Note

Intersex in the Mysid *Siriella japonica izuensis* Ii: the Possibility it is Caused by Infestation with Parasites

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Intersex is known in a variety of crustaceans, and is caused by some natural environmental factors, genetic abnormalities, endocrine disrupting chemicals, and infestation of parasites (cf. Reinhard 1956; Bulnheim 1975; Hastings 1981; Gins-burger-Vogel & Charniaux-Cotton 1982; Moore & Stevenson 1994). This phenomenon is reported also in mysids: two cases regarding masculinization of female *Neomysis integer* (Leach), and one for feminization in male *Acanthomysis mitsukurii* (Nakazawa) (Kinne 1955; Hough et al. 1992; Yamashita et al. 2001).

During our survey on zooplankton around Hachijo Island, southern Japan on April 11, 2002, a feminized male of the mysid *Siriella japonica izuensis* Ii was discovered. In addition the intersex individual was heavily infested by ectoparasites. We briefly describe the intersex individual of the mysid and tentatively identify the parasite, with notes on the parasitic effects on the intersex host. Mysids were collected from near the surface at night, by towing a conical plankton net (diameter: 30 cm; mesh size: 0.1 mm) around a water-proof fish collection light.

Two individuals of *S. japonica izuensis*, including one intersex individual, were infested by the ectoparasite. One of the mysids (body length 11.1 mm from the anterior tip of the rostrum to the posterior end of the telson, excluding spines) clearly exhibited intersexuality with both well-developed pleopods/antennulary processus masculinus (characteristic of normal male) and three pairs of "brood plates" (of normal female) (Fig. 1A~C). This intersex individual carried no eggs/embryos in the "marsupium". In addition, it bore a greatly expanded lobe originating from the protopod of the first pleopod

(Fig. 1D). This structure is superficially similar to those of the copulatory organs on the first pleopods in euphausiids and certain decapods, but the normal male mysid does not usually bear this kind of penis (Fig. 1I). The pseudobranchial rami of the first and second pleopods were less developed in the intersex specimen (Fig. 1D, E) than in the normal males (Fig. 1G, H). This intersex individual can be regarded as a feminized male due to the presence of the well-developed pleopods and antennulary processes masculinus, although it is internally castrated (see below). In contrast, the other infested individual was externally a normal mature female (body length 9.1 mm), in which there were no eggs/embryos in the marsupium.

The frequency of parasitism was low, 1.5% among mature and immature individuals of *S. japonica izuensis* (total individuals examined: 129). Although other mysids, *Siriella longipes* Nakazawa (1), *Australomysis hispida* Fukuoka & Murano (5), and *Anisomysis ryukyuensis* Murano (11) were also collected simultaneously, parasite infestation occurred only in adults of *S. japonica izuensis*.

The intersex individual and parasites attached to the brood plates were embedded in paraffin, sectioned (6 μm in thickness), and stained with hematoxylin-eosin in order to identify the parasite and to clarify its effect on the host. In addition, several individual parasites, detached from the other normal female host, were supplementarily used for transmission electron microscopy to aid identification. They were processed as outlined by Horiguchi & Ohtsuka (2001). Observations were made using a JEOL 100S transmission electron microscope.

All parasites on the two host mysids were attached to the inner sides of the "brood plates" with a perforation of about 15 μm on the mysid cuticle (Fig. 2C~E, arrowed). The parasites were variable in shape, ellipsoid (Fig. 2A, C) to column-like (Fig. 2B), tapering proximally with a semitransparent constricted part at the base (Figs. 2C, D), ranging approximately

