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Abstract

The morphology of the proventriculus may supply useful taxonomic characters for some species of crickets. This study evaluated if the proventriculus can be used to distinguish three species of Nemobiinae crickets: *Phoremia sp. n.*, *Zucchiella sp. n.*, and *Amanayara sp n.* In crickets the proventriculus presents six lobes, each one bearing eight appendices along its longitudinal axis. In *Phoremia sp. n.*, the central portion of the first appendix has a tooth like a spear, with two denticles; the central portion of the second appendix presents one plate formed by five denticles, whereas in *Zucchiella sp. n.*, the apex of the first appendix is U-shaped with denticles absent, and the second appendices differ from those of *Phoremia sp. n.* by the number of denticles. In *Amanayara sp. n.* the central portion of the first and second appendices form a long and sharpened tooth, the denticles are lacking. In the other proventricular appendices there was variation in the number of denticles and in their shape. These results indicate that the morphology of the proventriculus can provide auxiliary characters for taxonomy of Nemobiinae, especially useful for this subfamily, since for many species the external morphology is insufficient for characterization of genera and species.

Key words

taxonomy, scanning electron microscopy, *Phoremia*, *Amanayara*, *Zucchiella*, morphology, gut

Introduction

The proventriculus is an organ situated between the foregut and midgut, its lumen lined by cuticle with teeth-like projections. These, together with associated well-developed muscles, aid the food trituration process (Isely & Alexander 1949).

The proventriculus is comprised of several sclerotized plates (Chapman 1998), whose structure has been adaptively associated with insect diet (Gibbs 1967, Lebrun 1985, Lebrun & Lequet 1985, Caetano 1988, Bland & Rentz 1991). In addition, many studies suggest proventriculus morphology can provide useful taxonomic characters for Orthoptera (Judd 1948, Bland & Rentz 1991, Fontanetti & Zefa 2000, Fontanetti *et al.* 2002), Coleoptera (Judd 1947, Yahiro 1990), termites (Lebrun 1985, Lebrun & Lequet 1985), Trichoptera (Gibbs 1967), ants (Roche & Wheeler 1997) and bees (Serrão 2000, 2005, 2007).

The discovery of morphological characters that allow discrimination of taxonomically cryptic groups, whose morphology is otherwise very similar, is particularly important. Nemobiine crickets are taxonomically cryptic. These crickets are common in litter of tropical forests (Desutter-Grandcolas 1992, 1993; Ribas *et al.* 2004; Sperber 1999). However taxonomic determination is difficult, due to great similarity in external morphology and genitalia among related species or genera. The objective of the present study was to assess if proventricular structure could provide characters to distinguish three sympatric species of Nemobiine crickets.

Materials and Methods

The proventriculus of two adult males each of *Phoremia sp. n.*, *Zucchiela sp. n.* and *Amanayara sp. n.* were analyzed by scanning electronic microscopy (SEM). All individuals were collected in a remnant of Atlantic Forest in the region of Viçosa, Minas Gerais (lat 20°42'30"S, long 42°56'15"W) [see Sperber (1999)], and stored in 70% ethanol. Species descriptions are in preparation; voucher specimens were deposited at the Laboratório de Orthopterologia, affiliated with the Museu Regional de Entomologia of the Universidade Federal de Viçosa (UFVB).

Individuals were dissected in ethanol and the proventriculus isolated from the gut. A longitudinal section in the proventricular wall gave access to the proventricular lumen surface and its lining cuticle. The samples were dehydrated in a graded ethanol series (70%, 80%, 90% and 100%), 5 min in each bath; they were then transferred to hexamethyldisliazane (HMDS) for 5 min, air dried (Nation 1983), fixed onto aluminum stubs, gold coated and observed with a LEO VP1430 scanning electron microscope.

Results

In all three cricket species analyzed, the proventriculus has six longitudinal lobes, each with eight transverse sclerotized appendices arranged in longitudinal series (Figs 1A, 2A, 3A). The appendices of each lobe become progressively less elaborate posteriorly. Each appendix presents projections that angle posteriorly, *i.e.*, they point away from the mouth. The projections of each appendix are comprised of a median tooth (mt) (Figs 1B,C; 2B,C) and projections with lateral teeth (lt) to each side (Figs 1C, 2C, 3C).

Intimately associated with the median tooth are median denticles (md) (Figs 1C, 2C, 3C), which vary among the analyzed species in number, size and distribution, especially for the first two appendices, where the median teeth are more developed. The median tooth (mt) is absent from the final appendix of all species (Figs 1D, 2D, 3D, black arrow).

