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Histological Description of the Male and Female Gonads in *Tegula eiseni, T. funebralis, T. aureotincta, T. gallina* and *T. regina* from Bahia Tortugas, B.C.S., Mexico

Descripción Histológica de las Gónadas Masculinas y Femeninas en *Tegula eiseni, T. funebralis, T. aureotincta, T. gallina* y *T. regina* de Bahía Tortugas, B. C. S., México

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SUMMARY: Some of the gastropods of the Baja California peninsula are of commercial value, but the majority are not. Among the less commercially valuable species are members of the genus Tegula, which are ecologically important because of their abundance and the trophic level they occupy. Mature specimens of *T. eiseni, T. funebralis, T. aureotincta, T. gallina*, and *T. regina* were collected from two rocky reefs close to Bahía Tortugas, B.C.S. and were processed using a paraffin-embedding technique. All of these species are dioecious, without external sexual dimorphism; in adults, the female gonads are green moss colored while male gonads are cream colored. The gonadal tissue is limited externally by a single ciliated cylindrical epithelium with plentiful granular glandular cells and is located on a connective tissue and muscular fiber layer. This layer is invaginated towards the gonad core, creating radially distributed trabeculae where the germinal tissue is found. The size of the oogonia fluctuates between 10 and 30 µm, and that of the developing oocytes ranges between 30 and 140 µm. In this stage, the cells are pyriform with a peduncle linked to the trabeculae. Mature oocytes have an average size of 165 µm and present a well-defined chorion and a large quantity of vitelline platelets that occupy the whole cytoplasm. In the five species, the development in males is similar until the spermatid stage. In general, the average size of the spermatic cell nucleus is 2.5 µm, while the flagellum length varies from 35 to 45 µm. The species differ in the shape of the acrosome.

KEY WORDS: Gonadic histology; Baja California; Tegula.

INTRODUCTION

Gastropods of the Genus Tegula have a wide range of distribution in temperate and tropical marine environments throughout the world. On the American continents, this taxon is represented by diverse species on the coasts of the Atlantic and Pacific slopes (Turgeon *et al.*, 1998). *Tegula funebralis, T. gallina, T. eiseni, T. aureotincta*, and *T. regina* coexist on the rocky shore of the western coast of the Baja California peninsula in Mexico (Keen, 1971; Abbott, 1974; Morris *et al,* 1980). These species form part of a benthic community characterized by high bioproductivity (Guzmán del Próo *et al.,* 1991; Carreón-Palau *et al.*, 2003), which is a result of the oceanographic characteristics affecting this region (De la Lanza, 1991). Among the prominent studies on this genus are those on the anatomy and histology of the gonad in *T. atra* and *T. tridentata* by Coloma (1974) and Brown (1986), respectively, as well as the study by Watanabe (1984) that undertook an ecological analysis of the effect of predation and competition on population structure in *T. pulligo, T. montereyi* and *T. brunnea*. More recently, studies on larval development under laboratory conditions have been conducted on *T. funebralis* by Moran (1997) and Guzmán del Próo *et al*. (2006); on *T. rustica* by Kulikova & Omel'yanenko (2000); and on *T. eiseni* by research staff at the Northwest Center for Biological Research in La Paz, Baja California Sur, Mexico. Knowledge about the reproductive biology of these species is essential for a better understanding

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of the role each species plays, individually or in concert with other species, in community dynamics. This study presents the results of a histological analysis of the gonads in males and females of five gastropod species of the Tegula Genus.

MATERIAL AND METHOD

An average number of 10 adult organisms (the largest-sized specimens) of *T. eiseni, T. aureotincta, T. regina, T. funebralis,* and *T. gallina* were collected during the months of March and June 2002 and January 2003 in two localities near Bahía Tortugas (Fig. 1). *T. eiseni, T. aureotincta*, and *T. regina* were collected by SCUBA diving in the subtidal zones of two rocky reefs (Morro de Adentro, 27º 38' 57" N, 114º 52' 36" W, and Varadero at Clam Bay, 27º 37' 07" N, 114° 50' 32" W), while *T. funebralis* and *T. gallina* were collected in the intertidal zone of Varadero at Clam Bay. Each specimen was measured and shelled prior to sex determination by visual examination based on the criteria of gonad coloration used by various authors (Sevilla, 1971; Coloma; Belmar-Pérez *et al.*, 1991; Moran; Kulikova & Omel'yanenko) in other species of archaeogastropods. Sex determination results were subsequently confirmed during the histological analysis. Small portions removed from the gonad of each specimen were fixed in 10% formalin in seawater and processed by embedding in paraffin. Sections were cut at 8 µm thick for staining with hematoxylin-eosin and Masson's trichrome (Uría & Mora, 1996).

