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Macroscopic and Histological Structures of Testes in Three Different *Tentyria* Species

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ABSTRACT

The male reproductive organs in insects are typically composed of a pair of seminal vesicles and a pair of test tubes joined to the excretion channel on the median line. The samples used in the study were collected from İzmir Bozdağ region and from Turkish Republic of Northern Cyprus. All testis samples were fixed in Bouin's solution and Mayer's Haematoxylin-Eosin (H&E) was used to stain for tissue section. As a result of the macroscopic examinations, it was determined that the testis appearance of *Tentyria cypria* is different from other species used in the study. At the end of the histological examinations, the general appearance of the *T. cypria*'s testis was similar to a four-leafed clover. In all three species, spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa, which are the stages of spermatogenesis in all follicles, are clearly visible. In this study, morphological and histological differences in male reproductive organs were demonstrated for the first time in three different *Tentyria* species belonging to Tenebrionidae family.

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Üç Farklı Tentyria Türünün Makroskopik ve Histolojik Testis Yapıları

ÖZET

Böceklerde erkek üreme organları tipik olarak bir çift seminal vezikül ve medyan hatta bulunan boşaltım kanalıyla birleşen bir çift testisten oluşmaktadır. Araştırmada kullanılan örnekler İzmir Bozdağ bölgesinden ve Kuzey Kıbrıs Türk Cumhuriyeti'nden toplanmıştır. Disekte edilen testisler Bouin solüsyonunda fikse edilip, doku kesitlerinin boyanması için Mayer's Hematoksilen-Eozin kullanıldı. Makroskopik incelemeler sonucunda, *Tentyria cypria*'ya ait testis görünüşünün çalışmada kullanılan diğer türlerden farklı olduğu belirlenmiştir. Histolojik incelemeler sonunda *T. cypria*'ya ait testisin genel görünümünün 4 yapraklı bir yoncaya benzediği tespit edilmiştir. Her üç türde de bütün foliküllerde spermatogenezin safhalarına ait yapılar olan spermatogonyumlar, primer spermatositler, sekonder spermatositler, spermatidler ve spermler oldukça belirgin bir şekilde görülmektedir. Bu çalışmayla, Tenebrionidae familyasına ait üç farklı *Tentyria* türünde, erkek üreme organlarında morfolojik ve histolojik yapılar ilk kez gösterilmiştir.

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INTRODUCTION

The male reproductive system consists of a pair of testes with sperm tubes or follicles in most insect species (Omura, 1936; Resh and Cardé, 2009; Wu et al., 2017).

Towards the end of each follicle there are root cells called spermatogonium. These root cells undergo mitosis and increase their size and form sperms. Because of the mitotic spermatogonia, sperms are pushed towards the end of the follicle, the mature sperms pass through the

testis via short channels (vasa efferentia) and are deposited in the seminal vesicles, a somewhat wider region of the channels. Similar channels in vasa deferentia form an "ejaculatory" channel in the middle of the body. This channel opens to the male reproductive organ called "aedeagus". The male reproductive system may have one or more auxiliary glands (Jose et al., 2008). Some are located directly adjacent to the testis or reproductive vesicles, while others may be connected to the ejaculatory duct. These glands have two functions in general; formation of sperm fluid and the formation of spermatophore (Sehnal, 1985; Kılınçer and Bayram, 1999; Gillott, 2005; Klowden, 2008).

In this study, it is aimed to investigate whether there are morphological and histological differences in male reproductive organs of three different *Tentyria* species belonging to Tenebrionidae family taken from two different localities (Turkish Republic of Northern Cyprus [TRNC] and İzmir/Bozdağ).

MATERIAL and METHOD

Sample collection

The samples used in the study were collected in İzmir Bozdağ region (21 individuals) and (17 individuals) from Turkish Republic of Northern Cyprus with the help of pence through the floor, from the bottom of the stone and the bottom of the tree. The specimens collected from Bozdağ were diagnosed as *Tentyria rotundata* under the Zeiss Jena stereo microscope, and 15 of them were female and 6 of them were male individuals. In TRNC, two different species were identified *T. cypria* and *T. cylindrica*, 10 male and 5 female individuals from *T. cylindrica*, and 2 male individuals from *T. cypria* (Keskin, 2003; Keskin and Üzüm, 2017) (Fig 1).

Macroscopic Analyses

Before the samples were taken into the dissection, they were labelled containing the date, place and name of species. The samples fixed with a needle in a waxy petri dish and the dissection performed under a Zeiss Jena stereo microscope. First, the elytra lifted with the scissors, cut through to the pronotum, and with the help of pence, it was allowed to separate from the body without damaging the underlying membrane of elytra.

