Proc. Arthropod. Embryol. Soc. Jpn. 38, 13–17 (2003)

ISSN 1341–1527

Embryonic Development of a Proturan *Baculentulus densus* (Imadaté): Reference to Some Developmental Stages (Hexapoda: Protura, Acerentomidae)

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Abstract

Improving rearing techniques, we succeeded in obtaining six eggs of a proturan *Baculentulus densus*, different in developmental stage, under rearing conditions. By external observations of these eggs, we presented several features of the egg structure and embryogenesis. The egg is snow-white in color, nearly spherical with a long diameter of about 130 μm, and furnished with numerous, various-sized and -shaped protuberances on its surface. The newly formed blastoderm is extraordinarily thick. On the inner side of the blastoderm at the ventral side, a pair of cellular aggregations, which may be involved in the mesoderm segregation, is formed. The differentiated germ band is long enough to almost occupy the egg circumference. The blastokinesis is a simple flection of the embryo. The egg period is speculated to be about two months. The extraordinarily thick, early blastoderm and manner of mesoderm segregation shown in this species may be unique in Hexapoda. The long germ band and simple blastokinesis are features common to those of other entognathans.

Introduction

Protura represent one of the basal clades of Hexapoda, and are important for the reconstruction of the hexapod groundplan and phylogeny and for discussions on the hexapod origin. Aiming at the comparative embryological study, one of the most useful approaches for solving phylogenetic problems, the collection of proturan eggs had been attempted for some time. Eventually, Bernard (1976, 1979) succeeded in obtaining the proturan eggs from the field, which were at the stage just before hatching. Larink and Biliński (1989), being supplied with proturan eggs by Bernard, studied the egg membranes. However, collecting eggs from the field is considered destructive, and difficulties in the identification of species and in the specification of stages make egg collection under rearing conditions a prerequisite for the comprehensive, detailed embryological study of proturans. Due to the difficulty in rearing proturans, however, a number of attempts to collect their eggs under rearing conditions have failed, and no more information on proturan eggs and embryogenesis had emerged.

Recently, doubt has been cast on the hexapod phylogenetic system, currently the most accepted, or the "Entognatha-Ectognatha system" from some disciplines (Kukalová-Peck, 1987; Koch, 1997; Kraus, 1998) including our comparative embryological contribution (Ikeda and Machida, 1998a, 2001). Some evidence suggestive of closer affinity of the Myriapoda and Chelicerata (or a part of the former and part of the latter) has emerged (Aguinaldo *et al.*, 1997; Hwang *et al.*, 2001; Sakuma and Machida, 2002), so that the monophyletic status of Atelocerata (=Myriapoda+Hexapoda) should be reexamined as well. A contribution to the embryological research on Protura, being one of the basal clades of hexapods, is required more than ever.

We have been attempting to improve the rearing techniques for proturans, aiming at collecting eggs for embryological study. We finally succeeded in rearing *Baculentulus densus* for over a year, and obtained six eggs of the species, each differing in stage (Machida and Takahashi, 2003). In this paper, we report some features of the eggs and embryogenesis of the species, based on these six eggs.

Materials and Methods

Adults of *Baculentulus densus* Imadaté were extracted from the litter of a deciduous broadleaved forest dominated by *Quelcus serrata*, at Shinkoji, Sanada, Nagano Prefecture, with a Tullgren's funnel, and placed in newly developed rearing containers (see Machida and Takahashi, 2003), which were filled with pieces of leaf litter. The rearing containers were kept in an incubator: the temperature of the incubator was gradually, over two or three months, lowered from 15℃ (the soil temperature of the habitat in late summer), down to the range 0 to 5℃ [the soil temperature in late autumn to winter, that Takahashi (1998) had determined to be the peak of the breeding season of the species], and maintained. For details on the rearing techniques, see Machida and Takahashi (2003).

The inside of the rearing containers was periodically inspected, and six eggs were obtained. Five of them were incubated in a humid case at room temperature (*ca.* 20℃). The eggs were immersed in water and observed under a light microscope equipped with an extra-long-working-distance objective (Nikon ELWD 20X or 40X). The remaining egg was processed for SEM. It was fixed with Karnovsky's fluid for 30 min, dehydrated with a graded series of ethyl alcohol concentration, dried with a t-butyl alcohol freeze-dryer, coated with gold, and observed under an SEM (Topcon SM-300).

