

WITHDRAWN: The Male Reproductive Structure and Spermatogenesis of *Trypophloeus Klimeschi* Eggers (Coleoptera: Curculionidae: Scolytinae)

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Research

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EDITORIAL NOTE:

The full text of this preprint has been withdrawn by the authors while they make corrections to the work. Therefore, the authors do not wish this work to be cited as a reference. Questions should be directed to the corresponding author.

Abstract

Background

Trypophloeus Klimeschi Eggers (Coleoptera: Curculionidae: Scolytinae) is one of the most destructive pests of *Populus alba* var. *pyramidalis* (Bunge), resulting in significant losses in economic, ecological and social benefits in China's northwest shelter forest. But research of reproductive system, spermiogenesis and spermatozoon ultrastructure of *T. klimeschi* that is basis of phylogeny, reproductive biology and controlling is still black.

Results

The male reproductive organ of *T. klimeschi* is composed of testis, seminal vesicle, strand shaped accessory gland containing long branch of strand shaped accessory gland and short branch of strand shaped accessory gland, curly accessory gland, vas deferens and a common ejaculatory duct. The number of sperm per cyst is 350~512. Its spermatozoon is slender, measuring about 75 μm in length and 0.5 μm in wide and composed of a 3-layered acrosomal complex, a nucleus with two different states of aggregation, two mitochondrial derivatives with dark crystal, a 9+9+2 axoneme that run more or less parallel to mitochondrial derivatives, two crystalline accessory bodies with a big compact "puff"-like expansion. Especially in the seminal vesicle, its long flagella folded into several turns and the whole sperm is wrapped in a film.

Conclusion

The general morphology of male reproductive tract, the spermatogenesis and the spermatozoa of *T. klimeschi* are, for the most part, similar to the majority of the Curculionidae. However, some distinct differences were found: the low electron-dense band in the cytoplasm of spermatocytes; two different aggregation states of spermatozoon nucleus; especially the stored way of *T. klimeschi* spermatozoa.

Background

Curculionidae includes 60000 species that indicate it is difficult to provide a systematic arrangement based on phylogenetic considerations. Many characteristics of insects are used to study phylogeny, but undoubtedly comparative spermatology can significantly advance this work [1–4]. In addition, the strong reproductive capacity of insects is the key to its widespread transmission. Understanding the reproductive mechanisms of insects is the key to controlling the population of certain pests, and the study of spermatology lays a physiological foundation for understanding its reproductive mechanisms.

Previous research showing that the male reproductive system of Curculionidae composed of a pair of testes, seminal vesicle, vasa efferentia, vas deferens, accessory glands and ejaculatory duct [5]. The spermatozoa of Curculionidae display the classic structure, which is characterized by a head region formed by a 3-layered acrosome and nucleus; two enormous accessory bodies and a large and

crystallized mitochondrial derivatives support the tail region; a 9 + 9 + 2 axoneme pattern is the most common pattern within the Curculionidae [1, 2, 6, 7], only the Rynchiitidae exhibit an axoneme pattern of 9 + 9 + 0 [1].

Trypophloeus Klimeschi Eggers (Coleoptera: Curculionidae: Scolytinae) is one of the most destructive pests of *Populus alba* var. *pyramidalis* (Bunge). *T. klimeschi* was first recorded in the Kyrgyz Republic, which borders Xinjiang Uygur Autonomous Region in China [8]. Following an outbreak in 2003 in Xinjiang Uygur Autonomous Region, *T. klimeschi* spread rapidly to the adjacent areas. The widespread outbreak of this beetle has caused huge economic, ecological, and social losses in China's northwest shelter forest [9–12]. To protect *P. alba* var. *pyramidalis* from succumbing to the insect pest, populations of *T. klimeschi* must be maintained at manageable levels below the threshold. But so far, only a few aspects such as breeding preferences, frequency of oviposition, and the mating cycle of *T. klimeschi* have been studied [13, 14]. So, it is necessary to study the reproductive system, spermiogenesis and spermatozoon ultrastructure of *T. klimeschi* to explore their reproductive potential.

Results

Gross Morphology of the Male Reproductive Tract

The male internal reproductive tract in *T. klimeschi* is composed of two units. Each unit is formed by a bilobed testis comprising about 200 cysts, a seminal vesicle which becomes thicker as the maturation of sperm (Fig. 1B) and inserted in the testis depression, two accessory glands (curled gland and strand shaped gland) and vas deferens (Fig. 1A). The strand shaped accessory gland connected to the seminal vesicle while the curly accessory gland surrounded the vas deferens (Fig. 1A). Two units fuse at their ends flowing into an ejaculatory duct (Fig. 1A).