As part of a parallel line of study, small portions of the gonad of male specimens of *T. funebralis* were fixed in 2.5% glutaraldehyde in seawater, at pH 8, for 48 h, then postfixed with 1% osmium tetroxide (Buckland & Fu, 1986). The tissue was first dehydrated in ethanol and propylene oxide and then permeated with a propylene oxide/epoxy mixture and embedded in Epon 812. Very thin 0.5-µm–thick sections were obtained for staining with toluidine blue.

RESULTS

The five species in this study are dioecious and have a single, conical gonad which is coiled about itself, forming a spiral. Gonadal tissue, from base to apex, is in intimate contact with the digestive gland, forming the conical visceral mass. The shell completely encloses the conical visceral mass, which takes up all of the spire. In the adult stage, the female gonad is moss green in color while the male gonad is cream colored (Fig. 2A). Histological analysis showed that in all five species, the gonad is externally bounded by a single ciliated cylindrical epithelium, rich in granular glandular cells, which rests on a layer of connective tissue and muscle fibers. This layer is invaginated towards the center of the gonad, forming a radial arrangement of trabeculae on which the germinal tissue rests (Figs. 2B and 2C).

Oogenesis. The process of oogenesis was similar in all five species. Table I and Figs. 2D and 2E show the morphological

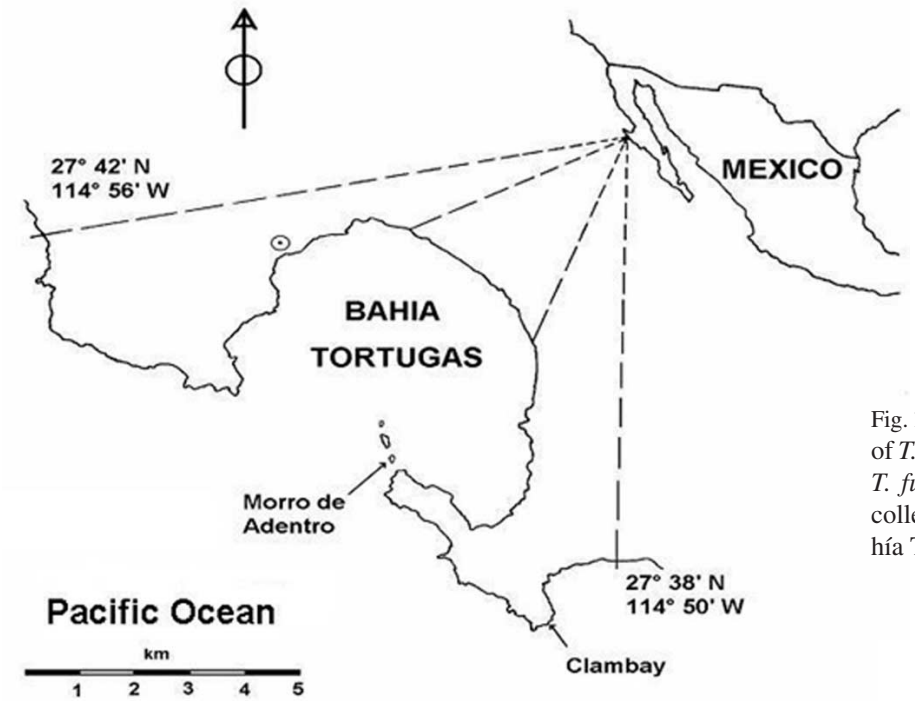

Fig. 1. Study area. The adult organisms of *T. eiseni, T. aureotincta, T. regina, T. funebralis,* and *T. gallina* were collected in two localities near Bahía Tortugas, Mexico.

