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Research Article

Light microscopic evidence of spermatogenesis of firefly, *Pyrocoelia tonkinensis* Olivier, 1886 (Coleoptera: Lampyridae)

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Abstract

The description of the firefly reproductive structure is relatively under-researched. Therefore, we initially observed the differentiating stages of spermatogenesis in the adult male firefly, *Pyrocoelia tonkinensis*, which could be the very first report in Thailand. All firefly specimens gathered from Nakhon Si Thammarat province, Thailand, were processed using the standard histological technique. It was divulged that a pair of testicular structure with several sperm tubules was seen. The stage of spermatogonia within testis follicle could be divided into three main zones, namely zone I (growth), zone II (maturation), and zone III (transformation). In this process, each zone contained the differentiating stages of spermatogenesis, which was classified into six stages based on size differences and the histological organization (especially in the chromatin organization) including spermatogonia, primary spermatocytes, secondary spermatocyte, spermatids with two sub-steps, and spermatozoa. The detailed information about male *P. tonkinensis* reproduction could generate not only a baseline data to be better known but also contribute to the future works regarding ultrastructure and physiology of the reproductive system. This would then be supportive of the conservation and management of the firefly in Thailand.

Keywords: firefly, histology, reproduction, sperm cell, Thailand

Introduction

Several investigations concerning the reproductive system of arthropod species have been exclusively observed because it is fundamental for ecology and physiology as well as the

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species conservation. Then, this information has been used in various documents, such as in the biological control and the outbreak of insect pests. Regarding previous studies, they speculated that the structure and ultrastructure of the reproductive insects were a complex process of cellular development and morphological changes (Gerber et al., 1971; Karakaya et al., 2012; Özyurt et al., 2013). The histology of the insect reproductive system was composed of two distinct parts including gonadal tissue and reproductive duct (Drecktrah, 1966; Özyurt et al., 2013). The similar pattern had been reported in dock bug, *Coreus marginatus* (Hemiptera: Coreidae) (Karakaya et al., 2012), shield bug, *Graphosoma lineatum* (Hemiptera: Pentatomidae) (Özyurt et al., 2013), and common emigrant, *Catopsilia pomona* (Lepidoptera: Pieridae) (Tongjeen et al., 2014); however, the knowledge of a basic structure of the reproduction in firefly is currently limited in Thailand and still has wide rooms for the further investigation.

Fireflies, also knowns as lightning bugs or glow-worms, are one of the beetles belonging to the family Lampyridae (order Coleoptera). They are considered being beneficial beetles in which their larvae can act as natural enemies for suppressing slugs and snails in natural or agricultural systems (Mckenna & Farrell, 2009; Branham, 2010). Despite their ecological importance, the reproductive biology regarding microscopic structure has been left unexplored. As part of on-going research on the reproductive system of Thai insect groups, the adult male firefly, *Pyrocoelia tonkinensis* Olivier, 1886, one of the most critical lampylids, their reproductive structure were chosen to study. This firefly is endemic to Southern Asia including Thailand (Jeng et al., 1999). In this regard, a quest for a description of the spermatogenesis of *P. tonkinensis* was initiated using the histological technique. The outcomes of this project could provide the in-depth understandings on the male reproductive structure for the purpose to support the reproductive cycle and management of the firefly in Thailand.

Materials and methods

Ten adult male *Pyrocoelia tonkinensis* fireflies with body length of 1.50±1.91 cm (mean±S.D.) were observed during breeding season from the rubber plantation during the rainy season (October to December 2016) at Chawang District, Nakhon Si Thammarat Province, Thailand (8°28'10" N, 99°29'45" E). They were euthanized by rapid cooling shock (Wilson et al., 2009) and fresh specimens were fixed with Davidson's fixative solution (~48 hr) for histological technique (Dietrich & Krieger, 2009).

According to a standard histological technique, the abdominal segments of *P. tonkinensis* were to be detached and processed (Presnell & Schreibman, 1997; Suvarna et al., 2013), embedded in paraffin, sectioned at 4 µm thickness, and finally stained with Harris's haematoxylin and eosin (H&E) (Presnell & Schreibman, 1997; Suvarna et al., 2013). After staining, a structure of the testicular structure and spermatogenesis of *P. tonkinensis* were investigated and photographed with a digital light microscope (Leica TE750-Ua, Boston Industries, Inc., USA). Each stage of the spermatogensis was performed with 50 randomized cell per slide under Leica light microscope (40X) by a standard program LAS version 4.9.

