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Original Article

Gonad morphology and histology of an endemic tooth-carp, *Aphanius sophiae* (Heckel, 1847) from Iran

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Abstract: This study presents the first details on morphological and histological characteristics of gonads and gonadal development stages of an endemic tooth-carp, *Aphanius sophiae* (Heckel, 1847) from a spring-stream system (south of Iran). The sampling was done from March 2012 to March 2013 using dip net, and a total of 223 individuals were collected. The gonads of specimens were removed, and then fixed in 10% formalin solution after checking their morphology and measuring their weights, lengths and widths. Based on the size, shape and weight of the gonads, degree of occupation of the body cavity, presence or absence of ripe oocytes or milt, diameter of the oocytes in the ovary, and histological observations, five stages of sexual maturation in females and males were determined by macroscopic and microscopic criteria. The results of the gonadal stages indicated that *A. sophiae* spawns at the beginning of spring.

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Introduction

The species of the old-world tooth-carps, *Aphanius* (Teleostei: Cyprinodontidae) typically thrive in costal and freshwater environments along the Mediterranean Sea, Red sea, Persian Gulf and Arabian sea (Clavero et al., 2007; Esmaeili et al., 2014, 2016). Most species tolerate a wide range of temperature and salinity regimes, and their small size permits viable population to persist in restricted habitats (e.g., Wildekamp, 1993). The genus *Aphanius* is the only genus of Cyprinodontidae available in Iran which is represented by 14 extant and one fissile species (Esmaeili et al., 2016, 2017). *Aphanius sophiae* is an endemic of Iran that inhabits in the Kor River basin, Fars Province, Southern Iran.

The reproductive biology and gonad histological changes of several species of cyprinodontid fishes had been studied by Leonardos and Sinis (1999), Keyvani and Soofiani (2004), Esmaeili and Shiva (2006), Monsefi et al. (2009), Güçlü and Küçük (2008) and Karsli and Aral (2010). The genus is sexually dimorphic (Berra, 2001; Esmaeili et al., 2012; 2014; 2016; Gholami et al., 2014, 2015a, b; Teimori et al., 2014). As no study on the gonad histology of *A. sophiae* has been published, therefore this study presents the first detailed description of gonad histology of this tooth-carp fish.

Materials and Methods

A total of 223 *A. sophiae* specimens were monthly collected during one year from March 2012 to March 2013 using 1 mm mesh-size dip net from Beiza spring-system in Denjan village (Beiza), Kor River basin, Fars Province (29˚57′47.5"N, 52˚24′12"E, alt. 1832 m asl). All collected specimens were deposited in the Zoological Museum, Collection of Biology Department, Shiraz University (ZM-CBSU). The biometry of specimens was carried out using a digital scale and a vernier caliper with accuracies of 0.001 g and 0.01 mm, respectively. For histological studies, the specimens were dissected and their ovary or testis were removed. The sexes and stages of sexual maturation were determined as possible as by naked eye examination and under a compound microscope

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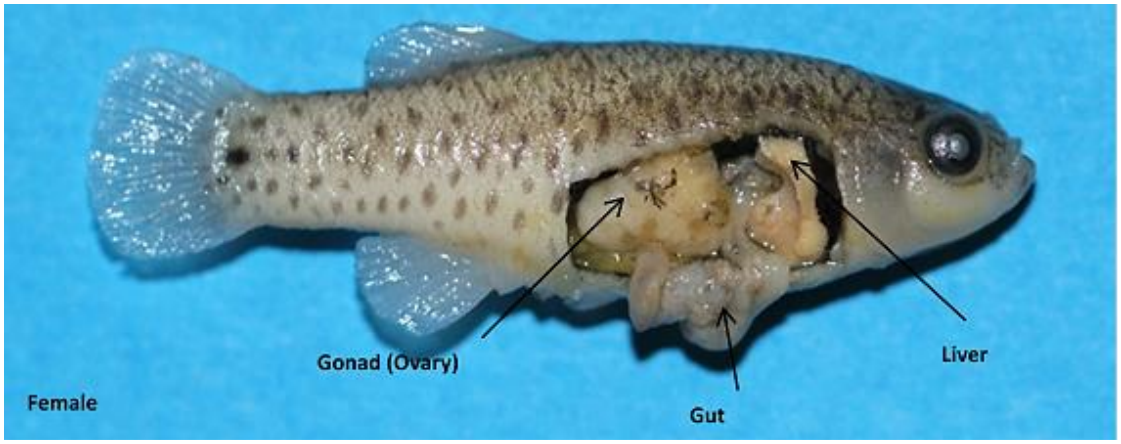

