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Egg structure and fertilization in six libellulid dragonflies (Insecta: Odonata)

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Abstract
The egg of the exophytic dragonflies *i.e.*, *Acisoma panorpoides*, *Crocothemis servilia*, *Neurothemis tullia*, *Orthetrum sabina* and *Rhyothemis variegata* and *Pantala flavescens* exhibit oval, subspherical or spindle shape. Whereas, egg of epiphytic *Brachythemis contaminata* is cylindrical in shape. The egg hatch in 7-15 days and turn dark within a few hours in water. The chorion is distinctly divided into an outer exochorion and an inner endochorion. In water, the exochorion expands into a transparent, thick, sticky jelly-like structure. A well-defined nipple shaped micropylar apparatus is located at the anterior end of the egg. It is composed of a small basal sperm storage chamber (atrium) and a median projecting stalk which possesses a pair of subterminal orifices.
For fertilization the mature eggs travels down the oviduct and enter the cuticular vagina. The pair of cuticular membranes present on the floor of the vagina slides the egg in the vagina where it is held by the lateral vaginal plates and the micropylar apparatus comes to lie under the fertilization pore. The campaniform sensillae of lateral vaginal plate transmit an impulse to the last abdominal ganglion which decodes and stimulates the muscles of bursa copulatrix and spermatheca to contract. This releases sperm material which reaches the tubular fertilization pore passing through the bursa communis. The sperm rushes down the passage of the fertilization pore and slithers inside the micropylar orifice located on either side of the micropylar stalk to reach the micropylar atrium for fertilization.

Keywords: chorion, egg, fertilization pore, micropylar apparatus, sperm

Introduction

In Odonata, two general modes of oviposition (and egg-types) exist. Endophytic eggs in which females insert their eggs into living or decaying plants using an ovipositor occur in the suborders Zygoptera, Anisozygoptera and the families Aeshnidae and Petaluridae in the Anisoptera (Corbet, 1999) [11]. Exophytic (Aphytic) eggs are laid freely into water or on wet land. The egg shell of Odonata comprises of the exochorion and endochorion. The exochorion of exophytic species undergoes structural modification to adapt to various physiological and survival needs. Typical exophytic mode of oviposition where the eggs during oviposition extrude from the genital opening, accumulate under the sub-genital plate and are then washed off by flicking the abdominal tip in water (Corbet, 1999) [11]. The exochorion expands into a transparent, thick, sticky jelly-like structure and the egg bloats. The sticky exochorion of adjacent eggs fuse in wet condition to form an egg-mass (Hinton, 1981; Trueman, 1990, 91; Andrew & Tembhare, 1992, 95, 96; Sahlen, 1994, 95; May, 1995; Andrew, 2002, 09; Gaino *et al*., 2008) [13, 18, 4, 16, 14, 2, 12].

Ando (1962) [1] reviewed the shape and external morphology of dragonfly and found that the shape of the eggs vary from spherical to tubular/ cylindrical Eggs that are laid endophytically are spindle shaped and several times longer than wide [*Calopteryx*, *Ceriagrion*, *Lestes*, *Polycanthagyna*, *Aeshna* (Ando,1962) [1], *Aeshna juncea* (Sahlen, 1994a) [16], *Anax guttatus* (Andrew & Tembhare, 1997] [7] whereas, exophytic eggs are spherical or subspherical [*Ictinogomphus rapax* (Andrew & Tembhare, 1992) [4], *Somatochlora metallica* (Sahlen, 1995) [17], *Bardinopyga geminata* and *Rhyothemis variegata variegata* (Andrew & Tembhare, 1996) [6] *Tramea virginia* (Andrew, 2002) [2], and *Libellula depressa* (Gaino *et al*., 2008) [12], *Orthetrum sabina sabina* and *Brachydiplax sobrina* (Andrew, 2009) [3]]. Epiphytic species have elongated/cylindrical eggs [*Tholymis tillarga* (Miller & Miller, 1985) [15], *Zyxomma petiolatum* (Andrew & Tembhare, 1995) [5], *Micrathyria dictynna* (Andrew & Foerster, 2015)].

