

# Description of *Mediomastus opertaculeus* sp. nov. (Annelida: Capitellidae) from Hokkaido, Northern Japan

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We describe a new species of capitellid polychaete, *Mediomastus opertaculeus* sp. nov., from an intertidal rocky shore in Hokkaido, northern Japan. This new species is distinguishable from all other 13 congeners by such characteristics as (1) staining pattern with methyl green (presumably indicating glandular cells in the epidermis), (2) absence of capillary chaetae in the abdomen, (3) absence of paddle-like chaetae in the thorax, and (4) shapes of the thoracic capillary chaetae and hooded hooks. Aligned partial sequences (624 bases) of cytochrome *c* oxidase subunit I gene from four paratypes of *M. opertaculeus* differed at 1–4 sites, corresponding to Kimura two-parameter (K2P) distances of 0.002–0.006. K2P distances were 0.216–0.218 between our sequences and that from an unidentified *Mediomastus* sp. from Darwin Harbor, Australia, indicating these are not conspecific.

**Key Words:** *Mediomastus*, Capitellidae, polychaete, Hokkaido, new species, COI.

## Introduction

Polychaetes in the genus *Mediomastus* Hartman, 1944 are free-living, mostly marine, benthic forms, occurring in all oceans of the world. Hartman (1944) established this genus for *M. californiensis* Hartman, 1944, with the following characters: a thorax of 11 segments, including a well-developed achaetigerous peristomial ring; segments two to five with capillary chaetae; and segments six to 11 with hooded hooks. Warren *et al.* (1994) reviewed the genus, which now contains 13 species (Hartmann-Schröder 1959, 1962; Day 1961; Hartman 1969; Rasmussen 1973; Ben-Eliahu 1976; Warren *et al.* 1994; Green 2002). In waters around East and Southeast Asia, two species are known, *viz.*, *Mediomastus warrenae* Green, 2002 from the Andaman Sea (Green 2002) and *M. californiensis* from Sagami Bay (Imajima 2006). In addition to those, unidentified species (as “*Mediomastus* sp.”) have been reported from Japan (Sato 2000; Kato *et al.* 2003; Suyama *et al.* 2003; Imajima 2006; Niki *et al.* 2006; Nishi and Tanaka 2007), Taiwan (Paxton and Chou 2000), Brunei (Chaw *et al.* 1992), and Thailand (Green 2002; Fujioka *et al.* 2007).

In collections made at Abashiri, Hokkaido, northern Japan, we found a number of capitellid specimens. Subsequent morphological observations and pattern of methyl-green staining on the body surface (probably representing glandular cells in the epidermis) indicated that these represented an undescribed species of *Mediomastus*. For supporting data in describing the species as new to science, we sequenced part of the cytochrome *c* oxidase subunit I (COI) gene for comparison with existing sequences.

## Materials and Methods

Worms were collected from sandy sediments among roots of the sea-grass *Phyllospadix iwatensis* Makino on an intertidal rocky shore at Abashiri, Hokkaido, Japan (44°05'N, 144°26'E) on 21 July 2012. For four of the 11 specimens collected, the anterior portion of the body (including about 11 segments) was fixed in 10% formalin seawater and later transferred to 70% ethanol after rinsing in deionized water, in order to observe morphology, and the posterior portion was preserved in 99% ethanol for DNA extraction. The other seven worms were fixed whole in 10% seawater formalin and later transferred to 70% ethanol after rinsing in deionized water.

Observations were made with a stereoscopic microscope, compound light microscope, and scanning electron microscope (SEM). For observation of chaetae, transverse sections of the body cut with a scalpel were mounted on glass slides and embedded in Hoyer's medium under a cover slip. For SEM observation, specimens were dehydrated in an ethanol series, critical-point dried with CO<sub>2</sub>, and coated with gold. Methyl green staining was performed with the method of Warren *et al.* (1994); specimens were submerged for 2 min in a solution of 0.5% methyl green in 80% ethanol, and then rinsed in 80% ethanol to eliminate excess stain.

