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Nakamura, Yoshi-Nori
Kuju Agricultural Research Center, Faculty of Agriculture, Kyushu University

Gotoh, Takafumi
Kuju Agricultural Research Center, Faculty of Agriculture, Kyushu University

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Comparative Ultrastructural Observation of the Cuticle and Muscle of an Enchytraeid (*Enchytraeus japonensis*) and an Oribatid species (*Tectocepheus velatus*) using Transmission Electron Microscopy

Yoshi–Nori NAKAMURA and Takafumi GOTOH*

Kuju Agricultural Research Center, Faculty of Agriculture, Kyushu University,
Kuju–cho, Oita, 878–0201, Japan
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We observed internal tissue of wet soil meso animal, *Enchytraeus japonensis* and dry soil meso animal, *Tectocepheus velatus* using optical and transmission electron microscopes.

Thin cuticle layer and thick epidermis covered the whole body, and there were epidermal gland cells of various forms in the cuticle of *E. japonensis*. The epidermis was formed of the bilayer, and thick body wall muscles of various travels were observed in the hypodermic. There were many lipid droplets (large and small size) under body wall muscles.

On the other hand, the mite body of *T. velatus* was surrounded by thick sclerites. The sclerites formed a thin epidermis (outer layer), a thick epicuticle (middle layer) and a thick procuticle (inner layer). The procuticle was formed of 5–6 thin layers. However, there was the position with the impossible discriminate in other place. The hypodermic muscles were connected to the procuticle, and were surrounded by many lipid droplets. There were lipid droplets (large and small sizes) in various places.

This study indicates that there are internal histological differences of soil animals according to different habitat and environment in soil.

Keywords: ultrastructure, cuticle, muscle, *Enchytraeus japonensis*, *Tectocepheus velatus*, transmission electron microscopy

INTRODUCTION

In soil, there are many soil animals which fitted in various environment, for example dry or wet condition. *Enchytraeus japonensis*, only one fragmenting enchytraeid in Japan, was discovered from a Japanese crop field soil under organic farming system (Nakamura, 1993) and was used as a new material for regeneration study (Myohara *et al*., 1999; Schmelz *et al*., 2000). On the other hand, *Tectocepheus velatus* (Michael, 1880), one of oribatid mites, is a common in Japan (Fujikawa *et al*., 1993) and occurs in fields, grasslands or forests (Fujikawa 1988, 1995). The former is covered with soft body wall and extracted by a wet funnel. The latter is covered with hard body wall and by a dry funnel.

Generally, an optical microscopy (OM) is used for observation of soil animals, in particular the taxonomical study. However, there are very small bodies of soil animal, for example about 0.1–1.6 mm in body length in oribatid mites. Therefore we need to use other microscopy, for example transmission electron microscopy (TEM), to investigate ultrastructure soil animal. In additon, until now, there are a few reports for internal morphological studies of soil animals. For example, in the family Enchytraeidae, Reichert *et al*. (1996) and Mothes–Wagner *et al*. (1996) reported the functional and ultrahistological investigation of the digestive system (especially gut) with feeding behaviour of *Enchytraeus coronatus*. On the other hand, Westheide (1999) and Schmelz and Westheide (2000) reported of the ultrastructure of spermathecae and oesophageal appendages of *Enchytraeus crypticus*. However, there is no report for morphological study of *E. japonensis* using TEM.

On the other hand, in oribatid mites, about 9,000 species are known world–wide and about 900 species are found in Japan (Fujikawa *et al*., 1993; Subías, 2004). However, there are few reports on the histology of oribatid mites using TEM (Smrž, 1995; Alberti *et al*., 1994, 2003). Smrž (1995) reported that there were conspicuous bundles of cells in the epimeral region of oribatid mites (*Scutovertex minutus, Trichoribates trimaculatus* and *Damaeus onustus*). In another study, Alberti *et al*. (2003) reported on the digestive system of an early–derivative oribatid mite (*Archegozetes longisetosus*). In addition, a few reports have been made regarding cell–morphological research of *T. velatus* using TEM (Iordansky and Stein–Margolina, 1993) and a chemical study of a congeneric species (*T. sarekensis*) (Mochnacka–Lawacz and Zyromska–Rudzka, 1977).

In this study, ultrastructure of enchytraeid (*E. japonensis*) and an oribatid (*T. velatus*), in particular cuticle and muscle structures, were observed by TEM to investigate body style in different condition in soil.

MATERIALS AND METHODS

Enchytraeids: Cultures (food: grounded oat meal) of *E. japonensis* (Annelida: Oligochaeta: Enchytraeidae) from a single worm were provided by Dr. Y. Nakamura of Ehime University.

