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AUTHOR(S):

Hoshino, Zen-ichiro; Nishikawa, Teruaki

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Taxonomic Studies of *Ciona intestinalis* (L.) and Its Allies

By

Zen-ichiro Hoshino

Department of Biology, Faculty of Education,
Iwate University, Morioka, 020, Japan

and

Teruaki Nishikawa

Biological Laboratory, College of General Education,
Nagoya University, Nagoya, 464, Japan

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Abstract The two species of the genus *Ciona*, *C. robusta* Hoshino et Tokioka and *C. intestinalis* (Linné) sensu Hoshino & Tokioka, 1967 from the Japanese waters are consistently distinguishable from each other by several minor but distinct features. On the other hand, the survey of literature and examination of many specimens collected from various localities in different years proved that European waters, the type locality of *C. intestinalis*, have been inhabited only by a single species indistinguishable morphologically from *C. robusta*. Thus, *robusta* should be regarded as a junior synonym of *intestinalis*. Further, it was also proved that *C. intestinalis* sensu Hoshino & Tokioka should be referred to *C. savignyi* Herdman originally collected off Kobe, Japan, during the Challenger Expedition, by reexamination of the type specimen of this species. In addition, brief references are made to other described species of the genus *Ciona*.

Introduction

Although a considerable number of species have been described in the genus *Ciona*, the specific criteria in separating respective species one another are seemingly of minor and rather unstable nature. Therefore, some researchers suggested even that the genus *Ciona* might be represented by only a single species *intestinalis* Linné and other described species might be its synonyms (for instance, see Berrill, 1950, p. 129). In such circumstances, it caused great excitement of the first author to find that there were two distinct forms of *Ciona* in the Japanese waters, that were separable consistently from each other by existence or absence of the endostylar appendage and by situation of the pharyngeo-epicardiac openings. Further, the test is always soft gelatinous in one form, while in the other it may be soft but very often considerably hard and cartilaginous as in some styelids. As these forms had been used widely and together as the material in various physiological and biological studies, it was urged

to describe these distinctively. Thus, in collaboration with Dr. Tokioka of the Seto Marine Biological Laboratory, the first author defined the form, with the test always soft, without the endostylar appendage, and with the pharyngeo-epicardiac openings close to the oesophageal opening, as *C. intestinalis* and the other, often with much harder test, with the endostylar appendage, and with the pharyngeo-epicardiac openings near the appendage, as a new species *C. robusta* (Hoshino & Tokioka, 1976). This was quite of a provisional nature, because the real figure of *C. intestinalis* described by Linné, checked at the same level of exactness as in separating these two Japanese forms, could not be recognized by the authors, for enough references and specimens of *C. intestinalis* from European waters were inaccessible to them at that time. The identification of the Japanese form with the test always soft with *intestinalis* was made only on the recognition of the authors, that the test of *intestinalis* is generally soft gelatinous in European waters. The definite conclusion of the taxonomic problem was, thus, left to the specialists in Europe at that time.

On the other hand, the distinction between these two forms has been proved in some other features else than morphology. For instance, it has been noticed embryologically (Pisanó & Rengel, 1972; but also see Rosati & De Santis, 1978), physiologically (Michibata & Yamazaki, 1981; Michibata, 1984) or biologically (Pisanó, Rengel & Bustuabad, 1971; Nakauchi & Kajiwarra, 1978). Thus, it has been urged again to make a final conclusion of the taxonomic problem of these two forms. Then, the first author started to do his efforts to draw the figure of *intestinalis* of Linné by checking the literature and by examining by his own eyes the specimens of *intestinalis* deposited at the institutes and museums in Europe and those actually collected by himself in various parts of European waters including type locality of this species (see Linné, 1767, p. 1087). Although the work was no easy, as not types of *intestinalis* remain at present and the specimens ever examined by Linné himself are entirely lost, the results revealed that European waters are inhabited by only a single form morphologically indistinguishable from *C. robusta*, namely *intestinalis* of Linné should be the same as *C. robusta*, and then *robusta* is to be a junior synonym of *intestinalis*.

Meanwhile, the last author was imposed to check the other species of the genus *Ciona*, else than *intestinalis* and began his researches mainly through the literature and reexamination of the specimens of described species of the genus *Ciona* as many as possible, that were still available at the institutes and museums in the world. And careful observation of the type specimen of *C. savignyi* Herdman, 1882 collected from off Kobe, Japan during the Challenger Expedition and deposited in the British Museum (Natural History) revealed that *C. intestinalis* sensu Hoshino & Tokioka, 1967 was quite the same as *savignyi*, thus the former should sink as a synonym of the latter.

The real taxonomic status of the two Japanese species of *Ciona* thus cleared has been published already but briefly at the 54th annual meeting of the Zoological Society of Japan (Hoshino & Nishikawa, 1983). However, the present studies seemingly show not only the taxonomic morphology of *C. intestinalis* more exactly than ever made, by comparing it with those of other species of the genus, but also the

actual existence of other valid species else than *intestinalis* in the genus *Ciona*. Thus, the present paper is prepared to give the full taxonomic data of *C. intestinalis* and *C. savignyi*, but the references are to be made as far as possible to other described species in the course of taxonomic discussion of the two species.

In describing the collections of ascidians of various museums and institutes, they are abbreviated as follows:

AMNH, American Museum of Natural History; **BMNH**, British Museum (Natural History); **HM**, Hancock Museum; **MNHN**, Muséum National d'Histoire Naturelle; **NMNH**, National Museum of Natural History, Smithsonian Institution; **OC**, Oka Collection deposited in the University of Tsukuba; **SMBL**, Seto Marine Biological Laboratory, Kyoto University; **SMNH**, Swedish Museum of Natural History; **TMBS**, Trondheim Marine Biological Station, University of Trondheim; **ZMB**, Zoological Museum, University of Bergen; **ZMK**, Zoologiske Museum, København.

Ciona intestinalis (Linné, 1767)

(Fig. 1, A–C; Figs. 2 and 4)

Limited synonymy: Numerous references have been made to "*Ciona intestinalis*", as well as to its various synonyms, but the majority lack information enough to identify precisely the therein described specimens with *intestinalis* defined here. Below listed are only the references having clear descriptions (and/or figures) as to the diagnostic characters such as the endostylar appendage (EA) and/or the pharyngo-epicardiac openings (PO), and those containing the descriptions of the specimens later reexamined (RE) and identified obviously by the present authors with this species.