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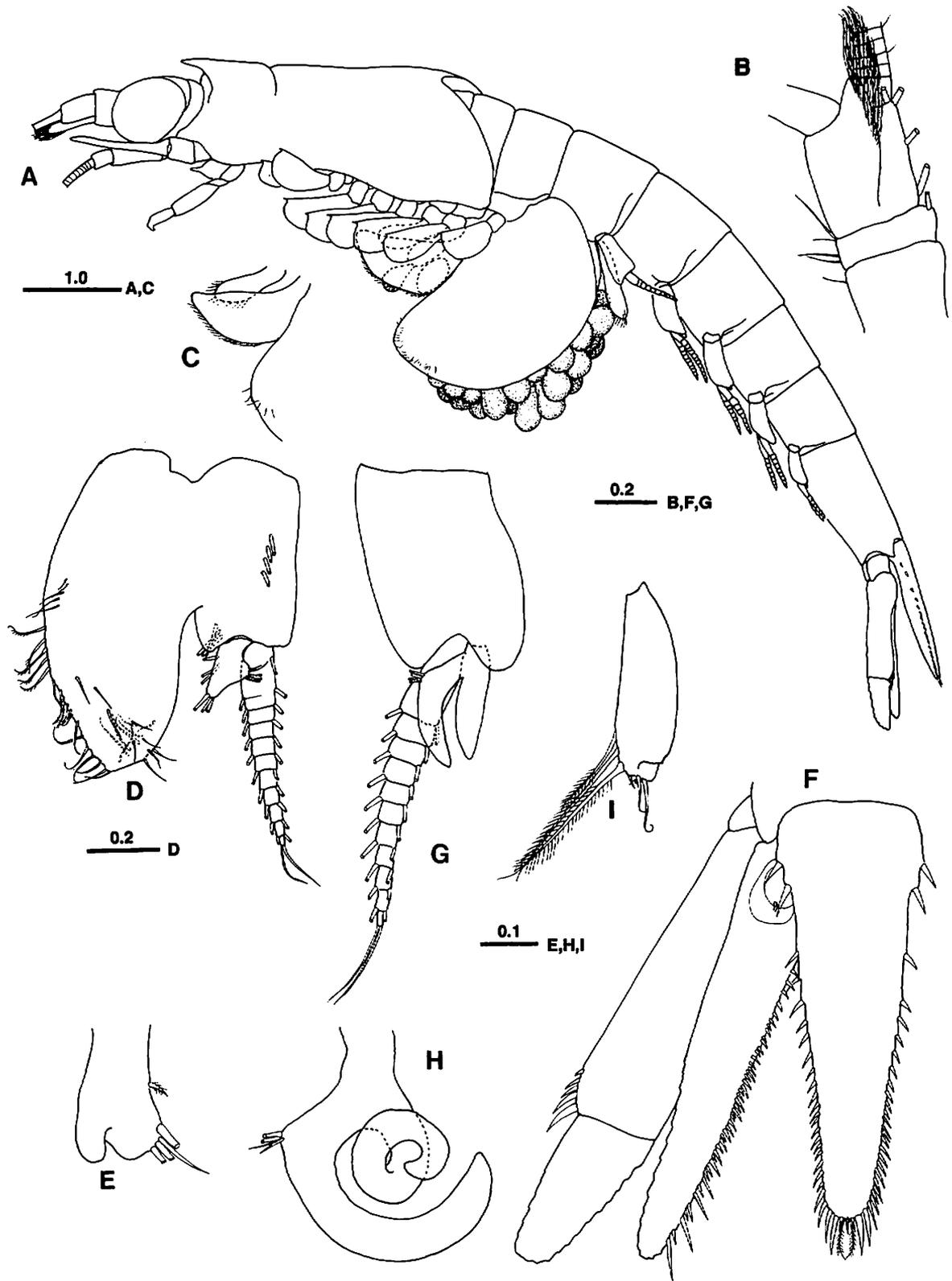E-mail: ohtsuka@hiroshima-u.ac.jp
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Fig. 1. *Siriella japonica izuensis*, intersex (A–F), normal male (G–I). A, Habitus, lateral view. Note well developed brood plates infested by parasites; B, Antennulary peduncle with processus masculinus; C, First and second brood plates, left lateral view; D, First pleopod with protopodal lobe abnormally developed: E, Pseudobranchial ramus of second pleopod; F, Telson and uropod, dorsal view; G, First pleopod; H, Pseudobranchial ramus of second pleopod; I, Penis. Scales in mm.