Results and discussion

Several investigations of the gonadal structure in the insect have previously reported (Bahadur, 1975; Bairati, 1968; Ferreira et al., 2004; Wheeler and Krutzsch, 1992); however, the records of histological observations showed that the study on reproductive histology of firefly is rarely known (Demary, 2005; South et al., 2008). Therefore, this study was conducted to continously observe the increased information on firefly reproduction under light microscopy.

All samples from our study showed that all insects were histologically considered to be a sexually mature testis because the testis of male *P. tonkinensis* was composed of several sperm tubules in the abdominal region (Figure 1A) where the mature sperms were stored (Figure 1B). It was similar to those observed in other insect groups (Adams, 2001; Freitas et al., 2010; Karakaya et al., 2012). The histological examination of the sperm tubule was enclosed by a thin layer of connective tissue (Figure 1C).

Based on the differential stage of male germ cell within the sperm tubule, it could be categorized into vitellarium and germarium (Figure 1D). At the overall histology of sperm tube, only spermatogonia frequency distribution was easily distinguished and produced within the vitellarium at the outer rim of the tubule near the adipose tissue (Figures 1C-1D). After the vitellarium region, the presence of the germarium continuously occurred and was classified into three sub-zones: growth, maturation, and transformation (Figure 1D). This feature was relatively similar to some insects (Tongjeen et al., 2014; Özyurt et al., 2014).

The observation on the growth zone indicated that it usually contained only spermatocytes (Figure 1D & 2A) whereas an apparent formation of the maturation zone consisted of the spermatids under two meiotic divisions (Figure 2B). Shreds of evidence yielded that the transformation zone finally located in the sperm tubule and usually presented in the spermatozoa (Figure 2B). This feature echoed the findings of the previous studies of predatory stink bug, *Podisus nigrispinus* (Lemos et al., 2005) and Italian striped bug, *Graphosoma lineatum* (Özyurt et al., 2013).

In this current study, a detailed information of the spermatogenenic stages of *P. tonkinensis* in the sperm tubules was classified into six successive stages according to size differences and histological organizations, especially the chromatin organizations including spermatogonium, primary spermatocyte, secondary spermatocyte, spermatid with two sub-steps and spermatozoon, as similarly related to some invertebrates such as three different *Tentyria* (İzzetoglu & Gűlmez, 2018) and giant river prawn, *Macrobrachium rosenbergii* (Sagi et al., 1988; Polijaroen, 2010).

Spermatogonium

The spermatogonium had a very largest size (12.87±0.98 µm in diameters) and appeared as a round shape (Figure 1C). A large centrally nucleus was stained as basophilic characterizes whereas the cytoplasm was mostly thin and had a basophilic stain (Figure 1F), which was similar to that of other spermatogonia in *Drosophila melanogaster* (Cooper, 1950; Hardy et al., 1979).

Primary spermatocyte

The primary spermatocyte was developed from spermatogonia by mitotic division. It appeared in the spermatocyst and formed as an oval or spherical shape with an average cell diameter 10.56±0.65 µm (Figure 2C). A prominent basophilic nucleolus, containing patchy heterochromatin, appeared (Figure 2C).

Secondary spermatocyte

According to the first meiotic division from primary spermatocyte, the formation of heterochromatin of the secondary spermatocyte was found increasing (Figure 1C). It remained in spermatocyst (Figure 2C). The size of this stage was about 5.98±0.56 µm in diameter (Figure 2C). This indicated a decrease of the cell size when compared to previous stages.

