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Surface ultrastructure of eggs chorion of *Andrallus spinidens* (Fabricius) (Heteroptera: Pentatomidae)

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Abstract
Scanning electron microscopy studies on eggs chorion of pentatomid bug *Andrallus spinidens* (Fab.). Eggs were barrel shaped, convex circular apex and flattened at the base. The anterior pole of the eggs contains a circular operculum that was lifted during hatching. It measured about 0.5 ± 0.06 mm in diameter. Egg chorion covered by hexagonal follicular cells with diameter 26.92 ± 0.02 µm. On the anterior pole of the egg, a long aero-micropylar process is arranged in circular fashion, around the operculum. Freshly laid eggs show a folded aero-micropylar process which was attached to the operculum. The average number of processes varied from 16 to 20. Each aero-micropylar process has a central canal for the entry of sperm and respiration.

Keywords: *Andrallus spinidens*, eggs, scanning electron microscopy, aero micropylar process

Introduction
The Pentatomid bugs are a widely distributed and economically important group of insects. These bugs may be a major crop pest, or a useful biological control agent (Carver *et al.*, 1991 [7]; Schaefer and Panizzi, 2000 and Triplehorn and Johnson, 2005) [18]. Eggs are cylindrical or barrel-shaped and always oviposited in clusters, in number of batches, (Esselbaugh, 1946 [9], Southwood, 1956 [16], Hinton 1981) [10]. Morphological character of eggs especially, the chorion protects the oocyte from environmental and mechanical stresses (Andrew and Tembhare, 1995, 1996 [1]; Belles *et al*., 1993, Pascual *et al*., 1990 [14]; Regier and Kafatos, 1985) [15]. The surface of chorion shows distinct patterns, smooth, granulated, reticulated hexagonal, spinose and salebrose (Esselbaugh, 1946 [9], Southwood, 1956 [16], Javahery, 1994 [11]; Wolf and Reid, 2001). An operculum present on the anterior pole of the egg, it is lifted during egg hatching and aeromicropylar process always present surrounded by operculum (Esselbaugh, 1946 [9], Southwood, 1956 [16], Brailovsky *et al.* 1992 [5]; Wolf and Reid, 2001). Among the Heteroptera, Pentatomidae are considered the best studied group concerning the earlier stage of development (Yonke, 1991) [20]. Several reports are available on morphology and ultra-structure of eggs of pentatomid bugs but, there is no report on egg chorionic structure and position of aero micropylar process of pentatomid bug *A. spinidens* (F) has been demonstrated.

Materials and Methods
A. spinidens (F) was collected from rice fields near by Bhandara district in Maharashtra, India and reared in laboratory of Department of Zoology RTM Nagpur University Nagpur. The fresh eggs were collected from the laboratory and proceeded for Scanning electron microscopy (SEM). This study was carried out in Sophisticated Analytical Instrument Facility (SAIF) of Indian Institution of Technology (IIT) Mumbai, India.

For SEM study eggs were cleared in xylene and dried at room temperature. Then materials were mounted on carbon-coated metallic stubs and coated with platinum coating in the JOEL coating unit. After coating materials were observed in a JOEL JSM- 7600.

Result and Observation
Andrallus spinidens deposit its eggs in two rows along the stem or upper side of leaves in natural field condition (Fig.1) but random egg laying was found in laboratory condition (Fig.2). The eggs were placed in contact with another egg in egg mass or substratum, by black colour sticky substance called meconium secreted by female bugs. Eggs were barrel shaped, convex circular apex and flattened base and it was measured about 1 ± 0.05 mm in length and 0.7 ± 0.03 mm in width (Fig.3 A). Freshly laid eggs were creamy white in colour and turned dark brown with a golden shine within an hour after egg laying. A day before hatching, the dark brown eggs colour changed into dark red. The anterior pole of the egg contains a circular operculum that was lifted during hatching and it was measured about 0.5 ± 0.06 mm in diameter. The hatching line was observed just on the inner side of the aero-micropylar process on the anterior pole of the egg (Fig.3 B). Chorion surface of egg covered by hexagonal follicular cells with diameter 26.92 ± 0.02 µm (Fig.3 C). On the anterior pole of the egg, the long aero-micropylar process arranged in circular fashion and average number of processes varied from 16 to 20 (Fig.3 D). The length of the aero-micropylar process was measured about 1.19 ± 0.03 µm. Freshly laid eggs show folded aero-micropylar process, that was attached to the operculum area of egg (Fig.3E) and after 4 to 5 hours, it erect out and apical portion of aero-micropylar become free from egg surface. The stalk of aero-micropylar was broad at base and gradually decreased up to apex and contained a circular apical portion with cross shape opening with crest border. The base of the aero-micropylar was measured about 31.73 ± 0.006 µm in diameter and the apical portion was measured about 19.13 ± 0.02 µm in diameter (Fig.3 F).

