Cleavage of the jumping bristletail, *Pedetontus unimaculatus* Machida (Hexapoda, Microcoryphia, Machilidae)

H. Ando, R. Machida* and T. Nagashima**

Introduction

It is well known that the cleavage of the winged insects (Pterygota) and silverfishes (Thysanura) is of the typical superficial type.[1, 2] That of another member of the true Insecta (Euentomata) (Microcoryphia + Thysanura + Pterygota) or jumping bristletails (Microcoryhia), which are accepted as the most ancestral within the Euentomata, has been also understood as the typical superficial type similarly to that of the Thysanura and Pterygota.[3, 4] Thus, it has been taken for granted that the Euentomata should perform the superficial cleavage. Our detailed study, however, revealed that the eggs of the jumping bristletail, *Pedetontus unimaculatus* Machida should undergo total cleaving in the early stage as reported for the myriapods or the other arthropods. It may be interesting in the phylogenetic accounts on the insects or on the arthropod higher taxa, i.e. the Antennata (insects + myriapods), to have confirmed here that the Microcoryphia should have the total pattern of cleavage in contrast to our previous knowledge.

Material and Method

The eggs of the jumping bristletail, *Pedetontus unimaculatus* Machid of Machilidae belonging to Microcoryphia were used.

Obtained eggs were incubated at room temperature[18], and fixed in the diluted (50%) Karnovsky's fixative. For sectioning, the eggs were embedded in a methacrylate resin Technovit 7100 (Kulzer) after dehydration. The sections of 2μm in thickness were stained with Schiff's reagent (Feulgen's reaction) and Fast Green FCF, and observed with a light microscope. Eggs were postfixed in 1% OsO_4 and embedded in Epon 812 after dehydration. Embedded eggs were sectioned into golden thickness, stained with uranyl

* Sugadaira Montane Research Center, University of Tsukuba, Sanada, Nagano 386-22, Japan

** Laboratory of Entomology, Tokyo University of Agriculture, Sakuragaoka, Setagaya, Tokyo 156, Japan

acetate and lead citrate and observed with a JEM 100-CX.

Results

Here we briefly report our observations on the cleavage of *P. unimaculatus*. Newly laid eggs, which assume distorted ellipsoid of approximately 1.3×0.7mm, are in the metaphase of the first maturation division. By 7-8 hr after oviposition, the maturation divisions finish by the production of only two polar bodies. The fertilized nucleus or synkaryon derived from the female and male pronuclei starts to divide mitotically at about 11-12 hr. The cell cycle during the following cleavages is approximately 3-4 hr, but it tends to be prolonged as the cleavage proceeds. The cleavage in earlier stages is holoblastic or total. In the 16-nucleus stage the first sign of cytoplasmic division or the furrows corresponding to nuclei in number is recognized on the egg surface between adjacent cleavage nuclei.In the following divisions the furrows gradually deepen (Fig. 1), to divide the yolk mass completely into yolk pyramids or blastomeres (Fig. 2).In this way, in the first three cleavages the cytoplasmic division is rather delayed compared to the nuclear division, but the fourth to eighth or ninth nuclear divisions are accompanied

