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Morphology and development of the female reproductive system of *Astacus leptodactylus* (Eschscholtz, 1823) (Decapoda, Astacidae)

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Abstract: In this study, the morphology and development of the female reproductive system in *Astacus leptodactylus* (Eschscholtz, 1823) were investigated. The trilobed ovary with 2 anterior lobules and only 1 posterior lobule lie dorsally to the gut on the large hepatopancreas. The oviduct extends laterally from the middle part of the ovary and its innermost layer produces 2 different types of secretion. The outermost layer of the ovary consists of a single layer of thin epithelium and a connective tissue under it. Longitudinally and annularly located collagen fibers are present in the connective tissue, and just under it lies a single layer of follicle cells, which surround the oocytes. The germarium containing oogonia is concentrated in the center of the ovarian lobe as a central germinal cluster.

Key words: Astacidae, crayfish, reproductive biology, ovary

Introduction

The freshwater crayfish *Astacus leptodactylus* (Eschscholtz, 1823), which is harvested as a human food item of high commercial value in Turkey and even more so in western Europe, has been introduced into 14 countries outside its natural distribution because it is more resistant to the crayfish plague fungus, *Aphanomyces astaci*, than is *Astacus astacus* (Erencin and Koksal, 1977; Koksal, 1988; Skurdal and Taugbol, 2001; Harlioğlu and Harlioğlu, 2004; Sağlamtimur, 2007). Natural stocks of the native freshwater crayfish species *Astacus leptodactylus* are severely reduced because of overfishing, water pollution, and plague in most of the inland aquatic areas (Baran and Soylu, 1989; Rahe and Soylu, 1989; Timur et al., 2009). Moreover, *A. leptodactylus* is in danger of extinction like many other crayfish species (Sutcliffe, 2002). Studies on basic information that will enable cultivation are required to support the natural stocks since it is not yet known whether the stocks will recover until the stage before the spread of the plaque (Sağlamtimur, 2007). Astacidea is an infraorder of Decapoda and includes 4 superfamilies: Parastacoidea, Enoplometapoidea, Nephropoidea, and Astacoidea (Hobbs et al., 2007). No study has been conducted on the female reproductive system of Enoplometapoidea yet, whereas there are various studies on the female reproductive system of *Nephrops norvegicus* (Farmer, 1974; Relini et al., 1998) and *Homarus americanus* (Talbot, 1981a, 1981b; Byard and Aiken, 1984), Nephropoidea members *Cherax quinquecarinatus* (Beatty et al., 2005) and

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Cherax quadricarinatus (Sagi et al. 1996; Abdu et al., 2000; Vazquez et al., 2008), Parastacoidea members *Procambarus clarkii* (Kulkarni et al. 1991; Ando and Makioka 1998) and *Orconectes limosus* (Kozak et al., 2007), and members of family Cambaridae included by Astacoidea. The relationship between egg size and female size (Harlioglu and Turkgulu, 2000), importance of sex, individual size and hide size in hide usage (Harlıoğlu and Aksu, 2002; Balık et al., 2005), reproductive efficiency (Berber and Mazlum, 2009), harvesting (Harlioğlu and Harlioğlu, 2004), meat yields (Harlioğlu and Holdich, 2001), morphometric characteristics of crayfish populations in various lakes in Turkey (Erdemli, 1983, 1985, 1987; Güner, 2006), and length-weight relationship (Güner and Balık, 2002) have been studied to date. On the other hand, there is no detailed study on the female reproductive system of *A. leptodactylus*, which is a member of the family Astacidae included by Astacoidea.

As Primavera (1985) and Quinitio et al. (1993) stated, detailed studies on the reproductive system are very important for the development of a complete culture technology and for fishery management (Courtney et al., 1995). The aim of this study was to describe the female reproductive system of *A. leptodactylus* in detail, to analyze the morphology and histology of the ovary and oviduct and the development of the oocytes histochemically, and to compare these features within the Astacidae in order to reveal information about the female reproductive system of this species and to cast light on future studies.

