

# A description of the free-living marine nematode *Euchromadora robusta* sp. n. (Nematoda: Chromadorida) with observations on the ultrastructure of the body cuticle

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**Summary.** *Euchromadora robusta* sp. n. found in meiobenthos samples from the Sea of Japan and the Sea of Okhotsk is described. The new species differs from other species in the genus by its long body (3.0-3.9 mm), thick cuticle (about 9  $\mu\text{m}$ ), and shape of spicules and gubernaculum. The ultrastructure of the body cuticle and transformation of the cuticle in the head end is reported.

**Key words:** *Euchromadora robusta* sp. n., nematode, cuticle, ultrastructure, Sea of Japan, Sea of Okhotsk.

Species of the genus *Euchromadora* de Man, 1886 have an intricate body cuticle devoid of a lateral differentiation, the stoma is enveloped by the pharyngeal musculature and specimens are equipped with a solid dorsal tooth, transverse rows of small denticles on stomatorhabdions, and a cylindrical oesophagus. The cephalic sense organs in *Euchromadora* occur as labial papillae, cephalic setae arranged in two circles and slit-like amphids. Currently 10, species of the genus have been described (Gerlach & Riemann, 1973; Kito, 1977). Specimens of a previously undescribed *Euchromadora* species were recovered from meiobenthos samples collected from the Sea of Japan and the Sea of Okhotsk. A description of this new species and observations of the ultrastructure of the body cuticle are presented in this paper.

## MATERIAL AND METHODS

Specimens of free-living marine nematodes were obtained from meiobenthos samples collected in the Bay of Peter the Great (Vostok Bay, August 1982, October 1993 and Boisman Inlet, June 1985) of the Sea of Japan, and in the Sea of Okhotsk (Sakhalin Island, in the vicinity of the settlement of Arsenievka, September 1993) at depths of 0.8-1.0 and 10 m. Nematodes were processed by slow evaporation to pure glycerin and mounted on slides. The anterior

and posterior body sections of selected specimens were removed and mounted for examination. Spinneret glands were revealed by methylene blue stain added to the mounting glycerin. For electron microscopy specimens were fixed with 2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH=7.2) and postfixed with 2% osmium tetroxide in similar buffer. After post-fixation specimens were dehydrated in an ethanol and acetone series and embedded in Araldite. Ultrathin sections, cut with a Reichert Ultracut E ultratome, were stained with uranylacetate and lead citrate. The stained sections were observed using a JEOL-JEM 100B and a Philips EM 300 electron microscopes. Body cuticle at the level of the posterior part of the oesophagus was examined, as was the body cuticle in the region of the head end. The nomenclature used to describe the cuticle structure is that of Maggenti (1979) who recognized four principal cuticular layers ("strata"): epicuticle, exocuticle, mesocuticle and endocuticle.

## DESCRIPTION

### *Euchromadora robusta* sp. n. (Figs. 1 & 2)

**Holotype male:** L = 3098  $\mu\text{m}$ ; a = 22.6; b = 7.3; c = 10.6.

**Paratype males (n=12):** L = 3014-3675  $\mu\text{m}$ ; a =

19.7-26.8; b = 6.4-8.5; c = 11.0-15.0.

**Paratype females** (n=11): L = 3062-3928  $\mu\text{m}$ ; a = 20.6-24.9; b = 6.9-8.2; c = 10.8-13.8; V% = 46.1-49.8.

**Adults.** Large nematodes. Body diameter in males increasing gradually towards the posterior part of the body, in the area of the cloaca equaling three-four head diameters. Female body more slender and tapering anteriorly and posteriorly from mid-body. Tail relatively short, conical, terminating with a tube with a terminal pore on the spinneret. Cuticle thick, in the genital region somewhat thinner than in the head and tail regions, with ornamentation and annulation visible from the level of the cephalic setae to almost the beginning of the tail tube. Width of cuticular annuli at the cephalic setae level = 2  $\mu\text{m}$ , at the middle of the oesophagus = 5  $\mu\text{m}$ , posterior from the beginning of the mesenteron = 6  $\mu\text{m}$ . Three layers of the cuticle distinguishable, each being different in ornamentation (Fig. 3A). Hexagonal structures visible in the external and middle layers. These structures are displaced longitudinally in the middle layer from those in the external layer by half the width of an annulus. Hexagonoids alter along the body somewhat. In the lower (= internal) cuticular layer a punctation is visible which is situated under the vertexes of the surface hexagonoids. In the lateral cuticle, from the middle of the oesophagus to the posterior third of the tail, the internal layer is present as transverse cuticular bulges situated between annuli. Near the dorsal and ventral lines in the anterior third of the oesophagus the cuticular ornamentation disappears, resulting in two longitudinal fields free of ornamentation which appear as two stripes which gradually widen toward the body mid-length and narrow in the tail part.

