

***Prosilocerus* Kieffer, 1923 shares morphological synapomorphies with Prodiamesinae**

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Abstract

In their comprehensive analysis Cranston et al. (2012) quite unexpectedly recovered a monophyletic Orthoclaadiinae, except that *Prosilocerus* Kieffer, 1923, was recovered as a representative of Prodiamesinae. In this short communication I show that *Prosilocerus* shares morphological synapomorphies with other Prodiamesinae – namely (i) an indirect tracheal connection of the pupal thoracic horn to the adult spiracle and (ii) a parallel-sided rami of female gonapophysis IX.

Introduction

In their comprehensive analysis of molecular data, Cranston et al. (2012) quite unexpectedly recovered a monophyletic Orthoclaadiinae, despite the seeming lack of any morphological synapomorphies, but excluding *Prosilocerus* Kieffer, 1923. The placement of this genus, recovered as sister group to Prodiamesinae (Cranston et al. 2012), “can be reconciled on the basis of some morphology” listing large plates of the submentum, fringed anal lobes of the pupal abdomen and “and diverse volsellae in the male hypopygium”. However all these features, as Cranston et al. (2012) note, are, symplesiomorphies, and thus cannot corroborate the affinity of *Prosilocerus* to Prodiamesinae.

Additionally, *Prosilocerus* lacks another common symplesiomorphy of the Prodiamesinae – the MCu crossvein. Cranston et al. (2012) note that absence of this character state in *Prosilocerus* renders morphological diagnosis of the Orthoclaadiinae even more problematic. It is also notable that *Prosilocerus* have only two precorneal setae while the rest of the Prodiamesinae for which immature stages are known, have three precorneals (Sæther 1986). Notably, Sæther (1986) had used the leg sheath arrangements and MCu presence and position in his key to the Prodiamesinae pupae, but not a type of the tracheal connection.

Coffman (1979) had examined the connection between the pupal thoracic horn and the thoracic spiracle of the pharate adult as a possible phylogenetically informative character in Chironomidae. He found all examined representatives of Prodiamesinae (*Monodiamesa* Kieffer, 1922, *Odontomesa* Pagast, 1947, *Prodiamesa* Kieffer, 1906) have an indirect connection between the thoracic horn and a spiracle (Coffman 1979: p.43). This type of connection is represented by a bundle of the thin tracheoles originating from the spiracle and approaching close to the base of the thoracic horn, in contrast to the direct connection by a trachea in Podonominae and Tanypodinae, or the absence of such a connection, as in Orthoclaadiinae (Coffman 1979) (Figs 1A, B). Sæther (1977) noted that parallel rami of gonapophyses IX in the female genitalia could be

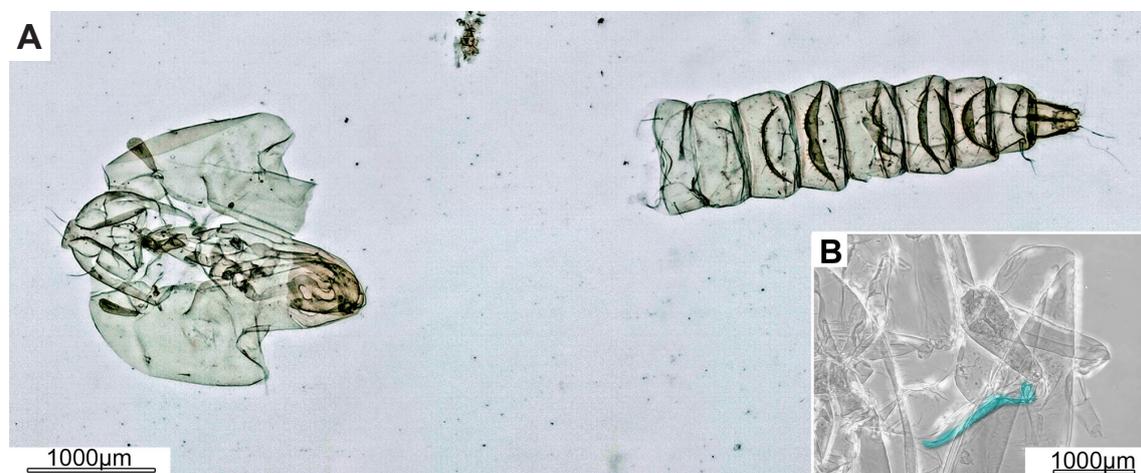


Figure 1. A. *Podochlus* sp. pupa, habitus (brightfield microscopy); B. same, thoracic horn, tracheal connection to the spiracle is marked in blue (phase contrast).

