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Histological and Electron Microscopical Observations on the Testis and Male Accessory Glands of *Poecilimon ataturki* Ünal, 1999 (Orthoptera, Tettigoniidae)

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ABSTRACT

Objective: The literature has many studies in the world about the morphology and histology of insect tissues, especially structures related to reproductive systems. However, there are no studies about the biology *of Poecilimon ataturki* Ünal, 1999 (Orthoptera, Tettigoniidae). For this reason, we aimed that the morphology and the structure of the testes and the accessory glands in *P. ataturki* are revealed.

Materials and Methods: In the present study, the histology and morphology of organs of the reproductive system in male *P. ataturki*, an endemic species in Turkey was examined by light microscope, scanning electron microscope, transmission electron microscope, and stereomicroscope.

Results: The reproductive system of male *P. ataturki* is composed of two testes, two vas deferens and short and long accessory glands. Each testis includes numerous follicles where the sperm generation occurs in. There are cysts in the follicles, one of which is in a particular sperm development. 3 different development stages are observed in follicles as growth zone, maturation zone and transformation zone. During these developmental stages, first spermatocytes reproduce by mitosis, and then they turn into spermatids by meiosis. Spermatozoa are also formed by the transformation of spermatids. Thus, the stages of spermatogenesis and spermiogenesis are completed. The accessory glands, whose main task is to produce secretion in order to facilitate the feeding of sperm and their transfer to the female, consist of many tubular structures, long and short. It is seen that it consists of single-layered epithelial tissue in cross sections of the accessory gland tubules.

Conclusion: As a consequence of this work, it has been evinced that male reproductive system elements belonging to *P. ataturki* show high similarity with the male reproductive systems of other species in the Tettigoniidae family.

Keywords: Male reproductive system, light microscopy, scanning electron microscopy, transmission electron microscopy, insect

INTRODUCTION

Studying the reproductive systems of the insects is central to bring out their population above threshold levels for endangered species. The male reproductive system has three main functions. These are to produce sperm by the male reproductive cells, to manufacture the protective fluid around the sperm via the glands, and to transfer the sperms that are produced to the female reproductive system (1-5).

Testes in order Orthoptera are made up of approximately 300-350 follicles which are tube or finger-shaped structure (2,4,6). There are cells in different stages of development in a testicular follicle. The germ cells which found at the distal side of follicles are called the sper-

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matocytes. The spermatocytes are comprised by the mitotic division and their numbers are increased. After that, spermatocytes located at the middle zone of testicular follicles undergo the meiosis and the spermatids occur. In the proximal side of the follicles, the spermatids are finally differentiated to the mature form, spermatozoa. The spermatozoa become mature and after that heads of sperm join together. This structure is named as spermatodesm (4,7,8).

The male accessory glands in insects play an important role for male fertility (9). It is the essential source of the production of proteinaceous secretions, called as accessory gland proteins (5,9,10). The accessory glands proteins are important for the competition between sperm during the mating. This situation is the major factor that affects to the male reproductive success (3,10).

Poecilimon ataturki Ünal, 1999 is an endemic species in Turkey belonging to the Tettigoniidae family (Orthoptera). Previous studies in the literature indicate that the *P. ataturki* is distributed in Bolu, Çankırı, Kastamonu, and Karabük Provinces (11-15). The name of *P. ataturki* is given by Prof. Dr. Mustafa Ünal in honor of Mustafa Kemal Atatürk, the founder of the modern Republic of Turkey in 1923 (11).

There are many literatures are found relevant to the taxonomy, the systematic, and the distribution of this species. However, there are no studies about the biology (such as alimentary system, reproductive organs of male and female individuals) of this species. For this purpose, the morphology and the structure of the testes that produce sperm and the accessory glands that manufacture seminal fluids those mix with sperm in *P. ataturki* are the subject of this paper.

MATERIALS AND METHODS

Supply of the Tissues

Adult individuals of *P. ataturki* were taken using a sweep net in Bolu province, Hamidiye Village in July 2019. Five male were anesthetized via the fumes of ethyl acetate. Some of the testes and male accessory glands were dissected in Na-PBS (pH 7.2) and some in 70% ethanol under stereomicroscope.

Light Microscope (LM) Analysis

Some of the specimens were fixed in formaldehyde (10%), dehydrated and blocked in paraffin. The sections (5-6 µm) were taken and were stained with Mallory's trichrome and routine staining, Hematoxylin-Eosin (H&E). Ultimately, the photos were shot with an Olympus BX51 photomicroscope in Gazi University.

