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THE POST-OVARIAN GENITAL COMPLEX IN *ANAX GUTTATUS* (BURMEISTER) (ANISOPTERA: AESHNIDAE) *

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It is situated in the mid-ventral region of the 8th abd. sternum, and is composed of a mid-anterior large bursa copulatrix, a mid-posterior tubular vagina and a pair of lateral elliptical spermathecae. These components are covered externally by the sternal and intrinsic muscles. A cuticular plate is embedded in the anterior region of the vagina and is connected to the seventh sternum by a pair of muscle bands. The spermathecae open into a bursa copulatrix through short spermathecal ducts anterodorsally. The bursa copulatrix contains a net-work of cuticular canals, and opens into the vagina through a distinct bursa communis. The spermatozoa dissociate from the spermiozeugma (Spermatodesms) and seminal fluid within the bursa copulatrix. The cuticular intima of the spermathecae is thin and lightly corrugated while that of the bursa copulatrix is thick and heavily folded. The cuticular intima of the lateral region of the vagina is thin but covered with overlapping dented scales. A pair of accessory sex glands are present in the 9th segment and open into the vagina. This is thought to be the first report of such glands in an anisopteran. – The eggs are elongate and cylindrical, with a complex micropylar apparatus. The egg-shell exhibits micromorphological modifications to cope with the endophytic mode of oviposition.

INTRODUCTION

Since the pioneer work of TILLYARD (1917), extensive work has been carried out on the odonate post-ovarian genital complex (POGC), particularly in relation to the mechanism of sperm transfer and sperm competition (WAAGE, 1984; SIVA-

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-JOTHY, 1987; MILLER, 1991; ANDREW & TEMBHARE, 1994). The available literature, however, reveals that studies on the functional anatomy of the POGC are confined to some Zygoptera and to libellulids, with no substantial information on aeshnids.

MATERIAL AND METHODS

Anax guttatus females were collected during late afternoons (13.30-16.00) and evenings (18.30--22.00) around the ponds and lakes of Brahmapuri and Nagpur during the monsoon and post-monsoon period in 1992-94. Ovipositing females were forced to lay eggs by dipping the abdomen in 500 ml beaker filled with water and lined with filter paper. The females oviposited by inserting the eggs into the paper. The eggs were collected by gently teasing the paper with fine-tipped forceps. Some eggs were also procured by dissecting the oviducts. The post-ovarian genital complex (POGC) was dissected in Ringer's solution, fixed in Bouin's fluid for 12-18 h, dehydrated, cleared in xylene, and embedded in paraffin (melting point 60-62°C). Serial sections, 4-6 μm thick, were cut and stained with Ehrlich's Haematoxylin-Eosin or Heidenhain's Iron-Haematoxylin-Orange G.

The eggs and fractured POGC were processed for Scanning Electron Microscopy (ANDREW & TEMBHARE, 1995) and examined in a 250 MK III. Cambridge Scanning Electron Microscope. Histological measurements were taken using a Lanometer (PZO Poland). At least 25 readings were taken to determine the mean value and standard error.