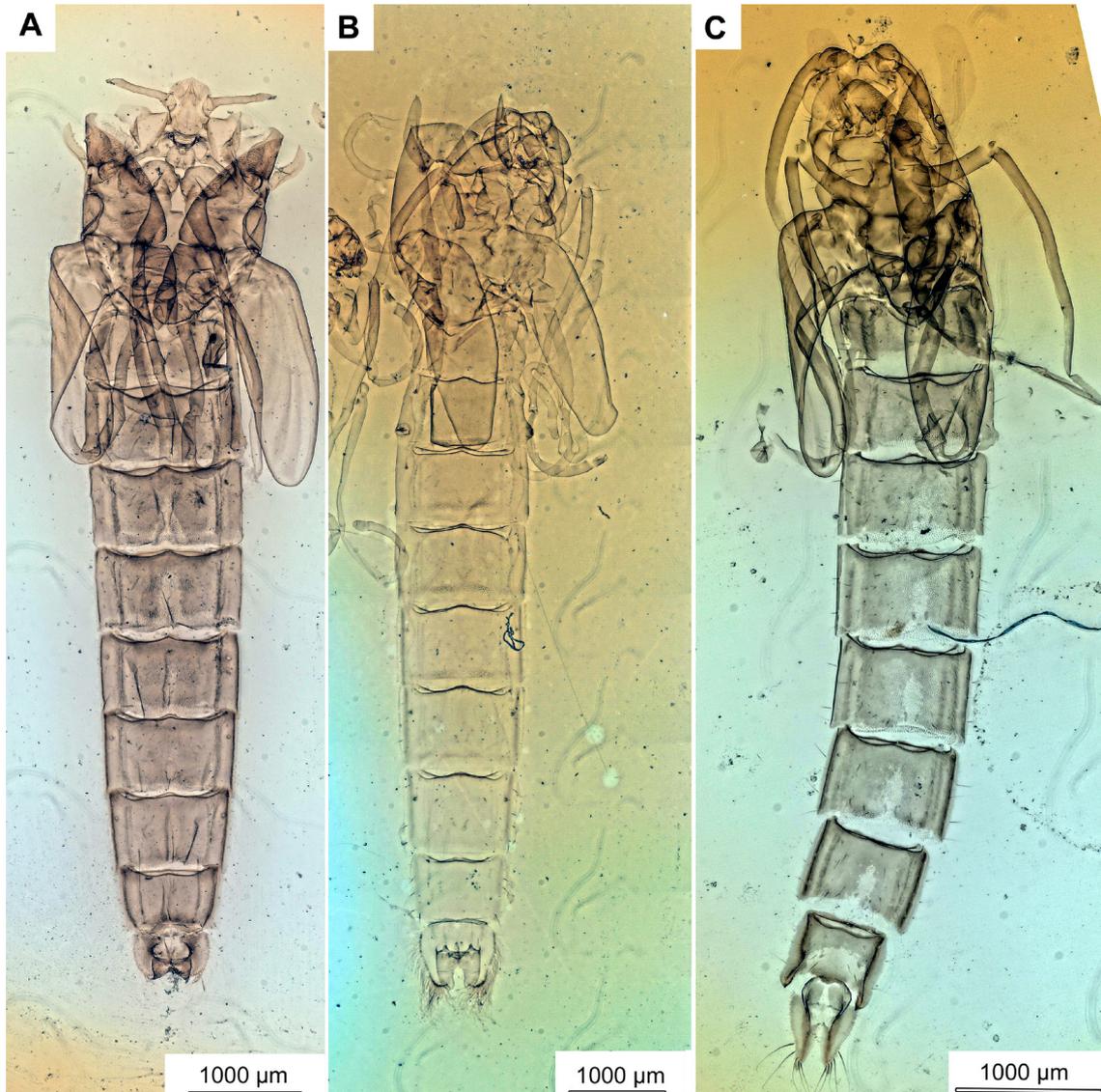


Figure 2. Pupal exuviae of Prodiamesinae. A. *Prodiamesa olivacea*; B. *Monodiamesa bathyphila*; C. *Odontomesa fulva* (brightfield microscopy).

considered as synapomorphic for the Prodiamesinae (also true for *Compteromesa* Sæther, 1981, for which only adults are known (Sæther 1985)).

