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NOTE

Infestation of the testes of the Japanese sea star *Asterias amurensis* by the ciliate *Orchitophyra stellarum*: a caution against the use of this ciliate for biological control

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ABSTRACT: Male infertility is a new phenomenon affecting populations of the Japanese sea star *Asterias amurensis*. The agent causing partial or total castration of the testes was identified to be *Orchitophyra stellarum*, a parasitic ciliate endemic to the north Atlantic. *A. amurensis* is a new host for *O. stellarum*, apparently due to the recent introduction of the ciliate to the Pacific Ocean. This ciliate disrupts the germinal layer and phagocytoses sperm. Male infertility of *A. amurensis* is largely effected by the phagocytic response of host's cells with each cell engulfing numerous sperm. *O. stellarum* is highly contagious in *A. amurensis* with 100% of the males in some populations infested. The absence of male sea stars in some populations is also linked to ciliate disease. This report of *O. stellarum* from the western Pacific Ocean completes the circumboreal distribution of this parasite. The contagious spread of *O. stellarum* in Japan and its ability to parasitize several asteroid genera cautions against its use for biological control of *A. amurensis* in Japan and of recently established pest populations of this asteroid in Australia.

KEY WORDS: Ciliate disease · *Orchitophyra stellarum* · *Asterias amurensis* · Biological control

Asterias amurensis is a locally abundant sea star in Japan and has long been an important research organism for biologists investigating fertilization and development (Kume & Dan 1968, Longo et al. 1995). In recent times, however, it has become difficult to obtain sperm from *A. amurensis* even during the peak spawning period of populations previously known to be a reliable source of gametes. This change in the reproduction of *A. amurensis* was first noted in 1990 when infertile males with discoloured and hardened testes were encountered. These testes were observed at a time in the reproductive cycle when copious amounts of sperm should have been present (Ino et al. 1955, Kim 1968). Since 1990, the incidence of male *A. amurensis* noted to exhibit this condition has increased and now male infertility is common in many populations.

The changes seen in the testes of *Asterias amurensis* are similar to those described for *Asterias* species from the north Atlantic infested with the parasitic ciliate *Orchitophyra stellarum* (Cépède 1907, 1910, Burrows 1936, Vevers 1951, Jangoux 1987). *O. stellarum* is specific to the Asteriidae and, within this family, is known to parasitize 4 genera and 6 species (Jangoux 1987, Leighton et al. 1991, B. J. Leighton pers. comm.). It usually infests male sea stars, where it causes regression of the germinal epithelium, resulting in partial or complete castration of the testis (Bouland & Jangoux 1988). *O. stellarum* was originally described at the turn of the century from the gonads of *A. rubens* from the northeast Atlantic Ocean (Cépède 1907) and was later found in asteroids from the northwest Atlantic and from the Mediterranean Sea (Burrows 1936, Smith 1936, Febvre et al. 1981). This long-standing Atlantic distribution of *O. stellarum* changed with the recent discovery of this parasite in the gonads of *Pisaster ochraceus*, a common asteroid in the northeast Pacific Ocean (Leighton et al. 1991). In addition to causing regression of the testes, the ciliate caused morbidity and mortality of *P. ochraceus*, symptoms not normally associated with the disease. The greater virulence of the disease in *P. ochraceus* was taken to suggest that this asteroid is a new host for *O. stellarum* (Leighton et al. 1991). The range of *O. stellarum* has increased in *P. ochraceus* populations and a second northeast Pacific species, *Evasterias troscheli*, has recently succumbed to the parasite (B. J. Leighton pers. comm.).

The aim of this investigation was to identify the agent affecting the testes of *Asterias amurensis*. Ultrastructural examination of the gonads revealed the presence of *Orchitophyra stellarum*. The effect of this ciliate on *A. amurensis* is described and the host's

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response is compared to that documented for other *Asterias* species (Vevers 1951, Bouland & Jangoux 1988). This report from Japan, together with the presence of *O. stellarum* in Canada (Leighton et al. 1991), shows that this species now has a circumboreal distribution. Our observations coincide with the dramatic increase of *A. amurensis* in Australia, where biological control of this recently introduced pest is being considered (Byrne 1996, Byrne et al. 1996, Viney 1996). In light of this new phenomenon, we present a cautionary assessment of *O. stellarum* as an agent for biological control.

