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[SHORT COMMUNICATION]

Oothecae and eggs of *Anaplecta japonica* Asahina, 1977 (Insecta: Blattodea, Anaplectidae)

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Blattodea, consisting of about 7,600 known species, is divided into three superfamilies: Blaberoidea, Blattoidea including termites (i.e., “Isoptera”), and Corydioidea (see Evangelista et al. 2019). Anaplectidae comprises two genera (*Anaplecta* and *Maraca*), including 115 described species (Beccaloni 2014), and its systematic placement within Blattodea has been a subject of discussion for a long time. Morphological studies of the genitalia and proventriculus affiliated Anaplectidae to Blaberoidea, and sometimes placed it in Blattellidae s.l. as one of the subfamilies (McKittrick 1964; Roth 1990, 1996, 2003; Grandcolas 1996). On the contrary, recent molecular phylogenomics has indicated that Anaplectidae represents one of major lineages within Blattoidea, with a likely alignment to subsocial Cryptocercidae + eusocial “Isoptera” (Djernæs et al. 2015; Wang et al. 2017; Bourguignon et al. 2018; Djernæs and Murienne 2022). Thus, Anaplectidae is one of the most significant groups that would enable the reconstruction of the phylogeny of Blattodea and the evolutionary history of the sociality developed in Blattodea. However, our biological knowledge regarding Anaplectidae is extremely limited because it is rare and difficult to procure. For these reasons, we initiated the embryological study of Anaplectidae using a Japanese anaplectid, *Anaplecta japonica* Asahina, 1977. As a first step of our study, we report the oothecae and eggs of *A. japonica*.

In September 2022, 111 adults were collected from Ainan Town, Minamiuwa District, Ehime Prefecture, and 23 adults were obtained from Uwajima City, Ehime Prefecture (Fig. 1A). They were reared in plastic cases (23 cm × 15 cm × 17 cm) that had a moistened leaf mold bottom, topped with dead leaves of deciduous-broadleaved *Quercus*, maintained at room temperature, and were fed a feed mixture as written in Fujita et al. (2011), a commercial food for goldfish (TetraFin,

Fig. 1 A. A sampling site in Ainan, Minamiuwa, Ehime Prefecture. Many *Anaplecta japonica* adults were collected from the undergrowth of path side. B. An *A. japonica* adult feeding on a feed mixture. Bar = 5 mm.

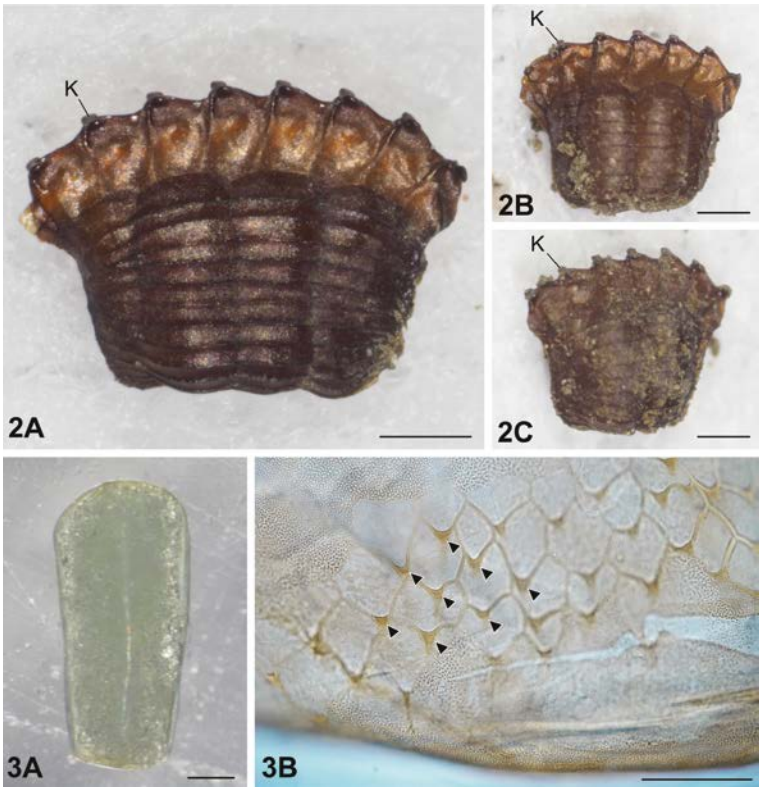
Spectrum Brands Japan, Kanagawa, Japan), or dead insects such as termites (Fig. 1B). Fifty-six oothecae were obtained in three months.