Each lateral tooth is comprised of two projections, the first more anterior and internal (i), larger than the second, external (e) (Figs 1C, 2C, 3C). Each lateral tooth presents a small rounded callosity, with the subsequent margin having two long apical lobes, also

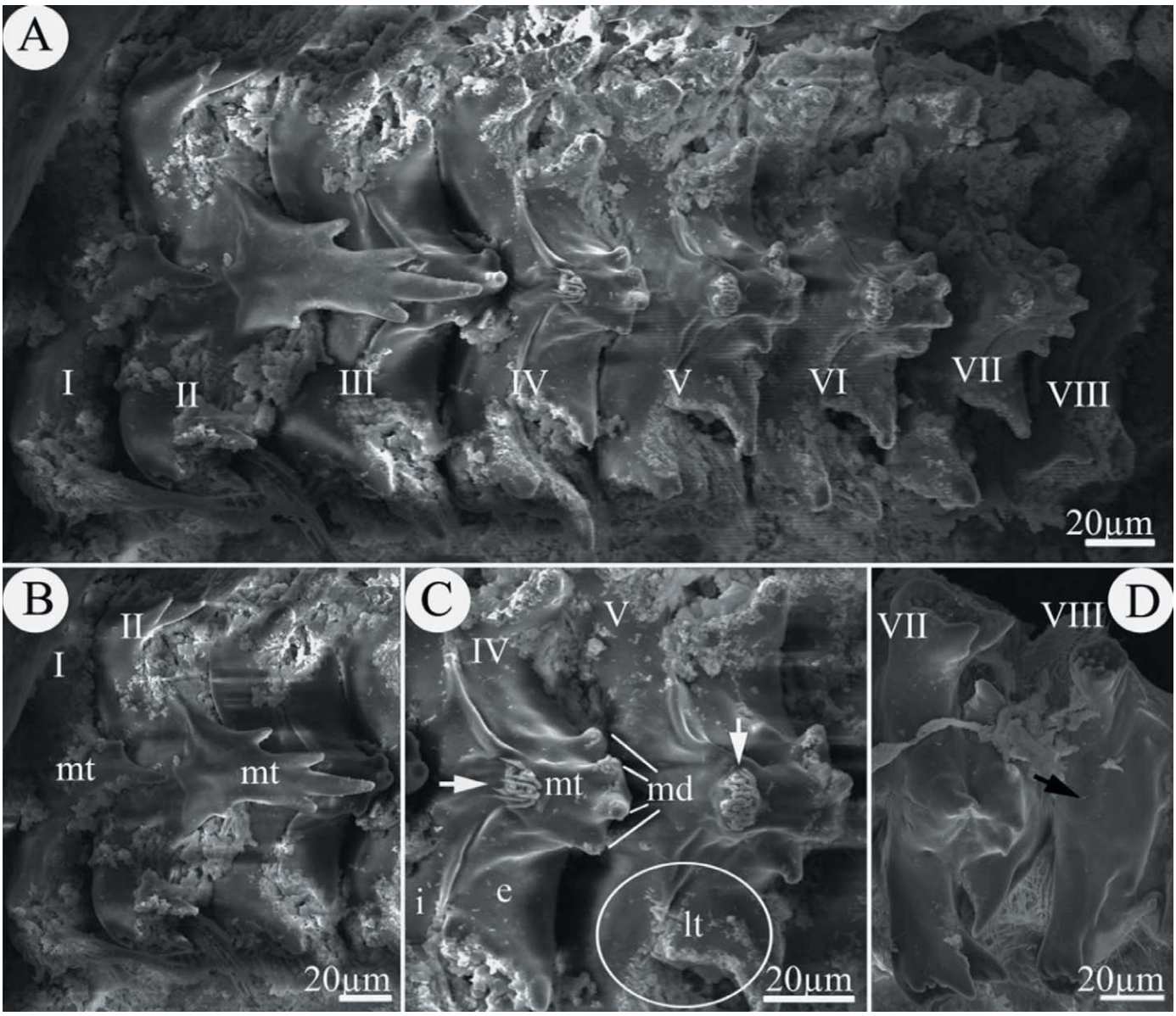

Fig. 1. Proventriculus of *Phoremia sp. n.* **A**) General view of the 8 appendices (I-VII) on one longitudinal lobe; **B**) median tooth of each of the first two appendices (mt); **C**) for appendix IV median tooth, lateral projections (lt), denticles (md), internal plate (i) and external plate (e); **D**) last appendix VIII; note absence of hair-like structures.

rounded. Occasionally, the appendices show hair-like projections associated with the median tooth (Figs 1C, 2C, 3C; white arrow).