* Corresponding author (Takafumi Gotoh, Ph.D. E–mail address: gotoh@farm.kyushu–u.ac.jp)

Mites: T. velatus (Acari: Oribatida) were originally collected from humus in a tree cave in Sekkei temple of Kochi Prefecture (857 Nagahama, Kochi city, 781–0270) on 2 Dec. 2004. The identification of the mite was made by Dr. Tokuko Fujikawa.

TEM: Adult specimens of both species were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 1 week. After being washed with the sample buffer, they were post–fixed with 1% osmic acid medium in 0.1M phosphate buffer (pH 7.4). Dehydration using a 50–to–100% ethanol series was performed before saturation with propylene oxide. After embedding in epoxy resin (Epon 812: Ohken Co., Tokyo, Japan), ultra–thin sections (90 nm thick) were prepared using an ultra–microtome (MT 6000 Sorvall Instruments: Du Pont Co., Delaware, USA). These were stained with 1% uranyl acetate and lead citrate and observed under a transmission electron microscope (TEM, H–800: Hitachi, Tokyo, Japan) with an accelerating voltage of 100 kV.

RESULTS AND DISCUSSION

Enchytraeid worms: The epidermis was covered with thin cuticle layer (**Figs. 1b and c**; E) as other family of Oligochaeta (Laverack, 1963; Jamiesen, 1981). Its epidermis was formed of bilayer of the almost equal thickness (approx. 500 nm per layer) (**Fig. 1d**; IE and OE). Westheide (1999) and Purschke (2003) reported a little deep cuticle layer and an epidermis which was not clearly layered of *Fridericia montafonensis*. In the epidermis,

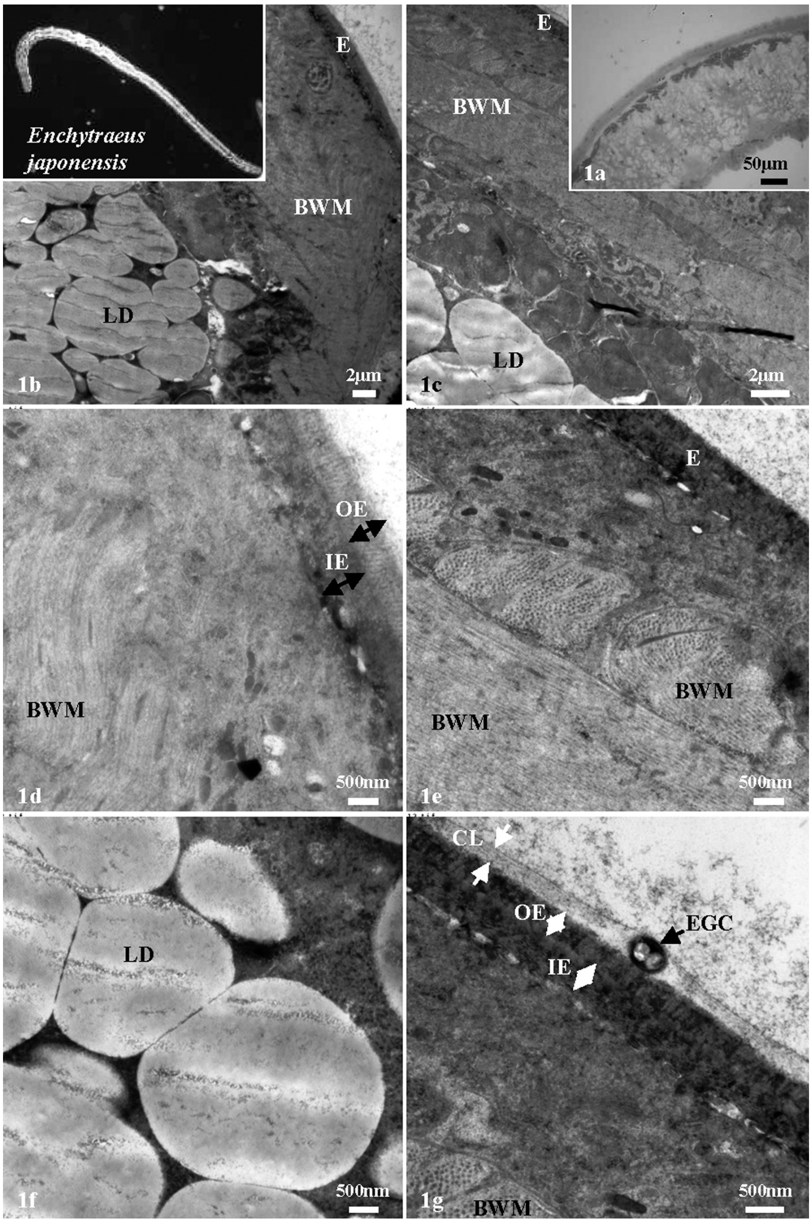

Fig. 1. An optical microscopic (OM) photograph of the subcutaneous tissues of the dorsal central part (the upper side) of *Enchytraeus japonensis* (1a). Transmission electron microscopic (TEM) photographs of the subcutis of *E. japonensis* (1b~1g). BWM: body wall muscle, CL: cuticle layer, E: epidermis, EGC: epidermal gland cells, IE: inner epidermis, LD: lipid droplet, OE: outer epidermis.