May (1995) [14] observed that there was a phylogenetic pattern of progressive reduction in the number of micropylar orifices from the most ancient family (Epiphlebiidae) having 12-14 to the most recent (Libellulidae) having only two micropylar orifices. According to Andrew (2002) [2] the micropylar stalk is modified for rapid fertilization during the process of oviposition. The present work has been undertaken to study the egg of seven libellulid dragonflies and to put forward the step wise process of fertilization based in tehe structure of the egg and the post ovarian genital complex.

Material and Methods

Collection of eggs: Egg-laying female dragonflies were collected during the post-monsoon period (July-Sept, 2018-19). The unwetted fertilized eggs were collected from the sub-genital plate by holding the wings flat and mimicking the dipping action Wet eggs were obtained by placing the abdominal tip in water containers to initiate egg shedding (Andrew & Tembhare, 1995) [5]. Some eggs were also obtained by dissecting the oviduct and vagina. The exochorion of a few eggs was removed using fine tipped forceps or 10% KOH treatment (Truemen, 1991). The eggs were processed for SEM by the methods described by Andrew & Tembhare (1992, 1995) [4, 5].

Histology: The dragonflies were dissected under the binocular stereo microscope (Magnus- MS 24) in Ringer's solution to remove the internal female genitalia. They were fixed in aqueous Bouin's fixative for one day and later washed in water, dehydrated in a series of ascending grades of aqueous alcohol, cleared in xylene, infiltrated in molten paraffin wax at 62ºC and blocks were prepared (Tembhare, 2012). The blocks were sectioned at 8 - 10 µm and stained with Delafield Haematoxylin Eosin, observed under Carl Zeiss microscope (Primostar- 37081) and photographed using Cat Cam Microscope Eyepiece digital camera (CC 130). Some POGC were treated with boiling 10 % KOH and stained with Eosin and processed for light microscopy as per the method detailed in Tembhare (2012) for whole mount preparation.

Observation

During oviposition the eggs pass through the median oviduct in the vagina where they are fertilized and move out of the gonopore and accumulate under the sub genital plate of the ninth segment. The female, releases the eggs from the subgenital by dipping the abdominal tip in water. In *Brachythemis contaminata* the eggs are smeared and glued on wet emerging aquatic vegetation.

Acisoma panorpoides

The egg is spindle-shaped with a broad midline. When released it is yellow in colour which turns brown within an hour. The egg is 0.38±0.08 mm long and 0.32±0.08 mm broad in the middle. In water the exochorion swells to form a soft sticky covering of 0.18 mm thickness. A prominent nipple-shaped micropylar apparatus is located at the tip of the anterior end. The eggs incubate in 12-15 days and the pro-larva hatches out through a longitudinal slit on the surface of the endochorion (Fig. A).

Brachythemis contaminata

The egg is comparatively long and cylindrical measuring 0.62±0.14 mm in length and 0.30±0.08 mm in width. The eggs do not separate easily in water but form a bunch. The exochorion is pitted and exhibits very slight swelling but with very high degree of adhesiveness. Light reticulations can be demarcated on the chorion. The micropylar apparatus is prominently differentiated at the anterior tip of the egg. The eggs incubate in 10-12 days (Fig. B).

Crocothemis servilia

The egg is oval in shape and light yellow in colour. The egg is 0.42±0.12 mm long and 0.38±0.08 mm broad in the middle. In water the exochorion uniformly swells around the egg and form a sticky covering of about 0.12 mm thickness. A tiny nipple-shaped micropylar apparatus is anteriorly placed. The chorion darkens within a few hours after oviposition. The eggs incubate in 07-11 days (Fig. C).

Neurothemis tullia tullia

The egg is very small and sub- spherical measuring 0.29±0.08 mm in length and is 0.27±0.06 mm wide in the middle. The egg changes from cream to brown within 20 minutes in water. The exochorion exhibits very slight swelling in water. The micropylar apparatus is triangular and tiny. The eggs incubate in 8-10 days (Fig. D).

Orthetrum sabina sabina

The egg is oval with a linear dimension of 0.37±0.04 X 0.32±0.02 mm. The exochorion exhibits moderate swelling in water. The micropylar apparatus is prominently placed at the anterior tip of the egg. In water the colour changes from light to dark brown. Incubation takes place within 07-10 days (Fig. E).

Rhyothemis variegata variegata

The egg is oval with a very prominent micropylar apparatus at the tip. It measures 0.42±0.12 mm in length and is 0.32±0.08 mm wide in the middle. The colour changes from light yellow to brown within an hour in water. The eggs incubate within 7-10 days (Fig. F).

