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Author(s)	TAJIKI, Ken-Ichi
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**A New Species of the Genus *Ciliocincta* Kozloff, 1965
(Mesozoa, Orthonectida) Parasitic in a Marine
Turbellarian from Hokkaido, Japan**

By

Ken-Ichi Tajika

Zoological Institute, Hokkaido University

(With 4 Text-figures and 1 Table)

In the course of my faunistic survey of marine interstitial turbellarians of Hokkaido, I found two individuals of coelognopodid turbellarians which were infected heavily with numerous mesozoan parasites. The analysis based upon both fresh and fixed materials proved this parasitic mesozoan to be a member of the order Orthonectida.

Since the 19th century orthonectids have been known as rather rare parasites of some turbellarians, nemerteans, annelids, molluscs and echinoderms (Caullery, 1961; Tokioka, 1961; Kozloff, 1965). Among turbellarians the orthonectid parasitism has been recorded in the three orders, Acoela (Westblad, 1942, p. 35), Polycladida (Keferstein, 1868, Taf. II, fig. 8; Nouvel, 1935a, p. 217) and Neorhabdocoela (cited from Lang, 1954, p. 604). Although Sopott (1973, p. 102) mentioned parasites, which she found in proseriat flatworms, as only Mesozoa, they also seem to be an orthonectid. The orthonectids parasitic in Polycladida have been relatively well-examined, but our knowledge on those of other turbellarians is still very poor.

In this paper morphology and systematics of the orthonectid mesozoan obtained are presented and some remarkable morphological features are discussed. This is the second report from the Pacific Sea.

Material and Methods

Only two infected hosts were obtained from living samples collected on 9 Aug. 1976 at Cape Aikappu near the Akkeshi Marine Biological Station, Hokkaido University, Akkeshi, east Hokkaido. One of the hosts was a fragment of the body and the other was of a normal body form with no any trauma. These were kept together in an electric refrigerator. A few days later the first mentioned host was

entirely damaged owing to the autolysis; accordingly only one host was available to the study.

Specimens were selected from those which were released through the body wall of the host when it was gently squashed for a vital observation. All of the animals released were female. Two living specimens released were spoiled after photographed. After the observation in the fresh condition, all the other eleven specimens released were fixed in Bouin's or 2% formalin fluids. The host still containing many individuals of this parasite was also fixed in Bouin's fluid and sectioned at 5 μm longitudinally.

Two specimens fixed in Bouin's fluid were mounted as a whole and stained with hematoxylin and eosin. Five of the rest fixed in 2% formalin were prepared for silver impregnation according to the Nouvel's method (1935b, p. 504; 1939, pp. 262-264). Two of the remaining specimens, set on a piece of a flower lip, were serially sectioned at 5 μm , longitudinally or transversally. As embedding medium "Paraplast Plus" (Sherwood) was used. All sections were stained with Delafield's hematoxylin and eosin.

Holotype: A released female (No. 4 specimen) which was mounted as a whole and stained with hematoxylin and eosin.

Allotype: A male (σ 1), which was found through 29-30th sections of a series of longitudinal sections of the host, is designated as the allotype.

Paratypes: Two released females sectioned transversally (No. 2) or longitudinally (No. 3). A released female (No. 5) prepared in the same manner as the holotype. A released female (No. 8) treated with the Nouvel's method for silver impregnation.

The type-locality: Cape Aikappu, Akkeshi, east Hokkaido, Japan (9-VIII-1976; Tajika leg.).

The other two females released from the host, fixed in 2% formalin fluid, have remained for a future study.

All the aforementioned specimens and the host are deposited in the Zoological Institute, Faculty of Science, Hokkaido University.

Rhopaluridae¹⁾
Ciliocincta Kozloff, 1965
Ciliocincta akkeshiensis spec. nov.
 (Figs. 1~4)

Description of adults (Figs. 1~3)

Female (Holotype). *Observations in the fresh condition*

The body is cylindrical, 128 μm long and about 16 μm wide (in the whole mounted preparation, measured exclusive of cilia), without remarkable coloration. Both the anterior and posterior extremities are of semi-spherical outline.

