Neotropical Entomology

ISSN: 1519-566X

journal homepage: www.scielo.br/ne

SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Characteristics of the Male Reproductive System and Spermatozoa of Leptophlebiidae (Ephemeroptera)

P Brito[1], FF Salles[2], H Dolder[1]

[1]Depto de Anatomia, Biologia Celular e Fisiologia, Instituto de Biologia, UNICAMP, Campinas, SP, Brasil
[2]Depto de Ciências da Saúde, Biológicas e Agrárias, Centro Universitário Norte do Espírito Santo, Univ Federal do Espírito Santo, São Mateus, ES, Brasil

Keywords
Morphology, sperm, aflagellate, sexual maturation

Correspondence
Heidi Dolder, Depto de Anatomia, Biologia Celular e Fisiologia, Instituto de Biologia, UNICAMP, CP 6109, 13083-863, Campinas, SP, Brasil; heidi@unicamp.br

Edited by Fernando L Cônsoli – ESALQ/USP

Received 24 February 2010 and accepted 23 August 2010

Abstract

This study describes morphological changes in the male reproductive system of *Miroculis amazonicus* (Savage & Peters) from mature nymphs to subimago stages. The sperm ultrastructure of *Massartela brieni* (Lestage), *Farrodes carioca* (Domínguez *et al*) and *Miroculis mourei* (Savage & Peters), as well as aspects of cell fragments observed in these species' subimagos deferent ducts were described. Sperm from the three species studied are aflagellated and immotile, while those from *F. carioca* and *Ma. brieni* are approximately spherical with a homogenous nucleus and acrosome. Sperm of *F. carioca* present two or three mitochondria located between the nucleus and the acrosome. In *Ma. brieni*, only one lateral mitochondria was found. Sperm from *Mi. mourei* are shaped as a number 'eight', with electron lucent spots inside the nucleus and two mitochondria above the acrosome. Large cell fragments containing degenerative vesicles and some sperm were observed in the deferent duct lumen of the three species. Testes of *Mi. amazonicus* are extremely reduced in the subimago stage, which suggests that these cell fragments originated from testes degeneration.

Introduction

Species from Leptophlebiidae are distributed around the world and can be found in very diverse aquatic environments. Recent studies show approximately 130 genera and 610 species described in this family (Barber-James *et al* 2008). In South America, it represents one of the two major families of Ephemeroptera (Pescador *et al* 2001), being represented by 40 genera and 150 species (Domínguez *et al* 2006). Species of Leptophlebiidae are grouped in three subfamilies: Leptophlebiinae, Atalophlebiinae and Habrophlebiinae. Only species from Atalophlebiinae are found in South America.

Sperm from Leptophlebiidae males are notably aflagellate (Soldán 1979a, Grimm 1985, Gaino & Mazzini 1991a), which differentiates them from other Ephemeroptera. However, only four species have been ultrastructurally described: *Habrophlebia lauta* (Eaton) (Grimm 1985), *Habroleptoides umbratilis* (Eaton), *Habrophlebia eldae* (Jacob & Sartori) and *Choroterpes picteti* (Eaton) (Gaino & Mazzini 1991a). Furthermore, little is known of the morphology of male reproductive system in Ephemeroptera, but it is considered simple. Male reproductive system is composed of a pair of testes where the sperm cells develop and the seminal ducts which also store the spermatozoa, without accessory glands or other specializations (Soldán 1979b). Secretions produced by accessory glands are usually reported to play

a role in the reproductive success of most insects (Chen 1984, Gillott 2003), but nothing is known about the possible consequences of the absence of these glands in the physiology of Ephemeroptera.

To provide new information on the Leptophlebiidae biology, this study describes morphological changes in the male reproductive system of *Miroculis* (*Atroari*) *amazonicus* (Savage & Peters) from their last instar to the subimago stage. The sperm ultrastructure of *Massartela brieni* (Lestage), *Farrodes carioca* (Domínguez *et al*) and *Miroculis* (*Ommaethus*) *mourei* (Savage & Peters) was also described, as well as aspects of cell fragments observed in these species' deferent ducts.

Material and Methods

Light microscopy

Miroculis amazonicus at the last nymph stage were collected from a river in Presidente Figueiredo, state of Amazonas, Brazil. Some specimens were dissected as nymphs and others were maintained until the first winged stage (subimago) and then dissected. Their reproductive systems were fixed in a 2.5% glutaraldehyde and 4% paraformaldehyde solution. They were placed on a histological slide and photographed unstained with an Olympus BX41 light microscope.