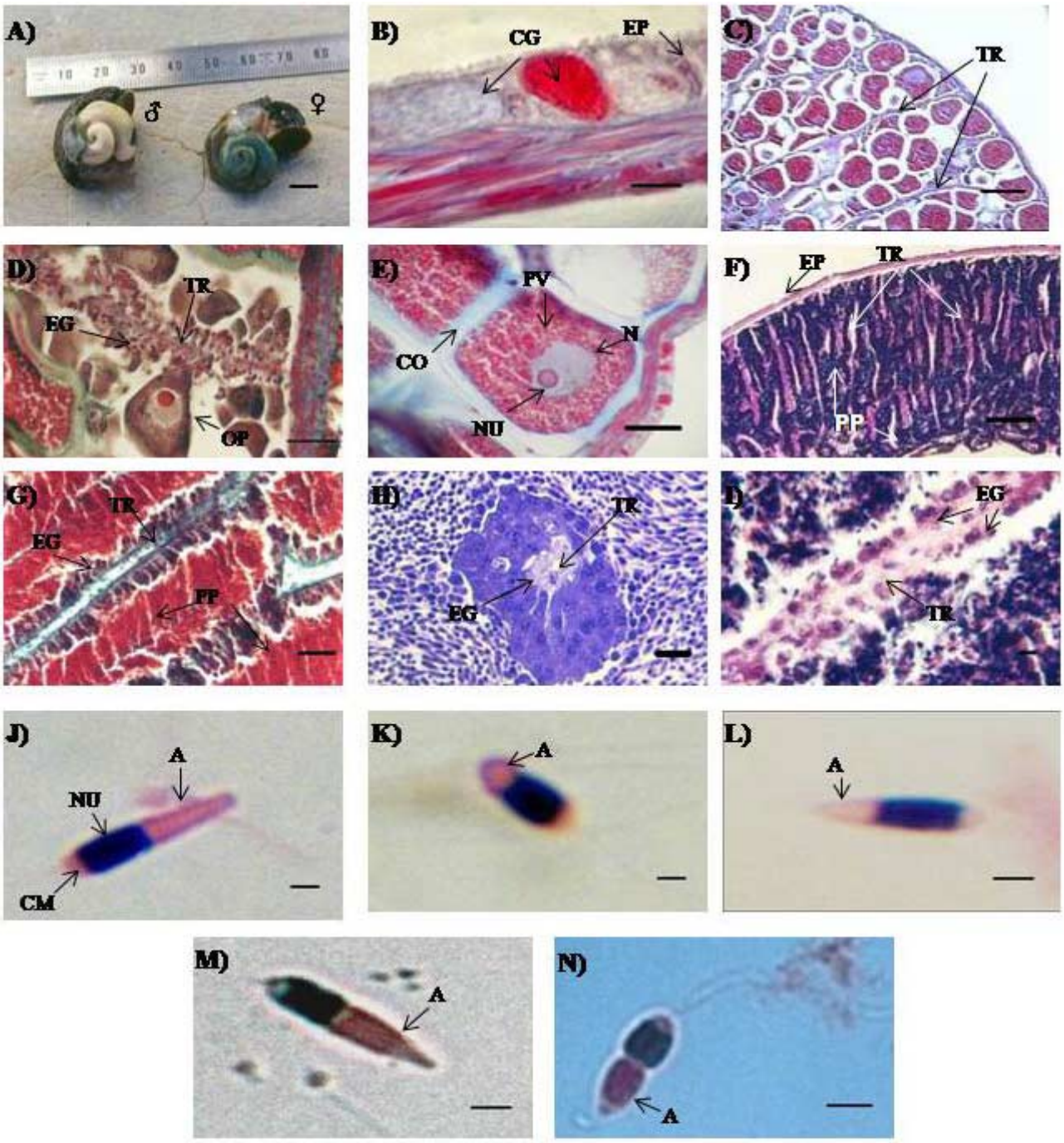

Figure 2. A) Gonad of *Tegula gallina*. Female (moss green) ♀ and male (cream-colored) ♂, bar=10 mm. B) *T. funebralis*. Ciliated cylindrical epithelium (EP) and glandular cells (CG), bar= 50 µm ;C) Ovary of *T. funebralis*. Radial anagement of trabeculae (TR), bar=200µm. D) *T. regina*. Trabeculae (TR), geminal epithelium (EG), pyriform oocyte (OP), bar=1 µm; E) Ovary of *T. gallina*. Mature Oocyte. Chorion (CO), vitelline platelets (PV), nucleus (N), nucleolus (Nu), bar=50 µm. F) Test of *T. aureotincta*. Ciliated cylindrical epithelium (EP), trabeculae (TR), bar=200 µm; G) *T. aureotincta*. Trabeculae (TR), geminal epithelium (EG), spermatozoa the testicle as feathery bundles (PP), bar=20µm; H) Semifine section of test de *T. gallina*. Trabeculae (TR), geminal epithelium (EG); I) Gonad of *T. regina*. Geminal epithelium (EG) and trabeculae (TR), bar= 200µ. J) Espermatozoon of *T. aureotincta*. Acrosome (A), Nucleus (N), mitochondrial collar (CM) ; K) *T. eiseni*; L) *T. regina*; M) *T. gallina* y N) *T. funebralis*, bar=1µm

and histological characteristics defining the different stages of this process.

