

# Morphology of Male Reproductive Systems in Ephemeroptera: Intrinsic Musculature

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## Keywords

Mating strategies, reproductive system, SEM, spermatozoa, TEM

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Edited by Roberto A Zucchi – ESALQ/USP

Received 6 November 2011 and accepted 15 March 2012

Published online 31 May 2012

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## Abstract

Although the Ephemeroptera have been studied over a long period of time, there are still few studies on the morphology of male reproductive system. The spermatic ducts are considered conserved among different Ephemeroptera groups. However, previous studies distinguished different organizational patterns of the spermatic duct intrinsic musculature. This study describes the morphology of the spermatic ducts, especially their musculature, in six species of Ephemeroptera, representing five families. We have observed variations in the organizational pattern of the spermatic ducts, even between species from the same family. Moreover, all species studied had intrinsic musculature in the spermatic ducts although with different organizational patterns. Thus, we believe that this musculature is important to move the spermatozoa along the ducts of all Ephemeroptera and not only of those with aflagellated spermatozoa (Leptophlebiidae). The variations in musculature organization must be related to differences in reproductive physiology (i.e., copula duration) and not only with spermatozoa characteristics.

## Introduction

Although Ephemeroptera internal morphology has been studied over a long period of time (Swammerdam 1681), studies on the morphology of the male reproductive system morphology of this group are still scarce. While taxonomists extensively studied the external genitalia, the internal seminal ducts are almost unknown. Moreover, these ducts are considered conserved among different Ephemeroptera groups (Brinck 1957).

The ground plan of the male reproductive system of Ephemeroptera is a pair of testis that elongate from the metathorax to the VI abdominal segment. Depending on the species, each testicle may be made up of a variable number of follicles, from 140 to 290. Each testicle empties its content into a spermatic duct, which acts also as a storage organ for sperm, after they leave the testes. Last instars have dilated spermatic ducts that empty into the gonopores between segments IX and X (Brinck 1957, Soldán 1979a).

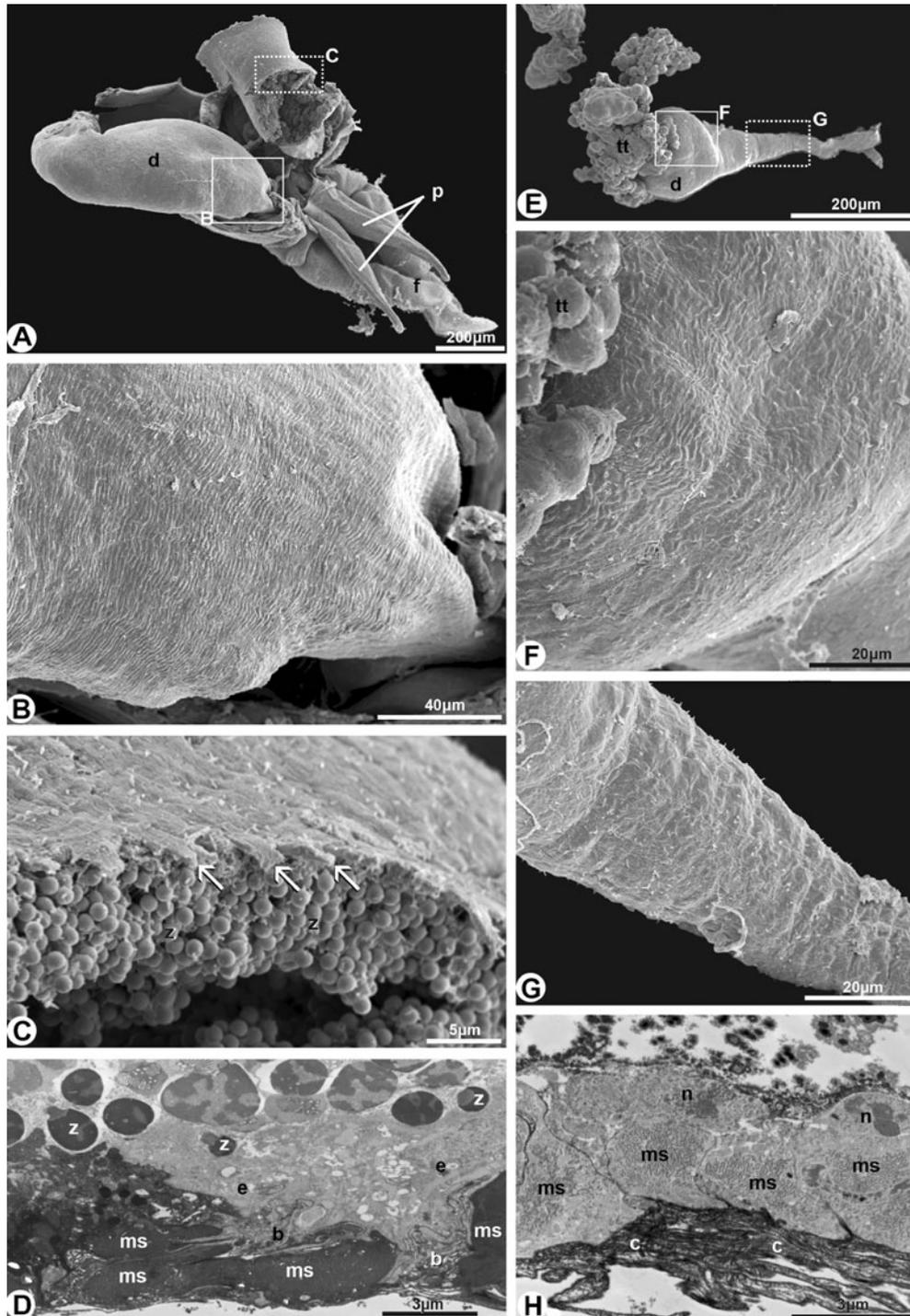
Although the male reproductive system of Ephemeroptera follows the basic plan described above, extensive observations made by Grimm (1985) allowed the identification of different patterns of the organization of the spermatic duct intrinsic musculature, concluding that (1) species from Leptophlebiidae have a strong ring musculature surrounding the ducts in order to pump their aflagellate and immotile spermatozoa, (2) some species from Baetidae do not present enough intrinsic duct musculature to pump the spermatozoa, and (3) the intrinsic musculature organization pattern is variable even between species from the same family.