In this short communication I examine the condition of the abovementioned characters in *Propiloscerus*, to assist in elucidation of its phylogenetic position.

Materials and methods

The following material was examined from the ethanol and slide collection of Zoologische Staatssammlung München (ZSM):

Propiloscerus lacustris Kieffer, 1923, from Kleiner Teufelsteich, Teichland, near Cottbus, Germany, collected in April 1962. No legator name. Numerous pupae and females preserved in ethanol.

P. lacustris pupal exuviae from Großer Plöner See, near Plön, Germany, collected by Lenz, April 24th, 1922. Canada balsam mounted pupal exuviae.

Prodiamesa olivacea (Meigen, 1818) from Schlitz, May 1st, 1985 collected by Hereberg (ZSM barcoding project number (“DIP 00254”). Numerous females.

P. olivacea from “Bäm”, Canada balsam mounted pupal exuviae, Thienemann collection, no collection date (as “*Prodiamesa praecox*”).

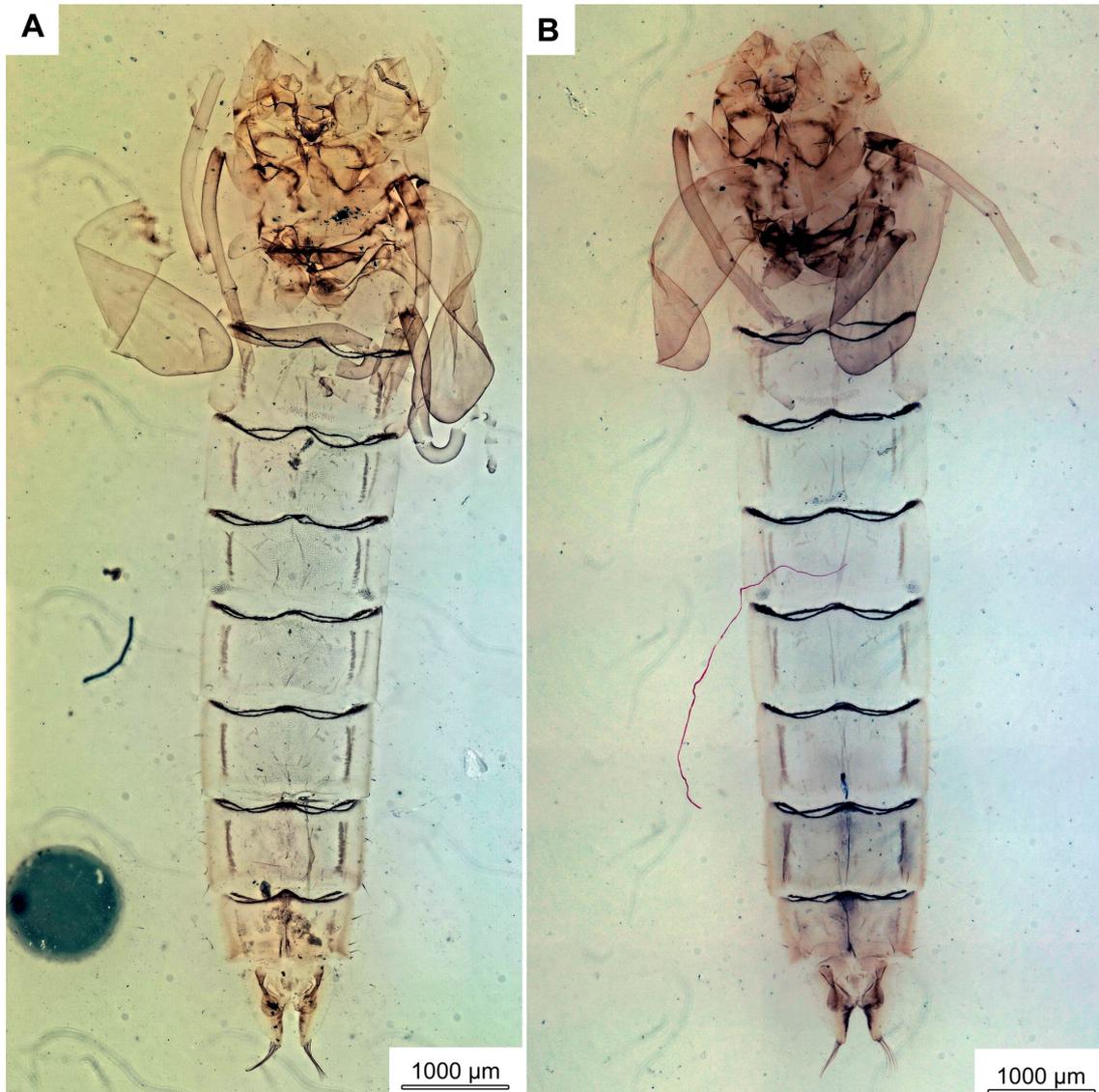


Figure 3. *Prosilocerus lacustris* pupal exuviae. A. specimen 1; B. specimen 2 (brightfield microscopy).

Monodiamesa bathyphila (Kieffer, 1918) Germany, 1937. Collected by Thienemann. Canada balsam mounted pupal exuviae.

Odontomesa fulva (Kieffer, 1919). Kalibach, Fliede, Hessen, Germany, May 1st, 1952, collected by E. J. Fittkau (# 387). Canada balsam mounted pupal exuviae.

Podochlus sp. South Chile, Lago Todos los Santos, Rio Bonito, December 5th, 1969. Collected by F. Reiss. Canada balsam mounted pupal exuviae.

Material was documented on a VHX-6000 digital microscope, following standard procedures (e.g. Haug et al. 2011). Ring light illumination was used with white background. Each image was recorded as a composite image combining images (“frames”) of different focal plains (“z-stack”) and several adjacent images to form a large panorama; processing was performed with the built-in software. Images were additionally recorded with several exposure times (HDR; Haug et al. 2011). Additionally, for the documentation of finer details, such as tracheal connections used BZX-900 fluorescent microscope, observing specimens using a brightfield, phase contrast microscopy as well as TRIC fluorescence (Haug et al. 2011). Individual photos subsequently stacked into the sharp composite using PICOLAY open software (www.picolay.de).

