

[ORIGINAL PAPER]

Comparative Study of Autotomic Structures in Centipedes (Arthropoda: Chilopoda)

Akihiro MATSUI¹⁾ and Kensuke YAHATA²⁾

¹⁾ College of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305–8572, Japan

Current address: Graduate School of Science, Osaka University, Toyonaka, Osaka 560–0043, Japan

²⁾ Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305–8572, Japan

E-mail: yahata@biol.tsukuba.ac.jp (KY)

Abstract

In the present study, autotomic structures of some centipede species are described. In each examined specimen of Scutigermorpha, Lithobiomorpha and Scolopendromorpha, discontinuous organization of exoskeletal cuticles was found within the trochanters of walking legs. The position of these exoskeletal rifts corresponded precisely with autotomizing sites in each species. In the scutigermorph, *Thereuonema tuberculata* (Wood, 1863), exoskeletal rifts were found around the entire circumference of the trochanters of walking legs near the trochanter-prefemural articulations. In the lithobiomorphs, *Bothropolys montanus* Verhoeff, 1938, *B. acutidens* Takakuwa, 1941, *B. richthofeni* Verhoeff, 1938 and *Monotarsobius holstii* (Pocock, 1895), exoskeletal rifts were present around two thirds of the circumference of trochanters, appearing from the dorsal side through to the ventral and posterior sides at the proximal skeletal annulus of the trochanter, which was located near the coxa-trochanteral articulations. In examined specimens of the scolopendromorphs, *Scolopocryptops rubiginosus* (L. Koch, 1878) and *S. quadristriatus* (Verhoeff, 1934), exoskeletal rifts were limited to the dorso-posterior half of the trochanter's circumference. In the geophilomorph, *Pleurogeophilus* sp., however, no discontinuity of exoskeletal cuticle was observed in any walking leg articles.

Introduction

Autotomy is a behavior seen in animals in which an organism severs a part of its body in order to escape a predator. Although many species are known to exhibit autotomic behaviors, studies of specific incidences of autotomy are limited in some representative groups, *e. g.*, chordates, echinoderms and decapod crustaceans (Fleming *et al.*, 2007). It has been shown that autotomies occur at specific sites, and in most cases, that particular structures designed to facilitate disconnection of distal body parts exist at these sites. Some of these autotomic structures are classified as autotomizers or autotomic rifts, which allow for the rapid detachment of distal parts from the body, while others are classified as post-autotomic provisions, which reduce the damage caused by autotomic injury.

Autotomic reactions are known to occur in many centipede species (chilopods), however, research into the autotomic structures of centipedes has been limited. The class Chilopoda is composed of five orders; the Craterostigmomorpha, the Scutigermorpha, the Lithobiomorpha, the Scolopendromorpha and the Geophilomorpha. In Japan, the latter four orders,

centipedes inhabiting. Among these centipede groups, limb autotomies are seen most frequently in scutigermorphs, relatively often in lithobiomorphs, rarely in scolopendromorphs, and very scarcely in geophilomorphs (Takakuwa, 1941). It has been observed that scutigermorphs autotomize distal parts of walking legs from the body at trochanter-prefemural articulations, whilst lithobiomorphs and scolopendromorphs do so at coxa-trochanteral articulations (Takakuwa, 1940, 1941).

Herbst (1891) found thick discoidal diaphragmatic connective tissue within the trochanteral segment of each walking leg in the scutigermorph species, *Scutigera coleoptrata*. According to his research, only a pedal nerve and two veins extended through the diaphragm, while neither muscle nor tracheae were present. These characteristics have led to scutigermorph diaphragms being identified as post-autotomic structures preventing the outflow of blood while aiding in damage recovery after limb autotomy. Herbst (1891) also noted the absence of diaphragmatic tissues in the walking legs of lithobiomorph centipedes. No previous studies have presented information regarding any autotomic structures in scolopendromorph centipedes, nor have they documented

the presence of autotomizers or autotomic rifts in any chilopodan orders.

From these background, we have been conducting a histological study of the walking legs of some centipede species, in order to reveal the morphological or histological mechanisms of autotomy (*i. e.*, autotomic structures and identified autotomic rifts). In this paper, detailed characteristics of centipede autotomic structures are described and discussed with respect to evolutionary transitions among chilopodan orders.

Materials and Methods

Centipede species used in the present study are summarized in Table 1. Specimens were collected from channels, underneath the bark of felled trees and leaf litter in Tsukuba and Sakuragawa Cities (Ibaraki Prefecture, Japan), from August to December, 2010.

To compare limb structure between legs that had been autotomized and those that had not, several specimens were made to autotomize some of their walking legs through artificial manipulation. In order to observe the skeletal elements of walking legs, several specimens that underwent autotomy and several that did not were fixed with 90% ethanol and then treated with 1% potassium hydroxide at 60°C for several days. Other specimens were fixed in Bouin's solution, dehydrated in a graded ethanol-*n*-butanol series and cleared with 100% methyl benzoate before being embedded in paraffin. Serial sections ranging between 5 and 7 μm in thickness were stained with Mayer's hematoxylin and eosin. Exoskeletal articulations of walking legs were distinguished based on their folding structures and stainability with eosin, as the sclerotized cuticle of each article was evidently more eosinophilic than the flexible inter-articular membranous cuticles. Names of muscles used in the present paper were based on Manton's nomenclature (Manton, 1965).

Technical problems encountered during histological examination restricted our analysis to the observation of the anterior walking legs, thereby preventing the elucidation of histological information regarding the posterior legs.

Results

Thereuonema tuberculata (*Scutigermorpha*)

In the skeletal materials of each walking leg in the scutigermorph, *Thereuonema tuberculata* (Wood, 1863), a melanized brownish streak was observed encircling the entire circumference of the trochanter at the section near the trochanter-prefemoral articulation (Fig. 1). In each autotomized walking leg, this brownish streak formed a rim around the sectional end of the remaining part of the leg (Fig. 2), suggesting that the distal part of the leg had been disconnected at the melanized streak.