The present study provides a collection of recent observations on the morphology of the male reproductive system of some Ephemeroptera. Special attention was directed to the musculature associated to the duct epithelium. Thus, we intend to complement the data available in the literature, providing new information about the subject.

## Material and Methods

Males from six species representing five families, at the imago, subimago or last instar stages were collected and processed for morphological analysis.

Last instars of *Miroculis (Atroari) amazonicus* Savage & Peters (Leptophlebiidae) were collected in Presidente Figueiredo, state of Amazonas, Brazil (2°1'5"S; 59°49'25.70"W), and maintained in river water up to subimago stage. Subimagoes of *Farrodes carioca* Dominguez,

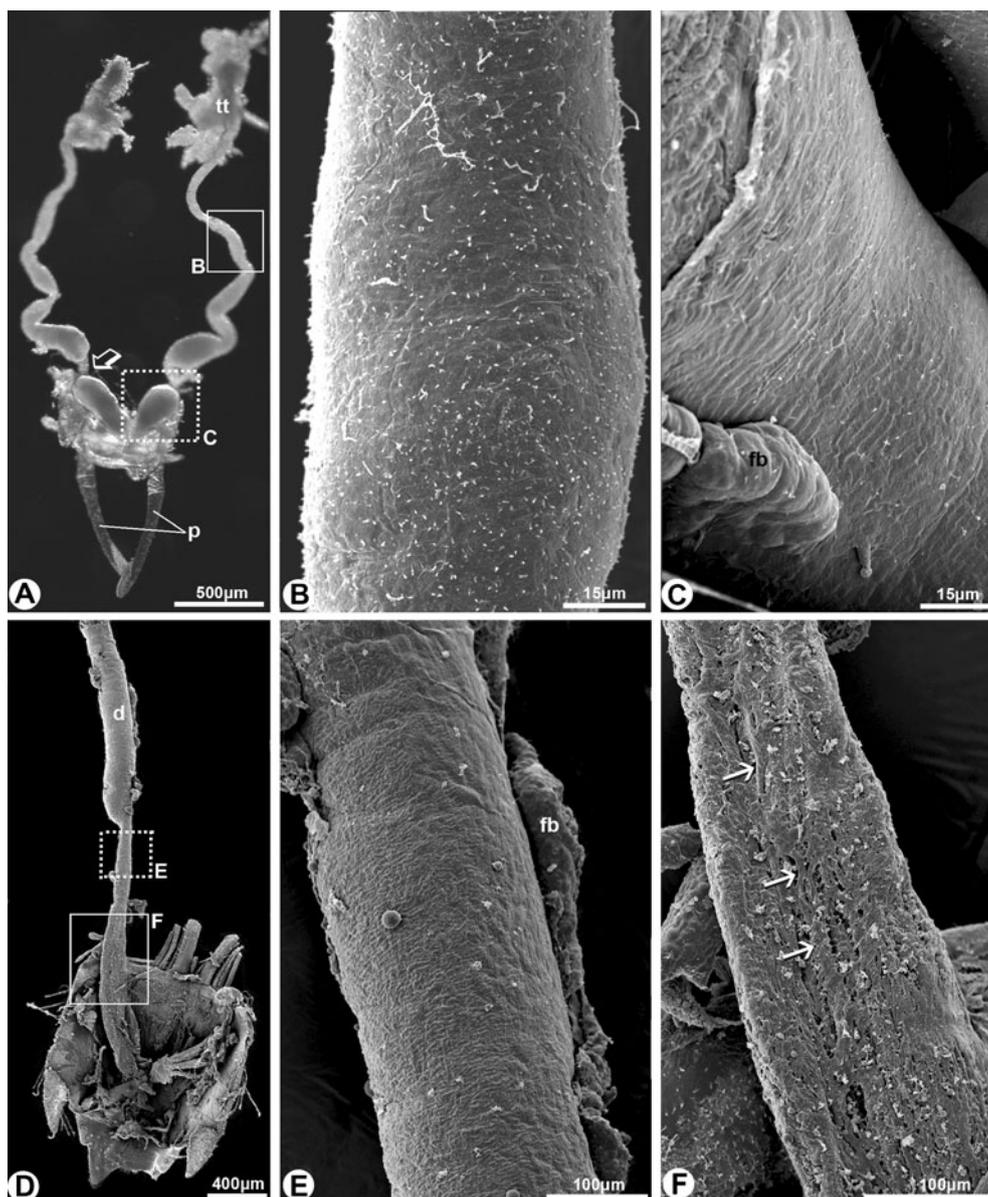


**Fig 1** **A–D** *Miroculis amazonicus*; **E–H** *Farrodes carioca*. **A, E** Reproductive systems visualized with SEM—(*d*) spermatic duct, (*tt*) reduced testes, (*p*) aedeagus, (*f*) forceps. **B, C, F, G** Higher magnification of boxed regions, (*arrows*) broken muscle fibers, (*z*) spermatozoa. **D, H** Transmission electron microscopy of the spermatic duct wall, (*ms*) muscles, (*e*) epithelium, (*b*) basal lamina, (*n*) epithelial cell nuclei, (*c*) conjunctive capsule.

