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Germ Band Formation of a Centipede *Scolopocryptops rubiginosus* L. Koch (Chilopoda: Scolopendromorpha)

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Introduction

"Myriapoda" (=Chilopoda+Diplopoda+Pauropoda+Symphyla) have been regarded as sharing a common ancestry and constituting a monophyletic taxon, Atelocerata, together with Hexapoda (*e. g.*, Dohle, 1988). However, recent molecular evolutional studies have suggested a closer affinity between Myriapoda and Chelicerata (*e. g.*, Hwang *et al.*, 2001), and phylogenetic relationships between higher taxa of Arthropoda, particularly those concerning Myriapoda, have yet to be clarified. Furthermore, a monophyly of Myriapoda and the affinities of the myriapodan classes themselves are still being argued.

Having many plesiomorphic characters, Chilopoda are the most controversial group in reconstructions of myriapod phylogeny (cf. Dohle, 1988). Therefore, Chilopoda are especially important to attemps to understand and reconstruct the groundplan and phylogeny of Myriapoda and of Arthropoda. The comparative embryological approach is one of the most promising methods for such a phylogenetic analysis. Although we have several studies (*e. g.*, Metschnikoff, 1875; Zograff, 1883; Heymons, 1901; Knoll, 1974), the embryology of Chilopoda is still not well understood. For this reason, we have been conducting a comparative embryological study on a scolopendromorph centipede, *Scolopocryptops rubiginosus* L. Koch. In this paper, we describe the external features of germ band formation in *Scolopocryptops rubiginosus* and refer to previous studies to characterize the germ band formation in chilopods.

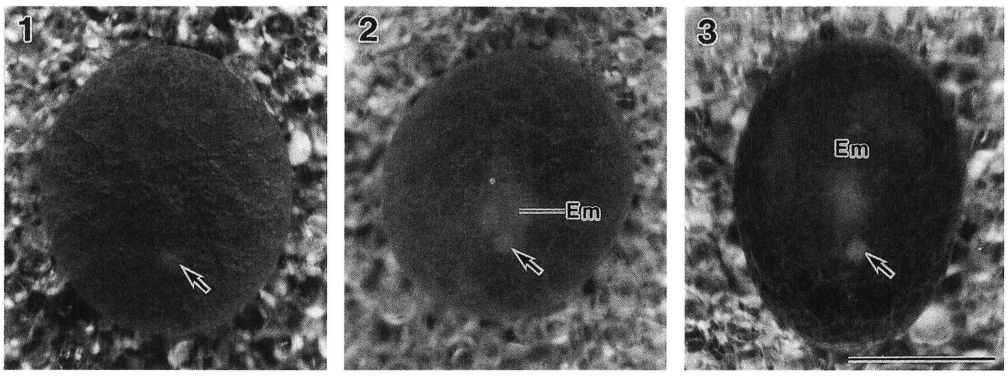
Materials and Methods

Adults of *Scolopocryptops rubiginosus* were collected at Minami-izu, Shizuoka Prefecture, and Ueda and Sanada, Nagano Prefecture, Japan. The centipedes were kept separately in plastic cases (120 mm×80 mm×60 mm) with a moistened plaster bottom at room temperature (about 20℃). They were fed fly larvae. Females deposited eggs in the form of an egg mass and cared for them.

Eggs, isolated from maternal care, were fixed in Bouin's fluid for 1 h and punctured with a fine needle. A small opening was made with fine forceps in the chorion, and the eggs were fixed again for 2 days and stored in 70% ethyl alcohol. The chorion was removed with forceps as needed. The embryos were observed under a stereomicroscope (Leica, MZ12).

