**The ciliate Orchitophrya stellarum viewed as a facultative parasite of asteriid sea stars**

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**Abstract:** Orchitophrya stellarum Cépède, 1907 is a ciliate that consumes sperm in the testes of male asteriid sea stars in the Pacific and North Atlantic oceans. Previous studies have reported its presence in smears and sections of testes, and we have also observed it in the spawn. This organism is easily cultured in seawater containing bacteria nourished by yeast extract or tissues from various marine invertebrates and the domestic chicken. During adaptation to culture conditions, the ciliates become smaller, the number of kineties is reduced, and the buccal cavity is shifted farther away from the anterior end. These changes are reversed if the ciliates are fed sperm of asteriid sea stars. Orchitophrya stellarum is therefore considered to be a facultative parasite that can live indefinitely in situations where it can feed on bacteria and tissue detritus. It probably enters the testes of reproductively mature male sea stars by way of the gonopores.

**Resumé:** Le cilié Orcitophyra stellarum vu comme un parasite possible des étoiles de mer astériide. Le cilié Orchitophrya stellarum Cépède, 1907, parfois trouvé dans les étoiles de mer asterides mâles dans les océans Pacifique et Atlantique Nord, se nourrit de spermatozoïdes. Les études précédentes ont démontré sa présence dans les frottis et les coupes des testicules et nous l’avons observé dans le frai. Ce cilié est facilement cultivé dans l’eau de mer contenant des bactéries alimentées avec des extraits de levure ou des tissus d’invertébrés marins ou de poulet. Pendant leur adaptation aux conditions de cultures, les ciliés deviennent plus petits, le nombre de leurs rangées de cinéties est réduit et la cavité buccale s’éloigne un peu plus loin de l’extrémité antérieure. S’ils sont nourris par les spermatozoïdes des astériides, ces changements sont réversibles. Orchitophrya stellarum est donc un parasite facultatif qui peut vivre indéfiniment dans l’eau de mer. Son entrée dans les testicules des étoiles de mer mâles se réalise probablement à travers les gonopores.

**Keywords:** Asteriid sea stars ● Ciliophora ● Facultative parasitism ● Orchitophrya stellarum

**Introduction**

Orchitophrya stellarum Cépède, 1907 is a ciliated protozoan that phagocytizes sperm in testes of sea stars of the family Asteriidae. It was first found in Asterias rubens in the Boulonnais region of France, but is now known to occur in this and several other sea stars in the North Atlantic and Pacific oceans. Male mortality and/or reduced sperm output have been observed or implied from a number of studies of localized collections of five species. Evaluation of the impact of the parasite on asteriid sea star survival has been...
based primarily on a decreased ratio of males to females in Asterias rubens (Claereboudt & Bouland, 1994), Pisaster ochraceus (Leighton et al., 1991; Stickle et al., 2001b), Evasterias troschelii (Stickle et al., 2001b), and Asterias amurensis (Byrne et al., 1997, 1998). In A. rubens (Claereboudt & Bouland, 1994), however, and also in four of the six collections of P. ochraceus and E. troschelii (Stickle et al., 2001b), males are on average smaller than females, so perhaps there is size-specific male mortality. In two of six collections of Leptasterias spp. (Stickle et al., 2001a,b) and in A. rubens (Lowe, 1978), sub-lethal stress is suggested by significantly smaller testis indices in parasitized males than in non-parasitized males.

Orchitophrya stellarum belongs to the scuticociliate family Paranophryidae, which is closely related to other groups that are endoparasitic, histiophagic, or ectocommensal or endocommensal associates of various invertebrates (Bouland et al., 1987; de Puytorac, 1994; Dragesco et al., 1995). Our observations on O. stellarum cultured in seawater containing bacteria show that the size and morphology of the ciliates of the free-living phase are very different from those parasitizing testes (Stickle et al., 2001a,b).

