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[SHORT COMMUNICATION]

Histological Study of the Ovarian Follicle Epithelium in *Hyleoglomeris* (Diplopoda, Glomerida, Glomeridae): Two Subpopulations in the Follicle Epithelial Cells

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The oocytes of most arthropods are surrounded by ovarian somatic tissue, i.e., follicle epithelium (Chelicerata: Makioka, 1979; Myriapoda: Yahata et al. 2018; Crustacea: Kubrakiewicz et al., 2012; Hexapoda: Büning, 1994). The follicle epithelium of the Hexapoda contains subpopulations of cells (Margaritis et al., 1980; Büning, 1994; Garbiec and Kubrakiewicz, 2012): for example, in *Drosophila melanogaster,* 11 subpopulations of follicle epithelial cells, such as posterior polar follicle cells, can be detected (Büning, 1994). These subpopulations of follicle epithelial cells differ from each other in their morphological features, i.e., cell-shape, organelle composition, and spatial position within the follicle epithelium (Margaritis et al., 1980; Tworzydlo and Kisiel, 2011), and have special functions that are important for oogenesis such as choriogenesis (Margaritis et al., 1980; Büning, 1994).

Although a number of studies have investigated the subpopulations of hexapod follicle epithelial cells (Büning, 1994; Tworzydlo and Kisiel, 2011), the cellular subpopulational construction of the follicle epithelium is yet to be clarified in arthropods other than Hexapoda. We discovered the cellular subpopulational construction of the follicle epithelium in two *Hyleoglomeris* (Diplopoda: Glomerida) species, and the histological and histochemical characteristics of these subpopulations of the cells are detailed herein.

Adults of *Hyleoglomeris japonica* Verhoeff, 1936 were collected in Mito and Tsukuba, Ibaraki Prefecture from May to October 2014 and from April to July 2015. Adults of *Hyleoglomeris yamashinai* Verhoeff, 1937 were collected in Uruma, Okinawa Prefecture in September 2014. Their ovaries were fixed either with Bouin's solution overnight, with 4% paraformaldehyde for 3 h or with Karnovsky's solution (2% paraformaldehyde + 2.5% glutaraldehyde) for 2 h. Some fixed specimens were dehydrated in a graded ethanol-*n*-butanol series and embedded in paraffin while others were dehydrated in a graded ethanol series and embedded in methacrylate resin (Technovit 7100: Külzer, Wehrheim, Germany). The 5-μm-thick paraffin sections and the 2-μm-thick resin sections were stained with Mayer's hematoxylin–eosin and then observed under a light microscope (BH-2: Olympus, Tokyo, Japan). To observe cells, the fixed ovaries were stained with rhodamine phalloidin for 40 min and 1 μmol/l 4',6-diamidino-2-phenylindole (DAPI) for 30 min, and observed under a fluorescence microscope (Eclipse Ni-U: Nikon, Tokyo, Japan) with excitation at 540 and 360 nm.

The *H. japonica* and *H. yamashinai* oocytes were surrounded by a flattened, single-layered follicle epithelium, which was connected to the ovarian epithelium. The follicle opened into the hemocoel at the follicle pore (Fig. 1A, D). The gross morphology of the ovaries in these species, as observed in the present study, was identical to that reported in previous studies of *H. japonica* (Yahata and Makioka, 1997) and other myriapods (Yahata and Makioka, 1994; Miyachi and Yahata, 2012; Yahata, 2012; Yahata et al., 2018).

In the *Hyleoglomeris* species examined, the follicle epithelial cells measured 1–2 μm in height while the cells opposite the follicle pore measured 2–10 μm in height (Figs. 1B, C, E, 2A). The epithelial cells opposite the follicle pore were therefore thicker than those located elsewhere (Fig. 1A, D) and could clearly be distinguished from the thinner cells according to their location within the follicle. The follicle epithelium of *Hyleoglomeris* was thus composed of two subpopulations of cells which can be distinguished by the cell-shape and spatial position within the follicle epithelium. The follicle epithelial region opposite the follicle pore composed of thicker cells is herein referred to as the "apical thick region," while the region composed of thinner cells is referred to as the "main-body region." Among Arthropoda, structurally different subpopulations of follicle epithelial cells have been described only in Hexapoda (Büning, 1994): hence, this is the first report of such subpopulations of follicle epithelial cells in arthropods other than Hexapoda.

In the apical thick region, 10 or more cells (Fig. 2A, A') were aligned in a rosette-like formation (Fig. 2B, B') with their nuclei located opposite to the center of the rosette (Fig. 2B'). The cells of the apical thick region were more eosinophilic than those of the main-body region (Fig. 1A, C–E). As acidophilic materials are known to show stainability to eosin, it is suggested that the apical thick region is rich in acidophilic materials such as proteins. Additionally, the cells of this region contained many lipid droplet-like vacuoles (Fig. 1C, E). These features indicate that the apical thick region has a secretory function. Strong phalloidin staining was also observed in this region (Fig. 2A'), especially in its central area (Fig. 2B'), suggesting a high inclusion of F-actin. Specific F-actin localization has previously been demonstrated in specialized subpopulations