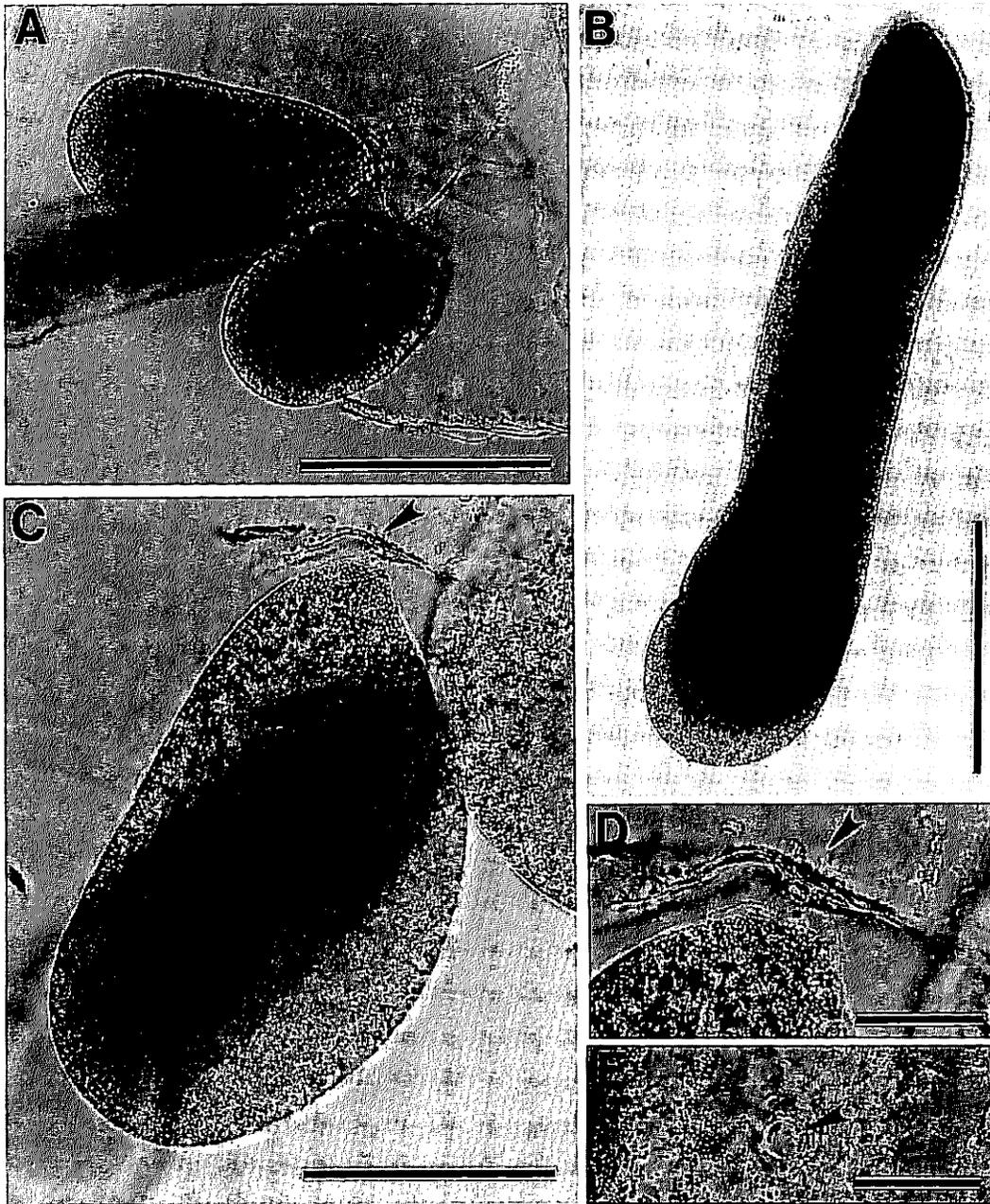
Fig. 2. Parasites on intersex specimen of *Siriella japonica izuensis*. A, Parasites attached to inside of second brood plate; B, Large-sized parasite attached to first brood plate; C, Enlargement of one parasite of "A", attachment site arrowed; D, Enlargement of attachment site of the parasite of "C", viewed laterally, arrowed; E, Attachment site, viewed from inner side of mysid marsupium, arrowed. Scales=0.4 mm (A, B); 0.2 mm (C); 0.05 mm (D, E).