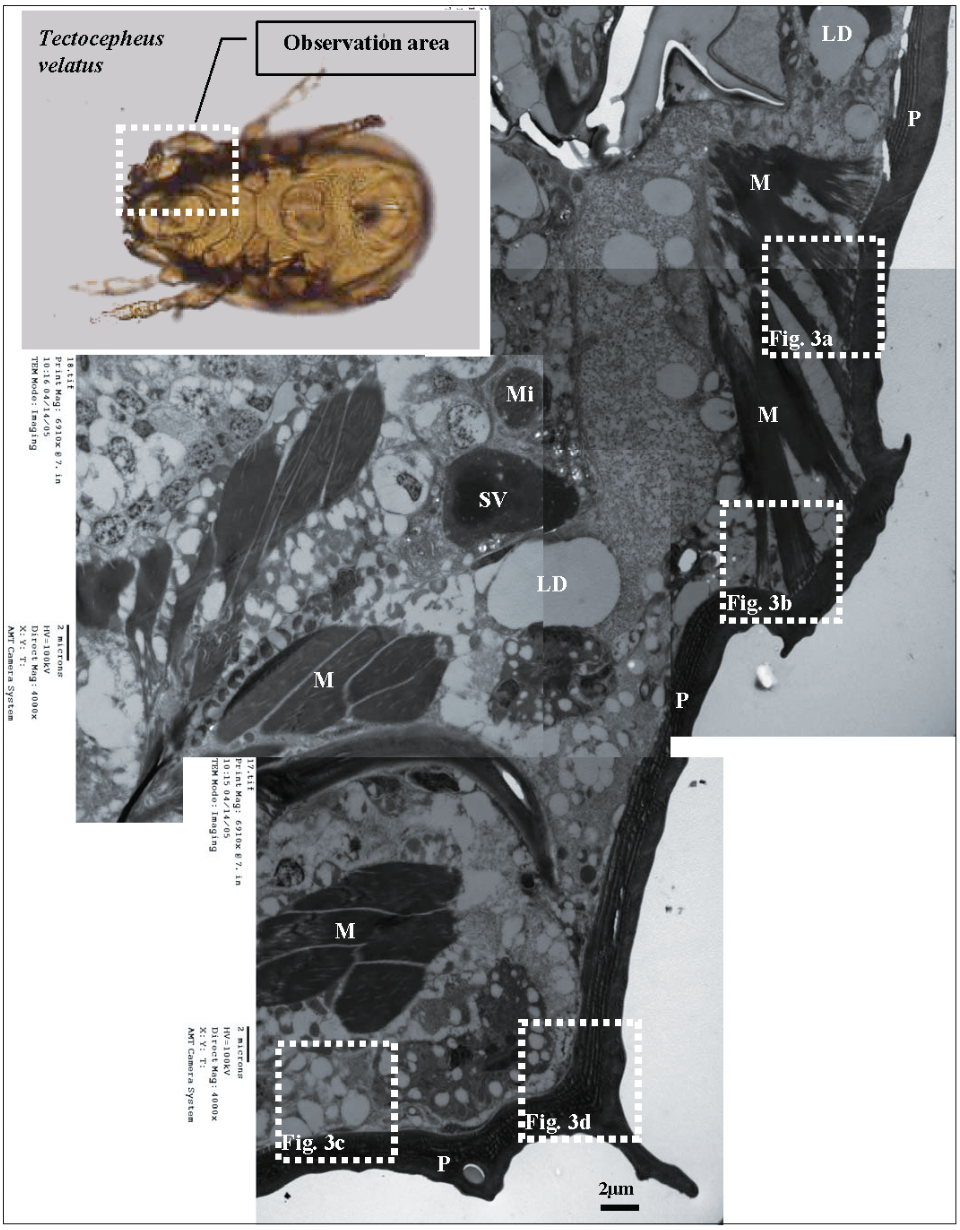

Fig. 2. OM and TEM photographs of the prodorsal lateral surface of *Tectocepheus velatus*. LD: lipid droplets, M: muscle, Mi: mitochondrium, P: prodorsal lateral sclerites.

there were many epidermal gland cells with different form (**Fig. 1g**; EGC). Such a granule was observed by Schmelz *et al*. (2000) and Myohara (2004). They reported that the granule was stained with an orcein. Thick body wall muscles were shown various travel and various clusters (**Figs. 1d and e**). The muscles had three shapes, namely the muscle layer which ran in parallel with the body (**Figs. 1c~e**; BWM), the aggregates of the muscle cells and the flabellate muscle layer (**Fig. 1e**). Under body wall muscles, there were many lipid droplets in large and small sizes, which were globularly (**Fig. 1f**). Similar lipid droplets were observed in other family (Yongean *et al*., 1998). Nakamura and Shiraishi (1999) described nickel nodules in the cavity between epidermis and intestine of *Enchytraeus buchholzia* cultured in the laboratory, and a possibility of detoxifying ability by autotomy.