Transmission electron microscopy

Farrodes carioca, *Ma. brieni* and *Mi. mourei* male subimagos were collected near a river in Santa Teresa, state of Espirito Santo, Brazil. The deferent ducts of the specimens were dissected and fixed in a 2% glutaraldehyde and 1% tannic acid solution, and post-fixed in 1% uranyl acetate solution. The material was dehydrated and embedded in Epon resin; ultrathin sections were contrasted with 3% uranyl acetate and lead citrate and analyzed in a Zeiss Leo 906 transmission electron microscope.

Results

The spermatozoa of *F. carioca* and *Ma. brieni* are approximately spherical and consist of a nucleus with uniformly condensed chromatin and an acrosome with median electron density (Fig 1a, b). Two or three spherical mitochondria are observed between acrosome and nucleus in *F. carioca* sperm (Fig 1a). *Massartela brieni* sperm cells generally present one spherical mitochondrion laterally observed between the nucleus and the acrosome (Fig 1b). Spermatozoa of *Mi. mourei* consist of a nucleus and an acrosome, but their shape is similar to a number 'eight', since they have a constriction at the acrosome base, near the nucleus. The nucleus is filled with compact chromatin containing electron lucent regions (Fig 1c). In general, spermatozoa of *Mi. mourei* present two spherical mitochondria located above the acrosome (Fig 1c). The longer axis measured from the acrosome tip to the nuclear base of the sperm is approximately: 1.4 μm in *F. carioca* and 1.6 μm in *Ma. brieni* and *Mi. mourei*.

The sperm are stored for copula in the deferent duct lumens of these species. Many cellular fragments were observed filling a large part of the volume of these ducts. These fragments are different among the species studied, but all three species have vesicles or degenerative vacuoles in their cellular fragments (Fig 1d-f). The cellular fragments observed in *F. carioca* presented a large vesicle partially filled with electron dense material and many small clear vacuoles in their cytoplasm (Fig 1d). Nuclei with partially condensed chromatin can still be recognized among these fragments. The cellular fragments observed in *Ma. brieni* made up most of the content of the deferent ducts. They presented vesicles partially filled with electron dense material and some interspersed spermatozoa (Fig 1e). There are two types of cellular fragments in *Mi. mourei*: one with variable electron density (Fig 1f, lower part of the figure), while the other type contained complex membranous structures derived from partially reabsorbed organelles (Fig 1f).

The male reproductive system of *Mi. amazonicus* undergoes profound changes from mature nymphs to subimagos. In mature nymphs, the testes were well developed (Fig 2a) while the deferent ducts were thin, approximately 52 μm in diameter, with empty lumens (Fig 2b). In subimagos, the testes were degenerated and the deferent ducts were dilated to approximately 124 μm in diameter, and their lumens were completely filled with sperm and cell fragments, as described earlier (Fig 2c).

Discussion

Data presented in this manuscript confirm aflagellate and non-motile sperm as an autapomorphy of Leptophlebiidae in the Order Ephemeroptera. Despite the apparent simplicity, the sperm morphology in this family allows us to distinguish each of the genera analyzed.

The presence of a *perforatorium* in the acrosome is a plesiomorphic characteristic of insects (Baccetti 1972, Jamieson *et al* 1999), which was lost in the main Ephemeroptera lineages (Baccetti *et al* 1969, Fink & Yasui 1988). This fibrous structure was described in the acrosome of *H. umbralitis* (Habrophlebiinae) and *C. picteti* (Atalophlebiinae) (Gaino & Mazzini 1991a). In *H. eldae* (Habrophlebiinae) (Gaino & Mazzini 1991a). However, in *F. carioca*, *Ma. brieni* and *Mi. mourei* (Atalophlebiinae) sperm, no *perforatorium* was observed. Probably, the presence of a *perforatorium* is related to the thickness of the egg chorion