Semi-thin sections taken from araldite blocks prepared in accordance with the procedure given below for transmission electron microscope (TEM) were stained with 1% methylene blue to reveal general histological organization.

Scanning Electron Microscope (SEM) Analysis

For the SEM investigations, the testes and male accessory glands were firstly fixed in 5% glutaraldehyde (pH 7.2) and were dehydrated. In following process, the specimens were dried with critical point dryer (Polaron CPD 7501). Dried specimens were coated with gold (Polaron SC 502). The testes and male accessory glands were finally examined with SEM (JEOL JSM 6060 LV, accelerating voltage 5-10 kV). The observations were done in Prof. Dr. Zekiye Suludere Electron Microscope Center in Gazi University.

Transmission Electron Microscope (TEM) Analysis

For the TEM observations, testis and accessory glands were first fixed twice: in 5% glutaraldehyde and then in 1% osmium tetroxide. After the fixation process, they were dehydrated and were embedded in Araldite blocks. Afterwards, the ultrathin sections were taken from the blocks with using ultramicrotome. Then, they were double stained with lead citrate and uranyl acetate dyes. The examinations with TEM (JEOL JEM 1400, accelerating voltage 80 kV) were done in Prof. Dr. Zekiye Suludere Electron Microscope Center in Gazi University.

RESULTS

The internal reproductive structures of male *P. ataturki* are made up of paired testes which are covered by a peritoneal sheath and aerated by several tracheoles, paired vas deferens, and as well as accessory glands (Figure 1). The testes which lay across the body cavity are elongated ovoid in form (Figure 1). Each testis is coated by an epithelium.

The cells in different stages of development are discriminated in several testicular follicles which are found each testis. The cysts consist of grouping of germ cells in the same growth stage (Figure 2A-B). The cells called spermatogonia are located in the germarium region at the distal end of the follicle. Other developmental stages are growth, maturation and transformation zones. The cells in here are spermatocytes, spermatids and spermatozoa in respectively. Spermatocytes are diploid cells with

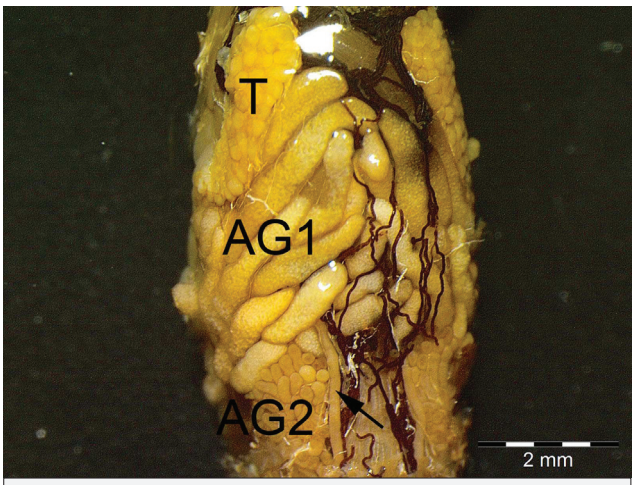

Figure 1. The general stereomicroscopic view of the male reproductive system in *P. ataturki*. T: testis, AG1: long accessory glands, AG2: short accessory glands, arrow: vas deferens, Scale bar=2 mm.

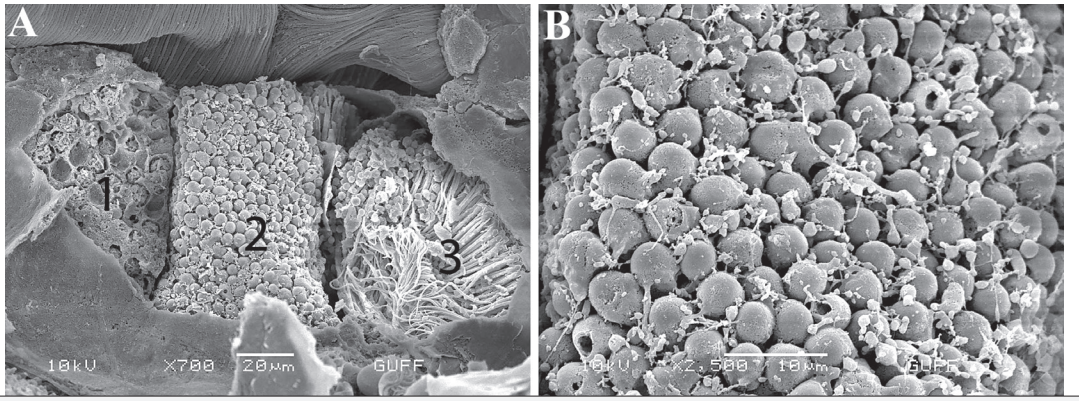

Figure 2. **A.** The SEM image of the cyst with different stages of development in the testicular follicle. 1: spermatocytes before the meiosis 2: early spermatids, 3: spermatozoa, Scale bar=20 µm; **B.** The SEM image of early spermatids with no tail, Scale bar=10 µm.