from 0.50 to 1.23 mm in length (less than 0.4 mm in width). There was no root-like structure at the attachment site (Fig. 2D). The number of parasites (cells) was 70 in total on the intersex individual (the number of parasites on the normal female host was unknown). The parasites fully occupied the space of the "marsupium". Transverse sections of the parasite showed that there were numerous nuclei (Fig. 3A). The parasite was coenocytic (single-cell with multiple nuclei), no internal walls or membranes to subdivide the cytoplasm have been observed, and each nucleus was spherical in shape (Fig. 3A). The nucleoplasm was homogeneous and contained a few dot-

like chromatin structures. No obvious nucleoli have been noted. Mitochondria were many and relatively small. Their profiles were spherical to oval. They contained the tubular type of mitochondrial cristae (Fig. 3B). The parasite was surrounded by a fibrous wall-like structure (Figs 3C, D). The plasma membrane was located beneath this wall. Many small vesicles were located just beneath the plasma membrane (Fig. 3C) and some of them seem to be fused with the plasma membrane (Fig. 3D). Other than nuclei and mitochondria, small vesicles of various sizes were present throughout the cytoplasm. Lipid-like granules were also present (Fig. 3E).
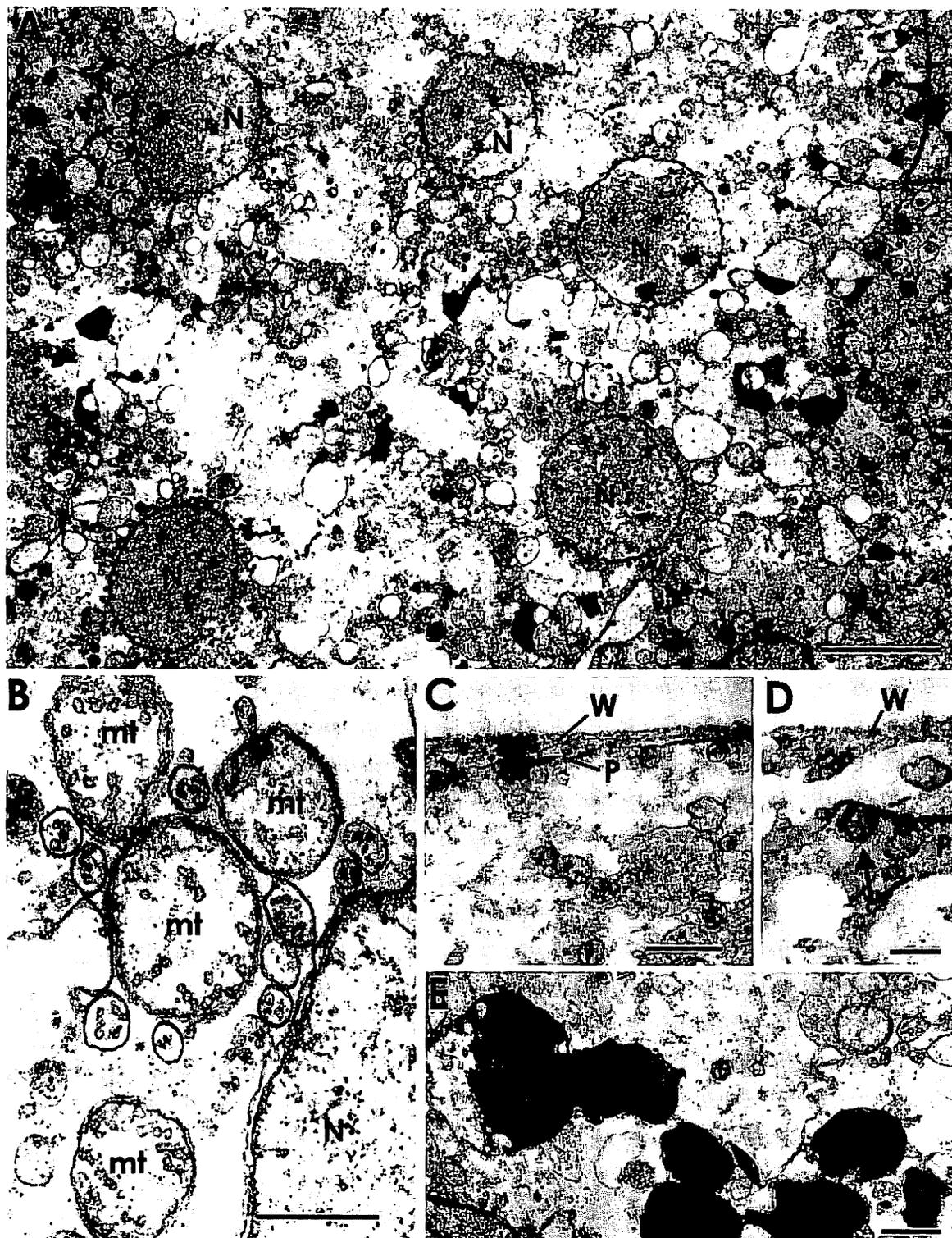
Fig. 3.   Transmission electron micrograph of a parasite on female *Siriella japonica izuensis*. A, Part of cytoplasm, showing coenocytic condition. (N) nucleus; B, Mitochondrial profiles (mt). Note the possession of tubular cristae; C, Part of cell, showing cell covering. The outermost wall-like structure (W) is composed of fibrous material. Beneath plasma membrane (P), small spherical vesicles can be seen; D, Close-up of cell covering. A small vesicle (arrow) is fused with the plasma membrane (P); E, Lipid-like structures (L) in the cytoplasm. Scales=5 μm (A); 500 nm (B); 500 nm (C); 100 nm (D); 1 μm (E)
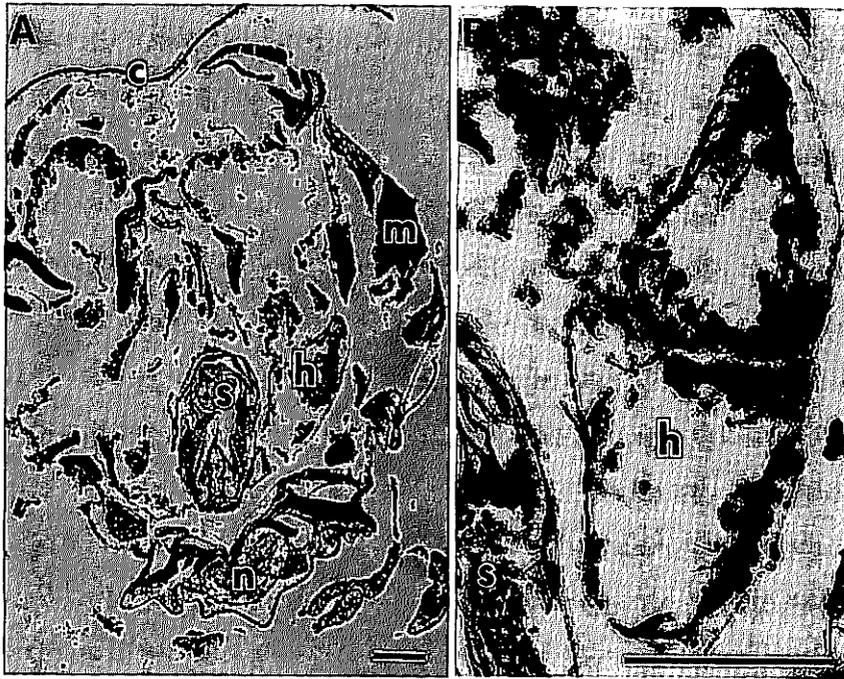
Fig. 4. Transverse section of intersex specimen of *Siriella japonica izuensis*. A, Position of posterior part of stomach, (c) carapace, (h) hepatopancreas, (m) muscles, (n) nerve cord, (s) stomach; B, Enlargement of hepatopancreas (h). Scales=0.1 mm (A, B).