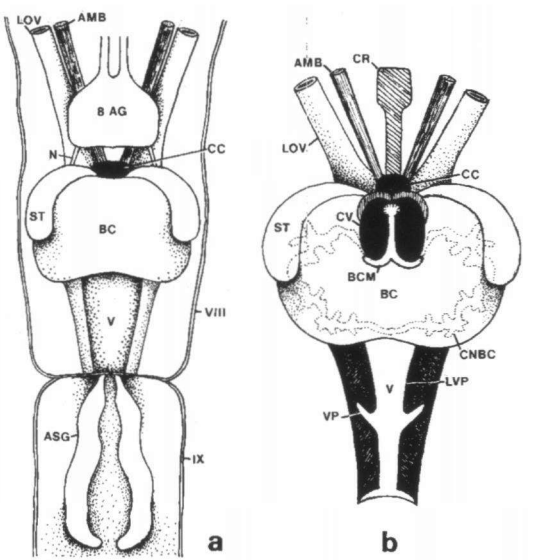
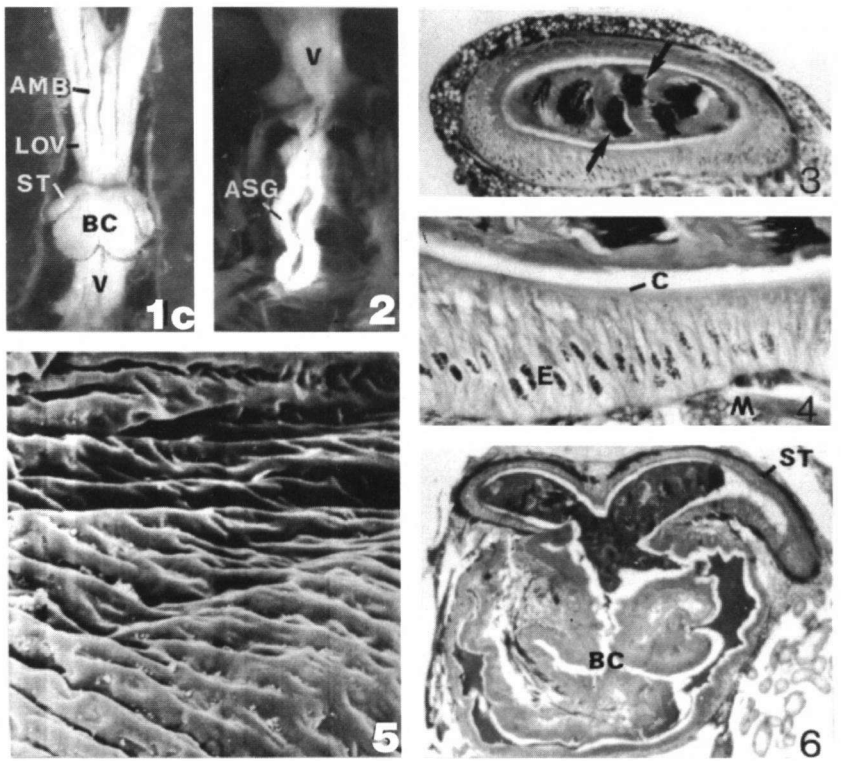


Fig. 1a-b. *Anax guttatus*: (a) diagram of the dissected 8th and 9th abdominal segments, exhibiting the post-ovarian genital complex (POGC) and accessory sex glands (ASG); – (b) diagram of the POGC gross anatomy. – [AMB = anterior muscle bands, – BC = bursa copulatrix, – CC = cuticular collar, – CNBC = cuticular network of bursa copulatrix, CR = cuticular rod, – CV = cuticular valve, – LOV = lateral oviduct, – LVP = lateral vaginal plate, – N = nerve, – ST = spermathecae, – V = vagina, – VP = vaginal pocket, – 8AG = 8th abd. ganglion, – VIII = 8th segment, – IX = 9th segment].

OBSERVATIONS

In *Anax guttatus* the POGC consists of a pair of lateral elliptical milky-white spermathecae (ST), a mid-dorsal spheroid bursa copulatrix (BC) and a pouch-like ventral vagina (Fig. 1). A thin external muscle coat invests the upper region of the ST and BC. Each ST communicates independently with the BC by a short spermathecal duct (STD). The BC opens into the vagina dorsally while the median oviduct enters the vagina