Orchitophrya stellarum has been suspected of having been introduced into the waters of southern British Columbia, where it parasitizes Pisaster ochraceus (Leighton et al., 1991), and to Japan, where it parasitizes Asterias amurensis (Byrne et al., 1997, 1998). Greater virulence of the parasite among sea stars in the Pacific Ocean has been attributed by Leighton et al. (1991) and Byrne et al. (1997, 1998) to its recent introduction from the Atlantic Ocean, but this hypothesis has not been tested. Nevertheless, significantly fewer and smaller males than females and smaller testis indexes of parasitized than non-parasitized male A. rubens from Port Daniel Bay, Quebec indicate virulence of O. stellarum at that location as well (Claereboudt & Bouland, 1994). Although it is possible that the parasite is an exotic species on the west coast of North America, it is more likely to have been associated with sea stars for a long time but only recently observed in most known host species. DNA sequencing data suggest that there is no observable genetic differentiation between O. stellarum isolated from sea stars in the Atlantic and Pacific oceans (Goggin & Murphy, 2000). Nucleotide sequence data from the internal transcribed spacers (ITS1 and ITS2) and the 5.8S gene from the ribosomal RNA gene cluster of isolates of O. stellarum collected from four species of asteroid hosts from the Atlantic and Pacific oceans were identical.

This report deals with changes undergone by O. stellarum as it adapts to a free-living, mostly bacteriophagic existence, and also as its morphology changes when it is fed sperm of an asteroid sea star.

Materials and Methods

Microscopy

Smear preparations of gonadal tissue in sea water revealed the presence or absence of ciliates in a particular host sea star and were also used for microscopic study of living specimens. For determining the distribution of ciliates within a parasitized testis, intact tissue was fixed in 10% buffered formalin adjusted in osmolality to equal 30‰ salinity, rinsed twice with 0.1 M sodium cacodylate in 3% sucrose, and stored in the same buffer at 5 °C until prepared for histology. The tissue was dehydrated in an ethanol series, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

For detailed light-microscope study of ciliates of the parasite phase, smears of parasitized testes on cover-glasses were impregnated with protargol after being fixed in Bouin’s or Hollande’s fluid, or impregnated with silver nitrate according to the Chatton-Lwoff method after double fixation in Champy’s and DaFano’s fluids. The same techniques of impregnation were applied to ciliates of the free-living phase, but it was necessary to concentrate the organisms by light centrifugation and, for the protargol method, to mix them with macerated mantle tissue from a marine gastropod or bivalve in order to make them adhere to a coverglass when this was dropped face down on the fixative. In general, silver nitrate preparations were more useful than protargol preparations, because fixation in Champy’s fluid caused less shrinkage and therefore also less congestion of buccal structures than Bouin’s and Hollande’s fixatives.

For transmission electron microscopy, ciliates were fixed for 1 hr in a solution of 2.5% glutaraldehyde, 1% freshly prepared formaldehyde (from para-formaldehyde), and half-strength seawater in 0.04M cacodylate buffer pH 6.8. After being rinsed in 0.2M buffer with 0.02 M glycine, the ciliates were enrobded in 1% low-melting-point agarase, then washed 5 times over 24 hr in buffer plus glycine, post-fixed for 1 hr in 2% osmium tetroxide, stained en bloc in the dark for 1 hr with 0.5% uranyl acetate, dehydrated in an ethanol series, infiltrated, and embedded in LR White resin. Thin sections were collected on collodion-coated grids, stained with Reynolds lead citrate, and imaged with a JEOL 100CX TEM.

Cultivation of ciliates of the free-living phase

Ciliates taken from the testis of Leptasterias spp. were transferred, with a piece of testis tissue about 3-4 mm in diameter, to small screw-cap jars containing about 15 ml of sterile seawater. The jars were kept in a refrigerator at 6°C. In a few days, by which time the ciliates had fed not only on sperm but also on bacteria that had multiplied in the culture,
subcultures were established in infusions of tissue taken from
the body wall of *Eudistylia polymorpha* (Polychaeta) and the
mantle of *Lottia pelta* (Gastropoda). The infusions were
prepared by dropping pieces of tissue, usually about 2-4 mm
in diameter, but sometimes larger, into boiling-hot seawater
in sterile screw cap jars. Eventually tissues taken from the
body wall of other polychaetes, the mantle, ctenidium, and
digestive gland of other molluscs, muscle of the domestic
chicken, and an 0.01% solution of yeast extract were used
with equal success. The cultures were kept in a refrigerator at
6°C. Bacteria, mostly bacilli, were the only foreign
organisms detected in the cultures.