Materials and methods

Eighty (20 for each development stage) adult female specimens of *Astacus leptodactylus* were collected from Lake Terkos, Turkey, at 3-month intervals between October 2009 and July 2010. Once in the laboratory, the total length (from the tip of the rostrum to the tip of the telson) of each specimen was measured to the nearest 0.001 mm with Vernier calipers and the total weight was measured with an accuracy of 0.001 g with a precision balance. Afterwards, the animals were killed by cold treatment (5 min, –20 °C). Lifting the dorsal carapace, the ovary and oviducts were dissected and the ovaries were weighed. The gonadosomatic index (GSI) was calculated (ovary weight / total weight × 100) according to the methods of McRae and Mitchell (1995) and Sagi et al. (1996). The ovary and oviducts were then fixed with Bouin's solution for 12 h at 20 °C and placed in 70% ethanol. The tissues were serially dehydrated in an ethanol series, cleared in xylene, and finally embedded in paraffin. The sections were cut at a thickness of 5 µm, placed onto microscope slides, and stained with hematoxylin and eosin (H&E) for observation of histological structure and with bromphenol blue, periodic acid–Schiff (PAS), and Alcian blue for histochemical analysis under light microscopy (Mazia et al., 1953; Pearse, 1960; Bancroft and Stevens, 1982). The diameter of 500–1000 oocytes from each stage was measured with ImageJ Software.

Results

The female reproductive system of *Astacus leptodactylus* is composed of an ovary and oviducts. The trilobed ovary is located within the cephalothorax and lies on the hepatopancreas dorsally (Figure 1A). The ovary is Y-shaped, consisting of a pair of anterior lobules and a single posterior lobule. From each base of the anterior ovarian lobules, a narrow oviduct protrudes laterally and runs straight to the genital pore on the coxa of the third walking legs (Figure 1B). No distinct seminal receptacles are present throughout the oviducts.

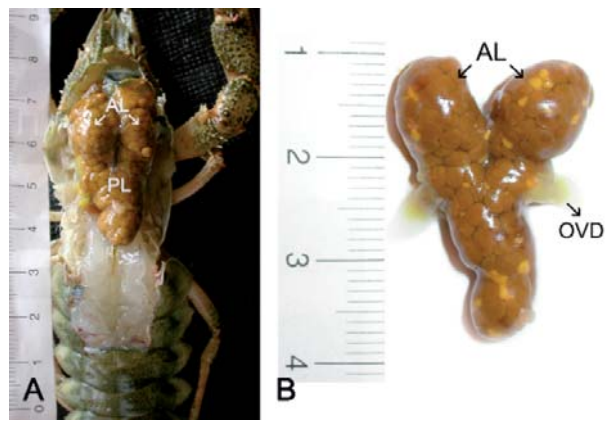

Figure 1. Dorsal view of *Astacus leptodactylus* (Eschscholtz, 1823): A) trilobed ovary is composed of 2 anterior lobules (AL) and a posterior lobule (PL); B) from each base of the anterior ovarian lobules (AL), a narrow oviduct (OVD) protrudes laterally.

The ovary is surrounded by a single layer of thin epithelium as an outermost layer. A connective tissue of monolayer lies under the epithelium. Longitudinally and annularly located collagen fibers are present in the connective tissue. Under it lies a single layer of follicle cells that begins to surround the oocytes in the early stages of development; in the final stage, the oocytes are fully surrounded by them (Figure 2).

The outermost epithelium and connective tissue also continue throughout the oviduct and surround it. The innermost layer of the oviduct is an epithelium composed of a monolayer of columnar cells, which produce 2 different types of secretion. One type, which is in a band form, lies throughout the epithelium that surrounds the lumen, stains with bromphenol blue, and also gives a PAS-positive reaction (Figure 3A). However, the other type, which is spread throughout the inside of the lumen, stains only with bromphenol blue and gives a PAS-negative reaction in contrast to the first type (Figure 3B). Neither the first type nor the second stains with Alcian blue.

The germarium, which is the proliferative zone containing mainly oogonia, is located all along the center of both the anterior and posterior ovarian lobes as a germinal cluster. In the germarium, only a couple of follicle cells begin to surround the oogonia.

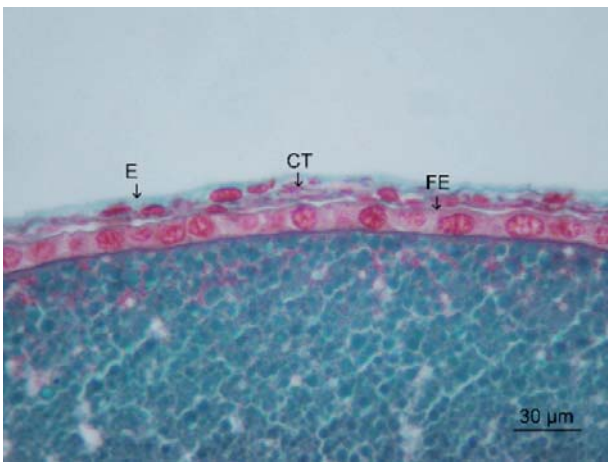

Figure 2. Section showing the layer that surrounds the oviduct of *Astacus leptodactylus* (Eschscholtz, 1823). The outermost layer of the ovary consists of a single layer of thin epithelium (E) and connective tissue (CT) under it. Collagen fibers are present in the connective tissue and under it lies a single layer of follicle epithelium (FE), which stains with Masson trichrome.

