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Seasonal Changes and Histological Studies of the Reproductive System of *Agabus biguttatus* Olivier, 1795 (Coleoptera: Dytiscidae) from Southern Iran

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ABSTRACT

Variations in the gross morphology and histology of internal reproductive systems of the male and female of *Agabus biguttatus* Olivier, 1795, an aquatic beetle, collected from a spring-stream habitat in Bamoo National Park, southwest of Iran in different seasons (mid-spring 2003 to mid-winter 2004), are described and illustrated. By studying the reproductive systems, two phases, developing and degenerating, can be proposed for them. In developing phase (mature, ready for mating) the length and diameter of gonads and accessory glands were larger than in degenerating phase. All the specimens collected in spring and summer demonstrate developing phase, while the autumn and winter specimens show the degenerating one.

Key words: Agabus biguttatus, reproductive system, biology, aquatic Coleoptera, Iran.

INTRODUCTION

Agabus Leach, 1817, predominantly Holarctic (Larson, 1985), is a medium-sized water diving beetle. The genus covers some 200 species (Williams & Feltmate, 1994), all found in most kinds of limnic environment, with a certain preference for smaller and stagnant water bodies (Nilsson & Holmen, 1995). They spend all their life stages, except the pupa, in water (Nilsson, 1990). *Agabus biguttatus* (Olivier, 1795) is a Palearctic species, ranging in size from 8.62 mm (female) to 8.89 mm (male). Despite a good amount of work on the external morphology of this species, attention to the anatomy and histology of the reproductive systems is very limited (Miller & Mumma, 1973; Dettner, 1979; Dettner *et al*., 1986; Schaff & Dettner, 2000; Drotz *et a*l., 2001; Drotz, 2003; Ribera *et al*., 2004; Eyre *et al*., 2005) and none on the Iranian ones. The present work is aimed to be a contribution to the Iranian species and in shedding some light on the biology of this species by focusing on the study of its reproductive organs.

MATERIAL AND METHODS

Sixty live specimens of *Agabus biguttatus* were collected seasonally from mid-spring 2003 to mid-winter 2004 from Cheshmeh-Ye-Ghanbari (Ghanbari spring), in Bamoo National Park. The park extends about 30 kms North to North-East of Shiraz (Fars, Iran, 52˚35′ and 52˚55′ E and 29˚35′ and 29˚55′ N). The live specimens were transferred to the laboratory in a bucket of water. About half of the specimens were quickly frozen for studies of reproductive system and the other half were fixed in Bouin-Brazil solution for histological analyses. The frozen specimens were then mounted (one at a time), dorsal side up, on the surface of solidifying paraffin in a dish and were dissected to expose the reproductive organs. Diluted methylen blue (1%) was constantly used during the operation to give more contrast to accessories and components of the system. After the organs were exposed, 5% KOH was used to solve excess fat around them (if there was any) and then they were washed with distilled water. Drawings were made and photographs were taken. Those specimens which were fixed in Bouin-Brazil were embedded in paraffin and were serially sectioned to the thickness of 7µm using a rotative microtome. Sections were then transferred to regular microscope slides for permanent preparation and stained with hematoxylin and eosin. The prepared slides were then studied and photographed. The microscope used for dissection, measurements (ocular micrometer), drawings (camera lucida attached on the microscope) and photography (camera, model 1 MPS 51, attached to the microscope) was stereo microscope, Zeiss, model SV6. Specimens, dissected organs and slides are kept at CBSU (Collection, Department of Biology, Shiraz University).

RESULTS

1. Gross morphology of the reproductive system

1.1. Male

The major components are: 1) gonads: pair of coiled tubular testes, 2) ducts: two *vasa deferentia* and a medium ejaculatory duct, and 3) a pair of accessory glands. Yellow spots were localized apically on the accessory glands. Accessory glands seemed milky white in spring and summer and translucent in autumn and winter. The average length of coiled testis was 3.78 mm, in spring; 3 mm, in summer; 2.3 mm, in autumn and 1.8 mm, in winter (Figs. 1, 3a).