Later, this membrane was cut and the intestines pulled to the side, allowing the gonads to be seen more easily. At this stage, it was differentiated whether the samples are male or female. A sample of testes from each species was taken with insect physiological water and photographed with an Olympus C-7070 camera on an Olympus SZX7 stereo microscope.

Histologic Analyses

All testis samples were fixed in Bouin's solution for 24 hours. Samples were embedded in paraffin. Tissues were sectioned at a thickness of 5-6µ by using the microtome. Mayer's Haematoxylin-Eosin (H&E) was used to stain for tissue section (Presnell and Schreibman, 1997; Öber, 2009). The preparations were photographed using a Zeiss Axio Scope A1 microscope and ZEN image analysis software.

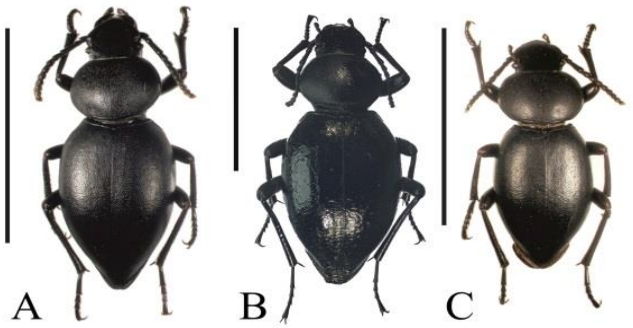

Figure 1. Species of genus *Tentyria.* A) *T. cypria*, B) *T. rotundata*, C) *T. cylindrica* (Bar: 1cm)

RESULTS and DISCUSSION

Macroscopy

As a result of the macroscopic examinations, it was determined *T. cypria*'s testis resembled bunch of grapes. Such a structure was not observed in *T. rotundata* and *T. cylindrical* (Fig 2).

Histology

At the end of the histological examinations, the general appearance of the *T. cypria*'s testis was similar to a four-leafed clover (Fig 3A). Each leaf corresponds to a follicle whereas the testes of *Dendroctonus armandi*, a primitive species of the Coleoptera order, has been examined and it was seen that each of testis is constituted 20 seminiferous tubules.

Follicles of *T. cypria* at the center were not separated by a connective tissue. In general, the outer connective tissue layer is thicker than in the *T. rotundata* (Fig 3B) and *T. cylindrica* (Fig 3C).

In *T. rotundata* and *T. cylindrica*, the general appearance is lobed and each lobe corresponds to a follicle. These follicles are separated from each other by a thin connective tissue (Fig 3).

The stages of spermatogenesis in the testes follicles of three species are clearly visible (Fig. 4). In all three species testes include germ cells, spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa (Fig. 3 and 4). On the base of their size, nuclei and cytoplasm, different spermatogenesis stages can be distinguished in three *Tentyria* species. Primary spermatocytes are formed from spermatogonia by mitotic division and measure 5,02 (±1,5) µm, 5,17 (±0,71) µm and 5,02 (±0,39) µm in *T. cypria*, *T. rotundata*, and *T. cylindrica*, respectively.