The cysts are full of testes (Fig. 1C), within which spermatogenesis occurs. The cyst cell with a big nucleus (Fig. 2A) constitutes a group of germ cells surrounded by an epithelium. The number of spermatids per cyst is 350 to 512. Most mature sperms are stored in the seminal vesicle.

Spermatogenesis

During spermatogenesis, spermatogonia undergo mitosis to produce spermatocytes that undergo meiosis to produce spermatids which to be spermatozoa eventually via spermiogenesis.

TEM allowed us to determine that the spermatogonia with a diameter of $5.67 \pm 0.58 \mu\text{m}$ are irregular cells and have an irregular and large nucleus ($3.45 \pm 1.04 \mu\text{m}$), homogeneously distributed granular chromatin and irregularly distributed high electron-dense heterochromatin lump (Figs. 2A, B). The cytoplasm contains abundant glycogen granules and mitochondria, as well as Golgi, a large lysosome and smooth endoplasmic reticulum.

The spermatocytes (Fig. 2C), originate from mitosis of spermatogonia, have a diameter of $4.69 \pm 0.43 \mu\text{m}$. The cells are characterized by their round shaped nucleus of $2.40 \pm 0.71 \mu\text{m}$ in diameter and a low electron-dense band comprised by smooth endoplasmic reticulum around the nucleus. Large lysosome, large mitochondria with well-defined crests, Golgi and abundant glycogen granules can be observed in their cytoplasm.

The spermatids (Fig. 2D) produced by meiotic division of the spermatocytes are $4.88 \pm 0.28 \mu\text{m}$ in diameter with a round nucleus of $3.31 \pm 0.22 \mu\text{m}$ in diameter. Nuclear chromatin exhibits different degrees of compactness and electron density and it is possible to observe the presence of synaptonemal complexes in some nuclei. The cytoplasm contains electron-lucent Golgi and many mitochondria.

Spermiogenesis

There are many changes in spermiogenesis. Nuclear chromatin begins to unhomogeneously concentrate and electron density is enhanced (Figs. 3B, C, 4G, H). At a more mature stage, nuclear chromatin will present two distinct regions (Figs. 4E, F), one is homogeneously compact and another is fibrillar. The posterior end of the nucleus forms a concavity where the axoneme occurring and elongate (Figs. 4A, G). Nearly round preacrosomal vesicle with $452.70 \pm 22.82 \text{ nm}$ in diameter is visible and close to the nucleus (Fig. 4B) and Golgi apparatus (Fig. 4C) and then became flat (Fig. 4D) as the elongation of nucleus.

At the same period, the spherical-shaped mitochondria that are found dispersed in the cytoplasm begin to migrate toward the basal pole of the nucleus (Fig. 3B). Then the globular mitochondria aggregate (Fig. 3C) and fuse into thin lines (Fig. 3D), which in turns become a coarse state, and then become thicker irregular but chimeric two mitochondrial derivatives (Figs. 3E, F). These two mitochondrial derivatives become slender as the cell's elongating (Fig. 4G, H). A pair of vertically arranged centrioles are separated and located at the ends of the mitochondrial derivatives (Figs. 4H, I). Shortly after, the centriolar adjunct appeared on both side of the origin of axoneme (Fig. 4L). During spermatogenesis, the sperm components are surrounded by microtubules (Figs. 4E, F) which are visible in spermatozoa (Fig. 6F).

Spermatozoa

The spermatozoa of *T. klimeschi* are slender (Fig. 5A), measuring about $75 \mu\text{m}$ in length and $0.5 \mu\text{m}$ in wide and composed of an acrosomal complex, a nucleus (Figs. 6A, D), an axoneme, two mitochondrial derivatives and two accessory bodies (Fig. 6F).

The 3-layered acrosome complex (Figs. 6A, B) made up of a dense extra acrosomal granular layer with low electron density material, a cup-like acrosomal vesicle and a rod-like conical perforatorium, measuring about $1.2 \mu\text{m}$ in length. The acrosome complex lies on a slightly concave nuclear face and is separated from nucleus by a thick basal lamina (Fig. 6A).