1.2. Female

The major components are: 1) pair of ovaries, each consisting of 16-20 ovarioles. The ovarioles contain three parts: a) terminal filament, with those of other ovarioles forming a single suspensory ligament per ovary, b) apical germarium and basal vitellarium that in an active ovariole is comprised of oocytes which are undergoing deposition of nutrients, a process referred to as vitellogenesis, and c) pedicel. Through pedicel, each ovariole opens into 2) a lateral oviduct uniting posteriorly to form 3) the common oviduct, connected to 4) the vagina which expands at its base dorsomedially, forming 5) an elongate *bursa copulatrix* and 6) the spermatheca joining the base of vagina through a duct. The length of each ovary in four seasons was the same (in average 3.5mm) (Figs. 2, 3b), but its average width varied, being 0.52mm, in spring ; 0.5mm, in summer; 0.31mm, in autumn and 0.24mm, in winter (Fig. 3c).

2. Histology of the reproductive system

2.1. Male

Into the testes there are spermatogenic cells in spring and summer. *Vasa deferentia* have columnar epithelial cells with oval nuclei in basal part of the cells, the epithelium apex covered with hair-like projections of the cuticle and surrounded by a layer of circular muscles. The accessory glands have secretory epithelium of columnar cells with elongated nuclei and some secretory vacuoles (Fig. 4). Reproductive system of males in spring and summer was characterized by: 1) larger diameter of testes, sperm duct and accessory gland, and 2) the presence of sperm in both testes (Figures 6a-d) and sperm ducts, but in autumn and winter was defined on the basis of: 1) lesser diameter of testes, sperm duct and accessory glands, and 2) lack of sperm in testes (Figs. 6e, 6f) and sperm ducts.

2.2. Female

The germarium region in each ovariole has an epithelium with cubic cells and large spherical nuclei occupying the greater part of each cell. The epithelium lies on a thin basal membrane surrounded by a thin layer of muscles. The common oviduct has a similar histological structure, but only with larger and darker nuclei (Fig. 5). Each oocyte in vitellarium has a follicular covering of flat cells and ovoid nuclei (Fig. 7a). Reproductive system of females (on the basis of histological studies) in spring and summer was characterized by: 1) high diameter of each ovariole (Fig. 7b), and 2) a linear sequence of oocytes (Fig. 7a) in various stages of maturation in each ovariole, but in autumn and winter was marked by lesser diameter of each ovariole (Fig. 7c).

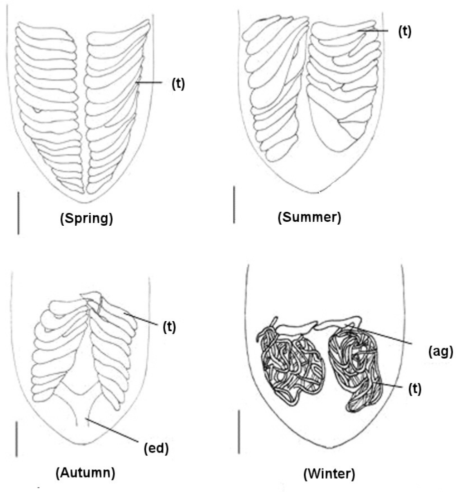


Fig. 1. Comparison of *Agabus biguttatus* male reproductive system in different seasons. Accessory gland (ag), ejaculatory duct (ed), testis (t). Scale: 1mm.

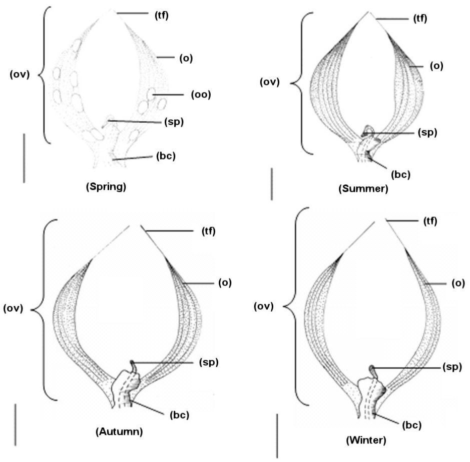
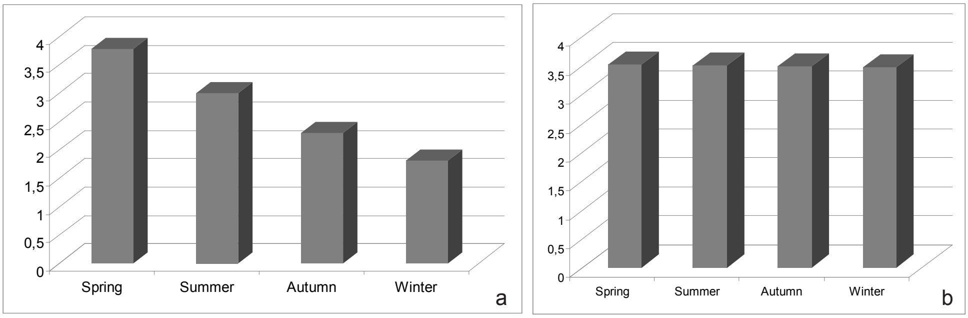