Figure 1. Position of the ovary in *Aphanius sophiae*, from Kor River basin, Fars Province, Iran.

(Olympus). Weight, length, width, color, and shape of each gonad were recorded and the maturity stage of them was recognized macroscopically based on Nikolsky (1963). The histological sections of ovary or testis of each maturing stages were prepared by routine histology method (Bancroft and Stevens, 1991; Mirghiyasi et al., 2016; Eagderi et al., 2013) as follow: they dehydrated in alcohol, cleared in xylene, imbedded in paraffin wax at 56°C melting point, sectioned at 5-7 μm thickness, and then the sections were stained by Hemotoxylin and Eosin (H&E). The histological slides were studied under a light compound microscopy and their pictures were taken by a compound microscope equipped to a digital camera.

Results

Characteristics of ovaries: The ovary is composed of 2 sac shaped parts extending along the body cavity in a dorsal position above the intestine (Fig. 1). In the mature individuals, eggs could easily be seen (beneath the ovarian membrane). Ovaries were in white color during non-spawning periods, and dirty yellow or gold yellow during the spawning period. The ovary composed of follicles that derived from the germinal epithelium and contain oogonia that develop into oocytes and ultimately ova, and the surrounding follicular epithelium (Guraya, 1988; Selman Wallace, 1989). Based on the histological characteristic, ovaries are classified into five stages as follow:

Stage I (Oogonia and chromatin nucleolar stage): In the chromatin nucleolus stage, oocytes were small spherical cells with a thin indistinct peripheral zone (primordial follicle) having strongly basophilic cytoplasm. The nucleus was spherical and large. The ratio of nucleus to cytoplasm was high, and a very thin layer of connective tissue originative from the ovarian, surrounded each oogonia (Fig. 2).

Stage II (Perinuclear stage): In perinuclear stage, the growing follicles exhibited a weak basophilic cytoplasm. The ovary was composed of nests of oocytes of chromatin nucleolus stage and perinuclear stage of different sizes which increased both their cytoplasmic mass and nuclear volume. The nucleoli appeared randomly at various depths in the ooplasm. This stage was representing the oocytes with average diameters of 0.122 mm (Fig. 2).

Stage III (Yolk vesicle (Cortical Alveoli) stage): The size of ovaries in this stage was enlarged. The cytoplasm became weekly basophilic and the nucleus still occupied a central position and it was contained a lot of nucleoli attached to the nuclear membrane; number and size of cortical alveoli increased arranging in two distinct layers; one close to the nuclear membrane and the other one located near the oocytes membrane. The yolk globules were formed in this stage with mostly oval or round in shape. In this stage, zona radiate was surrounded by zona granuloza and a thin external follicular layer of theca cells. The average diameter of oocytes was 0.202 mm (Fig. 3).