1) cf. Stunkard, 1937 p. 6; Caullery, 1961 p. 703

The body is entirely annulated and slightly constricted at two locations in the anterior half (Fig. 1 A, B). The annuli before the first constriction form an anterior cone. Two annuli between the constrictions, and the succeeding four of the somatoderm contain many refringent, nearly same in dimensions, granules. No such the granules are present in the rest of the annuli.

The somatoderm consists of one layer of relatively flat cells, though its constitution is not ascertained in the anterior third of the body. Inside the somatoderm there exists a column of oocytes which begins at the first constriction and extends to the posterior tip of the body. The oocytes with a large round nucleus are disposed in a single row anteriorly and zigzag posteriorly.

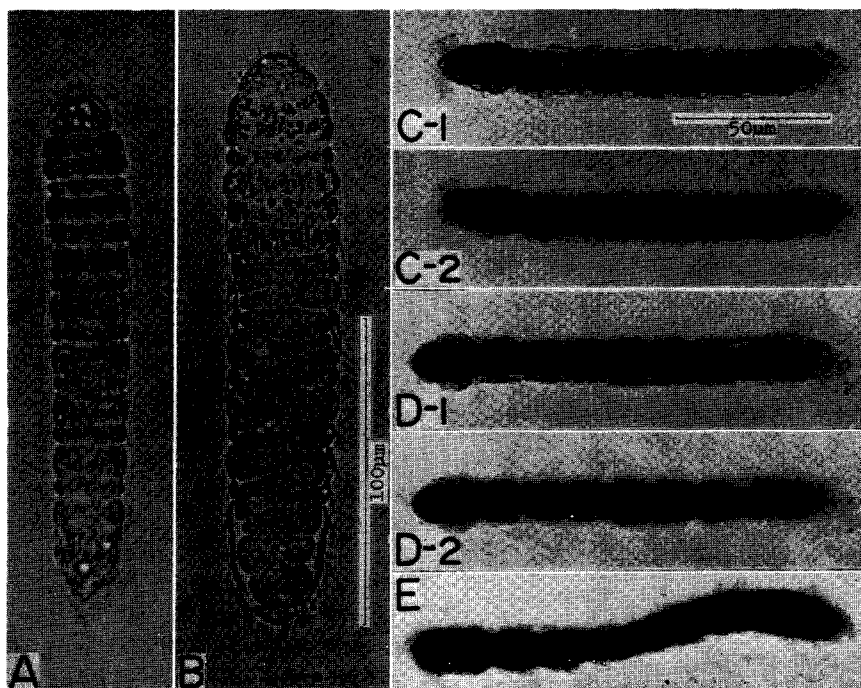


Fig. 1. *Ciliocincta akkeshiensis* spec. nov., Female. A. Living specimen (No. 6). B. Gently squashed living specimen (No. 7). C-1. No. 4 specimen (Holotype). C-2. Alternation of coloration on the somatoderm of the same. D-1. No. 5 specimen (Paratype). D-2. Alternation of coloration on the somatoderm of the same. E. No. 8 specimen (Paratype). (A~B as well as C-1~E in the same magnification. C-1~D-2 stained with hematoxylin and eosin. E treated with silver impregnation method).

Observations in the fixed and stained preparation

In the preparations stained and also treated with the Nouvel's method (l.c.), no refringent granules are present in the six annuli (Figs. 1 C-1~2, D-1~2, E; 2

A, B). These granules may have been lost during operations of making the preparations, as was observed in the male of *Rhopalura ophiocomae* Giard, 1877 (Caullery et Mesnil, 1901, p. 393) and *Stoecharthrum giardi* Caullery et Mesnil, 1899 (C. et M., 1901, p. 415).