Fig 1: Egg laying of *A. spinidens* in rice field

Fig 2: Egg laying of *A. spinidens* in laboratory condition

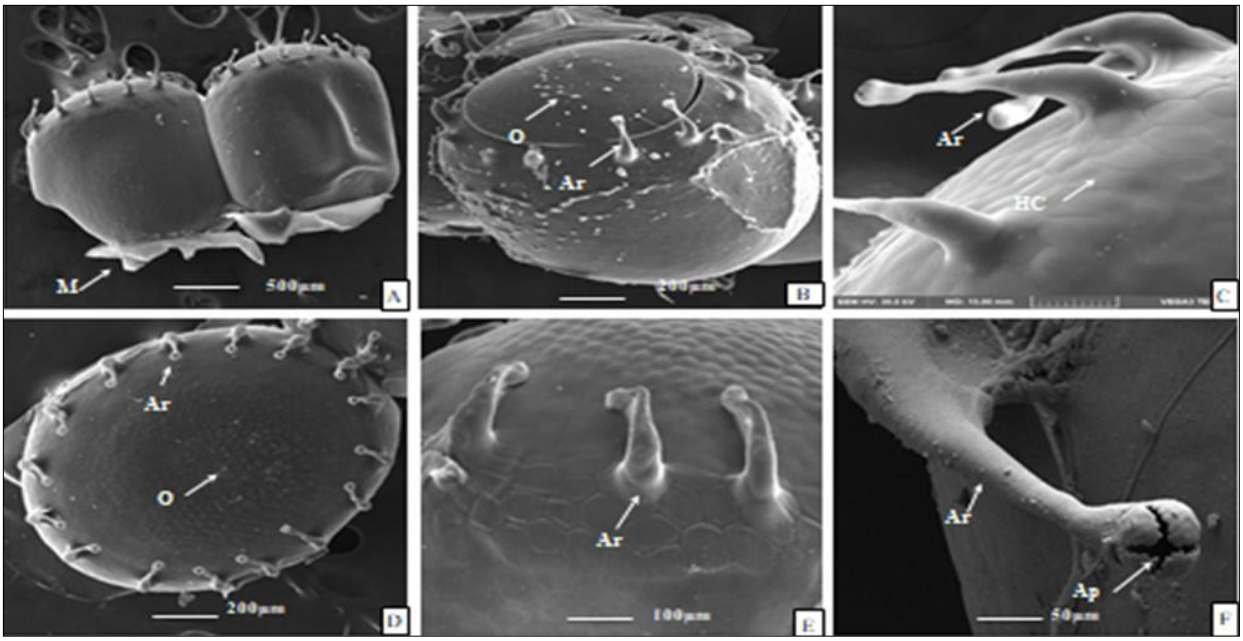

Fig 3: Scanning electron microscopic (SEM) structure of eggs of *Andrallus spinidens*. A: Lateral view of egg; B. Egg operculum; C. Egg showing hexagonal cell on surface, D: Anterior view of egg; E. Areo-micropylar process; F. Magnified view of aero-micropylar process, Abr.: Ar- Aero-micropylar, HC- Hexagonal cell, O-Operculum, AP – Apical pore and M –Meconium.

Discussion

Eggs of pentatomid bug *A. spinidens* laid in clusters generally on the upper surface of leaves. Eggs were barrel shaped, convex circular apex and flattened base attached to the substratum. The eggs were attached to each other by a sticky substance that was secreted by the female bug. Similar structure of eggs and cluster formation found in other pentatomid bugs (Esselbaugh, 1946 [9]; Southwood, 1956 [16]; Hinton, 1981 [10]; Candan *et al*., 2001 [6]; Matesco *et al.,* 2014,) [13]. Eggs of different Heteroptera especially from family Pentatomidae show changes in colour after egg laying and during embryonic development, generally fertile eggs change their colour during development (Kumar *et al*., 2002 [12], Barsagade and Salwe, 2020 [3], 2021) [2]. During the present study, it has been noticed that the eggs change their colour during development and confirmed by earlier findings in *A. spinidens* anterior pole of egg contains circular operculum that is lifted during hatching. During the hatching process, circular operculum is removed by young nymph and this circular cover at the anterior surface of the egg is known as "pseudo- operculum" reported by Southwood (1956) [16]. The egg surface of *A. spinidens* was covered by a polygonal cell. Polygonal cell mostly pentagonal and hexagonal follicular cell similar type of observation found in other bug *Podisus sagitta* (F.) (Wolf and Reid, 2004). The hatching line was observed on the anterior pole of egg just internal side to the aero-micropylar process in *A. spinidens,* similarly such hatching line was also found in other pentatomid eggs (Suludere *et al*., 1999 [17]; Candan *et al*., 2001 [6]; Wolf and Reid, 2004).

Aero-micropylar processes vary in number, length and shape according to the species present in all Pentatomid bugs, organized in a circular manner outside the hatching line (Esselbaugh, 1946 9[]; Southwood, 1956 [16]; Javahery, 1994) [11]. According to Esselbaugh (1946) [9], in Pentatomidae aero- micropylar processes number vary from 5 to 72 and very long in some genera, in present study, *A. spinidens* show 16 to 20 aero- micropylar processes. In *A. spinidens* apical opening is cross shape with crest border. According to Southwood (1956) [16], Cobben (1968) [8], Hinton (1981) [10] and Javahery (1994) [11], this apical opening is the central channel for sperm passage and for gaseous exchange of the embryo. The presence of apical opening of aero- micropylar process may be used for sperm passage and gaseous exchange in *A. spinidens.*

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