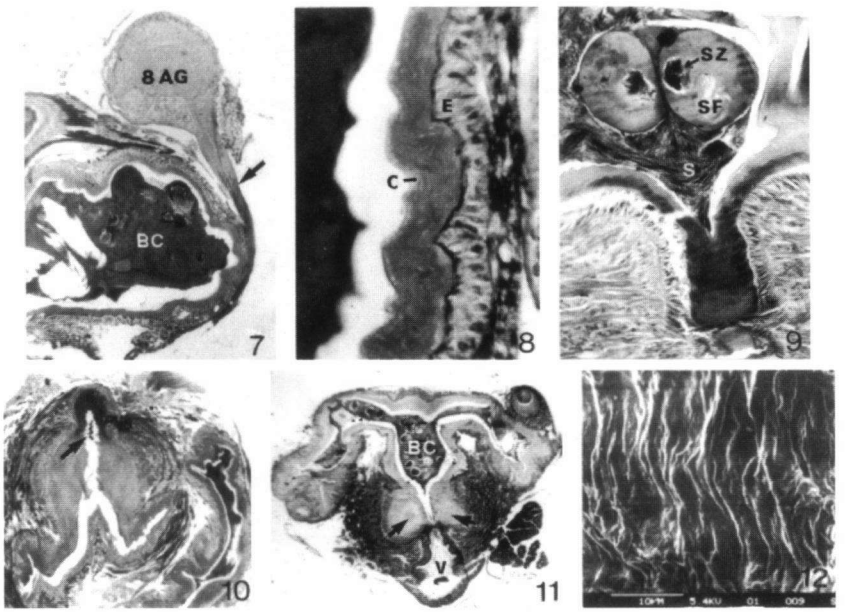


Figs 1c-6. *Anax guttatus*: (1c) post-ovarian genital complex (POGC) (× 5.4); – (2) tubular accessory sex glands (× 5.4); – (3) transverse section of spermatheca, showing spermiozeugma (arrow) (× 460); – (4) englarged view of part of Fig. 3 [c = cuticle, – E = epithelium, – M = mucsles] (× 710); – (5) SEM of a spermatheca (ST), showing corrugated cuticular lining; – (6) transverse section of the POGC, showing opening of the spermathecae (ST) into the bursa copulatrix (BC) (× 26).

anteriorly. The vagina is a large, long, laterally folded sac-like structure with many cuticular modifications. It terminates posteriorly in the ovipositor at the base of the 8th abdominal sternum. A cuticular plug is embedded in the anterior region of the vagina, which is connected to the seventh sternum by a pair of muscle bands. A pair of conical, tubular, slightly convoluted accessory glands are present in the ninth abdominal segment. They open through a very short duct in the posterior region of the vagina (Figs 1, 2).

The wall of all the components of the POGC (except the accessory glands) have the same structure, comprising an outer muscle layer and an inner epithelium. The epithelium rests on a basement membrane and is lined internally with cuticle.

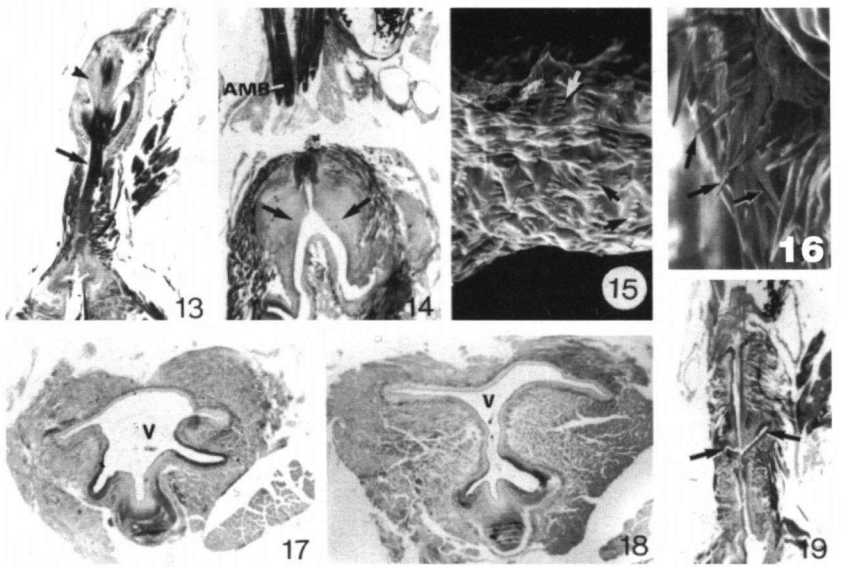
SPERMATHECAE. – Each one is 0.65±0.20 mm long and the epithelial layer of the wall is composed of tall columnar cells with basal nuclei. The cells are 12±3 μm long and 5±2 μm wide. The internal cuticular lining is corrugated and is 4±2 μm



Figs 7-12. *Anax guttatus*: (7) nerve (arrow) innervating the bursa copulatrix (BC) (× 35); – (8) transverse section of the bursa copulatrix, showing histological details [C = cuticle, – E = epithelium] (× 430); – (9) separation of sperm in the bursa copulatrix (BC) [S = spermatozoa, – SZ = spermiozeugma, – SF = seminal fluid] (× 70); – (10) bursa communis (note spines at the tip of its middle arm; arrow) (× 35); – (11) transverse section of bursa copulatrix, showing cuticular modifications (arrows) (× 70); – (12) SEM of the bursa copulatrix intima, showing corrugated cuticle [bar = 10 μm].

thick. The outer muscle coat is 15±3 μm thick. The lumen is 135 μm in diameter. The wall of the STD consists of a 2-3 μm thick cuticular intima and the epithelium is composed of small cuboidal/columnar cells 5±8 μm long and 3-4 μm wide. In post-copulated female, the ST, BC and STD are filled with male ejaculate containing spermiozeugma (spermatodesms) (Figs 3-6).