Results and Discussion

Baculentulus densus laid their eggs in October to December of 2001. All the six eggs obtained had been deposited on the pieces of leaf litter. The eggs are snow-white in color, and of a spheroid nearly spherical shape (Fig. 1A), with a long diameter of about 130 μm, and numerous, various-sized and -shaped protuberances are scattered on their surface (Fig. 1B). Larger protuberances are maldistributed in a small area of the egg (cf. Figs. 1–7), which might serve as a merkmal for designating the polarity of the egg. The cephalic region of the developing embryo was localized to the side opposite the area with larger protuberances, irrespective of whether the egg was in a pre- (Fig. 5) or postblastokinesis stage (Fig. 6), and so the area with larger protuberances might represent the posterior pole of the egg. The eggs of this species are consistent with hitherto known eggs of other proturans, *i. e.*, *Eosentomon* spp. (Bernard, 1979; Larink and Biliński, 1989), in shape and the presence of numerous protuberances on the surface, which may be the features of proturan eggs.

Among the five eggs which were incubated for observation, the youngest one had an extraordinarily thick blastoderm (Fig. 2). Such a thickened blastoderm may be unique among hexapods and a significant feature of the embyrogenesis in *Baculentulus densus*. Figure 3 shows the same egg after four days. The blastoderm has thinned a

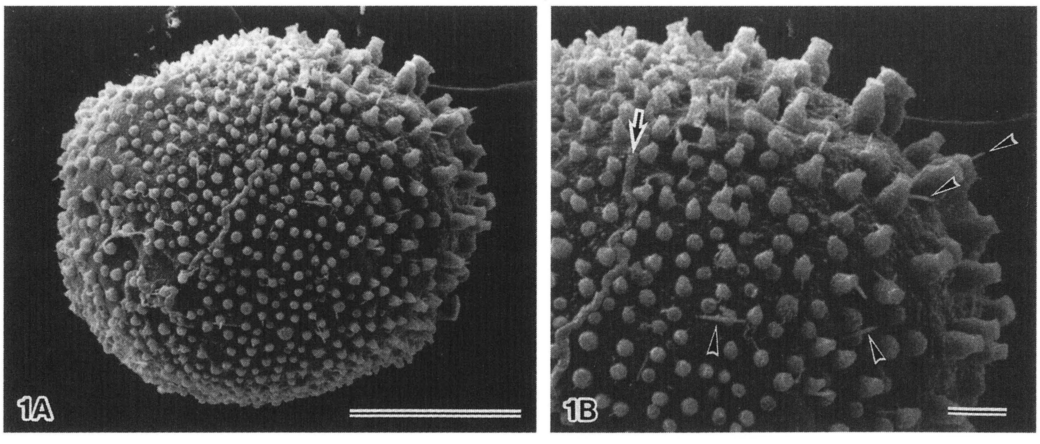
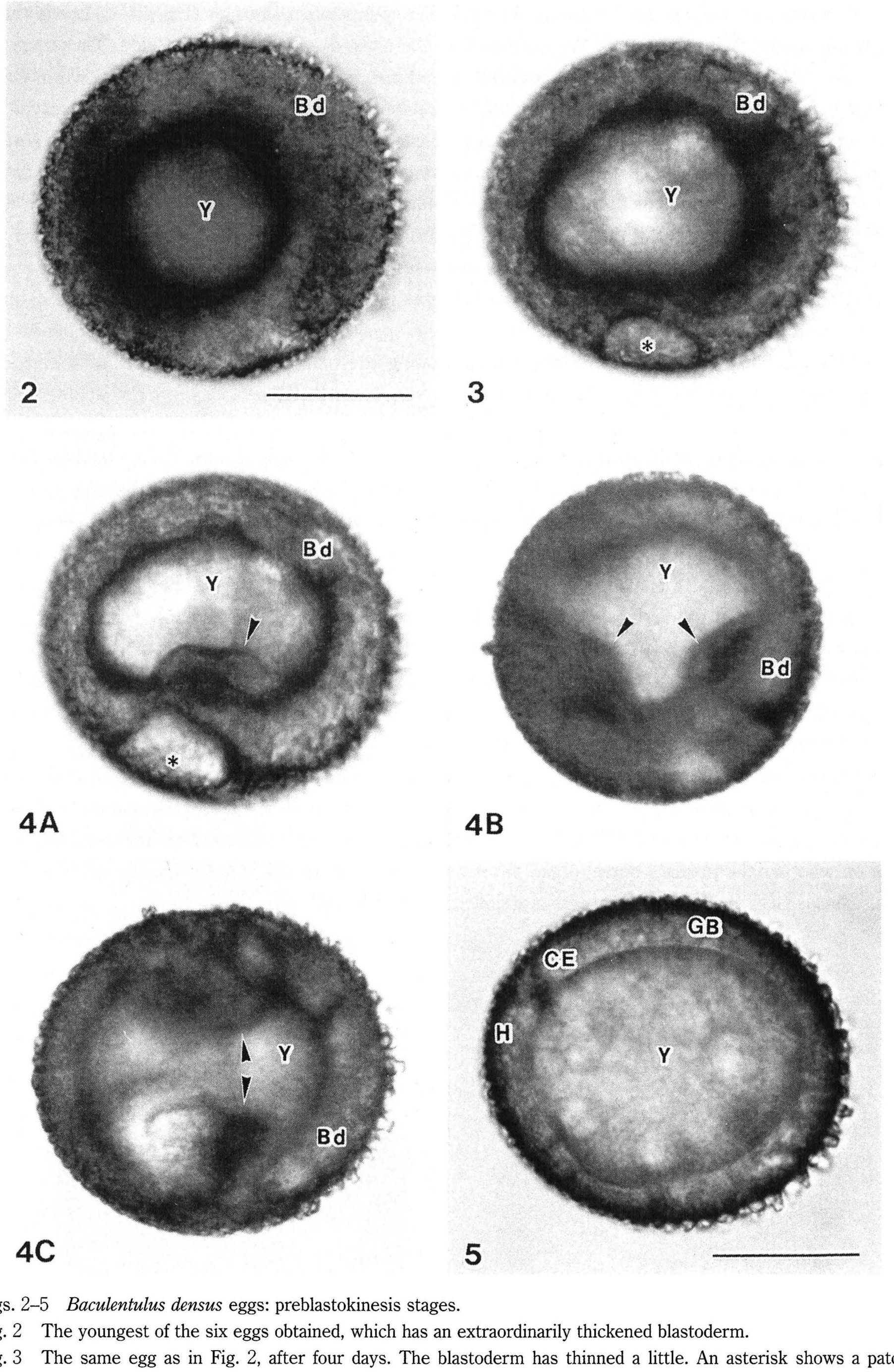