Ciona intestinalis —Kuppfer, 1875, p. 207 (North Sea and Baltic Sea, 10–50 fathoms, 1871–72; EA, PO); Heller, 1875, pp. 117–120, pl. 3, figs. 8–9, pls. 4–5 (Adriatic Sea; EA); Traustedt, 1882, pp. 454–455, pl. 33, figs. 3–5, pl. 34, fig. 2, pl. 35, figs. 1–2 (Naples, 1882; EA, PO); Roule, 1884, pp. 7–257, pls. 1–12, pl. 13, fig. 81 (Provence, Mediterranean; EA); Damas, 1899, pp. 1–25, pls. 1–3 (Naples and Belgian coast, 1885–86; EA, PO); Hartmeyer, 1903, pp. 297–301 (at least in part; Norway; RE); 1915, p. 321 (British waters; RE); Alder & Hancock, 1907, pp. 9–14, pl. 21, figs. 1–5, text-fig. 28 (at least in part; British waters; RE); Michaelsen, 1923, p. 37 (Rio de Janeiro, Brazil; RE); Millar, 1953, pp. 1–122, pls. 1–19, text-figs. 1–9 (British waters; EA, PO); 1963, p. 720 (Australia; RE).

Ciona intestinalis f. *typica* —Hartmeyer, 1924, pp. 90–98 (at least in part; Greenland and the Faroes; RE); Årnäck-Christie-Linde, 1934, pp. 15–24 (at least in part; Norway, North Sea, Baltic Sea and Western coast of Sweden; RE).

Ciona intestinalis form "*tenella*" —Van Name, 1945, p. 163 (at least in part; East Port, Maine; RE).

Ciona intestinalis forma *costata* —Millar, 1970, pp. 114–115, fig. 13 (Argentina; RE).

Ascidia intestinalis —Linné, 1767, p. 1087 (see Discussion below); Cuvier, 1815, pp. 32–33, pl. 2, figs. 4–7 (probably northern coast of France; EA).

Phallusia intestinalis —Savigny, 1816, pp. 107–109, 169–170, pl. 11, figs. 1.1–1.f (probably Mediterranean coast of Egypt; EA).

Ciona canina —Kuppfer, 1875, pp. 206–207, pl. 5, figs. 8–9 (Inner half of Baltic Sea, 1871–72; EA, PO); Traustedt, 1880, pp. 432–434 (at least in part; Denmark; RE).

Ascidia canina —Kuppfer, 1870, pp. 115–172, pls. 8–10 (Kiel Bay; EA).

Ciona fascicularis —Hancock, 1870, pp. 364–365 (Ireland; RE); Kuppfer, 1875, pp. 207–208, pl. 5, fig. 10 (Baltic Sea, 0–1 fathom, 1871–72; EA, PO); Alder & Hancock, 1907, pp. 15–18, pl. 22, figs. 1–8, text-fig. 29 (Ireland; RE); Hartmeyer, 1915, p. 321 (do.).

Ciona pulchella —Alder & Hancock, 1907, pp. 14–15, pl. 21, figs. 6–8 (at least in part; Channel

- Islands; RE); Hartmeyer, 1915, p. 321 (do.).
- Ascidia pulchella* —Alder, 1863, p. 157 (at least in part; Channel Islands; RE).
- Ciona robusta* —Hoshino & Tokioka, 1967, pp. 275–290, figs. 1–5, fig. 6 (right), fig. 8 (right), pl. 7 (Onagawa, Miyagi); Pisanó, Rengel & Bustuabad, 1971, pp. 1–10, figs. 2–5, 7, 10 (Mar del Plata, Argentina; EA, PO); 1972, pp. 179–183, figs. 2, 4 (do.); Nishikawa, 1980, tab. 1 (Tanabe Bay).
- Ciona gelatinosa* (?) —Monniot, 1969, pp. 1133, 1135, fig. 1, a–c (Bay of Biscay; RE).
- ?*Ciona intestinalis* var. *sydneyensis* —Kesteven, 1909, pp. 282–285, pl. 27, figs. 13–19 (Sydney; see Remarks below).
- ?*Ciona intestinalis* —Brewin, 1950, pp. 347–349, fig. 2 (New Zealand; see Remarks below).

Material examined:

- Europe (arctic waters). Greenland, date unknown, Möller leg.; **ZMK**; 20 spec.; described by Hartmeyer (1924), as well as probably by Traustedt (1880). Denmark Strait, Ingolf St. 9, 64°18'N and 27°W, 295 fathoms, WT 5.8°C, May 20, 1895; **ZMK**; 1 spec.; described by Hartmeyer (1924).
- Europe (boreal waters). Norway: **SMNH**; described by Ärnback-Christie-Linde (1934); Grötsund, Finmarken, 126 m, Oct., 1861, No. 396, 4 spec.; Karlsö, Finmarken, 54–72 m, June 13, 1864, No. 1894, 1 spec.; Kristiansand, 90 m, May 9, 1871, No. 829, 1 spec.; Ekersund, 90–108 m, Oct. 22, 1872, No. 334, 1 spec.; Off Jaedersen, 180–270 m, 1874, No. 359, 1 spec.; NW of Bergen, 160–300 m, 1878, No. 391, 1 spec. Norway: Moskenstrømmen, 90 m, **ZMB** No. 16011, 1 spec., described by Hartmeyer (1903); Tysfjord, 500 m, Mar. 28, 1899, **ZMB** No. 8578, 1 spec., do.; Florö, **BMNH** No. 1898–5–7–300, as *Ciona* sp., 1 spec.; Borgenfjorden C4, July 5, 1968, 11 m, **TMBS**, 2 spec.; Trondheimsfjorden Fänes (St. 72087, Flagruunem), June 29, 1972, 15–25 m, **TMBS**, 2 spec.; Locality unknown, gr. 225, 197?, **TMBS**, 2 spec.; Bergen, on ship, **MNH** No. P1–CIO–11, 5 spec.; Trondheim, shallow waters and aquarium (WT 8–10°C), Sept. 12, 1974, Hoshino coll., 6 spec. Faroes Islands: **ZMK**; described by Hartmeyer (1924); Thorshavn, Sept. 20, 1887, 1 spec.; Southern end of Nolso, 80 fathoms, June 20, 1899, 1 spec.; Nolso og Ostnos, 100 fathoms, June 9, 1899, 1 spec.; Trangivaag, Sept. 21, 1887, 1 spec. Off Shetland Islands, "Jutland Reef", 180–216 m, 1873, **SMNH** No. 304, 6 spec., described by Ärnback-Christie-Linde (1934).
- Europe (cold temperate waters). Western coast of Sweden: **SMNH**; described by Ärnback-Christie-Linde (1934); Koster Fjord, Bohuslän, 10–30 fathoms, 1865, No. 1562, 1 spec.; Ibid., 5–15 fathoms, 1871, No. 1397, 4 spec.; Syd-Koster, 10 fathoms, July 10, 1871, No. 800, 1 spec.; The Sound, Landskrona-Malmö, 10–16 m, July 22, 1878, No. 251, 3 spec.; Hallands Väderö, 18 m, July 15, 1878, No. 258, 4 spec. Denmark: **ZMK**; Vellerup, Isefjord, 1861, 2 spec., described probably by Traustedt (1880); Cilleleje, 2 spec., do.; Hellebaek, 2 spec., do.; Kalo Vig, 1887, 2 spec.; Hevringholm Flak, June 19, 1888, 2 spec.; Lillebaelt, Aug. 6, 1900, 2 spec.; Dana St. 2852, 56°49'N and 6°32'E, 48 m, Oct. 1, 1922, 2 spec. Denmark: Helsingør (Marine Biol. Lab., Univ. of Copenhagen), from the underground tanks, aquarium and West off the laboratory at a depth of 80 m, Sept. 9, 1974; Hoshino coll.; 11 spec. Kattegat or Odensefjord, Denmark; **HM** No. 2–2–7 and 2–2–8; as *Phallusia canina*; 5 spec. and 1 spec. respectively. Western part of Baltic Sea: Kiel or Rügen, date unknown, **SMNH** No. 1017, 1 spec., described by Ärnback-Christie-Linde (1934). British Islands: **BMNH**; Polperro, Cornwall, No. 1863–4–27–10, 2 spec.; Plymouth, No. 1896–12–3–14–5, 1 spec.; Loch Tyne, Scotland, No. 1898–5–7–296, as *Ciona* sp., 1 spec.; Clew Bay, Ireland, 1874, No. 1898–5–7–298, 2 spec.; Bertraghboy Bay, Ireland, No. 1898–5–7–299, 1 spec.; Ross-shire, Loch Alsh, No. 1905–1–20–1–2, 1 spec.; Clew Bay, Ireland, 1874, No. 1911–12–9–5 (part), 2 spec.; Plymouth Mar. Biol. Stat., No. 1913–4–23–2 and –3, 2 spec.; Holyhead, N. W. Wales, No. 1916–10–28–8, 1 spec.; Plymouth, No. 1926–9–9–6, 1 spec.; English Channel, St. 43, 118 m, No. 1949–12–29–127, 1 spec.; English Channel, St. 58, 30 m, No. 1949–12–29–129, 1 spec.; English Channel, St. 73, No. 1949–12–29–131, 1 spec.; Among weed on sand, 15 fathoms, SYRA (Cambridge Univ. Exp.), No. 1960–11–24–2, 1 spec.; SE of Millport, Isle of Cumbrae, 30 m, No. 1964–7–7–5, 1 spec.; Longston Hbr, Portsmouth, No. 1965–7–30–191, 1 spec.; Longston Hbr, Portsmouth, No. 1965–7–30–202–

- 205, 1 spec.; Loch Torridon Area, Mouth of Balgy River, Low Water Springs, July 30, 1973, No. 1973-10-3-2, 1 spec. British Islands: **HM**; Killkieran Bay, Connemara, Ireland, 1869, No. 1-6-2, as *C. fascicularis*, 13 spec. out of about 20 were identified with *C. intestinalis* without doubt, described by Hancock (1870), Alder & Hancock (1907) and Hartmeyer (1915); Hastings, England, No. 2-3-12, as *A. intestinalis*, 4 spec., reexamined by Hartmeyer (1915); Westport Bay, Ireland, 1871, No. 2-3-13, as *C. intestinalis*, 4 spec., do.; no data, No. 3-3-2, as *A. intestinalis*, 14 spec.; do., No. 3-3-3, as *A. intestinalis*, 10 spec.; at least a part of these specimens may probably have been described by Alder & Hancock (1907). Plymouth, from the underground tank, Sept. 5, 1974, Hoshino coll., 7 spec.
- Europe (warm temperate waters). Channel Islands, probably Guernsey, **HM** No. 4-3-7, as *A. pulchella*, 1 spec., described by Alder (1863), Alder & Hancock (1907) and Hartmeyer (1915). Guernsey, **BMNH** No. 1898-5-7-295, 1 spec. Lezardrieux, North France, **MNHN** No. P1-CIO-32, 5 spec. St. Vorst de la Hoyjue (St. 4c), North France, **BMNH** No. 1965-7-30-47, 1 spec. Thalassa St. U848, 44°11,2'N and 8°36.8'W, 640-630 m, **MNHN** No. P1-CIO-3, as *C. gelatinosa* (?), described by Monniot (1969), 1 spec. Thalassa St. W392, Sud Golfe de Gascogne, **MNHN** No. P1-CIO-4, as *C. gelatinosa*, 1 spec. Thalassa St. Z435, **MNHN** Nos. P1-61, 62, 63, as *C. gelatinosa*, 1 spec. on 3 slides. Mediterranean, **BMNH** No. 1890-5-19-2, 1 spec. Adriatic Sea, **BMNH** No. 1898-5-7-292, 1 spec. Naples: **BMNH** No. 1926-9-18-1, 1 spec.; **SMBL** Rare No. 146, 1 spec.; aquarium or bottom of boats in the Staz. Zool. Napoli, April to July, 1974, Hoshino coll., 13 spec.; the same station, date unknown, presented by F. Bentivegna, 12 spec. Off N. E. Chios, Greece, 20 m, **BMNH** No. 1966-7-20-29, 1 spec. Villefranche, South of France, **BMNH** No. 1960-10-10-5, 1 spec.
- Atlantic coast of North America. In the vicinity of Eastport, Maine, no data; **AMNH** No. 682, as *C. sociabilis tenella*; 13 spec., at least 4 of which are clearly identifiable with *C. intestinalis*, probably examined and referred to by Van Name (1945). Eastport, Maine, 1872; **AMNH** No. 740, as *C. sociabilis tenella*, one specimen probably referable to *C. intestinalis*, do.
- Atlantic coast of South America. Argentina: R. V. 'Vema' St. V-17-72, 39°21'S, 61°08'W, May 19, 1961, 15 m; **AMNH** No. 2271, as *C. intestinalis* forma *costata*, described by Millar (1970); 5 spec. Rio de Janeiro, Brazil, **SMNH** No. 217, Oct. 18, 1893, described by Michaelsen (1923), 13 spec.
- Pacific coast of North America. Almanitos Bay, Long Beach, California, from the boat floats, June 1979; Ju-Shey Ho coll.; 10 spec.
- Japan. Mone, Karakuwa, Miyagi Prefecture, Sept. 3, 1970; together with 1 spec. of *C. savignyi*; Hoshino coll.; 2 spec. Onagawa, Miyagi Prefecture, Aug. 5, 1976; together with 5 spec. of *C. savignyi*; Hoshino coll.; 5 spec. Yokohama Hbr, Dec. 26, 1902; **BMNH** No. 1907-9-1-15, as *C. japonica*; Owston coll.; 4 spec. In front of Sugashima Mar. Biol. Stat., Sugashima Is., attached to the cages, several meters deep, Feb. 18, 1981; together with 40 spec. of *C. savignyi*; Nishikawa coll.; 27 spec. Zaga, Ago Bay, from the oyster cages, Jan. 8, 1982; together with 7 spec. of *C. savignyi*; Oishi coll.; 2 spec. Osaka Bay, attached to vertical plates, ca. 5 m deep, June 10, 1980; Fuse coll., 12 spec. Tan'nowa Hbr, Osaka Bay, June 14, 1977; Yamanishi coll.; 4 spec. Tanagawa, Osaka Bay, from floating buoy, Aug. 11, 1980; Otani coll.; 2 spec. Kwarazu, Toyo City, Seto Inland Sea, June, 1978; Ishikawa coll.; 3 spec. Noto Mar. Biol. Stat., Noto-Ogi, Noto Peninsula, from the tanks, Apr. 5, 1975; Nishikawa coll.; 2 spec.
- Australia. Port Jackson, NSW, from bottom of HMS Alert; **BMNH** No. 1881-10-6-34-37; 1 spec. Swan River, W. Australia; **BMNH** No. 1962-1-12-26; 4 spec. Peppermint Grove, Swan River; **BMNH** No. 1962-1-12-27; 1 spec. All were described by Millar (1963).
- New Zealand. Lyttleton Hbr, South Island, wharf pile, ca. 20 feet, June, 1965; deposited in the Zool. Dept., Univ. of Canterbury and presented by A.C. Harris; 28 spec.