Close to the acrosome complex is the nucleus (Figs. 6A, B) with circular section, about 6 μm in length and 0.4 μm in diameter indicated by longitudinal section. The nucleus is not homogeneous and has two different states of aggregation (Fig. 6C), there are obvious boundary between thick filamentous nuclear condensation and filamentous nuclear condensation.

Longitudinal section showing (Fig. 6D) that the first to insert into the nucleus is the large mitochondrial derivative, followed by the small mitochondrial derivative, axoneme and two accessory bodies. The cross section indicates that the axoneme (Figs. 6F, G) has a diameter of 277.89 ± 10.58 nm with $9 + 9 + 2$ microtubules, the large mitochondrial derivatives with dark crystals accounted for about 60% of its circular cross section is always thinner than the axoneme and will become thinner along the axoneme until disappearing completely (Figs. 6F, H). The dark crystals accounted for about 40% of the drop shape cross section of the small mitochondrial derivative (Fig. 6F). The longitudinal section indicates that mitochondrial derivatives are comb-like (Figs. 5C, 6E). The axoneme and mitochondrial derivatives of *T. klimeschi* are not embracing each other, but rather run more or less parallel to each other (Fig. 5B).

Two accessory bodies are different (Fig. 6F). The completely crystalline crescent accessory body is smaller than a quarter of axoneme and close to the small mitochondrial derivative. The other consists of a small crystal that was mostly triangular and sometimes crescent and a big compact “puff”-like expansion.

At the end of the sperm, the first to disappear is the two accessory bodies, followed by small mitochondrial derivatives, large mitochondrial derivatives and finally is the disintegration and disappearance of axoneme (Fig. 6H).

In this study, bi- and multi-flagellate spermatids and spermatozoa were observed at a low rate (Figs. 6I, J).

The spermatozoa of *T. klimeschi* individually stored in the seminal vesicle, its long flagella folded into several turns and the whole sperm is wrapped in a film (Figs. 5D, E). Compare to slender spermatozoon, this state spermatozoon is more like a tadpole. The head of the “tadpole” is a circle surrounded by the middle of spermatozoon, in some circle form an “S” shape, and the tail is juxtaposed by the two ends of the spermatozoon.

Discussion

The general morphology of the male reproductive tract of *T. klimeschi* is similar to that reported for other Curculionidae [5, 15–18]. *T. klimeschi* have two testes like others; each testis is bilobed like *Dendroctonus armandi* [18], some are single lobed like *Sibinia arenariae* [19], or multilobed like one of Nemonychidae [5]; each lobe consists of a single large spherical follicle like *Nanophyes nigrovarius* [5], while *Allaeometrus breviceps* has no visible follicular structure and most have 3–50 follicles [5]. Each follicle of *T. klimeschi* has about 100 cysts that at various stages of development. Spermatozoa of a cyst are synchronous development like *Rhynchophorus ferrugineus* [3]. The number of sperm per cyst is varies

among different species, while that in *T. klimeschi* is between 350–512 that means this species is a primitive Coleopteran species [20].

The spermatogenesis of *T. klimeschi* occurs in cyst as in most insects [6, 7, 21–23]. Before spermiogenesis, the characters of *T. klimeschi* is the low electron-dense band comprised by smooth endoplasmic reticulum in the cytoplasm of spermatocytes, compared to *Euptoieta hegesia* [24], *Agraulis vanillae* [25], *Tribolium castaneum* [23] and *Timema poppensis* [26]. In addition, its spermatogonia has a large and irregular nucleus, that is similar to *T. poppensis* [26] but different from *A. vanillae* [25]. In the process of spermiogenesis, the nuclear chromatin undergoes different course of condensation in all species; sometimes the central zone, and other times the periphery, is the first to condense [2], both of these situations exist in this study. With the condensation of nuclear chromatin, two regions could be distinguished in this study that also be mentioned in other research [3], one showing the chromatin more homogeneously condensed and another showing the chromatin with a fibrillar aspect. In addition, homogeneous [27] and honeycombed [2, 17] nuclear chromatin were discovered. The centriolar adjunct of *T. klimeschi* appear in the spermatid stage but disappear in the mature sperm like *Rhynchophorus ferrugineus* [3, 17], *D. armandi* [18], *Sitophilus zeamais* and *Sitophilus oryzae* [2]. These researchers believe that the reason is that the centriolar adjuncts stabilize axoneme during spermiogenesis. Our research also shows a more detailed process of mitochondrial derivatives formation that is similar to previous research [28].