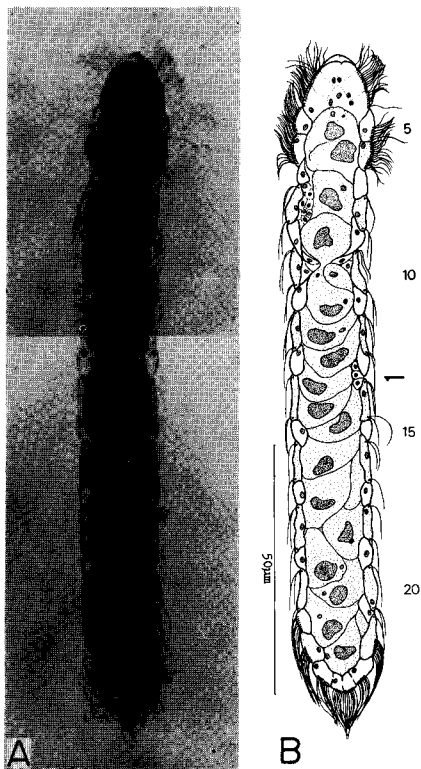


Fig. 2. *Ciliocincta akkeshiensis* spec. nov., Female. A and B. General organization of the No. 4 specimen (Holotype). The arrow indicates a presumable genital pore.

The phase-contrast-microscopy reveals 24 annuli of the somatoderm (Fig. 2 A, B). The preparation made with the silver impregnation method showed only some boundaries of the annuli, even in those of the best quality obtained (Fig. 1 E). If more detailed annulate pattern could be recognized, the number of the whole annuli probably increases.

The first and second constrictions correspond to the boundary between 4th and 5th annulus, and between 6th and 7th respectively (Fig. 2 A, B). Six annuli containing granules in life are accordingly regarded as the 5th to 10th annuli. In the anterior cone four annuli are detected faintly. From the 6th backwards a larger annulus alternates with a smaller one. Such the alternation becomes indefinite from the 21st. Generally the somatodermal cells of the larger annuli are flattened; those of the smaller annuli thicker. As most of the flat somatodermal cells contain very fine granulous inclusions which are rather feebly stained with hematoxylin, an alternation of coloration is embossed on the somatoderm (Fig. 1 C-1~2, D-1~2), as reported by Kozloff (1965, Figs. 3~5) in *Ciliocincta sabellariae* Kozloff, 1965.

Although the body seems to be entirely ciliated as far as observed in fresh preparation, this is not true in the whole mounted preparation stained with hematoxylin and eosin. This is due to the absence of the ciliation in the seven annuli, namely 7th, 9th, 11th, 13th, 15th, 17th, and 19th (Fig. 2 A, B). Ciliation of the foremost part of the body is uncertain. The annuli from the 2nd to the 6th bear dense cilia, most of which are oriented forwards. The cells of the even-numbered annulus from the 8th to the 20th have cilia along their anterior and

posterior margins. Thus a ciliated annulus alternates with a cilia-free one from the 7th backwards. But no such the alternation of the ciliation is present in those annuli from the 21st or 22nd. Each annulus from the 22nd to the hindmost has a rather wide row of cilia only near the posterior margin. The dense cilia on the posterior region of the body are oriented backwards in fixed as well as in living condition. The cilia are subequal in length (about 10 μm). Kinetosomes of the cilia are feebly stained with hematoxylin.

The axial column is composed of 19 oocytes, most of which contain one small body beside a large nucleus (Fig. 2 A, B), while two such bodies are also found. Both of these cell elements are rather deeply stained with hematoxylin. The column is, as in *Rh. leptoplanae* (Giard, 1877) (C. et M., 1901, p. 399 footnote), constricted at its anterior third by invasion of smaller cells. These small cells seem to be somatodermal. The oocytes appear to overlap one another, both anteriorly and posteriorly from the constriction.

Inside the anterior cone, before the anterior extremity of the column, there are a few small cells, whose nucleus is stained deeply with hematoxylin. Their coloration is similar to that of the somatodermal cells rather than of the oocytes. Similar cells are also found in part under the 8th somatodermal cells (Fig. 2 A, B).

