

MAURICIO DA SILVA PAULO

**ANATOMY AND HISTOLOGY OF THE MALE REPRODUCTIVE SYSTEM AND
SPERM MORPHOLOGY OF *Leptoglossus zonatus* (DALLAS, 1852)
(HETEROPTERA: COREIDAE)**

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-graduação em Biologia Celular e Estrutural, para obtenção do título de *Magister Scientiae*.

Orientador: José Lino-Neto

Coorientadora: Glenda Samara Dias Santos

**VIÇOSA - MINAS GERAIS
2023**

**Ficha catalográfica elaborada pela Biblioteca Central da Universidade
Federal de Viçosa - Campus Viçosa**

T

P331a
2023

Paulo, Mauricio da Silva, 1995-

Anatomy and Histology of the Male Reproductive System
and Sperm Morphology of *Leptoglossus Zonatus* (Dallas, 1852)
(Heteroptera: Coreidae) / Mauricio da Silva Paulo. – Viçosa, MG,
2023.

1 dissertação eletrônica (51 f.): il. (algumas color.).

Orientador: José Lino Neto.

Dissertação (mestrado) - Universidade Federal de Viçosa,
Departamento de Biologia Geral, 2023.

Inclui bibliografia.

DOI: <https://doi.org/10.47328/ufvbbt.2023.525>

Modo de acesso: World Wide Web.

1. Hemíptera - Espermatozoides - Desenvolvimento.
2. Pentatomomorpha. 3. Testículos - Morfologia. I. Lino Neto, José, 1959-. II. Universidade Federal de Viçosa. Departamento de Biologia Geral. Programa de Pós-Graduação em Biologia Celular e Estrutural. III. Título.

CDD 22. ed. 595.754

MAURICIO DA SILVA PAULO

**ANATOMY AND HISTOLOGY OF THE MALE REPRODUCTIVE SYSTEM AND
SPERM MORPHOLOGY OF *Leptoglossus zonatus* (DALLAS, 1852)
(HETEROPTERA: COREIDAE)**

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-graduação em Biologia Celular e Estrutural, para obtenção do título de *Magister Scientiae*.

APROVADA: 31 de julho de 2023.

Assentimento:



Documento assinado digitalmente
MAURICIO DA SILVA PAULO
Data: 28/08/2023 16:11:16-0300
Verifique em <https://validar.iti.gov.br>

Mauricio da Silva Paulo
Autor



Documento assinado digitalmente
JOSE LINO NETO
Data: 28/08/2023 16:04:04-0300
Verifique em <https://validar.iti.gov.br>

José Lino-Neto
Orientador

*Dedico esta dissertação à
minha mãe, Maria Aparecida
(in memoriam), meu melhor
exemplo de como ser
bondoso.*

AGRADECIMENTOS

Agradeço à Universidade Federal de Viçosa, o Programa de Pós Graduação em Biologia Celular e Estrutural e aos professores pelo ensino de qualidade.

Agradeço à CAPES pelo fomento à pesquisa.

Agradeço ao Prof^o. José Lino-Neto, pela orientação. Obrigado, inclusive, por me receber e me acolher como orientando, por ser sempre muito atencioso, presente, companheiro e por ter me ensinado tanto.

Agradeço a minha coorientadora Prof^a. Glenda Dias, pela amizade, companheirismo, pela ótima convivência, pelos inúmeros ensinamentos e por sempre ter me incentivado, mesmo quando eu achava que eu não era capaz. Nunca esquecerei disso!

Agradeço a Beth pela ajuda e esclarecimentos, além do excelente trabalho desempenhado no Programa de Pós-Graduação em Biologia Celular e Estrutural.

Agradeço à minha família, em especial à minha mãe, Maria Aparecida (*in memoriam*), que sempre foi a pessoa que mais acreditou em mim, sempre fez tudo que podia para que eu pudesse ser alguém na vida, nunca deixando de me apoiar. Ao meu pai, Silvio; minhas irmãs, Elisângela e Edna; aos meus irmãos, em especial o Jeovane e Elísio; aos meus sobrinhos e sobrinhas, em especial Giovanna, Anna Júlia, Felipe e Bernardo; à Jane, Charles. Obrigado por serem meu porto seguro e me impulsionarem a correr atrás dos meus sonhos.

Agradeço aos meus amigos que estiveram comigo desde o início da minha jornada em Viçosa, Bárbara e Pedro. Vocês foram e continuam sendo minha rede de apoio, presentes em todos os momentos em minha vida. Obrigado por serem tão especiais para mim e por tornarem meus dias mais felizes. Obrigado por tanto!

Agradeço aos amigos do Laboratório de Ultraestrutura Celular, em especial ao Paulo, Maura, Witallo, Ana Clara, Dayvson, Maísa, Camila, Henrique, Rômulo, Vinícius, Andreia, Vitória, Kerem, Ana Cris, Ane. Obrigado pelos momentos compartilhados no lab, aprendi muito com cada um de vocês.

Um agradecimento mais que especial ao Ronaldo, por estar sempre comigo, sendo companheiro, amigo, confidente, de modo que muitos momentos se tornaram muito mais fáceis de serem vividos.

Obrigado aos meus amigos de Barbacena, Pri, Thais, Wellington, Arlindo, Layane, Marlene, João Victor, por me apoiarem e por serem tão incríveis.

Agradeço também à Giovanna e Nicolle, por me acolherem em Viçosa, me trazendo calma num momento em que eu estava perdido.

Aos amigos da “Casinha”, Pedro, Alex, Bianca e Edileuza, com quem construí bons momentos nesses últimos meses. Obrigado pelo companheirismo e amizade.

Agradecimento especial à Prof^a Glenda, à Prof^a Helen, ao Prof^o Serrão e ao Prof^o Vinícius por aceitarem compor a banca.

“Não é sobre vencer, é sobre não desistir. Se você tem um sonho, lute por ele. Existe uma disciplina para a paixão, e não é sobre quantas vezes você é rejeitado ou quantas vezes você caiu e foi derrotado. É sobre quantas vezes você se levantou com coragem e seguiu em frente.”

(Lady Gaga, 2019)

RESUMO

PAULO, Maurício da Silva, M.Sc., Universidade Federal de Viçosa, julho de 2023. **Anatomia e Histologia do Sistema Reprodutor Masculino e Morfologia Espermática de *Leptoglossus Zonatus* (Dallas, 1852) (Heteroptera: Coreidae).** Orientador: José Lino-Neto. Coorientadora: Glenda Samara Dias Santos.

Coreidae é uma família diversa formada predominantemente por percevejos sugadores de plantas, contendo cerca de 520 gêneros, mais ou menos 3.100 espécies em todo o mundo, e 165 gêneros na região Neotropical. O gênero *Leptoglossus* geralmente passa por cinco ínstares ao longo de sua vida e tem distribuição mundial, principalmente nas Américas. *Leptoglossus zonatus* é um percevejo fitófago que se alimenta de culturas de Poaceae, Leguminosae, Rutaceae e Punicaceae, sendo o milho sua principal fonte alimentar. Dados relacionados a aspectos do sistema reprodutivo de Heteroptera são essenciais para entender a biologia da maioria de seus representantes, além de auxiliar na sistemática. Assim, os dados deste estudo podem fornecer informações úteis para a taxonomia e para a compreensão das relações desta espécie, ou gênero, com outros membros do grupo. Assim, descrevemos a morfologia do sistema reprodutor masculino e espermatozoides do percevejo *L. zonatus*. Indivíduos adultos foram coletados em Viçosa, MG – Brasil e seus sistemas reprodutivos foram processados para histologia de luz. Para morfometria dos espermatozoides, estes foram coletados das vesículas seminais dos machos, sendo utilizado o corante Giemsa para medir o comprimento total e o corante DAPI para medir o núcleo. *L. zonatus* tem um par de testículos e dois tipos distintos de glândulas acessórias enroladas. Cada testículo tem uma forma de pecten, como *Machtima crucigera* e *Coreus marginatus* e é circundado por uma bainha testicular altamente traqueolada e pigmentada de vermelho, como visto em outros Coreidae. Possui sete folículos alongados unidos por septos da bainha testicular. Os cistos são esféricos inicialmente e se alongam até a fase final da espermatogênese, com uma média de 850 espermatozoides por cisto (nove ciclos de divisão celular). A espermatogênese ocorre durante toda a vida adulta desse inseto, produzindo espermatozoides finos e alongados que medem em média 310 µm de comprimento, com núcleo de 36 µm. Não se agrupam nas vesículas seminais, sendo transferidos para a fêmea livremente durante a cópula. Características como o número de folículos por testículo e a produção de espermatozoides ao longo da vida adulta certamente refletem o sucesso

reprodutivo de *L. zonatus*, e esses achados, incluindo a morfometria espermática, podem ajudar em futuras pesquisas para entender a diversidade, taxonomia e sistemática do gênero *Leptoglossus*, assim como de outros Coreidae da subordem Heteroptera.

Palavras-chave: Hemiptera. Pentatomomorpha. Morfologia testicular. Desenvolvimento espermático. Glândulas acessórias.

ABSTRACT

PAULO, Mauricio da Silva, M.Sc., Universidade Federal de Viçosa, July 2023. **Anatomy and Histology of the Male Reproductive System and Sperm Morphology of *Leptoglossus Zonatus* (Dallas, 1852) (Heteroptera: Coreidae).** Adviser: José Lino-Neto. Co-adviser: Glenda Samara Dias Santos.

Coreidae is a diverse family formed predominantly by plant-sucking bugs, containing about 520 genera, more or less 3,100 species worldwide, and 165 genera in the Neotropical region. The genus *Leptoglossus* usually undergoes five instars throughout its life and has a worldwide distribution, especially in the Americas. *Leptoglossus zonatus* is a phytophagous bug that feeds on Poaceae, Leguminosae, Rutaceae and Punicaceae crops, with maize being its primary food source. Data related to aspects of the reproductive system of Heteroptera are essential to understand the biology of most of its representatives, in addition to assisting in the systematics. Thus, the data from this study could provide helpful information for taxonomy and for understanding the relationships of this species, or genus, with other group members. So, we described the morphology of the male reproductive system and spermatozoa of the bedbug *L. zonatus*. Adult individuals were collected in Viçosa, MG – Brazil and their reproductive systems were processed for light histology. For morphometry of the spermatozoa, these were collected from the seminal vesicles of males, and the Giemsa dye was used to measure the total length and the DAPI dye to measure the nucleus. *L. zonatus* has a pair of testes and two distinct types of coiled accessory glands. Each testis has a pecten-shaped, like *Machtima crucigera* and *Coreus marginatus* and is surrounded by a red-pigmented, highly tracheolated testicular sheath, as seen in other Coreidae. It has seven elongated follicles joined by testicular sheath septa. The cysts are spherical initially and elongate toward the final phase of spermatogenesis, with an average of 850 spermatozoa per cyst (nine cycles of cell division). Spermatogenesis occurs throughout the adult life of this insect, producing sperm thin and elongated that measures an average of 310 μm in length, with a 36 μm nucleus. They do not form bundles in the seminal vesicles, being transferred to the female freely during copulation. Characteristics such as the number of follicles per testis and sperm production throughout adult life certainly reflect the reproductive success of *L. zonatus*, and these findings, including sperm morphometry, may help in future research to

understand the diversity, taxonomy, and systematics of the genus *Leptoglossus*, as well as of other Coreidae in the suborder Heteroptera.

Keywords: Hemiptera. Pentatomomorpha. Testicular morphology. Sperm development. Accessory glands.

SUMÁRIO

1. INTRODUÇÃO GERAL.....	12
2. OBJETIVOS.....	14
3. RESULTADOS	15
3.1. Anatomy and histology of the male reproductive system and sperm morphology of <i>Leptoglossus zonatus</i> (Dallas, 1852) (Heteroptera: Coreidae).....	16
3.2. Abstract	16
3.3. Highlights.....	17
3.4. Introduction.....	17
3.5. Materials and methods	18
3.6. Results	19
3.7. Discussion	36
3.8. Final considerations.....	38
3.9. References	39
4. CONCLUSÃO GERAL.....	47
5. REFERÊNCIAS	48

1. INTRODUÇÃO GERAL

Hemiptera está entre as cinco ordens mais diversas de Insetos. Seu nome vem do grego (hemi = metade e pteron = asa), referindo-se às asas anteriores divididas em metade membranosa e metade coriácea (Grazia et al. 2012). Composta especialmente por percevejos, a ordem apresenta grande variedade entre seus representantes, seja em morfologia, reprodução ou alimentação. Ao longo de sua história evolutiva, os hemípteros adquiriram uma ampla gama de características morfológicas, reprodutivas e comportamentais, que certamente foram e são importantes para colonizarem ambientes aquáticos, semiaquáticos e terrestres (Grazia et al. 2012).