The endocupola is connected to the oesophagus in the region of the stomatoidal ring, with its base situated at the base of the cephalic setae. The amphids are frequently indistinct forming a broad slit with the front edge thicker than the posterior. Amphidial canals discernible at the dorsal edges of the amphids (clearly visible when the head is in the dorsal or ventral position). Labial papillae very small; cephalic sensilla arranged as two circles, with the anterior circle comprised of 6 papillae and the posterior circle comprised of 4 setae. Small somatic setae present over the whole body; two para-terminal setae at the base of the tail tube. Eyespots of yellow-brown pigmentation positioned in the anterior part of the oesophagus were observed only in specimens from Vostok Bay. Nerve ring encircling the mid-oesophagus.

Vestibulum supported by 12 ribs. The stoma

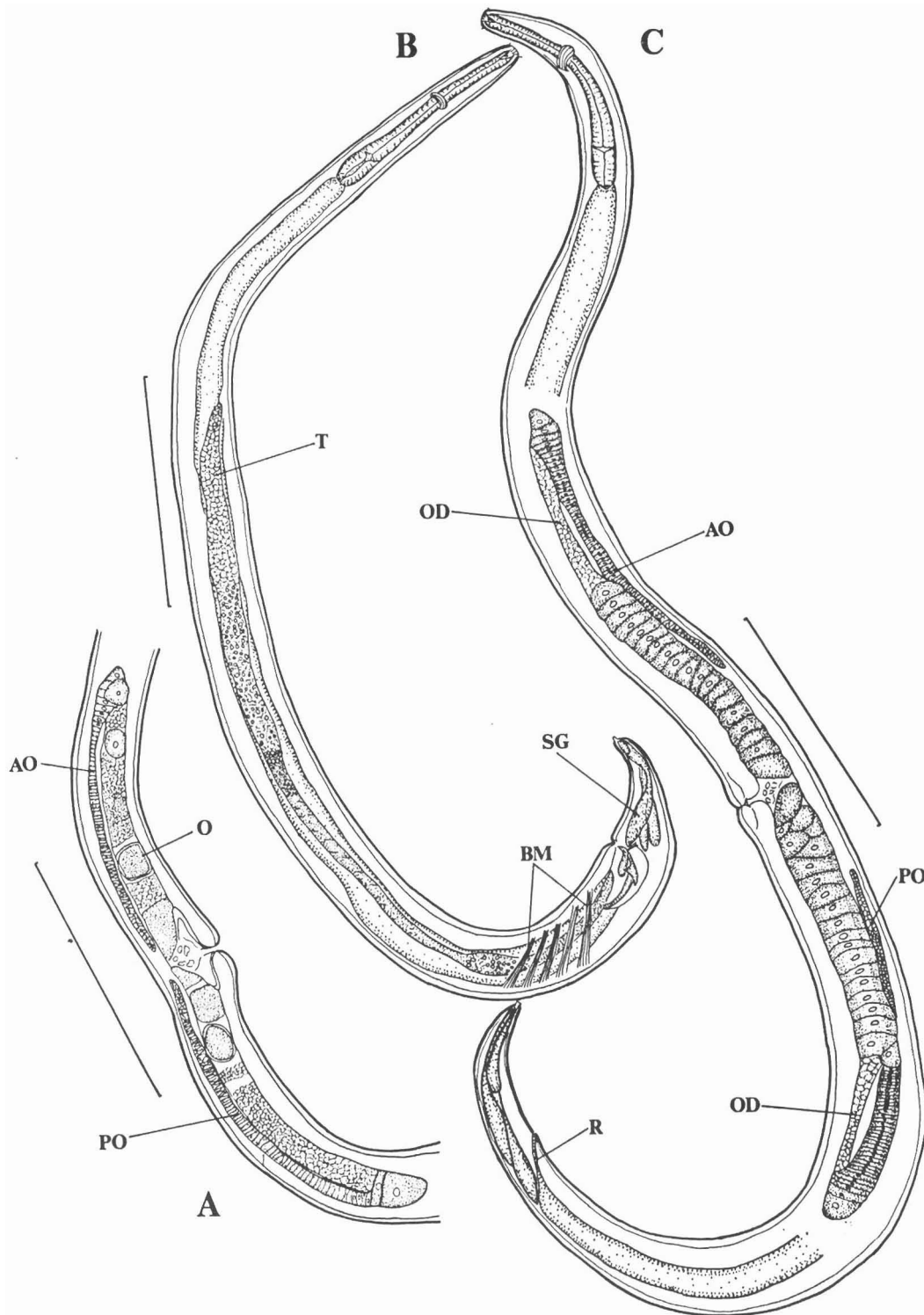
enveloped by the pharyngeal musculature and equipped with a solid dorsal tooth at the base of which, on the dorsal side, two rows of cuticular denticles are present. Lobe-like outgrowths with small denticles on their edges are present on the subventral walls of the stoma. Also small onchia present on the stomatorhabdions. Pharynx broadens at the stoma, forming a small bulb, with differentiated musculature. Cardial bulb not distinct and cardium small, indistinct. Nuclei of three glands situated in the base of the oesophagus with the dorsal gland duct opening at the base of the dorsal tooth. Ducts of the subventral glands opening into the stoma, almost at the level of the vestibulum base.