As they proceed into the previtellogenic stage, the number of follicle cells increases. When the oocytes reach the vitellogenic stage, they are completely enclosed by a single layer of follicle cells and move to the periphery of the ovary.

Determination of the ovarian development stages is based on the GSI, color and shape, histological structure, and the relative proportion of cellular types and the placement and content of these cells. Consequently, 4 ovarian development stages can be distinguished: proliferation, previtellogenic, vitellogenic, and mature.

Proliferation stage: At this stage, the total length of the individuals is 38.2 ± 4.39 mm, the total weight is 1.45 ± 0.39 g, and the size of the ovary is 0.291 ± 0.037 mm in diameter, on average. This stage represents the onset of ovarian differentiation. At this stage, the ovary is still a transparent sac-like structure that is composed of acini. The walls of the acini that adjoin the outermost epithelium of the ovary consist of a superficial layer of connective tissue surrounding a basement membrane, internal to which are generative cells along with follicle cells. The oogonia, which are generative cells, are 13–20 µm in diameter and are the most representative cellular type at this stage of development. The membrane of the oogonium is not well defined, but the centrally located nucleus, which contains basophilic chromatin material, has a well-defined membrane. Thread-like chromatin proceeds into granular chromatin in the nucleus of the oogonia and the cytoplasm is weakly eosinophilic. While most of the basophilic follicle cells are scattered throughout the acini, some of them begin to surround the oogonia (Figure 4A).

Previtellogenic stage: At this stage, the total length of the individuals is 52.2 ± 8.6 mm and the weight is 3.04 ± 1.23 g, on average. The mean size of the ovary is 1.8 ± 0.68 mm and the mean weight is 0.018 ± 0.005 g (GSI: 0.57). The germarium that includes oogonia and very early previtellogenic oocytes is concentrated as a central germinal cluster in the ovary. Early previtellogenic oocytes (EPVOs) (20–100 µm in diameter) and previtellogenic oocytes (PVOs) arrange radially around the germarium, as the EPVOs are located near the germarium and the PVOs are located peripherally and adjacent to the ovarian wall. The follicle cells begin to surround the

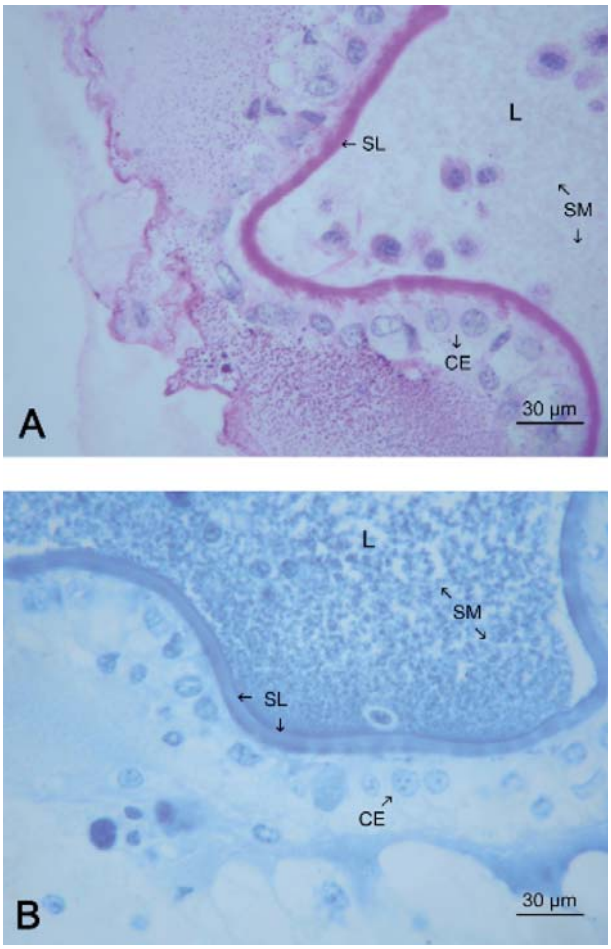

Figure 3. Histochemical structure of the oviduct of *Astacus leptodactylus* (Eschscholtz, 1823). Innermost layer of the oviduct is composed of a columnar epithelium (CE), which produces 2 types of secretion inside the lumen (L). A) The secretion layer (SL), as a band throughout the lumen internally, gives a PAS-positive reaction. Secretion material (SM), which is spread throughout the inside of the lumen, gives a PAS-negative reaction (scale bar: 30 µm). B) The secretion layer (SL) and the secretion material (SM) stain with bromphenol blue (scale bar: 30 µm).