Materials and methods. During the 1996 breeding season, *Asterias amurensis* was collected by SCUBA from populations in northern and central Japan. Specimens were collected from Tokyo Bay (35° 15' N, 139° 45' E) and Ise Bay (34° 30' S, 136° 50' E) in January and from Otuchi Bay (39° 20' S, 142° 00' E) in February. At each site, samples of 50 to 200 *A. amurensis* were collected at a depth of 2 to 3 m. The northern-most site, Otuchi Bay, is in northern Japan and the southern-most site, Ise Bay, is in central Japan. These sites are approximately 740 km apart and are separated by over 1000 km of shoreline. The specimens were dissected and examined for the presence of diseased gonads. Diseased gonads were examined by light (LM) and transmission electron microscopy (TEM). For histological examination, the gonads were fixed in 10% formalin or in Bouin's fluid, dehydrated in a graded series of ethanols, embedded in paraffin and sectioned (7 µm thick). The sections were stained with haematoxylin and eosin. For LM and TEM, pieces of gonad were fixed in 2.5% glutaraldehyde in 0.45 µm filtered seawater for 1 h, transferred to 70% alcohol and dehydrated in a graded series of ethanols. The tissues were then rinsed in acetone and embedded in Spurr's resin. Thick sections (0.7 µm) were stained with 1% toluidine blue in 0.5% ethanolamine. For TEM, ultrathin sections were stained with uranyl acetate and lead citrate and viewed in a Phillips 400 transmission electron microscope.

Results and discussion. Diseased gonads of *Asterias amurensis* were readily detected due to their atypical brown colour, shrivelled appearance and hard texture. In all cases the diseased specimens were male. Histological examination of the testes revealed the presence of small groups or extensive aggregations of ciliates in the lumen (Fig. 1a–c). Ultrastructurally, these ciliates were indistinguishable from the *Orchitophyra stellarum* described in the testes of *A. rubens* and *A. vulgaris* (Bouland et al. 1987, Bouland & Jangoux 1988, Claereboudt & Bouland 1994). *O. stellarum* is fusiform (40 µm long × 20 µm wide) and has a rounded posterior end and a pointed anterior end (Figs. 1c–d, 2a). A macronucleus, micronucleus, a Golgi complex and numerous vacuoles occupy the cytoplasm (Figs. 1c–d, 2a, b). Equally spaced longitudinal rows of cilia, called kineties, extend from the surface (Fig. 1e).

During the 1996 breeding season, ciliate infestation was widespread in 2 of the *Asterias amurensis* populations examined. At Tokyo Bay 100% of the males (n = 100) had parasitized gonads and at Otuchi Bay 38% of the males (n = 80) were infested. Male *A. amurensis* were not present in the Ise Bay sample (n = 50). Field surveys at Tokyo Bay revealed that ciliate disease is associated with a marked decrease in the proportion of male sea stars with sex-ratios of 1:1 observed at the beginning of breeding and ratios 10:1 or 20:1 in favour of females observed at the end of breeding. A similar decline in the male population is reported for other *Asterias* species infested by *Orchitophyra stellarum* (Claereboudt & Bouland 1994) and also for *Pisaster ochraceus* (Leighton et al. 1991). The decrease in the number of male *A. amurensis* in Tokyo Bay and the absence of males from Ise Bay indicate that ciliate disease may cause host mortality, as suggested for *A. vulgaris* and *P. ochraceus* (Leighton et al. 1991, Claereboudt & Bouland 1994). Although mortality of *A. amurensis* was not observed in the field, diseased specimens were more prone to autotomise their arms and die in aquaria, compared with uninfected specimens. *A. amurensis* migrates into shallow water for breeding and so the preponderance of females in the sampled populations may be due to mortality of males in deep water.

Orchitophyra stellarum was only evident in mature testes. The disease developed progressively. In the early stages of the disease, the ciliates were interspersed with sperm (Fig. 1a, c) and, in later stages, completely occluded the gonadal lumen (Fig. 1b). Early in the breeding season infected testes had an active spermatogenic layer and contained viable spermatozoa which could be used for fertilization. The lumen of diseased testes contained spermatozoa, ciliates and phagocytes (Figs. 1a–e, 2a–d). Disruption of the germinal layer was indicated by the presence of immature sperm in the lumen which had detached from a spermatocyte column (Fig. 2c). The ciliates appeared to disrupt the germinal layer by their locomotory activity. They occasionally inserted their anterior end between adjacent spermatocyte columns thereby causing the columns to separate (Fig. 1c). As the parasite population increased, the amount of sperm decreased until there were no germ cells evident. In the advanced stage of the disease, *A. amurensis* have hardened testes filled with ciliates and phagocytes. The symptoms exhibited by *A. amurensis* in response to *O. stellarum* infestation are characteristic of the disease in other *Asterias* species (Cépède 1910, Vevers 1951, Jangoux 1987, Bouland & Jangoux 1988).