Description: All the specimens examined in the present study are quite identical with the previous description of *C. intestinalis* given by Millar (1953) or of *C. robusta* by Hoshino & Tokioka (1967). As mentioned previously, the individual variations are discernible in the test consistency (soft gelatinous to more or less leathery, though

Fig. 1. Complicated ciliated groove in *Ciona intestinalis* (Linné) (A-C) and *C. savignyi* Herdman (D-G). A: 92 mm long specimen from California. B: About 165 mm long specimen from Naples. C: About 190 mm long specimen from Naples. D: 25 mm long specimen from Zaga, Japan. E: 49 mm long specimen from Sugashima, Japan. F: 64 mm long specimen from Onagawa, Japan. G: 67 mm long specimen from Sugashima, Japan. Enlarged to different scales.

not so hard and cartilaginous as once demonstrated by Hoshino & Tokioka in some Japanese specimens of *C. robusta*; however, the test is so hard that the animal can be held upward in a single specimen from New Zealand). The mantle musculature usually consists, on each side, of 6 longitudinal bundles, 4 converging to the branchial siphon and 2 to the atrial, but the number and arrangement of the bundles may be sometimes variable in some degree; in addition to these there are nearly always a pair of longitudinal muscle fascicles along the midventral line. The size of pharyngeo-epicardiac openings varies from minute to rather extensive, though this is partly due to artifact at fixation. The following additional notes may be noteworthy.

Rigidity of the test may be seemingly due mainly to its hardened thin surface layer, while the inner part is retaining more or less gelatinous consistency as already noted by Millar (1953, p. 9). Tentacles are usually well developed, ranging in number from ca. two dozens (in 8.5 mm long specimen from Plymouth) to 70 or more in larger specimens. Ciliated groove represented as a longitudinal slit in smaller specimens, while C-shaped sometimes with both horns rolled in, opened to the right side in the majority or anteriorly; it may be rather complicated in some larger specimens (Fig. 1, A-C). The endostylar appendage is usually very distinct, though rarely not so. Number of inner longitudinal vessels may seemingly change with growth as seen in Fig. 2. This figure may show that the specimens from Naples and the Japanese waters are somewhat different from those from northern European waters in the change rate of the number of inner longitudinal vessels with growth. Number of transverse vessels may increase with the body size, though rather difficult to be counted exactly; an example is given below in some specimens from Naples:

16.5 mm long individual	32 vessels
30 mm	46
36 mm	60
43 mm	70
81 mm	ca. 110
92 mm	ca. 130

The genital apertures are usually situated more or less far anterior to the anus which is located nearly in the middle of the branchial sac, in most cases opening at the level near the base of the atrial siphon. However, rarely they may be situated just close

to the anus as seen in a specimen of **MNHN** No. P1-CIO-3 (see Monniot, 1969, p. 1135), or slightly anterior to it as in the specimens of **MNHN** No. P1-CIO-4 and **ZMB** No. 8578, and a specimen from Ingolf St. 9. This feature may be reminiscent of *C. mollis* ever recorded from off California, 1100 fathoms deep (Ritter, 1907, p. 37) and *C. antarctica* from the Antarctic, 95 to 100 m deep (Monniot & Monniot, 1983, p. 49). It is noteworthy that all of these specimens with the genital apertures close to the anus, but *C. antarctica*, came from the deeper waters. The distal end of vas deferens is, so far as the authors are aware, always pigmented orange in living animals; this colouration still remains in many formalin-preserved specimens examined in the present study. Male genital apertures range in number from 5(?) to 30 in the European specimens, while 14 to 26 in the Japanese ones, and are opening one on each ellipsoidal papilla at the distal end of vas deferens; seemingly no correlation between the number and body size.