DNA was extracted from posterior segments by the silica method (Boom *et al.* 1990). PCR and sequencing were performed as described by Yoshihara *et al.* (2012); primers LCO1490 and HC02198 (Folmer *et al.* 1994) were used to PCR-amplify and sequence part of the mitochondrial COI gene. Sequence alignment and genetic distance calculations

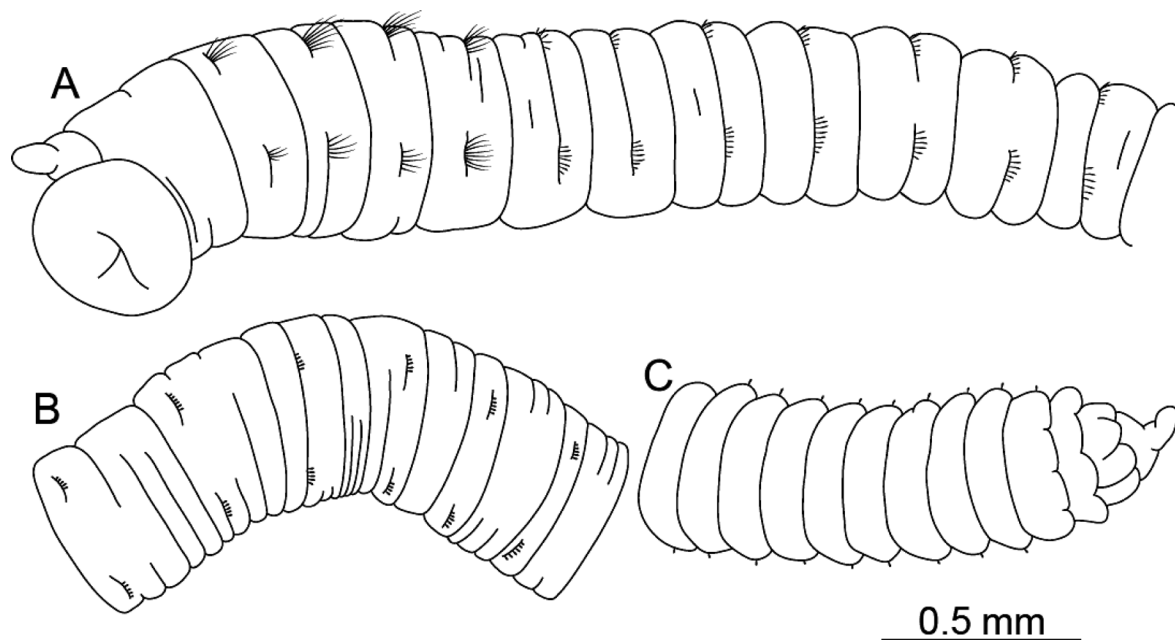


Fig. 1. *Mediomastus opertaculeus* sp. nov., holotype, ZIHU 4369. A, Anterior end of body, left lateral view; B, abdominal segments, left lateral view; C, posterior end, dorsal view.

were performed with MEGA 5.1 (Tamura *et al.* 2011). Sequences from the four paratypes (ZIHU 4372–4375) have been deposited in GenBank (accession numbers AB794985–AB794988).

The type specimens have been deposited in the Hokkaido University Museum, Sapporo, catalogued under the acronym ZIHU, referring to the former Zoological Institute, Hokkaido University.

Morphological terminology follows that of Warren *et al.* (1994).

***Mediomastus opertaculeus* sp. nov.**  
(Figs 1–5)

**Material examined.** Holotype: ZIHU 4369, whole specimen, fixed in 10% formalin seawater, preserved in 70% ethanol. Paratypes (seven specimens): ZIHU 4370, mounted on SEM stub; ZIHU 4371, some parts of body mounted on slides, remaining parts preserved in 70% ethanol; ZIHU 4372–4375, thoracic regions only, fixed in 10% formalin seawater, preserved in 70% ethanol; abdominal parts were used for DNA extraction; ZIHU 4376, whole specimen, fixed in 10% formalin seawater, preserved in 70% ethanol. Other material examined: ZIHU 4377–4379, three whole specimens, fixed in 10% formalin seawater, preserved in 70% ethanol.

**Description.** Holotype complete, sex uncertain, with 92 segments; 15.7 mm in length; 0.47 mm in maximum width. Body color red in life, whitish pink in ethanol. Epithelium smooth. Nephridiopores not visible. Branchiae absent. All segments cylindrical.

Prostomium (Fig. 1A) conical, with short palpode; everisible proboscis with numerous minute papillae (Fig. 2A). Peristomium 1.7 times as wide as long, slightly longer than

chaetiger 1, without eye spots, achaetigerous.