Spermatogenesis. Spermatogenesis proceeds in an outward direction from the cells of germinal epithelium attached to the trabeculae (Fig. 2F). Development is similar in the five species up to formation of spermatozoa, at which stage differences in size and shape become apparent (Figs. 2G–I). Table II shows the morphological and histological characteristics defining the different stages of this process.

Spermatozoa differ among the five species regarding the shape and size of the acrosome. Mean size of the nucleus is 2.5 µm, and the flagellum, seen as a fine refringent structure, varies in length from 35 to 45 µm. (Figs. 2J–N). Table III describes the differences found in each species with regard to the acrosome.

Table I. Morphological and histological characteristics of oogenesis.

Stage of development	Size interval (µm)	Histological description
Oogonia	10 to 30	Found attached to trabeculae. The nucleus is centrally located; chromatin is granular, and the nucleolus is eccentric.
Oocyte under development	30 to 130	Pyriform cells with a peduncle for attachment to the trabeculae. The cytoplasm is homogeneous and basophilic. The nucleus is centrally located while the eccentric nucleolus is intensely basophilic.
Mature oocyte	130 to 165	Cells characterized by presence of a chorion. The cytoplasm contains acidophilic vitelline platelets; chromatin is scattered throughout the nucleus which contains an eccentric, basophilic nucleolus.

Table II. Morphological and histological characteristics of spermatogenesis.

Stage	Mean size (µm)	Description
Spermatogonia	10	Spherical cells with basophilic cytoplasm. Spherical nucleus. Scattered, finely granulated chromatin takes up most of the cell.
Primary spermatocyte	7	Polarization of nucleus begins; cells are now elongated, and chromatin is condensed.
Secondary spermatocyte	5.5	Cells become smaller. Cytoplasm is scarce; the nucleus is located at one end of the cell; condensed chromatin migrates to peripheral areas of the nucleus.
Spermatid	4.0	The nucleus is eccentric and contains fully condensed chromatin.

Table III. Description of the acrosome in each species.

Species	*Tegula eiseni*	*T. funebralis*	*T. aureotincta*	*T. gallina*	*T. regina*
Shape of acrosome	Spherical structure	Cylindrical, with a border on its apical portion	Elongate cone with a slight widening at its apex	Cylindrical structure, its apex ending in a point	Triangular