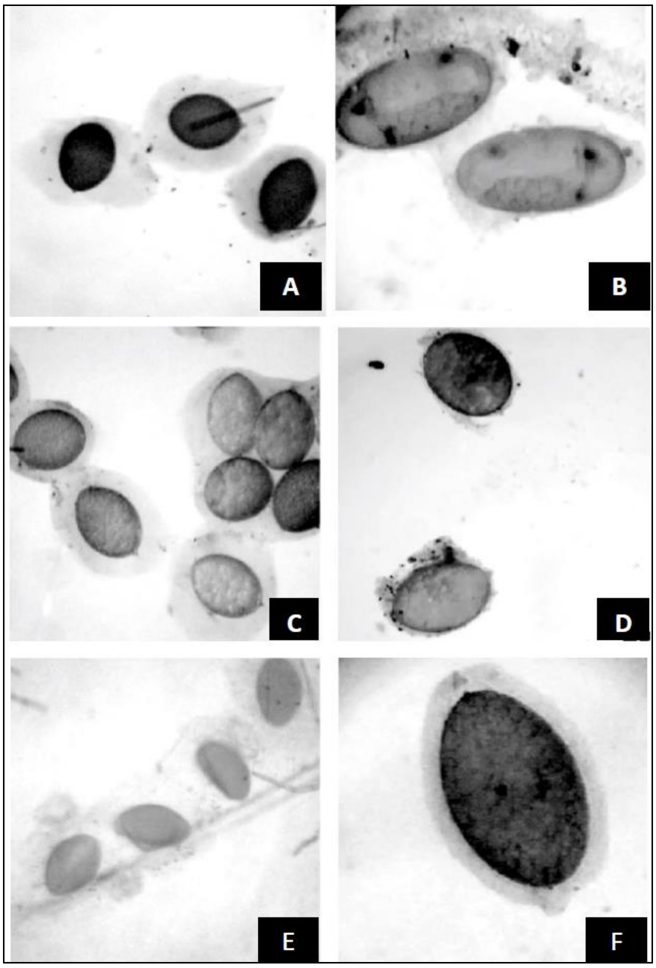
Pantala flavescens

Freshly laid unwetted eggs are pale yellow, oval, slightly sticky and measure about 0.72±20×0.53±10 mm. The chorion is distinctly divided into an outer exochorion and an inner endochorion. The exochorion forms a thin covering which can be easily detached from the endochorion. In water, the exochorion expands into a transparent, thick, sticky jelly-like structure**.** The sticky exochorion of adjacent eggs fuse in wet condition to form an egg-mass. A micropylar apparatus is located at the anterior end of the egg and is well defined from the exochorion by a circular depression. The micropylar apparatus is nipple shaped and is composed of a small basal sperm storage chamber (atrium) and a median projecting stalk. The stalk possesses a pair of sub terminal orifices (Andrew *et al*., 2011) [10] (Fig. 1B).

Egg Fertilization

After a thorough evaluation of the female reproductive system with special attention to the post ovarian genital complex and egg shell architecture of the seven libellulid dragonflies, the following steps are proposed to depict the process of fertilization.

The mature eggs post vitellogenesis travels down the oviduct and enter the cuticular vagina. The pair of cuticular membranes present on the floor of the vagina (Fig. 1A, 2A) helps the egg to slide down without tilting and losing its orientation. In the mid vaginal region, the lateral vaginal plates border the egg and orient the anterior region to face upwards so that the micropylar apparatus comes to lie under the fertilization pore hanging from the roof of the vagina (Fig. 1B). The lateral vaginal plates are studded with mechanoreceptors in the form of campaniform sensillae. These tactile receptors, with the weight and pressure of the egg get stimulated and initiate a receptor potential, giving sensory input about the position and orientation of the egg. The innervated sensory neurons in these receptors respond to this compression probably generating a tonic response. The impulse is transmitted to the last abdominal ganglion located just above the post ovarian genital complex (Fig. 1C). The impulse is perceived by the ganglion (at the position and orientation of egg in the vagina) and it transmits (through the ascending inter-neuron) this impulse to the muscles of bursa copulatrix and/or spermathecae which results in contraction of these sperm storage organs. This causes the release of sperm material from the sperm storage organs into the cuticular cancliculi and the sperm ultimately reached the tubular fertilization pore passing through the bursa communis (Fig. 2B, 2C). At this stage, the stalk of the micropylar apparatus is gripped by the lateral walls of the fertilization pore. The sperm rushes down the passage of the fertilization pore and slithers inside the micropylar orifice located on either side of the micropylar stalk and reaches the micropylar atrium. Later, the sperm penetrates the underlying vitelline membrane to fertilize the egg (Fig. 3A).