Thoracic chaetigers biannulate. Whip-like capillary chaetae without wing (Fig. 3A) present on chaetigers 1–4; noto- and neurochaetae each 5–10 in number per fascicle (Fig. 2B). Chaetigers 5–10 with hooded hooks, but without paddle-like chaetae. Notopodial hooded hooks with obvious fang (Fig. 3B); hood with small opening; fang with 4 teeth; constriction distinct (Fig. 3B, arrowed); 5–8 hooks per fascicle (Fig. 2C). Neuropodial hooded hooks without opening (Fig. 3C); fang with 7 teeth; constriction slight (Fig. 3C, arrowed); 5–8 hooks per fascicle (Fig. 2D).

Abdominal segments 1.4 times as wide as long (Fig. 1B); posterior segments becoming narrower and longer than anterior chaetigers; with hooded hooks only. Notopodial hooded hooks with obvious fang (Fig. 3D); hood with opening wider than that of thoracic notopodial hooded hooks; fang with 4 teeth; with distinct constriction (Fig. 3D, arrowed); 2–5 hooks per fascicle (Fig. 2E). Neuropodial hooded hooks with pointed fang (Fig. 3E); hood with opening wider than that of thoracic notopodial hooded hooks; fang with 4 teeth; with distinct constriction (Fig. 3E, arrowed); 2–5 hooks per fascicle (Fig. 2F).

Transition from thorax to abdomen marked by alteration of shape of hooded hooks; neuropodial hooded hooks in thorax without opening on hoods, while those in abdomen with opening on hoods.

Pygidium with short, rounded-cylindrical caudal cirrus (Fig. 1C).

**Methyl-green staining.** Among nine specimens observed, methyl-green staining resulted in several patterns, three of which are diagrammed in Fig. 4. All patterns showed numerous minute spots densely and uniformly covering segments 1–4, though in some individuals, spots completely covered segment 5 as well (Fig. 4A). All pat-