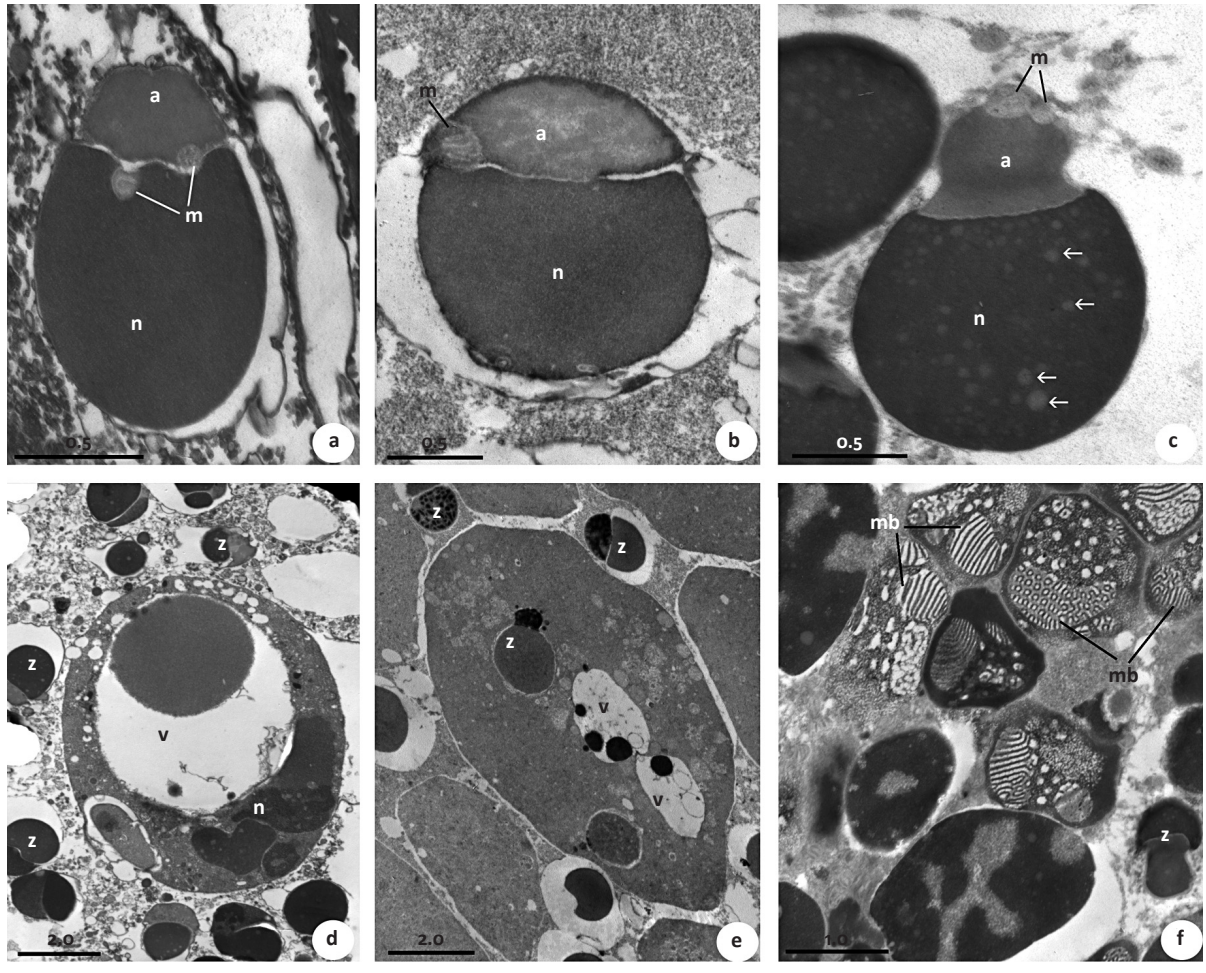

Fig 1 Transmission electron microscopy of subimagos. Sperm and cell fragments with degenerative vesicles from deferent duct lumens of *Farrodes carioca* (a, d), *Massartela brieni* (b, e), *Miroculis mourei* (c, f), respectively. (n) nucleus, (m) mitochondria, (a) acrosome, (z) sperm, (v) vacuole, (mb) membranous structures, (arrows) electron lucid chromatin regions in *Mi. mourei* spermatozoa. All bars are in µm.

for these different species as suggested by Gaino and Mazzini (1991b), and does not seem to have a phylogenetic significance in Leptophlebiidae, since this characteristic is not shared among species of the same subfamily.

Mitochondrion position is another variable characteristic among Leptophlebiidae sperm. A mitochondrion was observed beneath the nucleus in *H. umbralitis* (Gaino & Mazzini 1991a), laterally located between the nucleus and the acrosome in *C. picteti* (Gaino & Mazzini 1991a) as well as in *F. carioca*, but different from *Ma. brieni*, where it occurs laterally in the nucleus/acrosome contact region, and from *Mi. mourei,* where it is found above the acrosome. Interestingly, no visible mitochondrion was observed in *H. eldae* sperm (Gaino & Mazzini 1991a). Most Ephemeroptera sperm present a single, enlarged mitochondrial derivative with a separated paracrystalline body that extends along the tail (Baccetti *et al* 1969, Phillips 1969, Fink & Yasui 1988, Gaino & Mazzini 1991b).