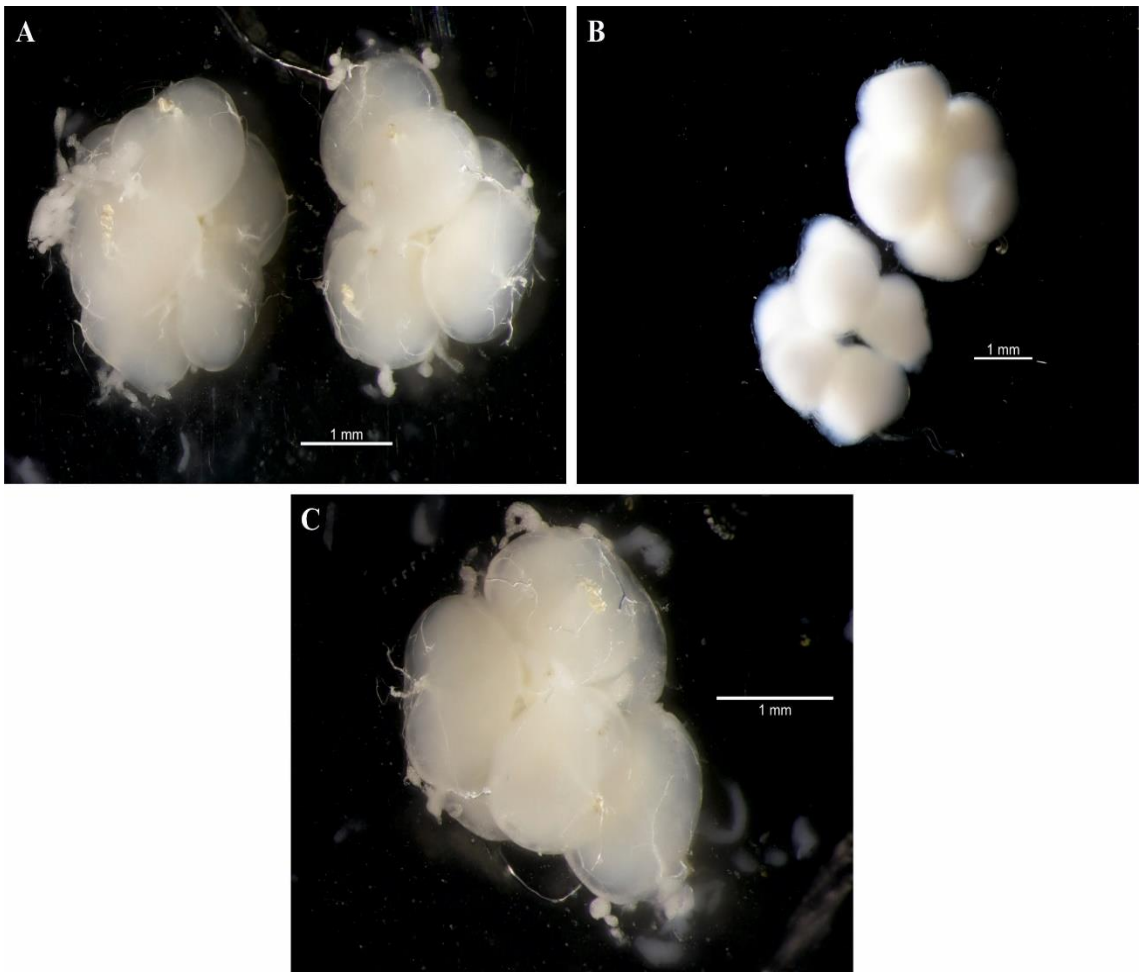

Figure 2. General morphology of testes A) *T. cypria*, B) *T. rotundata*, C) *T. cylindrica*

The secondary spermatocytes were larger than primary spermatocytes. Such *Tentyria* as *cypria* (18,13 [±3,15] µm), *rotundata* (15,47 [±0,92] µm), and *cylindrica* (11,73 [±0,40] µm) (Fig. 4).

Similar to *T. cypria, T. rotundata, and T. cylindrica*, in most insects, testicular tubules of sexually mature individuals are full of sperm cysts where spermatogenesis occurs (Wu et al., 2017). The development of germinative cells in *D. armandi* occurs in cysts and mature sperm are stored in seminal vesicles (Phillips, 1970; Wu et al., 2017). The absence of any deterioration in sperm of mature individuals shows that sperm production may continue until mature (Wu et al., 2017).

CONCLUSION

In this study morphology and histology of testes in *T. cypria*, *T. rotundata* and *T. cylindrica* species were determined for the first time and such studies are important because it will provide support to the basic sciences and help researchers who want to work in this area.

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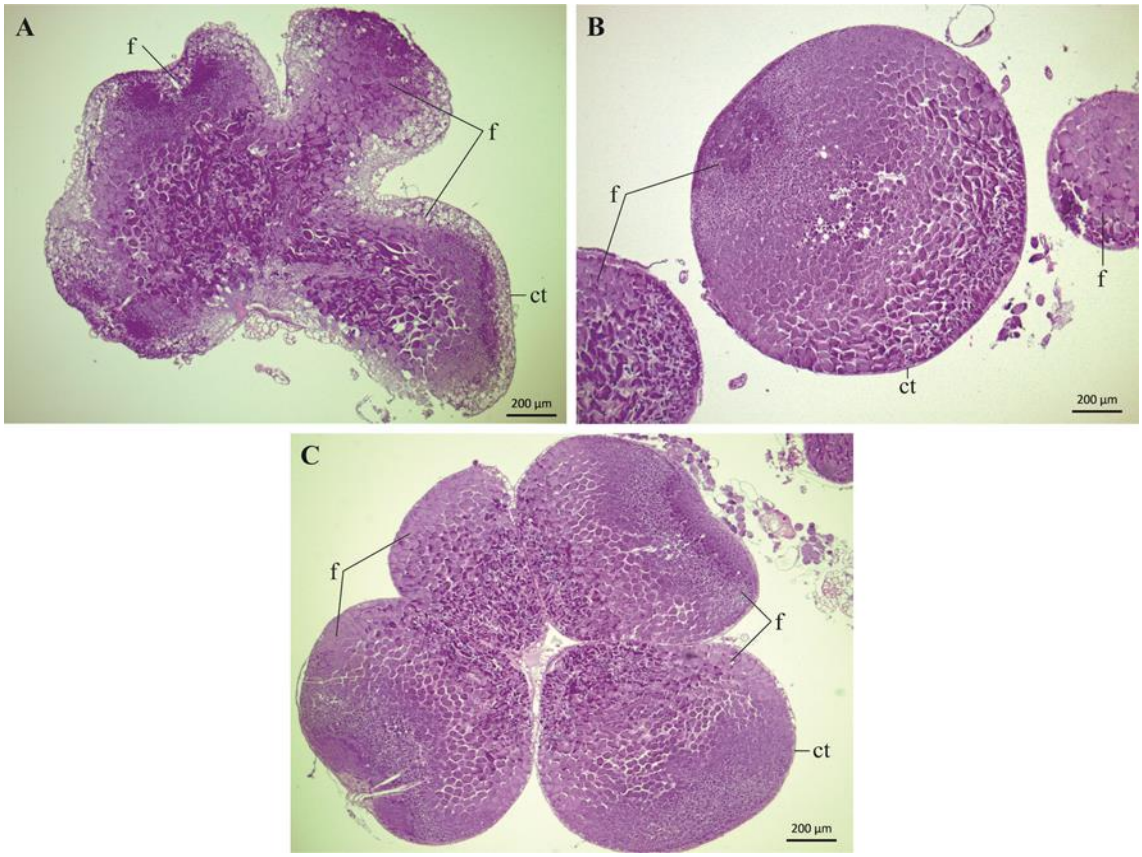