Results

In the eggs of eight days after oviposition, a small circular mass of cells, about 100 μm in diameter, appears in the blastoderm (Fig. 1). Ten days after oviposition, an embryo or embryonic anlage about 300 μm long forms just anterior to the cellular mass (Fig. 2). The embryo soon starts to elongate (Fig. 3). Eleven days after oviposition, the embryo has acquired the form of a germ band (Fig. 4), and paired head lobes and antennal segment are recognizable (Fig. 4A); the cellular mass is clearly visible at its original position just posterior to the germ band (Fig. 4B). Twelve days after oviposition, the germ band further elongates (Fig. 5). All cephalic, *i. e.*, preantennal, antennal, intercalary, mandibular, first maxillary and second maxillary, and a few anterior trunk segments differentiate (Fig. 5A), but the following trunk segments are still indistinct (Fig. 5B). At this stage, the cellular mass, which was originally observed to be just to the

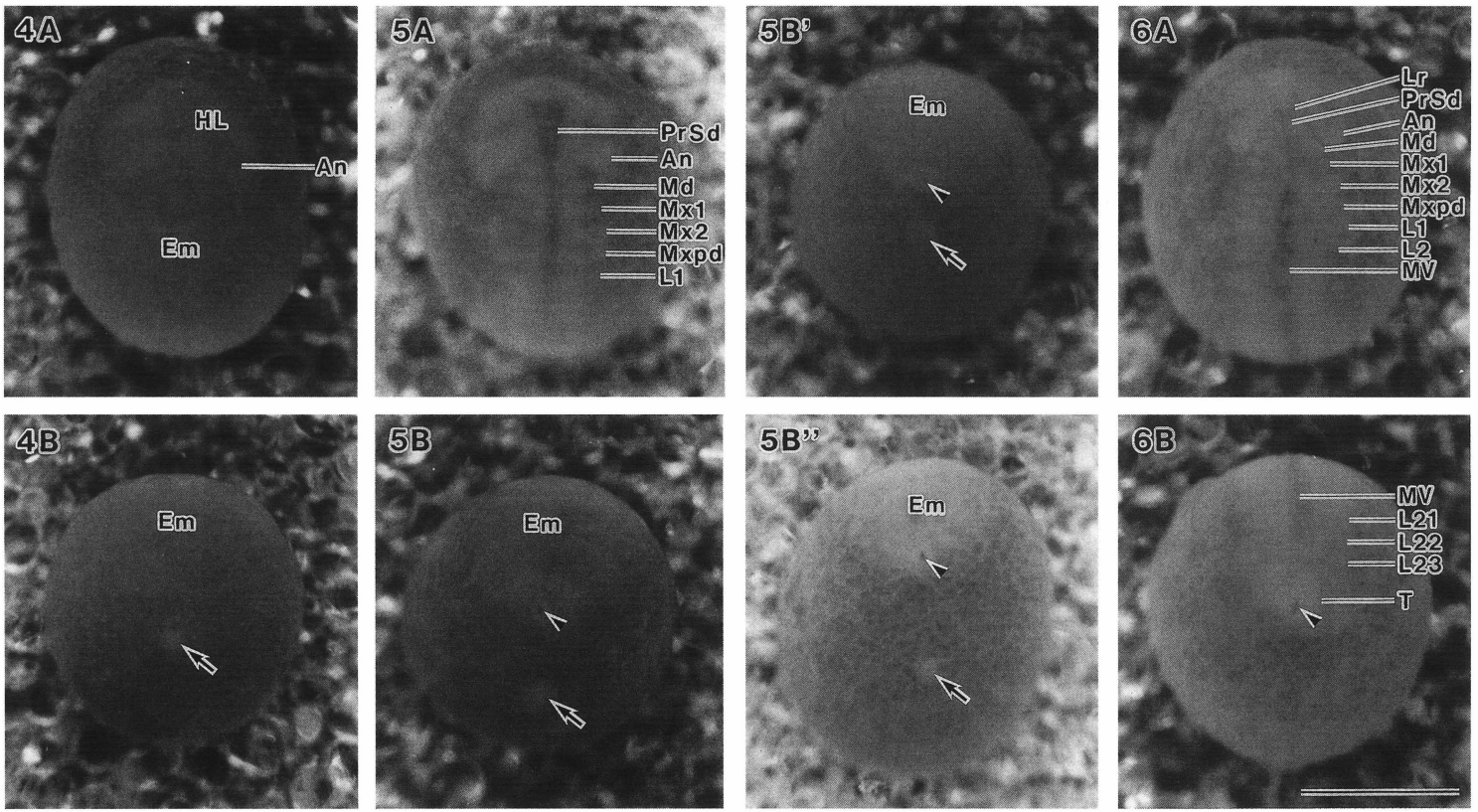


Figs. 1–3 Eggs of *Scolopocryptops rubiginosus*, eight and 10 days after oviposition. Arrows show the cellular mass.
Fig. 1 Egg, eight days after oviposition. The cellular mass appears in the blastoderm.
Fig. 2 Egg, 10 days after oviposition. The embryo (or embryonic anlage) appears anteriorly adjacent to the cellular mass.
Fig. 3 Egg, 10 days after oviposition. The embryo begins to elongate.
Em: embryo. Scale=500 μm.

rear of the germ band (Figs. 2, 3, 4B), starts to move posteriorly. Figure 5B, B', B" shows the migration of the mass: B', starting; B, on the way; B", migrated much far away from the germ band. Whereas the stomodaeum has not yet differentiated and its precursor is recognized as a narrow-inverted triangular area with a low cell density (Fig. 5A), the proctodaeum differentiates and is clearly observed as a small well-defined invagination at the center of the presumptive telson (Fig. 5B, B', B"). Thirteen days after oviposition, the undeveloped trunk segments, except for the pregenital and genital segments, which do not acquire the definitive segmental construction until the postembryonic period, and the telson with the proctodaeum at its center, differentiate. The germ band, 1.5–1.8 mm long, is composed of six cephalic segments, a maxillipedal segment and 23 leg-bearing segments (Fig. 6A, B). The anlagen of an unpaired labrum and paired preantennae, antennae, mandibles, first maxillae and second maxillae are developed, but the stomodaeum is less apparent (Fig. 6A). At this stage, the dissociation of the germ band into left and right halves characteristic of chilopods (see *e. g.