A ordem é dividida em quatro subordens: Heteroptera, Sternorrhyncha, Coleorrhyncha e Auchenorrhyncha (Grazia et al. 2012). Heteroptera compreendem cerca de 40.000 espécies descritas distribuídas em cerca de 89 famílias (Henry, 2009; Weirauch & Schuh, 2011). A maioria das espécies é fitófaga, mas famílias como Reduviidae e Cimicidae possuem hábitos predadores e hematófagos (Panizzi, 2000; Panizzi et al., 2012; Weiler et al., 2012; Cioato et al., 2015). A subordem Heteroptera se distingue de outros insetos por usar como defesa as secreções produzidas pelas glândulas odoríferas (Eliyahu et al., 2012), sendo considerada uma autapomorfia do grupo (Schuh & Slater, 1995). Tais estruturas se localizam no metatórax dos adultos e no dorso abdominal das ninfas.

Os Heteroptera estão distribuídos em sete infraordens: Cimicomorpha, Dipsocoromorpha, Enicocephalomorpha, Pentatomomorpha, Gerromorpha, Leptopodomorpha e Nepomorpha (Schuh & Slater, 1995). A infraordem Pentatomomorpha, formada por 40 famílias, representa mais de 14.000 espécies. É o segundo maior grupo entre as sete infraordens de Heteroptera (Weirauch & Schuh, 2011). Pentatomomorpha está distribuído em cinco superfamílias (Aradoidea, Coreoidea, Lygaoidea, Pentatomoidea e Pyrrhocoroidea). Até o momento, existem poucas análises cladísticas para as superfamílias Aradoidea, Pyrrhocoroidea e Coreoidea, especialmente a última. A escassez de informações é resultado da falta de um consenso na classificação do grupo. Assim, as classificações diferem consideravelmente em número e composição de subfamílias.

Três famílias pertencentes à superfamília Coreoidea são encontradas na região Neotropical: Coreidae, Rhopalidae e Alydidae. A família Coreidae é formada por um grupo diverso de percevejos, predominantemente fitófagos. Contém em torno de 520 gêneros com 3.100 espécies espalhadas por todo o mundo. Desses, 165 gêneros são encontrados na região Neotropical (Packauskas, 1994, 2010; Oliveira et al, 2017). O gênero *Leptoglossus* tem distribuição mundial, principalmente nas Américas; *L. zonatus* (Dallas, 1852), *L. gonagra* (Fabricius, 1775) e *L. stigma* (Herbst, 1784) são as espécies mais encontradas no Brasil (Allen, 1969; Pires et al., 2012). Na primeira espécie, os indivíduos adultos apresentam coloração acastanhada, duas manchas ovoides amareladas no pronoto. Também apresentam dimorfismo sexual, com fêmeas de *L. zonatus* geralmente maiores que os machos (Allen, 1969; Panizzi, 1989; Matrangolo & Waquil, 1994).

Leptoglossus zonatus utiliza uma ampla gama de fontes alimentares e Panizzi (1989, 2004) destaca o milho como especialmente nutritivo para esta espécie. Os adultos e ninfas se alimentam de grãos dessa cultura, prejudicando a produção (Pires et al., 2012). Sawazaki et al. (1989) observaram que, além de se alimentarem, contaminavam as espigas do milho com fungos, como *Fusarium moniliforme*, *Penicilium sp.* e *Cephalosporium sp.*, sendo mais um contribuinte para os prejuízos na produção deste alimento (Matrangolo & Waquil, 1994). Eles também são importantes pragas de pomelos, laranjas e pêssegos e muitas outras espécies de Poaceae, Leguminosae, Rubiaceae, Rutaceae e Punicaceae (Matrangolo & Waquil, 1994; Pires et al., 2012).

Em geral, em Heteroptera, o sistema reprodutor masculino consiste em um par de testículos, um par de ductos deferentes, um par de vesículas seminais, um par de glândula acessória, um bulbo ejaculatório seguido por um ducto ejaculatório (Pendergrast, 1956, 1957; Özyurt et al., 2013). Os testículos podem ter de um a oito folículos, variando conforme a espécie, sendo os folículos preenchidos por cistos, onde ocorre a espermatogênese (Phillips, 1970). As diferenças encontradas na organização dos testículos, como o número de folículos por testículo, por exemplo, podem ser utilizadas na sistemática (Phillips, 1970; Schuh & Slater, 1995). Os folículos são preenchidos por cistos com células germinativas nas diferentes fases de desenvolvimento: espermatogônias, fase inicial da espermatogênese, até os espermatozoides maduros no final da espermiogênese. Em Hemiptera, o tamanho dos espermatozoides varia entre 20 µm (Báo et al., 1997) a 15.000 µm de comprimento

(ver Taylor et al., 1982). Essas células são fundamentais para perpetuar a espécie, além disso, os espermatozoides maduros podem apresentar uma morfologia muito diversa entre as espécies, seja no tamanho, formato, número de componentes e/ou na organização ultraestrutural destes (Jamieson et al., 1999). Assim, seu conjunto de caracteres pode auxiliar no entendimento da organização sistemática de um grupo, bem como para a discriminação de uma espécie. Ademais, os dados ultraestruturais combinados com dados moleculares e da morfologia interna e externa, podem contribuir para a maior compreensão das relações filogenéticas entre os diversos grupos.

Como ainda não há chaves de identificação para vários grupos importantes em Coreidae, os estudos de taxonomia ainda são fragmentados e precisam ser revistos. Da mesma forma, dados relacionados aos aspectos do sistema reprodutivo de Heteroptera podem contribuir para o entendimento da biologia da maioria de seus representantes. Assim, os dados deste estudo podem fornecer informações úteis para a taxonomia e para a compreensão das relações da espécie *L. zonatus*, ou gênero, com outros membros do grupo.

2. OBJETIVOS

Descrever a anatomia e histologia do sistema reprodutor masculino, assim como a morfologia dos espermatozoides de percevejos da espécie *L. zonatus* (Heteroptera: Coreidae).

3. RESULTADOS

PAULO, Mauricio da Silva; REZENDE, Paulo Henrique; DIAS, Glenda; LINO-NETO, José. **Anatomy and histology of the male reproductive system and sperm morphology of *Leptoglossus zonatus* (Dallas, 1852) (Heteroptera: Coreidae)**. Trabalho submetido à revista *Microscopy Research and Technique*, em julho de 2023.

3.1. Anatomy and histology of the male reproductive system and sperm morphology of *Leptoglossus zonatus* (Dallas, 1852) (Heteroptera: Coreidae)

Mauricio da Silva Paulo¹; Paulo Henrique Rezende²; Glenda Dias¹; José Lino-Neto^{1*}

¹Departamento de Biologia Geral

Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil.

²Departamento de Entomologia

Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil.

*José Lino-Neto, ¹Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa, Minas Gerais 36570-900, Brasil.

E-mail: linoneto@ufv.br

3.2. Abstract

Leptoglossus zonatus (Heteroptera: Coreidae) is a phytophagous bug that feeds on Poaceae, Leguminosae, Rubiaceae, Rutaceae, and Punicaceae crops, with maize being its primary food source. Information regarding the anatomy and histology of the male reproductive system of Coreidae is scarce. Also, no data on the sperm morphometry of *L. zonatus* has been available so far. Aiming to provide the data mentioned above, we described the reproductive system morphology of *L. zonatus*. Adult individuals were collected at the Federal University of Viçosa, MG – Brazil and their reproductive systems were dissected, processed, and embedded in historesin for light histology. Sperm were collected from the seminal vesicles of males to do morphometry. *L. zonatus* has a striking red colouration in the paired testes and two distinct pairs of coiled accessory glands with high secretory activity. Spermatogenesis occurs throughout the adult life of this insect, producing sperm that measure an average of 310 µm in length, with a 36 µm nucleus. The anatomy, histology, and morphometric data of *L. zonatus* sperm may help understand the reproductive biology of this species and taxonomy in Heteroptera.

3.3. Highlights

- The *L. zonatus* sperm are slender and long, about 310 µm in total length and 36 µm in nucleus length.
- Spermatogenesis occurs throughout the adult life and equally in the seven testicular follicles.
- Accessory glands of ectodermal and mesodermal origin are coiled and highly developed.
- The ectodermal gland produce a filamentous secretion, whereas in the ectodermal sac the secretion is globular.

Keywords: Hemiptera; Pentatomomorpha; Testicular morphology; Sperm development; Filamentous secretion

3.4. Introduction

Coreidae is a diverse family formed predominantly by plant-sucking bugs, containing about 520 genera, more or less 3,100 species worldwide, and 165 genera in the Neotropical region (Packauskas, 1994, 2010; Oliveira et al, 2017). The genus *Leptoglossus* usually undergoes five instars throughout its life and has a worldwide distribution, especially in the Americas. In Brazil, *L. zonatus* (Dallas, 1852), *L. gonagra* (Fabricius, 1775), and *L. stigma* (Herbst, 1784) are the most commonly found (Allen, 1969; Pires et al., 2012). In the first species, adult individuals (Fig. 1A,B) have a brownish colouration, two yellowish ovoid spots on the pronotum. Also, they show sexual dimorphism, with females of *L. zonatus* generally bigger than males (Allen, 1969; Panizzi, 1989; Matrangolo & Waquil, 1994).

Panizzi (1989, 2004) mentions that *L. zonatus* uses a wide range of food sources and highlights maize to be especially nutritious for this species, where adults and nymphs feed on maize grains, impairing the production (Pires et al., 2012). Sawazaki et al. (1989) observed that in addition to feeding on maize crops, they also contaminated the ears with fungi, such as *Fusarium moniliforme*, *Penicillium sp.* and *Cephalosporium sp.*, being one more contributor to the damage in the production of

this food (Matrangolo & Waquil, 1994). They are also important crop pests of pomelos, oranges and peaches and many other species of Poaceae, Leguminosae, Rubiaceae, Rutaceae and Punicaceae (Matrangolo & Waquil, 1994; Pires et al., 2012).

In general, in Heteroptera, the male reproductive system consists of a pair of testes, a pair of deferent ducts, a pair of seminal vesicles, a pair of accessory gland, an ejaculatory bulb followed by an ejaculatory duct (Pendergrast, 1956, 1957; Özyurt et al., 2013). The testes can have of one to eight follicles, varying according to the species, and the follicles being filled by cysts, where spermatogenesis occurs (Phillips, 1970). Differences found in testes organisation, such as the number of follicles per testis, for example, can be used in systematics (Phillips, 1970; Schuh & Slater, 1995).

Taxonomy studies on Coreidae has been fragmented and needs to be revised, as there are still no identification keys for several important groups. Likewise, data related to the reproductive system aspects of Heteroptera are essential to understand the biology of most of its representatives. Thus, the data from this study could provide helpful information for taxonomy and for understanding the relationships of this species, or genus, with other group members. Therefore, we describe the morphology of the male reproductive system of *L. zonatus* and their spermatozoa. This data may help evolutionary studies of said characteristics and understand the reproductive biology of this bug of great agricultural importance.

3.5. Materials and methods

Adult individuals of *L. zonatus* were collected between June and August of 2022 at the Federal University of Viçosa, -20.766119, -42.869443, Viçosa, Minas Gerais, Brazil. The bugs, pre and post-mating, were cryoanesthetized, and their reproductive systems were dissected in 0.1M phosphate-buffered saline – PBS, pH 7.2 and subsequently fixed in 2.5% glutaraldehyde + 3% sucrose for 2 hours.

After dissecting the male and female reproductive systems, drops of seminal fluid from the seminal vesicle (male) and spermatheca (female) were spread on histological slides and photographed. Two staining technics were used to measure the spermatozoa; a fluorescence marker, the DAPI dye (0.2 mg/mL), was used for marking the nucleus. Slides were stained for 20-30 minutes, washed in distilled water and mounted with 50% sucrose. After being photographed, these slides were stained with

Giemsa (Eosin + methylene blue) for 15-30 minutes and washed in tap water. Sperm images were taken on an Olympus BX-60 photomicroscope with a digital camera and, for DAPI staining, equipped with a blue fluorescence laser. The measurements were made using the Sperm Sizer-1.6.6 software (McDiarmid et al., 2021).

For the histological analysis, the male reproductive systems were fixed with 2.5% glutaraldehyde + 3% sucrose and washed for 1 hour in distilled water at 10 minutes intervals. After washing, the organs were post-fixed with 1% osmium tetroxide for 2 hours, rewashed with distilled water and dehydrated in increasing alcoholic solution series (30%, 50%, 70% and 100%). Then, the material was infiltrated and included in Histoiresin® plastic resin (Leica Histoiresin, Heidelberg, Germany) and polymerised at 60 °C for 12 hours.