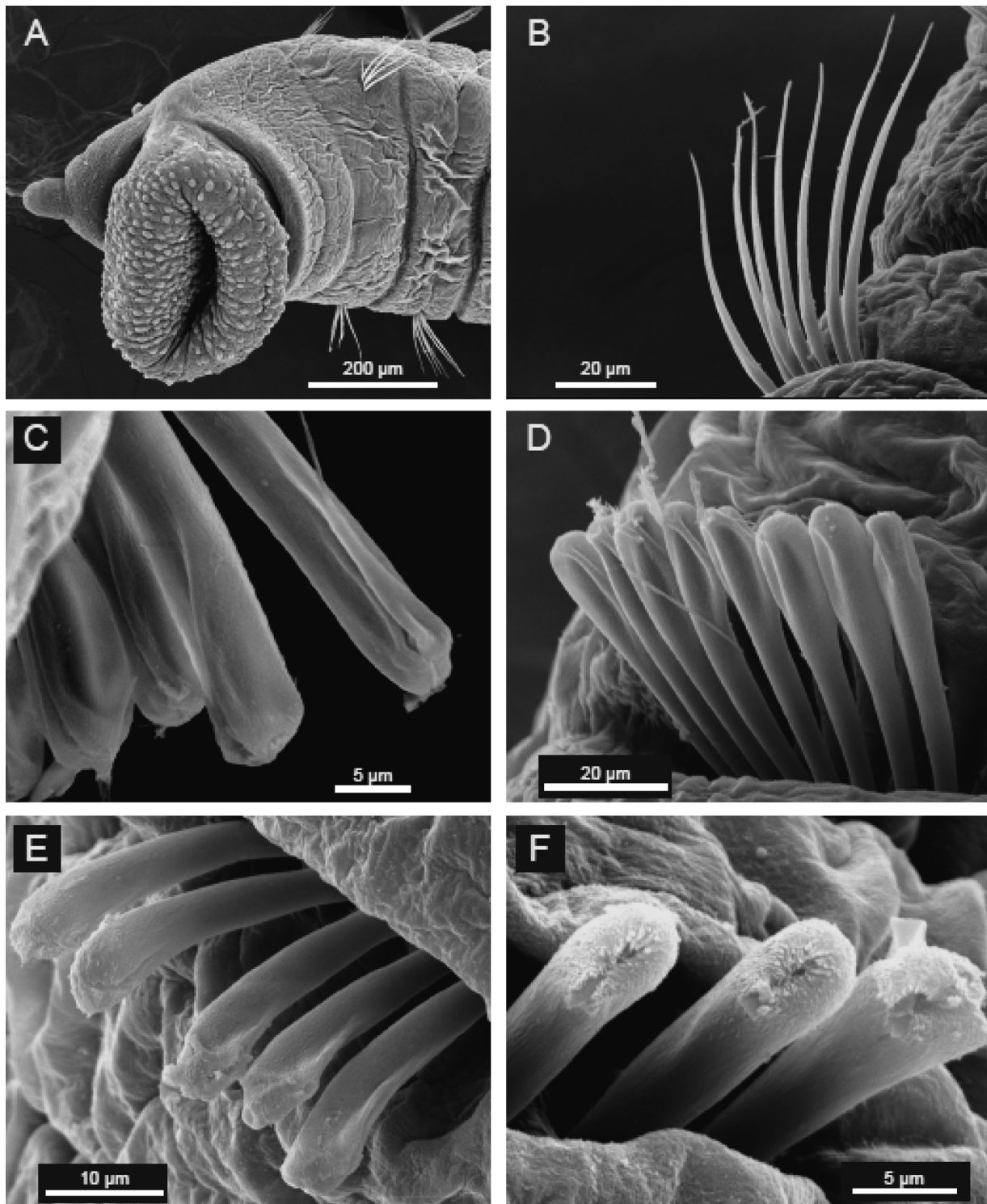


Fig. 2. *Mediomastus opertaculeus* sp. nov., paratype, ZIHU 4370, SEM images. A, Anterior end of body, ventral view; B, capillary chaetae on segment 4; C, notopodial hooded hooks on segment 6; D, neuropodial hooded hooks on segment 6; E, notopodial hooded hooks on segment 12; F, neuropodial hooded hooks on segment 20.

terns showed, immediately posterior to the completely spotted segments, at least two (Fig. 4A, C) and sometimes three (Fig. 4B) segments that had spots densely and uniformly distributed only in the anterior half of each segment. Finally, all patterns showed, immediately posterior to the half-spotted segments, at least two (Fig. 4A, B) but up to four (Fig. 4C) segments having a narrow band of dense spots in the middle of each segment. Fig. 5 shows a stained worm with

a pattern most similar to that illustrated in Fig. 4B, except that only segment 8 appears to have a middle band of deeply stained spots.

**COI sequences.** The four sequences obtained from the paratypes differed from each other at 1–4 sites within the aligned 624 bases, giving Kimura (1980) 2-parameter (K2P) distances of 0.002–0.006. The four paratypes were identical in the translated amino-acid sequences (208 residues).