Stage IV (Vitellogenic stage): The most perennial phase of oogenesis was the vitellogenic phase. The ovary was filled mainly with previtellogenic and vitellogenic oocytes in different stage of yolk

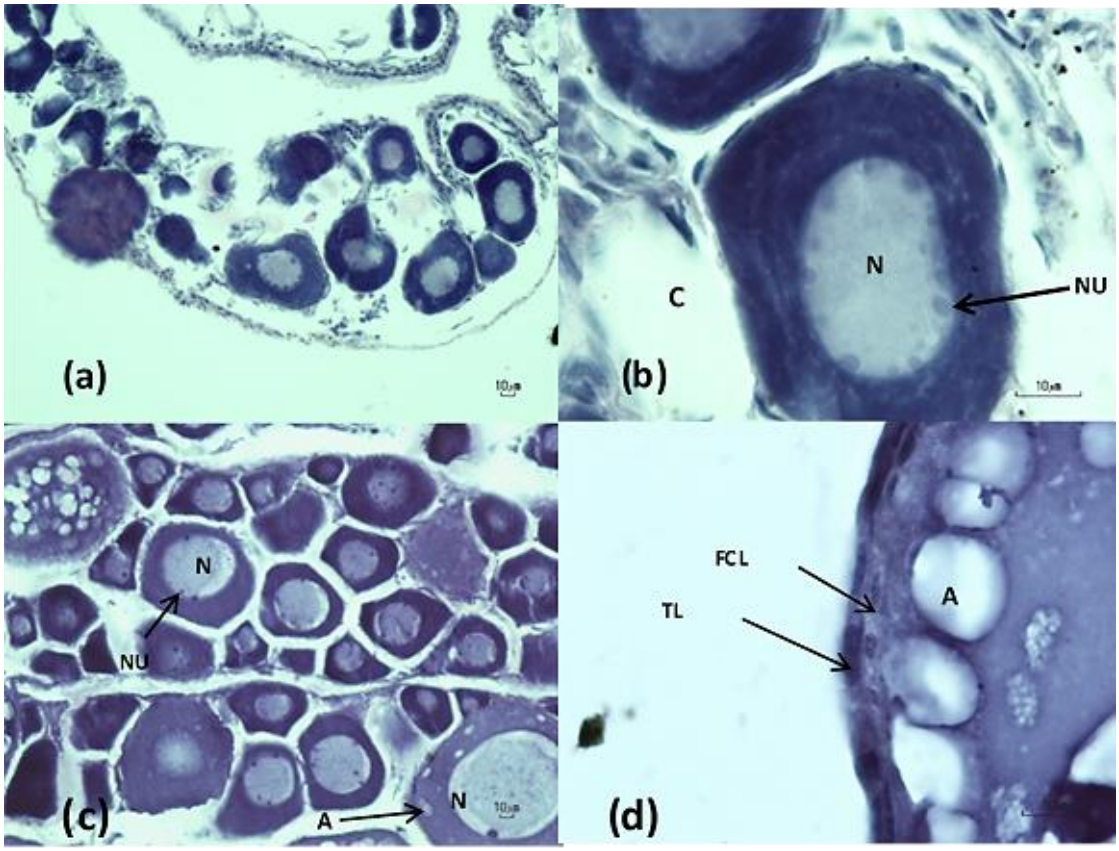

Figure 2. Microphotographs of the ovaries in *Aphanius sophiae* in different stages. (a and b) stage I (Chromatin nucleolar phase), and (c and d) stage II (Perinucleolar phase). N: Nucleus, Nu: Nucleoli, A: Alveoli, TL: Theca Layer, FCL: Follicular cell Layer, ZR: Zona Radiata.