Semi-thin sections (1.0-1.5 µm) were obtained with a Leica RM 2255 microtome and glass knife and stained with Giemsa. Subsequently, they were analysed and photographed using a Q-Color3 digital camera attached to the Olympus BX-60 microscope. For DAPI-stained slides, a BP 360-370 nm excitation filter was added. For the testis (Fig. 3), e.g., there were 544 sequential photos (frames), which showed 30% to 40% compatibility between each one. The photos were taken at 40x or 100x magnification and mounted using the Photomerge function of the Adobe Photoshop 2022 software. Thus, to increase image quality and allow a high-resolution power for each mounted histological image. In the end, 1200 to 1300 photos were taken for the assembly of each structure, and 134 photos of spermatozoa, in Giemsa and DAPI stained.

3.6. Results

The male reproductive system of *L. zonatus* is formed by a pair of testes, followed by deferent ducts, seminal vesicles, and two distinct types of accessory glands; a close to the seminal vesicles and the other the other associated with the ectodermal sac, surrounding the common ejaculatory ducts. This latter, is dilated and extends from the fusion of the lateral ejaculatory ducts to the aedeagus (Fig. 1C). The pecten-shaped testes are isolated, and each is surrounded by a red-pigmented, highly tracheolated testicular sheath (Fig. 1C-D). Each testis has seven vertically elongated follicles joined

by testicular sheath septa (Fig. 1C-D; 3A, 5A). Numerous cysts at different stages of spermatogenesis are visible in each follicle (Fig. 3A).

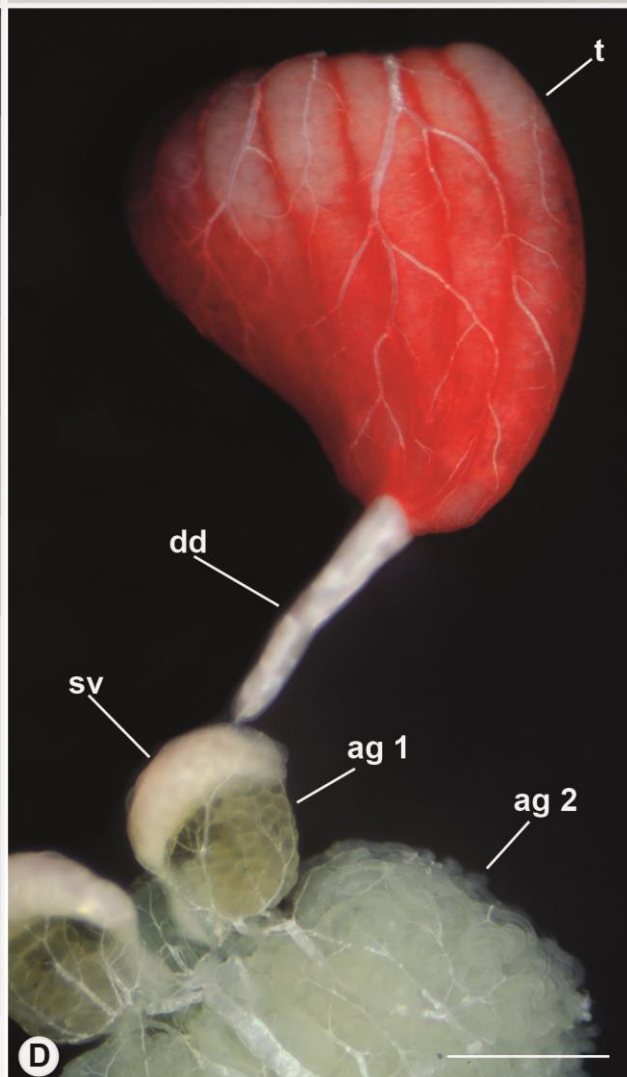
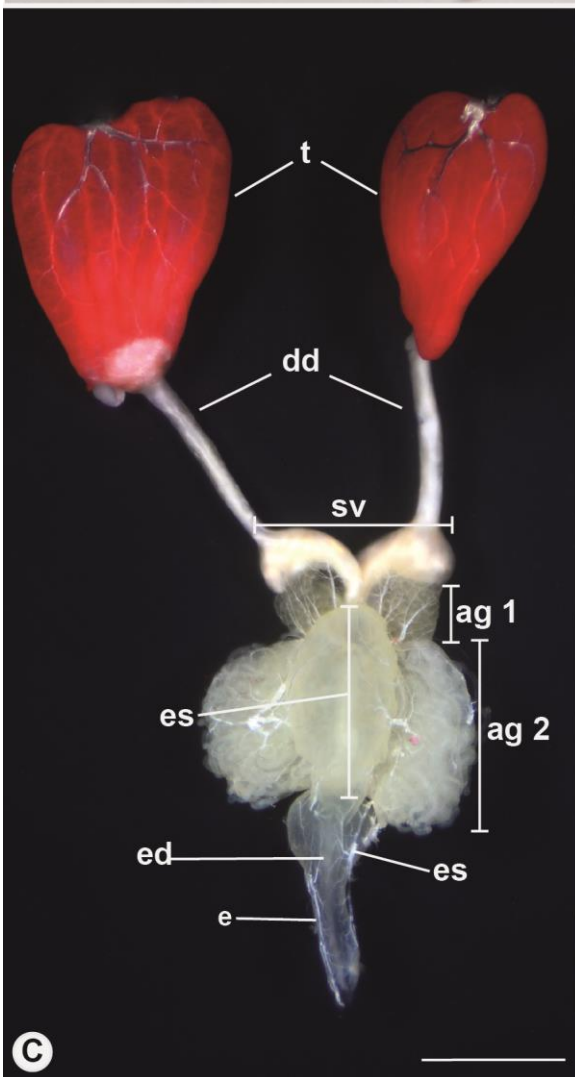
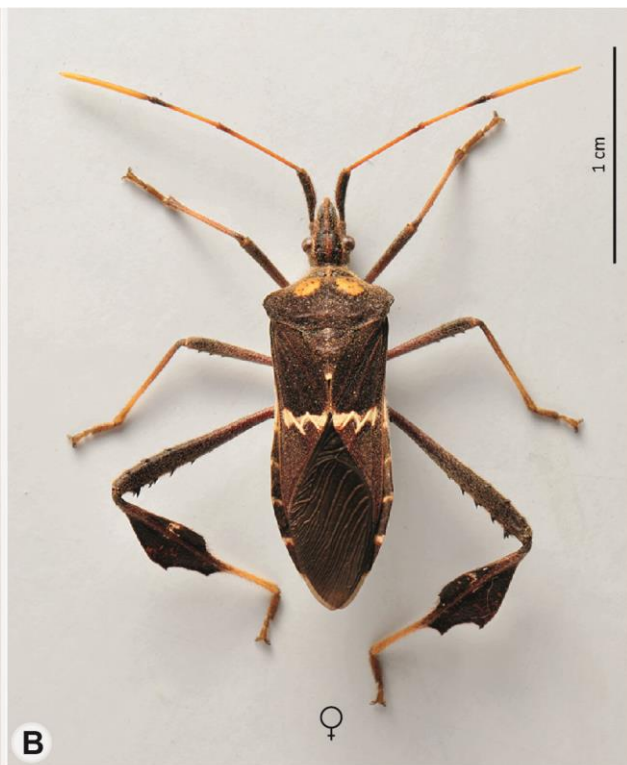


Figure 1. Male and female individuals and male reproductive system from *Leptoglossus zonatus*. A: male and B: female. C and D: t: testicle; dd: deferens duct; sv: seminal vesicle; ag1: accessory gland 1; ag2: accessory gland 2; ed: ejaculatory duct; es: ectodermal sac; e: aedeagus. Scales: A and B: 1 cm; C and D: 1,5 mm.

The spermatozoa from the seminal vesicles and the spermatheca (in females) had no significant differences. They are thin and elongated and do not form bundles in the seminal vesicles, being transferred to the female freely during copulation. The total sperm length was about 310 μm (Fig. 2A). The nucleus region, stained by DAPI (Fig. 2B), was approximately 36 μm .

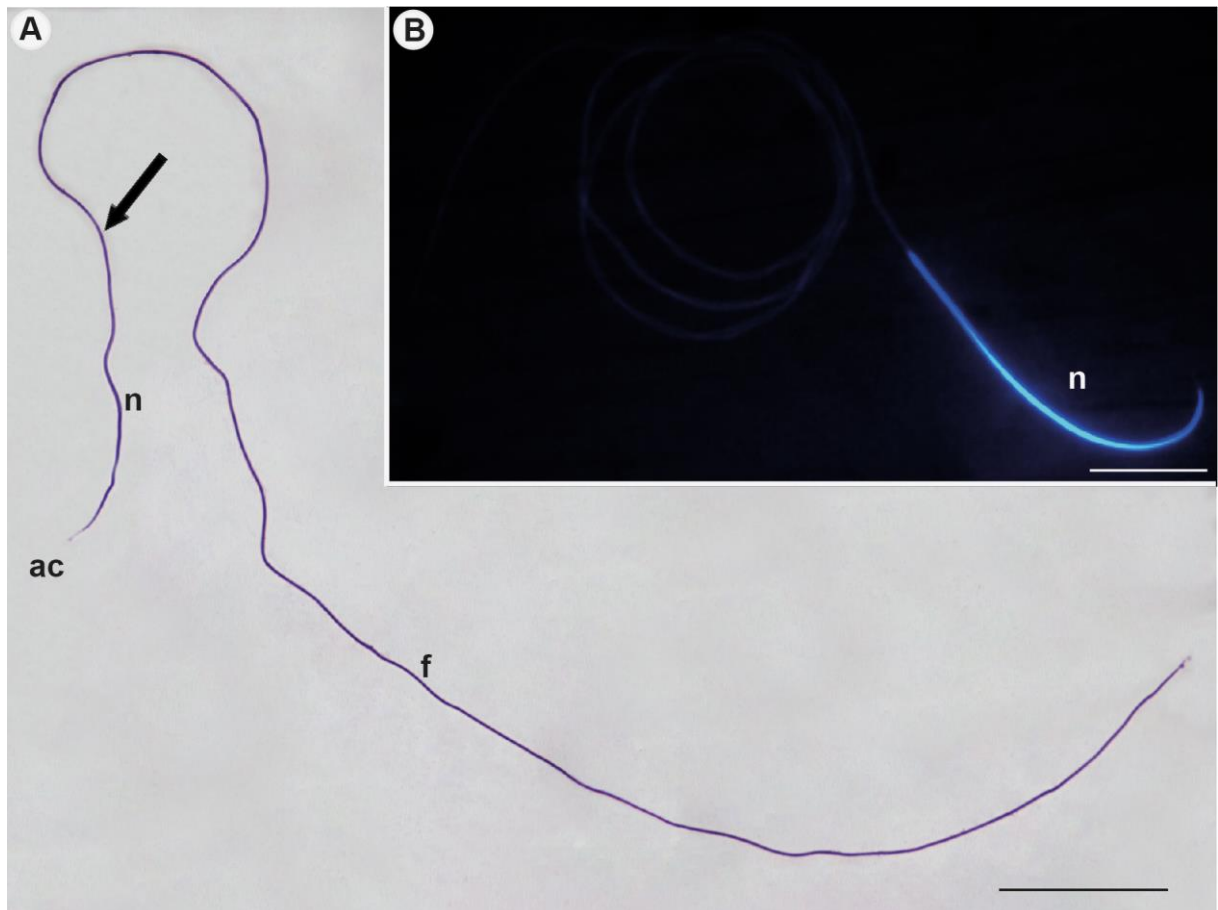


Figure 2. *Leptoglossus zonatus* spermatozoa. A: total length as evidenced by Giemsa stain and B: nucleus stained with fluorescence by DAPI. ac: acrosome; n: nucleus; f: flagellum; arrow: middle piece. Scales: A: 20 μm , D: 10 μm .

Spermatogenesis similarly takes place in all seven follicles, where the different stages of sperm development are seen. So that five zones can be distinguished in

each follicle: (I) growth zone, where groups of spermatogonia multiply by mitosis, being organised in cysts surrounded by the cystic cells (Fig. 3A); (II) zone of spermatocyte development, marked by the fusion of mitochondria into a neberkerns and the elongation of this until the formation of two mitochondrial derivatives (Fig. 3A, 4A-C); (III) zone of spermatid differentiation into spermatozoa (spermiogenesis), with elongation of the nuclei and flagella (Fig. 3A, 4E); (IV) sperm maturation zone, where there is the final elongation of sperm and elimination of cell debris (Fig. 3A, 5B); and (V) the efferent duct, the final portion of the follicles filled with cell debris and mature sperm that will be directed to the deferent duct (Fig. 3A). The cysts are spherical initially and elongate toward the final phase, with an average of 850 spermatozoa per cyst (Fig. 4D), corresponding to nine cycles of cell division.