Although the proventriculus of the three species shows the same basic morphology, there is variation among species in the number and shape of the denticles, in median tooth morphology, in the shape and location of both the hair-like projections and the lateral projections.

In *Phoremia sp. n.*, the median tooth (mt) (Fig. 1C) of the first appendix is a spear-shaped structure with two small and globular denticles laterally; whereas the central portion of appendix II is a long and widened structure with five denticles, one median, long and rounded, and two lateral pairs, small and rounded (Fig. 1B).

In *Zucchiella* sp. n., although the median tooth (mt) of the first appendix is similar to that of *Phoremia sp. n.*, its apex is U-shaped, with an absence of lateral denticles (Fig. 2B). The second appendix is more developed than in *Phoremia sp. n.*, showing six rounded denticles: one central, four lateral and one on the median surface of the structure (Fig. 2B; Fig. 2A black circle). In *Amanayara sp. n.*, the central portion of the first and second appendices comprises a long and sharpened tooth (mt) without denticles (Fig. 3B).

In all other six appendices of the proventriculus, the central portion varies in number and location of denticles (md). *Phoremia sp. n.*, has 3 to 5, *Zucchiella sp. n.*, 3 to 6, and *Amanayara sp. n.*, presents a maximum of four denticles (Figs 1A,C, 2A,C, 3A,C). None of the studied species bear teeth on the last appendix (Figs 1D, 2D, 3D, black arrow).

The lateral projections (lt) are present from the second to the seventh appendix in *Phoremia sp. n.*, and *Zucchiella sp. n.*, whereas in *Amanayara sp. n.*, these projections are present in all appendices (Figs 1A, 2A, 3A). *Phoremia sp. n.*, shows gradual reduction in the size of these lateral projections, but without morphological simplification (Fig. 3A). In *Zucchiella sp. n.*, only the second appendix differs from the others, with a subtle division into two plates and with a long projection arising in the more internal plate (Fig. 2A).