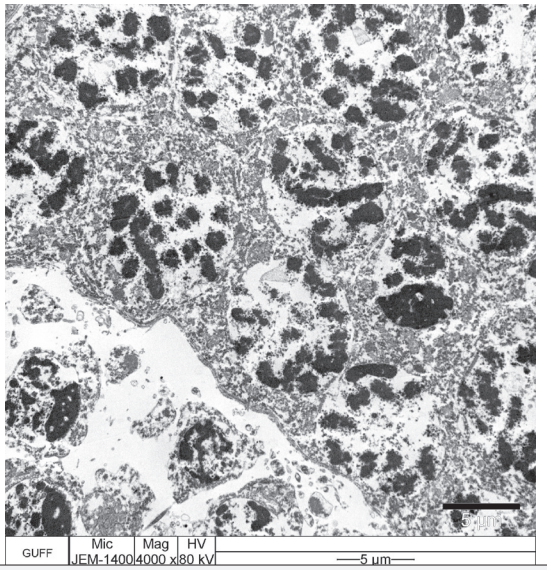

Figure 3. The TEM image of the cyst with spermatocytes, Scale bar=5 µm.

large spherical nuclei without tails that reproduce by mitosis in cysts in the growth zone. Before meiosis, the nucleus of cells looks as if to be fragmented TEM micrograph (Figure 3). When it comes to the maturation zone, spermatocytes form spermatids as a result of meiosis, which are haploid. The transformation of spermatids is characterized by tail formation. The spermatids in the early stage look like cells with elliptical nucleus which is located in the midst of the cell and no tail (Figures 2B, 4A-B). In the late stage of spermatids, the thin tail structure starts to be apparent and cells have a head beginning to elongate and an elongated tail (Figures 4C, 5). When the transformation of the spermatids is finished in the transformation zone, the spermatozoa occur. During the transformation of spermatids into sperm cells or spermatozoa, the head of the sperm completes elongation (Figure 6A-B). The axoneme and two mitochondrial derivates appear along the flagella in the cross sections of the mature sperm cells (Figure 4C).

The acrosome region is seen in the apex of the sperm head. This region is shirt collar in shape and the hole can be observed close to the tip of the acrosome (Figure 6A-B). Sperm heads are

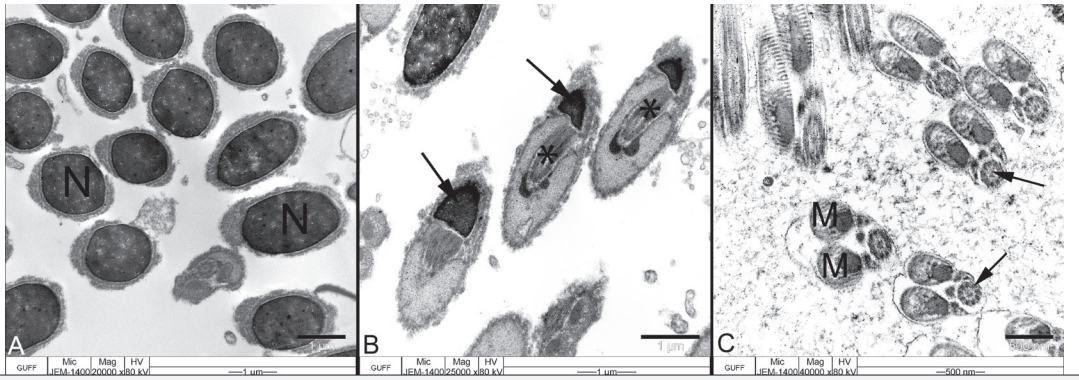

Figure 4. **A.** The TEM image of the beginning of tail formation in spermatids, N: nucleus, Scale bar=1 µm; **B.** The TEM image of the beginning of tail formation in spermatids, arrow: acrosome, asterisk: centriol, Scale bar=1 µm; **C.** The axoneme (arrows) and two mitochondrial derivatives (M) in the TEM image, Scale bar= 500nm.