DISCUSSION

The general characteristics of the gonads are similar to those reported by other authors (Sevilla; Coloma; Tutschulte & Connell, 1981; Hickman, 1992; Voltzow, 1994; Apisawetakan *et al*., 2001; Muñoz, 2003; Noriega, 2003) in other prosobranchs. The pattern of gonad coloration, moss green in females and cream colored in males, corresponds exactly to observations in other archaeogastropods (Sevilla; Coloma; Belmar-Pérez *et al*., 1991; Belmar-Pérez & Guzmán del Próo, 1992). Similarly, the cylindrical epithelium, rich in glandular cells, which bounds the gonad externally and the connective tissue to form the trabecular system, has been described by these authors in species such as *Haliotis fulgens, Astraea undosa, Tegula atra, T. eiseni, T. aureotincta*, and *T. regina*. Apisawetakan *et al*. pointed out that in *Haliotis asinina*, the trabeculae function as a guide and support for germinal cells. The pattern of gametogenic development in both males and females is also in exact agreement with earlier observations in *Astraea undosa* (Belmar-Pérez *et al.,* 1991), *Haliotis fulgens* (Sevilla; Belmar-Pérez & Guzmán del Próo, 1992), *H. rufescens* (Tutschultte & Connell), and *H. asinina* (Apisawetakan *et al*.), as well as in different species of the same genus, such as *Tegula atra* (Coloma), *T. tridentata* (Brown), and *T. rustica* (Kulikova & Omel'yanenko). The size of mature oocytes in this study (165 µm) is within the size interval found by other authors in these as well as other species. Moran reported a diameter of 165 µm in mature oocytes of *T. funebralis* while Kulikova and Omel'yanenko reported 145 µm in *T. rustica*. Belmar-Pérez & Guzmán del Próo reported an interval of 160 to 250 µm for the diameter of mature oocytes of *Haliotis fulgens*, while Padilla (2004) described a mean size of oocytes in *Megathura crenulata* varying from 133 to 140 µm. The histological structure of the testis follows the same pattern of development as spermatogenic cells, i.e. in an outward direction from connective tissue trabeculae. This observation is similar to reported descriptions for *Tegula atra* (Coloma), *T. tridentata* (Brown), *T. rustica* (Kulikova & Omel'yanenko), and other archaeogastropods such as abalone and other snails (Young & De Martini, 1970; Sevilla; Apisawetakan *et al*.; Belmar Pérez *et al*., 1991). Once mature, spermatozoa remain distributed throughout the testis as feathery bundles. The general histological characteristics of the different types of spermatogenic cells are similar in all five species and are in exact agreement with reports on other species, except for spermatozoa which, as stated in the results section, differ in regard to the acrosome. Measurements are similar to those reported by other authors in these and other species. Moran reported a head size of 9 µm and a flagellum of 40 µm in *T. funebralis.* In *Megathura crenulata*, Padilla found that the spermatozoon head measures 5.6 µm and the flagellum 40 µm. Head length in *Haliotis fulgens* and *Astraea undosa* is 6 µm (Belmar-Pérez & Guzmán del Próo, 1992). Franzén (1956), Healy (1988), and Hickman pointed out that species with external fertilization, such as most of the archaeogastropods, have this type of spermatozoa, which characterizes them as primitive. The acrosome in gastropod spermatozoa shows great variation as to size, composition, and morphology and has therefore been considered a determinant, species-specific character in the taxonomy of the different groups of gastropods. At the specific level, lysins in the acrosomic vesicle dissolve the chorion of mature oocytes, interacting with the lysin receptor on the surface of the oocyte, with which they are associated by coevolution. These factors are determinant for genetic isolation between sibling species coexisting in very close areas (Dohmen, 1983; Haino-Fukushima *et al.*, 1999).

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ORTÍZ-ORDOÑEZ, E.; MENDOZA-SANTANA, E. L.; BELMAR-PÉREZ, J. & PADILLA-BENAVIDES, T. D. Descripción histológica de las gónadas masculinas y femeninas en T*egula eiseni, T. funebralis, T. aureotincta, T. gallina*, y *T. regina* de Bahía Tortugas, BCS, México. ***Int. J. Morphol., 27(3)***:691-697, 2009.

RESUMEN: La costa oriental de la Península de Baja California se caracteriza por ser una zona de alta productividad biológica en la que coexiste un elevado número de gastrópodos, algunos de alto valor comercial y otros, la gran mayoría, no. Tal es el caso de diversas especies del Género *Tegula*, las cuales, ya sea por su abundancia y/o por el nivel trófico que ocupan dentro de la comunidad, resultan relevantes. Ejemplares adultos de *Tegula eiseni, T. funebralis, T. aureotincta, T. gallina* y *T. regina*, fueron colectados en dos arrecifes rocosos próximos a Bahía Tortugas, B.C.S. y procesados mediante la técnica de inclusión en parafina. Las especies estudiadas son dioicas sin dimorfismo sexual externo; la gónada de las hembras adultas es color verde musgo, mientras que la de los machos es color crema. La gónada se encuentra limitada externamente por un epitelio cilíndrico simple ciliado con

abundantes células glandulares granulares, que se asienta sobre una capa de tejido conjuntivo y fibras musculares. Esta capa se invagina formando trabéculas de disposición radial donde se desarrolla el tejido germinal. Las ovogonias presentan un tamaño que fluctúa entre 10 y 30 µm, en tanto que el de los ovocitos en desarrollo varía entre 30 y 140 µm; en este estadio, las células son piriformes con un pedúnculo, por medio del cual se unen a las trabéculas. Los ovocitos maduros tienen un tamaño promedio de 165 µm, presentan un corion bien definido y una gran cantidad de plaquetas vitelinas que ocupan la totalidad del citoplasma. En los machos el desarrollo es similar en las cinco especies, hasta la etapa de espermátide. En general, el tamaño promedio del núcleo de la célula espermática es de 2,5 µm, mientras que el flagelo tiene una longitud que fluctúa entre 35 y 45 µm. La forma del acrosoma es diferente para cada especie.

PALABRAS CLAVE: Baja California; Tegula; Ciclo gonádico.

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