The spermatozoa of *T. klimeschi* are linear and slender and are similar to the general description for other Curculionidae sperm [2, 3, 17, 18, 29, 30]. Its length is about 75 μm and is very short compared to other Curculionidae that spermatozoa are 110 to 300 μm in length [1, 17]. But its structure is similar to most Curculionidae [1, 2, 4, 17, 18, 29–31]: 3-layered acrosome with a cup-like acrosome vesicle, but its roundly hooked extra acrosomal layer is similar to few of Curculionidae in which most is conical; a 9 + 9 + 2 axoneme; two comb-like mitochondrial derivatives of different sizes and crystallization; two accessory bodies and one big compact “puff”-like expansion. Of course, these differences, which will be mentioned below, deserve our attention.

At present, there are two storage method of spermatozoon in seminal vesicle: in bundles or individually [32–34]. The bundled spermatozoa are in cyst and their heads are inserted into the extracellular matrix. But there seems no description of how individually spermatozoon to store. In this study, we find that in seminal vesicle *T. klimeschi* spermatozoa folded their long flagella into several turns and wrapped in a film. By this way spermatozoa may make it not easy to knot and increase its survival rate.

Nuclear chromatin of most insect spermatozoa is dense and uniform, only few are unhomogeneous. The cross section of *Dryomyia lichtensteini* (Cecidomid: Lasiopteridi) spermatozoa showing that elliptical high electron density chromatin envelops round circular low electron density chromatin [35]. The cross section of *Blaptostethus pallescens* (Heteroptera: Anthocoridae) spermatozoa [36] showing irregular lower density chromatin. There are similar phenomena in *Hypanthidium foveolatum* (Hymenoptera: Apidae: Megachilinae) [21]. In this study, the nucleus of *T. klimeschi* spermatozoa is characterized by the

two different states. Obviously, this feature of insect spermatozoa nucleus is not extensive, and its formation should be related to different living habits of different species, but the specific reasons need to be explored in subsequent experiments.

Conclusions

The general morphology of male reproductive tract, the spermatogenesis and the spermatozoa of *T. klimeschi* are, for the most part, similar to the majority of the Curculionidae. However, some distinct differences were found: the low electron-dense band in the cytoplasm of spermatocytes; two different aggregation states of spermatozoon nucleus. Especially the spermatozoa of *T. klimeschi* individually stored in the seminal vesicle, its long flagella folded into several turns and the whole sperm is wrapped in a film. This study provides important theoretical basis for the phylogeny of Curculionidae and the reproductive biology and controlling of *T. klimeschi*.

Methods

Insects

The *T. klimeschi* (larvae and pupae) collected from the bark of infested *P. alba* var. *pyramidalis* in Dunhuang City (40°06'50.61" N, 94°36'10.24" E), Gansu Province, China, were reared on 24-hole plates with feed containing *P. alba* var. *pyramidalis* bark powder in an artificial climate incubator (14L: 10D, 25 ± 1 °C, 65 ± 5% relative humidity) [10]. On the first day, the eighth and the sixteenth day of eclosion, 30 males were taken for later use. For the anatomical analyses, 10 males' reproductive systems were observed with an OLYMPUS SZ2-ILST stereomicroscope and photographed with an OLYMPUS DP25.

Scanning electron microscopy

The reproductive systems of 10 males *T. klimeschi* were dissected and fixed in 2.5% glutaraldehyde and buffered to pH 7.2 with 0.1M phosphate solutions for 12 h at 4 °C. After washing the testes in phosphate buffer saline (PBS), pH 7.2, dehydrated through a graded series of alcohol and isoamyl acetate, finally critical point dried with liquid CO₂ and sputtered coated with gold. Samples were then examined using the HITACHI S-4800.

Transmission electron microscopy

The 10 fixed reproductive systems were rinsed with PBS, and post-fixation was performed in 1% osmium tetroxide for 1 h at 4 °C. After other four times 15 min rinse in the same buffer, the samples were dehydrated through a graded ethanol series, and embedded in 14381-UC LR WHITE. Semithin sections were obtained with a glass knife on a LEICA RM2265 Semi-thin slicer, stained with toluidine blue, and observed with an OLYMPUS BX43F microscope. Ultrathin sections were obtained with a diamond knife on

an ultramicrotome (LEICA ULTRACUT UCT), routinely stained with uranyl acetate and lead citrate, and observed with a HITACHI HT7700 transmission electron microscope.