Figs: A-F. Eggs of libellulid dragonflies. Fig. A: *Acisoma panorpoides* (Spindle shaped with thick exochorion). Fig. B. *Brachythemis contaminata* (Cylindrical with thinner exochorion). Fig. C. *Crocothemis servilia* (Subspherical with thick sticky exochorion forming clusters). Fig. D. *Neurothemis tullia* (Subspherical, sticky exochorion). Fig. E. *Orthetrum sabina* (Exochorion sticking to form a clutch). Fig. F. *Rhyothemis variegata* (Uniform thin exochorion).

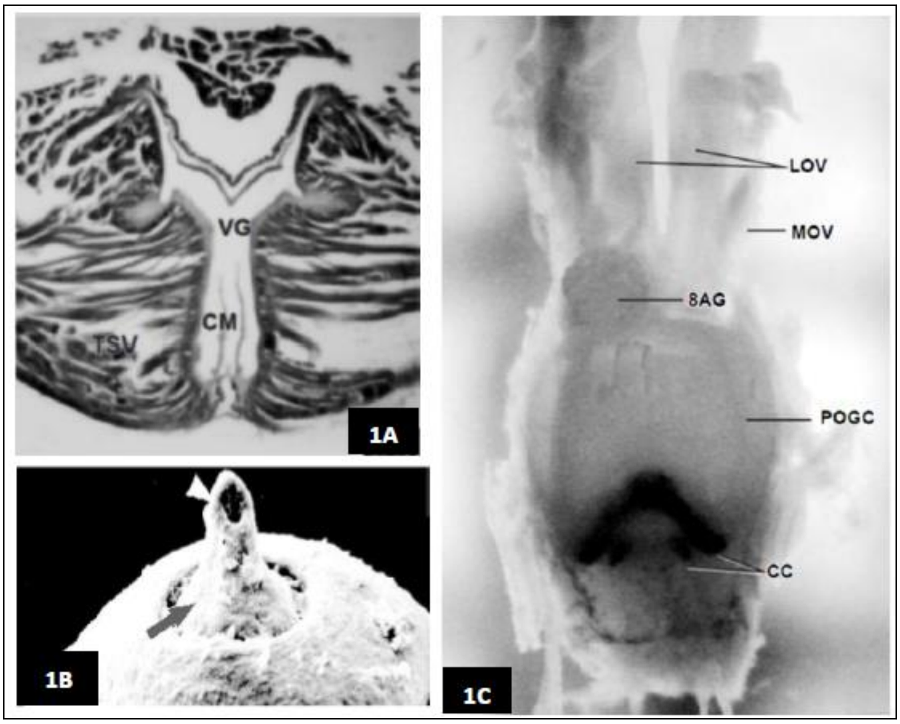


Fig. 1A. *Rhyothemis variegata*: Transverse sections of vagina showing cuticular membrane along the floor of vagina‘ (VG), which is surrounded by vaginal muscles (TSV) (x150). Fig. 1B. *Pantala flavescens*: Scanning electron micrograph of micropylar apparatus exhibiting a tiny atrium (arrow), cylindrical micropylar stalk with subapical micropylar orifice (white arrow head). Fig. 1C. *Acisoma panorpoides*: Whole mount of the postertion abdominal region, showing the eighth abdominal ganglion (8AG) above the post ovarian genital complex (POGC) embedded with cuticular collars (CC). Note the descending lateral and median oviducts (LOV, MOV) (x40).