Orchitophyra stellarum phagocytoses the sperm of *Asterias amurensis* and may also utilise nutrients pre-

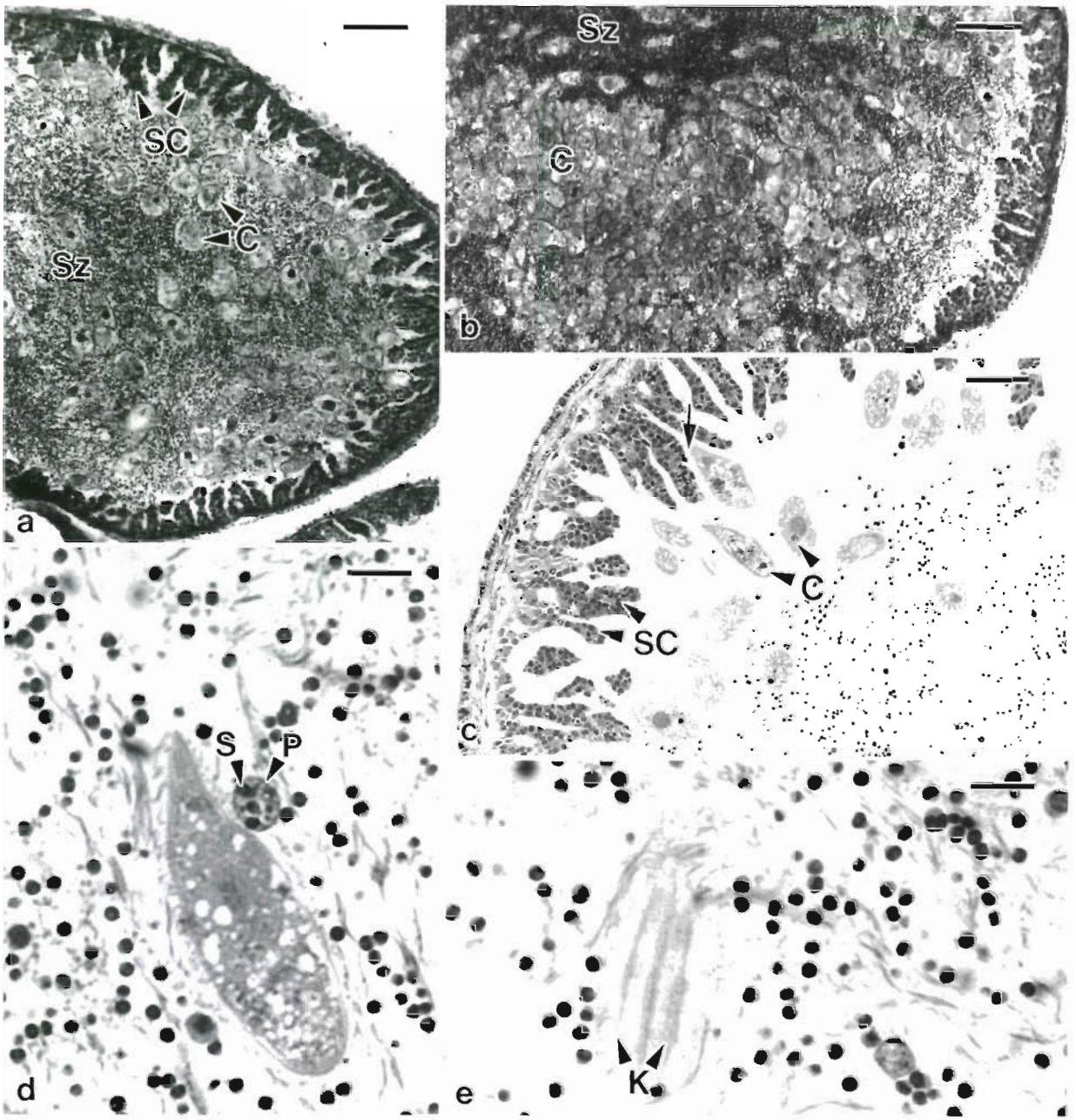

Fig. 1. *Asterias amurensis*. Light micrographs of parasitized testes. (a) Cross section of a mature testis containing *Orchitophyra stellarum* (C) and spermatozoa (Sz). The germinal epithelium contains columns of developing sperm (SC). (b) Mature testis with ciliates (C) occluding the lumen. Sz: spermatozoa. (c) *O. stellarum* (C) scattered in the testis lumen, one of which has its anterior end (arrow) inserted between adjacent spermatocyte columns. (d) *O. stellarum* and an adjacent host phagocyte (P) which contains sperm (S). (e) Grazing section of *O. stellarum* showing 3 kineties (K). Scale bars: (a) = 50 µm; (b) = 30 µm; (c, d, e) = 8.0 µm