Exoskeletal cuticles of the trochanters had thicknesses ranging about 20–30 μm , thicker than the cuticles associated with other articles, which were only 15 μm in thickness (Figs. 3a, 4). In the proximal part of each trochanter, the skeletal cuticle formed the proximal skeletal annulus of the trochanter (PSAT), reached thicknesses of 40–120 μm , and was especially thick on the ventral side (Figs. 3a, 4). In the distal part of the trochanter, the skeletal cuticle reached thicknesses of 30–40 μm (Figs. 3–6).

In the trochanter of each walking leg, the epidermal layer extended continuously from the proximal part to the distal part of the leg, while the extension of the exoskeletal cuticular layer was interrupted at the thickened distal section immediately proximal to the trochanter-prefemoral articulation constriction (represented by the arrowheads in Figs. 3–6). These exoskeletal rifts encircled the entire circumference of each trochanter. That the outer edges of these rifts were melanized was apparent due to their brownish coloration (Figs. 3–6), which was the same as that of the melanized streaks observed in the skeletal elements (Figs. 1–2).

Within the trochanter of each walking leg, thick discoidal diaphragmatic connective tissue was found at a position immediately proximal to the exoskeletal rift and immediately distal to the PSAT, to which the proximal trochanteral muscles were adhered (Figs. 3a, 4–6). The diaphragm adhered tightly to the epidermal epithelium, and the epidermal epithelial cells adhering to the diaphragm were concentrated and extended (Figs. 3a, 4, 6).

Table 1. Species of centipedes examined in the present study with corresponding collection data.

Species	Collection data
Order Scutigermorpha <i>Thereuonema tuberculata</i> (Wood, 1863)	Tsukuba City, August 2010; Sakuragawa City, October–December 2010
Order Lithobiomorpha <i>Bothropolys montanus</i> Verhoeff, 1938 <i>B. acutidens</i> Takakuwa, 1941 <i>B. richthofeni</i> Verhoeff, 1938 <i>Monotarsobius holstii</i> (Pocock, 1895)	Sakuragawa City, October 2010 Sakuragawa City, October 2010 Sakuragawa City, October 2010 Tsukuba City, December 2010
Order Scolopendromorpha <i>Scolopocryptops rubiginosus</i> (L. Koch, 1878) <i>S. quadristriatus</i> (Verhoeff, 1934)	Tsukuba City, December 2010 Sakuragawa City, October 2010
Order Geophilomorpha <i>Pleurogeophilus</i> sp. (species unidentified)	Tsukuba City, December 2010

The pedal nerve cord was observed running through the diaphragm (Fig. 6).

The proximal trochanteral muscles, which consisted of the *retractor trochanteris* and *depressor trochanteris*, adhered to the proximal part of the PSAT (partly shown in Figs. 3a, 4, 6), while the distal muscles, the *flexores unguiculi prefemoris*, *depressor femoris* and *depressor femoris anterior*, adhered to the distal end of the trochanteral or prefemural sclerites (partly shown in Figs. 3a, 4, 6; two superficial muscles, the *flexores unguiculi prefemoris* and *depressor femoris anterior*, were omitted from present figures). The *flexores tibiae* to trochanter was the only distal muscle adhered to the apodeme from the trochanter portion of the anterior coxa-trochanter pivot (Figs. 4, 5). The apodeme was located at a position immediately distal to the exoskeletal rift and the diaphragm (Figs. 4, 5).

The edges of sectional ends of autotomized walking legs were encircled by the melanized proximal borders of exoskeletal rifts exteriorly and terminated at the diaphragms interiorly (as indicated by the arrowheads in Fig. 7). The diaphragms of autotomized legs shrank along the distal-proximal axis, but not along the transverse axis (Fig. 7). The pedal nerve cord was torn at the site of limb autotomization (Fig. 7).

Bothropolys species and Monotarsobius holstii
(*Lithobiomorpha*)

Autotomic structures observed in the lithobiomorph species examined [*Bothropolys montanus* Verhoeff, 1938, *B. acutidens* Takakuwa, 1941, *B. richthofeni* Verhoeff, 1938, and *Monotarsobius holstii* (Pocock, 1895)] were similar to each other, with no substantial differences observed between them.