*, Heymons, 1901) begins. In Figure 6A, B, a narrow cleft is visible along the median line of the germ band: this is the rudimentary membrana ventralis [nomenclature according to Heymons (1901)]. The cellular mass, which had moved posteriorly away from the germ band, degenerates and disappears.

Discussion

This paper revealed that in *Scolopocryptops rubiginosus*, a relatively small embryonic anlage forms and segmentation proceeds sequentially from the anterior. It was reported, however, that in another scolopendromorph, *Scolopendra cingulata*, middle trunk segments differentiate first, the following segments next, and the cephalic segments last (Heymons, 1901). Furthermore, the proctodaeum differentiates much earlier than the stomodaeum in *Scolopocryptops rubiginosus*, whereas in *Scolopendra cingulata* the reverse is true: the proctodaeal differentiation is much delayed. It is worth noting the close resemblance between the cephalic regions of *Scolopocryptops rubiginosus* early germ bands (Figs. 4, 5) and the caudal regions of *Scolopendra cingulata* early germ bands described by Heymons (1901: *e. g.*, Figs. 8, 12), including the developmental features of the stomodaeum and proctodaeum. Assuming that the anteroposterior axis is reversed in the description of *Scolopendra cingulata* early germ bands, the observational/interpretational contradiction between *Scolopocryptops rubiginosus* and *Scolopendra cingulata* could be resolved. Hence, we suggest that Heymons (1901) described *Scolopendra cingulata* early germ bands with their anteroposterior axes reversed. We thus generalize the germ band formation of scolopendromorph centipedes as follows: i) a relatively small embryonic anlage forms, ii) the anlage gradually elongates, iii) segmentation proceeds from the anterior, with elongation of the germ band, and iv) the proctodaeum appears in the telson, but the differentiation of the stomodaeum is retarded. From i) to iii), the germ band of Scolopendromorpha can be categorized as a short-germ type (cf. Krause, 1939; Sander, 1984). Also in *Scutigera coleoptrata*, belonging to Scutigeromorpha, the germ band, which is initially a relatively small embryonic anlage, is categorized as a short-germ type: the cephalic and anterior trunk segments



Figs. 4–6 Eggs of *Scolopocryptops rubiginosus*, 11 to 13 days after oviposition. Arrows and arrowheads show the cellular mass and proctodaeum, respectively.