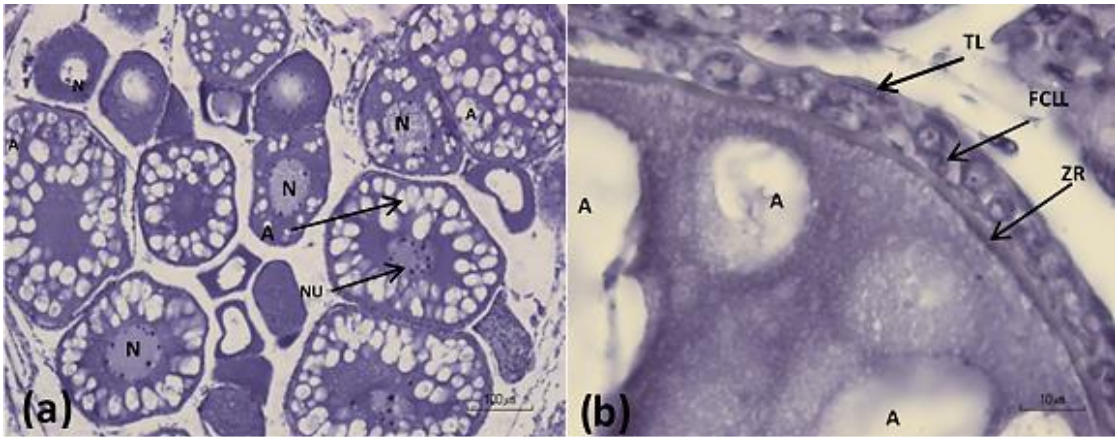

Figure 3. Microphotographs of the ovary in *Aphanius sophiae* in stage III (cortical alveolar phase). N: Nucleus, Nu: Nucleoli, A: Alveoli, TL: Theca Layer, FCL: Follicular cell Layer, ZR: Zona Radiata.

deposition, and the nucleus was slightly displaced from the central position to the animal pole. At the end of this stage, cytoplasm started to fill-up with yolk granules (proteins) and formation of fat vacuoles that reached their maximum size. The zona radiate was more conspicuous. The oocyte average diameter was 0.405 mm (Fig. 4).

Stage V (Maturation stage): This stage was distinguished by migration of the nucleus to the animal pole where it remained, but the nucleus membrane was disintegrated. During the migration of nucleus, it began to liberate its substances into the cytoplasm. The hydrated oocytes were spherical in shape and contain single yolk mass and large lipid droplets. The development of the eggshell was completed with the completely thickness of the zona radiate, and the outer layer (follicular epithelium) became ruptured. The oocyte average diameter reached 0.526 mm which was