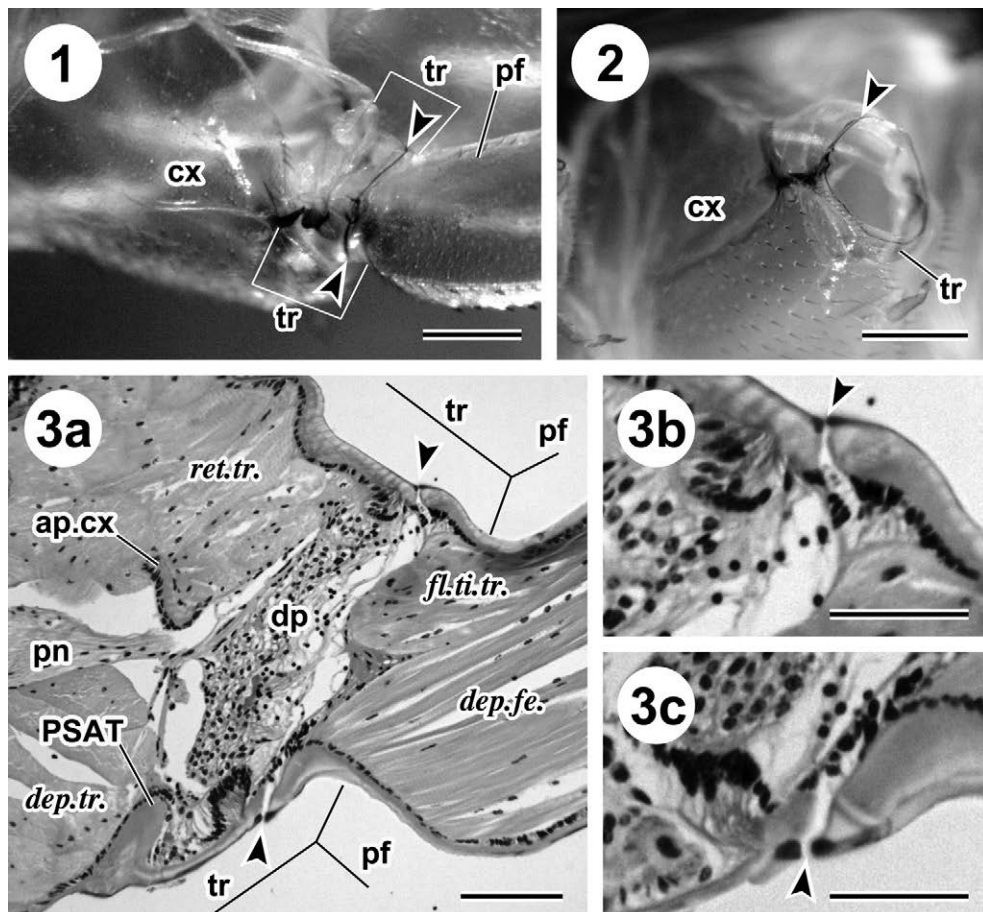


Fig. 1 Lateral view of the exoskeletal preparation of the proximal part of the 13th walking leg in *Thereuonema tuberculata*. Dorsal and anterior sides are oriented towards the top and left sides of the image, respectively. Arrowheads indicate a ring consisting of a melanized streak within the trochanter, immediately proximal to the trochanter-prefemural articulations. cx: coxa, pf: prefemur, tr: trochanter. Scale = 500 μ m.

Fig. 2 Antero-lateral view of the exoskeletal preparation of the proximal part of the autotomized 13th walking leg in *Thereuonema tuberculata*. Dorsal and anterior sides are oriented towards the top and left sides of the image, respectively. Sectional end is encircled by a ring consisting of a melanized streak within the trochanter (indicated by the arrowhead). cx: coxa, tr: trochanter. Scale = 500 μ m.

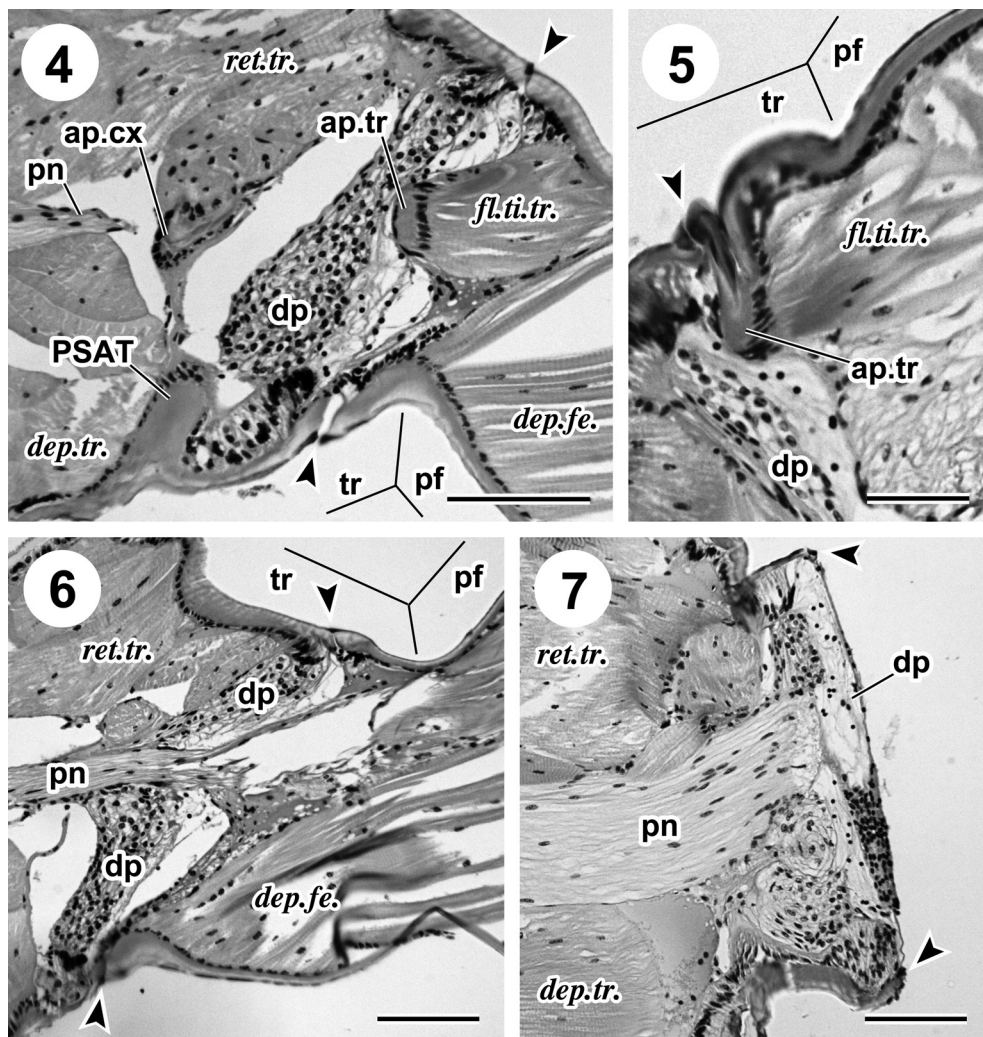
Fig. 3 a. Sagittal-section of the 3rd walking leg in *Thereuonema tuberculata*. Dorsal and proximal sides are oriented towards the top and left sides of the image, respectively. The boundary between the trochanter and prefemur was distinguished based on other sections. Arrowheads indicate the exoskeletal rift located at the position immediately proximal to the trochanter-prefemural articulation constriction. Thick discoidal diaphragmatic connective tissue (dp) can be observed immediately proximal to the exoskeletal rift and immediately distal to the proximal skeletal annulus of the trochanter (PSAT). b and c. Extended parts of dorsal and ventral exoskeletal rift shown in a. Outer edge of the rift is melanized. ap.cx: apodeme of coxa, pf: prefemur, pn: pedal nerve, tr: trochanter; *dep.fe.*: *depressor femoris*, *dep.tr.*: *depressor trochanteris*, *fl.ti.tr.*: *flexores tibiae* to trochanter, *ret.tr.*: a group of *retractor trochanteris*. Scales = 100 μ m in a, and 50 μ m in b and c.