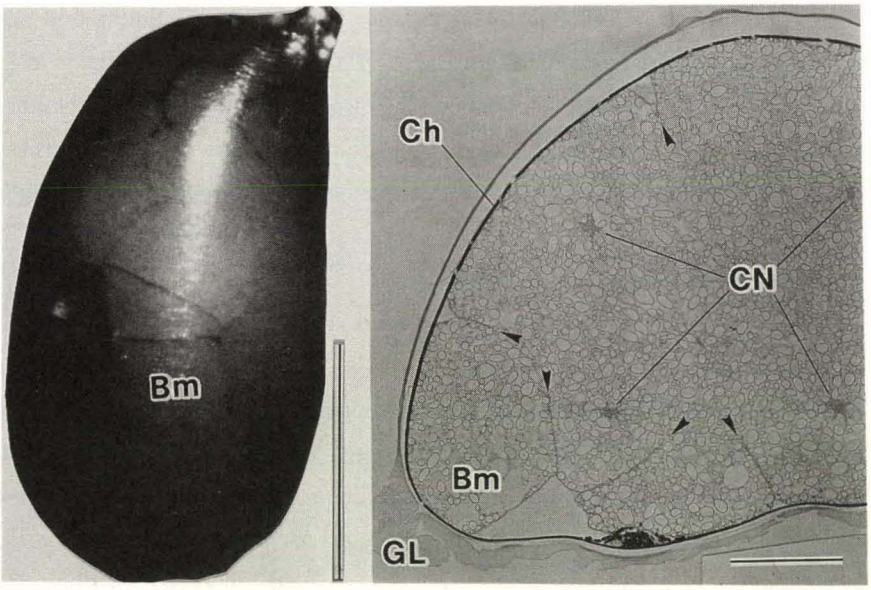

Fig. 1 Eggs of *Pedetontus unimaculatus* in the 32-nucleus stage: Whole (*a*) and sectioned (*b*) eggs. On the egg surface, furrows defining the blastomeres (Bm) are seen (*a*). Cytoplasmic divisions are in progress (arrow heads) (*b*) . Ch, chorion ; CN, cleavage nucleus, GL, gelatinous layer. Scales = 500 μm (*a*), 200 μm (*b*).

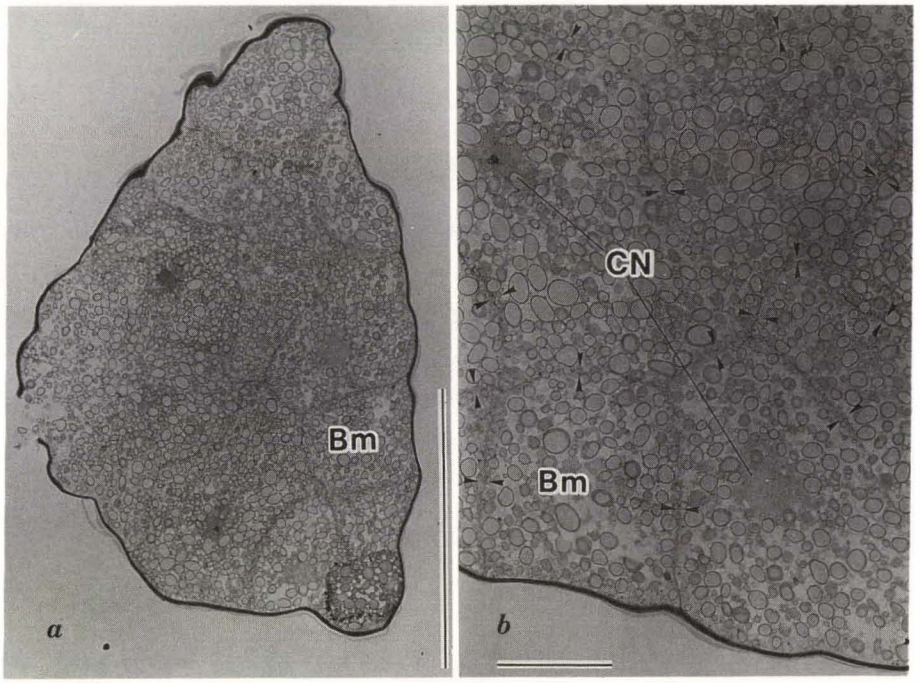

Fig. 2 Section of *Pedetontus unimaculatus* egg in 128-nucleus stage. (b, enlargement). Egg is completely divided into blastomeres (Bm). Each blastomere contains a single cleavage nucleus (CN), and such conditions are visually demonstrated for two blastomeres in b. Arrow heads in b show the boundaries of blastomeres. Scales = 500 μm (*a*), 100 μm (*b*).