Three pairs of small cells are arranged longitudinally between the 13th and 14th annuli (Fig. 2 A, B). Their nuclei show the same coloration as that of the somatoderm. Judging from their constitution and position, they may be concerned in a genital pore.

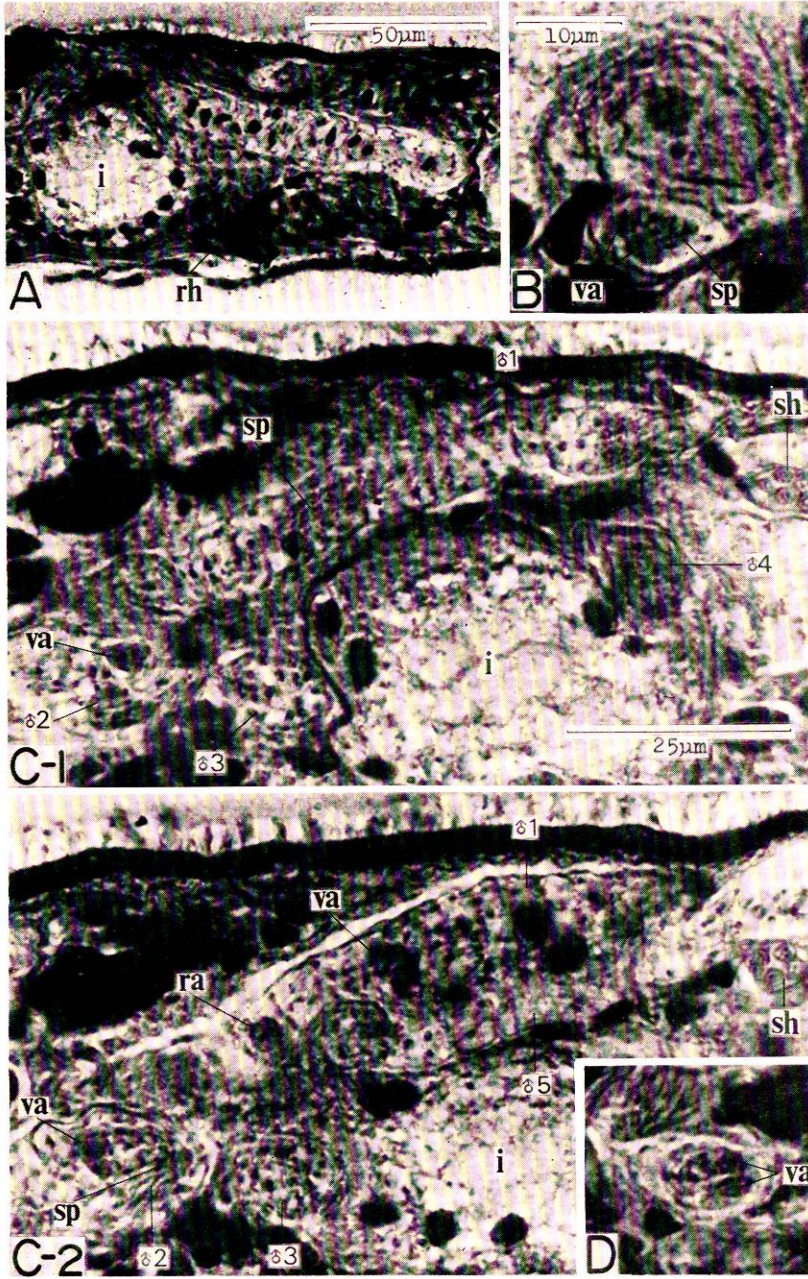
Male (Allotype).

As already mentioned, no males were found in those released from the host. Fortunately males as well as females were found embedded in the sectioned host (Fig. 3 A~D). The following description is based upon one (Fig. 3 C-1~2, ♂ 1) of those embedded. The male was sectioned longitudinally.