Skeletal materials in the walking legs of each lithobiomorph species examined consisted of a trochanter composed of a small ring (Fig. 8). Furthermore, no obvious melanized streak was observed within the examined trochanters. In each autotomized walking leg, the sectional end of the limb was not visible as it had been drawn into the coxa (Fig. 9).

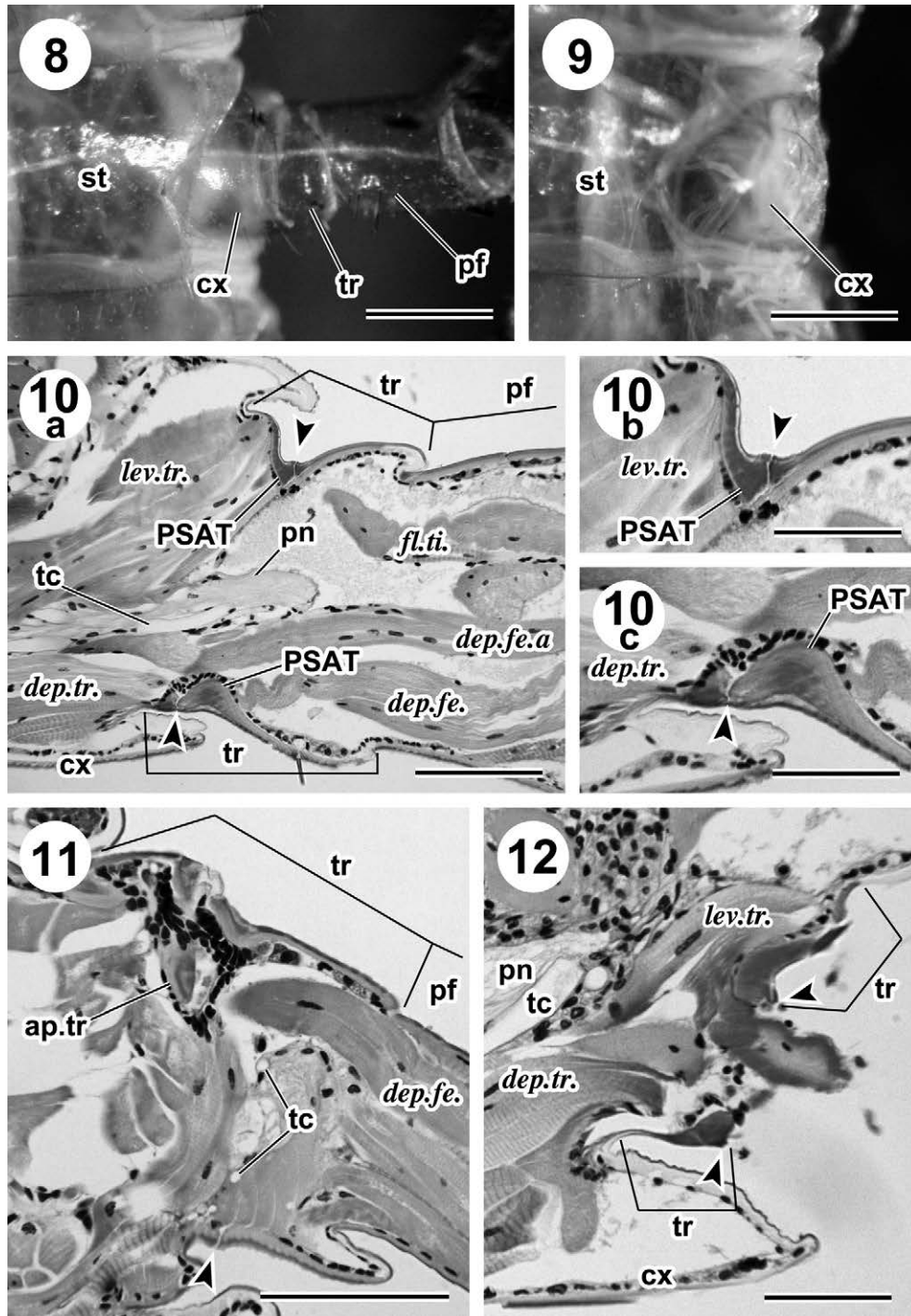
Exoskeletal cuticles of the trochanters were of the same thickness as those found in the other articles (Figs. 10a, 11). In the trochanters of *Bothropolys acutidens*, the majority of exoskeletal cuticles were approximately 10 μm in thickness. The proximal part of each trochanter formed

the PSAT, while the skeletal cuticle ranged 20–25 μm in thickness and was especially well developed on the ventral side (Figs. 10, 11). Increased thickness of the distal part of the skeletal cuticle was not observed.

In the trochanter of each walking leg of the lithobiomorphs examined, an exoskeletal rift similar to the one observed in the scutigermorph, *Thereuonema tuberculata*, was observed (indicated by the arrowheads in Figs. 10, 11). The position of the exoskeletal rifts in the lithobiomorphs examined differed from the position observed in *T. tuberculata*, as they were located near the top of the PSAT, immediately distal to the coxa–



- Fig. 4 Sagittal-section of the 3rd walking leg in *Thereuonema tuberculata*. Dorsal and proximal sides are oriented towards the top and left sides of the image, respectively. Arrowheads indicate the exoskeletal rift. ap.cx: apodeme of coxa, ap.tr: apodeme of trochanter, dp: diaphragm, pf: prefemur, pn: pedal nerve, PSAT: proximal skeletal annulus of the trochanter, tr: trochanter; dep.fe.: depressor femoris, dep.tr.: depressor trochanteris, fl.ti.tr.: flexores tibiae to trochanter, ret.tr.: a group of retractor trochanteris. Scale = 100 μm .
- Fig. 5 Horizontal-section of the 1st walking leg in *Thereuonema tuberculata*. Anterior and proximal sides are oriented towards the top and left sides of the image, respectively. Arrowhead indicates the exoskeletal rift, which is located at the position immediately proximal to the apodeme of the trochanter (ap.tr) and immediately distal to the diaphragm (dp). pf: prefemur, tr: trochanter; fl.ti.tr.: flexores tibiae to trochanter. Scale = 50 μm .
- Fig. 6 Sagittal-section of the 3rd walking leg in *Thereuonema tuberculata*. Dorsal and proximal sides are oriented towards the top and left sides of the image, respectively. Arrowheads indicate the exoskeletal rift. Pedal nerve (pn) crosses through the diaphragm (dp). pf: prefemur, tr: trochanter; dep.fe.: depressor femoris, ret.tr.: a group of retractor trochanteris. Scale = 100 μm .
- Fig. 7 Horizontal-section of an autotomized 2nd walking leg in *Thereuonema tuberculata*. Anterior and proximal sides are oriented towards the top and left sides of the image, respectively. Autotomic sectional end is closed by the diaphragm (dp) and encircled by the remaining half of the exoskeletal rift (as indicated by the arrowheads). Pedal nerve (pn) is severed. dep.tr.: depressor trochanteris, ret.tr.: a group of retractor trochanteris. Scale = 100 μm .