The oval body of the renette situated in the posterior oesophagus, not discernible in all specimens. Excretory pore observed only in two specimens, positioned at the level of the mid-oesophagus. Three spinneret glands present, each having a cone-type retentor. Secretions of the glands released through a terminal pore, formed by the inner layers of the cuticle, in the end of the tail tube.

**Males.** Body diameters: level of stoma = 35-45  $\mu\text{m}$ , base of oesophagus = 77-102  $\mu\text{m}$ , cloaca = 112-147  $\mu\text{m}$ ; maximum diameter (anterior to the cloaca) = 131-156  $\mu\text{m}$ . Thickness of cuticle: head and tail regions = 10-11  $\mu\text{m}$ , genital region = 9-10  $\mu\text{m}$ . Length of cephalic sensilla in the first circle = 2-5  $\mu\text{m}$ , in the second circle = 12-16  $\mu\text{m}$ . Length of oesophagus = 412-548  $\mu\text{m}$ , renette body = 78-103  $\mu\text{m}$ , tail = 259-283  $\mu\text{m}$ . Amphid width = 27-29  $\mu\text{m}$ . Distance from the anterior end of the body to: amphid = 6.4  $\mu\text{m}$ , cephalic setae = 13-14  $\mu\text{m}$ , nerve ring = 161-175  $\mu\text{m}$ , renette body = 485-515  $\mu\text{m}$ .

A single, straight testis situated on the right side of the intestine. Length of genital tube = 1547-1804  $\mu\text{m}$ . Spicules with indiscernible heads, thickened in the mid-part; in the posterior third a geniculate bend present. Spicules truncate at the distal ends, 122-165  $\mu\text{m}$  long with a span 113-145  $\mu\text{m}$  long. Gubernaculum complex, lateral part L-shaped (as occurs in all euchromadorins, but unlike other members of the genus *Euchromadora*) with well developed "heel" protrusions. Central part of gubernaculum in form of lamellae, each with a ventro-lateral wing overlapping the L-shaped lateral part. Length of gubernaculum lateral parts = 73-105  $\mu\text{m}$ . There are 5-6 pairs of bursal muscles in front of the cloaca. Length of the bursal field = 270-400  $\mu\text{m}$ .