sent in gonadal fluid though uptake of fluid into the numerous vacuoles seen in the cytoplasm (Figs. 1d, 2a, b). The mean number of sperm engulfed by the ciliates was 0.1 (SE = 0.07, range = 0 to 1; n = 20). *O. stellarum* also phagocytoses the sperm of *A. rubens* (Bouland et al. 1987, Bouland & Jangoux 1988).

Phagocytic activity by the host's cells was prevalent in diseased testes with amoebocytes observed in the process of engulfing sperm (Figs. 1c, 2d). These cells contained numerous phagocytosed sperm ($\bar{x}$ = 4.3, SE = 0.84, range = 0 to 10, n = 10). This host's phagocytic response is largely responsible for the marked decrease and eventual absence of sperm in parasitized *Asterias amurensis*. Male infertility in *A. amurensis* is thus induced by the disruptive activity of the ciliate and is largely effected by the phagocytic response of

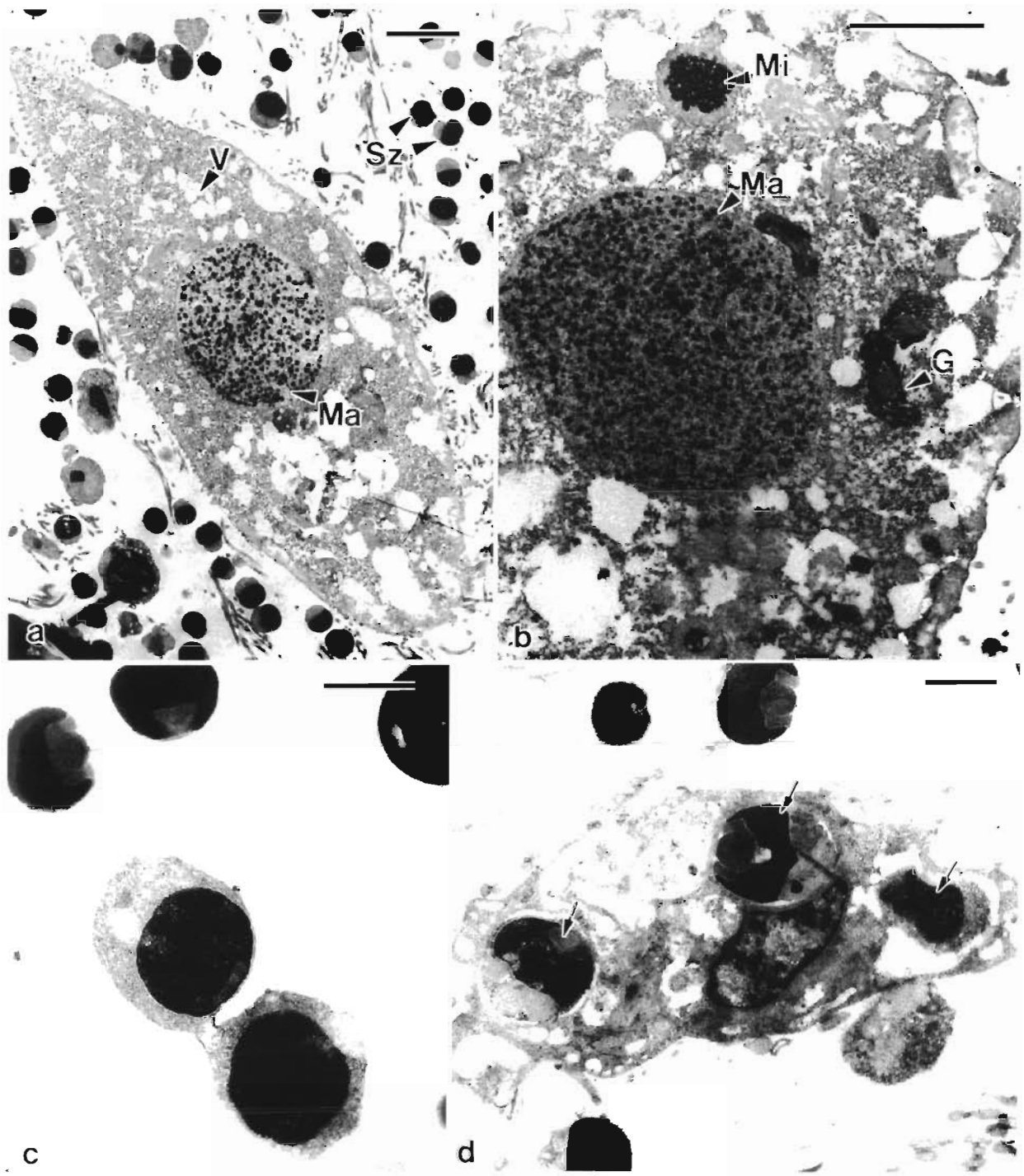

Fig 2. *Asterias amurensis* Electron micrographs of parasitized testes (a) *Orchitophyra stellarum* has a fusiform profile and contains a macronucleus (Ma) and numerous vacuoles (V) Sz spermatozoa (b) Detail of macronucleus (Ma) and micronucleus (Mi). G Golgi complex (c) Spermatids in the lumen (d) Host's phagocyte containing 3 engulfed sperm (arrows). Scale bars: (a, b) = 4 0 µm, (c, d) = 1.0 µm

the host's cells. Disruption of the germinal layer by *O. stellarum* followed by massive invasion of phagocytes into infested gonads is also reported for *A. rubens* (Bouland & Jangoux 1988). The phagocytes of *A. amurensis* did not attack the ciliates. As noted for *A. rubens*, it is surprising that these cells do not recognise the ciliates as foreign inclusions to be engulfed (Bouland & Jangoux 1988).

The high infestation rate of *Orchitophyra stellarum* in *Asterias amurensis*, contrasts with the incidence of ciliate

disease in the Atlantic Ocean, where infestation rates are generally low (0 to 1%), with occasional exceptions reported in the literature (Jangoux 1987). Widespread infestation of male *A. amurensis* in Japan over a broad geographical range indicates that the disease is highly contagious in this asteroid. Aquarium observations suggest that the spread of *O. stellarum* is mediated by seawater or by direct contact. The more contagious and debilitating aetiology of ciliate disease in *A. amurensis* and *P. ochraceus* compared with the disease in Atlantic sea stars is probably because these asteroids are new hosts for *O. stellarum* (Leighton et al. 1991).