- Fig. 8 Ventral view of the exoskeletal preparation of the proximal part of the 2nd walking leg in *Bothropolys richthofeni*. Anterior and proximal sides are oriented towards the top and left sides of the image, respectively. cx: coxa, pf: prefemur, st: sternite, tr: trochanter. Scale = 500 μ m.
- Fig. 9 Ventral view of the exoskeletal preparation of the proximal part of the autotomized 2nd walking leg in *Bothropolys richthofeni*. Anterior and proximal sides are oriented towards the top and left sides of the image, respectively. Autotomic sectional end is drawn into the coxa and the trochanter is not outwardly visible. cx: coxa, st: sternite. Scale = 500 μ m.
- Fig. 10 a. Sagittal-section of the 2nd walking leg in *Bothropolys acutidens*. Dorsal and proximal sides are oriented towards the top and left sides of the image, respectively. Arrowheads indicate the exoskeletal rift within the proximal skeletal annulus of the trochanter (PSAT), at a position immediately distal to the coxa-trochanteral articulation. b and c. Extended parts of the dorsal and ventral exoskeletal rift shown in a. cx: coxa, pf: prefemur, pn: pedal nerve, tc: trachea, tr: trochanter; *dep.fe.*: depressor femoris, *dep.fe.a.*: depressor femoris anterior, *dep.tr.*: depressor trochanteris, *fl.ti.*: flexores tibiae, *lev.tr.*: a group of levator trochanteris. Scales = 100 μ m in a, and 50 μ m in b and c.
- Fig. 11 Horizontal-section of the 3rd walking leg in *Monotarsobius holstii*. Anterior and proximal sides are oriented towards the top and left sides of the image, respectively. Arrowhead indicates the exoskeletal rift, which can be observed on the posterior side but not on the anterior side. ap.tr: apodeme of trochanter, pf: prefemur, tc: trachea, tr: trochanter; *dep.fe.*: depressor femoris. Scale = 100 μ m.
- Fig. 12 Sagittal-section of an autotomized 2nd walking leg in *Bothropolys acutidens*. Dorsal and proximal sides are oriented towards the top and left sides of the image, respectively. Autotomic sectional end is encircled by the remaining half of the exoskeletal rift (as indicated by the arrowheads). Pedal nerve (pn) and tracheae (tc) are severed due to autotomization. cx: coxa, tr: trochanter; *dep.tr.*: depressor trochanteris, *lev.tr.*: a group of levator trochanteris. Scale = 50 μ m.

trochanteral articulations (Figs. 10a, 11). The presence of these exoskeletal rifts was restricted to two thirds of the circumference of the trochanters, ranging from the dorsal to the ventral, and through to the posterior side.

The proximal trochanteral muscles, which consisted of a group of *levator trochanteris* and a group of *rotator trochanteris*, as well as the *retractor trochanteris* and *depressor trochanteris*, adhered to the proximal part of the PSAT, just proximal to the exoskeletal rift (partly shown in Fig. 10a), while two distal muscles, the *flexores tibiae* and a part of the *depressor femoris*, adhered to the distal part of the PSAT, immediately distal to the exoskeletal rift (e.g., Fig. 10a). Three other distal muscles, the *depressor femoris anterior* and *posterior*, and the coxa-femur muscle, however, ran across the trochanter and adhered to the coxal sclerite (partly shown in Fig. 10a). No diaphragmatic structures were found within the trochanters of walking legs.

In the lithobiomorph species examined, the sectional ends of autotomized walking legs were extremely narrow and were encircled by exoskeletal rifts (Fig. 12). These observations suggested that the distal parts of walking legs were disconnected from the proximal parts at the exoskeletal rifts within the trochanter during autotomization. Three cross-boundary muscles, the pedal nerve cord, and some tracheae that extended across the autotomic border were severed during autotomization (Fig. 12).

Scolopocryptops rubiginosus and *S. quadristriatus* (*Scolopendromorpha*)

The two scolopendromorph species examined, *Scolopocryptops rubiginosus* (L. Koch, 1878) and *S. quadristriatus* (Verhoeff, 1934), exhibited no substantial differences in both general limb structures and structures related to autotomization.

Skeletal elements in the walking legs of each scolopendromorph species examined consisted of a short trochanter (Fig. 13), which was particularly short on the dorsal side. No obvious melanized streak was observed within trochanters. In each autotomized walking leg, the sectional end of the limb was not visible as it had drawn into the coxa (Fig. 14).

In *Scolopocryptops rubiginosus*, exoskeletal cuticles of trochanters were thicker than those observed in other articles, with thicknesses ranging 15–20 μm . Exoskeletal cuticles of trochanters were up to 40 μm thick in the dorsal region and up to 80 μm thick in the ventral region (Fig. 15a). The entire trochanter comprised the PSAT (Fig. 15a).

In the trochanter of each walking leg in the *Scolopocryptops* species examined, an exoskeletal rift similar to those observed in the former two groups was found (indicated by the arrowheads in Figs. 15, 16). Exoskeletal rifts were limited to the dorso-posterior half of the circumference of walking leg trochanters. Position

of the exoskeletal rifts seen in *Scolopocryptops* species differed from those observed in the scutigermorph, *Thereuonema tuberculata*, and the lithobiomorphs, *Bothropolys* spp. and *Monotarsobius holstii*, as they were located somewhat proximally to the top of the PSAT, at a position located roughly in the middle of the trochanter (Figs. 15, 16). Outer edges of these rifts were melanized, as indicated by their brownish coloration (Fig. 15b).