The body is cylindrical, about 66 μm long and 10 μm wide (Fig. 3 C-1~2). Both the anterior and posterior extremities bear cilia, of which orientation is the same as in the females. The ciliation of the other body parts is uncertain. An annulate pattern appears indistinctly; the annuli measure at least 14.

The relative loose body is divisible into four parts. The foremost and hindmost semi-spherical parts are filled with very fine eosinophile granules (Fig. 3 C-1~2, ♂ 1, ♂ 4). In the anterior half of the rest a few small undifferentiated cells are surrounded only by the somatodermal cells (Fig. 3 C-1~2, ♂ 1, ♂ 3). In the posterior half they are substituted for fibril-like strands which lie in antero-posterior direction (Fig. 3 B, C-1~2, ♂ 1, ♂ 2). These strands are maybe tails of sperms, though whose head parts are not detected clearly.

The male has two kinds of special apparatus as well as the fibril-like strands. One of the apparatus is present in the middle of the body (Fig. 3 C-2, ♂ 1). This represents a posteriorly unfolded V-shaped form, which consists of two pear-like lobules. The form measures eight micra by five. The other is a round flat body



(about 4 μm in diameter) which is laid at the rear of the former. Both apparatus are feebly stained with hematoxylin. Neither the detailed morphology nor the function of these apparatus is ascertained. It is, however, suggested that they play no little roles in producing male gametes, because the position where the fibril-like strands are present is at the one side of the V-shaped apparatus and furthermore between these two apparatus.

On the other hand the transversal sections of the other males reveal that the apparatus are situated not in the center but somewhat close to the somatoderm. Figures (Fig. 3 B, D) indicate this deviation of the V-shaped apparatus, even if it were sectioned just a little obliquely to the rectangular plane of the body. This will be more certain if two serial longitudinal sections of the ♂ 1 (Fig. 3 C-1~2) are taken into consideration. It is, therefore, suggested that the bilateral symmetry is differentiated in the present species.

Additional observations of the other specimens

The largest female released was 187 μm long in live preparation. Some larger vacuole-like spaces were present in the anterior cone or partially in the somatoderm of two females (Fig. 1 A, B). Although the column of the oocytes is clearly constricted in the holotype described, the constriction is not so conspicuous in the other specimens (see Fig. 1 D-1~2).

Descriptions of plasmodium and agamont development (Figs. 3~4)

The parasites affect the parenchym of the host (Fig. 3). The adults as well as seemingly preadult individuals generally exist near the intestinal canal in large numbers. The females seem to be superior in number to the males. While the females as well as the males usually aggregate with the individuals of the same sex, both sexes are also co-exist in a group (Fig. 3 B). Consequently plasmodia of this species may possibly produce both of sexes, though it is difficult to decide the sexuality of the plasmodia from these evidences.

Many agamont developmental stages are detected rather just under the dermal muscle layer of the host (Fig. 4). Smaller young plasmodia are rarely found. They contain a number of small eosinophilous cells which bear also fine basophilic granules. Among these cells are present a few larger cells, maybe germ cells. Most of the germ cells and embryos in various stages, however, seem to be scattered independently in the parenchym; there clear boundaries of plasmodia do not appear (Fig. 4 A~C).

Fig. 3. *Ciliocincta akkeshiensis* spec. nov. A. Female embedded in the host. Longitudinal section. B. Embedded female (upper) and male (lower). Transverse section. C-1. A few males embedded in the host. C-2. The same in the next side of sections. D. Male (♂ 3) exposed also in C-1 and C-2. Transverse section. (i: intestine, rh: rhammit gland, sh: tubular gland (Schlauchdrüse), sp: sperms, ra: round apparatus, va: v-shaped apparatus. C-1~2 and D in the same magnification. All stained with hematoxylin and eosin).

Although I was unable to make a direct observation of the agamont development, each stage could be safely followed by examining the specimens of different developmental stages found in the preparations.