Orchitophyra stellarum was first observed in the eastern Pacific Ocean in the mid 1980s (Leighton et al. 1991) and was first noted in Japan in 1990. The method of introduction of *O. stellarum* to the Pacific Ocean is not known. Its introduction to Japan may have been mediated through a seed source of *O. stellarum* in populations of *Pisaster ochraceus* in Canada. *Asterias amurensis* occurs across the north Pacific, and it would be interesting to determine the incidence of *O. stellarum* in these asteroids where they co-occur.

Extensive searches for mature male *Asterias amurensis* over the last 5 yr at geographically distant sites indicate that ciliate infestation has resulted in a marked drop in sperm production by Japanese populations of this asteroid. This reduction in male reproductive potential has most likely resulted in a decrease in the fertilization success of *A. amurensis* with potential long-term effects on its recruitment and population biology. The epidemiology of ciliate disease in Japan highlights the need to monitor the spread of *O. stellarum* in *A. amurensis* and in other potential asteroid hosts from the family Asteriidae. Considering the role that asteriid sea stars play as top predators in many benthic communities (Jangoux 1982), this new situation in Japan may also have serious ecological implications.

The dietary preference of *Asterias* species for bivalve molluscs often makes them a pest for fisheries and aquaculture and several unsuccessful attempts have been made to use *Orchitophyra stellarum* as an agent for biological control of *Asterias* (Cépède 1910, Piatt 1935, Burrows 1936). Although the virulence of ciliate disease in the Pacific might rekindle interest in the use of *O. stellarum* for biological control of *A. amurensis* in Japan, and of recently established pest populations of *A. amurensis* in Australia (Byrne 1996, Byrne et al. 1996, Viney 1996), the lack of species specificity of *O. stellarum* cautions against its use. The apparently rapid spread of *O. stellarum* in Japan and its ability to parasitize several asteroid genera indicates that the use of this parasite for biological control in Japan, or elsewhere in the Pacific, might result in the infestation of a range of endemic asteroids in the family Asteriidae with serious consequences for their population biology.

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