Oribatid mites: The thick sclerites, which surrounded the body, formed three layers, namely the thin epiermis of an outer layer (**Fig. 2**; P), the tick epicuticle of a middle layer (**Fig. 3d**; Ep) and the thick procuticle of inner layer (**Fig. 3d**; P). Iodansky and Stein–Margolina (1993) reported a similar structure of sclerites in *T. velatus*. However, there was a position with an impossible discrimination in other place (**Figs. 3a and b**). In addition, the place in double layers (**Figs. 2 and 3**; Ep and P) was thick (2.2–2.6 μm) in comparison with the position which was not layered (**Fig. 3**; S, 1.5–1.8 μm). Therefore, we consider that there is a structural difference according to the positions of the sclerites of *T. velatus*. In other species, the body cuticle (ventral position) of *Archegozetes longisetosus* (Oribatida) was not clearly layered (Alberti *et al*., 2003). Baker (1997) reported that there was a pore canal in the dorso–lateral

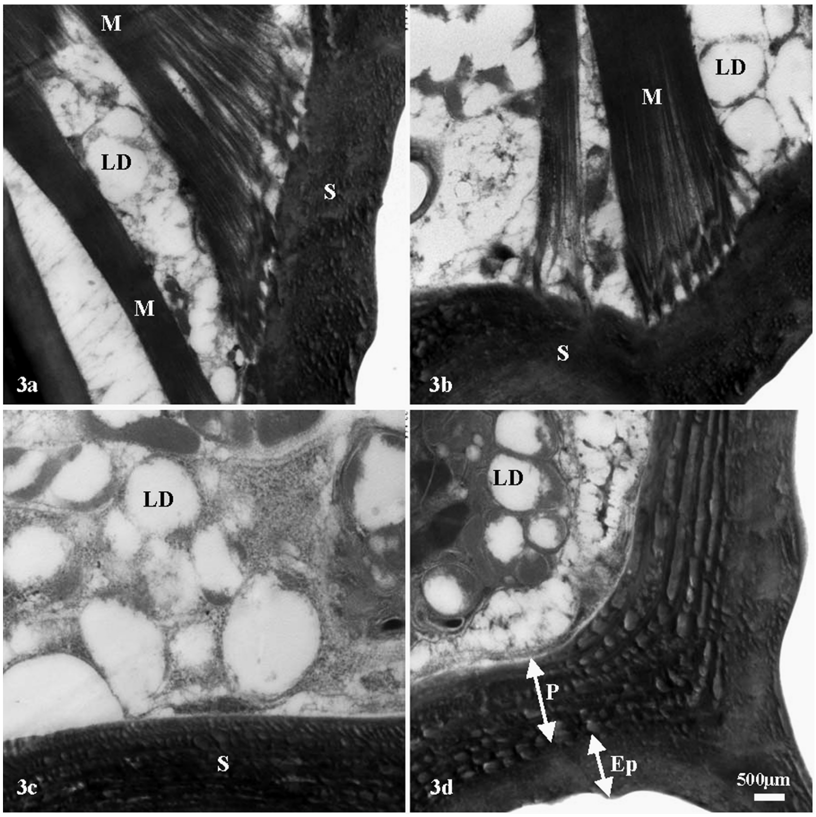

Fig. 3. TEM photographs of the prodorsal lateral surface of *T. velatus*. Ep: epicuticle, LD: lipid droplets, M: muscles, P: procuticle, S: sclerites.

sclerites (no layer) of the legs of *Rhipicephalus sanguineus* (Ixodida). Tarba and Semenova (1976) distinguished various types of cuticle of some Oribatei. However, the type in the present study did not resemble either of these. The cuticles of the prodorsal lateral surface of *T. velatus* constituted a thin epidermis and developed epi– and pro–cuticles (**Figs. 2 and 3**). On the other hand, the hypodermic muscles of *T. velatus* were directly joined together (**Figs. 2 and 3**; M). In addition, the hypodermic muscles were surrounded by many lipid droplets. Moreover, many lipid droplets (large and small sizes) were scattered in various places (**Figs. 2 and 3**; LD).

In conclusion, this study indicates that there are internal histological differences of soil animals according to different habitat and environment in soil.

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