The germ cell increases its cytoplasm which is deeply stained with hematoxylin and eosin (Fig. 4 A). An unequal holoblastic cleavage of the cell now takes place (Fig. 4 B). The blastomeres are always of a water-drop shape, at least to the 5-cell stage (Fig. 4 B). They develop into a slightly slender cluster at latest by about a dozen cell stage; their cell boundaries are observed clearly (Fig. 4 C).

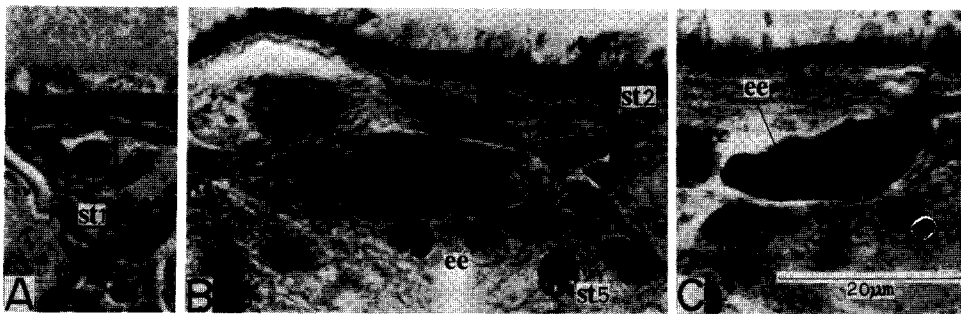


Fig. 4. *Ciliocincta akkeshiensis* spec. nov. A. Germ cell in one cell stage (st. 1). B. Two cell stage (st. 2), five cell stage (st. 5, a part) and early embryo (ee, longitudinal section). C. Early embryo (ee, longitudinal section). (All in the same magnification and stained with hematoxylin and eosin).

As the number of the blastomeres increases, involutions of cells take place; it becomes difficult to detect outlines of cells (Fig. 4 B). At this embryonic stage differentiations to the somatoderm and the inner mass may arise. The late embryos have not yet any ciliation; the sexuality is not distinguishable.

As embryos become larger, the bodies are stained more eosinophilously and a ciliation appears at both extremities.

A younger female has a loose body and its somatoderm becomes distinguishable from the inner mass of oocytes. While nuclei of the oocytes are stained still eosinophilously, their cytoplasm is light in color. Thereafter an annulate pattern is definitely organized in the somatoderm (Fig. 3 A).

The bodies of younger males also become loose exclusive of both extremities. But two more densely stained regions remain in the middle and posterior parts of the body. They may correspond to two problematic apparatus of the adult male. At a slightly more advanced stage, males have sperms and their somatoderm is constricted as annuli (Fig. 3 C-1 ~2).

Host problem

The present host, a coelogyonopod, is infected so seriously that any reproductive organ disappears and the pharynx is transformed into an accumula-

tion of cells in spite of its longer body (about 8 mm). Accordingly, only two kinds of dermal glands, namely, tubular (Schlauch-) and rhammit glands (Rhammitendrüsen) are available for the identification of the host (Fig. 3 A, C-1 ~2). As the coelogyneporids bearing such glands, *Invenusta* sp., *Vannuccia* sp. (both unpublished) and *V. tripapillosa* have been so far found by me (Tajika, 1977) at the same locality as the host was obtained; therefore, the host is assumed to be one of these species.

Discussion

According to Caullery (1961), two genera *Rhopalura* Giard, 1877 and *Stoecharthrum* Caullery et Mesnil, 1899 constitute the family Rhopaluridae. The third genus *Ciliocincta* has been recently proposed by Kozloff (1965). *Ciliocincta* as well as *Stoecharthrum* is monotypic.

The present new species is related closely to *Ciliocincta sabellariae* Kozloff, 1965 as far as judged from the three characters, namely, the cylindrical body, the linear disposition of oocytes and the general appearance of ciliation. Incidentally, *C. sabellariae* is recorded as a parasite of *Sabellaria cementarium* (Polychaeta) collected at San Juan Archipelago, Washington (USA) (Kozloff, l.c.).