EPVOs, forming a single layer, and the PVOs, which are larger than 100 µm in diameter, are completely enclosed in their own follicle epithelium. Both the EPVOs and PVOs have a large, round, and centrally located nucleus, which contains granular chromatin material. At this stage, accumulation of PAS-positive material at the periphery of cytoplasm, in the intermediate zone beyond the perinuclear region, can be observed in PVOs (Figure 4B).

Vitellogenic stage: At this stage, the total length of the individuals is 80.8 ± 5.08 mm and the total weight is 13.57 ± 2.05 g, on average. The mean size of the ovary is 1.8 ± 0.68 mm and the mean weight is 0.086 ± 0.056 g (GSI: 0.63). The most characteristic cellular type of this stage is the vitellogenic oocyte, which represents the onset of sexual maturity. At this stage, the ovary consists of PVOs (50–150 µm in diameter) situated close to the center and vitellogenic oocytes (200–800 µm in diameter), which are recognized with their basally located nucleus, situated peripherally. Fine yolk granules first appear in the periplasm of the earliest vitellogenic oocytes (about 200–300 µm in diameter). The yolk granules increase in size and number, filling the ooplasm except in the perinuclear zone. These yolk granules are PAS-positive and also stain with bromphenol blue, but do not stain with Alcian blue (Figures 4C and 4D). A number of small lipidic droplets, which are PAS-negative and do not stain with H&E, bromphenol blue, or Alcian blue, also occur in the ooplasm of the vitellogenic oocytes for the first time. As vitellogenesis proceeds, the cytoplasm of the oocytes becomes more heterogenic.

Mature stage: At this stage, the total length of the individuals is 141.3 ± 7.2 mm and the total weight is 60.72 ± 7.94 g, on average. The mean size of the ovary is 3.74 ± 0.42 mm and the mean weight is 3.83 ± 7.94 g (GSI: 6.3). The ovary reaches maximum size just before spawning. At this stage, the ovary is composed of mature oocytes only. The mean size of the mature oocyte is 1512.13 ± 297.38 µm in diameter. Fewer oocytes can be observed in the ovary compared to the other stages of development. The germarium is remarkably reduced in the mature ovary in consequence of developing oocytes. Compared to other stages, larger and more plentiful yolk granules and lipid droplets can be observed in the ooplasm of the mature oocytes. The yolk granules are situated peripherally, closer to the oocyte membrane, while the lipid droplets are spread throughout the entire cytoplasm. As a result of this, the cytoplasm of the mature oocytes is much more heterogenic than the cytoplasm of the oocytes of the earlier stages. Dense yolk granules in the cytoplasm are PAS-positive and stain strongly with bromphenol blue (Figures 4E and 4F).