In autotomized limbs sectional ends were encircled by exoskeletal rifts on the dorso-posterior side (indicated by the arrowhead in Fig. 17), while on the ventro-anterior side, they terminated at the trochanter-prefemur articulations, at a position immediately distal to the PSAT (Fig. 17). These observations in *Scolopocryptops* spp. suggested that the autotomizing plane of their walking legs slant across the trochanter.

Proximal trochanteral muscles, consisting of a group of *levator trochanteris* and the *depressor trochanteris*, adhered to the proximal part of the PSAT, proximal to the exoskeletal rift in the dorso-posterior region (partly shown in Fig. 15a). On the other hand, the distally located ventral part of the *depressor femoris*, adhered to the distal part of the PSAT, immediately proximal to the trochanter-prefemur articulation (partly shown in Fig. 15a). Five other distal muscles, the *depressor femoris anterior*, *flexores unguiculi* to trochanter, *protractor femoris longus*, *retractor femoris*, and coxa-femur muscle ran across the trochanter and adhered to the proximal end of either the trochanteral sclerite or the coxal sclerite (partly shown in Fig. 16), crossing the autotomic boundary. In autotomized limbs, these cross-boundary muscles were severed, their remnants having been observed in the examined specimens (e.g., *dep.fe.* in Fig. 17). In addition, a pedal nerve cord and some tracheae crossed the autotomic boundary in each walking leg (Figs. 15a, 17). No diaphragmatic structures were observed within the trochanters of walking legs.

Pleurogeophilus sp. (*Geophilomorpha*)

Walking legs of the unidentified *Pleurogeophilus* species examined were individually torn at several inter-articular parts due to artificial manipulation (e.g., femur-tibia, tibia-tarsus; Fig. 18). The sclerotized exoskeletal cuticular layer of each limb article was smooth and continuous throughout the entire limb, from coxa to tarsus (Fig. 19), and after histological examination, no autotomic structure, such a diaphragm or exoskeletal rift, could be identified in specimens' walking legs.

Discussion

In the trochanters of walking legs in the scutigermorph, *Scutigera coleoptrata*, the presence of diaphragmatic connective tissue, which serves as a post-autotomic structure, has been observed (Herbst, 1891). In the present study, similar diaphragms were found in another

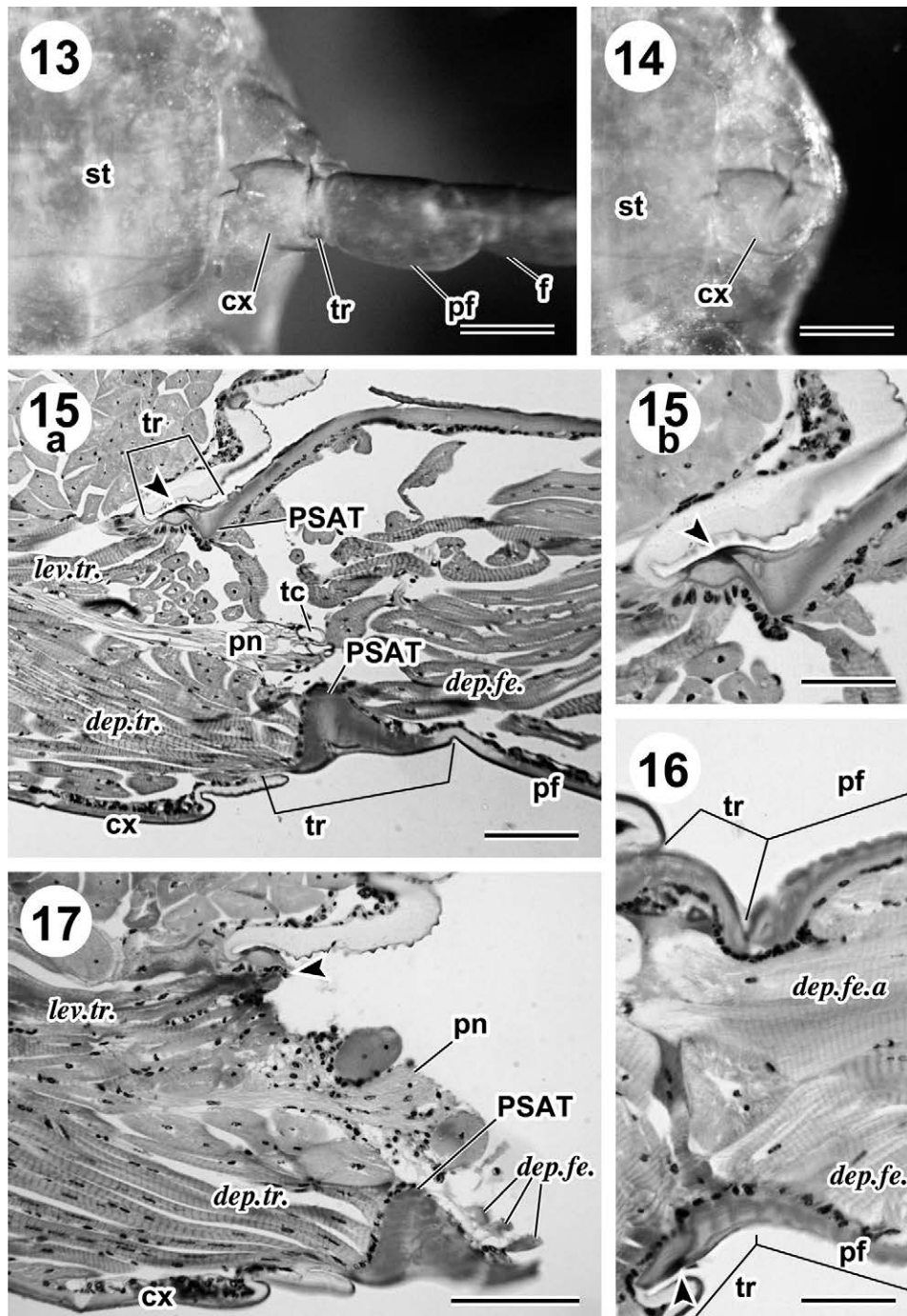


Fig. 13 Ventral view of the exoskeletal preparation of the proximal part of the 6th walking leg in *Scolopocryptops rubiginosus*. Anterior and proximal sides are oriented towards the top and left sides of the image, respectively. cx: coxa, f: femur, pf: prefemur, st: sternite, tr: trochanter. Scale = 500 μ m.