Remarks: If the orange pigmentation at the distal end of vas deferens can be taken as a significant diagnostic character for *C. intestinalis* (but also see Remarks of *C. savignyi*), *C. intestinalis* var. *sydneyensis* described by Kesteven (1909) and *C. intestinalis* described by Brewin (1950) may safely be assignable to *C. intestinalis* defined here. According to their descriptions, "at its (=vas deferens) termination, just below anus, there is a brilliant vermillion swelling" (p. 285) in var. *sydneyensis*, and "oviduct-opening at base of atrial siphon, red and deeply lobed" (p. 348) in Brewin's *intestinalis*. The position of the "swelling" in the former seems, however, quite unusual for this species. It was tried to examine Kesteven's specimens that are probably deposited in the Australian Museum, but so far in vain. Further, according to the personal communication of Drs. A. Harris and R. A. Savill, Brewin's specimens might seemingly be lost. However, all the specimens from Lyttelton, New Zealand, where Brewin obtained her specimens, that were examined in the present study, are clearly identified with *intestinalis* here defined. Consequently, var. *sydneyensis* and Brewin's *intestinalis* may be included in *C. intestinalis* defined here, though some doubts still remain.

Ciona intestinalis longissima Hartmeyer and *C. intestinalis gelatinosa* Bonnevie have been recorded from the Arctic and the boreal waters of the North Atlantic. These forms are both provided with the postabdominal extension of mantle body (see Ärnback-Christie-Linde, 1934, pp. 18-24; Van Name, 1945, pp. 162-163). Although Hartmeyer (1899, text-fig. L) showed the endostylar appendage in his original description of *C. longissima*, nothing was mentioned as to the feature of the pharyngeo-epicardiac openings. Fortunately the last author was given a chance to examine 22 specimens of *C. intestinalis longissima* collected from Baffin Bay, 450-1200 m deep, that were described by Lützen (1959) and now are deposited at the Zoologiske Museum, København. Their taxonomic morphology is as follows;

Test is thick, but soft, gelatinous, transparent and colourless except in the posterior part, where it is somewhat tough, nearly opaque and white or slightly yellowish white. The endostylar appendage is rather indistinct, while the pharyngeo-epicardiac openings are discerned near the base of the appendage, but usually very small.

Fig. 2. Correlation between the total number of inner longitudinal vessels and the body length in the well-preserved specimens of *Ciona intestinalis* (Linné) from Greenland (solid triangle), Norway (inverted solid triangle), the Faroes and off the Shetlands (open triangle), Denmark and Western coast of Sweden (inverted open triangle), Plymouth (solid circle), Naples (open circle), Brazil (solid square) and Japan (open square), as well as those of *Ciona intestinalis longissima* from Baffin Bay (semi-open circle).

Genital apertures are situated anterior to, though not so far apart from, the anus; this feature may be reminiscent of the deep-water specimens of *C. intestinalis* mentioned previously (p. 67). Number of inner longitudinal vessels in well-preserved specimens is given in Fig. 2. This figure seems to suggest a closer relation between the specimens of *longissima* from Baffin Bay and the specimens of typical *intestinalis* from the boreal or the cold temperate waters as to the number of inner longitudinal vessels. Tentacles are much fewer than in typical *intestinalis*; up to 30–40 even in larger specimens. Number of male genital apertures ranges from 8 to 21.

In addition, the last author also examined two specimens of "*Ciona gelatinosa*" deposited at the Zoological Museum, Oslo and registered as No. H212. These specimens, ca. 55 mm and 67 mm long respectively, were collected at St. 270 of "Nordhav Expedition" (72°27'N and 35°01'W; June 30, 1878) and identified by Bonnevie, the author of this taxon. Neither of them can be the holotype, as the type was collected "on June 30th, 1878 in 72.38°N. Lat. and 33.50°E. Long.--" (Bonnevie, 1896, p. 3). These specimens are of the following morphology:

Test thin but tough, translucent and white in alcohol. Mantle body much deteriorated, though postabdominal extension is clearly discerned. Endostylar appendage is very indistinct, while a pair of pharyngeo-epicardiac openings are

barely discernible near the endostyle. Genital apertures are situated slightly (ca. 3.5 mm) anterior to the anus, as the original description shows as "the oviduct--ending in a club-shaped body which extends a few millim. above the anus" (loc. cit.). Male genital apertures are about 20. Judging mainly from the position of pharyngeo-epicardiac openings, "*longissima*" and "*gelatinosa*" are considered to be closely related to *C. intestinalis*, but never to *C. savignyi* described below. Thus, *longissima* and *gelatinosa* are provisionally assigned in the present study to a subspecific rank in *C. intestinalis*.

Michibata, Vincentiis & Gualtieri (1983) reported that in the content of vanadium *C. intestinalis* from Naples is quite similar to *C. savignyi* from Japan, but different from *C. intestinalis* from Japan, while in that of iron *C. intestinalis* from Naples resembles *C. intestinalis* from Japan. The taxonomic significance of the difference between the specimens of *intestinalis* from Japan and Naples is still an open question. *Distribution*: Europe, Atlantic coast of North and South America, Australia, New Zealand, California and Japan.

Ciona savignyi Herdman, 1882

(Fig. 1, D-G; Fig. 3)

Ciona savignyi —Herdman, 1882, pp. 236-237, pl. 35, figs. 1-2 (Kobe, Japan; RE); Traustedt, 1885, pp. 10-11 (without new records).

Ciona aspera —Herdman, 1886, pp. 416-418, pl. 49, figs. 11-13 (Kobe, Japan; RE).

Ciona intestinalis —Ritter, 1913, p. 500 (Alaska: RE); Oka, 1935, pp. 464-466, fig. 35 (at least in part; Mutsu Bay, Japan; RE); Hoshino & Tokioka, 1967, pp. 286-289, fig. 6 (left), fig. 8 (left) (Miyagi Pref., Japan); Pisanó, Rengel & Bustuobad, 1971, figs. 1, 3, 6, 8-9 (Mar del Plata, Argentina); 1972, figs. 1 and 3 (Ibid.); Nishikawa, 1980, tab. 1 (Tanabe Bay and Sugashima Is., Japan).

?*Ciona intestinalis* —Tokioka, 1953, pp. 3-6, figs. 2-3 (in part; Seto Inland Sea, Japan; RE).

non *Ciona savignyi* —Roule, 1884, pp. 195-197, pl. 9, fig. 82 (Marseille).