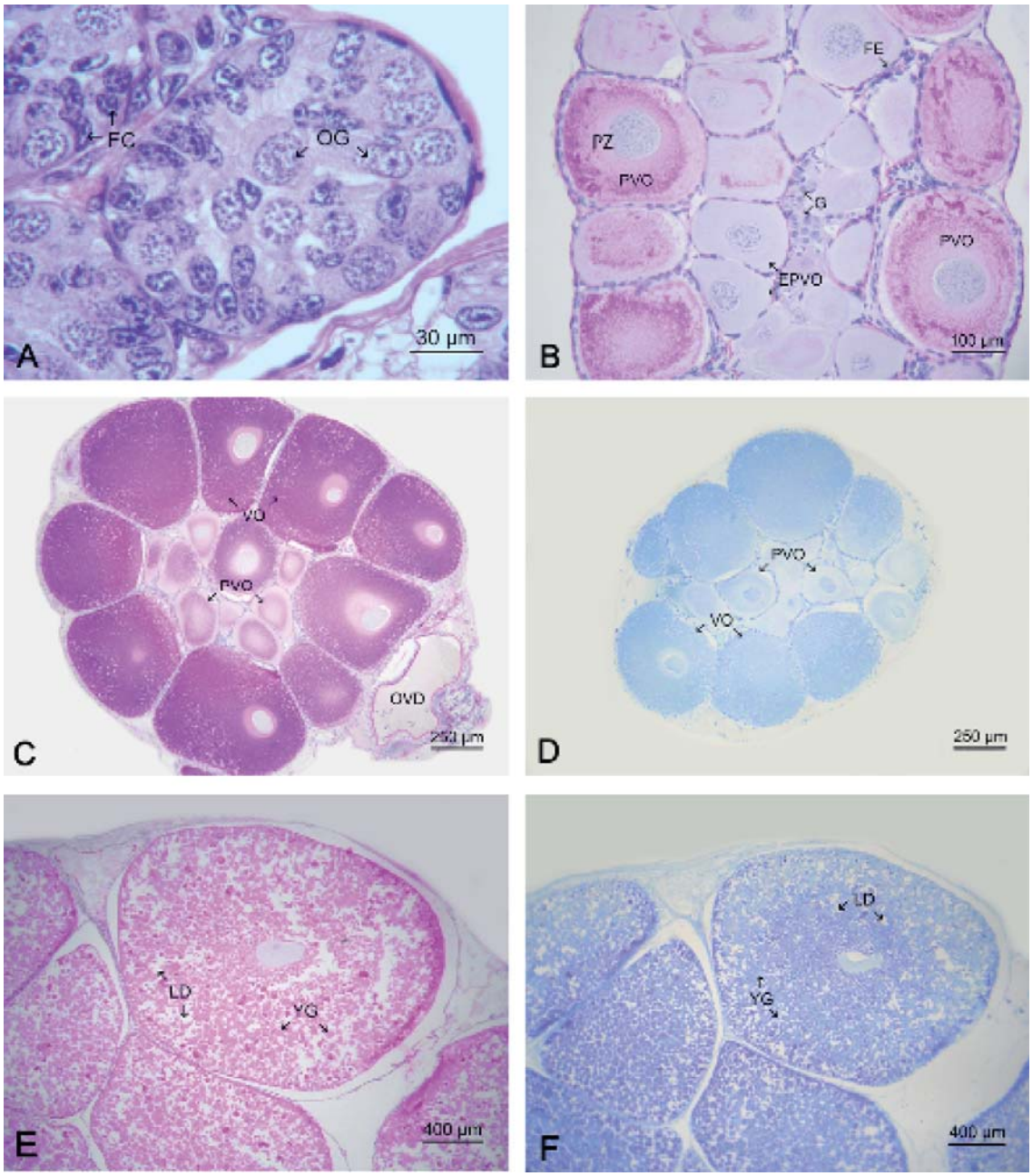

Figure 4. Development stages of the ovary of *Astacus leptodactylus* (Eschscholtz, 1823). A) In the first stage, the ovary, which is composed of oogonia (OG) and follicle cells (FC), shows acinar structure and stains with H&E (scale bar: 30 µm). B) In the second stage the ovary is composed of germarium (G), early previtellogenic oocytes (EPVO), and previtellogenic oocytes (PVO). Only the previtellogenic oocytes (PVO) contain PAS-positive material in their cytoplasm peripherally. The perinuclear zone (PZ) can be distinguished as a PAS-negative ring around the nucleus (scale bar: 100 µm). C) In the third stage, the ovary is composed of previtellogenic oocytes (PVO) and vitellogenic oocytes (VO). Different from the previtellogenic oocytes (PVO), the vitellogenic oocytes (VO) contain PAS-positive material throughout the cytoplasm, except the perinuclear zone. Oviducts (OVD) extend laterally from the middle part of the ovary (scale bar: 250 µm). D) The previtellogenic oocytes (PVO) stain weakly whereas the vitellogenic oocytes (VO) stain strongly with bromphenol blue (scale bar: 250 µm). E) In the fourth stage, the fully mature ovary is composed of only mature oocytes, which contain PAS-positive yolk granules (YG) and PAS-negative lipid droplets (LD) in their cytoplasm (scale bar: 400 µm). F) While the yolk granules (YG) stain with bromphenol blue, lipid droplets (LD) do not stain with bromphenol blue (scale bar: 400 µm).

Discussion

The ovary of *Astacus leptodactylus* is located dorsally on the hepatopancreas, on the ventral side of the heart as in *Procambarus clarkii* of the family Cambaridae (Ando and Makioka, 1998) and *Cherax quadricarinatus* of the family Parastacidae (Vazquez et al., 2008), and is limited to the cephalothorax.