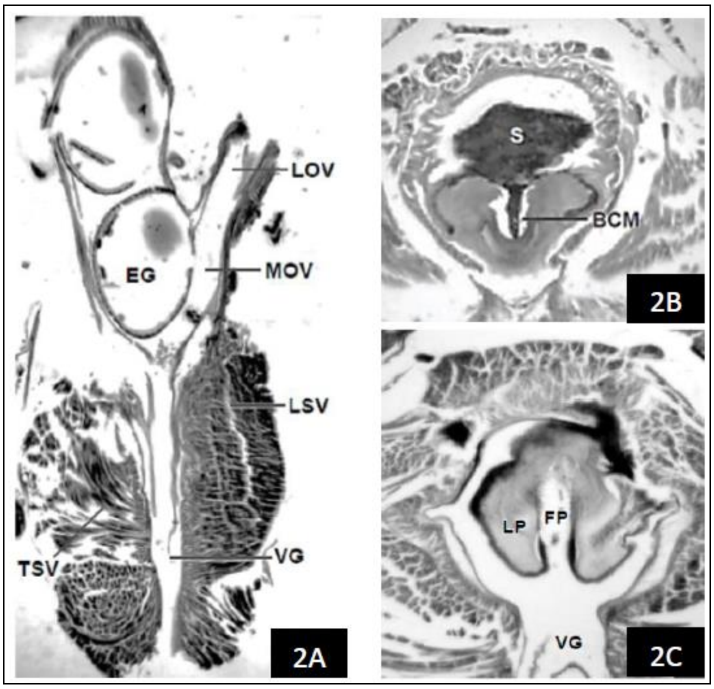


Fig 2A. *Acisoma panorpoides*: Section showing the lateral and median oviducts filled with eggs and opening in the vagina (VG) which is surrounded by vaginal muscles (TSV & LSV) (x80). Fig. 2B. *Neurothemis tullia*: Section passing through the female genitalia showing sperm material in the bursa copulatrix (BC) and bursa communis (BCM) (x200). Fig. 2C. *Acisoma panorpoides*: Sections showing the fertilization pore formed from lateral plates (LP) and hanging from the roof of vagina (VG) (x150).