Declarations

Ethics approval

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JG: Writing - original draft, Methodology, Investigation; LD: Writing - review & editing; GG and JW: Investigation; HC: Supervision, Funding acquisition, Project administration, Resources. All authors read and approved the final manuscript.

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References

1. Burrini AG, Magnano L, Magnano AR, Scala C, Baccetti B. Spermatozoa and phylogeny of Curculionoidea (Coleoptera). *Int J Insect Morphol*. 1988; 17:1-50.
2. Name KP, dos Reis GP, Báo SN. An ultrastructural study of spermiogenesis in two species of *Sitophilus* (Coleoptera: Curculionidae). *Biocell*. 2007; 31:229-236.

3. Alzahrani AM, Abdelsalam SA, Elmenshawy OM, Abdel-Moneim AM. Ultrastructural characteristics of spermiogenesis in *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae). Fla Entomol. 2013; 96:1463-1469.
4. Dallai R, Gottardo M, Beutel RG. Structure and evolution of insect sperm: new interpretations in the age of phylogenomics. Annu Rev Entomol. 2016; 61:1-23.
5. Calder AA; Gross morphology of the soft parts of the male and female reproductive systems of Curculionoidea (Coleoptera). J Nat Hist. 1990; 24:453-505.
6. Gassner III G, Childress D, Klemetson DJ. Spermiogenesis in boll weevil, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae). Int J Insect Morphol. 1975; 4:115-125.
7. Werner M, Simmons LW. Ultrastructure of spermatozoa of *Onthophagus taurus* (Coleoptera, Scarabaeidae) exhibits heritable variation. Sci Nat-Heidelberg. 2011; 98:213-223.
8. Eggers VOH. *Trypophloeus klimeschi* nov. spec. Entomol. Blatter. 1915; 25:7-9.
9. Gao G, Gao J, Hao C, Dai L, Chen H. Biodiversity and Activity of Gut Fungal Communities across the Life History of *Trypophloeus klimeschi* (Coleoptera: Curculionidae: Scolytinae). Int J Mol Sci. 2018; 19:2010.
10. Gao G. Occurrence and Host Selection Mechanism of *Trypophloeus Klimeschi* Eggers. Northwest A&F University, Forestry Entomology; 2018.
11. Gao G, Dai L, Gao J, Wang J, Chen H. Volatile organic compound analysis of host and non-host poplars for *Trypophloeus klimeschi* (Coleoptera: Curculionidae: Ipinae). Russ J Plant Physiol+. 2018; 65:916-925.
12. Gao G, Dai L, Gao J, Wang J, Chen H. Electroantennogram, behavioural responses, and field trapping of *Trypophloeus klimeschi* (Coleoptera: Curculionidae: Scolytinae) to eight host volatiles. Can Entomol. 2019; 151:236-250.
13. Cao Y, Luo Z, Wang S, Zhang P. *Trypophloeus Klimeschi* Eggers - a New Insect Pest to Xinjiang Poplar. Journal of Tarim University. 2003; 15:9-11.
14. Cao Y, Luo Z, Wang S, Zhang P. Bionomics and control of *Trypophloeus klimeschi*. Entomol Knowl. 2004; 41:36-38.
15. Cerezke HF. The Morphology and Functions of the Reproductive Systems of *Dendroctonus monticolae* Hopk.(Coleoptera: Scolytidae). Can Entomol. 1964; 96:477-500.
16. José DRG, Alex EBP, Luis FVE, José RAZ, Pablo BM. Alimentary canal and reproductive tract of *Hypothenemus hampei* (Ferrari)(Coleoptera: Curculionidae, Scolytinae). Neotrop Entomol. 2008; 37:143-151.
17. Paoli F, Dallai R, Cristofaro M, Arnone S, Francardi V, Roversi PF. Morphology of the male reproductive system, sperm ultrastructure and γ -irradiation of the red palm weevil *Rhynchophorus ferrugineus* Oliv. (Coleoptera: Dryophthoridae). Tissue Cell. 2014; 46:274-285.
18. Wu Y, Wei L, Torres AM, Zhang X, Wu S, Chen H. Morphology of the male reproductive system and spermiogenesis of *Dendroctonus armandi* Tsai and Li (Coleoptera: Curculionidae: Scolytinae). J

Insect Sci. 2017; 17:1-9.