Numerous clonal cultures, also maintained at 6°C, were
established by pipetting a few ciliates from a bacterized
culture into a drop of sterile seawater, then micropipetting
them through a succession of drops until it was clear, at a
magnification of 25X, that single ciliates had been isolated.
These were transferred into jars containing infusions of
tissue prepared according to the protocol described above.
Seven of the clonal cultures were used for monitoring
changes in size, general appearance, and numbers of
kineties in ciliates as they adapted to favorable nutrition
and later to near starvation. These same lines were also
used for following changes taking place when small,
poorly-nourished ciliates were introduced into rich
infusions of living sperm from non-parasitized *Leptasterias*
spp. and two other sea stars, *Pisaster ochraceus* and
*Pycnopodia helianthoides* (the latter is not known to be
parasitized by *O. stellarum*).

**Results**

*Morphological characteristics of the parasitic phase*

Sections of reproductively mature testis tubules of asterid
sea stars show that non-parasitized tubules are full of sperm
(Fig. 1A). In parasitized individuals, however, the concen-
tration of *O. stellarum* typically eventually becomes so
dense that sperm appear to have been completely replaced
(Fig. 1B, C).

Cépède (1907, 1910) classified *O. stellarum* as an
astomatous ciliate. It was not until Bouland et al. (1987)
carefully studied this organism from *Asterias rubens* that
the morphology of the species was accurately described.
These authors showed that it had a functional buccal cavity
of the type characteristic of the class Scuticociliata.
Leighton et al. (1991) confirmed that the ciliate parasitizing
*Pisaster ochraceus* in British Columbia was *O. stellarum*.
Ciliates we have taken from *Pisaster ochraceus*, *Evasterias
troschelii*, and species of *Leptasterias* collected in
Washington conform to the redescription given by Bouland
et al.
The length and width of *O. stellarum* from *Asterias rubens* were stated by Bouland et al. to be, respectively, about 50 and 16 µm; in specimens from *A. amurensis*, the length was 35 to 65 µm (Goggin & Bouland, 1997). The size of 20 living specimens from one *Leptasterias* ranged from 47 by 15 µm to 55 by 17 µm, but in smears from other host individuals ciliates as small as 36 by 14 µm and as large as 62 by 24 µm were observed. These measurements

**Figure 2.** *Orchitophrya stellarum*, parasitic phase. *A, B.* Protargol impregnations, showing caudal cilia. *C-F.* Silver nitrate impregnations. *C.* Ventral view, showing buccal cavity close to anterior apical region. *D.* Lateral view from left side. *E.* Anterior apical view, showing paroral membrane, membranelles, and short suture formed by several kineties lateral and anterior to buccal cavity. *F.* Posterior portion, showing kinetosome of caudal cilium (arrowhead) in apical area bordered by terminal portions of somatic kineties; only a small part of the cilium is in focus. *m1, m2, m3:* buccal membranelles. *ma:* macronucleus. *mi:* micronucleus. *pm:* paroral membrane. *s:* scutica.

are in general agreement with those of Cépède (1907, 1910) who stated that the length of ciliates from Asterias rubens ranged from 35 to 65 µm and the width from 12 to 26 µm.

In specimens freshly taken from the testis of a parasitized Leptasterias, the anterior one-fourth or one-third of the body is noticeably narrower than the rest (Fig. 2A, B). The buccal cavity is difficult to see in living specimens, partly because it lies in an area where the somatic kineties are converging toward the rather sharply-pointed anterior end, and partly because its own membranelles help to obscure it. Specimens impregnated with silver nitrate, however, generally show at least the paroral membrane (Fig. 2C, D, E) and sometimes also the membranelles and a distinct pre-buccal suture formed by a few kineties approaching the apex (Fig. 2E).