BURSA COPULATRIX (Figs 7-12). – The BC is 1±0.25 mm long and 0.85±0.15 mm wide. The epithelial layer of the dorsal wall region is composed of small cells (16×20 μm) while those of the lateral and ventral walls are tall, measuring 55±5 μm long and 15±5 μm wide. The epithelium is lined internally with a 60-70 μm thick cuticular intima. In the BC, the STD opens antero-dorsally, and the bursa communis (BCM) opens ventrally. The BCM is a highly modified inverted 'Y'-shaped structure (Fig. 10). The median arm is highly sclerotized, spiny (20±2 μm long) and lies below the opening of the STD, and it is surrounded by a heavily sclerotized cuticular valve. The two paired lateral arms form winding, hollow, tubular pockets (network) which are either empty or filled with the seminal fluid. The posterio-dorsal region of the BC is a spheroid cavity with a bifid posterior

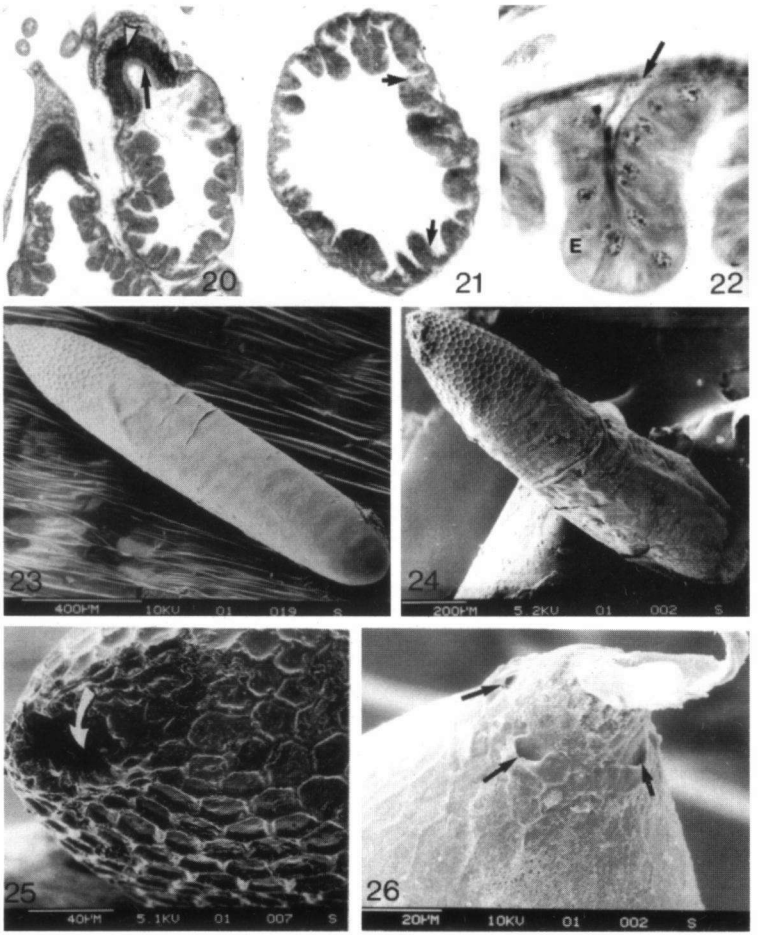


Figs 13-19. *Anax guttatus*, vagina: (13) cuticular rod of vagina (arrow), with flat spoon-shaped apical end (arrow-head) (× 25); – (14) cuticular valves of the vagina (arrow) and anterior muscle band (AMB) (× 25); – (15) SEM of the vagina intima, showing denticulate scales (arrows) (× 1025); – (16) SEM of bursa communis exhibiting spines (arrows) (× 625); – (17-18) transverse section of the posterior region (17) and posterior end region (18) of the vagina, to show the 'T'-shaped lumen (× 30); – (19) longitudinal section of the vagina, to show the cuticular folds in the mid-region (arrow) (× 15).

area.