**Females.** Body diameter at the level of: stoma = 38-50  $\mu\text{m}$ , base of oesophagus = 84-107  $\mu\text{m}$ , anus = 70-85  $\mu\text{m}$ ; maximum body diameter (in the vulva region) = 123-175  $\mu\text{m}$ . Thickness of cuticle: head



**Fig. 1.** *Euchromadora robusta* sp. n. A: Female reproductive system; B: Male; C: Female. Scale bars: 500  $\mu$ m. (Abbreviations: A - amphid, AC - amphidial canal, AO - anterior ovary, BM - bursal muscles; CS - cone of spinnerete, DG - duct of dorsal oesophageal gland, DT - dorsal tooth, E - endocupola, G - gubernaculum, O - ovum, OD - oviduct, OG - subventral oesophageal gland duct, OS - lobe-like outgrowths of stoma, PB - pharyngeal bulbus, PO - posterior ovary, PS - paraterminal setae, R - rectum, S - spicule, SG - spinneret glands, SR - stomatoidal ring, T - testis, TP - terminal tail pore, VLW - ventral-lateral wing).

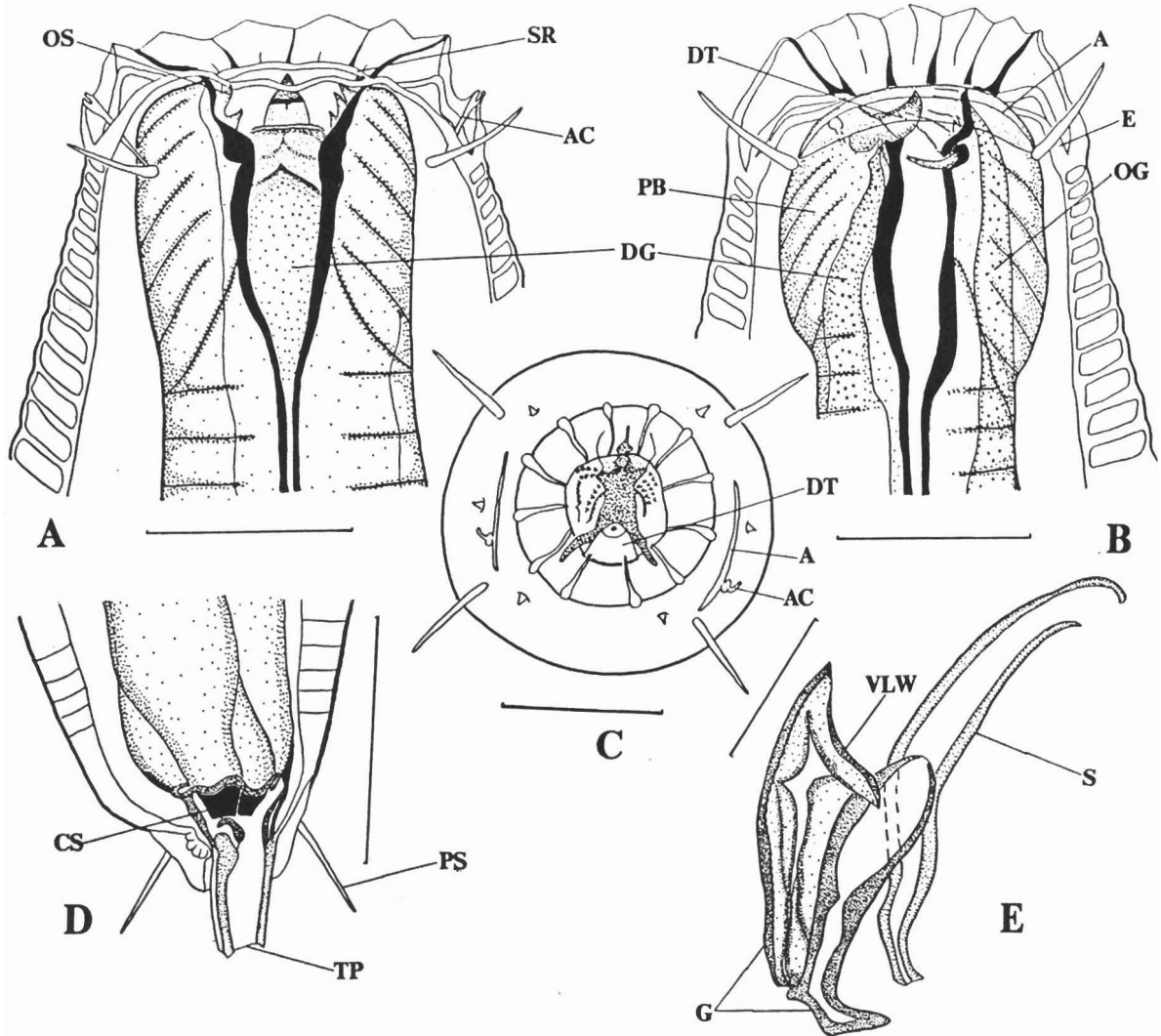