Molineri & Peters (Leptophlebiidae) were attracted by a light trap near a river in Santa Teresa, state of Espírito Santo, Brazil (19°52'31.60"S; 40°31'49.10"W). Imagoes of *Asthenopus curtus* (Hagen) (Polymitarcyidae) were collected by a light trap at Catalão lake, Manaus, state of Amazonas (3°9'13.16"S; 59°54'56.40"W). Last instars of *Lachlania* sp. (Oligoneuriidae) were collected at Dores do Rio Preto, state of Espírito Santo (20°37'30.37" S; 41°49'26.46" W), and last instars of *Callibaetis jocosus* (Navás) (Baetidae) were collected in a lake at "Serra do Japi", Jundiá, state of São Paulo, Brazil (23°14'18.30"S; 46°56'27.25"W), and maintained in the laboratory up to the imago stage.

The reproductive systems of the collected or reared specimens were dissected in 0.1 M phosphate buffer, pH 7.2, and processed with routine techniques for scanning electron microscopy (SEM) techniques (fixed in 2.5% glutaraldehyde, dehydrated in alcohol, critical point dried and sputter-coated with gold). For transmission electron microscopy techniques, the material was fixed in 1% tannic acid in 2.5% glutaraldehyde solution in the same buffer (Dallai & Afzelius 1990).