VAGINA (Figs 13-19). – It is a tapering, sac-like structure 1.35±0.15 mm long. The epithelial layer consists of tall (20±5 µm) columnar cells with prominent basal nuclei. The anterior region of the vagina is anchored on a flat cuticular rod, which is horizontally placed in between the two muscle bands just below the median oviduct (Fig. 13). The rod is 300 µm long and 15±5 µm wide with a flattened (80 µm wide) anterior end. The valve of the BCM continues into the vagina and guards the opening of the median oviduct (Fig. 14). Antero-dorsally, it continues as a small square-shaped, heavily sclerotized plug (collar), providing a site of attachment to the anterior pair of muscle bands. The mid-posterior region of the vagina contains thin lateral vaginal plates along the lateral wall. The vaginal plates are covered with multiramous denticulate scales. Each scale contains three to five 1.5-2.5 µm long spines (Fig. 15). The opening of the BCM into the vagina is inlaid with sharp tapering spines (Fig. 16). The vagina tapers posteriorly, bearing a 'T'-shaped lumen before opening to the exterior through the ovipositor at the base of the 8th sternum (Figs 17, 18). The wall of the mid-region of the vagina exhibits a large lateral fold or pocket (Fig. 19).

ACCESSORY SEX GLANDS (ASG). – These are 1.85±35 mm long and 700±50 µm



Figs 20-22. *Anax guttatus*, accessory sex glands: (20) longitudinal section of gland and duct (arrow), with thick muscular layers (arrow-head) (× 65); – (21) transverse section of gland, showing folded layer (arrows) of secretory cells (× 85); – (22) enlarged view of part of Fig. 21 (note absence of cuticular intima and thin muscle layer; arrow) (× 435).

Figs 23-26. *Anax guttatus*, egg morphology: (23) SEM of the newly laid eggs [bar = 400 µm]; – (24) SEM of 6 day old egg [bar = 200 µm]; – (25) SEM of collar, to show the hexagonal reticulation and apical aperture (arrow) [bar = 40 µm]; – (26) micropylar orifices (arrrows) [bar = 20 µm].

wide. The wall of ASG is composed of an inner, folded epithelium lying on a basement membrane, and an outer 2-2.5 µm thick unfolded muscle layer. The epithelial cells are 25-30 µm tall and 5-8 µm wide and contain prominent nuclei. The anterior region of the ASG is narrow and composed of an inner epithelial (4-5 µm) layer lined internally with a 2-3 µm thick cuticle and externally covered with

a (5-8 μm) muscle layer. It forms the duct of the glands. The lumen of the ASG gland is filled with a creamy white secretion (Figs 20-22).

EGGS. – The eggs of *A. guttatus* are cylindrical, with a pointed anterior and a rounded posterior end (Figs 23, 24). Each egg measures 1.65±0.5 mm long and 0.35±0.02 mm wide at the mid-region. The egg chorion is composed of an outer exochorion (EX) and an inner endochorion (EN). The exochorion is well developed and thick at the anterior end but decreases to 2.5 μm in the mid-region. It bears 18-20 tiers of distinct hexagonal reticulations as the imprints of the follicle cells at the anterior end. This reticulated region is loosely attached and generally slips up to cover the anterior-most tip of the egg during oviposition (Fig. 25). The endochorion is of almost uniform thickness (1.8±0.3 μm) and bears faint hexagonal reticulations at the anterior end. It is circumscised by five micropylar orifices (MO) about 20 μm below the anterior pole (Fig. 26). These orifices are 8±1.5 μm in diameter and 60±10 μm apart. The eggs incubate in about 12-18 days at 25±5°C and the larvae emerge after splitting the eggs vertically from the anterior end.

DISCUSSION

SIVA-JOTHY (1987) and MILLER (1991) asserted that the anisopteran females have paired spermathecae attached to a bursa copulatrix lying dorsal to the vagina, and that the shape and size of these organs vary between genera. In *Anax guttatus*, the spermathecae open without forming a common duct and thus differ from those of *Epiophlebia superstes* (ASAHINA, 1954), *Brachythemis leucosticta, Zyxomma petiolatum, Tholymis tillarga* and *Potamarcha congener* (MILLER, 1982, 1988, 1991). MATSUDA (1976) also reported the opening of the spermathecae directly into the vagina.