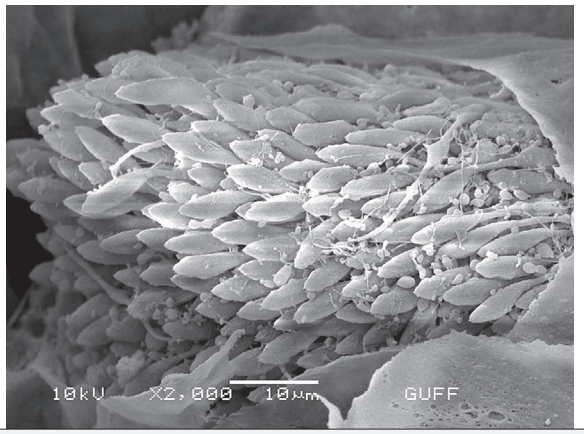

Figure 5. The late stage of spermatids with thin tail and a head beginning to elongate (SEM), Scale bar=10 µm.

19,2±1,7 µm in length. The tail is quite thin and long compared to the head of the sperm. We detected many granules among the spermatozoa (Figure 6A).

The mature spermatozoa are packed together from the head to the holders to form bundles in cysts. This structure is called spermatodesm (Figure 7).

The accessory glands are tubular structures with closed end (Figures 1, 8). The male accessory glands of this species, *P. ataturki* consist of two different types of glandular tubule groups: the first group consisting of short and thin tubules and the second group consisting of long and thick tubules. The long tubes generally arise in front of the hindgut. Its outer surface is quite flat and surrounded by a trachea network. The epithelial layer is seen as a single-layered in cross sections in both groups of tubules. The difference between them is related to tubule lengths and di-

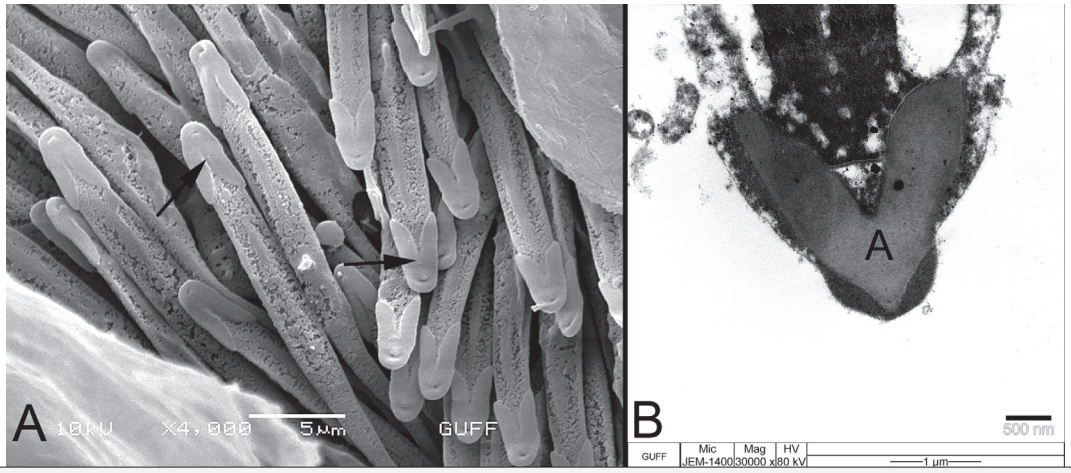

Figure 6. **A.** The SEM image of the mature sperm cell with head which completes elongation and long tail. Arrows: acrosome, Scale bar=5 µm; **B.** The TEM image of the sperm head with acrosome (A), Scale bar= 1 µm.

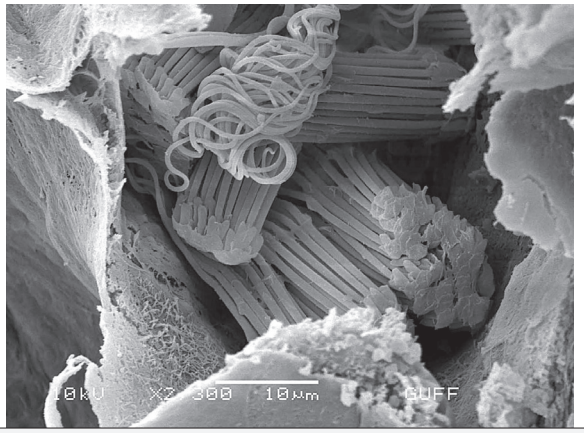

Figure 7. The SEM image of the mature spermatozoa packed together called spermatodesm, Scale bar= 10 µm.

