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A Comparison of Female Reproductive System in Agabus Biguttatus Olivier, 1795 and Agabus Conspersus Marsham, 1802 (Coleoptera, Dytiscidae) in Different Seasons from Southern Iran

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Abstract: Agabus *biguttatus* and *Agabus conspersus* have been selected as a model in order to compare the seasonal variations of female reproductive system. Specimens were collected from a spring-stream from southern Iran between mid-spring 2003 to mid-Winter 2004. The female internal reproductive system of *A. conspersus*, showed no outstanding difference in different seasons when compared with *A. biguttatus* in regards to general shape and structure. The greatest difference was in the size and shape of bursa copulatrix. The bursa copulatrix (a part of female reproductive system) has a cuticular feature with some parallel lines on it in *A. conspersus*. However, the bursa copulatrix has a soft feature without any lines on it. In addition, by studying the reproductive systems in different seasons, two phases, developing and degenerative 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 degenerative phase. All the specimens collected in spring and summer demonstrate developing phase, while the autumn and winter specimens show the degenerative one.

Key words: *Agabus biguttatus*, *Agabus conspersus*, Coleoptera, Dytiscidae, female reproductive system, Iran, seasonal changes

INTRODUCTION

Genus Agabus Leach, 1817, as redefined by Nilsson (2000), is one of the largest among the family Dytiscidae, with approximately 180 species (Millan, 2001). It has a predominantlyHolarctic distribution (Larson, 1985), with some species in eastern and southern Africa (Nilsson, 1992). The species are found in most kinds of limnic environment, with a certain preference for smaller and stagnant water bodies. No single species spends its whole life in water and normally the pupal stage is spent out of the water (Nilsson, 1990; Nelson, 1996). Despite a wide range of studies on the external morphology of these two species (Larson and Nilsson, 1985; Carr and Nilsson, 1988; De Marzo and Nilsson, 1993; Ribera *et al*., 2004; Nilsson and Petrov, 2006), work on the anatomy and comparison of their reproductive systems is very few. The aim of this paper is to present the results of a study of the basic design of female reproductive system in *A. biguttatus* Olivier, 1795 and *A. conspersus* Marsham, 1802 in different seasons and also the comparison of that between these two species.

MATERIALS AND METHODS

Sixty live specimens of *A. biguttatus* and *A. conspersus* were collected seasonally from mid-spring 2003 to mid-winter 2004 from Cheshmeh-Ye-Ghanbari (Ghanbari spring), in Bamoo National Park, Iran. The park extends about 30 km North to North-East of Shiraz (Fars, Iran, 52º35′ and 52º55′ E and 29º35′ and 29º55′ N). The live specimens were transferred to the laboratory in a bucket of water and quickly frozen for studies of internal reproductive system. The frozen specimens were then mounted (once 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. The microscope used for dissections, 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.

RESULTS

The primary components of the female reproductive system are ovaries, lateral oviduct, common oviduct, spermatheca and its duct, bursa copulatrix and vagina.

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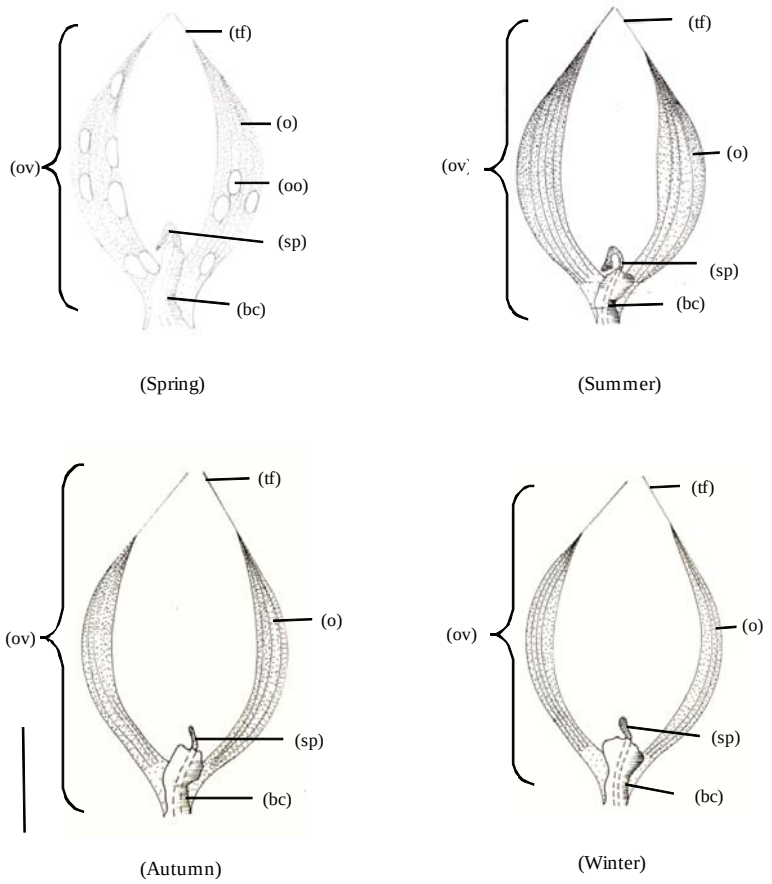