The presence of small, simple mitochondria in Leptophlebiidae sperm is a derivation of the typical pattern described for this order. Since the mitochondrial derivative is associated with the flagellum and its movement, these immotile species would have very different energetic needs and therefore their mitochondria were not modified in the same manner. To confirm a probable phylogenetic significance of different localizations of the mitochondria in the spermatozoa of Leptophlebiidae, more species must be studied. A phylogenetic inference based on the available data would be premature.

The presence of large cell fragments in the deferent duct lumens has not been previously reported in Ephemeroptera. Since the testes of *Miroculis amazonicus* were greatly reduced and the enlarged seminal duct in the subimago stage, the cell fragments observed in this duct probably originated from the degenerated testes. The presence of some spermatozoa (as observed in *Ma. brieni*) and of degenerative vesicles inside these

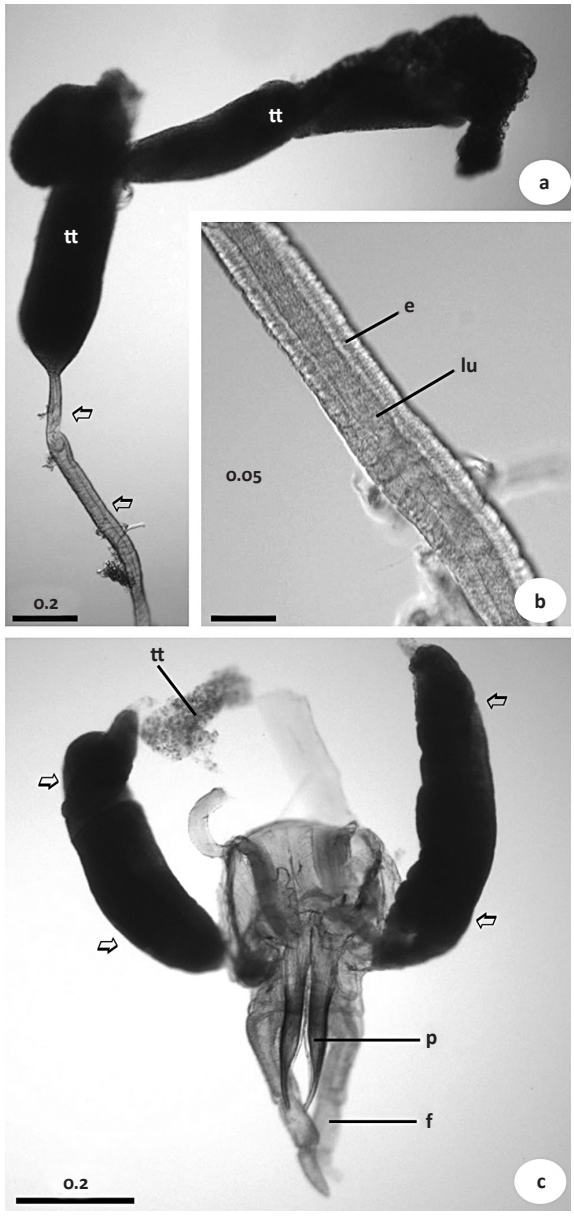

Fig 2 a, b) Male reproductive system of a *Miroculis amazonicus* mature nymph, (tt) testes, (open arrows) still empty deferent ducts; b) High magnification of the deferent duct, (e) epithelium, (lu) lumen; c) Male reproductive system of a *Mi. amazonicus* subimago, (tt) degenerated testes, (open arrows) deferent ducts full of spermatozoa and cell fragments, (p) penises, (f) forceps. All bars are in mm.

cell fragments seem to confirm this hypothesis. Some studies have reported the presence of holocrine and apocrine secretion in the ducts of male reproductive systems of different insects (Leopold 1970, Perotti 1971, Riemann 1973, Almadoss 1990, Brito *et al* 2010). Since Leptophlebiidae, as all other Ephemeroptera, do not present accessory glands in the male reproductive system, the cell fragments observed in this study could act as functional secretions inside the deferent ducts or in the female reproductive tract. They could also provide nutrients for sperm nutrition or function as a protective medium for sperm storage, or modulate post copula behavior in females. However, this hypothesis must be confirmed by future investigations.

