena Hulst, 1896 is a New World genus of Boarmiini (Lepidoptera: Geometridae: Ennominae) that comprises more than 40 species described so far, of which over 30 occur in the Neotropics (Pitkin, 2002). Natural history and external morphology of immature stages of *Glena* still remain poorly studied (Rindge, 1965, 1967). Plants of the Aceraceae, Ericaceae, Pinaceae, Rosaceae, Salicaceae and Tamaricaceae families are recorded as hosts for Nearctic representatives of *Glena*, while the host records for the Neotropical fauna include plants of the Asteraceae, Clusiaceae, Cupressaceae, Erythroxylaceae, Fabaceae, Myrtaceae and Pinaceae families (Osorio, 2005; Marconato et al., 2008; Robinson et al., 2010; Méndez-Abarca et al., 2014). However, these records are mostly based on a few polyphagous species, whereas many others have never been reared from larvae.

Glena mielkei Vargas, 2010 is the only species of the genus currently described for Chile. To date, the literature on *G. mielkei* includes a description based on the adult stages plus information about its distributional range, which has so far been restricted to the province of Arica (Northern Chile) (Fig. 1A). Although in the laboratory, larvae of *G. mielkei* are able to feed on three species of Asteraceae, namely *Trixis cacalioides* (Kunth), *Pluchea chingollo* (Kunth) and *Tessaria absinthioides* (Hook. & Arn.), the only host plant recorded in the field has been *T. cacalioides* (Vargas, 2010; Méndez-Abarca et al., 2014) (Fig. 1B). We describe and illustrate the external morphology of the last instar larva and pupa of *G. mielkei* for the first time based on specimens collected in the type locality with the

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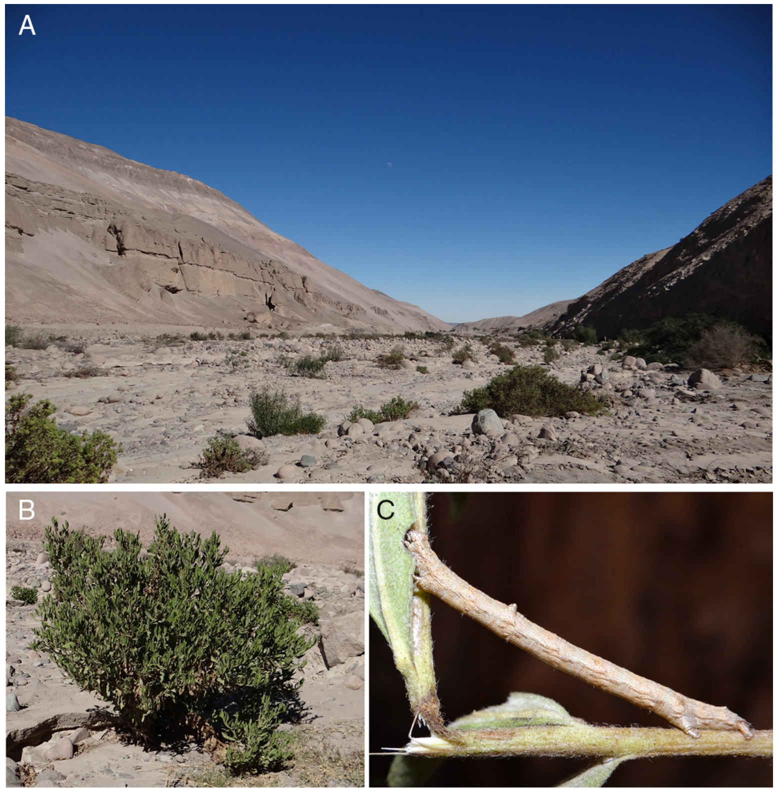

Figure 1. Details of the natural history of *G. mielkei* in the Atacama Desert of northern Chile. (A) Type locality in the Azapa Valley, Arica Province, northern Chile. (B) Host plant *T. cacalioides* (Asteraceae) at the type locality. (C) Last instar larva of *G. mielkei* feeding of *T. cacalioides* at the type locality.

aim of contributing to further comparative studies on morphology of immature stages of Boarmiini. In addition, the first DNA barcode (sensu Hebert et al., 2003) sequences of *G. mielkei* are provided.