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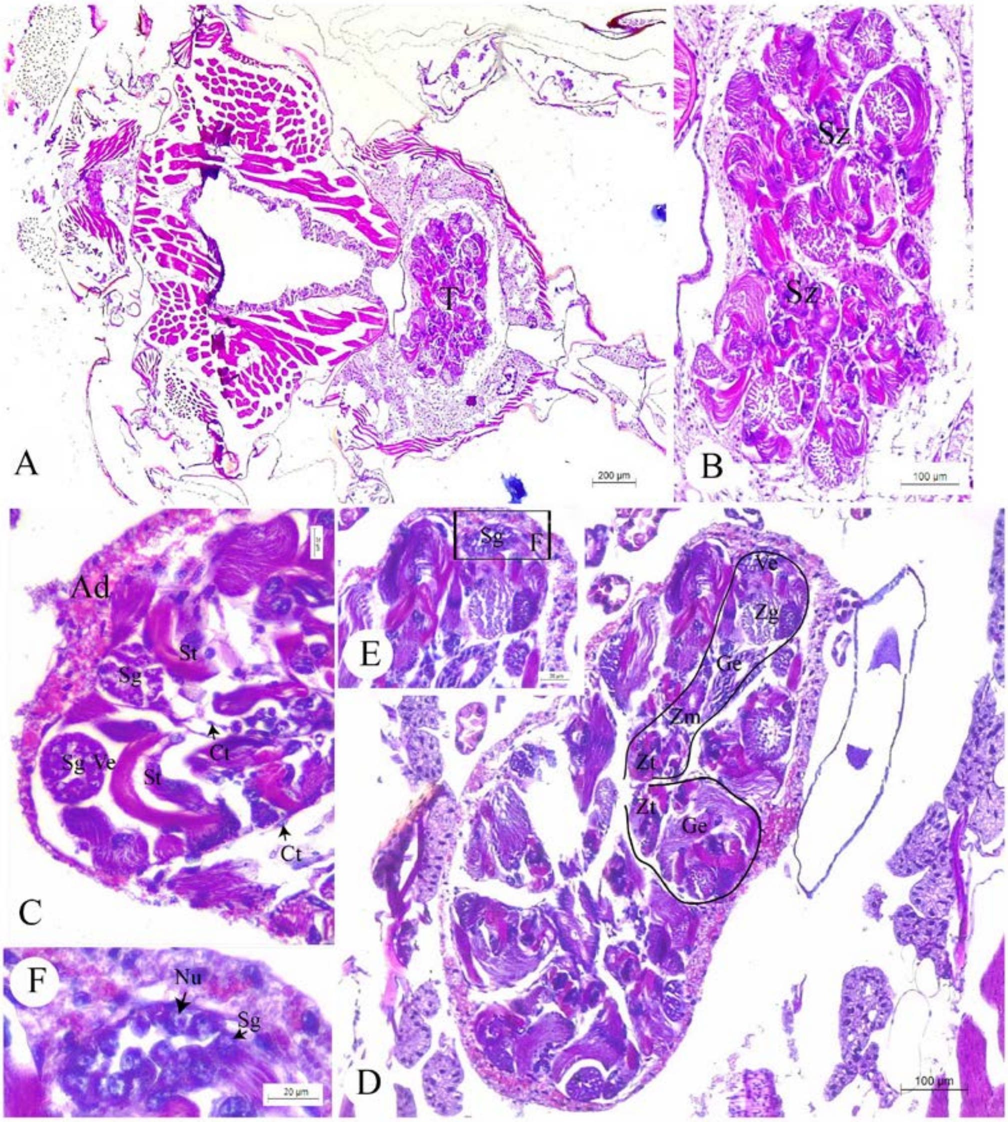

Figure 1. The light photomicrograph of the testicular maturation of *Pyrocoelia tonkinensis*. A-B: Localization of the testis (T) containing in the sperm (Sz) within sperm tube. C: sperm tube was enclosed with the connective tissue (Ct). D: Two vitellarium (Ve) and germarium (Ge) of the sperm tubule was classified, which germarium could be divided into three main zones, zone of growth (Zg), zone of maturation (Zm) and zone of transformation (Zt). E-F: Spermatogonia (Sg) was observed in the vitellarium. Note: Ad = adipose tissue.

Spermatid

The prominence of the spermatid could be divided into two sub-steps (early and late spermatids as shown in Figures 2D & 2E). Early spermatid showed that the nucleus enlarged in size, but it had a smaller size than the secondary spermatocytes, approximately 4.45±0.98 µm in diameter (Figure 1D). A strong heterochromatin condensation of this stage was centrally distinguished (Figure 1D). Afterwards, it transformed into the late spermatid. This stage completely condensed the heterochromatin and was in the beginning to produce the tail (Figure 2E).