The female reproductive system of the species included in the Decapoda comprises a pair of ovaries and a pair of oviducts attached to the ovaries. The oviducts open to the exterior at the gonopores located at the base of the third pair of legs, and the eggs are released from this location (Krol et al., 1992). The ovary of the crayfish included in families Astacidae, Cambaridae, and Parastacidae is Y-shaped (Adiyodi and Subramoniam, 1983; Krol et al., 1992), while the ovary of *H. americanus* is H-shaped (Talbot, 1981a). On the other hand, during its development stages, the ovary of *C. quadricarinatus* (Vazquez et al., 2008), belonging to the family Parastacidae, is first shaped like 2 parallel bands, is then H-shaped due to the formation of a bond between the bands in the middle, and is finally Y-shaped in the most mature stage due to the partial fusion of the posterior lobes. However, in the present study, it was observed that the ovary of *A. leptodactylus* is Y-shaped from the first stage to the mature stage of development, as in *P. clarkii* (Ando and Makioka, 1998).

When the structure of the ovarian wall is analyzed from the exterior to the interior, it is seen that the ovary of Astacidae species is surrounded by an epithelium from the exterior and there is connective tissue of one layer or more under the epithelium, and the ovarian wall generally lacks muscles. However, while in *C. quadricarinatus* (Vazquez et al., 2008) and *P. clarkii* (Ando and Makioka, 1998) the ovary is surrounded by a thin muscular layer, the ovary of *H. americanus* is surrounded by a developed muscular net (Talbot, 1981a). On the other hand, no muscles exist in the ovary of *A. leptodactylus*, and the outermost layer consists of a single layer of thin epithelium under which there is a connective tissue, which consists of longitudinally and annularly located collagen fibers among the cells. This structure reflects the one generally seen in Astacidae species (Beams and Kessel, 1963; Talbot, 1981a; Adiyodi and Subramoniam, 1983; Ando and Makioka, 1998; Vazquez et al., 2008). These layers, which generally surround the ovary from the exterior to the interior in Decapoda, continue also in the oviduct and surround the whole ovary, as in *A. leptodactylus*.

In Decapoda, the innermost layer of the oviduct facing the lumen generally consists of an epithelium comprising secreting cells. In addition, in the oviduct of many species, there is a spermatheca or seminal receptaculum that enables long-term sperm storing and fertilization (Adiyodi and Subramoniam, 1983; Krol et al., 1992). Many species belonging to the infraorder Astacidea do not include a spermatheca; however, the oviduct of *C. quadricarinatus* includes an epithelium that produces a high level of secretion, provides lubricity during the transit of oocytes, and eases spermatophore destruction (Adiyodi and Subramoniam, 1983; Vazquez et al, 2008). Ando and Makioka (1998) stated that this epithelium may be related to the fertilization that may occur in the oviduct. However, since fertilization in *A. leptodactylus* occurs outside the female reproductive system, as in *C. quadricarinatus*, the present study suggests that the role of oviduct secretion is not related to internal fertilization. Therefore, it is thought that the oviduct epithelium secretion in *A. leptodactylus* not only provides lubricity for the oviduct during the transit of oocytes, but that this secretion, which is thrown out with eggs, may also play such roles as helping the destruction of the spermatophore and the protection of eggs against microorganisms (Erkan et al., 2009). In *A. leptodactylus*, the secretion found in a band shape in the part of the oviduct epithelium facing the lumen stains strongly with bromphenol blue and also gives a PAS-positive reaction, which indicates that the secretion contains protein and carbohydrate. The fact that the secretion that forms the content of the lumen outside this band stains only with bromphenol blue indicates that there are substances that contain protein in this part. Since the secretion layer and secretion substance in the lumen do not stain with Alcian blue, it is thought that neither contains sulfated acid mucopolysaccharide.

The oogonia are first observed in the germinal epithelium included in the connective tissue (King, 1948; Adiyodi and Subramoniam, 1983; Bell and Lightner, 1988; Krol et al., 1992). In decapods, the location of the regions where the germinal

epithelium exists in the ovary varies among species (Adiyodi and Subramoniam, 1983). Five types of germarium regions are observed: 1) throughout the ovary periphery, 2) in the shape of a thin band in the lateral periphery or ventral periphery, 3) in the form of a germinal band in the center, 4) in the form of germ clusters and scattered throughout the ovary, and 5) in the form of clusters only in the ovary periphery. The germarium mostly exists in the form of clusters in the periphery or center of the ovary. In *A. leptodactylus*, the germarium is located along the ovarian lobes in the form of a central band, as in *H. americanus* (Kessel, 1968), *Nephrops norvegicus* (Farmer, 1974), and *P. clarkii* (Ando and Makioka, 1998). Where *C. quadricarinatus* is concerned, during the development of the ovary the bridging part that links the lobes of the H-shaped ovary lacks a germarium and there exists only oocytes in this part (Vazquez et al., 2008).