C. akkeshiensis spec. nov. accords with *C. sabellariae* in the principal nature of the ciliation pattern (cf. Kozloff, 1971), but is distinguishable from the latter as is mentioned below. The female of the new species has 24 annuli, of which the anterior six contain refringent granules in life, while the female of *C. sabellariae*, whose body ($260 \times 21 \mu\text{m}$) consists of 38 or 39 annuli, has no granules (not granulous inclusions) (Kozloff, 1965; 1971). The male of the new species, on the other hand, has the characteristic apparatus as already described, whilst it seems that *C. sabellariae* has no such apparatus as far as can be seen in Kozloff's figures.

If the patterns of ciliation are disregarded, since older descriptions on this character are occasionally questionable as already stressed by Kozloff (1965, pp. 37-38), *C. akkeshiensis* also bears some resemblance to five *Rhopalura*-species. The five are those species that are subgrouped as having one or two linear rows of oocytes by C. et M. (1901, p. 419) and Caullery (1961, pp. 703-704)¹. The differences among the species mentioned are presented in Table 1.

Meinkoth reported an orthonectid which is similar to the present new species (1965, p. 308). His material ($126 \times 18 \mu\text{m}$), however, is composed of 19 to 23 annuli, all of which contain refringent granules exclusive of six small alternating annuli and are entirely ciliated.

The present new species also differs from *Stoecharthrum giardi* Caullery et Mesnil 1899 in the number of the whole annuli as well as the granule-containing

1) *Rh. philinae* should be included in the *Rh. ophiocomae* group rather than in the *Rh. leptoplanae* group, because the oocytes of the species in question form a compact mass as in the original description (Lang, 1954, p. 606).

Table 1. Comparison among some Rhopaluridae-species.

	Male		Female				Host
	Length × width in μm	No. of annuli	Length × width in μm	No. of annuli	No. of oocytes	No. of annuli with large granules	
<i>Rh. leptoplanae</i> (Giard)	115×20	23 (28) ¹⁾	155×50	20 (26) ¹⁾	15~20	—	<i>Leptoplana tremellaris</i> (Turbellaria, Polycladida)
<i>Rh. linei</i> (Giard)	45×?	?	(120×20) ³⁾	20 (22) ²⁾	?	?	<i>Lineus gesserensis</i> (Nemertea)
<i>Rh. metchnikovi</i> Caullery & Mesnil	40×30	7 or 8	120~140 ×25~30 (130×25) ³⁾	20	12~14 (17)	—	<i>Spio martinensis</i> (Annelida, Polychaeta) & <i>Tetrastemma flavidum</i> (Nemertea)
<i>Rh. pelseneeri</i> Caullery & Mesnil		♀	120~150 ×30	17<	20~25	successive 2, alternating 5, & successive 4.	<i>Tetrastemma flavidum</i> (Nemertea)
<i>Rh. julini</i> Caullery & Mesnil	100×25	about 20	200×25	35~40	8~10	—	<i>Scolecopsis fuliginosa</i> (Annelida, Polychaeta)
<i>St. giardi</i> Caullery & Mesnil		♀	700~800 ×15	70~80	?	successive 4 only.	<i>Scoloplos armiger</i> (Annelida, Polychaeta)
<i>C. akkeshiensis</i> sp. n.	about 66×10 ⁴⁾	14<	128×16 ⁴⁾	24	19	successive 6 only.	Coelogygnoporidae sp. (Turbellaria, Proseriata)

This table is mainly based upon a monograph of Caullery et Mesnil (1901). 1) According to Nouvel (1939).
2) Nouvel (1935b). 3) Caullery (1961). 4) Measurement of a fixed material, see in text.

annuli (Table 1). In *C. akkeshiensis* the sexes are separate, while *St. giardi* is hermaphroditic.

On the other hand, Kozloff (1965) pointed out that the ciliation pattern is of a particular importance as the taxonomic character within the family Rhopaluridae. In connection with this, four different patterns of ciliation are discernible among the known forms of the family as follows.