Material examined:

Japan. About 1500 m off Shirogasawa, Shimokita Peninsula, Mutsu Bay, Aug. 11, 1926; **OC** No. 254 (M407); described by Oka (1935); 1 spec. Off Tsubakiyama, Mutsu Bay, July 17, 1927; **OC** No. N3 (M467); do.; 1 spec. Asamushi, Mutsu Bay, Aug. 3, 1975; Hoshino coll.; 3 spec. Mone, Karakuwa, Miyagi Pref., Sept. 3, 1970; together with 2 spec. of *C. intestinalis*; Hoshino coll.; 1 spec. Onagawa, Miyagi Pref., July 3, 1967 and Aug. 5, 1976; 10 spec. Tateyama, May 4, 1924; **OC** No. 325 (M365), as *C. intestinalis*; 1 spec. Enoura, Suruga Bay, Apr. 2-8, 1904; **OC** No. 387 (M113), as *C. intestinalis*; 6 spec. In front of Sugashima Mar. Biol. Stat., Sugashima Is., from cages, several meters deep, Feb. 18, 1981; together with 27 spec. of *C. intestinalis*; Nishikawa coll.; 40 spec. Zaga, Ago Bay, from oyster cages, Jan. 8, 1982; together with 2 spec. of *C. intestinalis*; Ooishi coll.; 7 spec. Off Kobe, 34°35'N and 135°10'E, 8 and 50 fathoms, May 17-19, 1875; type specimen of *C. savignyi*; **BMNH** No. 1887-2-4-183. The same as the type locality of *C. savignyi*, 50 fathoms, May 19, 1875; type specimen of *C. aspera*; **BMNH** No. 1887-2-4-184. Inland Sea (=Seto Inland Sea), 8-15 fathoms, R. Gordon-Smith coll.; **BMNH** No. 1902-12-24-1-10, as *Ciona* sp.; 4 spec. Mukaishima Is., Seto Inland Sea, intertidal zone, Apr. 14, 1934; **SMBL** Rare No. 5, labelled as *Ciona pulchella*; 1 spec.; described by Tokioka (1953, pp. 4-6, fig. 3); posterior part of endostyle missing, but pharyngeo-epicardiac openings(?) barely discernible. Tsukumo Bay, Japan Sea, several meters, Aug. 20-21, 1975; Nishikawa coll.; 6 spec. Tsukumo Bay, 25 m, Mar. 17, 1976; Shin'ya et al. coll.; 18 spec.

Off Tateishi, Wakasa Bay, 50 m, June 16, 1981; Yasuda coll.; 1 spec.
 Pacific coast of North America. Loring, Alaska, April 29, 1903; **NMNH** No. 5633; described by
 Ritter (1913); 1 spec. Stuart Is., British Columbia, on float, April 9, 1937; **AMNH** No. 1427,
 as *C. intestinalis*; 1 spec.

Description: The morphology of this species is given below in comparison with that of *C. intestinalis*:

- 1) Test soft, gelatinous, transparent to translucent and white. Hardening of the superficial layer of test as seen in *C. intestinalis* is usually indistinct. In some of old preserved specimens in alcohol such as the type specimens of *C. savignyi* and *C. aspera*, however, test is thin, transparent and white, but has become tough.
- 2) Tentacles are rather variable in number, but seemingly a little fewer than in *C. intestinalis*; never over about 50. In 19 specimens of *C. savignyi*, 35 to 75 mm long, and 15 specimens of *C. intestinalis*, 51 to 84 mm long, collected together on Feb. 18, 1981 in the vicinity of Sugashima, Japan, tentacles range from 11 (in 56 mm long specimen) to 38 (in 60 mm long one) in the former, while from 55 (in 62 mm long one) to 80 or more (in 94 mm long one) in the latter.
- 3) Ciliated groove simply C-shaped, or complicated (Fig. 1, D-G).
- 4) In the number of inner longitudinal vessels (Fig. 3), *C. savignyi* is similar to *C. intestinalis* from the Japanese waters or some parts of European waters (cf. Fig. 2).

Transverse vessels in some specimens from Onagawa, Japan are:

33 mm long specimen	ca. 90 vessels
44 mm	ca. 100
64 mm	ca. 160

- 5) Endostylar appendage completely absent.

Fig. 3. Correlation between the total number of inner longitudinal vessels and the body length in the well-preserved specimens of *Ciona savignyi* Herdman from Japan (solid circle) and British Columbia (open circle).

- 6) A pair of pharyngeo-epicardiac openings are situated close to the oesophageal opening.
- 7) Genital apertures are usually situated far anterior to the anus; in a 60 mm long specimen from British Columbia (**AMNH** No. 1427), however, they are located only slightly anterior to it.
- 8) Male genital apertures opening at the distal end of vas deferens, though never accompanied with any ellipsoidal papillae such as those seen in *C. intestinalis* (see p. 67). They range from 5(?) (in the type specimen of *C. savignyi*) to 20 (in a 52 mm long specimen from Wakasa Bay) in number, but seemingly less than in *C. intestinalis*. The distal end of vas deferens is, so far as the authors' limited observations of living specimens are concerned, never pigmented orange.

Remarks: According to the original description of *C. aspera*, "a red spot--placed on the extremity of the vas deferens, which is sometimes so conspicuous in *Ciona intestinalis*" (p. 418). This type specimen (**BMNH** No. 1887-2-4-184) is, in other features, quite identical with *C. savignyi* and, therefore, included in the synonymy of this species in the present study. The taxonomic significance of the pigmentation in the original description may be left in question.

Roule (1884) recorded *C. savignyi* from Marseille. In spite of his carefulness to figure the endostylar appendage of *C. intestinalis* (pl. 3, fig. 29), he made no special references to this structure in his brief description of *C. savignyi*. This may suggest that Roule's *C. savignyi* was also provided with the appendage. If this is true, Roule's *savignyi* can never be assigned to *C. savignyi* defined here. The authors tried to examine Roule's specimens to confirm this supposition, but in vain. In such circumstances, it seems better to exclude Roule's *savignyi* from the synonymy of *C. savignyi*. Thus at present, European waters are seemingly inhabited only by *C. intestinalis*.

Distribution: Japan, Pacific coasts of Canada and Alaska, and Argentina.