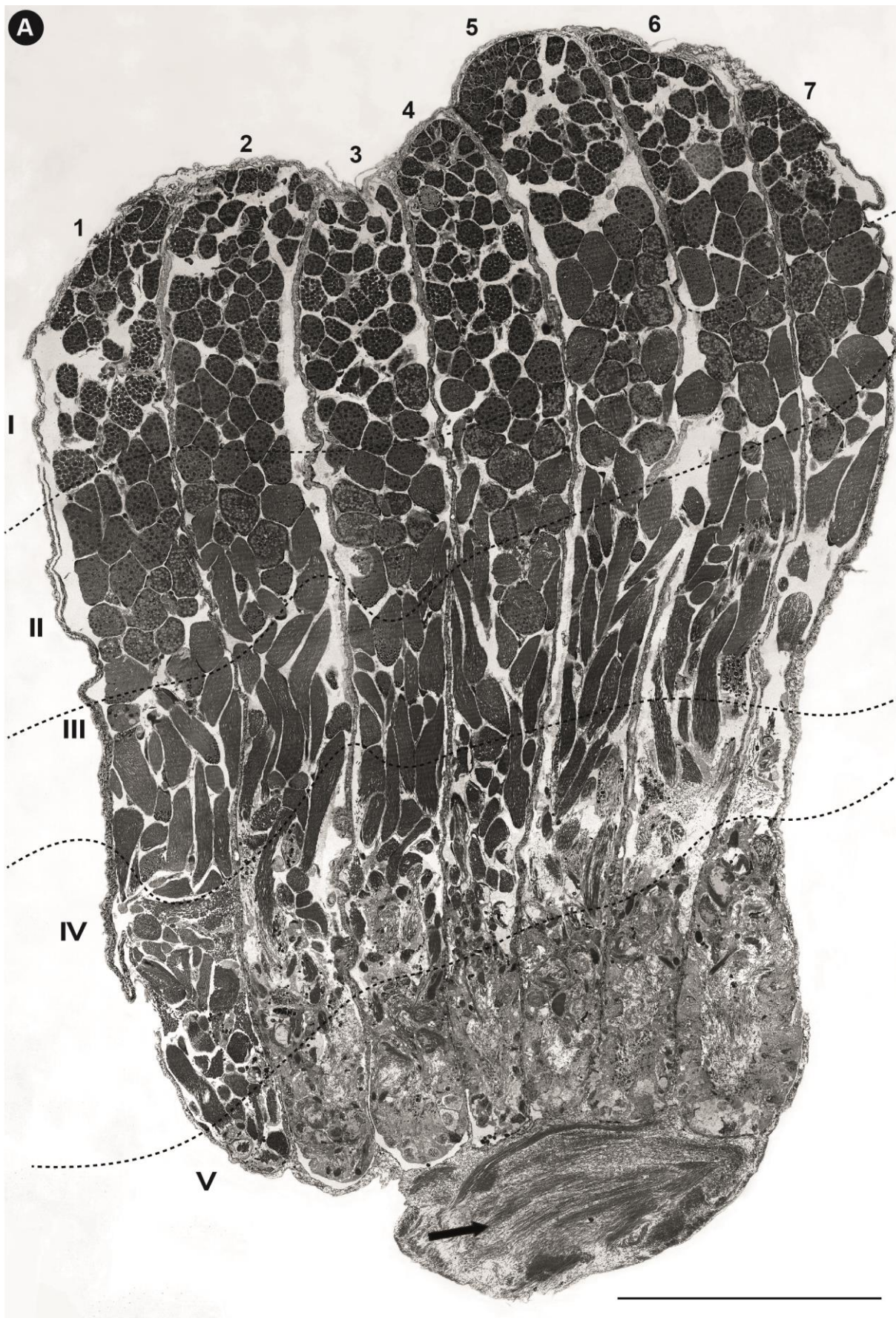


Figure 3. Histology of the testis of *Leptoglossus zonatus*. A: Longitudinal section of the testicle showing the seven testicular follicles (1-7). I: growth zone; II: zone of spermatocyte development; III: zone of spermatid differentiation; IV: sperm maturation zone; V: efferent duct; arrow: spermatozoa in the deferent duct. Scale: 500 μm .

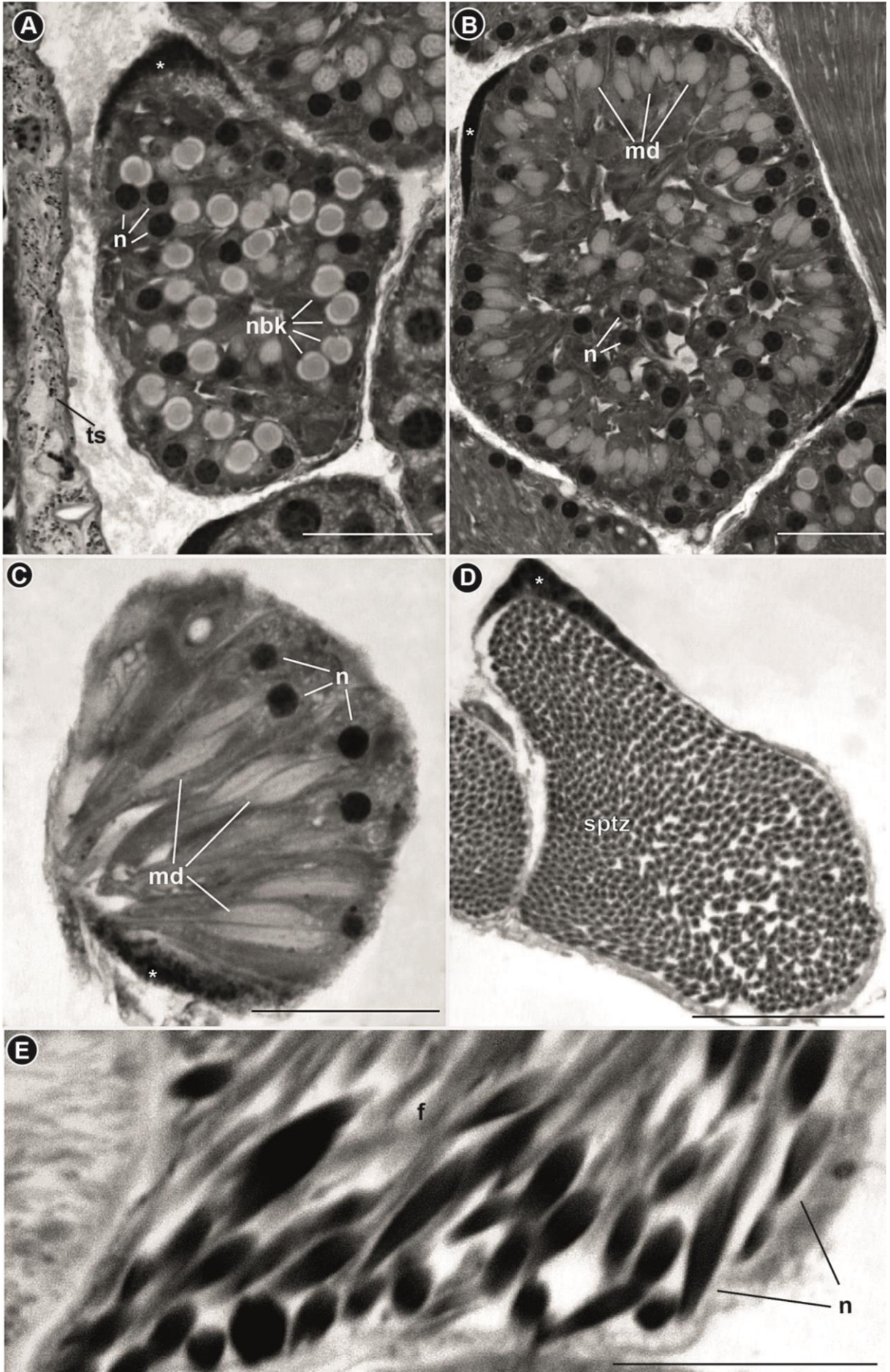


Figure 4. Histology of the testis of *Leptoglossus zonatus*. A: cyst in a spermatocyte development zone. n: nuclei; nbk: nebenkern; ts: testicular sheath; asterisk: cystic cell nucleus. B and C: development and elongation of mitochondrial derivatives. n: nuclei; nbk: nebenkerns; md: mitochondrial derivatives; asterisk: cystic cell nucleus. D: Cross-section of the cyst. sptz: spermatozoa; asterisk: cystic cell nucleus. E: zone of spermatid differentiation. Observe the nuclei (n) and flagella (f) as they elongate. Scales: 20 μ m.



Figure 5. Histology of the testis of *Leptoglossus zonatus*. A: Cross-section of the testicle showing the seven testicular follicles (1-7). B: *L. zonatus*'s spermatozoa (sptz) in the final stage of sperm maturation. Scales: A: 100 μ m, B: 20 μ m.

The efferent ducts, formed by a simple epithelium of cuboidal cells with spherical nuclei, are short and flow into the deferent duct (Fig. 3A). Each deferent duct is formed by similar epithelial cells, visualized in longitudinal and transverse sections (Fig. 6B-D). It is also lined externally by a thin, non-pigmented peritoneal layer. The seminal vesicles have a simple epithelium of cubic cells with spherical and slightly flattened nuclei basally (Fig. 6A). Their lumen is filled with a large volume of spermatozoa (Fig. 6A). In the final portion of each vesicle, a well-developed musculature is evident (Fig. 6E-F), which continues posteriorly to cover the common ejaculatory ducts (Fig. 7C, 9D). Surrounding each seminal vesicle is a convoluted accessory gland of mesodermal origin. These latter have simple epithelium formed by cubic cell of rounded nuclei with

decondensed chromatin (Fig. 6G). These epithelial cells, whose surface is covered by stereocilia, secrete substances that fill the glandular lumen.