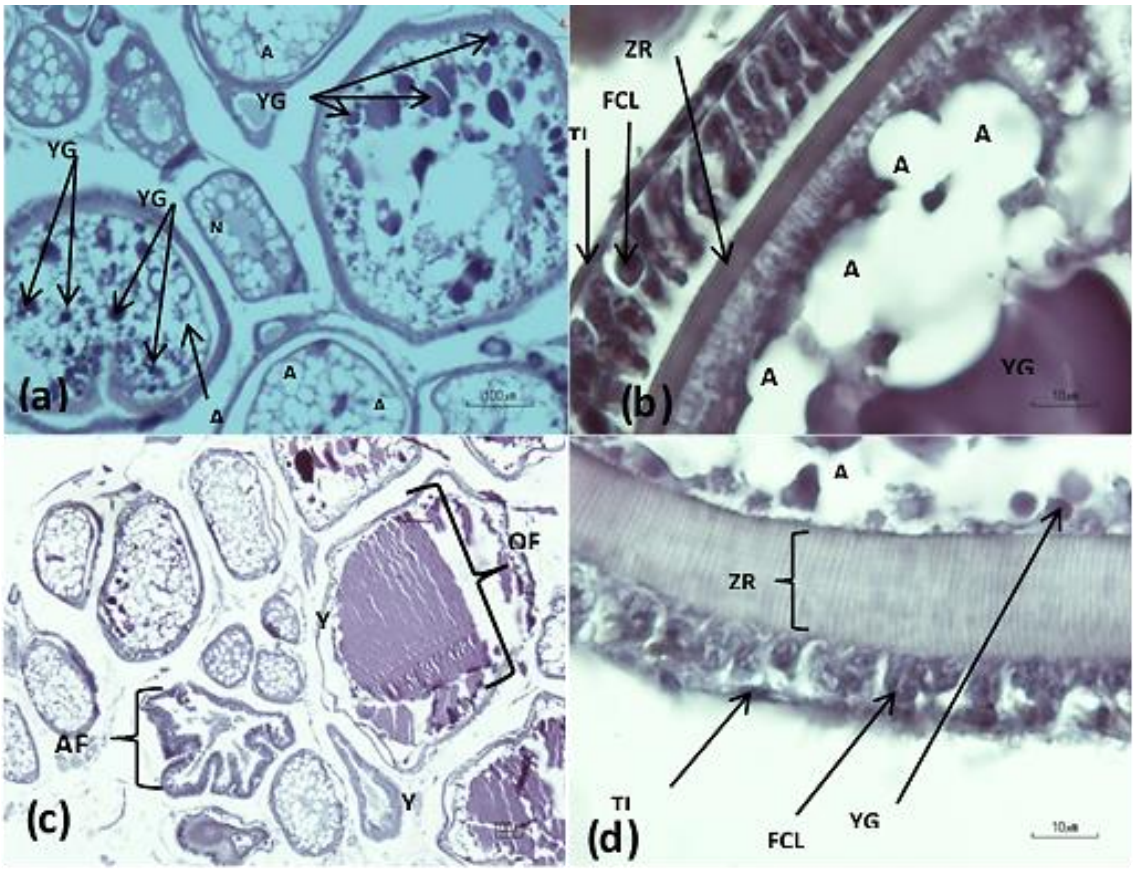

Figure 4. Microphotographs of the ovary in *Aphanius sophiae*. (a and b) stage IV (vitellogenic phase) and (c and d) stage V (Maturation phase). N: Nucleous, Nu: Nucleoli, A: Alveoli, TL: Theca Layer, FCL: Follicular cell Layer, ZR: Zona Radiata, YG: Yolk Globule, AF: Atretic Follicle, HYM: Homogeneous Yolk Mass.

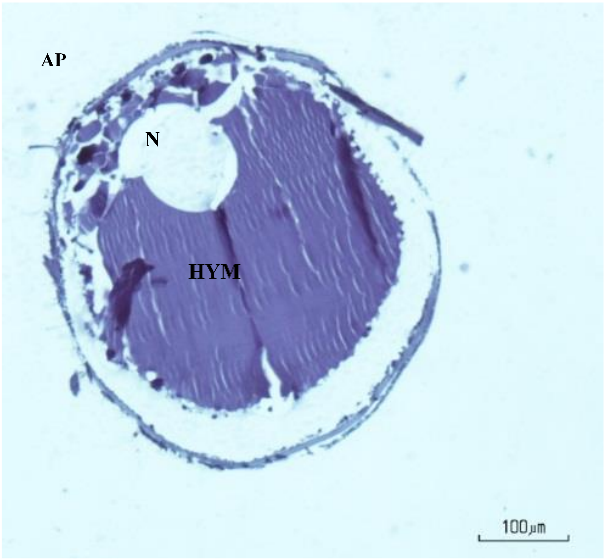

Figure 5. Microphotographs of a single mature oocyte of *Aphanius sophiae*. Nucleus migrated to the animal pole and oocyte contained single yolk mass. AP: Animal Pole, HYM: Homogeneous Yolk Mass, N: Nucleus.

the maximum size of the oocytes during the oogenesis (Fig. 4).

Characteristics of testes: The pair testes of *A. sophiae* are an elongated milky white organ that have two lobes composed of numerous lobules that connective tissue surrounding the testicular surface as capsule (Fig. 6). The immature fish have a very thin, steak like organ which become steadily thicker and bigger as the mature. In mature fish, the organ becomes creamy in color. Based on the histological characteristic, testes are classified into five stages.