Brief History of *Ciona intestinalis*

Linné (1767, p. 1087) firstly defined *Ascidia intestinalis* only as "A. (=Ascidia) laevis alba membranacea" and denoted as "Habitat in Oceano Europaeo", with bibliographical references to Bohadsch (1761), Baster (1760) and "Act. nidros." (=Gunnerus' paper published in 1765). Among these, Baster's species was later regarded as a doubtful junior synonym of *Molgula manhattensis* by Hartmeyer (1923, p. 66; but also see Hartmeyer, 1924, p. 93). The description of Bohadsch's "*Tethyum fasciculatum*" (p. 132, pl. 10, fig. 4) is never enough to determine its taxonomic identity. Gunnerus "gave a clear description of an ascidian species from Trondheimsfjorden which he named *Tethyum sociabile*" (Snæli & Gulliksen, 1975, p. 127), but it cannot be supposed that the description may have information regarding the above-mentioned diagnostic characters requested now in the classification of *Ciona*; recently Snæli & Gulliksen (loc. cit.) proposed the suppression of the name *sociabile*, validating the name *intestinalis*. The authors of the present paper have had no

Fig. 4. Dissected mantle body of *Ciona intestinalis* from Europe, from Cuvier (1815, pl. 2, fig. 7), reproduced (left) or partly enlarged (right). Note the endostylar appendage (EA). See the text below.

opportunities to examine the type specimens of Bohadsch's, Baster's or Gunnerus' species up to now. And unfortunately enough, the type or other specimens of *intestinalis* examined by Linné himself are no longer found in the Swedish Museum of Natural History, Zoological Institute of Uppsala University or the Linnean Museum (Å. Franzén, person. comm.), nor in the Linnean Society of London so far as the first author inspected there. Thus it seems that the question cannot be answered primarily what may really represent *C. intestinalis* established by Linné. According to Alder & Hancock (1907, pp. 6-7), Dominicus Vandelli well described and figured *C. intestinalis* in the year 1761 as "*Holothurium laeve, dichotomum, fucis marinis alligatum*". His figures, reproduced by them (fig. 27 on p. 8), show that his "*Holothurium*" is provided with 6 muscle bundles on each side of mantle body and very easily referable to the genus *Ciona*; however, they lack information completely as to the endostylar appendage and pharyngeo-epicardiac openings that are taken up in the present paper as the most important taxonomic clues.

Then in the literature, Cuvier (1815) is the first to show the occurrence of the form of *Ciona* provided with the appendage (pl. 2, fig. 7; reproduced in Fig. 4 in the present paper), from European waters, and followed by many authors listed above. On the other hand, the other form, with the pharyngeo-epicardiac openings situated close to the oesophageal opening and without the endostylar appendage, has not yet been recorded there. However, this doesn't necessarily mean that the latter form has never inhabited European waters, for its diagnostic features may have been overlooked very easily. The possibility cannot be denied that the specimens referable to the latter form were confused with those of the former or described

under the other names else than *intestinalis* without sufficient information about these diagnostic characters. The authors have made their efforts to find out such specimens in any of European museums or other institutes, and were fortunately given chances to examine some of such specimens as shown above, though failed to meet a number of important specimens that were ever described by various authors under the specific name of *intestinalis*, as well as the type or any identified specimens of *Ascidia corrugata* O.F. Müller, 1776, *A. viridescens* Bruguière, 1789, *A. membranosa* Renier, 1807, *A. virescens* Pennant, 1812, *Pleurociona edwardsi* Roule, 1884, *Ciona roulei* Lahille, 1887 and *C. intestinalis* var. *lutea* Harant, 1928, all of which were recorded from Europe. All the specimens examined by the present authors are proved assignable to one of the two forms, which is provided with the endostylar appendage and the pharyngeo-epicardiac openings close to it and is defined as *intestinalis* in this paper; among them, the oldest ones were collected in 1861.

On the basis of the results given by survey of the literature and actual examination of many specimens, it may well be supposed that European waters have been inhabited only by one of the two forms mentioned above. Thus, *intestinalis* of Linné should be automatically represented by that form with endostylar appendage and the pharyngeo-epicardiac openings near its base. This form was already described intimately and thoroughly by Millar (1953).

Other Described Species of *Ciona*

(1) The other form (= *C. intestinalis* sensu Hoshino & Tokioka, 1967) was proved to be referable to *C. savignyi* Herdman, 1882 through the reexamination of the type specimen of this species. However, there remains a possibility that the specific name *savignyi* might be replaced in future according to the Law of Priority by the following three species of *Ciona* that were originated before 1882, but without any references to the above-mentioned diagnostic features: *Ascidia ocellata* Agassiz, 1850 and *A. tenella* Stimpson, 1852 from the North Atlantic coasts of North America, and *A. tubifera* Stimpson, 1855 from China. The first two were later synonymized with *C. intestinalis* by Hartmeyer (1903, p. 301), while the last was regarded as an uncertain species of the genus *Ciona* by Hartmeyer (1909-11, p. 1414). All these three species still remain uncertain as to their exact affiliation, because of the insufficient descriptions and (temporal?) missing of the type specimens.

(2) *C. edwardsi* —Very recently *Ciona edwardsi* (Roule, 1884) was redefined by Copello, Devos & Lafargue (1981), basing on many specimens collected at a depth of up to 40 m at Banyuls-sur-Mer in the Mediterranean. By the courtesy of Dr. Lafargue, the last author of the present study examined four intact specimens of this species collected by Lafargue herself on August 31, 1983 at a depth of 30 m in the mentioned region. They were proved to share the main features with the above-mentioned species with the endostylar appendage. Brief description of these specimens will be made below.

In the four specimens mentioned above, body 78 to 124 mm long, attached to

the substratum by left posterior half. Test soft, gelatinous and transparent in the anterior one-third of body, while rather tough, though not so hard, somewhat leathery and translucent in the rest. Mantle musculature consists basically of 4 longitudinal bundles converging to the branchial siphon and 2 bundles to the atrial on each side, and a pair of longitudinal muscle fascicles along the midventral line. Tentacles 21–35; ciliated groove as a simple C-shaped in the larger two specimens, while split into 2 (in 97 mm long specimen) or 5 (in 78 mm long one) simple pieces; endostylar appendage distinct; a pair of pharyngeo-epicardiac openings close to the endostyle; inner longitudinal vessels 45–47 on each side; genital apertures situated more or less near the base of atrial siphon, and far apart from the anus located nearly in the middle of the branchial sac; male genital apertures, very lightly pigmented dark brown or yellow, opening one on each ellipsoidal papilla at the distal end of vas deferens, their number ranging from 15 (in 97 mm long specimen) to 26 (in the smallest one); gonad matured, eggs in oviduct about 200 μ m in diameter. These specimens examined are indistinguishable from *C. intestinalis*, only excepting that they are provided with markedly more inner longitudinal vessels than in *C. intestinalis* from any localities (ref. Fig. 2). The taxonomic significance of this difference, and then the specific distinctness of *C. edwardsi* may be better left pending here for the future studies.