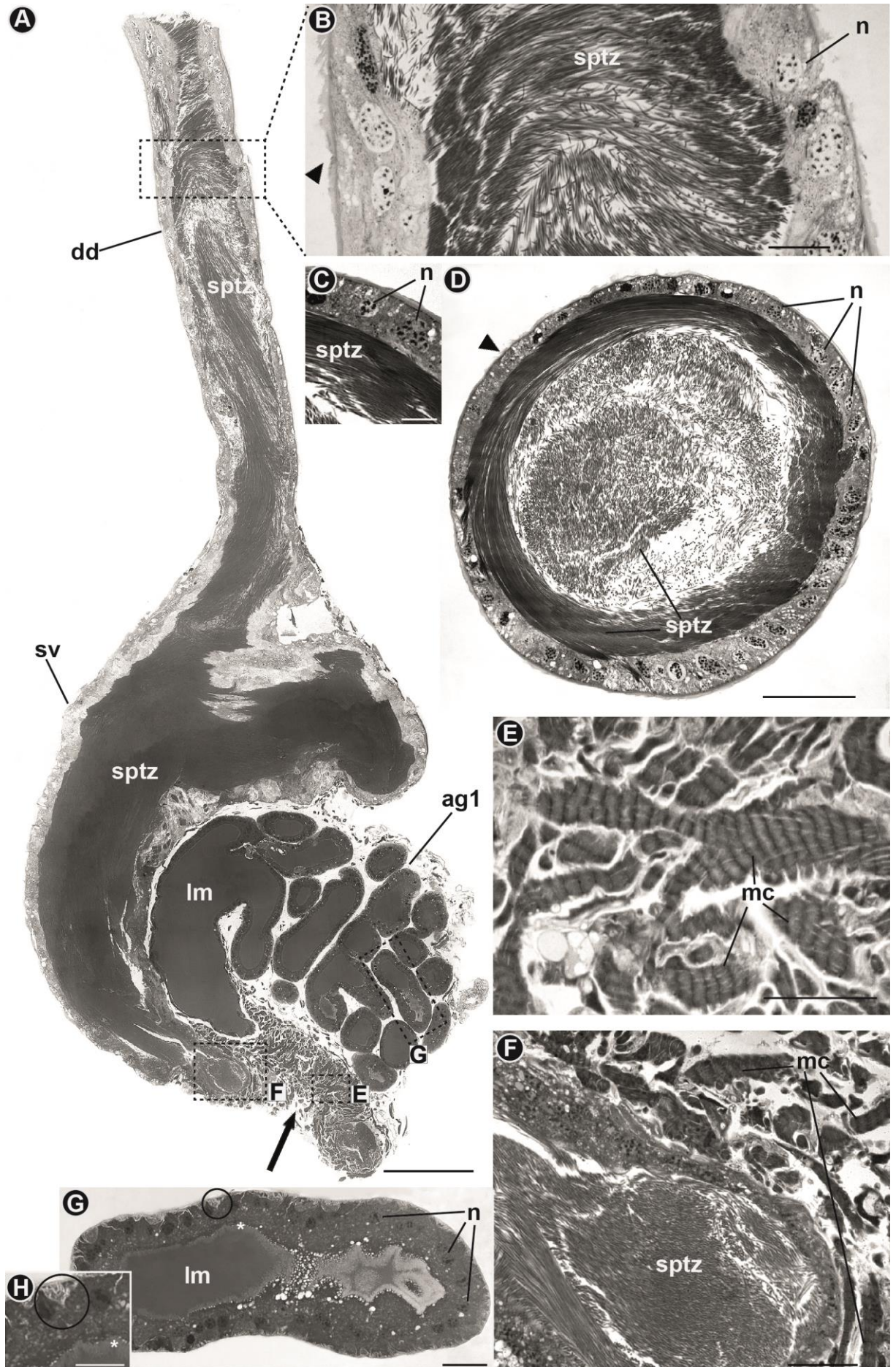


Figure 6. Histology of the ejaculatory duct, seminal vesicle and first accessory gland of *Leptoglossus zonatus*. Longitudinal section of the deferent duct (dd), seminal vesicle (sv) and accessory gland 1 (ag1). (A). Deferens duct (dd) and seminal vesicle (sv) are full of sperm (sptz); accessory gland 1 (ag1) contains secretion in the lumen region (lm); the arrow points to the beginning of the musculature in the final portion of the seminal vesicle (sv). Longitudinal (B) and cross-section (C and D) of the deferent duct, lined by simple cubic epithelium with spherical nuclei (n); the dd is externally covered by a thin, non-pigmented peritoneal layer (arrowhead). E and F: detail of the musculature (mc) that begins its coating in the final portion of the seminal vesicle. G and H: accessory gland 1, covered by simple cubic epithelium, with spherical nuclei (n), containing extensions (circle) and stereocilia (asterisk) projected to the gland lumen (lm). Scales: A: 200 μ m; B, E, F and G: 20 μ m; C and H: 10 μ m, D: 50 μ m.

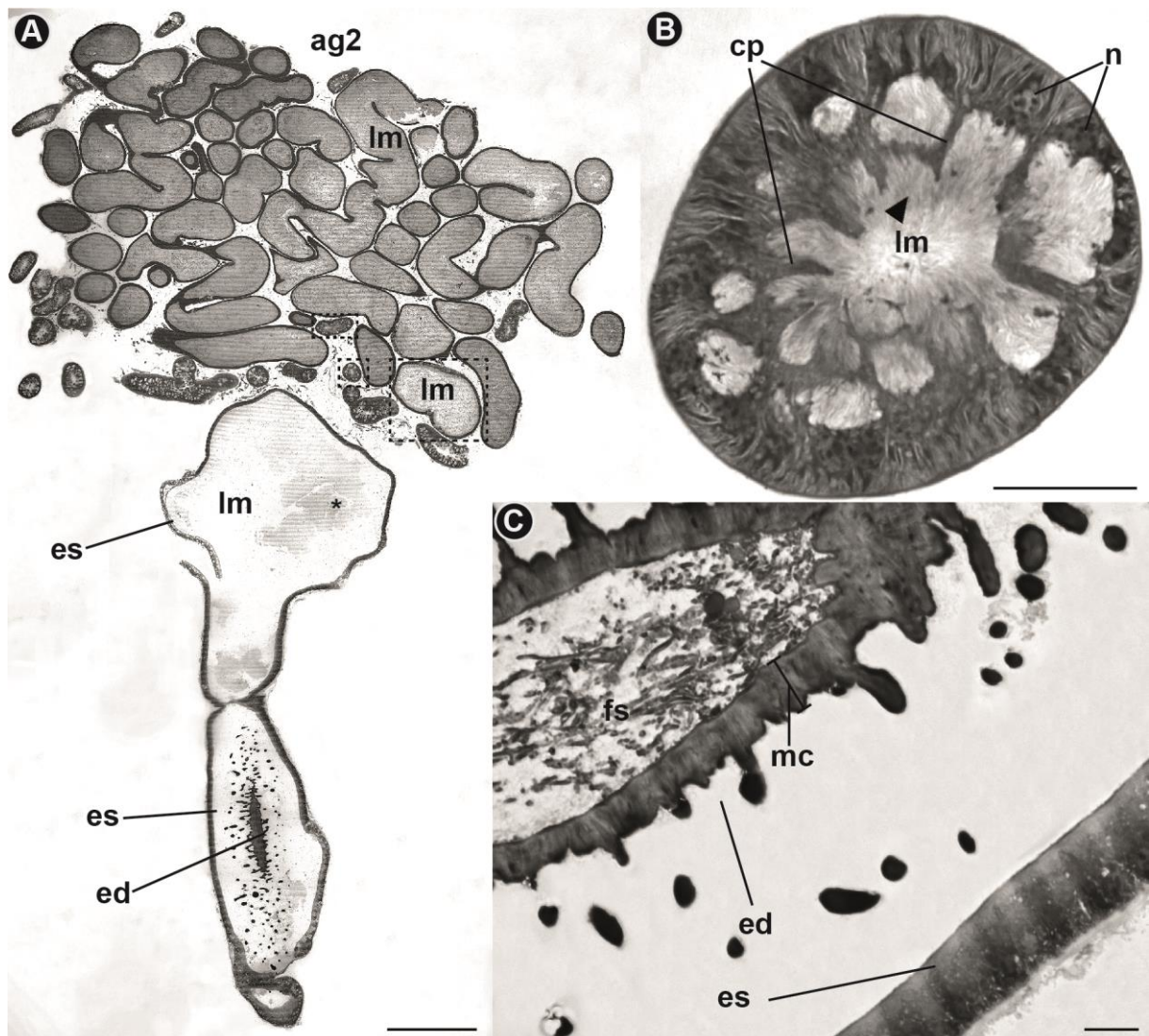


Figure 7. Histology of the ejaculatory duct, the second accessory gland, and ectodermal sac of *Leptoglossus zonatus*. Longitudinal section of accessory gland 2 (ag2), ectodermal sac (es) and ejaculatory duct (ed) of *Leptoglossus zonatus*. A: ag2 is coiled with a lumen (lm) full of secretion; ectodermal sac (es) lines the ejaculatory duct and has secretion (asterisk) in the lumen region (lm). B: transverse section of the lateral ejaculatory duct after seminal vesicle, covered by simple cubic epithelium with spherical nuclei (n), note the presence of cytoplasmic projections (cp) and stereocilia (arrowhead) towards the lumen (lm). C: detail of the ejaculatory duct (ed), covered by thick muscles (mc); in the region of the lumen of the ejaculatory duct, it is possible to observe the filamentous secretion (fs) produced by accessory gland 2 (ag2); the ectodermal sac (es) surrounds the structures. Scales: A: 300 μm ; B and C: 20 μm .

The second type of gland has very long and densely tangled (Figs. 7A). The secretory portion is the distal, longer, thicker, and well-dilated lumen. Its epithelium is simply formed by two types of cubic cells with stereocilia, spherical nuclei of decondensed chromatin (Fig. 7A, 9A-C). In this portion, almost the entire epithelium is formed by lower cells, uniformly blushed, and the nuclei are situated in their median region (Fig. 9A-C). However, there are small regions where the epithelium is formed by one or some larger cells with quite vacuolized cytoplasm, especially in the lower half, and nuclei near the apical region (Fig. 9A-B). Its secretion is made up of well-stained particles and mainly by filamentous structures evenly distributed throughout the lumen, especially in the secretory portion (Fig. 9A-B). At a certain point, the epithelium of its duct merges with the epithelium of the ectodermal sac, however, the secretion produced by the gland was not observed in the lumen of the ectodermal sac. (Fig 9C,D). A thin layer of secretory cells forms the epithelium of the sac ectodermal and fills its lumen with secretion (Fig. 7A, 9C-D).

The two lateral ejaculatory ducts are formed from the junction of the two post-vesicular vasa deferentia with the respective ducts of the first type of accessory glands, of mesodermal origin. In these ducts, the epithelium is also simple, but with a remarkable difference, compared to the vas deferens that is the presence of long apical cytoplasmic projections and innumerable stereocilia covering in epithelia cells (Fig. 7B, 8A-D). In the initial region, after the seminal vesicles, the cytoplasmic projections are smaller (Fig. 8C) and spermatozoa are observed in the lumen region (Fig. 8B). The

two lateral ejaculatory ducts merge after the fusion of the second accessory gland epithelia with the ectodermal sac (Fig. 8D-E, 9C), forming a common ejaculatory duct (Fig. 9D).

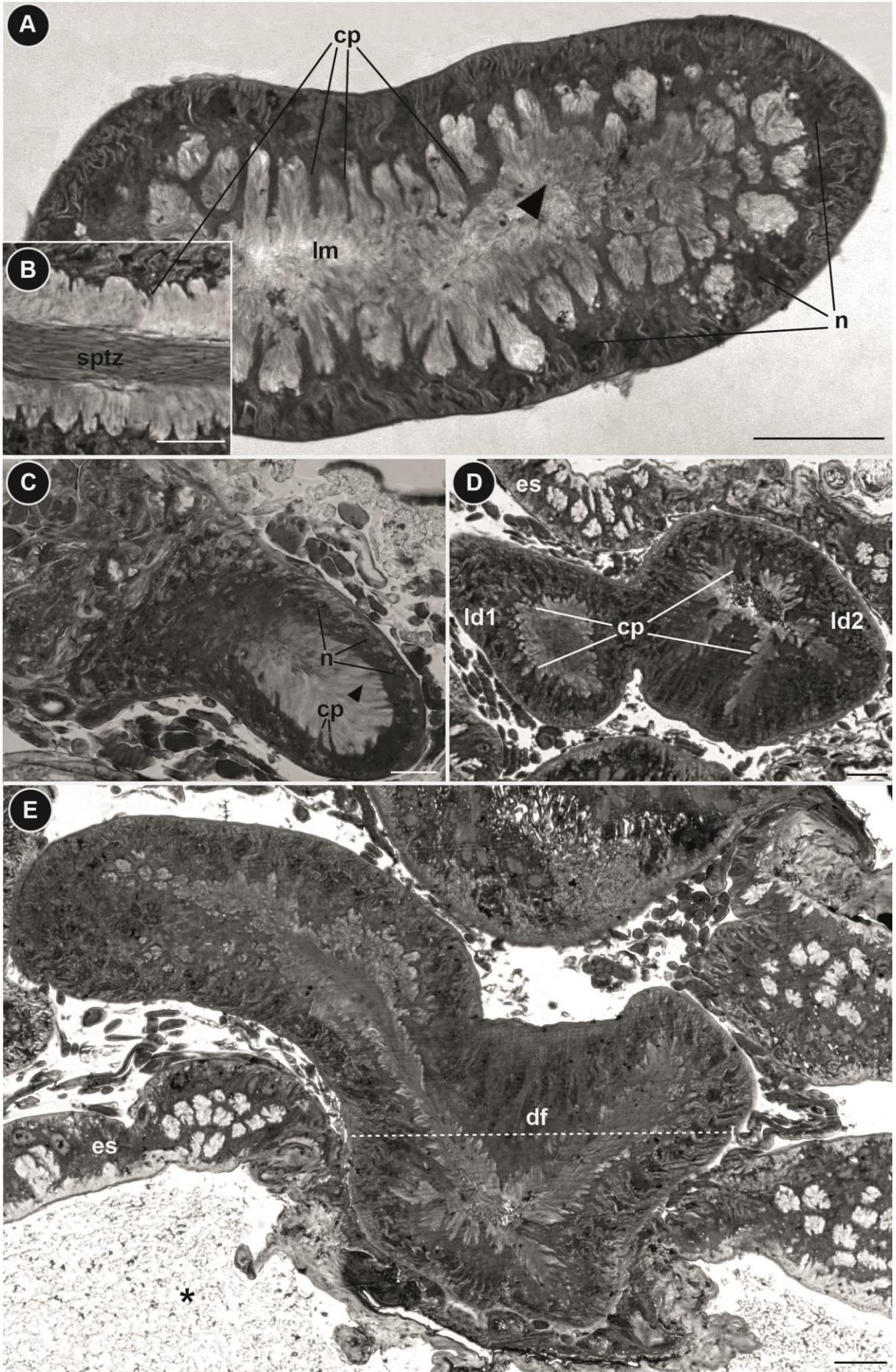


Figure 8. Histology of the lateral ejaculatory duct after seminal vesicle of *Leptoglossus zonatus*. A: longitudinal section showing a simple cubic epithelium with spherical nuclei (n), note the presence of cytoplasmatic projections (cp) and stereocilia (arrowhead) towards the lumen (lm). B: sperm (sptz) in the lumen. C: beginning of formation of the lateral ejaculatory duct after seminal vesicle. nuclei (n), cytoplasmatic projections (cp) and stereocilia (arrowhead). D: lateral ejaculatory duct 1 (ld1) and 2 (ld2) before fusion in common ejaculatory duct. cytoplasmatic projections (cp), es: ectodermal sac. E: fusion of lateral ejaculatory duct in common ejaculatory duct. Df: duct fusion, es: ectodermal sac, globular secretion (asterisk). Scales: A, C, D and E: 20 μm ; B: 10 μm .