19. Aslam NA. An assessment of some internal characters in the higher classification of the Curculionidae *S.L.* (Coleoptera). T R Entomol Sci Lond. 1961; 113:417-480.
20. Virkki N. Sperm bundles and phylogenesis. Cell Tissue Res.1969; 101:13-27.
21. Gracielle IMS, Fiorillo BS, Lino-Neto J, Bao SN. Morphology of the male reproductive system and spermiogenesis in *Hypanthidium foveolatum* (Alfken, 1930) (Hymenoptera: Apidae: Megachilinae). Micron. 2009; 40:419-425.
22. Xie S, Hua B. Sperm ultrastructure in two species of *Panorpa* and one *Bittacus* (Mecoptera). Micron. 2010; 41:622-632.
23. Dias G, Lino-Neto J, Mercati D, Dallai R. The sperm ultrastructure and spermiogenesis of *Tribolium castaneum* (Coleoptera: Tenebrionidae) with evidence of cyst degeneration. Micron. 2015; 73:21-27.
24. Mancini K, Dolder H. Dichotomic spermiogenesis in *Euptoieta hegesia* (Lepidoptera: Nymphalidae). Braz J Morphol Sci. 2004; 21:13-23.
25. Mari IP, Gigliolli AA, Sinopolis, Nanya S, de Brito Portela-Castro AL. Histological and electron microscopy observations on the testis and spermatogenesis of the butterfly *Dione juno* (Cramer, 1779) and *Agraulis vanillae* (Linnaeus, 1758) (Lepidoptera: Nymphalidae). Micron. 2018; 109:11-21.
26. Gottardo M, Mercati D, Dallai R. The spermatogenesis and sperm structure of *Timema poppensis* (Insecta: Phasmatodea). Zoomorphology. 2012; 131:209-223.
27. Yaszumi G, Ishida H: Spermatogenesis in Animals as Revealed by Electron Microscopy. IV. Fine Structure of Spermatid Nuclei of Grasshopper in Early Stage of Maturation. J Electron Microsc. 1957; 5:38-42.
28. Baccetti B: Insect Sperm Cells. Advances in Insect Physiology. Elsevier; 1972. P. 315-397.
29. Grodner ML. Aberrant spermatogenesis in hybrid progeny of sub-species of the boll weevil *Anthonomus grandis* Boheman (Coleoptera: Curculionidae). Int J Insect Morphol. 1975; 4:107-114.
30. Dallai R, Lino-Neto J, Dias G, Nere PHA, Mercati D, Lupetti P. Fine structure of the ladybird spermatozoa (Insecta, Coleoptera, Coccinellidae). Arthropod Struct Dev. 2018; 47:286-298.
31. Jamieson BGM, Dallai R, Afzelius BA. Insects: their spermatozoa and phylogeny. Science Publishers, Inc; 1999.
32. Mojica JM, Bruck DL. Sperm bundle coiling: Transporting long sperm bundles in *Drosophila dunnii dunnii*. J Insect Physiol. 1995; 42:303-307.
33. Moreira J, Brito P, Mancini K, Dolder H, Linoneto J. The descriptions of new microanatomical structures of the male reproductive system and sperm of *Myschocyttarus cassununga* (Hymenoptera: Vespidae). Micron. 2012; 43:292-297.
34. Oliveira CM, Moreira J, Gomes LF, Camargo-Mathias MI, Lino-Neto J. Sperm Bundles in the Seminal Vesicle of the *Crematogaster victima* (Smith) Adult Males (Hymenoptera: Formicidae). Neotrop Entomol. 2014; 43:201-208.

35. Ciolfi S, Mencarelli C, Dallai R. The evolution of sperm axoneme structure and the dynein heavy chain complement in cecidomid insects. *Cytoskeleton*. 2016; 73:209-218.
36. Santos ABR, Lino-Neto J. Sperm morphology of predatory pirate bugs *Amphiareus constrictus* and *Blaptostethus pallescens* (Heteroptera: Anthocoridae) with phylogenetic inferences. *Micron*. 2018; 105:18-23.