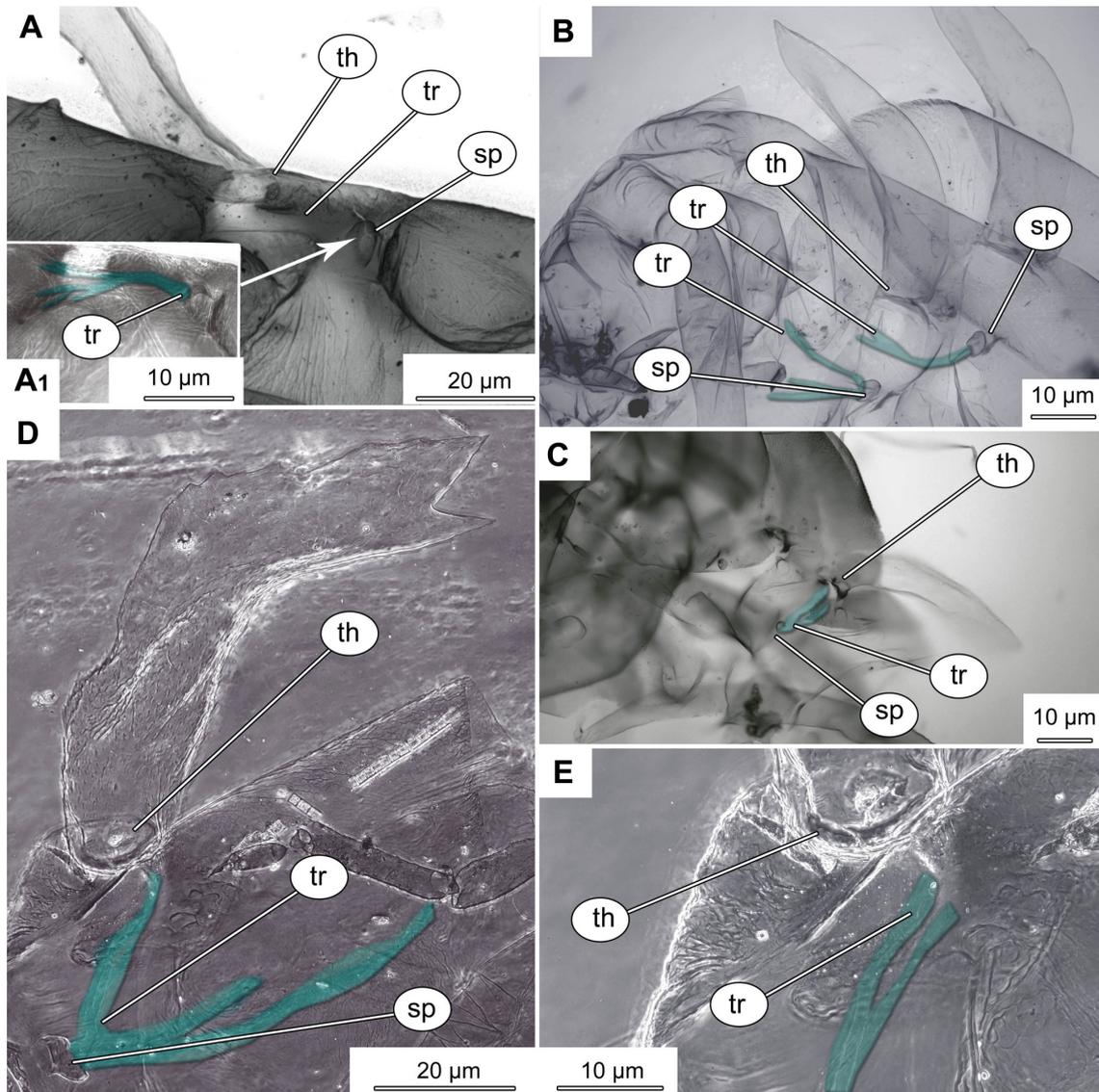


Figure 4. Indirect tracheal connections in Prodiamesinae pupae. A and A1. *Prodiamesa olivacea* (brightfield microscopy); B. *Monodiamesa bathyphila* (brightfield microscopy); C. *Odontomesa fulva* (brightfield microscopy); D, E. *Propsilocerus lacustris* (phase contrast). Abbreviations – *th*: thoracic horn; *tr*: tracheoles; *sp*: spiracle.

Results

My examination of the material revealed that *Propsilocerus* shares both of the abovementioned apomorphic character states with all other Prodiamesinae examined (Figs 2A-C, 3A-B, 4A-F, 5A-D).

Examination of the base of the thoracic horn and the opening of the adult spiracle in *Propsilocerus lacustris* pupae and pupal exuviae revealed a loose bundle of tracheoles extending between the opening of the spiracle and almost to the very base of the thoracic horn, as in all examined Prodiamesinae (Figs 4A-F).

In the female genitalia, the paired rami of the gonapophyses IX of *P. lacustris* were distinctly parallel, similarly to examined *P. olivacea* and in accordance with the literature concerning other Prodiamesinae (Sæther 1977: fig. 35.). It is notable that rami in *Propsilocerus* appear more weakly sclerotized than in other Prodiamesinae (Figs 5A-D, Sæther 1977: fig. 35.).

Discussion

Examination shows that *Propsilocerus* is resolved not only as an ingroup-Prodiamesinae based on the multigene phylogeny of Cranston et al. (2012) but also shares important morphological synapomorphies with Prodiamesinae, thus further cementing location of *Propsilocerus* within this monophyletic subfamily.