Materials and methods

Larvae of *G. mielkei* were collected on *T. cacalioides* in the Azapa Valley (type locality) in June 2017. Larvae were placed in plastic vials with leaves of the host plant and towel paper at the bottom and brought to the laboratory. The vials were cleaned and leaves of *T. cacalioides* were provided periodically until larvae finished feeding. Eight larvae (last instar) and six pupae were kept in ethanol 70% to carry out the morphological analysis. The integument of the larvae was cleaned in hot KOH 10% for a few minutes and structures were slide mounted either on glycerine or Euparal to observe morphological details using a Leica M125 stereomicroscope and an Olympus® BX51 optical microscope. Two pupae were kept in ethanol 95% at −20 °C until DNA extraction. Four pupae were kept in the plastic vials to obtain adults to confirm the taxonomic identification based on the morphology of the genitalia.

Genomic DNA was extracted from two pupae of *G. mielkei* following the procedures described in Huanca-Mamani et al. (2015). Amplification and sequencing of the DNA barcode region (sensu Hebert et al., 2003) were undertaken at Macrogen, Inc. (South Korea) with the primers LCO-1490 and HCO-2198 (Folmer et al., 1994) following the amplification programme described by Escobar-Suárez et al. (2017). The sequence alignment was performed by the Clustal W method in the software MEGA6 (Tamura et al., 2013), and a search for close sequences was performed in BOLD (Ratnasingham and Hebert, 2007).

Results

Last instar larva (Figs. 1C, 2A–E, 3A–F).

Head well-developed, hypognathous, brown-greyish with irregular darker stains; thorax and abdomen brown-greenish, with two tuberiform dorsolateral projections on A2; prolegs present on A6 and A10 (Fig. 1C).

Head. Two groups of six subcircular stemmata located dorsally from the antennal socket (Fig. 2A and B). Antennae trisegmented: first segment ring-like; second segment cylindrical, width similar to first segment, length about twice the width, pore and sensillum on lateral surface, four sensilla on distal surface; third segment cylindrical, length about one-third that of second segment, width slightly smaller than length. Mouthparts adapted to chew. Labrum bilobulated with straight dorsal margin. Sides slightly convex. Ventral margin with a central cleft. External surface sclerotized, slightly convex with 12 setae and 4 pores. Internal surface slightly concave, membranous with two pores located in the central area and six sclerotized flattened projections by the ventral margin (Fig. 3B and C). Mandibles (Fig. 3D) strongly sclerotized with a pair of bristles nearby the basal area on the external surface (M1, M2), apical margin serrated with seven teeth-like projections. Maxilla (Fig. 3E) with galea and palp well-differentiated; maxillary palp trisegmented; third segment with digitiform sensillum and two pores laterally, distal surface with eight sensilla; galea with six sensilla on distal surface. Labium (Fig. 3F) with cylindrical spinneret located on the apex; labial palpi approximately half the spinneret length.

Thorax (Fig. 2C). Three segments clearly differentiated. Prothorax with a dorsal plate slightly sclerotized and divided along the medial line. Two ellipsoidal spiracles present laterally on prothorax, with conspicuous filtering structures.

Legs (Fig. 2E). Coxa wide, mainly membranous, with eight bristles, three of them very reduced and the remaining five elongated. Trochanter reduced to a slim strip compressed between the coxa and the femur, with three pores and only one highly reduced bristle. Femur cylindrical, with most of its surface highly sclerotized, membranous towards its medial area, with two bristles.