(3) In addition to those described species mentioned above, several others have also been so far published under the generic name *Ciona*, such as *flemingi* Herdman, 1882, *abdominalis* Sluiter, 1898, *indica* Sluiter, 1904, *mollis* Ritter, 1907, *antarctica* Hartmeyer, 1911 and *imperfecta* Monniot et Monniot, 1977. Among these, *C. abdominalis* is clearly assignable to the genus *Rhopalaea* mainly on the basis of the feature of the branchial papillae (see Sluiter, 1898, pp. 8–10, pl. 1, figs. 3–8) as already so treated by Van Name (1921, pp. 370–372).

(4) *C. flemingi* and *C. indica* —Further, the last author reexamined the type specimens of *C. flemingi* (BMNH No. 1887–2–4–182) and *C. indica* (Zoological Museum in Amsterdam, TU 332; see Spoel, 1969, p. 163). The examination revealed that these two species may safely be referred also to the genus *Rhopalaea*. In these the thorax is clearly constricted off from the abdomen below it (though in the type specimens of *flemingi* the abdominal part is missing, the constriction is well illustrated in the figure 7 of plate 34 in the original description), the branchial papillae are almost (in *flemingi*) or utterly (in *indica*) indiscernible, instead of projecting so conspicuously from the mesial surface of inner longitudinal vessels as in *C. intestinalis* and *C. savignyi*, though clearly denoted as such in their original descriptions (Herdman, 1882, p. 235, pl. 34, fig. 8; Sluiter, 1904, p. 25, pl. 4, fig. 2). The thoracic musculature consists of about a dozen longitudinal bundles in the former or 20 in the latter, markedly ramified posteriorly on either side. On the other hand, another type specimen of *Ciona indica* collected at a depth of 274 m in the Flores Sea (Zoological Museum in Amsterdam, TU 333) differs utterly from both the original description of the species and the type specimens registered as TU 332 mentioned above. In the specimen from the Flores Sea, approximately 21 mm long and 14

mm thick and with 11 mm long posterior extension of test, the mantle body is strongly contracted and rather heavily injured in former examination, and therefore, close reexamination was impossible. It is obvious, however, that the present specimen may safely be assignable to the genus *Ciona* in that it shares such generic features as the body never divided into thorax and abdomen, the mantle musculature consisting mainly of thick longitudinal bundles, the apparent existence of "perivisceral cavity", the characteristic position of heart and pericardium as well as the structure of gonad. Nevertheless, the present specimen is quite different from *C. intestinalis* and *C. savignyi* in the structure of branchial sac. In this specimen, the inner longitudinal vessels are supported above the sac surface by tall branchial papillae at or near their distal tip; thus the tips of papillae are never projecting out markedly beyond the vessels. In contrast with this, in *C. intestinalis* and *C. savignyi* the vessels are located virtually on the sac surface and large and conspicuous papillae are mostly projecting out beyond the vessels. In the specimen in question, tentacles about 20, ciliated groove as an oval opening elongated longitudinally, inner longitudinal vessels about 40 or more on each side, endostylar appendage indiscernible, a pair of pharyngeo-epicardiac openings (?) situated close to the posterior end of endostyle, and genital apertures opening adjacent to the anus. Further affiliation of this specimen from the Flores Sea may be better left pending at present till more material is available in future. *C. indica* was also recorded from Sagami Bay, Japan by Hartmeyer (1906, p. 26), with brief description. The authors tried to examine those specimens of Hartmeyer, probably deposited in the Museum für Naturkunde, Berlin; unfortunately, however, Dr. D. H. H. Kühlmann of the museum kindly showed them that these specimens are not found there.

(5) *Ciona mollis*, ever recorded from off California, 1100 fathoms deep, may seemingly be very closely related to *C. intestinalis* and *C. savignyi*. Ritter's original description (1907, pp. 36–39) can not be brief, but lacks completely the information regarding the endostylar appendage and pharyngeo-epicardiac openings. As the type material is now kept in neither American Museum of Natural History nor National Museum of Natural History, Smithsonian Institution, the real taxonomic status of this species remains uncertain.

(6) *Ciona antarctica* was established by Hartmeyer (1911, pp. 471–472), basing on two specimens collected at a depth of 350 m and 385 m respectively in the Kaiser Wilhelm II Land, Antarctic Sea, but without any information about the above-mentioned diagnostic features either. The authors have not yet been given a chance to examine the type of this species. Very recently Monniot & Monniot (1983, pp. 47 and 49) rediscovered this species from the western coast of the Antarctic Peninsula, 95–100 m deep. Though their description also lacks the same information, *C. antarctica* may seemingly be somewhat different from *C. intestinalis* and *C. savignyi* in that the ovary is flattened, leaf-like and more or less diffused, instead of usually forming a compact pear-shaped mass in the latter. Conclusive taxonomic discussions on *C. antarctica* may be left pending at present. On the other hand, *C. imperfecta*, recorded from NE Atlantic, 3992–4300 m deep, by Monniot &

Monniot (1977, pp. 698–700), is obviously unique.

(7) In conclusion, the present authors are of the opinion that at least the following taxa belonging to the genus *Ciona* (sensu Monniot & Monniot, 1973, p. 33) may be taken as certain and valid ones at present:

- A) Provided with endostylar appendage, and pharyngeo-epicardiac openings situated neat it.
 - a) Without postabdominal extension of mantle, but very young animals.
C. intestinalis (Linné, 1767)
 (= *C. robusta* Hoshino et Tokioka, 1967)
 - b) With postabdominal extension of mantle even in adults.
C. intestinalis longissima Hartmeyer, 1899
C. intestinalis gelatinosa Bonnevie, 1896
- B) Without the appendage, and the openings situated near the oesophageal opening.
C. savignyi Herdman, 1882 (= *C. aspera* Herdman, 1886;
C. intestinalis sensu Hoshino & Tokioka, 1967)
- C) Abyssal species.
C. imperfecta Monniot et Monniot, 1977
- D) Antarctic species.
C. antarctica Hartmeyer, 1911

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