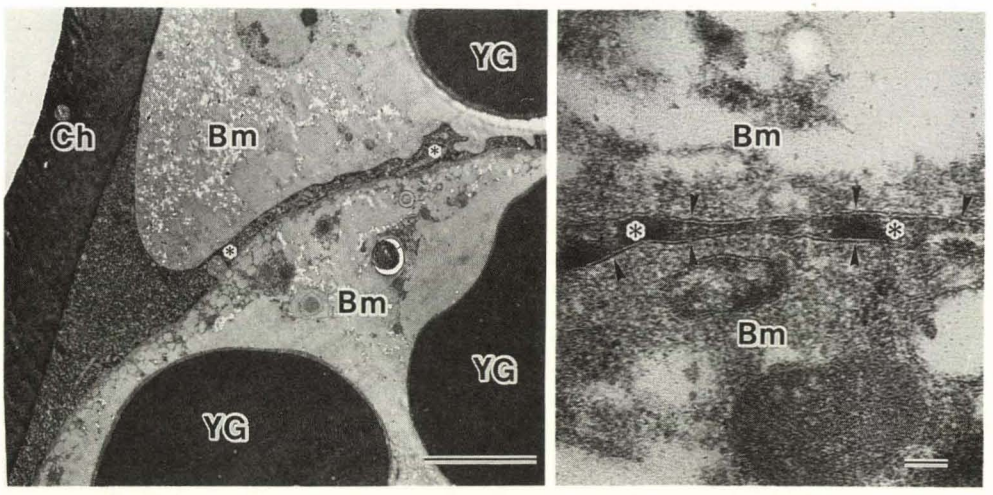

Fig. 3 Electronmicrographs (TEM) of *Pedetontus unimaculatus* egg in 32-nucleus stage, illustrating the adjacent condition of two blastomeres (Bm) (b, enlargement). Asterisks show the interspace between the blastomeres. Each blastomere is defined by the cell membrane (arrow heads in b). Ch, chorion ; YG, yolk globule. Scales = 5μm (*a*), 0.1 μm (*b*).

by cytoplasmic ones almost synchronously. Each resulting blastomere contains a single cleavage nucleus and it is surrouned by the cell membrane (Fig. 3).

Exclusively the nuclear divisions in earlier stages are performed radially (*i.e.* the spindle axes of the divisions run parallel to the egg surface), and the resulting blastomeres confront the egg surface. From the seventh cleavage onward, however, tangential divisions (*i.e.* the spindle axes perpendicular to the egg surface), which are rather less in frequency than the radial ones, take place, resulting in the formation of blastomeres localizing away from the egg surface. The nuclei of these off-surfaced blastomeres are future primary yolk nuclei, and they proliferate mitotically. In the surfaced blastomeres each cleavage nucleus migrates centrifugally, and by the 250-nucleus stage all of them settle on the egg surface in each blastomere.

After 250-500 nucleus stage (*ca.* 40 hr after oviposition), no cytoplasmic division is

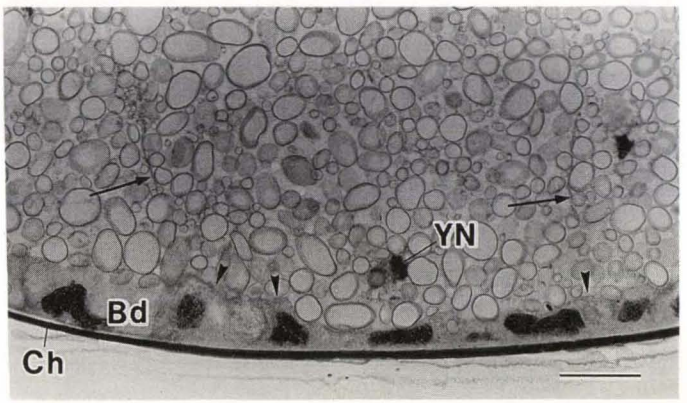

Fig. 4 Section of *Pedetontus unimaculatus* egg in the stage of completion of blastoderm (Bd). Arrow heads and arrows respectively show the inner wall of blastoderm and the boundaries of blastomeres heading for disappearance. Ch, chorion; YN, yolk nucleus. Scale = 20μm.

observed any more except in the egg periphery, and the cleavage transits "from the total into the superficial". The cleavage nuclei settled on the egg surface continue to proliferate mitotically, accompanied by cytoplasmic divisions. Consequently, on the egg surface a continuous cell layer or blastoderm is formed (Fig. 4). At the same time, in the blastoderm a circular thickening about 200 μm in diameter appears, then it is condensed into 50-100 μm in diameter to form the rudiment of embryo or germ disc. A small part of blastoderm cells liberates into the yolk mass, and they are the secondary yolk nuclei. In the course of the formation of blastoderm and germ disc, the boundaries of blastomeres inside the egg gradually fade out to vanish (Fig. 4). By the time of the formation of germ disc, the nucleus number reaches about 3,000-4,000 through the cleavage and proliferation of blastoderm cells and yolk nuclei.

Discussion and Conclusion

As described above, the eggs of *P. unimaculatus* undergo the holoblastic or total cleavage at least in the earlier stages. The similar pattern of cleavage was also observed in the other five microcoryphian species belonging to two subfamilies, four genera (Machilinae : *Haslundichilis,* Petrobiinae: *Pedetontus, Pedetontinus, Petrobiellus)* (unpublished data). Based on the previous studies for *Petrobius brevistylis* (Petrobiinae)[4] , the cleavage pattern of microcoryphians has been accepted as the typical superficial similarly to that found in the Thysanura and Pterygota, but these may be misleadingly brought about from the inadequateness of techniques and from the insufficiency of materials, therefor the reexaminations need on the same species which the studies made on.