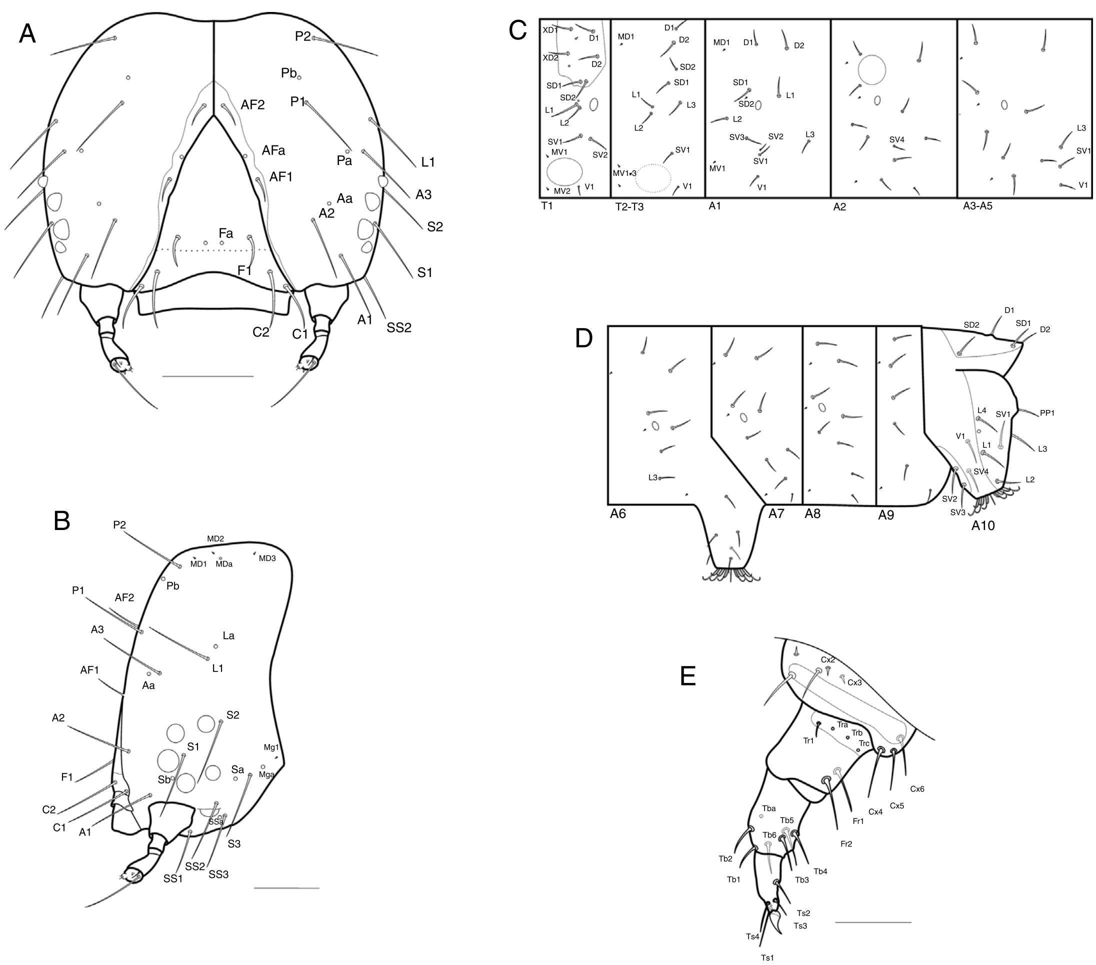

Figure 2. Chaetotaxy of the last instar larva of *G. mielkei*. (A) Head—frontal view. Scale bar = 0.5 mm. (B) Head—lateral view. Scale bar = 0.5 mm. (C) Thorax and anterior segments of the abdomen; circle on A2 indicates the tuberiform dorsolateral projections. (D) Posterior segments of the abdomen. (E) Prothoracic leg. Scale bar = 0.3 mm.

Tibia cylindrical, highly sclerotized, with six bristles and one pore. Tarsus conical, highly sclerotized with four bristles. Tarsus with its base widened becoming pointed towards the apex. Tarsal claw curve with a slightly expanded base, with pointy apex.

Abdomen (Fig. 2C and D). Ten clearly differentiated segments (A1–A10), with a pair of dorsolateral tuberiform projections on A2. A pair of prolegs associated to segments A6 and A10. Prolegs with crochets curved towards their apex. A pair of ellipsoidal spiracles associated to segments A1–A8. Chaetotaxy of the last instar larva as shown in Fig. 2.

Pupa (Fig. 4A–E)

Obtecta type. Dark brown coloured.