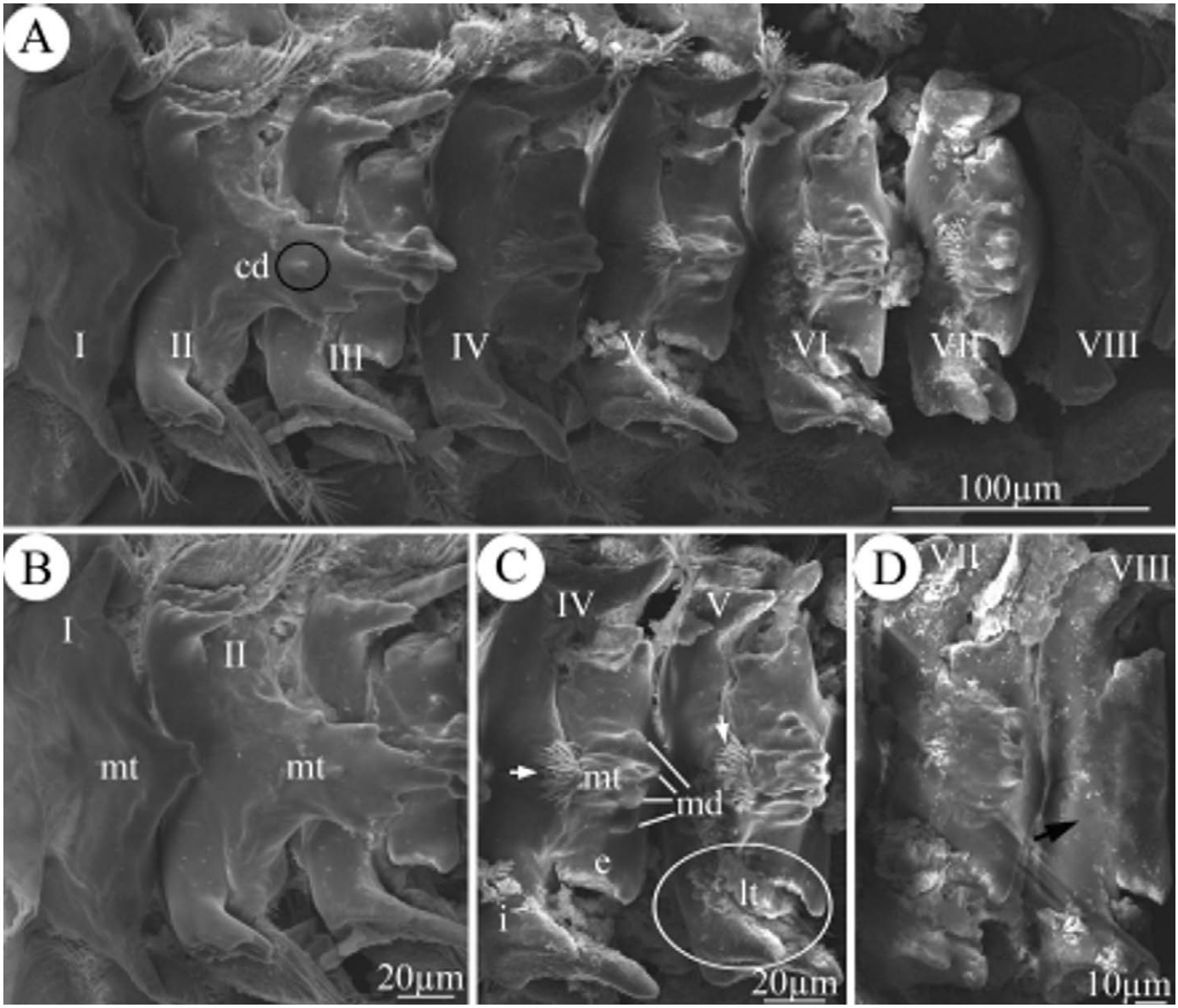

Fig. 2. Proventriculus of *Zucchiela sp. n.* **A)** General view of the eight appendices (I-VII) of one longitudinal lobe; **B)** median tooth of each of the first two appendices (mt); **C)** for appendix IV median tooth, lateral projections (lt), denticles (md), internal plate (i) and external plate (e); **D)** last appendix VII; note absence of hair-like structures.

In *Phoremia sp. n.*, and *Amanayara sp. n.*, the third and four appendices have a hair-like structure on the base of the median tooth (Figs 1C, 3C), but this structure was reduced in the fifth, sixth and seventh appendices, whereas in *Zucchiella sp. n.*, the reduction occurs only on the third appendix (Fig. 2C).

Discussion

A direct relation of proventriculus morphology with diet is established for termites: these wood feeders show a proventriculus armed with stronger teeth and strongly sclerotized appendices, compared to noncellulose feeders, where the proventriculus is less sclerotized and presents reduced appendices (Gibbs 1967, Lebrun 1985, Lebrun & Lequet 1985, Caetano 1988, Bland & Rentz 1991). In *Endecous* (Grylloidea: Phalangopsidae) the presence of large teeth positioned at the median and lateral portions of the proventricular appendices, can provide these insects with an apparatus effective in the trituration of several food sources (Fontanetti *et al.* 2002).

The Nemobiinae species here analyzed have several morphological characters of the proventriculus that may be associated with omnivore feeding habits: i) long lateral denticles in the anterior appendices and small denticles on the posterior appendix, ii) robust and truncated median teeth, iii) rounded denticles on the lateral projections and iv) hair-like structures on the central portion of median teeth.

The proventriculus of crickets has been suggested as a powerful tool for taxonomic separation in Oecanthidae, Trigonidiidae, Eneopteridae, Mogoplistidae and Gryllidae (Judd 1948), Gryllacrididae (Bland & Rentz 1991), Gryllidae (Fontanetti & Zefa 2000) and Phalangopsidae (Fontanetti *et al.* 2002). These studies detected morphological differences among families and subfamilies (Judd 1948, Bland & Rentz 1991), but small or no differences among