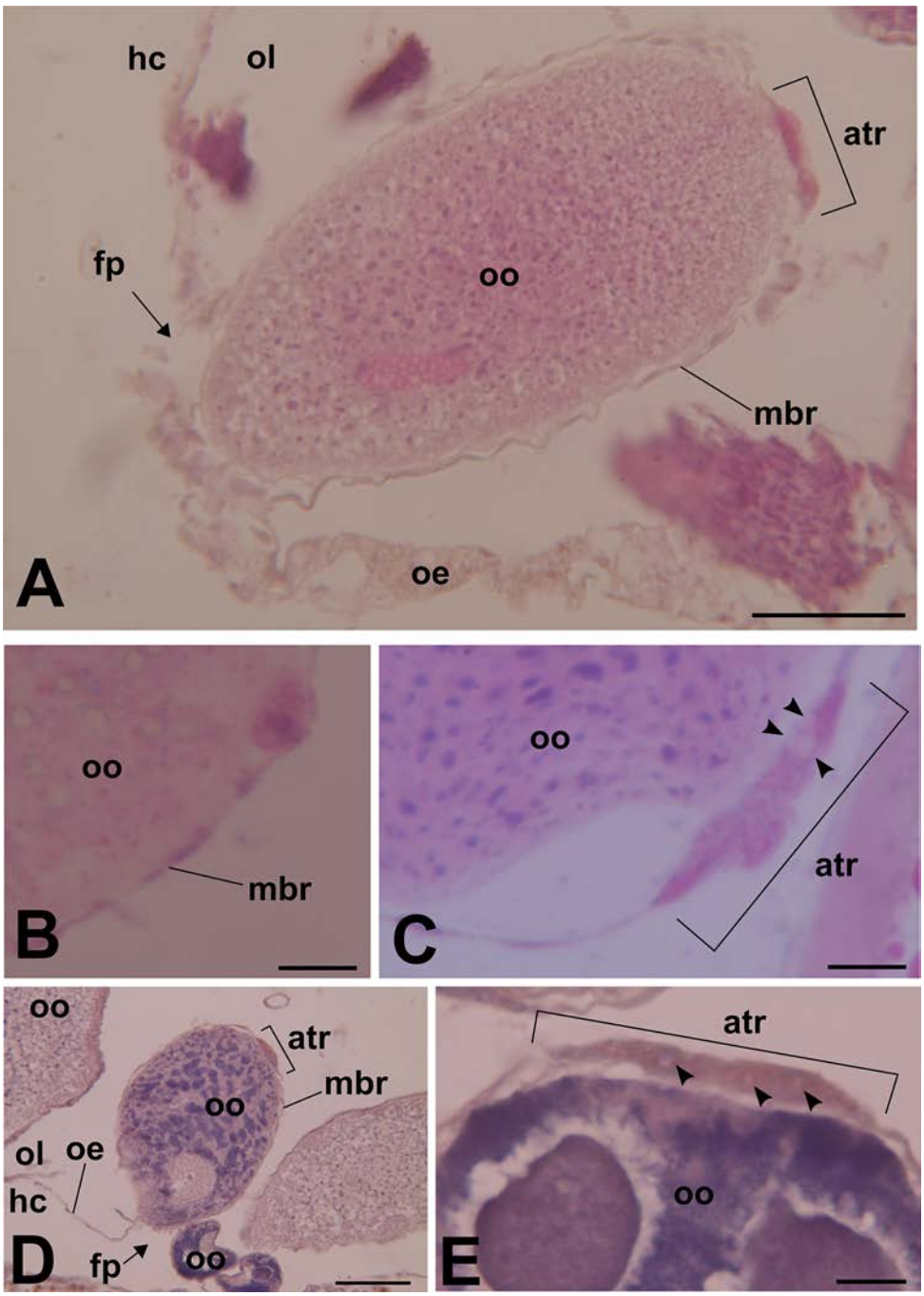

Fig. 1 The ovarian follicles and follicle epithelia of *Hyleoglomeris japonica* and *H. yamashinai*. A. The ovarian follicle of *H. japonica* showing two distinct regions, i.e., the apical thick region and the main-body region. Paraffin. B. Main-body region of *H. japonica* follicle epithelium. The main-body region constructs a major part of follicle epithelium with cells measured 1–2 μm in height. Methacrylate resin. C. Apical thick region of *H. japonica* follicle epithelium. The apical thick region is composed of follicle epithelial cells measuring 2–10 μm in height. The cells of this region intensely stained by eosin, are rich in lipid droplet-like vacuoles (arrowheads). Methacrylate resin. D. The ovarian follicle of *H. yamashinai*. As in *H. japonica*, the apical thick and main-body regions of the follicle epithelium can be distinguished. Paraffin. E. Detail of the apical thick region of the *H. yamashinai* follicle epithelium. Paraffin. atr: apical thick region, fp: follicle pore, hc: hemocoel, mbr: main-body region, oe: ovarian epithelium, ol: ovarian lumen, oo: oocyte, arrowhead: lipid droplet-like vacuole. Scales = A, D: 50 μm; B, C, E: 10 μm.