Head. Tegument smooth. Anterior margin rounded. Antennae flat extended from the posterior margin of the compound eye to the posterior margin of the abdominal segment A4; limited at the medial area by the legs and laterally delimited by the mesothoracic wings (Fig. 4A and B). Frontoclypeus limited in its posterior area by the labrum, and laterally flanked by both compound eyes. Compound eyes surrounded by the antennae in the dorsal area, the galea in the ventral-front and prothoracic legs in the laterofrontal area (Fig. 4A and B). Galeae located in the central ventral area of the pupa.

Thorax. Covered with smooth tegument. Prothorax visible from a laterodorsal perspective. Dorsal area with a narrow transversal band with acute lateral projections. Prothoracic spiracles visible, from dorsal and lateral perspectives, on both sides of the prothorax. Mesothorax larger than the prothorax and metathorax. Mesothoracic wings covering most of the thorax and abdomen.

Abdomen (Fig. 4B). With 10 abdominal segments clearly visible. Segments A1–A7 subtriangular when observed dorsally (Fig. 4C). A1 spiracles not visible, hidden behind the wings. A2–A8 spiracles visible. Final end of A10 modified as a cremaster, with an inverted "Y" shape, slightly expanded laterally in its basis. Male's genital pore located on the ventral area of A9, with two small lobules on its side (Fig. 4D). Female's genital pore ventrally located between segments A8 and A9 (Fig. 4E). A1–A8 segments show an indented tegument with a number of structures similar to small craters or concavities randomly distributed on all the tegument of the abdominal segments, except for the last two.

DNA barcodes

Two identical sequences of 658 base pairs of DNA barcodes were obtained and deposited in GenBank (accession number: MH025796; MH025797). A search in BOLD unveiled the closest match (92.75% similarity) with a sequence of *Physocleora* Warren, 1897 not identified at the species level.

Discussion

Biological and ecological interactions of geometrid moths with their environment are important in natural and human-modified environments of the Neotropics, as their larvae are mostly host-specialist phytophagous with a narrow range of host plants and can be a food source of many insectivorous organisms (Méndez-Abarca