Specimens were observed in a ZEISS LEO 906 transmission electron microscope and in a JEOL JSM5800LV scanning electron microscope. The spermatic ducts of *A.*



**Fig 2** A–C *Asthenopus curtus*, D–F *Lachlania* sp. **A** Male reproductive system, with reduced testes (*tt*) and a pair of long spermatic ducts with constrictions (*open arrow*), (*p*) aedeagus. **B,C** SEM of indicated regions, (*fb*) fat body. **D** SEM of a spermatic duct (*d*) associated to the subgenital plate. **E, F** Higher magnifications of boxed regions, (*arrows*) longitudinal muscular fibers.

*curtus* were also photographed with an Olympus BX41 photomicroscope.

## Results

The male reproductive system of *M. amazonicus* features an approximately cylindrical, dilated spermatic duct (Fig 1A), with reduced testicle vestiges (not shown). At the scanning electron microscope, parallel grooves forming rings are observed along the entire spermatic ducts (Fig 1B). A duct was broken showing that the grooves represent muscle fibers of the spermatic duct intrinsic musculature (Fig 1C). The muscle fibers are strongly stained by tannic acid and are not uniformly distributed, externally to the epithelium, as seen in ultrathin sections (Fig 1D).

The spermatic duct of *F. carioca* is pear shaped, dilated at the proximal portion, juxtaposed to the testes (Fig 1E). The testes are reduced and globular (Fig 1E, F), coated by a thin conjunctive layer. Some grooves are observed in both the dilated and the tapered portion, near the aedeagus (Fig 1F, G). However, these grooves do not seem to follow an organized pattern. Observed in ultrathin sections, the muscle fibers make up a thicker layer, weakly stained by the tannic acid, covered by a thin epithelium that lines the lumen and a complex external conjunctive coat (Fig 1H).

The male reproductive system of *A. curtus* is long (Fig 2A), extending from thorax, where testes vestiges are observed, leading to the spermatic ducts that run along the abdomen. The spermatic ducts are thinner near the testes, increasing their volume near the aedeagus, where a constriction is observed (Fig 2A). At the scanning electron microscope, few grooves are observed (Fig 2B); while near to the aedeagus, many grooves are observed ringing the duct (Fig 2C).

Spermatic ducts of *Lachlania* sp. can be divided into three distinct regions (Fig 2D). The proximal region is thicker and has several grooves forming rings around the duct. The thinner region of the duct is divided into two portions. The first portion has the same groove pattern (Fig 2E), while the portion near the gonopore is characterized by strong muscle bundles running parallel to the duct (Fig 2F). This region is also identified by the brownish color in fresh specimens, due to a chitin cuticle at the epithelial surface (not shown).

The spermatic ducts of *C. jocosus* have a 4.6- $\mu\text{m}$ -thick epithelial layer with many degenerative vesicles in the cytoplasm. This pseudostratified epithelium has nuclei in different positions, varying from basal to apical. A thin basal lamina separates a uniform muscle layer, which is approximately 1.9  $\mu\text{m}$  thick (Fig 3A).

The spermatic ducts of *Traverhyphes yuati* have an epithelial layer, approximately 2  $\mu\text{m}$  thick, with basal