Stage 1: Spermatogonia and primary spermatocytes were the dominant cells. The number of primary spermatogonia had increased which was parallel to the testicular development. Spermatogonia had a light cytoplasm and a large nucleus. Primary spermatocytes were smaller than spermatogonia and had a dense nucleus covered with small and pale cytoplasm. The secondary spermatocyte was smaller than primary spermatocyte (Fig. 7a).

Stage 2: Spermatogonia, primary and secondary spermatocytes were more remarkable in the histological sections. Secondary spermatocytes were

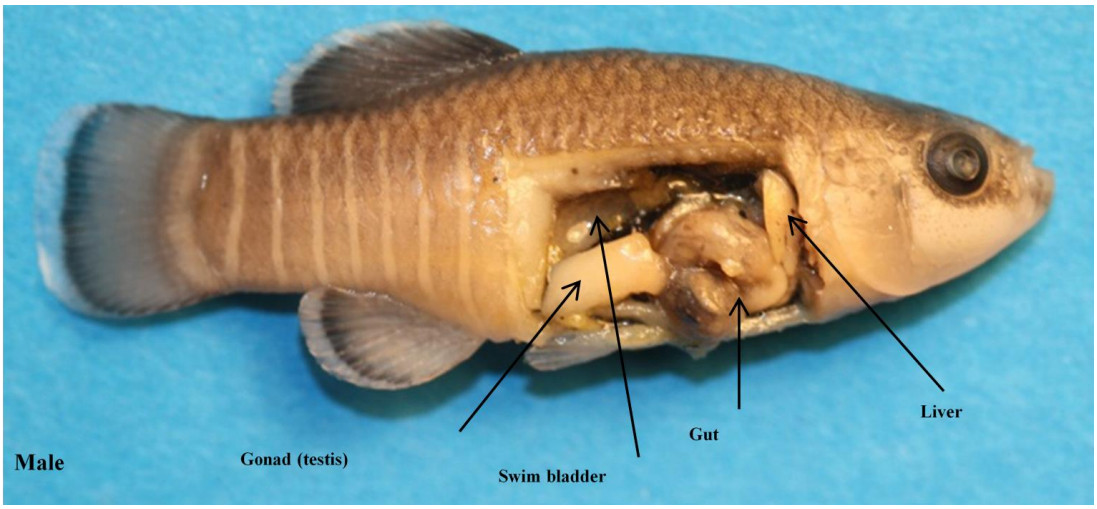

Figure 6. Position of testes in *Aphanius sophiae*, from Kor River basin. Fars Province, Iran.

similar to the primary spermatocytes but smaller. Few spermatids were also observed in some lobules. Spermatid was the smallest cell in lobules appeared during the spermiogenesis (Fig. 7b).

Stage 3: The lobule diameter increased in this stage and spermatocytes were predominate, but the clusters of spermatozoa and spermatid were present. Spermatocytes were recognized by their smaller nucleus and darkly staining chromatin material (Fig. 7c).

Stage 4: In this stage, the gonad had well-defined lobules with the large numbers of small spermatids and spermatozoa (Fig. 7d).

Stage 5: Tubules were characterized by presence of all developing germ cells. The predominant cells were spermatozoa with dark blue stain related to their nucleus. They were the smallest spermatogenic cells. A small number of spermatogonia were evident around the subcapsular lobules (Fig. 7e).