Fig. 2. Comparison of *Agabus biguttatus* female reproductive system in different seasons. Bursa copulatrix (bc), oocyte (oo), ovariole (o), ovary (ov), spermatheca (sp), terminal filament (tf). Scale: 1mm



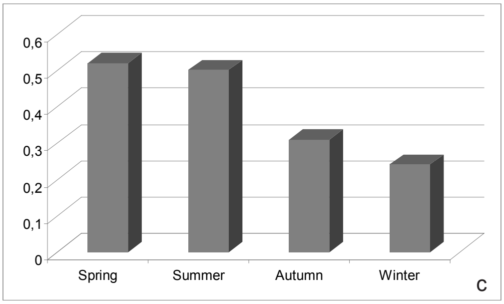


Fig. 3. Comparison of testis length (a), ovary length (b) and ovary width (c) of *Agabus biguttatus* in different seasons.

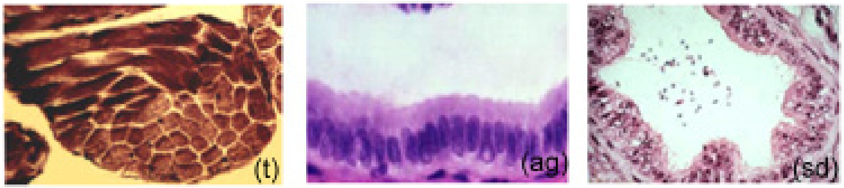


Fig. 4. Cross sections of testis (t)(×88), wall of accessory gland (ag)(×88) and sperm duct (sd)(×35) of *Agabus biguttatus* in spring.

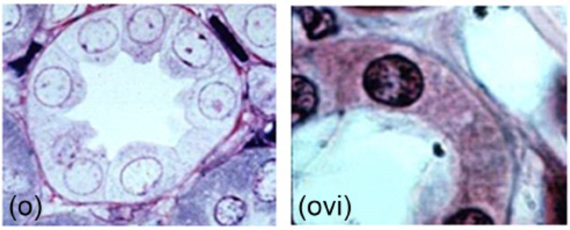


Fig. 5. Cross sections of ovary (o)(×49) and oviduct (ovi)(×88) of *Agabus biguttatus* in spring.