Fig. 2. *Euchromadora robusta* sp. n. A: Head region, dorsal view; B: Head region, lateral view; C: Head region, *en face* view; D: Tip of tail, elements of spinneret; E: Spicula with gubernaculum. Scale bars: A-D = 20  $\mu$ m, E = 50  $\mu$ m. Abbreviations see Fig. 1.

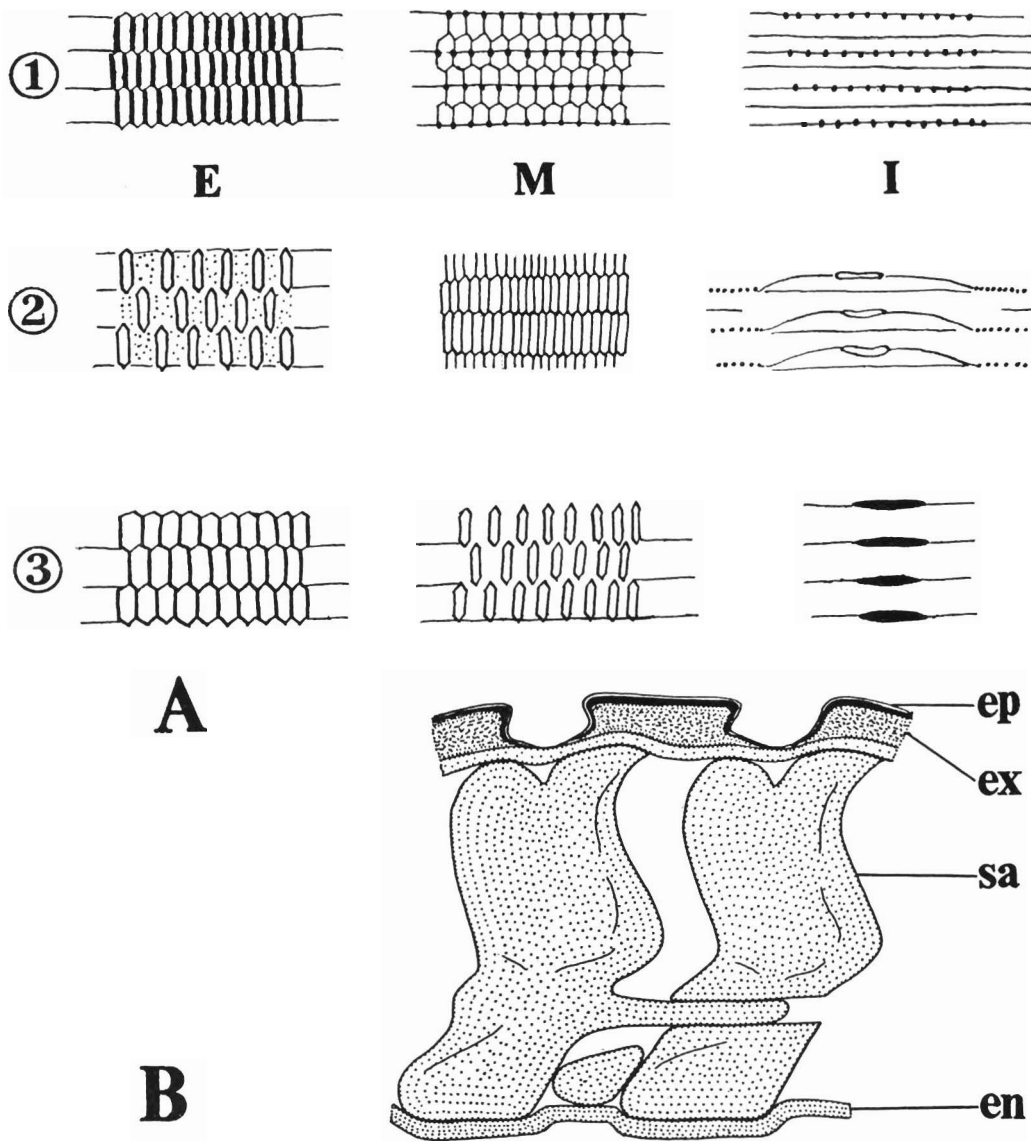
and tail regions = 10-11  $\mu$ m, genital region = 9-10  $\mu$ m. Length of cephalic sensilla in the first circle = 2  $\mu$ m, in the second circle = 10-14  $\mu$ m. Length of oesophagus = 410-555  $\mu$ m, tail = 250-353  $\mu$ m. Amphid 27-29  $\mu$ m wide. Distance from the anterior end of the body to: amphid = 6.4  $\mu$ m, cephalic setae = 12-13  $\mu$ m, nerve ring = 177-270  $\mu$ m, vulva = 1153-1950  $\mu$ m.