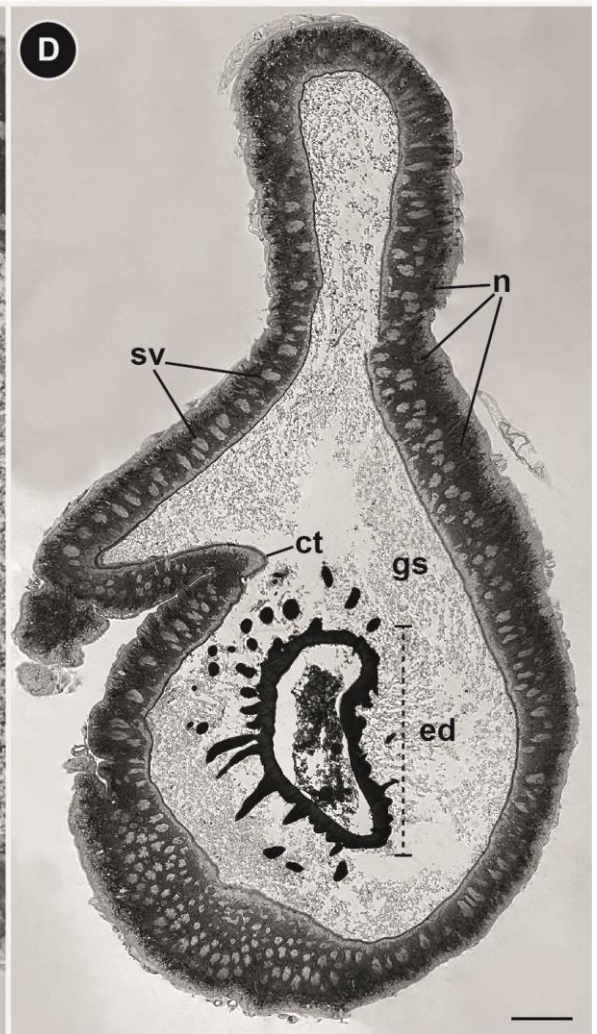
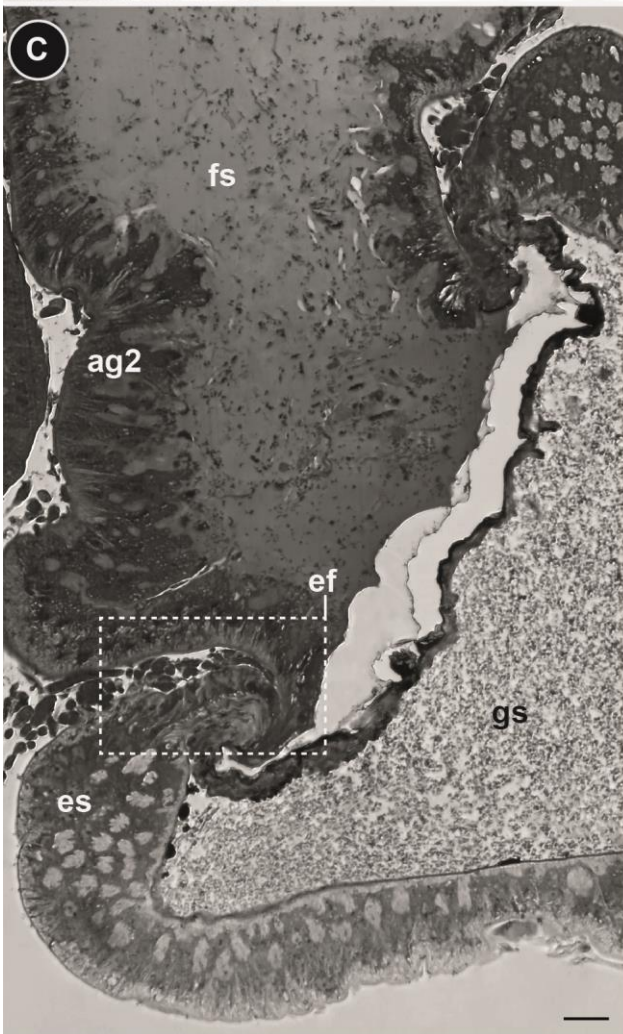
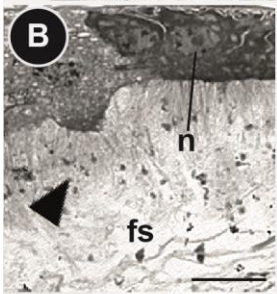


Figure 9. Histology of the second accessory gland, the common ejaculatory duct and ectodermal sac of *Leptoglossus zonatus*. A and B: Section of the central portion of accessory gland 2 (ag2), covered by simple cubic epithelium and spherical nuclei (n); the presence of stereocilia (arrowhead) towards the lumen (lm) is also observed; filamentous secretion (fs) fills the lumen. C: Detail of the fusion (ef) of accessory gland 2 (ag2) and ectodermal sac (es) epithelium. Note the two types of secretion, filamentous secretion (fs) by the accessory gland 2 and globular secretion (gs) by the ectodermal sac. D: transverse section of the common ejaculatory duct and the ectodermal sac (es) surrounds the structure. n: nuclei; sv: secretory vesicles; ct: cuticle; ed: common ejaculatory duct. Scales: A, C and D: 20 μm ; B: 10 μm .

3.7. Discussion

The anatomical and histological analysis showed that the male reproductive system of *L. zonatus* follows the general characteristics found in other Heteroptera. Some groups have fused follicles (Nijhout, 1998) in an elongated/oval shape, such as in Pentatomidae, Gerridae, and Lygaeidae (Souza et al., 2007a; Castanhole et al., 2008, 2010; Souza & Itoyama, 2010, 2011; Gomes et al., 2013). However, as observed in other Coreidae like *Machtima crucigera* (Fabricius, 1775) and *Coreus marginatus* (Linnaeus, 1758), in *L. zonatus*, the testes are longitudinally elongated and pecten-shaped and are not fused (Karakaya et al., 2012; Gomes et al., 2013; Oliveira et al., 2021).

It is common for insect testes to be covered by a usually colorless sheath. This is also true for some aquatic heteropterans (Pendergrast, 1957; Castanhole et al., 2008, 2010; Gomes et al., 2013; Novais et al., 2017; Candan et al., 2018). However, in Pentatomomorpha such as Pentatomidae (Souza et al., 2007a; Ozyurt et al., 2015), Lygaeidae (Souza et al., 2007b), Pyrrhocoridae (Castanhole et al., 2008), Coreidae (Oliveira et al., 2021), Reduviidae and Cimicomorpha (Jahnke et al., 2006), this sheath is pigmented. It can exhibit a significant color variation, such as orange, yellow, blue, green, and intense red, as in the species of this study. The color of this pigmentation can be influenced by the individual's diet or metabolism (Araújo et al., 2020).

The number of follicles per testis can range from one to nine in Heteroptera, but it tends to be consistent in a species, genus, or even within a family (Matsuda, 1976;

Freitas et al., 2010; Mróz & Wojciechowski, 2011; Karakaya et al., 2012; Özyurt et al., 2013, 2014, 2015; Gapon et al., 2021; Rezende et al., 2021; Grozeva et al., 2022). Seven follicles, as occurs in *L. zonatus*, have been considered the modal number for heteropterans, as it has been the most commonly observed number in the order. (Pendergrast, 1957; Freitas et al., 2010; Kaur & Patial, 2012, 2016; Karakaya et al., 2012; Gomes et al., 2013; Özyurt et al., 2014, 2015). In all individuals of *L. zonatus* studied, we observed that all follicles were filled with cysts at different stages of spermatogenesis, suggesting continuous sperm production. The adult phase of individuals of this species is approximately 40 days., with many chances of mating throughout this phase (Matrangolo & Waquil, 1994; Jamieson et al., 1999; Pires et al., 2007). So, the continuous production of spermatozoa should contribute to a better chance of fertilization of eggs in many females.

The accessory glands are tubular and densely tangled; the pair associated with the seminal vesicles and deferent ducts, is smaller and of mesodermal origin. In contrast, the single gland, associated with the lateral ejaculatory duct and common ejaculatory duct, is more prominent and of ectodermal origin. In Heteroptera, both glands can vary significantly in their dimensions and shape, but they usually occur in pairs (Pendergrast, 1957; Mrós, 2011, 2012; Rezende et al., 2021; Vélez, et al., 2020), different from what was observed in *L. zonatus*.

The ectodermal sac, as described in *Coreus marginatus* by Karakaya (2012), is present in other Coreidae. This primary role of this structure is to coat the ejaculatory duct, but it can also produce fluid that fills the organ. We have observed in *L. zonatus* the presence of secretory vesicles in the epithelium of the ectodermal sac, indicating a secretory activity. A globular-type secretion increases as the male enters the mating stage, as observed after the dissection of recently mated individuals. Similar activity was also described in the coreid *Machtima crucigera* (Oliveira et al., 2021), with the same glandular type as *L. zonatus* but considerably reduced size. The products of the accessory glands constitute semen and can have many functions. In the male, they are linked with maintaining sperm viability, lubrication of ducts for transport and activation of male gametes, and spermatophore formation in some taxa (Chen, 1984; Davey, 1985; Gillott, 1988, 2003; Raina et al., 1994; Freitas et al., 2010). After mating, they can affect the female, such as nutrition, influencing oviposition, and even behavioral modulation to diminish or prevent the female from remating, thus avoiding sperm competition (Raina et al., 1994; Gillot, 2003), (Pendergrast 1957; Jahnke et al.,

2006). Concerning the secretion of these structures, they can increase the female's refractory period, thus reducing the chance of competing with other males. In addition, these secretion can form a mating plug when transferred to females during copulation, promoting the sealing of their genital opening.

3.8. Final considerations

In conclusion, the studies referring to the anatomy and histology of the male reproductive system, added to the morphometric data of the spermatozoa of *Leptoglossus zonatus*, make up a collection of essential information for understanding the species reproductive biology. Differences in the organization, size, and type of secretion of the accessory gland of ectodermal origin bring a new perspective on this structure. It is evident that such findings reinforce its importance for the reproductive success of *L. zonatus* and may help in future research to understand diversity, morphology, and reproduction in the genus *Leptoglossus*, in other Coreidae, and the suborder Heteroptera.

AUTHOR CONTRIBUTIONS

MSP: investigation, writing — original draft, writing — review and editing; PHR: investigation, writing—review and editing; GD: investigation, writing—review and editing, visualisation and supervision.; JL-N: conceptualisation, methodology, investigation, writing—review and editing and supervision.

ACKNOWLEDGEMENTS

This work was supported by the Brazilian funding agencies CAPES (process number 88887.677850/2022-00 – MSP) and CNPq (Process 140905/2020–0 - PHR), and the research grant number 306486/2019–9 - JLN). We want to thank Dr. Jocélia Grazia (Universidade Federal do Rio Grande do Sul - UFRS) and Dr. José Antônio Marin Fernandes (Universidade Federal do Pará - UFPA) for confirming the identification of the specimens.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

DATA AVAILABILITY STATEMENT

This published article includes all the data generated or analyzed during this study.

ORCID:

Paulo, M.S.: <http://orcid.org/0000-0002-3017-3987>

Rezende, P.H.: <https://orcid.org/0000-0003-2919-0218>

Dias, G.: <https://orcid.org/0000-0002-1455-6398>

Lino-Neto, J.: <https://orcid.org/0000-0002-2172-1654>

3.9. References

Allen. RC (1969). **A revision of the genus *Leptoglossus* Guerin (Hemiptera: Coreidae)**. Entomologica Americana. 45:35-140.