Oothecae were dark brown and exhibited a well-developed, serrated keel on their dorsal median line. Four to six eggs were included in each ootheca, although in most cases, the number of eggs in the ootheca was six (5.7 ± 0.6, n = 25) (Fig. 2A – C). For fixation, cutting off the keel of the ootheca, the eggs were punctured at their exposed anterior part with a fine tungsten needle using Karnovsky's fixative (2% paraformaldehyde + 2.5% glutaraldehyde in a 0.1 M HCl-sodium cacodylate buffer, pH 7.2) and fixed for 24 h. The oothecae containing fixed eggs were soaked in a 10% solution of a dishwasher detergent (JOY, P&G Japan, Kobe, Japan) for half a day to soften the oothecal wall, following which, the eggs were carefully taken out of the ootheca. The eggs, which were approximately 1.2 mm in length and 0.6 mm in width, were whitish yellow in color (Fig. 3A).

The chorion was torn open at the dorsal side of the egg, and the yolk inside was removed for observing the micropyles. The opened chorion, following cleaning with an ultrasonic cleaner in a 10% water solution of dishwasher detergent, was mounted with glycerin, and observed using a biological microscope (Optiphot-2, Nikon, Tokyo, Japan) equipped with a high-resolution objective (Plan Apo 60x / N.A. = 1.40 Oil DIC, Nikon, Tokyo, Japan) under a bright field. The micropylar area, which displays on a connected-polygons pattern, is at the posterior ventral side of the egg as observed in Nocticolidae (see Fujita et al., 2020, Fig. 4D, E), and a funnel-shaped micropyle opens at the posterior corner of each polygon (arrowheads in Fig. 3B). Thirty to 40 micropyles are in the micropylar area.

The oothecae of Anaplectidae were reported for two Panamanian species, *Anaplecta hemiscotia* Hebard, 1920 and *Anaplecta lateralis* Burmeister, 1838 by Hebard (1920). In accordance with this report, the ootheca of these species possesses a very heavy sutural ridge, i.e., keel, similar to that observed in the present study for *Anaplecta japonica*.

Fujita and Machida (2017) and Fukui et al. (2018) suggested that "micropyles grouped on the ventral side of the egg" are a groundplan feature of Dictyoptera, and that this is likely an apomorphic groundplan feature of this lineage. The present study revealed that the micropyles of *A. japonica* are likewise distributed on the ventral side of the egg. Fujita et al. (2020) recognized several varieties with respect to the position of the micropylar area in Blattodea. For cockroaches, i.e., "Blattaria", the micropylar area is: 1) in the anterior region of the ventral side of the egg



Figs. 2, 3 Oothecae and eggs of *Anaplecta japonica*.

Fig. 2 Oothecae. Oothecae, bearing a well-developed, serrated keel along its dorsal median line, contain mostly six eggs (A), rarely five (B) or four (C). K: keel. Bars = 500 μm.

Fig. 3 Eggs. A. An egg taken out of the ootheca, ventral view, anterior to the top. B. Micropylar area, anterior to the top. Arrowheads show micropyles. Bars = A: 200 μm, B: 50 μm.

in *Blattella germanica* (Linnaeus, 1767) (Blaberoidea, Blattellidae s.s.) (Fujita 2016); 2) in the middle region in *Periplaneta fuliginosa* Serville, 1838 (Blattoidea, Blattidae) and *Cryptocercus punctulatus* Scudder, 1862 (Blattoidea, Cryptocercidae) (Thipaksorn and Machida unpublished); and 3) in the posterior region in *Eucorydia yasumatsui* Asahina, 1971 (Corydioidea, Corydiidae) (Fujita and Machida 2017) and *Nocticola* sp. (Corydioidea, Nocticolidae) (Fujita et al. 2020). With regard to termites, i.e., "Isoptera" , which are subordinated to Blattoidea, the micropylar area is: 1) in the posterior region of the ventral side of the egg in *Kalotermes flavicolis* (Fabricius, 1793) (Kalotermitidae) and in the middle region in *Zootermopsis nevadensis* (Hagen, 1874) (Archotermopsidae) (Striebel 1960; Mukerji 1970). The present study has demonstrated that the micropylar area in *A. japonica* of Anplectidae, the phylogenetic affiliation of which within Blattodea has long been argued, is located in the posterior region of the ventral side of the egg. Phylogenetic placement of Anaplectidae cannot be determined solely based on the distribution type of micropylar area, but further survey of micropylar distribution, including that in more number of lineages of Blattodea, is required.

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