Since we have only limited information regarding the parasite, it is difficult to identify the organism precisely. We have no information on its life cycle or motile cell morphology. However, the fact that the organism is an ectoparasite on a crustacean (mysid) and that it possesses a balloon-like cell body suggests that the organism is a member of the family El-lobiopsidae, most likely to be a member of the genus *Ellobio-cystis* Coutière, 1911 (cf. Chatton 1920; Kane 1964; Wing 1975; Shields 1994). Shields (1994) defined the genus as an el-lobiopsid consisting of a single trophomere and one or more gonomeres with neither a root system for attachment nor penetration for nutrient absorption. However the present parasite shows no external division in the body, and has a minute basal pore via which nutrients may be absorbed from the host. Although several species of another ellobiopsid genus *Thalas-somyces* are found on the bodies of both shallow- and deep-water mysids (Collard 1966; Vader 1973; Wing 1975; Shields 1994), this is easily distinguished from *Ellobiocystis* by (1) the presence of a root system for attachment to the host, and (2) a large cluster of gonomeres developing from the numerous branching trophomeres (cf. Kane 1964; Wing 1975; Shields 1994). Transmission electron microscope observations revealed that the parasite is characterized by (1) being coeno-cytic and (2) possession of tubular mitochondrial cristae. It is not possible to identify the organism solely based on these features, but these characteristics further support the possibility of ellobiopsid affinity of the organism (Whisler 1990). However, exact identification of the parasite must await the accumulation of more data.

Transverse sections of the intersex individual point to the possibility that the male reproductive organs were castrated by the parasite. The hepatopancreas and testes of the intersex individual seemed to be entirely reduced (Fig. 4). Some el-lobiopsids are known to castrate their hosts (Kane 1964; Shields 1994). Considering the specific attachment site of the parasite, the development of the marsupium in the intersex individual is likely to have been induced by parasitism. Although parasites such as Microspora (Protista) and Rhizocephala (Crustacea) affect molt, maturation, and sex determination of their crustacean hosts (cf. Reinhard 1956; Bulnheim 1975; Hastings 1981), the present finding may be the first record of intersex induced by ellobiopsilids. Some species of the el-lobiopsid genus *Thalassomyces* are also found within the marsupium of hyperiid amphipods (Kane 1964; Ohtsuka unpublished data). This attachment site may be an adaptation to avoid detachment due to grooming by these crustaceans (cf. Acosta & Poirrier 1992). According to Kane (1964), the shape and size of the ellobiopsid resemble those of eggs of the host amphipod, and it occupied the space that eggs would normally fill in an ovigerous female host, suggesting that it may mimic the eggs and embryos of the host.

Acknowledgments

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