Fig. 1 A. *Baculentulus densus* egg. Larger protuberances are localized in a small area to the right. B. Enlargement. The surface is occupied by numerous, various-sized and -shaped protuberances. An arrow and arrowheads respectively show the fungal mycelium and bacteria adhered to the egg. Bars = A: 50 μm; B: 10 μm.



Figs. 2–5 *Baculentulus densus* eggs: preblastokinesis stages.

Fig. 2 The youngest of the six eggs obtained, which has an extraordinarily thickened blastoderm.

Fig. 3 The same egg as in Fig. 2, after four days. The blastoderm has thinned a little. An asterisk shows a patch artificially made on the surface.

Fig. 4 The same egg as in Figs. 2, 3, after another two days. Three dimensional views (A: lateral, B: anterior/posterior, C: ventral) reveal that a pair of cellular aggregations (arrowheads) have differentiated on the inner side of the blastoderm, which may be involved with the segregation of the mesoderm. An asterisk in A, the same as in Fig. 3.

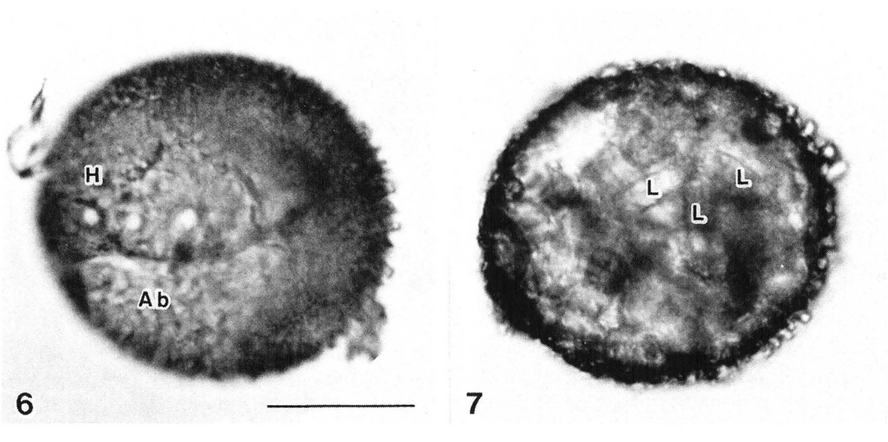
Fig. 5 Egg with an early germ band, which is long enough to almost occupy the egg circumference, lateral view. The cephalic region is localized to the side opposite the area with larger protuberances on the surface.