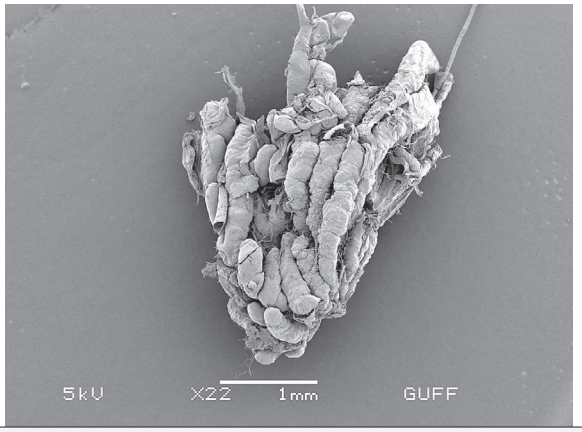

Figure 8. The SEM image of the long accessory glands, Scale bar= 1 mm.

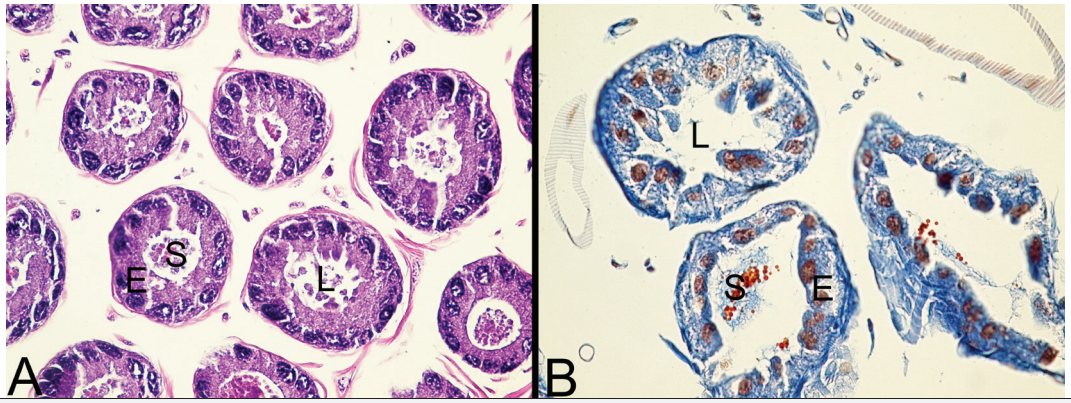

Figure 9. **A.** The light microscopic image of the cross sections of long accessory glands. E: epithelial cell layer, L: lumen, S: secretion of the accessory gland cells, H&E staining, X400; **B.** The light microscopic image of the cross sections of long accessory glands. E: epithelial cell layer, L: lumen, S: secretion of the accessory gland cells, Mallory's trichrome staining, X400.

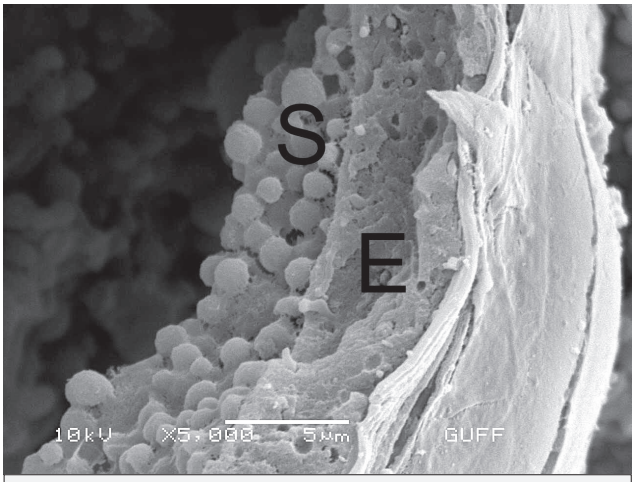

Figure 10. The SEM image of different sizes of secretory granules (S) in the lumen of the accessory glands. E: epithelial cell layer, Scale bar= 5 µm.

ameters. No significant difference was observed ultrastructurally. The shape of the cells in the epithelial layer is cubic or pyramidal (Figure 9A-B). Different sizes of secretory vesicles are observed in the lumen of the accessory gland tubules (Figure 10).