While there are studies on the ovary and oocyte development in Astacidea, it is seen that the staging criteria for the development of the ovary is different from one study to another (Abdu et al., 2000; Kozak et al., 2007; Cabiddu et al., 2008). In this study, considering the staging criteria for the ovary and oocytes in *A. leptodactylus* and the forms of staging inferred from the studies conducted on the species belonging to the infraorder Astacidae (Charniaux-Cotton, 1974; Sagi et al., 1996; López et al., 1997), 4 main stages of development have been determined by investigating not only the morphological features such as general ovarian size, shape, color, GSI, and oocyte diameter, which provide limited information about the reproductive system and its development, but also histological and histochemical findings that give more detailed information (Cuzin-Roudy and Amsler, 1991; Courtney et al., 1995; McRae and Mitchell, 1995; Sagi et al., 1996; Abdu et al., 2000).

In *A. leptodactylus*, at the first developmental stage the ovary is in the form of a small transparent sac, as in *C. quadricarinatus* (Vazquez et al., 2008), and oogonia exist in clusters in the ovary (Abdu et al., 2000; Vazquez et al., 2008). Likewise, the oogonia in *P. clarkii* (Beams and Kessel, 1963; Ando and Makioka, 1998) and *C. quadricarinatus* (Abdu et al., 2000) contain thread-like and granular basophilic chromatin material in their very large nuclei and round basophilic follicle cells are scattered among the oogonia, which is very similar to *A. leptodactylus*. In addition, at this stage, the movement of the chromatin material with a filamentous pattern toward the chromatin with a granular structure and the weak eosinophilic nature of the cytoplasm are similar to *C. quadricarinatus* (Abdu et al., 2000). In the study by Vazquez et al. (2008) on *C. quadricarinatus*, at the first stage of the ovarian development the existence of PVOs is mentioned, while in *A. leptodactylus* no PVOs are present at the first stage; there exist only oogonia, which are in the reproduction phase, and young follicle cells. The fact that the oocytes and follicle cells at this stage gives PAS-negative reactions and do not stain with bromphenol blue further indicates that they do not contain carbohydrate and protein yet.

At the second stage of development, in the ovary of *C. quadricarinatus* vitellogenic oocytes begin to appear (Vazquez et al., 2008), while the largest cells seen in the ovary of *A. leptodactylus* at the same stage are still PVOs. The size of the oocytes at this stage is similar to the oocytes at the same development stage of both *C. quadricarinatus* (Abdu et al., 2000; Vazquez et al., 2008) and *P. clarkii* (Ando and Makioka, 1998). The oocytes determined as early and late perinuclear stage oocytes were defined as early and late perinuclear oocytes by Abdu et al. (2000). In *A. leptodactylus*, at this stage there is an annular region around the nucleus of the oocytes that gives a PAS-negative reaction. This region is called the perinuclear zone in *Procambarus* sp. (Beams and Kessel, 1963) and in *C. quadricarinatus* (Abdu et al., 2000), as well. In addition, the fact that the PVOs of *A. leptodactylus* at this stage give PAS-positive reactions but do not stain with bromphenol blue indicates that these cells begin to store carbohydrate before protein and lipid, as in *C. quadricarinatus* (Abdu et al., 2000).

It was reported that at the third developmental stage, the number of PVOs and the number of vitellogenic oocytes in the ovary of *C. quadricarinatus* were equal but that the number of mature oocytes was very low (Vazquez et al., 2008). There are a small number of PVOs and a large number of early and late vitellogenic oocytes in the ovary of *A. leptodactylus* at the same stage and no mature oocytes were observed. This stage of development represents the beginning