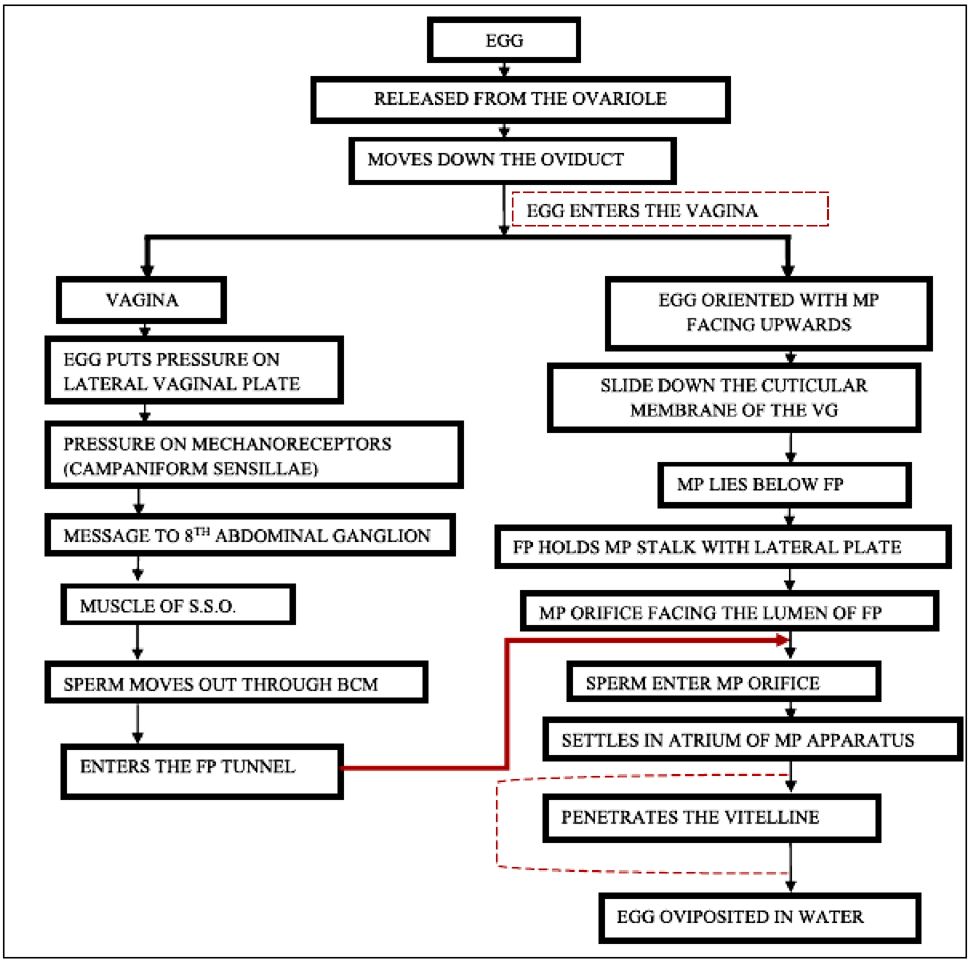


Fig 3A: Schematic diagram of proposed steps undertaken during egg fertilization in a libellulid dragonfly. (BCM- Bursa communis, FP- Fertilization pore, MP- Micropylar apparatus, MP ORIFICE- Micropylar orifice, SSO- Sperm storage organ, VG- Vagina)

Discussion

Acisoma panorpoides, *Crocothemis servilia*, *Neurothemis tullia*, *Orthetrum sabina*, *Rhyothemis variegata* and *Pantala flavescens* exhibits a typical exophytic mode of oviposition (Corbet, 1999) [11], where the eggs during oviposition extrude from the genital opening, accumulate under the sub-genital plate and are then washed off by flicking the abdominal tip in water. The egg of these dragonflies are oval/spheroid, or spindle shaped which is a typical feature of exophytic eggs (Miller & Miller, 1985; 91; Ivey *et al*., 1988; Trueman, 1990, 91; May, 1995; Sahlen, 1995; Andrew & Tembhare, 1996; Andrew, 2002, 09; Gaino *et al*., 2008) [15, 18, 14, 17, 6, 2, 12]. This shape helps to facilitate quick sinking of the egg in the water body and to avoid being consumed by fishes and other aquatic animals. Further, these eggs can easily settle in small recesses on the floor of the water body. The exochorion of these exophytic dragonflies swells into a sticky spongy jelly-like mass in water. The expanded sticky exochorion not only protects the growing embryo but firmly anchors the egg to the substrate. It also provides an extended area for tiny particles to stick to it and forms a protective camouflage. The swelling of the exochorion of *Neurothemis tullia* is not as pronounced and thick as that of *Orthetrum sabina* and *Rhyothemis variegata.*

Although sculptured endochorion has been reported in *Landona deplanta* (Ivey *et al*., 1988) and *Bradinopyga geminata* (Andrew & Tembhare, 1996; Andrew *et al*., 2006) [6, 9] the endochorion of the dragonflies observed in the present study is unsculptured as found in many other libellulids (Andrew and Tembhare, 1996; Andrew, 2009) [6, 3]. The micropylar apparatus of all these dragonflies is typically libellulid (Trueman, 1991; May, 1995) [19, 14] nipple shaped with a tiny atrium (sperm storage chamber) and a short stalk possessing a pair of apical micropylar orifices. The micropylar apparatus of *Tramea virginia* and *Brachydiplax sobrina* possess a large dome shaped atrium (Andrew & Tembhare, 1996; Andrew, 2002, 09) [6, 2]. The micropylar stalk is cone shaped in *Bradinopyga geminata*, humped in *Brachydiplax sobrina* and cylindrical in *Zyxomma petiolatum* (Andrew &Tembhare, 1995, 96; Andrew 2009) [5, 3]. The micropylar stalk of these are the ‘concave cone’ type, also found in the dragonfly, *Idionyx yolanda*, *Idomacromia proavita*, *Neocordulia batesi* and *Uracis imbuta* (May, 1995) [14]. The stalk is modified in accordance with the shape and size of the fertilization pore of the vagina. A circular band of depression, demarcating the exochorion around the micropylar apparatus of *Pantala flavescens* and *Rhyothemis variegata* is also reported in the libellulid Tramea *virginia* and *Brachydiplax sobrina* (Andrew & Tembhare, 1996; Andrew, 2009) [6, 3]. Species specific modification in the micropylar apparatus of the egg corresponds to the modification in the cuticular fertilization pore, present at the roof of the vagina (Verma, 2015, 2016, 2017, 2022) [20, 21, 22, 23]. These modifications help to rapidly process the egg clutch for fertilization as it moves down the tract of the female genitalia for oviposition

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