Fig. 4 Eggs, 11 days after oviposition. A. Anterior half of the germ band. The head lobes and antennal segment differentiate. B. Posterior half of the germ band shown in A. The cellular mass is just to the rear of the germ band.

Fig. 5 Eggs, 12 days after oviposition. A. Anterior half of the germ band. The cephalic and anterior trunk segments differentiate. B. Posterior half of the germ band shown in A. The cellular mass is located far from the germ band. B', B". Posterior halves of germ bands, differing in degree of migration from the cellular mass. (See text).

Fig. 6 Eggs, 13 days after oviposition. A. Anterior half of the germ band. The dissociation of germ band into left and right halves begins, and a narrow membrana ventralis appears. B. Posterior half of the germ band shown in A. The telson differentiates. The cellular mass is no longer visible.

An: antennal segment, Em: germ band, HL: head lobe, L1, 2, 21–23: 1st, 2nd and 21st to 23rd leg-bearing segments, Lr: labrum, Md: mandibular segment, MV: membrana ventralis, Mx1, 2: first and second maxillary segments, Mxpd: maxillipedal segment, PrSd: presumptive stomodaeum, T: telson. Scale=500 μm.

differentiate first, then the following trunk segments differentiate sequentially towards the rear (Knoll, 1974).

In Geophilomorpha, although segmentation proceeds anterior to posterior as in Scolopendromorpha and Scutigeromorpha, a germ band long enough to account for about three quaters of the circumference of the egg is formed initially (Metschnikoff, 1875; Zograff, 1883), unlike in Scolopendromorpha and Scutigeromorpha in which a relatively small embryonic anlage is formed. We suggest, however, that in Geophilomorpha, a smaller embryonic anlage would exist (that is, in these previous studies on geophilomorph embryogenesis, the stage prior to the appearance of the initial germ band failed to be examined) and that the geophilomorph germ band might initially form as a relatively small embryonic anlage, like in Scolopendromorpha and Scutigeromorpha, which respectively represent one of the derived chilopod clades and the most ancestral clade (cf. Kraus, 1997; Giribet *et al.*, 1999). The germ band fomation in the Scolopendromorpha (as well as that in Scutigeromorpha) may therefore be representative of the Chilopoda: the generalization made for the scolopendromorph germ band fundamentally applies to all the Chilopoda. The groundplan for chilopod germ band formation is in sharp contrast with that of the other myriapods, typically of a long-germ or semilong-germ type (cf. Tiegs, 1940, 1947; Dohle, 1964) . This should be discussed further in light of the phylogeny.

Heymons (1901) suggested a resemblance between the cellular mass situated just to the rear of the chilopod embryonic area and the cumulus posterior (cumulus primitivus or secondary/caudal thickening) in the posterior region of the embryonic anlagen of chelicerates, in which germ band formation is categorized as a short-germ or semilong-germ type (cf. Sekiguchi, 1960, 1973). This paper revealed that the chilopod cellular mass and chelicerate cumulus posterior resemble each other also in behavior: they both migrate posteriorly as the morphogenesis of the germ band proceeds, and eventually degenerate. If a correlation between these structures in chilopods and chelicerates is established, a closer affinity of these animals may be deduced. A structure similar to the chilopod cellular mass is reported in diplopods (Dohle, 1964).

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Appendix: After the submission of paper, we found an excellent paper of Hertzel (1984) on the germ band formation of a lithobiomorph centipede *Lithobius forficatus*, in which he demonstrated the lithobiomorph germ band to be of a short-germ type. Recently, Hughes and Kaufman (2002), in their developmental biological study for another lithobiomorph *Lithobius atkinsoni*, made a similar observation.

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