Didelphic and amphidelphic. Reflected and antitromous ovaries lie on the right side of the intestine. Length of anterior ovary = 480-611  $\mu$ m, posterior ovary = 368-637  $\mu$ m. Anterior and posterior genital

tubes 1186-1375  $\mu$ m and 1042-1424  $\mu$ m long, respectively.

**Type habitat.** The specimens were found among green and brown algae at 0.8-1 m depth in the Sea of Japan (Bay of Peter the Great) and in the Sea of Okhotsk (near the settlement of Arsenievka, Sakhalin Island). Also, in a druse of *Crenomytilus grayanus* at 10 m depth in the Sea of Japan (Vostok Bay).

**Type material.** Holotype male (slide n° 2030), 6 male and 6 females paratypes deposited in the nematological collection of the Department of



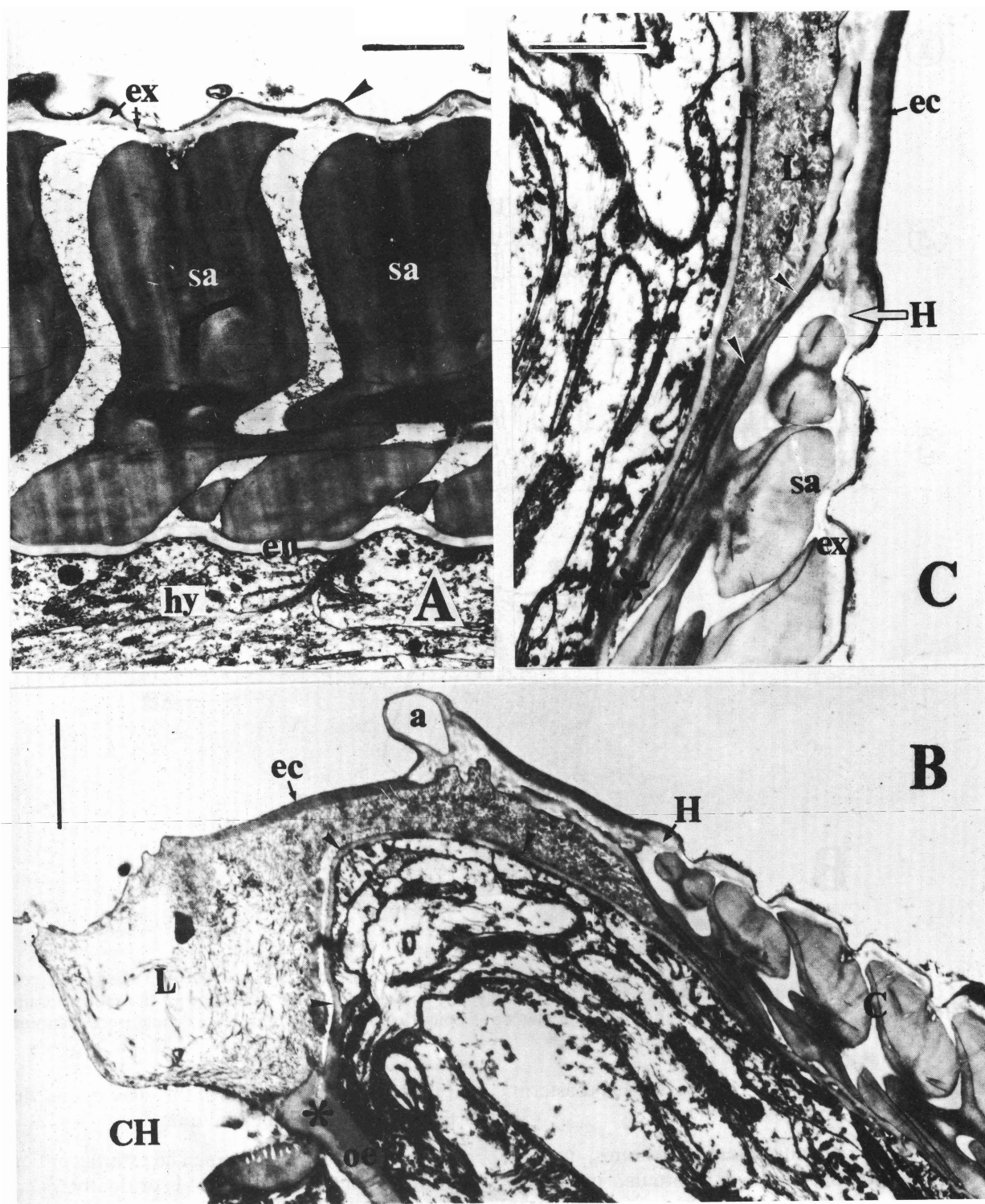
**Fig. 3.** *Euchromadora robusta* sp. n. A: Light microscope surface view of the body cuticle in (1) head; (2) genital and (3) tail regions (E - external, M - middle, I - internal layers); B: Schematic representation of the ultrastructure of the body cuticle of the head region (en - endocuticle; ep - epicuticle; ex - exocuticle; sa - sclerotized annule of mesocuticle).