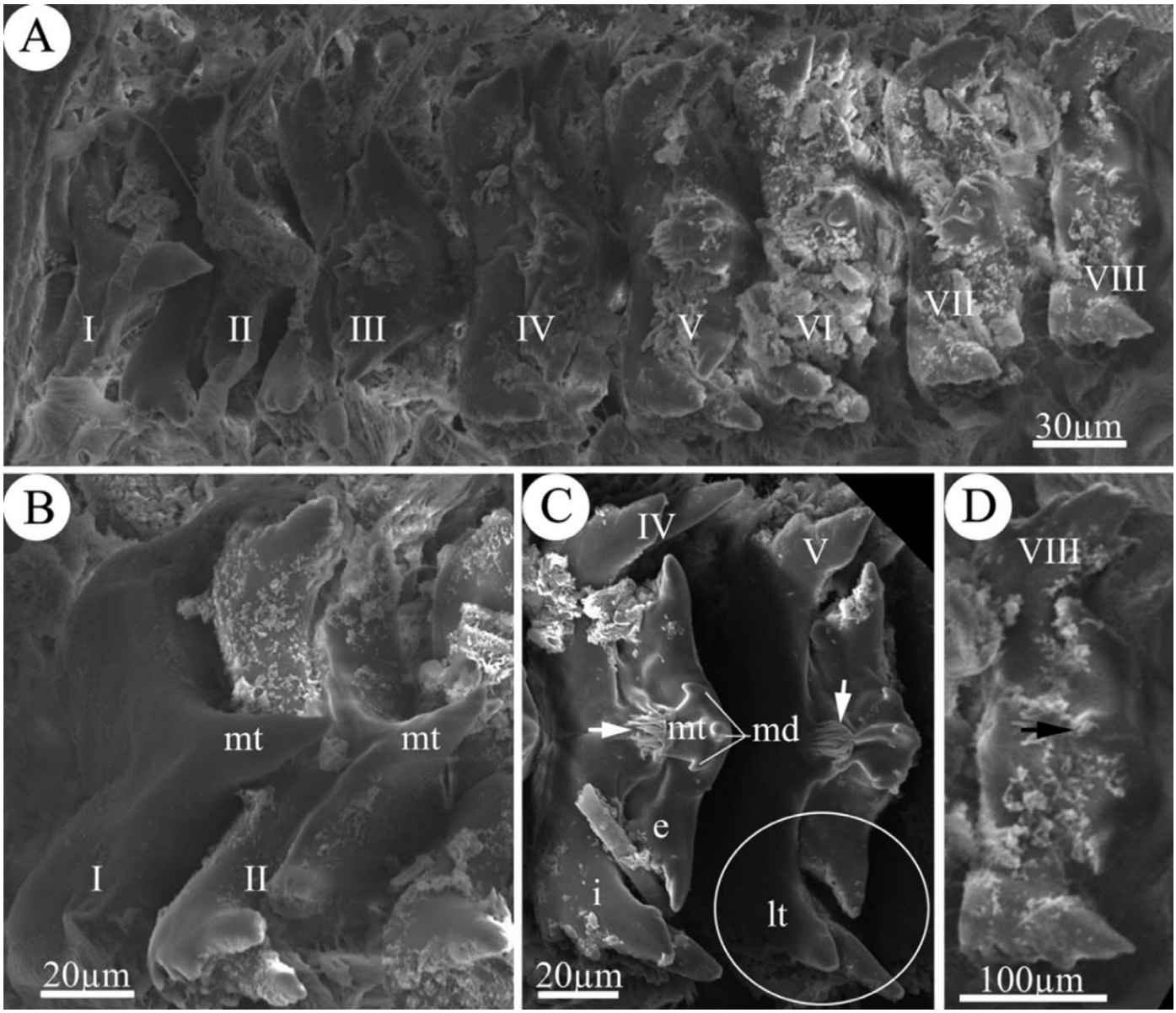

Fig. 3. Proventriculus of *Amanayara sp. n.* **A**) General view of the eight appendices (I-VII) of one longitudinal lobe; **B**) median tooth of each of the first two appendices (mt); **C**) median tooth, lateral projections (lt), denticles (md), internal plate (i) and external plate (e); **D**) last appendix; note absence of hair-like structures.

species of the same genus (Fontanetti & Zefa 2000, Fontanetti *et al.* 2002).

Our results show conspicuous differences in the proventriculus among the analyzed species, suggesting its taxonomic usefulness in these genera *Phoremia sp. n.* and *Zuchiella sp. n.* have the lowest number of distinct proventricular characters between them, while *Amanayara sp. n.* has the most discrepant proventriculus morphology. This coincides with external morphology of these species: *Phoremia sp. n.* and *Zuchiella sp. n.* are externally more similar than *Amanyara sp. n.* (Pereira 2008). Subsequent studies will need to evaluate if the differences among the species studied here are maintained among genera, or if there is intra-generic morphological variation.

We conclude that proventriculus morphology provides useful taxonomic characters to distinguish Nemobiinae species. Further studies will evaluate interspecific variation within genera, so as to establish which characters are genus-specific.

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