Araújo, V.A.; Bacca, T.; Dias, L.G. (2020). **Anatomy of male and female reproductive organs of stink bugs pests (Pentatomidae: Heteroptera) from soybean and rice crops**. Biota Neotropica. <https://doi.org/10.1590/1676-0611-BN-2020-1045>

Araújo, V.A.; Lino-Neto, J.; Ramalho, F.S.; ZANUNCIO, J.C.; SERRAO, J.E. (2011). **Ultrastructure and heteromorphism of spermatozoa in five species of bugs (Pentatomidae: heteroptera)**. Micron 42, 560-677. <https://doi.org/10.1016/j.micron.2011.02.001>

Araújo, V.A.; Moreira, J.; Lino-Neto, J. (2010). **Morphology of the male reproductive system of the social wasp, *Polistes versicolor versicolor*, with phylogenetic implications**. Journal of Insect Science. 10 (71), 1-10. <https://doi.org/10.1673/031.010.710>

- Bowen, R.H. (1920). **Studies on Insect Spermatogenesis - I. The History of the Cytoplasmic Components of the Sperm in Hemiptera**. Department of Zoology, Columbia University. Biological Bulletin. <http://www.jstor.org/stable/1536656>
- Bushrow, E.S.; Fuller, C.L.; Cowan, D.P.; Byrd, C.A. (2006). **Anatomy of the male reproductive system and sperm morphology in the caterpillar-hunting wasp *Ancistrocerus antilope* (Hymenoptera, Vespidae)**. Invertebrate Biology. 125, 354-362. <https://doi.org/10.1111/j.1744-7410.2006.00067.x>
- Candan, S.; Ozyurt, N.; Suludere, Z. (2018). **Morphological and histological structure of the male reproductive system of the water strider *Gerris lacustres* (Linnaeus 1758) (Gerridae, Heteroptera)**. Microscopy Research & Technique. 1-11. <https://doi.org/10.1002/jemt.23035>
- Castanhole, M.M.U.; Pereira, L.L.V.; Souza, H.V.; Bicuda, HEMC; Costa, L.A.A.; Itoyama, M.M. (2008) **Heteropicnotic chromatin and nucleolar activity in meiosis and spermiogenesis of *Limnogonus aduncus* (Heteroptera, Gerridae): a stained nucleolar organising region that can serve as a model for studying chromosome behavior**. Genetics and Molecular Research. 7 (4), 1398-1407. <https://doi.org/10.4238/vol7-4gmr527>
- Castanhole, M.M.U.; Pereira, L.L.V.; Souza, H.V.; Itoyama, M.M. (2010). **Spermatogenesis and karyotypes of three species of water striders (Gerridae, Heteroptera)**. Genetics and Molecular Research. 9 (3), 1343-1356. <https://doi.org/10.4238/vol9-3gmr841>
- Chen, P.S. (1984). **The functional morphology and biochemistry of insect male accessory glands and their secretions**. Annual Review of Entomology. 29, 233-255. <https://doi.org/10.1146/annurev.en.29.010184.001313>
- Cioato, A.; Bianchi, F.M.; Eger, J.; Grazia, J. (2015). **New species of *Euschistus* (*Euschistus*) from Jamaica, *Euschistus* (*Mitripus*) and *Ladeaschistus* from southern South America (Hemiptera: Heteroptera: Pentatomidae: Pentatominae)**. Zootaxa. <http://dx.doi.org/10.11646/zootaxa.3937.3.4>
- Dallacqua, R.P.; Cruz-Landim, C. (2003). **Ultrastructure of the Ducts of the Reproductive Tract of Males of *Melipona bicolor bicolor* Lepeletier (Hymenoptera, Apinae, Meliponini)**. Anatomia, Histologia, Embryologia. 32, 276–281. <https://doi.org/10.1046/j.1439-0264.2003.00484.x>

- Dallai, R. (2014). **Overview on spermatogenesis and sperm structure of Hexapoda**. *Arthropod Structure & Developments*. 43, 257–290.
<https://doi.org/10.1016/j.asd.2014.04.002>
- Davey, K.G. (1985). **The male reproductive tract**. *Comprehensive Insect Physiology, Biochemistry and Pharmacology*. Oxford. Pergamon Press, Oxford, England, pp. 1-14.
- Freitas, S.P.C.; Gonçalves, T.C.M.; Serrão, J.E.; Costa, J.; Santos-Mallet, J.R. (2010). **Male reproductive system structure and accessory glands ultrastructure of two species of *Triatoma* (Hemiptera, Reduviidae, Triatominae)**. *Micron*. 41, 518-525. <https://doi.org/10.1016/j.micron.2010.01.008>
- Gapon, D.A.; Kuznetsova, V.G.; Nadachowska, A.M. (2021). **A new species of the genus *Rhaphidosoma* Amyot et Serville, 1843 (Heteropteran, Reduviidae), with data on its chromosome complement**. *Comparative Cytogenetics*. 15(4): 467–505. <https://doi.org/10.3897/CompCytogen.v15.i4.78718>
- Gillott, C. (1988). **Arthropoda - Insecta**. In: *Reproductive Biology of Invertebrates*. Vol.3. New York: Wiley, pp.319–371.
- Gillott, C. (2003). **Male accessory gland secretions: modulations of female reproductive physiology and behaviour**. *Annual Review of Entomology*. 48, 163-184. <https://doi.org/10.1146/annurev.ento.48.091801.112657>
- Gomes, M.O.; Castanhole, M.M.U.; Souza, H.V.; Murakami, A.S.; Firmino, T.S.S.; Saran, P.S.; Banho, C.A.; Monteiro, L.S.; Silva, J.C.P.; Itoyama, M.M. (2013). **Morphological aspects of the testes of 18 species of terrestrial of Heteroptera from Northwestern Sao Paulo (Brazil)**. *Biota Neotropica*. 13, 132-135. <https://doi.org/10.1590/S1676-06032013000300016>
- Grazia, J.; Cavichioli, R. R.; Wolf, R. R. S.; Fernandes, J. A. M.; Takiya, D. M. (2012). **Insetos do Brasil: diversidade e taxonomia. Hemiptera. Linnaeus, 1758**. Ribeirão Preto: Holos Editora. p. 347-405.
- Grozeva, S.; Stoianova, D.; Konstantinov, F.; Simov, N.; Kuznetsova, V.G. (2022). **A synopsis of the numbers of testicular follicles and ovarioles in true bugs (Heteroptera, Hemiptera) – sixty-five years of progress after J. Pendergrast's review**. *ZooKeys*. 1136: 71–123.
<https://doi.org/10.3897/zookeys.1136.96431>

- Henry, T. J. (2009). **Biodiversity of Heteroptera**. Chapter 10. Pp. 223–263 In: Footitt, R.G.; Adler, P.H. (eds). *Insect Biodiversity: Science and Society*. Wiley-Backwell, Oxford, UK, 656 pp.
- Jahnke, S.M.; Redaelli, L.R.; Diefenbach, L.M.G. (2006). **Internal reproductive organs of *Cosmoclopius nigroannulatus* (Hemiptera: Reduviidae)**. *Brazilian Journal of Biology*. 66 (2A), 509-512. <https://doi.org/10.1590/S1519-69842006000300017>
- Jamieson, B.G.M.; Dallai, R.; Afzelius, B.A. (1999). **Insects: their spermatozoa and phylogeny**. Science Publishers, Inc, Enfield, NH, USA.
- Karakaya, G.; Ozyurt, N.; Candan, S.; Suludere, Z. (2012). **Structure of the male reproductive system in *Coreus marginatus* (Linnaeus, 1758) (Hemiptera: Coreidae)**. *Turkish Journal of Entomology*. 36, 193-204. <https://dergipark.org.tr/en/download/article-file/65165>
- Kaur, H.; Patial, N. (2016). **Internal male reproductive organs in five species of Heteroptera (Insecta: Hemiptera)**. *Indian Journal of Fundamental and Applied Life Science*. 6 (3), 30-35. <http://www.cibtech.org/jls.htm>
- Kaur, H.; Patial, N. (2012). **Internal male reproductive organs of ten species of Heteroptera (Insecta: Hemiptera)**. *Indian Journal of Fundamental and Applied Life Science*. 2 (1), 317 e 324. <http://www.cibtech.org/jls.htm>
- Klowden, M.J. (2013). **Physiological Systems in Insects**. 3rd ed. Academic Press, San Diego, CA.
- Matrangolo, W.J.R.; Waquil, J.M. (1994). **Biologia de *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) alimentado com milho e sorgo**. *Anais da Sociedade Entomológica do Brasil* 23 (3)
- Matsuda, R. (1976). **Morphology and Evolution of the Insect Abdomen**. Oxford: Pergamon Press, Oxford, England, p. 544.
- McDiarmid, C. S.; Li R.; Kahrl, A. F.; Rowe, M.; Griffith, S.C. (2021) **Sperm Sizer: a program to semi-automate the measurement of sperm length**. *Behavioral Ecology Sociobiology* 75:84. <https://doi.org/10.1007/S00265-021-03013-4>
- Mróz, E. (2012). **The structure of the male reproductive system of the genus *Psallus* Fieber (Hemiptera: Heteroptera: Miridae)**. *Polish Journal of Entomology*. 81:107–118. <https://doi.org/10.2478/v10200-011-0070-8>
- Mróz, E.; Wojciechowski, W. (2011). **The systematic position of the tribe *Stenodemini* (Heteroptera: Cimicomorpha: Miridae: Mirinae) in the light of**

- the male internal reproductive system.** Journal of Natural History. 45 (25e26), 1563-1588. <https://doi.org/10.1080/00222933.2011.559595>
- Nijhout, H.F. (1998). **Reproduction. Insect Hormone.** Princeton: Princeton University. pp. 142–159.
- Novais, A.M.; Dias, G.; Lino-Neto, J. (2017). **Testicular, spermatogenesis and sperm morphology in *Martarega bentoi* (Heteroptera: Notonectidae).** Arthropod Structure & Development. 46 (4), 635 e 643. <https://doi.org/10.1016/j.asd.2017.04.002>
- OLIVERA, L.; MELO, M.C.; DELLAPÉ, P.M. **First records of leaf-footed bugs (Heteroptera: Coreoidea: Coreidae) from Argentina.** Revista de la Sociedad Entomológica Argentina. 76 (1-2): 68-70. 2017. <https://doi.org/10.25085/rsea.761212>
- Oliveira, M.S.; Serrão, J.E.; Dias, G.; Martins, L.C.B.; Araújo, V.A. (2021). **Anatomy and histology of the male reproductive tract of *Machtima crucigera* (Fabricius, 1775) (Heteroptera: Coreidae).** Zoologischer Anzeiger 293, 156-162. <https://doi.org/10.1016/j.jcz.2021.06.009>
- Özyurt, N.; Candan, S.; Sulidere, Z. (2013). **The Morphology and Histology of The Female Reproductive System of *Graphosoma lineatum* (Heteroptera: Pentatomidae) based on light and scanning electron microscope studies.** International Journal of Scientific Research. <https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.672.3020&rep=rep1&type=pdf>
- Özyurt, N.; Candan, S.; Sulidere, Z. (2014). **The morphology and histology of the male reproductive system in *Apodiphus amygdali* (Germar, 1817) (Hemiptera: Heteroptera: Pentatomidae).** Life: The Excitement of Biology. 2 (1). [https://doi.org/10.9784/leb2\(1\)ozyurt.01](https://doi.org/10.9784/leb2(1)ozyurt.01)
- Özyurt, N.; Candan, S.; Sulidere, Z. (2015). **Ultrastructure of Male Reproductive System of *Eurydema ventrale* Kolenati 1846 (Heteroptera: Pentatomidae).** Microscopy Research and Technique. Volume 78, Issue 8 p. 643-653. <https://doi.org/10.1002/jemt.22514>
- Packauskas, R. J. (1994). **Key to the subfamilies and tribes of the New World Coreidae (Hemiptera), with a checklist of published keys to genera and species.** Proceedings of the Entomological Society of Washington. 96: 44–53.