Type-I: Both of the foremost and hindmost annuli are entirely ciliated. Many annuli of the body also bear cilia thickly. Between these annuli a cilia-free smaller annulus is inserted. This pattern is typical in *Rh. ophiocomae* Giard, 1877 (Kozloff, 1969). The following three species at least have probably a similar pattern: *Rh. intoshii* Metschnikoff, 1881, *Rh. granosa* Atkins, 1933 and *Rh. philinae* Lang, 1954. These species are already subgrouped as ones having a compact mass of oocytes by C. et M. (1901, p. 419) and Caullery (1961, p. 703).

Type-II: Cilia of the first annulus are restricted in its anterior a third, whilst posterior a third in the last annuli. In the rest of the body ciliation is limited exclusively in certain very small annuli. *Rh. pterocirri* St. Joseph, 1896 presents this pattern.

Type-III: The first annulus has cilia at its base. In the greater (or a half) part of the body, a smaller cilia-free annulus alternates with a larger one which bears cilia only at its anterior and posterior margins. In the rest including the last annulus cilia arise from each posterior margin of the annuli. *Ciliocincta sabellariae* (Kozloff, 1965; 1971) and *C. akkeshiensis* are of this type.

Type-IV: The fourth type appears in *Stocharthrum giardi*. According to Caullery et Mesnil (1901) most of the annuli of *St. giardi* consist of two series of cells and each annuli bears cilia exclusively on the posterior margin. So cilia-free somatodermal cells alternate with those cells having cilia on the posterior margin. The first annulus has cilia at its base. Ciliation is uncertain at the hindmost annulus.

If the taxonomic relationships among the genera within the family Rhopaluridae are considered based upon this typification, the two genera, *Stocharthrum* and *Ciliocincta* are clearly definable. Furthermore it suggests that the genus *Rhopalura* is heterogeneous. In connection with this, the subgroups by C. et M. (1901) based upon the ciliation patterns and condition of oocytes also suggest the heterogeneity of the genus *Rhopalura*. In these respects the systematic position of *Rh. pterocirri* within the family still remains an open question. The systematic position of each of the other five *Rhopalura*-species (presented in the Table 1) is also questionable. On the other hand, Kozloff (1969, 1971) intends to resurrect the genus *Intoshia* for *Rh. leptoplanae* (Giard) and *Rh. linei* (Giard), though the genus *Intoshia* originally established by Giard (1877 and also 1878, 1880) was recognized as an invalid taxon by Metschnikoff (1881) and C. et M. (1901). His taxonomic treatment for the genus must be also put under future discussion, inasmuch as he did show no clear reasons.

As already mentioned, infections by the Rhopaluridae-species are spread over the marine invertebrates in five phyla. The morphological relationship among Rhopaluridae-species does not seem to be particularly correlated with the phylogenetic relations of the host animals (cf. for annelid host in the Table 1). This may be connected with the facts that a parasite can affect two different hosts as in *Rh. metchnikovi* C. et M., 1899 (Caullery, 1961, p. 703) and *Rh. ophiocomae* (Fontaine, 1968) or vice versa as in *Tetrastemma flavidum* (Nemertea) infected by both *Rh. metchnikovi* and *Rh. pelseeneeri* C. et M., 1901 (Caullery, 1961, p. 703).

The orthonectids are regarded as being of radial symmetry by Metschnikoff (1881, p. 298). But they have been recently incorporated together with dicyemids into a group of bilateral symmetry by Beklemishev (1969, xxiv), though he explains nothing why he does so. In this connection, it is noticeable that the male of *C. akkeshiensis* clearly represents a bilateral symmetry. The problematic apparatus of the present new species may be comparable with two genital pores in the male of *Rh. linei* reported by Nouvel (1939, p. 269). The function of the problematic apparatus as well as the phylogenetic significance of the bilateral symmetry of *C. akkeshiensis* is a subject for a future study.

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