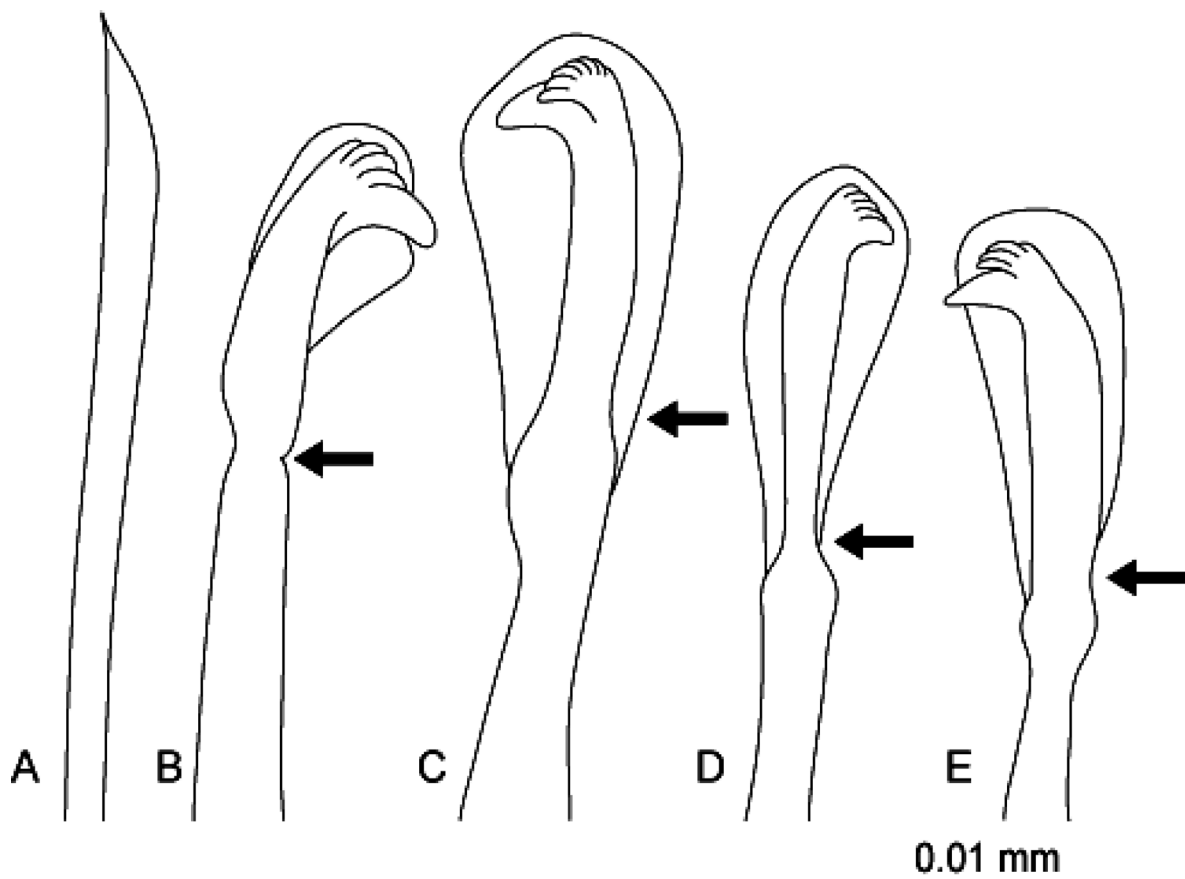


Fig. 3. *Mediomastus opertaculeus* sp. nov., paratype, ZIHU 4371. A, Capillary chaeta from segment 3; B, notopodial hooded hook from segment 9; C, neuropodial hooded hook from segment 9; D, notopodial hooded hook from segment 19; E, neuropodial hooded hook from segment 19; arrows indicating constrictions.

As of writing, GenBank contained only one COI sequence (EU835672) for any species in *Mediomastus*, an unidentified species from Darwin Harbor, Australia (M. J. Neave *et al.* unpublished data). The sequences from *M. opertaculeus* differed from that of the Australian *Mediomastus* sp. at 116–117 sites, giving K2P distances of 0.216–0.218, which are far greater than 0.0043, an average intraspecific K2P distances in 11 species of *Hydroides* Gunnerus, 1768 (Serpulidae) from the Pacific (Sun *et al.* 2012), indicating that *M. opertaculeus* and the Australian *Mediomastus* sp. are not conspecific. Sun *et al.* (2012) reported the average inter-specific K2P distance between 11 species of *Hydroides* was 0.262, a value slightly greater than that observed between *M. opertaculeus* and the Australian *Mediomastus* sp., possibly strengthening the adequacy of the generic placement of our new species. The amino acid sequence differed at five sites between *M. opertaculeus* and the Australian *Mediomastus* sp.

**Etymology.** The specific name is a noun, from the Latin participle *opertus* (“hidden”) and the Latin noun *aculeus* (“thorn”), referring to the species’ diagnostic hooded hooks, in which the hood completely covers the fang.

**Remarks.** Among 13 species of *Mediomastus*, nine have been reported to lack both paddle-like chaetae and abdominal capillary chaetae as in *M. opertaculeus* sp. nov.; these are *M. australiensis* Warren, Hutchings and Doyle, 1994; *M.*

*californiensis*; *M. cirripes* Ben-Eliahu, 1976; *M. calliopensis* Warren, Hutchings and Doyle, 1994; *M. capensis* Day, 1961; *M. deductus* (Pillai, 1961); *M. fragilis* Rasmussen, 1973; *M. thomassini* Warren, Hutchings and Doyle, 1994; and *M. warrenae* (Warren *et al.* 1994; Green 2002). *Mediomastus opertaculeus* differs from these nine species in the staining pattern with methyl green; Warren *et al.* (1994) presumed that this method would indicate the presence of glandular cells. In *M. opertaculeus*, segments 1–4 stain with numerous spots, while in the latter nine species the corresponding segments do not stain, except in *M. warrenae* (only known from the type locality, the Andaman Sea), in which segment 1 remains unstained and segments 2–4 stain variably with numerous spots, or remain unstained. *Mediomastus opertaculeus* also differs from *M. warrenae* in the staining pattern in segments 6–11. In *M. opertaculeus*, the overall staining pattern is variable across segments 6–11, with dense, deeply stained spots occupying the anterior half or middle of each segment; in *M. warrenae*, each of segments 6–11 stains with numerous, uniformly distributed spots (Warren *et al.* 1994; Green 2002).