Discussion

Histology can be powerful tool, especially when used in association with measurements of reproductive factors such as vitellogenin and morphological studies. Classification keys of ovary development was gain with used of indices like color, size of egg, and degree of occupation of body cavity in teleost fishes (see Mirghiyasi et al., 2016). Based on interspecies similarities, maturity stages of ovary in fishes were divided into different steps between 5 and 8 stages (West, 1990; Salem et al., 1999; Ünver and Ünver-Saraydin, 2004; Monsefi et al., 2007; Mirghiyasi et al., 2016). This study provided the details on gonad morphology and histology of *A. sophiae*, an endemic fish of Fars Province that was found in the Kor River basin. Based on diameter of the oocytes in the ovary and yolk vesicle oocytes, five stage of ovarian development were observed. Similar five developmental stages have also been reported in the ovary of *A. persicus* (Monsefi et al., 2007) which be similar to many species such as *Thunnus albacares*, *Cyprinus carpio* and *Lutjanus fulviflamma*. Zona radiata (ZR) of *A. sophiae* was observed at the cortical alveolar stage and became thicker along with progress of oocyte development and yolk deposition. It can be concluded that ZR has no functional features at early oocyte development. The reproductive cycle of male *A. sophiae* is characterized by relative testicular size and developmental stage. Five stage of testicular development according to the most advanced type of germ cell were observed in lobular testis: spermatogonia (stage 1), spermatogonia and spermatocytes (stage 2), spermatogonia, spermatocytes and spermatids (stage 3), spermatogonia, sprrmatocytes, spermatids and spermatozoa (stages 4 and 5).

The results of the present study showed that *A. sophiae* spawns in the spring. Percentage of late gonad maturation stages (IV and V) and high frequency of large oocyte confirmed the spawning season. The season of spring reported as the spawning