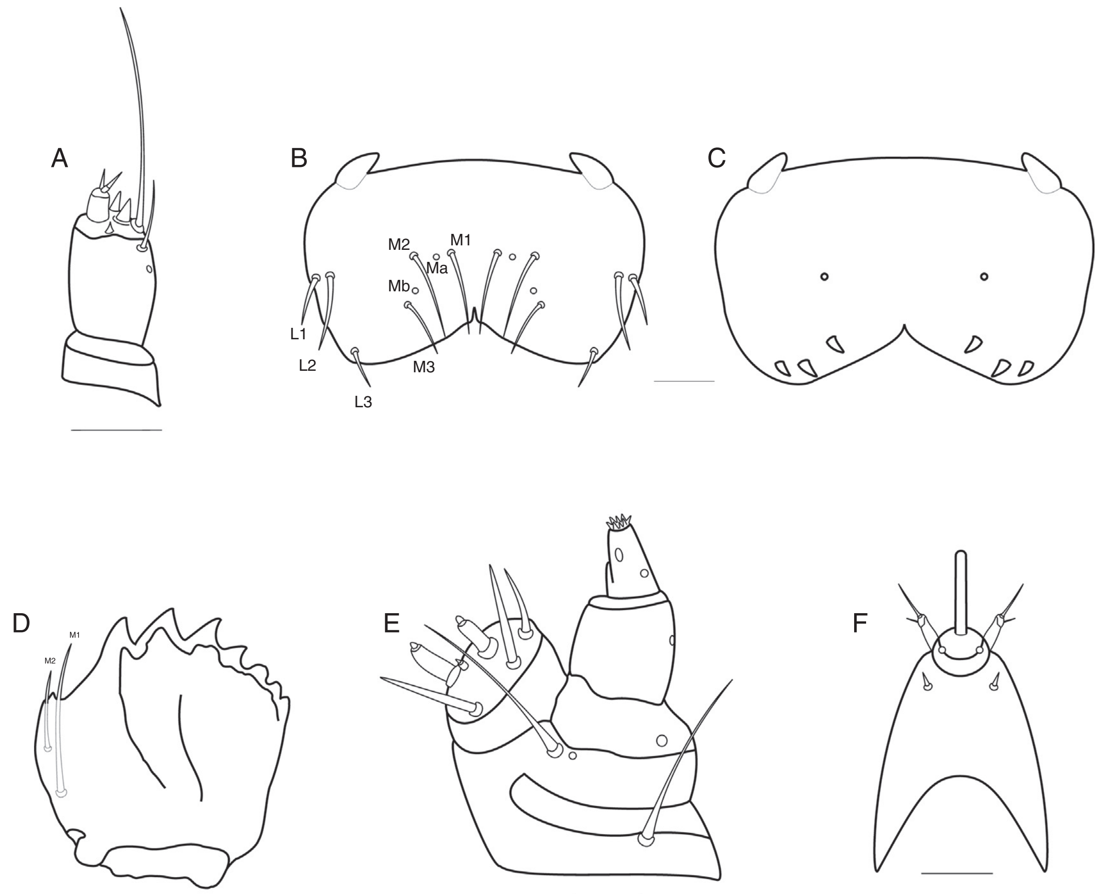

Figure 3. Cephalic appendages of the last instar larva of *G. mielkei*. (A) Antenna. Scale bar = 0.1 mm. (B) External view of the labrum. (C) Internal view (epipharynx) of the labrum. Scale bar = 0.1 mm. (D) Left mandible—mesial view. Scale bar = 0.05 mm. (E) Left maxilla in ventral view. Scale bar = 0.05 mm. (F) Labium—ventral view. Scale bar = 0.05 mm.

et al., 2012; De Sousa-Lopes et al., 2016; Vargas, 2016). In spite of their importance, however, studies on the natural history and morphology of the immature stages of the Neotropical geometrid species are, generally speaking, scarce (Vargas et al., 2017).

In the case of the Neotropical representatives of the genus *Glena*, McGuffin (1967) provides descriptions and illustrations of the larva and pupa of the species *G. interpunctata interpunctata* (Barnes and McDunnough, 1917) based on specimens collected in Durango, Mexico. A close comparison between the results of that study with ours suggest that the chaetotaxy of the SV group of A1–A3 and the morphology of the cremaster could be useful tools for species identification based on last instar larva and pupa, respectively. At the larval stage, the SV group has two setae in A1 and three setae in A2 and A3 in *G. interpunctata interpunctata*, whereas in *G. mielkei* this group exhibits three setae in A1 and four setae in A2 and A3 (Fig. 2C). On the other hand, at the pupal stage, the cremaster of *G. interpunctata interpunctata* is triangular with the apex slightly cleft, whereas the cremaster of *G. mielkei* is basally widened with the apex widely cleft. In addition, in the case of these two species, the presence of the tuberiform dorsolateral projections of A2 also makes it possible to recognize *G. mielkei* as these structures are absent in *G. inerpunctata interpunctata*. Similar projections, although variable in number and shape, have been described for other representatives of Boarmiini (Vargas and Parra, 2013).

It is known that the morphological attributes of immature stages can be either conserved or variable between congeneric species of Geometridae (e.g., Bolte, 1990). Accordingly, the usefulness of morphological characters, either for species identification or for systematic studies, must be assessed for each genus separately. In the case of the Neotropical fauna, this is a difficult task, mainly due to the lack of knowledge of the host plants used by many geometrid species, which makes it difficult to collect larval stages. In this scenario, the comparisons provided in the present study have to be considered as preliminary results, as the external morphology of the immature stages of *Glena* and other Boarmiini genera are still poorly known. In this regard, the description of additional morphological characters of immature stages should be considered a priority when advancing our knowledge of the host plants.

In general, the analysis of DNA barcodes provides a good approximation to the generic status of species of Geometridae (Hausmann et al., 2011). Thus, it was surprising to find that although sequences of species of both Nearctic and Neotropical *Glena* are deposited in BOLD, the closest match was found to be with a representative of *Physocleora*, a genus that also belongs to the tribe Boarmiini (Pitkin, 2002). Interestingly, a few morphological features of the male and female genitalia of *G. mielkei* were already mentioned as atypical for the genus *Glena* in the original description of this species (Vargas, 2010). Hence, the absence of a close match of the DNA barcode of *G. mielkei* with any representative of *Glena* highlights the need of further integrative studies to assess the generic placement of this species of the Atacama Desert.