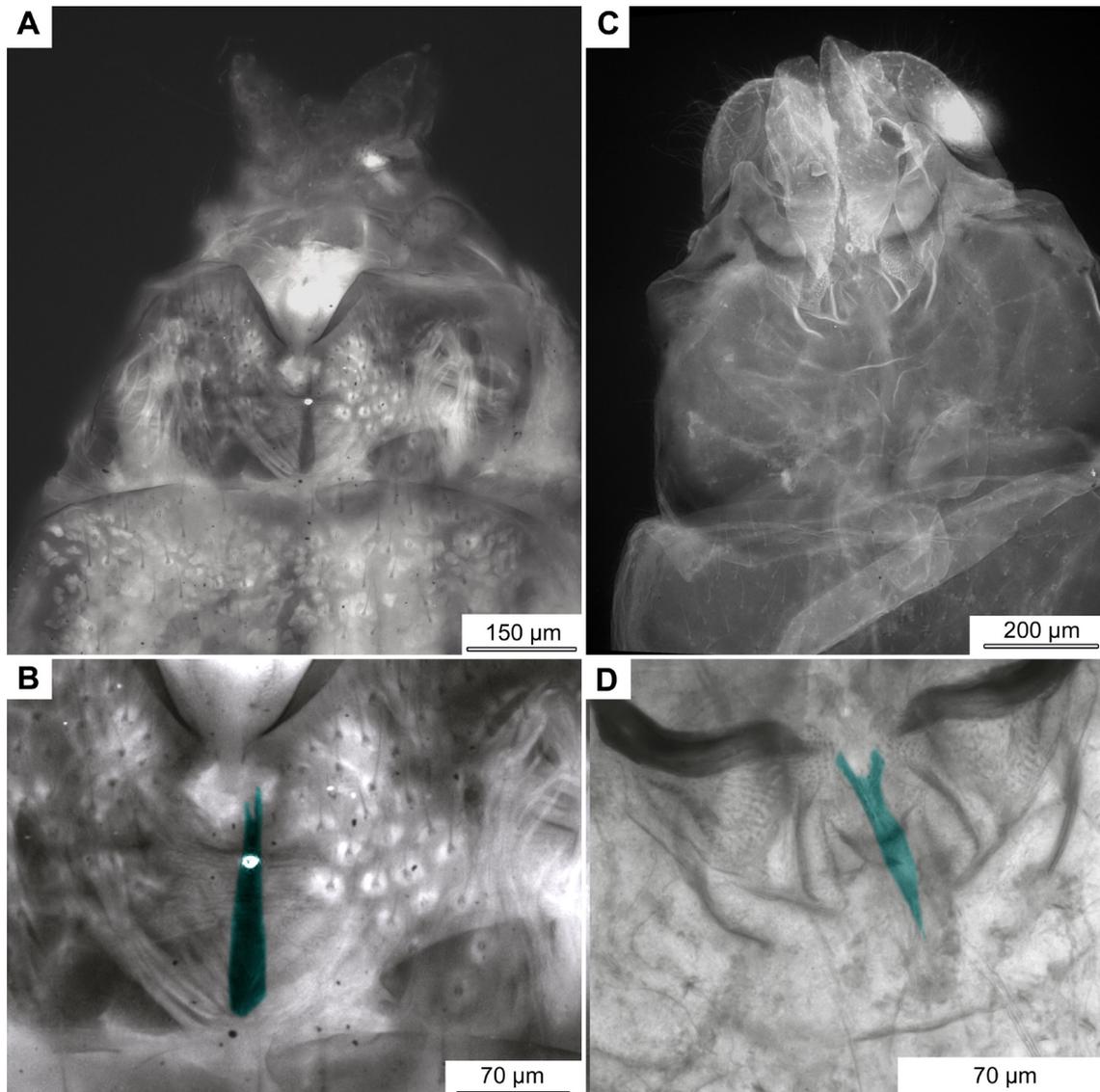


Figure 5. Female genitalia of Prodiamesina. A, B. *Prodiamesa olivacea* (TRIC Fluorescence); C. *Propsilocerus lacustris* (TRIC fluorescence); D. *Propsilocerus lacustris*. Rami of gonapophyses marked in blue (bright-field microscopy).

Status of the *Propsilocerus* as an ingroup-Prodiamesinae sheds light on the persistence of morphological analysis in the age of molecular systematics. It also shows the danger of implicit definition of the groups by plesiomorphies. For example, in most fossil Prodiamesinae (I.e. *Cretadiamesa* Veltz, Azar et Nel, 2007, *Lebanodiamesa* Veltz, Azar et Nel, 2007) their purported affinities with Prodiamesinae appears based on plesiomorphies such as the presence and location of veins R_{2+3} and MCu, rather than any meaningful synapomorphies (Veltz et al. 2007, Baranov et al. 2019). By defining fossil Prodiamesinae using such characters, we are in danger of including in Orthocladiinae some fossil representatives of Prodiamesinae that lack crossvein MCu. The fact that Orthocladiinae (as of now) still lack morphological synapomorphies (Sæther 1977; Cranston 2000; Cranston et al. 2012) confuses the matter. Future studies of extant material through all the ontogenetic stages, as well as fossils, may eventually resolve this problem, and help us to locate morphological support for the unexpected Orthocladiinae monophyly (Cranston et al. 2012).

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