Fig. 1: Comparison of *Agabus biguttatus* female reproductive system in different seasons. Bursa copulatrix (bc), oocyte (oo), ovariole (o), ovary (ov), spermatheca (sp), terminal filament (tf). Scale: 1 mm

Agabus biguttatus: Ovaries are paired and place laterally to the alimentary tract. Each ovary consists of a number of ovarioles. The ovarioles divide into three parts: 1) terminal filament at the apex of each ovariole joins to other terminal filaments of other ovarioles forming a single suspensory ligament per ovary 2) apical germarium and basal vitellarium, and 3) pedicel. Apical germarium contains the primary female germ cells, the oogonia, which divide mitotically and eventually give rise to primary oocytes. The germarium also contains prefollicular tissue, which forms the follicular epithelium in the vitellarium. Vitellarium in an active ovariole contains oocytes that are undergoing the deposition of nutrients (vitellogenesis). Each developing oocyte is surrounded by a follicular epithelium, the oocyte and its associated epithelial layer comprises a follicle. Through pedicle, each ovariole opens into the lateral oviduct. Lateral oviduct is very short comparing to the system as a whole. The lateral oviducts united posteriorly froming the common oviduct. The latter is also short and is connected to the vagina. The vagina expands at its base dorsomedially, forming an elongate bursa copulatrix. The spermatheca is clublike and connects to the base of vagina by means of a duct, but not with the bursa copulatrix.

The internal sexual organs of female specimen of *A. biguttatus* provided a great deal of information with respect to the age structure. Females of *A. biguttatus* in degenerative phase had undifferentiated ovarioles which were coalescent and translucent. There were no developing oocytes in the vitelarium. The average length of each ovary in this phase was 3.42 mm (Fig. 1, 3a) and its width 0.27 mm (Fig. 1, 3b). Bursa copulatrix and spermatheca were translucent. The ovarioles in developing phase exhibited a noteworthy increase, mainly in diameter. The length of each ovary in this phase was in average 3.43 mm (which is approximately the same as in degenerative phase) (Fig. 1, 3a) and its average width was 0.52 mm (which increased approximately two times of the degenerative phase) (Fig. 1, 3b). There were oocytes in different stages of development in this phase. The bursa copulatrix and spermatheca were creamy white in color.

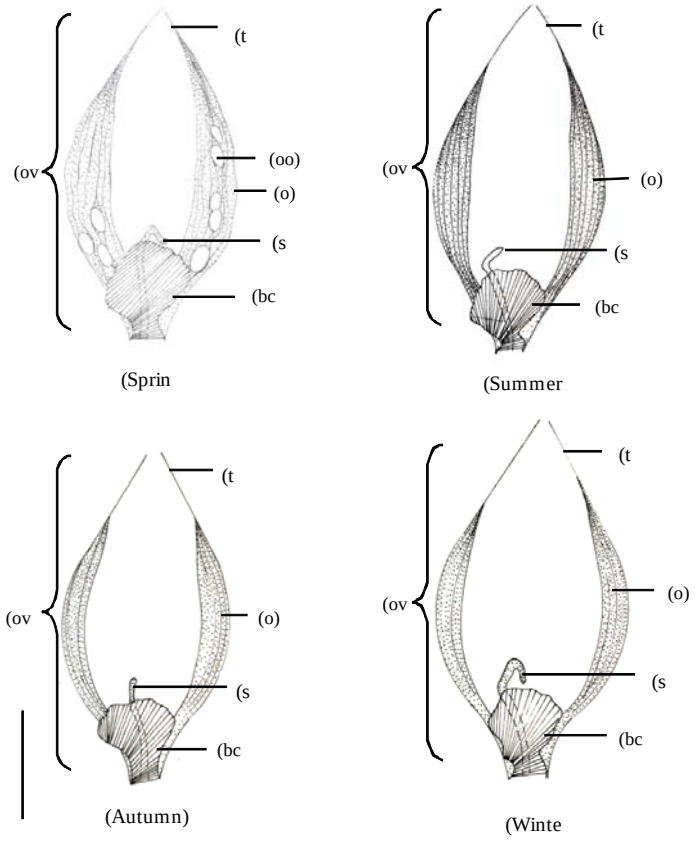

Fig. 2: Comparison of *Agabus conspersus* female reproductive system in different seasons. Bursa copulatrix (bc), oocyte (oo), ovariole (o), ovary (ov), spermatheca (sp), terminal filament (tf). Scale: 1 mm