Conflicts of interest

The author declares no conflicts of interest.

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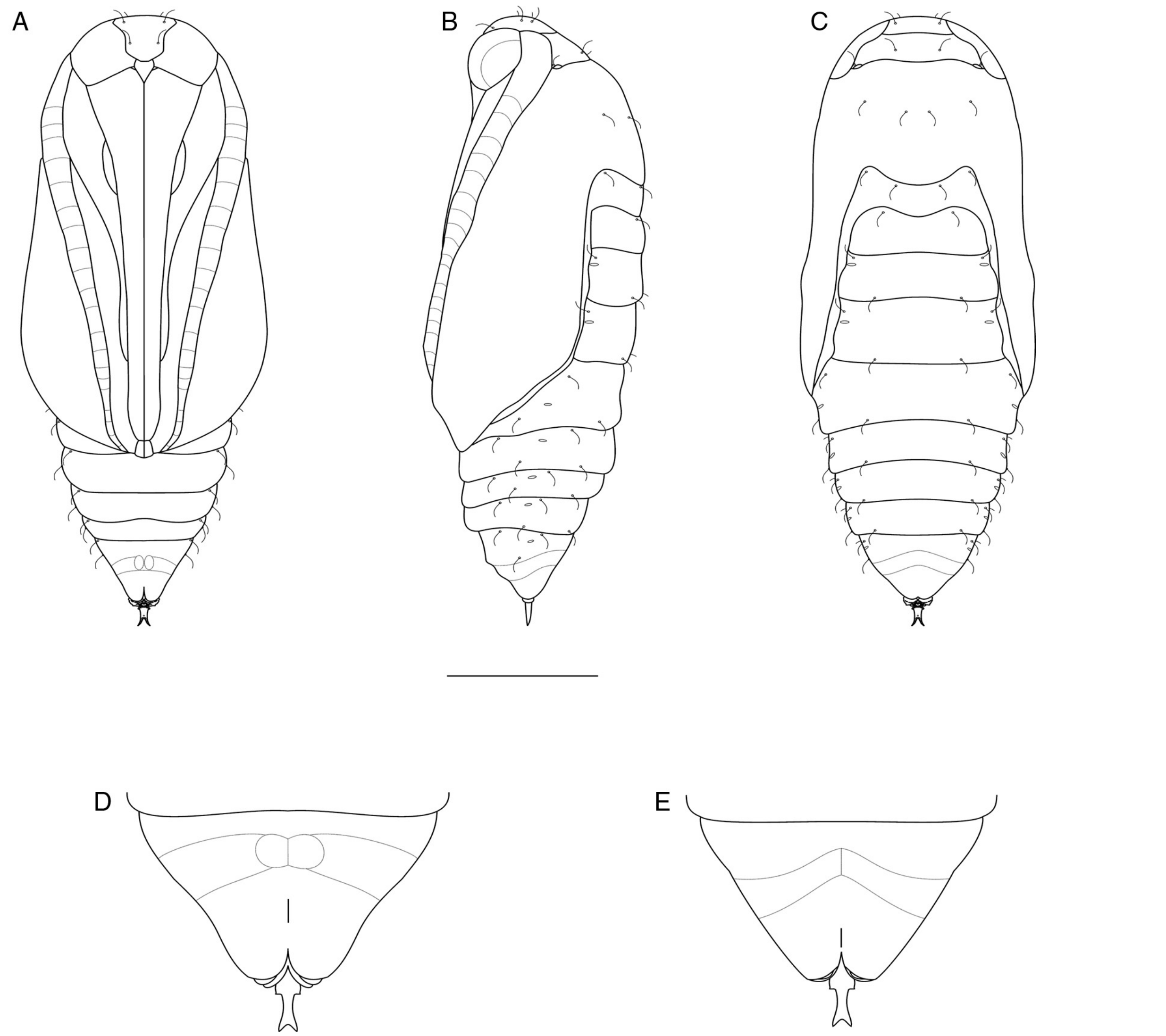

Figure 4. Pupa of *G. mielkei.* (A) Ventral view. (B) Lateral view. (C) Dorsal view. (E) Male terminalia—ventral view. (F) Female terminalia—ventral view. Scale bar = 0.5 cm.

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