- Packauskas, R. J. (2010). **Catalog of the Coreidae, or Leaf-Footed Bugs, of the New World**. Fort Hays Studies. Fourth Series. Number 5. 2010.
<https://doi.org/10.58809/HCWH1818>
- Panizzi, A.R. (2004). **A possible territorial or recognition behavior of *Leptoglossus zonatus* (Dallas) (Heteroptera, Coreidae)**. Revista Brasileira de Entomologia. 48(4): 577-579.
- Panizzi, A.R. (1989). **Desempenho de ninfas e adultos de *Leptoglossus zonatus* Dallas(Hemiptera: Coreidae) em diferentes alimentos**. Anais da Sociedade Entomológica do Brasil 18:375-389.
- Panizzi, A.R. (2000). **Heteroptera of economic importance**. Edited by Carl W. Schaefer and Antônio Ricardo Panizzi. International Standard Book Number.
<https://doi.org/10.1201/9781420041859>
- Panizzi, A.R.; Bueno, A.F.; Silva, F.A.C. (2012). **Insetos que Atacam Vagens e Grãos**. Soja: manejo integrado de insetos e outros artrópodes-praga. Brasília: Embrapa.
- Pendergrast, J.G. (1956). **The Male Reproductive Organs of *Nezara viridula* (L.) with a Preliminar Account of Their Development. (Heteroptera; Pentatomidae)**. Auckland University College. Transactions of the Royal Society of New Zealand.
- Pendergrast, J.G. (1957). **Studies on the reproductive organs of the Heteroptera with a consideration of their bearing on classification**. Transactions of the Entomological Society of London. 109. PT. 1.
- Phillips, D.M. (1970). **Insect sperm: their structure and morphogenesis**. Journal Cell Biology.
- Pires, E.M.; Ferreira, P.S.; Guedes, R.N.; Serrão, J.E. (2007). **Morphology of the phytophagous bug *Platyscytus decempunctatus* (Carvalho) (Heteroptera: Miridae)**. Neotropical Entomology. 36 (4), 510-513.
<https://doi.org/10.1590/S1519-566X2007000400004>
- Pires, E.M.; Ruffato, S.; Manica, C.L.M.; Soares, M.A.; Lacerda, M.C. (2012). **Novas plantas hospedeiras oara o percevejo fitófado *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae)**. EntomoloBrasilis. 5 (3): 249-252.
- Raina, A.K.; Kingan, T.G.; Giebultowicz, J.M. (1994). **Mating-Induced loss of Sex Pheromone and Sexual Receptivity in Insects with Emphasis on**

- Helicoverpa zea* and *Lymantria dispar***. Archives of Insect Biochemistry and Physiology 25:317-327. <https://doi.org/10.1002/arch.940250407>
- Rezende, P.H.; Dias, G.; Folly, C.; Lino-Neto, J. (2021). **Male reproductive system and sperm morphology of Eccritotarsini plant bugs (Heteroptera: Miridae)**. Zoomorphology. 140:257–267. <https://doi.org/10.1007/s00435-021-00525-5>
- Sawazaki, E.; Rossetto, C.J., Fantini, G.M.; Petinelli, A.Jr. (1989). ***Leptoglossus zonatus* (Dallas, 1852) nova praga do milho**. Arquivos do Instituto Biológico. 56 (Sup1.): 22.
- Schuh, R.T., Staler, J.A., (1995). **The Bugs of the World (Hemiptera: Heteroptera)**. Classification and Natural History. Cornell University Press, Ithaca, New York, USA.
- Souza, H.V., Itoyama, M.M. (2010). **Comparative study of spermatogenesis and nucleolar behavior in testicular lobes of *Euschistus heros* (Heteroptera: Pentatomidae)**. Psyche: A Journal of Entomology. 10: 1-10. <https://doi.org/10.1155/2010/428673>
- Souza, H.V., Itoyama, M.M. (2011). **Comparative analysis of heteropyknotic chromatin and silver-stained material in *Platycarenum umbractulatus* (Pentatomidae: Discocephalinae)**. Invertebrate Reproduction & Development. 55 (2): 65-72. <https://doi.org/10.1080/07924259.2010.548637>
- Souza, H.V.; Bicudo, H.E.M.C.; Costa, L.A.A.; Itoyama, M.M. (2007a). **A study of meiosis and spermatogenesis in different testicular lobes of *Antiteuchus tripterus* (Heteroptera: Pentatomidae)**. European Journal of Entomology. 4: 353-362. <https://doi.org/10.14411/eje.2011.041>
- Souza, H.V.; Bicudo, H.E.M.C.; Itoyama, M.M. (2007b). **Study of chromosomal and nucleolar aspects in testes of *Nysius californicus* (Heteroptera: Lygaeidae)**. Genetics and Molecular Research. 6, 33-40. <http://www.geneticsmr.com/year2007/vol6-1/pdf/gmr0249.pdf>
- Vélez, M.; Dias, G.; Morales, I.; Guedes, R.N.C.; Lino-Neto, J. (2020). **Morphology and histology of the male reproductive system of *Collaria oleosa* (Distant, 1883) (Heteroptera: Miridae)**. Revista Bionatura. 5:1122–1125. <https://www.revistabionatura.com/files/2020.05.02.7.pdf>
- Weiler, L.; Bianchi, F.M.; Grazia, J. (2012). **Percevejos-do-mato (Hemiptera, Heteroptera, Pentatomoidea) da Floresta Nacional de São Francisco de Paula, RS, Brasil**. Revista Brasileira de Biociências – Brazilian Journal of

Biosciences. Porto Alegre. v.10(2)

<https://www.seer.ufrgs.br/rbrasbioci/article/view/115588>

Weirauch, C.; Schuh, R. T. (2011). **Systematics and evolution of Heteroptera: 25 years of progress**. Annual Review of Entomology, v. 56, p. 487–510.

<https://doi.org/10.1146/annurev-ento-120709-144833>

4. CONCLUSÃO GERAL

Os dados apresentados neste trabalho evidenciam um apanhado de características anatômicas e histológicas comum em várias famílias de insetos. *L. zonatus* apresenta o formato dos testículos em “pecten shaped”, que é similar aos de outros representantes da família Coreidae, variando a cor da bainha peritoneal que reveste externamente esta estrutura. *L. zonatus* possui sete folículos testiculares e cistos em diferentes fases da espermatogênese, que também são características compartilhadas com outros representantes de Coreidae. Ademais, tais características atreladas a produção de espermatozoides ao longo de toda a vida adulta, refletem na reprodução de *L. zonatus*.

Em conclusão, os estudos referentes à anatomia e histologia do aparelho reprodutor masculino, somados aos dados morfométricos dos espermatozoides de *L. zonatus*, compõem um acervo de informações essenciais para o entendimento da biologia reprodutiva da espécie. Com esta pesquisa foi possível observar que há diferenças na organização, tamanho e tipo de secreção da glândula acessória de origem ectodérmica, quando comparada a outros Coreidae. Portanto, esses dados trazem uma nova perspectiva sobre essa estrutura, visto que sua alta atividade secretora está relacionada, principalmente, à produção continuada de espermatozoides. É evidente que as características descritas do sistema reprodutor dos machos de *L. zonatus* constadas neste trabalho, reforçam o evidente o sucesso reprodutivo desta espécie e podem auxiliar em pesquisas futuras para entender a diversidade, morfologia e reprodução no gênero *Leptoglossus*, em outros Coreidae, e na subordem Heteroptera.

5. REFERÊNCIAS

- ALLEN, R.C. **A revision of the genus *Leptoglossus* Guerin (Hemiptera: Coreidae).** Entomologica Americana. 45:35-140. 1996.
- BÁO, S.N. et al. **Spermiogenesis in three species of whitefly (Hemiptera, Aleyrodidae).** Acta Zoologica, v. 78, n. 2, p. 163-170, 1997. ISSN 1463-6395. <http://dx.doi.org/10.1111/j.1463-6395.1997.tb01135.x>
- CIOATO, A.; BIANCHI, F.M.; EGER, J.; GRAZIA, J. **New species of *Euschistus* (*Euschistus*) from Jamaica, *Euschistus* (Mitripus) and *Ladeaschistus* from southern South America (Hemiptera: Heteroptera: Pentatomidae: Pentatominae).** Zootaxa. Vol. 3937 No. 3: 26 mar. 2015. <http://dx.doi.org/10.11646/zootaxa.3937.3.4>
- ELIYAHU, D.; CEBALLOS, R.A.; SAEIDI, V.; BECERRA, J.X. **Synergy versus pontency in the defensive secretions from nymphs of two Pentatomomorphan families (Hemiptera: Coreidae and Pentatomidae).** Journal of Chemical Ecology. nov ; 38(11): 1358–1365. 2012. doi:10.1007/s10886-012-0200-0.
- GRAZIA, J.; CAVICHIOLI, R.R.; WOLF, R.R.S.; FERNANDES, J. A. M.; TAKIYA, D. **M. Insetos do Brasil: diversidade e taxonomia. Hemiptera.** Linnaeus, 1758. Ribeirão Preto: Holos Editora. p. 347-405. 2012.
- HENRY, T.J. **Biodiversity of Heteroptera.** Chapter 10. Pp. 223–263 In: Foottit, R.G.; Adler, P.H. (eds). Insect Biodiversity: Science and Society. Wiley-Backwell, Oxford, UK, 656 pp. 2009.
- JAMIESON, B.G.M.; DALLAI, R; AFZELIUS, B.A. **Insects: their spermatozoa and phylogeny.** Science Publishers, Inc, Enfield, NH, USA. 1999.

- MATRANGOLO, W.J.R.; WAQUIL, J.M. **Biologia de *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) alimentado com milho e sorgo**. Anais da Sociedade Entomológica do Brasil 23 (3), 1994.
- OLIVERA, L.; MELO, M.C.; DELLAPÉ, P.M. **First records of leaf-footed bugs (Heteroptera: Coreoidea: Coreidae) from Argentina**. Revista de la Sociedad Entomológica Argentina. 76 (1-2): 68-70. 2017.
<https://doi.org/10.25085/rsea.761212>
- ÖZYURT, N.; CANDAN, S.; SULIDERE, Z. **The Morphology and Histology of The Female Reproductive System of *Graphosoma lineatum* (Heteroptera: Pentatomidae) based on light and scanning electron microscope studies**. International Journal of Scientific Research. 2013.
<http://dx.doi.org/10.15373/22778179/DEC2013/14>
- PACKAUSKAS, R.J. **Key to the subfamilies and tribes of the New World Coreidae (Hemiptera), with a checklist of published keys to genera and species**. Proceedings of the Entomological Society of Washington. 96: 44–53. 1994.
- PACKAUSKAS, R.J. **Catalog of the Coreidae, or Leaf-Footed Bugs, of the New World**. Fort Hays Studies. Fourth Series. Number 5. 2010.
<https://doi.org/10.58809/HCWH1818>
- PANIZZI, A.R. **Desempenho de ninfas e adultos de *Leptoglossus zonatus* Dallas (Hemiptera: Coreidae) em diferentes alimentos**. Anais da Sociedade Entomológica do Brasil 18:375-389. 1989.
- PANIZZI, A.R. **Heteroptera of economic importance**. Edited by Carl W. Schaefer and Antônio Ricardo Panizzi. International Standard Book Number. 2000.
<https://doi.org/10.1201/9781420041859>
- PANIZZI, A.R. **A possible territorial or recognition behavior of *Leptoglossus zonatus* (Dallas) (Heteroptera, Coreidae)**. Revista Brasileira de Entomologia. 48(4): 577-579. 2004.

- PANIZZI, A.R.; BUENO, A.F.; SILVA, F.A.C. **Insetos que Atacam Vagens e Grãos. Soja: manejo integrado de insetos e outros artrópodes-praga.** Brasília: Embrapa. 2012.
- PENDERGRAST, J.G. **The Male Reproductive Organs of *Nezara viridula* (L.) with a Preliminar Account of Their Development. (Heteroptera; Pentatomidae).** Auckland University College. Transactions of the Royal Society of New Zealand. 1956.
- PENDERGRAST, J.G. **Studies on the reproductive organs of the Heteroptera with a consideration of their bearing on classification.** Transactions of the Entomological Society of London. 109. PT. 1. 1957.
- PHILLIPS, D.M. **Insect sperm: their structure and morphogenesis.** Journal Cell Biology. 1970.
- PIRES, E.M.; RUFFATO, S.; MANICA, C.L.M.; SOARES, M.A.; LACERDA, M.C. **Novas plantas hospedeiras para o percevejo fitófago *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae).** EntomoloBrasilis. 5 (3): 249-252. 2012.
- SAWAZAKI, E.; ROSSETTO, C.J.; FANTINI, G.M.; PETINELLI, A.JR. ***Leptoglossus zonatus* (Dallas, 1852) nova praga do milho.** Arquivos do Instituto Biológico. 56 (Sup1.): 22. 1989.
- SCHUH, R.T., STALER, J.A. **The Bugs of the World (Hemiptera: Heteroptera).** Classification and Natural History. Cornell University Press, Ithaca, New York, USA. 1995.
- TAYLOR, V. A.; LUKE, B. M.; LOMAS, M. B. **The giant sperm of a minute beetle.** Tissue and Cell, v. 14, n. 1, p. 113-123, // 1982. ISSN 0040-8166. <http://www.sciencedirect.com/science/article/pii/0040816682900118>
- WEILER, L.; BIANCHI, F.M.; GRAZIA, J. **Percevejos-do-mato (Hemiptera, Heteroptera, Pentatomoidea) da Floresta Nacional de São Francisco de**

Paula, RS, Brasil. Revista Brasileira de Biociências – Brazilian Journal of Biosciences. Porto Alegre. v.10(2). 2012.
<https://www.seer.ufrgs.br/rbrasbioci/article/view/115588>

WEIRAUCH, C.; SCHUH, R.T. **Systematics and evolution of Heteroptera: 25 years of progress.** Annual Review of Entomology, v. 56, p. 487–510. 2011.
<https://doi.org/10.1146/annurev-ento-120709-144833>