Impregnation with protargol often clearly demonstrates the caudal cilium (Fig. 2A, B), as well as the kinetosome from which it originates; this is located in the area bordered by the ends of the kineties at the posterior tip of the body (Fig. 2F). Specimens with two caudal cilia (Fig. 2B) are occasionally encountered.

TEM images show digestive vacuoles, some containing heads of sperm (Fig. 3A, B). Putative acidosomes appear to be concentrated anterior to the nuclei and to be more scattered posterior to the nuclei (Fig. 3A).
Transition from the parasitic phase to the free-living phase

Ciliates cultured in infusions of fresh testis tissue continue to feed on living sperm as long as these are available (usually 3-4 days at 6°C), then on dead sperm and bacteria that have become abundant. The ciliates multiply rapidly by fission and will continue to do so if the infusion is not so rich that bacterial putrefaction takes place. They also multiply after they have been transferred to infusions of tissue from other invertebrates, meat from the domestic chicken, and 0.01% yeast extract. All they need, apparently, is a source of bacteria, although they also metabolize tissue detritus to some extent.

The size of the ciliates becomes smaller once they are no longer feeding almost entirely on sperm, but it generally stabilizes at about 35 by 14 µm to 45 by 18 µm, unless the culture is allowed to become so old that insufficient food is available. In that case, the size range of most ciliates is usually about 20 by 7 µm to 30 by 11 µm, but the size of some may drop to as low as 15 by 5 µm. Nevertheless, a few starving cultures remained viable for more than 11 months after they were established.

In cultures, the number of somatic kineties declined more or less in accordance with size. Ciliates about 30-35 µm long generally have 11-12 kineties; ciliates about 25 µm long have 10 or 11 kineties. In spite of the fact that hundreds of silver-impregnated specimens have been studied with respect to the number and arrangement of kineties, short rows of kinetosomes that could tentatively be interpreted as remnants of kineties were only rarely noted (Fig. 4A).

The general appearance of *O. stellarum* changes gradually after it has been established in cultures. By the time the length has dropped to about 40 µm, the ciliates are proportionately wider and no longer pointed at the anterior end. The buccal cavity, now more easily observed than it is

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**Figure 4.** *Orchitophrya stellarum*. Photomicrographs of specimens from bacterized cultures, impregnated with silver nitrate. A. Nearly ventral view, showing buccal cavity displaced nearly to middle of body. A few isolated kinetosomes (arrowheads), probably remnants of a kinety that has mostly disappeared, can be seen between two normal kineties. B. Lateral view, showing 11 somatic kineties converging at anterior apical region. C. Posterior apical view showing kinetosome of caudal cilium. D. Specimen from a culture to which fresh sperm of *Pycnopodia helianthoides* had been added, ventral view, showing short suture formed by 3 somatic kineties anterior to buccal cavity. **m1, m2, m3**: membranelles. **pm**: paroral membrane.
in the parasitic phase, is closer to the middle of the body (Fig. 4A, B). The pre-apical suture has disappeared, and all kineties, including those closely adjacent to the buccal cavity, now terminate around a circular area at the anterior end (Fig. 4C). The caudal cilium is usually 9 or 10 µm long, about the same length as it is in ciliates of the parasitic phase, but much longer in proportion to body length. In favorably-oriented silver-impregnated specimens, the kineties of the caudal cilium is usually distinct in the circular area bordered by the posterior ends of the somatic kineties; sometimes it appears to be part of the terminal portion of a kinety that intrudes into this area (Fig. 4C).

Transition from the free-living phase to the parasitic phase

If an infusion of fresh testis tissue of *Pisaster ochraceus*, *Pycnopodia helianthoides*, or a species of *Leptasterias* is inoculated with a rich concentration of cultured ciliates about 25-35 µm long, these immediately begin to ingest sperm, and within three days some of them are close to the maximum size of ciliates found in a parasitized testis. The changes undergone by the ciliates as they become adapted to culture conditions are now reversed. As they grow, they add kineties, and the buccal cavity is gradually displaced to a more anterior position. A few kineties lateral to the buccal cavity converge in such a way that they form a pre-apical suture (Fig. 4D) similar to that of ciliates of the parasitic phase. We have not determined where new kineties are formed.