Some workers have reported the presence of secretory cells in the wall of the spermatheca (ASAHINA, 1954; PRASAD & SRIVASTAVA, 1961; MIDTTUN, 1976). These were not found in *A. guttatus*, and this supports the observations in other dragonfly species (BJÅNES, 1974; SIVA-JOTHY, 1987; ANDREW & TEMBHARE, 1994). In *A. guttatus*, the cuticle of the spermathecae and their ducts is corrugated to form ridges and furrows, presumably to facilitate movement of the male ejaculate, as reported in *Somatochlora arctica* (MIDTTUN, 1976) and *Tramea virginia* (ANDREW & TEMBHARE, 1994).

The odonate bursa copulatrix is generally a simple and undivided, single structure (ANDREW & TEMBHARE, 1994). PRASAD & SRIVASTAVA (1961) reported a bilobed bursa copulatrix in *Pantala flavescens*. However, in *A. guttatus*, it is a complex structure, consisting of a large central lumen and a long tubular network of canals which are probably developed for (i) removal of seminal fluid from male ejaculate and (ii) initiating the breakdown and release of sperm from the sperm-bundle, i.e. spermiozeugma or spermatodesms. The seminal fluid is stored in the canals, while the released individual sperm are stored in the lumen.

The post-ovarian genital complex (POGC) of all libellulids contains a special tubular fertilization pore (SIVA-JOTHY, 1987; ANDREW & TEMBHARE, 1994). However, this is lacking in *A. guttatus* and its function is carried out by the bursa communis. A similar situation has been observed in the Zygoptera (SRIVASTAVA & SRIVASTAVA, 1992). The wall of the bursa communis in *A. guttatus* bears extensively modified cuticular structures (valves) to churn out and separate the seminal fluid and release sperm from the spermiozeugma. The vaginal spoon-shaped cuticular rod in *A. guttatus* is similar to that found in *Somatochlora arctica* (MIDTTUN, 1976). The valve of the bursa communis of *A. guttatus* is similar to those found in Zygoptera (MILLER, 1987) and seems to be a modification associated with the endophytic ovipositing habit of these dragonflies. In *A. guttatus*, the modified scales of the lateral vaginal plates may help to orientate the egg in order to facilitate fertilization and oviposition.

The eggs of *A. guttatus* are long and spindle-shaped, exhibiting endophytic characteristics similar to those of *Aeshna juncea* (SAHLEN, 1994) and *Gynacantha millardi* (pers. obs.). The distinct polygonal reticulation observed at the anterior end of the eggs of *A. guttatus* differs from *Gynacantha tibiata* (MAY, 1995) and *Ictinogomphus rapax* (ANDREW & TEMBHARE, 1992), in which the whole egg surface is hexagonally reticulated. The reticulation probably helps to anchor eggs laid in thin plant tissue and may also help the vagina to hold the egg firmly during fertilization. The structure of the micropylar complex of *A. guttatus* is similar to that of other aeshnids, although the number of micropylar orifices varies between species (SAHLEN, 1994).

In *A. guttatus* the anterior exochorionic "collar" is formed as a protective covering over the exposed region of the egg, since the egg is inserted in the plant tissue (CORBET, 1962). The endochorion is smooth and devoid of hexagonal reticulation in most aeshnids (SAHLEN, 1994), but in *A. guttatus*, faint hexagonal reticulations are also observed at the anterior end of the endochorion.

Accessory sex glands in Odonata have only been reported in the Zygoptera; this is the first investigation exploring their presence in an anisopteran. In the Zygoptera these glands contain well-demarked glandular and ductal regions and the epithelium is unfolded and completely lined with cuticular intima (SRIVASTAVA & SRIVASTAVA, 1992). This differs from the condition in *A. guttatus*, which indicates that the origin of the accessory sex glands probably differs in the two suborders. Furthermore, in Zygoptera the glands cover the eggs with a frothy sticky secretion prior to oviposition. This helps to fix the inserted egg to the submerged vegetation (SRIVASTAVA & SRIVASTAVA, 1992). Such extra-chorionic coating ("spumalin"; MILLER, 1987) was not found by us in the eggs of *A. guttatus*, and no morphological changes were observed in the chorion of pre- and post-oviposited eggs. The secretion may act as a lubricant during oviposition.

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