Bd: blastoderm, CE: caudal end, GB: germ band, H: cephalic region, Y. yolk. Bars = 50 μm.

little. Figure 4 shows the same egg after another two days. Three dimensional images (Fig. 4A–C) reveal that a pair of cellular aggregations has differentiated on the inner side of the blastoderm at the ventral side. These paired cellular aggregations may be involved in the segregation of the mesoderm. If correct, the segregation shown in *Baculentulus densus* is unique among hexapods. Alternatively, it might correlate with the mesoderm segregation observed in Diplura (Uzel, 1898; Ikeda and Machida, 1998b), in which a doughnut-shaped cellular thickening, recognizable as a mesodermal precursor, occurs in the blastoderm at the ventral side: views of a dipluran egg in this stage, taken from any direction perpendicular to the dorsoventral axis of the egg, yield a similar image [*e. g.*, Fig. 17 of Uzel (1898) or Fig. 1c of Ikeda and Machida (1998b)] to that from the anterior or posterior view of a *Baculentulus densus* egg in the same developmental stage (Fig. 4B). Information on the mesoderm segregation in *Baculentulus densus* must be absolute, and so a critical examination is desired. On the surface of the eggs incubated, microorganisms such as fungi and bacteria proliferated markedly. They hindered close observation of the eggs, and the surface had to be cleaned every two or three days. This was very difficult, and all five eggs including the one shown in Figures 2 to 4 were regretfully punctured and failed to be incubated up to hatching. For this reason, we could not follow the development any further.

The remaining four eggs were of approximately the same developmental stage. The surface cellular layer, which should be now called the germ band, further thins (Fig. 5), and the cellular aggregations inside the blastoderm shown in Figure 4 are no longer observed: the mesoderm may have already spread over the dorsad of the germ band as an inner layer. The germ band is very long, almost occupying the circumference of the egg, and closely resembles that shown in other entognathans, *i. e.*, collembolans and diplurans (Collembola: Jura, 1972; Uemiya and Ando, 1987; Diplura: Uzel, 1898; Ikeda and Machida, 1998a). In the following two weeks, similar conditions to those shown in Figure 5 continued, and then blastokinesis occurred. The blastokinesis is a simple flection of the embyro, as in other entognathans, *i. e.*, collembolans and diplurans (Collembola: Jura, 1972; Uemiya and Ando, 1987; Diplura: Uzel, 1898; Ikeda and Machida, 1998a). As a result of blastokinesis, the germ band, which had elongated along the egg circumference, became bent and took a jackknife posture (Fig. 6). We could incubate only a single egg, for another ten days, up to just before hatching: within it was a full-grown embryo compactly packed (Fig. 7).

We have presented the information drawn from the six *Baculentulus densus* eggs collected. Provided that it takes about two weeks to reach the stage shown in Figure 2 and that stages shown in Figures 4 and 5 differ in time only by several days, the egg period of *Baculentulus densus* is presumed to be about two months. We could not obtained any information on whether the primary dorsal organ develops in the proturan embryogenesis. We are now rearing more *Baculentulus densus* than ever, with the intention of collecting more eggs.



Figs. 6, 7 *Baculentulus densus* egg: postblastokinesis stages.

Fig. 6 The same egg as in Fig. 5, after two weeks, lateral view. Blastokinesis, which is a simple flection of the embryo, is completed. The head remains to be localized at the side opposite the area with larger protuberances on the surface. Ab: abdomen, H: head. Bar = 50 μm.

Fig. 7 The same egg as in Figs. 5, 6, after another ten days, in which a full-grown embryo is observed to be compactly packed. L: thoracic legs.

Acknowledgments: We thank Prof. Em. Dr. H. Ando for constant encouragement and Dr. T. Tsutsumi for assistance in surveying materials. This study was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (10640677) and from the University of Tsukuba Project Research, to R.M.

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