The Microcoryphia are a hexapod (insect) group regarded as the nearest to the ancestors of Euentomata, and the group assumes a weighty part in the phylogenetic reconstruction of Hexapoda, and further for the discussion on the evolution of the Antennata. Our observation revises the widely and unconditionally accepted knowledge that the pattern of cleavage in the Euentomata should be the typical superficial one except for some holometabolous pterygote groups of which cleavage could be easily understood to have been secondarily altered from the original superficial type to the total in association with the acquisition of parasitic or polyembryonal habits.[1,5]

Although there are some problems in the application of embryological data to the phylogenetic accounts[6], we offer a view of ours in the following. The cleavage of Microcoryphia described above closely resembles that of springtails, the Collembola[7,8] which are currently treated as a hexapod group independent of the Euentomata[9,10] and of myriapods (Chilopoda[11], Symphyla[12], Pauropoda[13] and Diplopoda[14]). The review through over the Arthropoda may lead us to an idea that this type of cleavage in itself found in the Microcoryphia, Collembola and myriapods should be acceptable as the fundamental type in the Arthropoda or as its slightly modified derivatives[15-17] On the other hand, given the argument defined to the Antennata for the sake of simplifying the discussion, the cleavage type found in the euentomate groups other than the Microcoryphia (*i.e.* the Thysanura and Pterygota) — that is, the genuinely superficial type resulting from the restriction of cytoplasmic divisions to the egg periphery — may be interpreted as a specialized one within the Antennata. Accordingly, the feature could be entitled as one of the characters enough to integrate the Thysanura and Pterygota into a single natural taxon, and the Microcoryphia not possessing the feature should be excluded from it. Our view is coincident with the current phylogenetic reconstruction in which the Euentomata

should be divided into two major groups, the Microcoryphia and the Thysanura−Pterygota[9,10]. Also our view would, as a matter of course, provide and additional base for the denial of a traditional taxon, order Thysanura *s. lat* (Microcoryphia + Thysanura *s. str.*).

More detailed description on the early embryogenesis of *P. unimaculatus* and the full discussion of phylogeny bsed on it will be given in the near future.

REFERENCES

1. Johannsen, O. A. & F. H. Butt, 1941 : *Embryology of Insects and Myriapods*,McGraw-Hill, New York.
2. Schwalm, F., 1988 : *Insect Morphogenesis*, Karger, Basel.
3. Heymons, R. & H. Heymons, 1905 : *Verh. dtsch. zool. Ges.*, 15, 123-135.
4. Larink, O., 1969 : *Helgoländer wiss. Meeresunters.* 19, 111-155.
5. Ivanova-Kasas, O. M., 1972 : in *Developmental Systems* : *Insects, vol.*1, (eds S. J. Counce & C. H. Waddington) 243-271, Academic Press, London.
6. Seidel, F., 1960 : *Zool. Anz.*, 164,245-305.
7. Jura, Cz., 1972 : in *Developmental Systems* : *Insects, vol.* 1, (eds S. J. Counce & C. H. Waddington) 49-94, Academic Press, London.
8. Jura, Cz., A. Krzysztofowicz, & E. Kisiel, 1987 : in *Recent Advances in Insect Embryology in Japan and Poland* (eds H. Ando & Cz. Jura)77-124, Arthropod. Embryol. Soc. Jpn, Tsukuba.
9. Kristensen, N. P., 1975 : *Z. zool. Syst. Evolut.-forsch.*, 13, 1-44.
10. Boudreaux, H. B., 1978 : *Arthropod Phylogeny with Special Reference to Insects* (Wiley-Interscience, New York.
11. Metschnikoff, E., 1875 : *Z. wiss. Zool.*, 25.313-322.
12. Tiegs, O. W., 1940 : *Quart. J. microsc. Sci.*, 82,1-225.
13. Tiegs, O. W., 1947 : *Quart. J. microsc. Sci.* 88,165-267.
14. Dohle, W., 1964 : *Zool. Jb. Anat.*, 81,241-310.
15. Wzgin, V. L., 1964 : in *Problemy Sovremennoy Embryologiy* (ed V. V. Popov) 208-216, Moscow Univ. Press, Moscow.
16. Sharov, A. G., 1966 : *Basic Arthropodan Stock with Special Reference to Insects*, Pergamon Press, London.
17. Anderson, D. T., 1973 : *Embryology and Phylogeny in Annelids and Arthropods*, Pergamon Press, London.
18. Machida, R., 1981 : J. Morphol. 168,339-355.