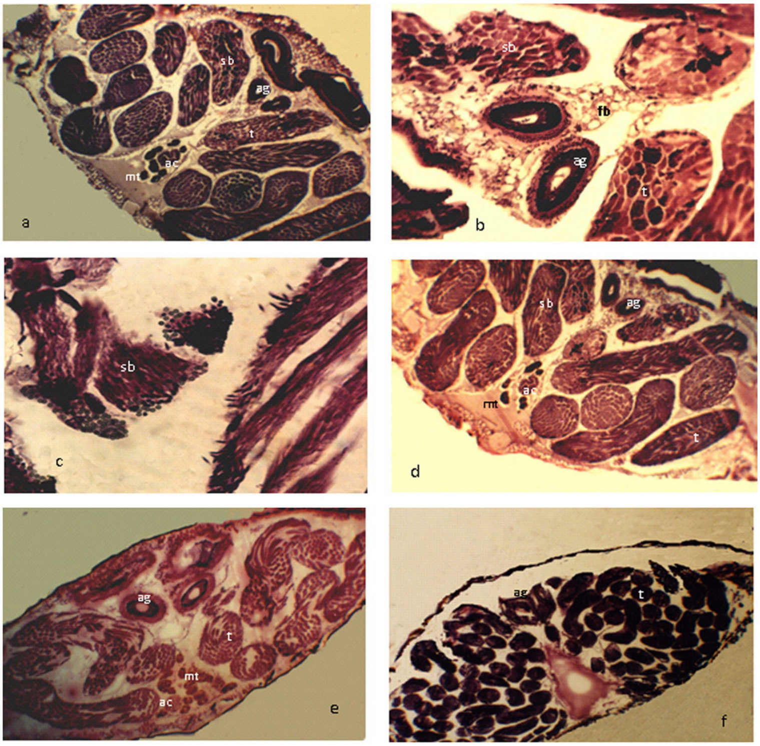


Fig. 6. Cross sections of male reproductive system in spring (a)(×35), (b)(×49) and (c)(×200); summer (d)(×35); autumn (e)(×35); winter (f)(×35) of *Agabus biguttatus*. Testis (t), accessory gland (ag), alimentary canal (ac), fat body (fb), Malpighian tubules (mt) and sperm bundles (sb).

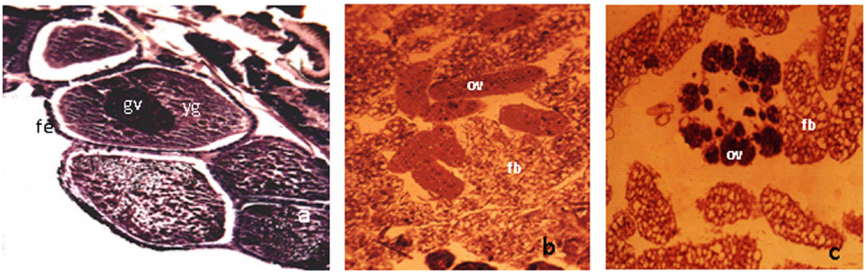


Fig. 7. Cross sections of oocyte in spring (a)(×108) and female reproductive system in summer (b)(×88) and winter (c)(×88) of *Agabus biguttatus*. Ovariole (ov), fat body (fb), follicular epithelium (fe), germinal vesicle (gv), yolk granule (yg).

DISCUSSION

Researches of this type which deal with primary and such basic necessary data have not yet been conducted in Iran and not much in other places on these taxa. Thus, there is a broad gap in world data in this part. Just few papers show some work on the anatomy of reproductive organs of some Coleoptera like Dettner, 1986; Nasserzadeh, 2005. In order to Dettner (1986) among male members of Hydroporinae, *Vasa deferentia* and testes are rolled up and closely connected. Within *Agabus bipustulatus*, *Agabus paludosus* and *Platambus maculatus*, *Vasa deferentia* and testes are distinctly separate. In representative of this study, *Vasa deferentia* and testes are also distinctly separate. In accordance with Burmeister (1976) the spermatheca of female members of Agabini like *Platambus maculates* is saclike and connected with the oviduct but not with the bursa copulatrix. Whitin genera of Hydroporinae such as *Deronectes* and *Oreodytes*, the spermatheca originates close to the oviduct and is connected with the bursa copulatrix by means of a long duct. In females of *Agabus biguttatus* is the same as in other Agabini. Two age phases were observed in gross anatomy and histology of the reproductive systems of male and female of *Agabus biguttatus*: developing and degenerating. All collected specimens in spring and summer showed developing phase of reproductive system and in autumn and winter degenerating one. In males, the length of testes were conspicuously larger in developing phase than in degenerating one, paralleled with the presence of spermatoza in the former phase and lack of them in the latter. There were yellow spots, localized apically on the accessory glands in developing phase that maybe related to their mucus secretion activity (Snodgrass, 1935). In females, in both phases the length of each ovary was approximately the same, but the width in developing phase was approximately two times than that in degenerating phase. Also, in developing phase there were several oocytes in the ovaries, whereas in degenerating phase there were fewer or none. The major annual cycles in habitat favorability and climatic condition could be important factors for generating the observed suggested phases. The habitat favorability is viewed against the axis of a seasonally varying supply of prey organisms for these carnivore beetles (both larvae and adults), paralleled with variations in temperature. The main environmental factor is water temperature since other chemical factors such as PH are quite stable. It is assumed that a high productivity (developing phase) is reflected in high abundance of prey organisms. Adults of *Agabus biguttatus* are found in springs and springfed streams. Their main foods are most abundant in spring and summer. Favorite conditions in these two seasons apt the beetles to reach to the developing phase and allow exploration and dispersal. The contrary to this is the not-agreeable condition in autumn and winter, the degenerative phase. The effect of these seasonal variations are, of course, true for all organisms, but not studied for insects prior to the present work.

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