of sexual maturity, as in *C. quadricarinatus* (Abdu et al., 2000; Vazquez et al., 2008). At this stage, the vitellogenic oocytes in *A. leptodactylus* are similar to those in *P. clarkii* (Ando and Makioka, 1998) in terms of size and are larger than those in *C. quadricarinatus* (Abdu et al., 2000; Vazquez et al., 2008). The fact that the yolk material that accumulates in the cytoplasm of the vitellogenic oocytes in *A. leptodactylus* during vitellogenesis stains with bromphenol blue and gives a PAS-positive reaction indicates that it is of protein and carbohydrate origin. It is thought that the droplets that are seen in *A. leptodactylus* for the first time at this stage, which do not stain with bromphenol blue or Alcian blue and give a PAS-negative reaction, may have lipid-like characteristics (O'Donovan et al., 1984; Cuzin-Roudy and Amsler, 1991; Kozak et al., 2007). While the observation of lipid droplets first in the cytoplasm of the oocyte represents the beginning of primary vitellogenesis in *Penaeus japonicus* (Yano, 1988) and *Ranina ranina* (Minagawa, 1993), it indicates the beginning of late vitellogenesis in *Penaeus indicus* (Sunilkumar and Diwan, 1994) and the last stage of primary vitellogenesis in *C. quadricarinatus* (Abdu et al., 2000). Kulkarni et al. (1991) did not mention lipid droplets in the definition of vitellogenesis in *P. clarkii.* However, Ando and Makioka (1998) stated that lipid droplets were present in the cytoplasm of the largest PVOs in *P. clarkii.* As a result, lipid is mostly observed first in the vitellogenesis stage, which is consistent with the results of the present study.

As stated in numerous studies, the fact that the vitellogenic oocytes give more intense reactions with PAS compared to the oocytes in earlier stages indicates that the accumulation of substances that contain carbohydrate is more intense compared to the earlier stages, and the fact that the PVOs do not stain with bromphenol blue while the vitellogenic oocytes stain with it indicates that protein accumulation starts at this stage. As the oocytes at this stage do not stain with Alcian blue, they therefore do not contain sulfated acid mucopolysaccharide. Abdu et al. (2000) studied how the oocytes of *C. quadricarinatus* stained with bromphenol blue at this stage when vitellogenesis began and stated that the parts of the oocyte cytoplasm that were close to the ovarian wall stained darker; this might suggest that yolk precursors were taken to the oocyte through endocytosis. Since a similar form of staining is seen in the same type of vitellogenic oocytes of *A. leptodactylus* at the same stage, the present authors think that the yolk precursors in *A. leptodactylus* may be taken to oocytes through endocytosis.

Compared to the other stages of development, the fourth stage shows more similarity to *C. quadricarinatus* (Abdu et al., 2000; Vazquez et al., 2008) and *P. clarkii* (Ando and Makioka, 1998) in terms of morphology and histology. In *A. leptodactylus*, the dominant cell type in the ovaries at this stage is the mature oocyte and the germarium is no longer observed. The oocytes at the fourth stage of development represent the fully mature ovary, store yolk intensely, and are ready for spawning, as in *C. quadricarinatus* (Abdu et al., 2000; Vazquez et al., 2008) and *P. clarkii* (Ando and Makioka, 1998). At this stage, the mature oocytes of *A. leptodactylus* have a similar size to the mature oocytes of *Virilastacus araucanius* (Rudolph and Rojas, 2003), *Orconectes limosus* (Kozak et al., 2007), and *C. quadricarinatus* (Abdu et al., 2000; Beatty et al., 2005; Vazquez et al., 2008). However, *A. leptodactylus* is most similar to *P. clarkii* (Ando and Makioka, 1998) with respect to this feature. It was observed that in the mature ovary of *A. leptodactylus*, as in *C. quadricarinatus* (Abdu et al., 2000), in the cytoplasm of the mature oocytes the yolk granules are larger in size and number compared to the oocytes at the earlier stages. The yolk granules are intensely present in the periphery of the oocytes' cytoplasm, closer to the cell membrane as in *C. quadricarinatus* (Abdu et al., 2000; Vazquez et al., 2008). It is seen that in *A. leptodactylus* some yolk granules in the cytoplasm of the oocytes at this stage give PAS-positive reactions and stain more strongly with bromphenol blue compared to the earlier stages, as in *C. quadricarinatus* (Abdu et al., 2000) and *P. clarkii* (Ando and Makioka, 1998). Moreover, GSI analyses show that the development of oocytes and the weight of the ovary are in harmony with each other at every stage of development and the GSI of gonads reaches the maximum value at the most mature stage, which suggests that the growth and quality of the oocyte are not affected by any external factors (Courtney et al., 1995; Rodríguez-González et al., 2006).

The new information that we have gained through this study about the structure and development of the

ovary in *A. leptodactylus*, which is an economically important local species, will contribute to the understanding of the basic structure and function of the ovary in Decapoda and will help explain the phylogenetic relations of *A. leptodactylus* by throwing light on future studies.

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