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**Diagnosis.** Seven characteristic features, including stoma arming, amphid contours, cuticular ornamentation, L-shaped lateral parts of the gubernaculum and the shape of the oesophagus, enable this species to be identified as a representative of the genus *Euchromadora*. *Euchromadora robusta* sp. n. can be distinguished from other species of the genus by its large size, long body length (3.0-3.9 mm as compared with 2.3-3.0 mm, length of the longest species, *E. ezoensis*, in the genus), ornamentation

and thickness (about 9  $\mu\text{m}$ ) of the cuticle, and shape of the spicules and gubernaculum.

**Body cuticle ultrastructure.** The external cuticular layer of the cuticle (epicuticle) is distinctly tri-laminated (Figs. 3B & 4A). The stratum corresponding to exocuticle is composed of a finely granulated substance. The bulk of the cuticle is formed by strongly sclerotized transversal annules 8-9  $\mu\text{m}$  thick and 3-4  $\mu\text{m}$  wide, situated in the intra-cuticular cavity. These annules may be considered as representing the mesocuticle. Each annule is composed of external (6.0  $\mu\text{m}$  thick) and internal (2.0  $\mu\text{m}$  thick)



**Fig. 4.** *Euchromadora robusta* sp. n., ultrastructure of the cuticle. A: Longitudinal section of the body cuticle in the head region (arrowhead indicates the epicuticle); B: Longitudinal section of the head end (arrowheads indicate the endocupola, asterisk indicates the junction of lip (body)-cuticle with that of the oesophagus); C: High magnification of the region of the head seam (arrowheads indicate the intracuticular septum delimiting the body cuticle and the lip (mesocuticular) cavity, asterisk indicates the beginning of the endocupola). Scale bars: A, B = 2  $\mu$ m; C = 1  $\mu$ m. (Abbreviations: a - amphid groove; C - body cuticle; CH - cheilostome; E - endocupola; en - endocuticle; ec - exocupola; ex - exocuticle; H - head seam; hy - hypoderm; L - lip mesocuticle; oe - cuticle of oesophagus; sa - sclerotized annules of mesocuticle).

sub-annules joined by radially oriented bridges. Long processes originating from the bridges stretch anteriorly and penetrate into the space between similar bridges on the next ring and this is repeated in turn. Relatively small sclerotized annules are positioned between the basal parts of the main annules of the cuticle. The endocuticle is a thin (0.2  $\mu\text{m}$ ) but distinct electron-light layer of uniform thickness which underlies the mesocuticular annules and delimits the mesocuticular cavity and the hypodermis.