Acknowledgments

The authors would like to acknowledge Dr Neusa Hamada and Paulo V Cruz for their support for specimen collection. Some specimen collection expeditions were supported by PRONEX/CNPq/FAPEAM. This research was funded by the Brazilian agencies Fapesp and Capes (Proex).

References

Almadoss G (1990) Fine structure of the regional differentiation of the ductus ejaculatorius simplex (EJ1, EJ2, EJ3) along with the role of their secretions in sperm activation and motility in two Noctuid species, *Heliothis armigera* (Hübner) and *Spodoptera litura* (Fabricius) (Lepidoptera). II. Fine structure and function of simplex 2 (EJ2). Proc Indian Acad Sci 99: 19-38.

Baccetti B (1972) Insect sperm cells. Adv Insect Physiol 9: 315-397.

Baccetti B, Dallai R, Giusti F (1969) The spermatozoa of Arthropoda. VI Ephemeroptera. J Ultra Res 29: 343-349.

Barber-James HM, Gattlliat JL, Satori M, Hubbard MD (2008) Global diversity of mayflies (Ephemeroptera, Insecta) in freshwater. Hydrobiologia 595: 339-350.

Brito P, Zama U, Dolder H, Lino Neto J (2010) New characteristics of the male reproductive system in the Meliponini bee, *Frisella schrottkyi* (Hymenoptera: Apidae): histological and physiological development during sexual maturation. Apidologie 41: 203-215.

Chen P S (1984) The functional morphology and biochemistry of insect male accessory glands and their secretions. Annu Rev Entomol 29: 233-255.

Domínguez E, Molineri C, Pescador ML, Hubbard MD, Nieto C (2006) Ephemeroptera of South América, 646p. In Adis J, Arias JR, Rueda-Delgado G, Wantzen KM (eds) Aquatic biodiversity in Latin America, Vol. 2. Sofia-Moscow, Pensoft.

Fink TJ, Yasui LS (1988) Ultrastructure of the sperm of *Dolania americana* Edmunds and Traver (Ephemeroptera: Behningiidae). Int J Insect Morphol Embryol 17: 447-454.

Gaino E, Mazzini M (1991a) Aflagellate sperm in three species of Leptophlebiidae (Ephemeroptera). Int J Insect Morphol Embryol 20: 119-125.

Gaino E, Mazzini M (1991b) Spermatozoon of mayflies (Ephemeroptera): an ultrastructural approach, p. 27-38. In Alba-Tercedor J, Sanchez-Ortega A (eds) Overview and strategies of Ephemeroptera and Plecoptera, Gainesville, The Sandhill Crane Press, 588p.

Gillott C (2003) Male accessory gland secretions: Modulators of

female reproductive physiology and behavior. Annu Rev Entomol 48: 163-184.

Grimm VR (1985) Vergleichend-anatomische Untersuchung der abdominalen Muskulatur und der Gonoducte männlicher Ephemeroptera-Imagines (Insecta). Stuttgarter Beiträge zur Naturkunde (Serie A) 59: 1-59.

Jamieson BGM, Dallai R, Afzelius BA (1999) Insects: their spermatozoa and phylogeny. Enfield, Science Publisher, 555p.

Leopold RA (1970) Cytological and cytochemical studies on the ejaculatory duct and accessory secretion in *Musca domestica*. J Insect Physiol 16: 1859-1872.

Perotti ME (1971) Microtubules as components of *Drosophila* male paragonia secretion: An electron microscopic study, with enzymatic tests. J Submicrosc Cytol 3: 255-282.

Pescador ML, Hubbard MD, Zuñiga MC (2001) The status of the taxonomy of the mayfly (Ephemeroptera) fauna of South America, p.37-42. In Domínguez E (ed) Trends in research in Ephemeroptera and Plecoptera. New York, Kluwer Academic Plenum Publishers, 478p.

Phillips DM (1969) Exceptions to the prevailing pattern of tubules (9+9+2) in the sperm flagella of certain insect species. J Cell Biol 40: 28-43.

Riemann JG (1973) Ultrastructure of the ejaculatory duct region producing the male housefly accessory material. J Insect Physiol 19: 213-223.

Soldán T (1979a) A comparative study of spermatozoa of some Central European Ephemeroptera. Acta Entomol Bohemoslov 76: 223-230.

Soldán T (1979b) The structure and development of the male internal reproductive organs in six European species of Ephemeroptera. Acta Entomol Bohemoslov 76: 22-33.