Figure 3. General histology of testes A) *T. cypria*, B) *T. rotundata*, C) *T. cylindrica*. ct: connective tissue, f: follicule.

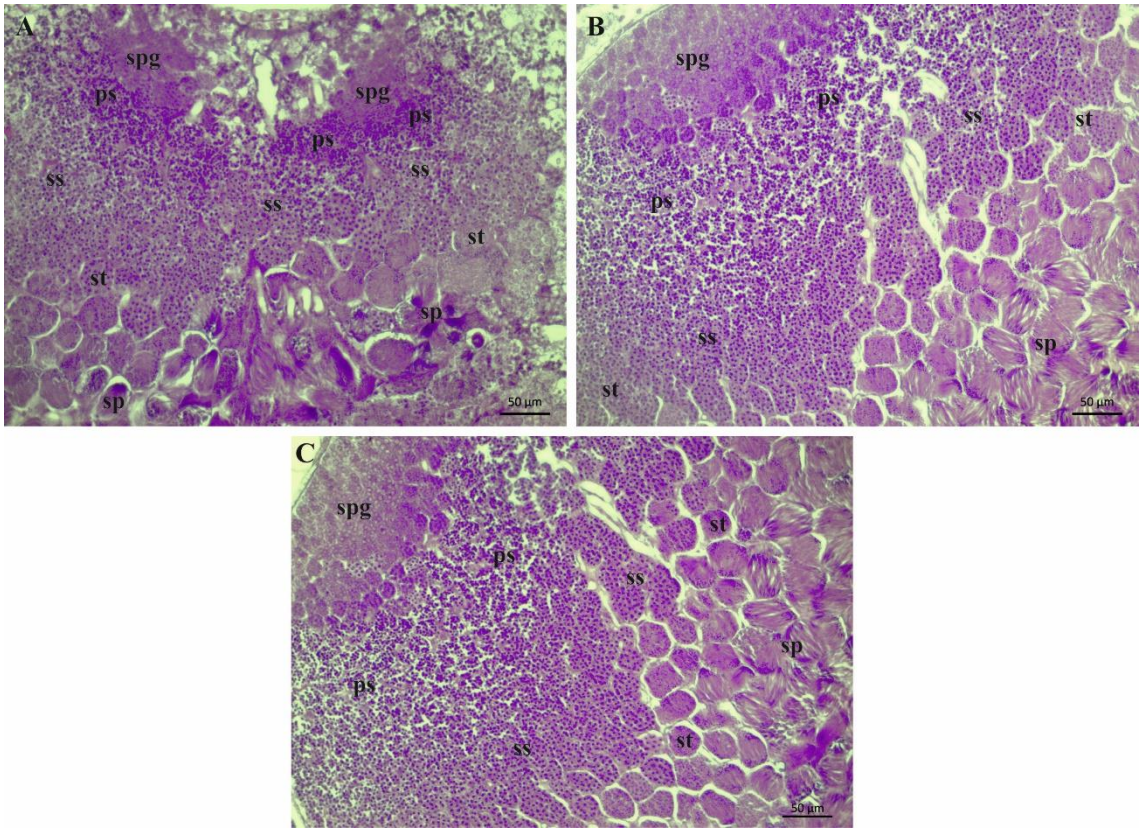

Figure 4. The stages of spermatogenesis in a follicle of testis. A) *T. cypria*, B) *T. rotundata*, C) *T. cylindrica*. ps: primary spermatocyte, sp: spermatozoa, spg: spermatogonium, ss: secondary spermatocyte, st: spermatid.

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