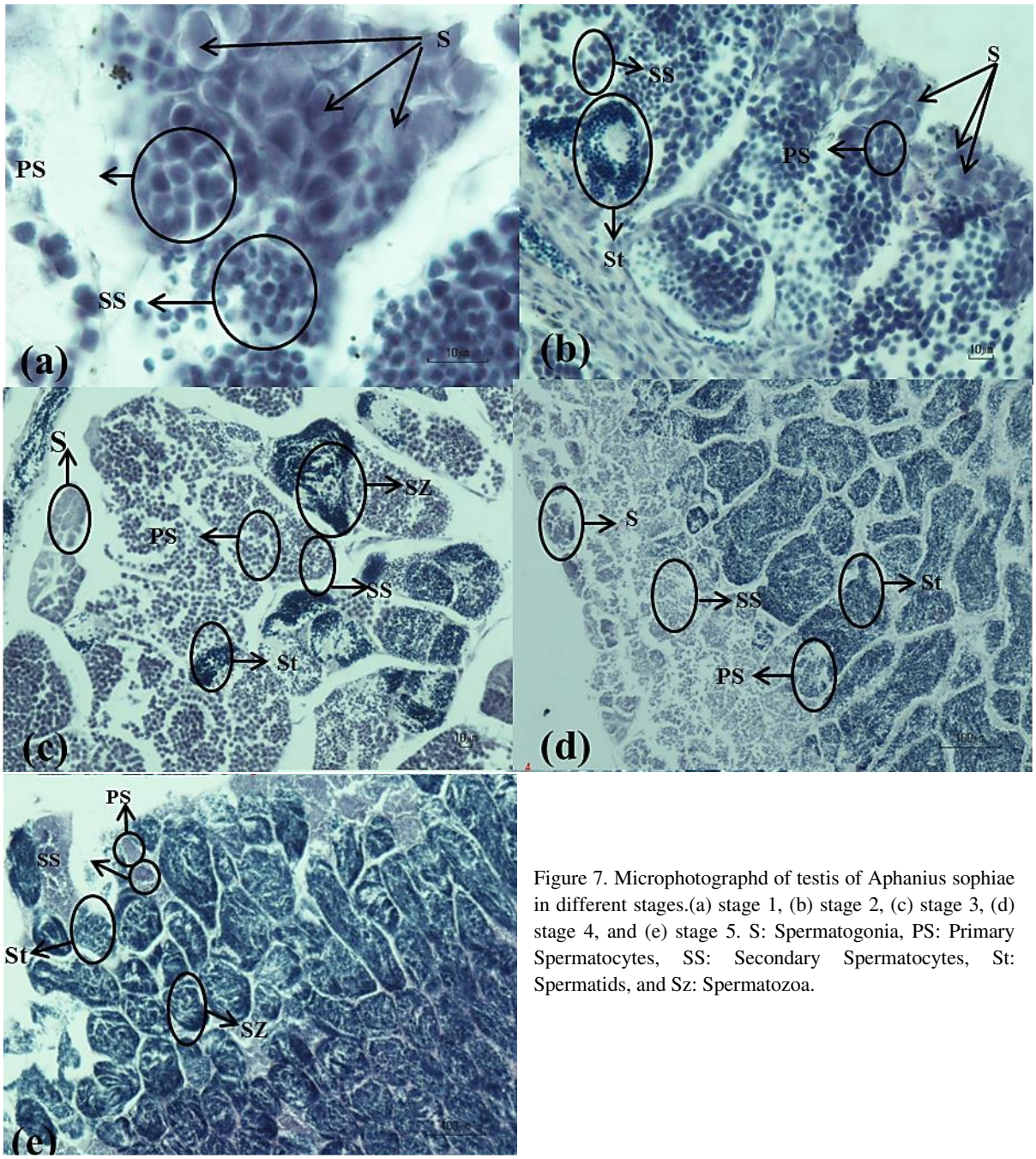

Figure 7. Microphotographd of testis of Aphanius sophiae in different stages.(a) stage 1, (b) stage 2, (c) stage 3, (d) stage 4, and (e) stage 5. S: Spermatogonia, PS: Primary Spermatocytes, SS: Secondary Spermatocytes, St: Spermatids, and Sz: Spermatozoa.

season for some other *Aphanius* such as, *A. fasciatus* (Lenardos and Sinis, 1998), *A. mento* (Güclü and Kücük, 2008), and *A. disaper disaper* (Bibak et al., 2012). In the spring, the ecological factors, such as temperature, photoperiod and nutrition increased fat storage in oocyte and thus increase fish size and to increase the size of gonads. Based on histological section in which oocytes are in different stage of development, *A. sophiae* is asynchronous. Batch spawning of an individual in a spawning season is an advantage for this fish that lives in unstable and changeable environments, such as temporary lagoon or a very small pool. This strategy of spawning allows relatively large eggs to be laid, which have a greater chance of survival (Wooton, 1990; Leonardos and Sinis, 1998).

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چکیده فارسی

ریخت‌شناسی و بافت‌شناسی غدد جنسی گورماهی کر (Heckel, 1847) *Aphanius sophiae* در ایران

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چکیده:

در این مطالعه، ویژگی‌های ریختی، بافتی و مراحل بلوغ جنسی گنادهای نر و ماده گورماهی کر *Aphanius sophiae* (Heckel, 1847)، که یک گونه بومزاد ایران است، ارائه شده است. نمونه‌برداری از ماه مارچ ۲۰۱۲ تا مارچ ۲۰۱۳، به‌وسیله تور دستی انجام شد و در کل تعداد ۲۲۳ قطعه ماهی از چشمه جویباری در حوضه رودخانه کر جمع آوری گردید. گنادها از بدن ماهی خارج و پس از بررسی ریختی، اندازه‌گیری وزن، طول و عرض آن‌ها، در محلول فرمالین ۱۰ درصد تثبیت گردید. بر اساس اندازه، شکل و وزن گنادها، اندازه گناد نسبت به طول حفره شکمی، وجود و یا عدم وجود تخمک در گناد ماده و یا مایع شیری رنگ میلت در گناد نر، قطر تخمک در تخمدان و مشاهدات بافت‌شناسی، ۵ مرحله بلوغ جنسی برای افراد نر و ماده منظور گردید. نتایج بررسی مراحل بلوغ جنسی گنادهای نر و ماده گورماهی کر نشان داد که این ماهی درآغاز بهار تخمریزی می‌کند.

کلمات کلیدی: تولیدمثل، بافت‌شناسی گناد، بلوغ جنسی، نمایه گنادی-بدنی.