***Agabus conspersus*:** The ovaries are paired and situate laterally to the digestive tract. As in *A. biguttatus*, each ovary consists of a number of ovarioles that are associated with lateral oviduct. Each ovariole is divided into three zones that contain 1) terminal filament; 2) two broad zones, the germarium and vitellarium and 3) pedicel. Lateral oviducts are short and join to each other to form common oviduct which is also short and is connected to the vagina. Vagina expands at its base dorsomedially and formes a saclike bursa copulatrix. The spermatheca is cylindrical and connects by a duct to the base of vagina.

Age dependent changes in internal reproductive organs in female specimens of *A. conspersus* were found in the form of two phases: degenerative and developing. In degenerative phase, as in *A. biguttatus* had undifferentiated and colorless ovarioles. There were no developing oocytes in them. The average length of each ovary in degenerative phase in *A. conspersus* was 3.49 mm (Fig. 2, 3a) and its width 0.29 mm (Fig. 2, 3b). Bursa copulatrix and spermatheca were translucent. In developing phase, specimens had reproductive organs which were approximately the same as in degenerative phase, but increased in their width. The length of each ovary in developing phase was 3.5 mm (this was nearly the same as in degenerative phase) (Fig. 2, 3a) and its width in average was 0.53 mm (which increased approximately two times in compare with degenerative phase) (Fig. 2, 3b). There were several oocytes in the vitellarium and in some ovaries. The bursa copulatrix and spermatheca were milky.

DISCUSSION

Female internal reproductive system of two species of water beetles (*A. biguttatus*, *A. conspersus*) and their changes in four seasons have been studied in order to perform a comparison and also define age classes with respect to their structure. Female reproductive system in *A. biguttatus* has nearly the same structure and shape with that in A. conspersus. The whole anatomy of internal reproduvtive system in A. conspersus is larger than *A. biguttatus* (Fig. 3a). A specific difference has been found

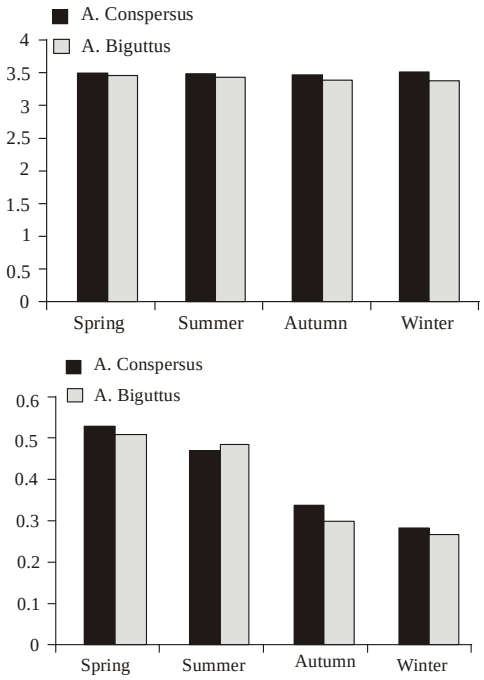

Fig. 3: Comparison of ovary length (a) and ovary width (b) of Agabus conspersus and Agabus biguttatus in different seasons.

among reproductive organs of these two species. Bursa copulatrix in *A. conspersus* was larger with some parallel lines on it (Fig. 2) and a cuticular feature, but in *A. biguttatus* bursa copulatrix had a soft feature without any lines on it (Fig. 1). As well as, two age phases can describe in each of these two species: developing phase and degenerative phase. In both phases, the length of each ovary was the approximately the same, but its width was changed. The width of each ovary in developing phase was approximately two times comparing with those in degenerative phase. In addition, in developing phase, there were several oocytes in the ovaries. All collected specimens in spring and summer showed developing phase of reproductive system, but specimens collected in autumn and winter demonstrated degenerative phase. The major annual cycles in habitat favorability and climatic condition could be important factors for generating the observed suggested phases. Favorite conditions in spring and summer 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.

CONCLUSION

By using comparative method, some developmental and reproductive aspects of two species of Coleoptera have been studied in four seasons. The results of this work indicate that the whole anatomy of female reproductive system shows two age phases in each of these two species: developing phase (in spring and summer) and degenerative phase (in autumn and winter). Also, a significant difference has been found among the female reproductive organs of these two species which was the shape and size of their bursa copulatrix.

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