*Mediomastus opertaculeus* differs from subsets of congeners in other characters: from *M. australiensis*, *M. californiensis*, *M. capensis* in the absence of nephridiopores (Hartman 1944; Day 1961; Warren *et al.* 1994); from *M. ambiseta* and *M. cirripes* in the shape of the capillary chaetae [the



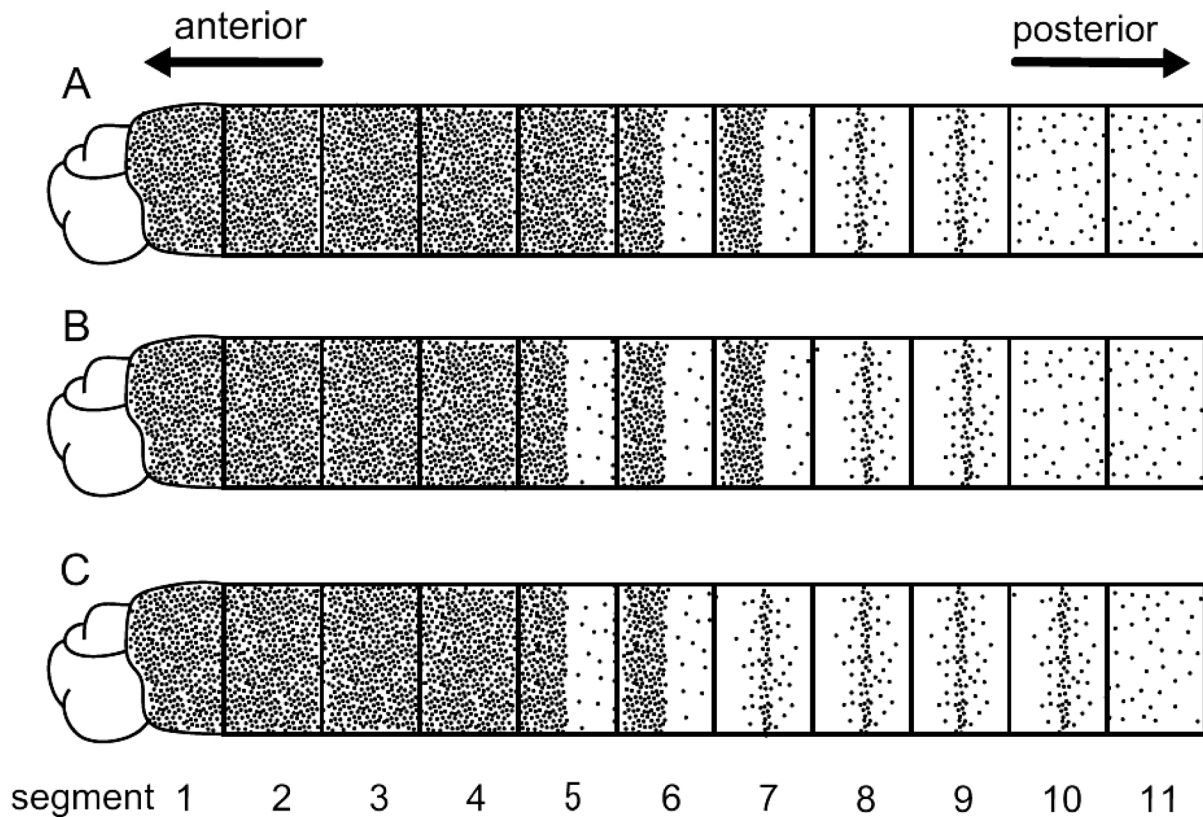


Fig. 4. Diagram showing three of the methyl-green staining patterns observed in the thorax (consisting of 11 segments) among nine specimens of *Mediomastus opertaculeus* sp. nov. A, Holotype, ZIHU 4369; B, paratype, ZIHU 4376; non-type, ZIHU 4377.



Fig. 5. Photograph of the thorax of *Mediomastus opertaculeus* sp. nov., paratype, ZIHU 4376, showing the methyl-green staining pattern, with the segment numbers and segmental boundaries labeled.

capillary chaetae in *M. opertaculeus* lack a wing, whereas those in *M. ambiseta* and *M. cirripes* possess a broad wing (Warren *et al.* 1994)]; from *M. acutus*, *M. ambiseta*, *M. branchiferus*, *M. calliopensis*, *M. californiensis*, and *M. fragilis* in the lack of an opening in the thoracic neuropodial hooded hooks (Warren *et al.* 1994); and from *M. warrenae* in the absence of constriction of hooded hooks on thorax (Green 2002).

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