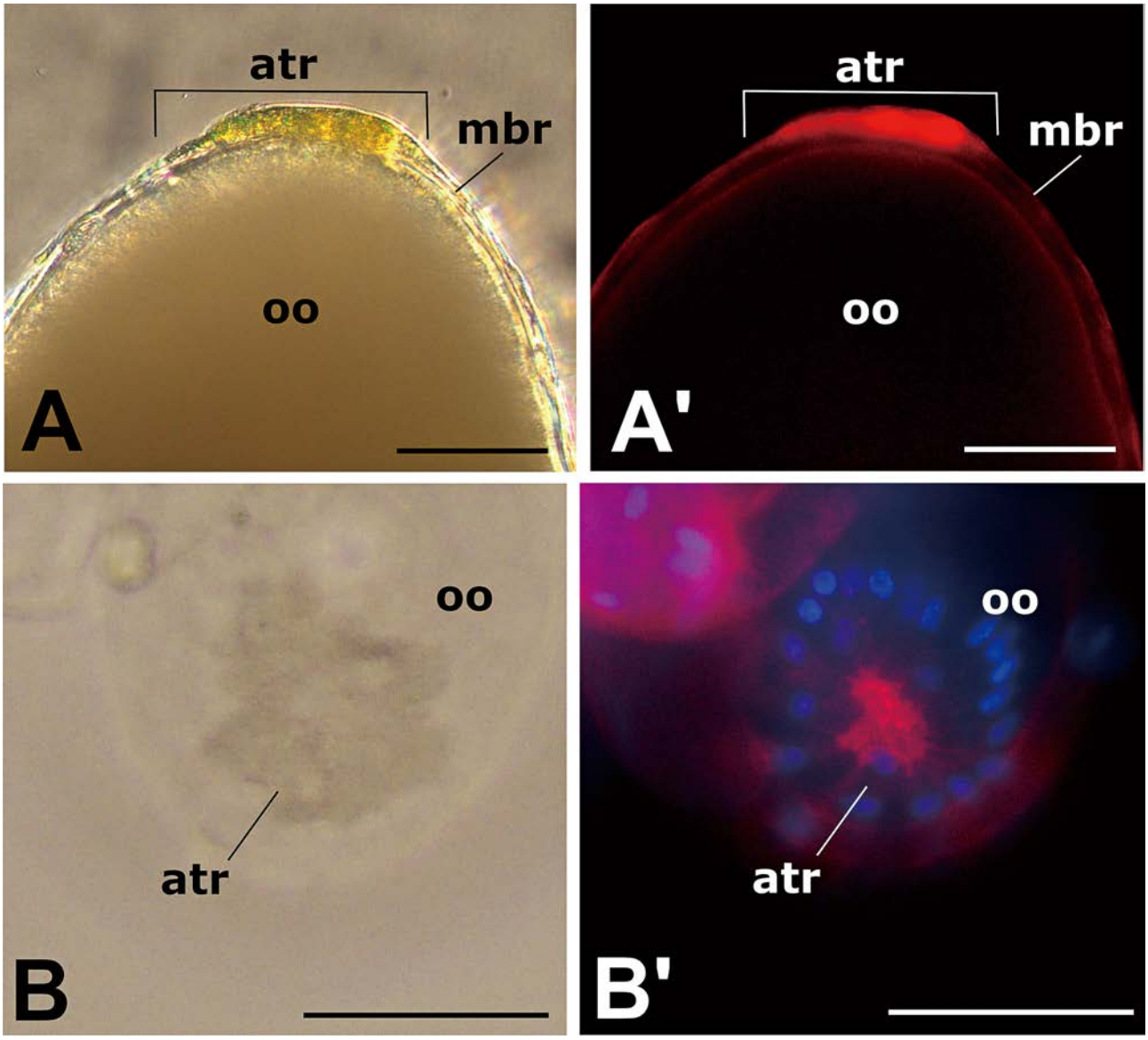

Fig. 2 Whole-mount images of the ovarian follicle of *Hyleoglomeris japonica* A, A'. DIC (A) and fluorescence microscope (A') images of the apical thick region of the follicle epithelium, stained by phalloidin, lateral view. B, B'. DIC (B) and fluorescence microscope (B') images of the apical thick region of follicle epithelium, stained by phalloidin and DAPI, apical view. atr: apical thick region, mbr: main-body region, oo: oocyte. Scales = 50 μm.

of epithelial cells in the follicles of hexapods (Blattodea: Zhang and Kunkel, 1992; Diptera: Büning, 1994; Phthiraptera: Zawadzka et al., 1997). These specialized subpopulations of the follicle epithelial cells with high F-actin levels are known to play important roles in oogenesis, such as formation of the micropyle (e.g., Zhang and Kunkel, 1992; Kubrakiewicz et al., 2005; Zawadzka et al., 1997). F-actin richness in the apical thick region in *Hyleoglomeris* suggests that it has a possible special function related to oogenesis.

To fully understand the apical thick region in *Hyleoglomeris* follicles, intensive ultrastructural, histochemical and developmental studies are needed, which will demonstrate the diversity of ovarian somatic tissue in myriapods and arthropods.

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