DISCUSSION

Male reproductive organs are generally similar in insect groups. However, some minor or major differences in these structures can be observed between different taxa. Many studies support the idea that many of the differences in these structures can be used as distinctive characters (4,5,16-19).

The elements of the reproductive system are usually testis, vasa deferentia, seminal vesicles, accessory glands, and ejaculatory duct in male insects, despite some differences. In accordance with this rule, two testes, two vas deferens, and ejaculatory duct have been observed in some insect species, such as *Drepanosiphum platanoidis* (Schrank), *Euceraphis betulae* (Koch), *Macrosiphoniella tanacetaria* (Kaltenbach), and *Myzocallis walshii* (Monell) from Aphididae (Homoptera) (20), *Melanoplus sanguinipes* (Fabricius, 1798) (18) and *Pseudochorthippus parallelus parallelus* (Zetterstedt, 1821) from Acrididae (Orthoptera) (4), *Gryllus sigillatus* (Walker, 1869) from Gryllidae (Orthoptera) (21). On the other hand, in addition to these structures, seminal vesicles are also found in some insect species such as *Balclutha brevis* Lindberg, 1954 from Cicadellidae (Hemiptera) (22). In *P. ataturki*, the situation is like the species in the first group, the seminal vesicle is absent.

Insects are the animal group with the most species diversity and high reproductive and survival ability (23, 24). Although insects generally have high reproductive capacity, the number of follicles in each testicle varies between various groups (25). Some species such as *P. ataturki, P. parallelus parallelus* (4), *Poecilimon cervus* Karabağ, 1950 (Orthoptera, Tettigoniidae) (26), and *M. sanguinipes* (18) has a large number of follicles in their testis, while some species has much less follicle number. For example, *Orphullela punctata* (De Geer, 1773) (Orthoptera, Acrididae) (2), larval *Dione juno* (Crameri 1779) (Lepidoptera, Nymphalidae) and larval *Agraulis vanilla* (Linnaeus, 1758) males have four follicles (27), *Martarega betoi* (Heteroptera, Notonectidae) (28) and *Furcatopanorpa longihypovalva* (Hua and Cai, 2009) (Mecoptera, Panorpidae) males have two follicles (29) and *Tuberculatus eggleri* Börner, *E. betulae, D. platanoidis, M. walshii, and M. tanacetaria* (Homoptera, Aphididae) males have three follicles (20) in their testes. One of the most important points we observed in this study we conducted is related to the sperm production capacity of each testis which has a big size. Because, we found a large number of follicles in each testis of *P. ataturki*. Similarly, the number of cysts in the follicles was also quite high. Pitnick and Markow also stated that daily sperm production is generally directly proportional to testicular size (30).

In addition to differences in testicular and follicle structure, sperm structure can also vary greatly between species. This

is due to the fact that the sperm can have a suitable function and structure for successful fertilization of the insect (24,31,32). These differences in sperm structures can be phylogenetically determinant (33).

The size, morphological and histological structure, and the results of histochemical staining of the male reproductive glands in insects may vary greatly (34). For example, there are different subtypes of accessory glands according to the diameter and length of the tubules in various orthopteroid families, such as three or nine in some Acrididae species, six in some Gryllidae species and two in some Tettigoniidae species (21,35,36). The accessory glands of *P. ataturki* include two different types as long and short tubules, as in other Tettigoniidae species, such as *P. cervus*, *Steropleurus elegans* (Fischer), *Bolivarius siculus* (Fischer), *Rhacocleis annulata* Fieber, *Tylopsis liliifolia* Fabricius 1793, and *Platycleis intermedia* (Serville, 1838) (17,26,37). For this reason, the accessory glands of *P. ataturki* also show high similarity morphologically with the accessory glands of other Tettigoniidae species.

CONCLUSION

When all these results are evaluated, it can be said that the male reproductive system of *P. ataturki*, with some minor differences, closely resembles that of other species in the Tettigoniidae family. We hope that this study will serve as a basis for future studies on insect groups, especially species belonging to the order Orthoptera.

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Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- D.A.P., I.P., Z.S.; Data Acquisition- D.A.P., I.P.; Data Analysis/Interpretation- D.A.P., I.P., Z.S.; Drafting Manuscript- D.A.P., I.P., Z.S.; Critical Revision of Manuscript- D.A.P., I.P., Z.S.; Final Approval and Accountability- D.A.P., I.P., Z.S.

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