Fig. 14 Ventral view of the exoskeletal preparation of the proximal part of the autotomized 6th walking leg in *Scolopocryptops rubiginosus*. Anterior and proximal sides are oriented towards the top and left sides of the image, respectively. Autotomic sectional end is drawn into the coxa and the trochanter is not outwardly visible. cx: coxa, st: sternite. Scale = 500 μ m.

Fig. 15 a. Sagittal-section of the 4th walking leg in *Scolopocryptops rubiginosus*. Dorsal and proximal sides are oriented towards the top and left sides of the image, respectively. Arrowhead indicates the exoskeletal rift within the dorsal section of the proximal skeletal annulus of the trochanter (PSAT). b. Extended part of the dorsal exoskeletal rift shown in a. Outer edge of the rift is melanized. cx: coxa, pf: prefemur, pn: pedal nerve, tc: trachea, tr: trochanter; *dep.fe.*: depressor femoris, *dep.tr.*: depressor trochanteris, *lev.tr.*: a group of levator trochanteris. Scales = 100 μ m in a and 50 μ m in b.

Fig. 16 Horizontal-section of the 11th walking leg in *Scolopocryptops quadristriatus*. Anterior and proximal sides are oriented towards the top and left sides of the image, respectively. Arrowhead indicates the exoskeletal rift, which can be observed on the posterior side of trochanter. pf: prefemur, tr: trochanter; *dep.fe.*: depressor femoris, *dep.fe.a.*: depressor femoris anterior. Scale = 50 μ m.

Fig. 17 Sagittal-section of an autotomized 4th walking leg in *Scolopocryptops rubiginosus*. Dorsal and proximal sides are oriented towards the top and left sides of the image, respectively. Autotomic sectional end is encircled by the remaining half of the exoskeletal rift (indicated by the arrowhead) dorsally and by the proximal skeletal annulus of the trochanter (PSAT) ventrally. Pedal nerve (pn) is severed. Torn depressor femoris (*dep.fe.*), remains at the distal side of the ventral PSAT. cx: coxa; *dep.tr.*: depressor trochanteris, *lev.tr.*: a group of levator trochanteris. Scale = 100 μ m.

scutigermorph species, *Thereuonema tuberculata*, however, they were not observed in the other centipede species examined. As such diaphragms seems to be a unique characteristic of scutigermorph centipedes. Scutigermorphs also have a kind of specialized plasma cell which plays a role in hemolymph clotting (Hilken *et al.*, 2003); these cells may also function in the autotomization of scutigermorph limbs.

In the present study, discontinuous organization of exoskeletal cuticles within trochanters were identified in the centipede species examined except for the geophilomorph species. These exoskeletal rifts were shared among centipedes which exhibited autotomization behaviors, scutigermorphs, lithobiomorphs and scolopendromorphs, but absent in geophilomorphs, which are not known to autotomize limbs. The position of these exoskeletal rifts corresponded with the autotomizing sites of each species. With these results in mind, it was concluded that exoskeletal rifts are the prerequisite structure for limb autotomization in centipede species.

Previous authors have noted that autotomization of distal parts in the walking legs of scutigermorphs occurs at the trochanter-prefemural articulations, while, in lithobiomorphs and scolopendromorphs, it occurs at the coxa-trochanteral articulations (*e. g.*, Takakuwa, 1940, 1941). The present study, however, revealed that autotomies occur at exoskeletal rifts within the trochanter of all examined scutigermorph, lithobiomorph and scolopendromorph species, without exception. These results clearly suggest that past authors misidentified the positions of autotomizing sites.

Exoskeletal rifts in scutigermorph species are located near the trochanter-prefemural articulations and most parts of the trochanters remain visible after

autotomization. Exoskeletal rifts in lithobiomorphs, on the other hand, are located near the coxa-trochanteral articulations. In the lithobiomorph limb, coxal sclerites overhang the proximal parts of trochanters at the location of exoskeletal rifts. Trochanters in scolopendromorphs are very small, and their dorsal sides are hidden by pleurites. Due to these characteristics, trochanters in lithobiomorph and scolopendromorph autotomized limbs remained only as small pieces, difficult to detect by outward observation.

Results suggested that frequency of autotomization events was correlated with the relative sizes of exoskeletal rifts. Based on our observations, scutigermorphs, which autotomized limbs more frequently than the other centipede groups examined, exhibited exoskeletal rifts along the entire circumference of the trochanter; scolopendromorphs, which rarely underwent limb autotomization, exhibited exoskeletal rifts along only half of the trochanteral circumference; and geophilomorphs, which are not known to autotomize limbs at all, exhibited no exoskeletal rifts on the trochanter.

Results also suggested that frequency of autotomies correlated with characteristics of the internal structures of the walking legs, such as the organization of both muscles and tracheae. Autotomy in lithobiomorph and scolopendromorph centipedes, which rarely undergo limb autotomization, requires tearing of several muscles and tracheae at the autotomic boundary, while in scutigermorphs, which autotomize more frequently, it does not require any muscle tearing whatsoever. Scutigermorphs are the only centipede group known to use hemocyanin in order to transport oxygen (Mangum *et al.*, 1985), a possible explanation for the lack of tracheae within their walking legs.