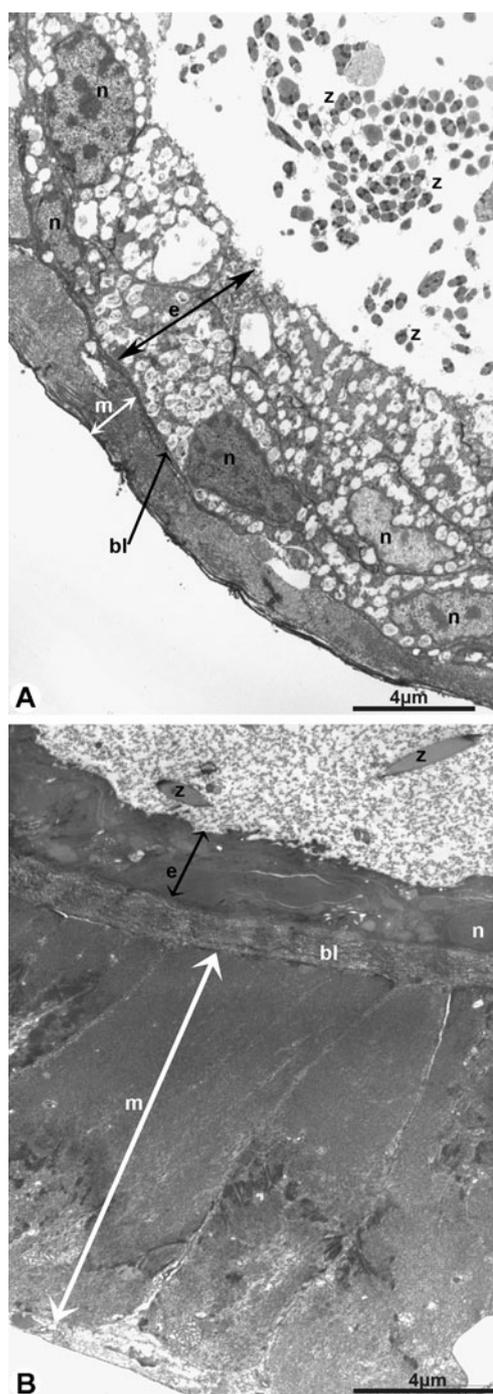


Fig 3 Transmission electron microscopy. **A** Spermatic duct wall of *Callibaetis jocosus*; **B** Spermatic duct wall of *Traverhyphes yuati*—(e) epithelium, (m) muscle layer, (bl) basal lamina, (z) spermatozoa, (n) epithelial cell nuclei.

nuclei. Externally, a fibrous basal lamina separates the well-developed muscle layer, which is approximately 11  $\mu\text{m}$  thick (Fig 3B). However, this muscle layer surrounds only half of the duct's perimeter, while the other half has no musculature (not shown).

## Discussion

Previous studies describe the spermatozoa of Ephemeroptera species as flagellated and motile (Baccetti *et al* 1969, Jamieson *et al* 1999), with exception of the species from Leptophlebiidae, which has aflagellate and immotile spermatozoa (Soldán 1979b, Gaino & Mazzini 1991, Brito *et al* 2011).

According to Grimm (1985), Leptophlebiidae species should have ringed musculature surrounding the spermatid ducts. This characteristic would be important to move the sperm, since their spermatozoa are aflagellate (Soldán 1979b, Grimm 1985, Gaino & Mazzini 1991, Brito *et al* 2011). The morphology observed in the present study for *M. amazonicus* confirms Grimm's (1985) conclusions. On the other hand, although *F. carioca* spermatid ducts have a well-developed musculature, it is not organized in rings. Thus, this is not a characteristic common to all Leptophlebiidae.

The intrinsic musculature of the *A. curtus* spermatid ducts is well developed and organized, especially near to the aedeagus, which should be related to sperm ejaculation. Muscular sphincters were described along the spermatid ducts of different species of Ephemeroptera (Grimm 1985); therefore, the constrictions observed near to the aedeagus of *A. curtus* are probably sphincters.

Ephemeroptera ejaculatory ducts are ectodermic invaginations with a chitin cuticle covering the epithelium (Quadri 1940). Thus, the caudal portion of the spermatid ducts of *Lachlania* sp. with longitudinal muscle bundles is the ejaculatory duct. These muscles are responsible for the duct eversion described during copulation of Oligoneuriidae species (Pescador & Peters 1980).

The well-organized and evenly distributed musculature along the spermatid ducts of *C. jocosus* does not support the conclusions of Grimm (1985), since this species is flagellated and should not need a strongly muscular duct. Another unusual occurrence is the thick muscle layer surrounding only half of the spermatid duct in *T. yuati*, which has not been previously observed in Ephemeroptera.

We have observed variations in the organizational pattern of the spermatid ducts, even between species from the same family (i.e., *M. amazonicus* and *F. carioca*). Moreover, even with different patterns, all species studied showed intrinsic musculature in the spermatid ducts. According to Werner & Simmons (2008), the contraction

of the spermatid duct musculature seems to be essential to move spermatozoa along the ducts in major insect groups. Therefore, we believe that this musculature is important to move the spermatozoa along the ducts of all Ephemeroptera and not only those with aflagellate spermatozoa (Leptophlebiidae). The diverse organization patterns of this musculature must be related to differences in reproductive physiology (i.e., copula duration) and not only with characteristics of the spermatozoa.

**Acknowledgments** The authors would like to acknowledge Dr. Neusa Hamada, Paulo V. Cruz, and Rafael Boldrini for their support provided for specimen collection. The specimen collection expeditions were partially supported by PRONEX/CNPq/FAPEAM. This study was financed by the Brazilian agencies Fapesp and Capes/PROEX. FFS is a fellowship holder of CNPq (Brazilian Research Council).

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