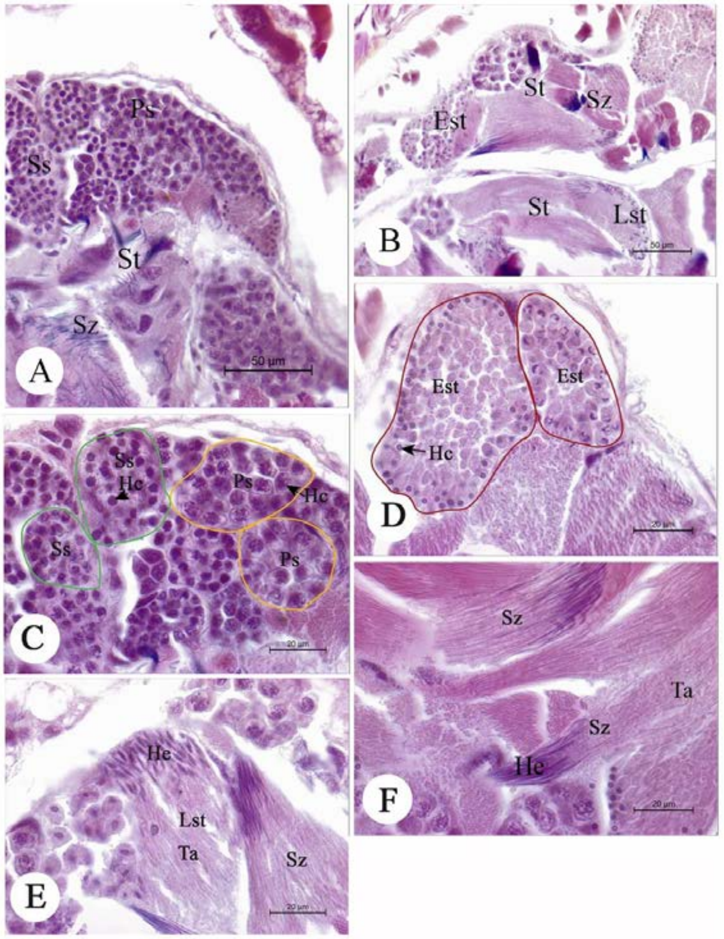

Figure 2. The light photomicrograph of the spermatogenesis in *Pyrocoelia tonkinensis*. A-B. Differenting stages of spermatogenesis [such as primary spermatocyte (Ps), secondary spermatocyte (Ss), early spermatid (Est), late spermatis (Lst) and spermatozoa (Sz)] within the sperm tubules (St). C. Primary spermatocytes (Ps) and secondary spermatocytes (Ss). D. Early spermatids. E. Late spermatid. F. Spermatozoa. Hc = heterochromatin, He = head, Ta = tail.

Spermatozoa

During the spermatozoon process, the mature spermatozoon was the last one of spermatogenesis, which was formed in two distinct regions: 1) a packed head (quite close to the elongated shape and thin (about 15-20 µm): 2) a tail region (flagellum) in the sperm tubule (Figure 2F).

Consequently, the spermatozoa migrated into the vasa efferentia (Figure 3). The structure of the vasa efferentia was located in the middle area of the testicular parenchyma (Figure 3A), which it was surrouned by simple cuboidal epithelium (Figure 3B). They were separated into individual spermatozoa (Figure 3B), as similar seen in *G. lineatum* (Özyurt et al., 2013)

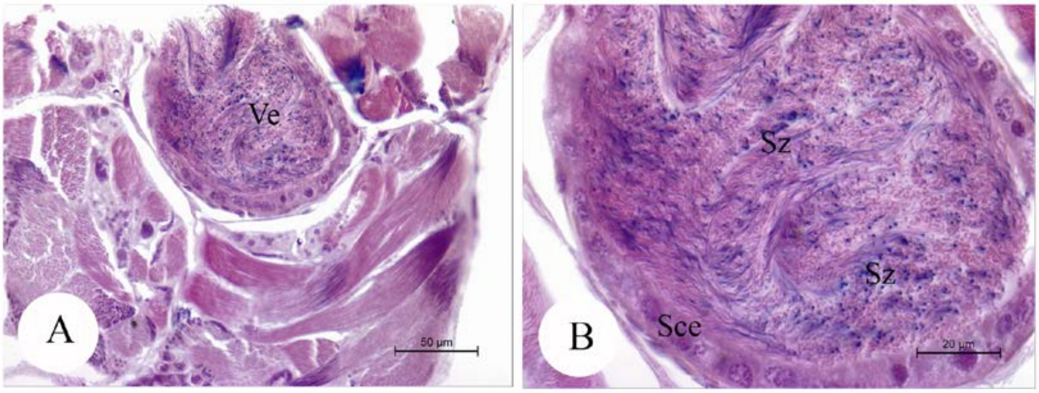

Figure 3. The light photomicrograph of the occurrence of spermatozoa in the vasa efferent (Ve) in *Pyrocoelia tonkinensis*. Note: Sce = simple cuboidal epithelium.

Conclusion

Our research indicated that the male reproduction system had a testicular structure with several sperm tubules, as likely seen in other insects. The accuracy of information form this research could provide the background knowledge of *P. tonkinensis* reproduction, which would lead to insights for further in-depth studies such on the ultrastructure and physiology of the reproductive system. Hopefully, this currently would then construct greater understandings to support the conservation and management of the firefly in Thailand.

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