Muscular systems of centipede limbs have been

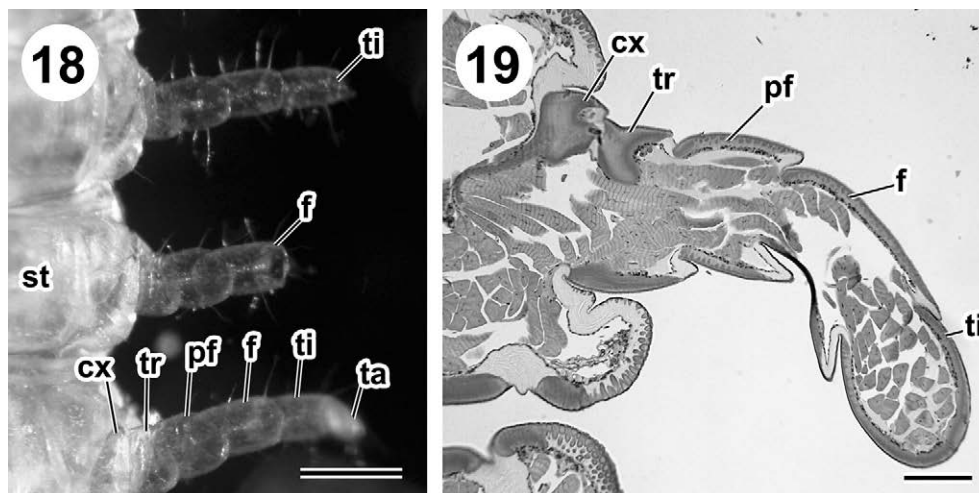


Fig. 18 Ventral view of the exoskeletal preparation of the proximal part of 2nd–4th walking legs in *Pleurogeophilus* sp. Anterior and proximal sides are oriented towards the top and left sides of the image, respectively. The 2nd and walking leg was torn at tibia-tarsal articulation, while the 3rd at femur-tibial one. cx: coxa, f: femur, pf: prefemur, st: sternite, ta: tarsus, ti: tibia, tr: trochanter. Scale = 500 μ m.

Fig. 19 Horizontal-section of the 12th walking leg in *Pleurogeophilus* sp. Anterior and proximal sides are oriented towards the top and left sides of the image, respectively. Within the walking leg, neither an exoskeletal rift nor a diaphragm can be observed. Tarsus is not shown in this section. cx: coxa, f: femur, pf: prefemur, ti: tibia, tr: trochanter. Scale = 100 μ m.

documented in detail by Manton (1965) with consideration paid to mechanisms of locomotory function. Muscular systems of scutigermorph limbs seems to be specialized, and are distinctive among centipedes (see Table 4 in Manton, 1965). Scutigermorphs are the only centipedes that have no muscle running across the trochanter. Results of the present study suggested that the distinctiveness of the muscular system in scutigermorph limbs may correlate with the autotomic structures. It is possible that evolutionary transition of the muscular system in limbs could have allowed scutigermorphs to shift the site of autotomization on the trochanter distally, allowing the impregnable diaphragms to serve as an autotomic damage reducer.

The class Chilopoda is divided into two subclasses, the Notostigmophora and the Pleurostigmophora. The former consists of a single order, Scutigermorpha. Among the pleurostigmophoran orders, the Lithobiomorpha is the most basal clade while the Scolopendromorpha and the Geophilomorpha form a terminal clade. This system of classification has been supported by several studies based on morphological data (*e. g.*, Dohle, 1985; Shear and Bonamo, 1988) and molecular data (*e. g.*, Giribet *et al.*, 1999). According to this system, exoskeletal rifts seen in various centipede species should share a common evolutionary origin, while the diaphragm reported only in Scutigermorpha among myriapod groups should be considered an autoapomorphic feature. In addition, it is possible that the Geophilomorpha lost their exoskeletal rifts secondarily, a possible cause being their body shape, which is specialized for interstitial life. Geophilomorphs are characterized by a long body with many diminutive legs which is suitable for interstitial life, makes escape from predators unlikely. These results suggested as follows; while scutigermorphs adapted their specialized

characteristics for frequent autotomies in order to avoid predators inhabiting ground, geophilomorphs secondarily lost their autotomic structures due to their interstitial lifestyles.

References

- Dohle, W. (1985) Phylogenetic pathways in the Chilopoda. *Bijdragen tot de Dierkunde*, **55**, 55–66.
- Fleming, P. A., D. Muller and P. W. Bateman (2007) Leave it all behind: a taxonomic perspective of autotomy in invertebrates. *Biological Reviews*, **82**, 481–510.
- Giribet, G., S. Carranza, M. Riutort, j. Bagnà and C. Ribera (1999) Internal phylogeny of the Chilopoda (Myriapoda, Arthropoda) using complete 18S rDNA and partial 28S rDNA sequences. *Philosophical Transactions of the Royal Society of London, Series B*, **354**, 215–222.
- Herbst, C. (1891) Beiträge zur Kenntnis der Chilopoden (Drüsen; Coxalorgan; Gefäßsystem und Eingeweidenervensystem). *Bibliotheca Zoologica*, **3**, 1–43.
- Hilken, G., C. Brockmann and L. Nevermann (2003) Hemocytes of the centipede *Scutigera coleoptrata* (Chilopoda, Notostigmophora) with notes on their interactions with the tracheae. *Journal of Morphology*, **257**, 181–189.
- Mangum C. P., J. L. Scott, R. E. L. Black, K. I. Miller and K. E. Van Holde (1985) Centipedal hemocyanin: its structure and its implications for arthropod phylogeny. *Proceedings of the National Academy of Sciences of the United States of America*, **82**, 3721–3725.
- Manton, S. M. (1965) The evolution of arthropodan locomotory mechanisms. Part 8. Functional requirements and body design in Chilopoda, together with a comparative account of their skeleto-muscular systems and an Appendix on a comparison between burrowing forces of annelids and chilopods and its bearing upon the evolution of the arthropodan haemocoel. *Journal of the Linnean Society of London, Zoology*, **45**, 251–484.
- Shear, W. A. and P. M. Bonamo (1988) Devonobiomorpha, a new order of centipeds (Chilopoda) from the Middle Devonian of Gilboa, New York State, USA, and the phylogeny of centipede orders. *American Museum Novitates*, **2927**, 1–30.
- Takakuwa, Y. (1940) Shinsokurui Seikeirui Omukademoku. In Y. Okada *et al.*, (eds.), *Nihon Doubutsu Bunrui*, Vol. 9-8 (2). Sanseido, Tokyo. (In Japanese).
- Takakuwa, Y. (1941) Shinsokurui Kaikeirui Ishimukademoku. In Y. Okada *et al.*, (eds.), *Nihon Doubutsu Bunrui*, Vol. 9-8 (3). Sanseido, Tokyo. (In Japanese).