Figures



Figure 2

The male reproductive organ of *T. klimeschi*. (A) The male reproductive organ is composed of testis (T), seminal vesicle (SV), strand shaped accessory gland containing long branch of strand shaped accessory glands (LG) and short branch of strand shaped accessory glands (SG), curly accessory gland (CG), vas deferens (VD) and a common ejaculatory duct (EJ); (B) On the first day of feathering, the seminal vesicle is a thin tube (white arrow); (C) The cross section of testis showing many cysts (CY).



Figure 4

The spermatogenesis of *T. klimeschi*. (A) A cyst with spermatogonia showing the cyst cell nucleus (black arrow); (B) The longitudinal section of spermatogonia containing many mitochondria (M), Golgi (G), abundant glycogen granules (black arrow), smooth endoplasmic reticulum (SER), lysosome (L) and a big nucleus (N); (C) The spermatocytes of *T. klimeschi* showing many mitochondria (M), Golgi (G), abundant glycogen granules (black arrow), lysosome (L) and smooth endoplasmic reticulum (SER) forming a low electron density band; (D) The spermatids of *T. klimeschi* showing synaptonemal complexes (SC).



Figure 6

The spermiogenesis of *T. klimeschi*. (A) The Golgi (G), different from that of spermatogenesis period, next to the layers of high electron density substances (black arrow); (B) Mitochondria (M) gather together; (C) Mitochondria begin to fuse (black arrow); (D) Mitochondria fuse into thin lines (black arrow); (E) Mitochondria fuse into two mitochondrial derivatives (black arrow) that are thicker, irregular and chimeric; (F) Two mitochondrial derivatives begin to elongate (black arrow).



Figure 8

The spermiogenesis of *T. klimeschi*. (A) Stretching axoneme (AX) from centriole (C); (B) Preacrosomal vesicle (PAV) next to the nucleus (N) and axoneme (AX); (C) Golgi (G) next to the preacrosomal vesicle

(PAV) and axoneme (AX); (D) As the nucleus elongates, the preacrosomal vesicle (PAV) becomes flat; (E-F) The sperm components are surrounded by microtubules (black arrow) and the nucleus chromatin has different states of aggregation: homogeneously compact chromatin (HCC) and fibrillar chromatin (FC); (G) The axoneme (AX) start elongating from the concavity of nucleus. Mitochondrial derivatives (MD) are located on both sides of the axoneme (AX) and parallel to the axoneme (AX); (H) There is a centriole (C) at each end the mitochondrial derivatives (MD); (I) A pair of vertically arranged centrioles; (L) The centriolar adjunct (CA) appear in the spermatid stage. Nucleus (N), Axoneme (AX).



Figure 10

SEM micrograph of *T. klimeschi* spermatozoa. (A) The appearance of spermatozoa; (B) Detail of spermatozoa showing the arrangement of axoneme (AX), large mitochondrial derivative (LMD) and small mitochondrial derivative (SMD); (C) Detail of spermatozoa showing comb-like mitochondrial derivative (LMD and SMD) of *T. klimeschi* spermatozoa. Axoneme (AX); (D-E) SEM micrograph showing the long flagella folded into several turns and the whole sperm is wrapped in a film. The ruptured film exposes the ringed spermatozoa (black arrow).



Figure 12

The spermatozoa structure of *T. klimeschi*. (A-B) The 3-layered acrosome complex, composed of extra acrosomal layer (E) with low electron density material (black arrow), acrosomal vesicle (A) and perforatorium (P), lies on nuclear (N) face and is separated from nucleus by a thick basal lamina (BL); (C) The nucleus has different states of aggregation: thick-filamentous nuclear condensation (TNC) and filamentous nuclear condensation (FNC); (D) The longitudinal section showing the junction of nucleus (N), axoneme (AX) and large mitochondrial derivative (LMD). Centriole (C); (E) The comb-like mitochondrial derivatives (black arrow); (F) The cross section of the middle of the spermatozoa of *T. klimeschi* showing axoneme (AX), the crescent accessory body (AB), the other accessory body composed of triangular crystal (white arrow) and "puff"-like expansion (PF), large mitochondrial derivative (LMD), small mitochondrial derivative (SMD) and microtubules (black arrow); (G) The axoneme of *T. klimeschi* with 9+9+2 microtubules; (H) The cross section showing that the axoneme disintegrate (black arrow) at the end of the spermatozoa; (I-J) The bi- and multi-flagellate spermatids (black arrow) and spermatozoa (white arrow) of *T. klimeschi*.