Conjugation was observed frequently in cultures; whenever it was noted, many pairs were usually involved at the same time. We are unable to connect conjugation with any particular stage in the history of a culture. Cépède (1910) once observed a thick-walled cyst in a putrefying parasitized testis, but this is not likely to have been of *O. stellarum*.

Discussion

Although *O. stellarum* in the testis of a sea star is viewed as a parasite, the fact that it can subsist on bacteria in infusions of yeast extract and animal tissue strongly suggests that it is probably of widespread occurrence in situations where organic substances are undergoing decomposition. Cépède (1910) reported that ciliates from *Asterias rubens* could live in seawater for 16 days, and Goggin & Bouland (1997) stated that ciliates from *A. amurensis* survived seawater culture for 14 days, during which time their length decreased to 29 µm. We have shown that *O. stellarum* can live indefinitely in cultures, even those in which there is minimal food. We therefore view it as a facultative parasite that is probably widely distributed in the world oceans.

The changes in general appearance and number of kineties of *O. stellarum* after it has been removed from a testis and established in culture is somewhat similar to the way another ciliate, *Tetrahymena limacis*, inhabiting tubules of the digestive gland of certain terrestrial slugs and snails, changes when it is cultured in tissue infusions (Kozloff, 1956). The parasitic phase of *T. limacis* differs from the cultured phase in being, on average, appreciably larger and noticeably pointed at the anterior tip of the body; it also has a proportionately smaller buccal cavity and more kineties. The parasitic phase, however, is decidedly unlike that of *O. stellarum* in that it rarely shows digestive vacuoles; it probably subsists mostly on soluble or colloidal nutrients provided by its host. When cultured axenically in 1‰ yeast extract, it resembles the parasitic phase in being slightly pointed at the anterior end and not forming obvious digestive vacuoles.

Early reports of *O. stellarum* as a parasite of sea stars in the European north Atlantic were followed by accounts of its presence on the east coast of North America, then the Pacific Ocean. We do not know where its parasitism of sea stars evolved, but perhaps it could have been in the Pacific, in association with *Asterias amurensis*. This sea star is thought to have arrived in the North Atlantic from the Pacific during the trans-Arctic interchange, about 3.5 million years ago, and to have evolved into *A. forbesii* and *A. rubens* (Wares, 2001). *Pisaster ochraceus* appears to cluster closely with *Asterias* spp.; *Leptasterias* spp. are somewhat more distant in both of the morphological consensus tree constructed by Mah (2000). Could *O. stellarum* have invaded the North Atlantic during the trans-Arctic exchange or by some other route? Only genetic analyses will explain the geographic origin and probable trajectory of invasion of *O. stellarum* into asteriid sea stars. To at least some extent, however, the matter of where and in what host it first became parasitic may be moot, because the ciliate could have had an almost worldwide distribution as a free-living, bacteriophagic and/or histiophagic organism long before it entered the testis of a sea star.

As a phagocytic consumer of bacteria and sloughed-off epidermal tissue, *O. stellarum* perhaps may reside on the surface of sea stars throughout the year and invade the testes only when these are in an advanced state of development. On the west coast of North America, its parasitism of *Leptasterias* spp. could conceivably alternate with parasitism of spring- and summer-spawning *Evasterias troschelii* and *Pisaster ochraceus*; on the Atlantic coast, ciliates emerging from *Leptasterias* spp. could perhaps enter the testes of *Asterias forbesii* and *A. rubens*.

The mode of transmission of *O. stellarum* is not known with certainty, but it is likely to enter the testes through gonopores. This is suggested by the fact that the number of parasitized testes and the extent of damage to the testes varies from one male sea star to the next (Burrows, 1936;
Ververs, 1951; Bouland & Jangoux, 1988; Goggin & Bouland, 1997).

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