**Head region.** Near the head-end the annulated body cuticle gradually tapers and stops abruptly at the head seam (Fig. 4B). Thin intracuticular septum delimits the body mesocuticle and the wide cavity of the lips (Fig. 4C). Two cuticular layers build the contours of the lips. The external one (exocupola) is the continuation of the exocuticle (Fig. 4B). The slit-like groove of the amphid is situated in this layer. The exocuticle continues anteriorly, arches over the lip region and turns inward into the stoma, where it lines the cheilostome and connects to the cuticle of the oesophagus. The internal layer, which may correspond to the endocupola of Belogurov & Belogurova (1975), is a continuation of the body endocuticle. The endocupola is connected to the cuticle of the oesophagus to form the line delimiting the cheilostome and the oesophastome. The prominent cavity of the lips is filled with loose fibrous material which may be referred to as the mesocuticle.

## DISCUSSION

The cuticle of representatives of the family Chromadoridae appears to be uniform in its general ultrastructure. Epicuticle covers a relatively thin exocuticle, which, in turn, covers the solid transverse annules of the mesocuticle. The endocuticle of chromadorids is thin but is a consistently distinguishable layer. The mesocuticular annules of chromadorids show considerable variability of configuration but in all cases they are the main feature of the cuticle (Inglis, 1964, 1969; Watson, 1965; Lippens, 1974; Yushin & Malakhov, 1994). Very different and diverse surface appearances of the body cuticles of chromadorids, including *E. robusta* sp. n., are due to the structure of the annules (Inglis, 1969).

The cuticle of *E. vulgaris* described by Watson (1965) includes thick mesocuticle consisting of overlapping sclerotized plates which differ significantly in their profiles from the mesocuticular annules of *E. robusta* sp. n. and *E. ezoensis* (Yushin & Malakhov, 1994). Also, Watson (1965) described the dorsal and ventral cuticles which, as shown by Inglis (1969), are of different structure. The lateral parts of the cuticular annules in each of the *Euchromadora* spp. have similar configuration (Inglis, 1964, 1969; Yushin &

Malakhov, 1994), and this was confirmed for *E. robusta* sp. n. in the present study.

Malakhov & Voronov (1981) reported that the cuticle covering the head end of nematodes is continuous with that covering the remainder of the body, but the ultrastructure of the cuticle in these two regions is quite different. In the head end there is a thickening of the layer of the mesocuticle, resulting in separation of the layers of exo- and endocuticle with the endocuticle joining to the stoma walls and forming the internal skeleton of the head end, the endocupola (Belogurov, 1985; Tchesunov, 1990a, b; Van de Velde & Coomans, 1991; Malakhov, 1994). Our observations of *E. robusta* sp. n. revealed the cuticle of the head end undergoing the same structural changes. The endocuticle (endocupola) of the head does not change, but exocuticle (exocupola) tends to be thin and weak (especially in the cheilostome), unlike the very thick mesocuticle which appears to be filled with filamentous material embedded in a liquid substance. This abrupt changeover of the cuticle facilitates free movement of the lips.

Several examples have been described where the mesocuticle in the head region tapers so that for a short distance the endocuticle and exocuticle are adjacent to one another and finally may connect at the line of the head seam (Hope, 1982; Tchesunov, 1990b; Malakhov, 1994). Consequently, a discontinuity between the mesocuticles of the body and head is formed. In *E. robusta* sp. n. the developed septum delimits the body annules and the intracuticular space of the lips. A similar septum was described for another chromadorid nematode, *Halichoanolaimus sonorus* (Cyatholaimina, Selachinematidae) (see Fig. 12d in Malakhov, 1994). It appears probable that when an abrupt changeover of the cuticular structure in the head occurs a mechanical separation of the mesocuticular layers is required.

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**Куликов В., Дашенко О.И., Колос Т.В., Юшин В.В.** Описание свободноживущей морской нематоды *Euchromadora robusta* sp. n. (Nematoda: Chromadoria) и наблюдения по ультраструктуре стенки тела этих нематод.

**Резюме.** Описан новый вид *Euchromadora robusta* sp. n. из мейобентосных проб, собранных в Японском и Охотском морях. Новый вид рода отличается от известных видов большей длиной тела (3,0-3,9 мм), толстой кутикулой (9 мкм), формой спикул и рудька. Изучена ультраструктура кутикулы тела и головного конца.

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