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Oodinium inlandicum sp. nov. (Blastodiniales, Dinophyta), a new ectoparasitic dinoflagellate infecting a chaetognath, *Sagitta crassa*

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Abstract: A new ectoparasitic dinoflagellate, *Oodinium inlandicum* Horiguchi et Ohtsuka sp. nov. infesting a neritic chaetognath, *Sagitta crassa* Tokioka is described from the Seto Inland Sea, western Japan. This is the first record of the genus *Oodinium* in the western North Pacific. The new species is readily distinguished from other congeners by: (1) host specificity; (2) the shape and location of the nucleus; and (3) the morphology of the peduncle. The present study has revealed that as development proceeds, the nucleus and cytoplasm change markedly. The cytoplasm of the mature trophont consists of a central endoplasm, rich in cytosol and a highly membranous exoplasm. The nucleoplasm is uniform and lacks electron dense structures, such as chromosomes. In contrast, the young trophont has an elongated dinokaryotic nucleus, which almost completely occupies the anterior half of the cytoplasm. The peduncle penetrates host tissue, but mechanical damage caused by the parasite seems not to be extensive. The comparative ultrastructure and ecology of the new parasite are also discussed.

Key words: dinoflagellate, *Oodinium inlandicum* sp. nov., ectoparasite, chaetognath, *Sagitta crassa*

Introduction

A large number of dinoflagellates are known to parasitize marine vertebrates and invertebrates (e.g., Chatton 1920; Cachon & Cachon 1987; Shields 1994; Ohtsuka et al. 2000). Those parasitic dinoflagellates only displaying a typical dinokaryotic nucleus during a part of their life cycle are classified into the order Blastodiniales, class Blastodiniphyceae (Fensome et al. 1993). Currently, six families are recognized in the order Blastodiniales. One of these, the family Oodiniaceae, has members which are characterized by being ectoparasitic and possessing suboval to fusiform cells with well-developed absorption apparatuses in the trophont stage. This results in very atypical dinoflagellate morphology, unlike the trophonts of the Protoodiniaceae, which are clearly recognisable as belonging to this division. The type genus of the family, *Oodinium* was established by Chatton (1912) and consists of ectoparasites of metazoans such as annelids, appendicularians, and chaetognaths (Chatton 1912; Cachon 1964; McLean & Nielsen 1989).

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During our survey of parasites of zooplankton in the Seto Inland Sea, western Japan, the most common neritic chaetognath encountered was *Sagitta crassa* Tokioka, and it was heavily infested by an *Oodinium*-like dinoflagellate. Light and electron microscopical observations have revealed that this is an undescribed species of the genus *Oodinium*. The taxonomy of parasitic dinoflagellates in Japanese waters is poorly understood (Ohtsuka et al. 2000), and thus biodiversity studies centering on parasitic dinoflagellates are badly needed.

Materials and Methods

Sampling

Host zooplankton were collected on three separate occasions (September and October 1999 and October 2000) by nocturnal surface net tows using a conical net (diameter 30 cm; mesh size 0.1 mm) in the center of the Seto Inland Sea, western Japan, (34°19′N, 132°55′E). Chaetognaths with attached dinoflagellates collected on 9 September 1999 were used for the current descriptions and measurements.

Light microscopy

Chaetognaths infested by *Oodinium* cells were fixed in 10% neutralized formalin/sea-water and observed using a Nikon Eclipse E600 microscope. For observation of the thecal plates, fixed dinoflagellate trophonts were stained with 1% Fluorescent Brightener 28 (Sigma) and observed with a Nikon Optiphoto-2 epifluorescent microscope, equipped with a Nikon EFD2 epiflourescent device and with an UV-2A filter set). The nucleus was visualized by staining fixed cells with DAPI (4′-6-Diamidino-2-phenylindole) (0.5 g ml^{-1}) and observing them with the same epifluorescent microscope. Measurement of the body length of *Sagitta crassa*, from the anterior tip of the head to the posterior end of the caudal fin, was made on all undamaged individuals in the sample collected on 9 September 1999. The degree of infection relative to host size and the sites of attachment of *Oodinium* to the body of *S. crassa* were noted. The frequency of each attachment site in each size class of the host was calculated as a percentage of the total number of sites.

Transmission electron microscopy (TEM)

Parasitized specimens of *Sagitta crassa* were fixed in the field in 2.5% glutaraldehyde made up with seawater, and then transported at about 5°C to the laboratory. The fixed specimens were rinsed with seawater and postfixed in 2% Osmium tetroxide for 1 h. These were dehydrated through an ethanol series and finally embedded in Spurr's resin (Spurr 1969). The chaetognaths were individually mounted in a small amount of resin between two sheets of overhead projector (OHP) transparency film prior to polymerization so as to obtain thin flat embedded samples. After polymerization, *Oodinium* cells were located using a light microscope and excised (size 1×2 mm), together with a small piece of the host from the thin resin plate using a razor blade. These excisions were then stacked on to a plastic block for sectioning. Observations were made with a JEOL 100S transmission electron microscope.

Results

Description

***Oodinium inlandicum* Horiguchi et Ohtsuka sp. nov.**

Ectoparasita chaetognathae, *Sagittae crassae*. Cellula vegetativa (trophont) ellipsoidea vel bacilliformis, 30–150 μm longa; cytoplasma luteoli-brunneola granulataque; pedunculus bulbiformis vel bacilliformis, 6–500 μm longus et fere hyalinus, cum striis longitudinalibus numerosique; nucleus cellulae maturae non-dinokaryonticus hemisphaericusque, in parte extremitas supera cellulae situs; nucleus in cellula immatura, dinokaryonticus ellipsoideaque; thecam habens; sine chloroplasti et stigmate.

Ectoparasite on chaetognath, *Sagitta crassa*. Vegetative cell (trophont) oval or rod-shaped, 30–150 μm in length; cytoplasm yellow-brown, granular in appearance; peduncle bulbous to rod-shaped, 6–500 μm in length, almost transparent, with many longitudinal striations; nucleus in mature cell non-dinokaryotic, hemispherical in shape, situated in the upper extremity of the cell; nucleus in immature cell di-

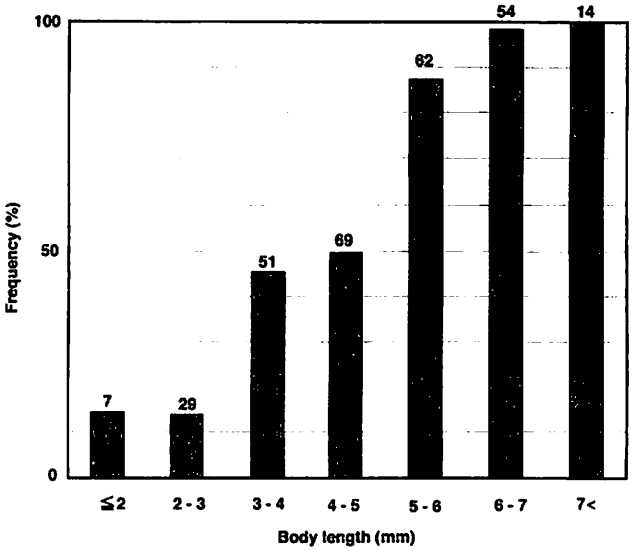

Fig. 1. Relationships between prevalence of infection of parasite *Oodinium inlandicum* sp. nov. and body length of host *Sagitta crassa*. Number of chaetognaths examined is shown above each column.

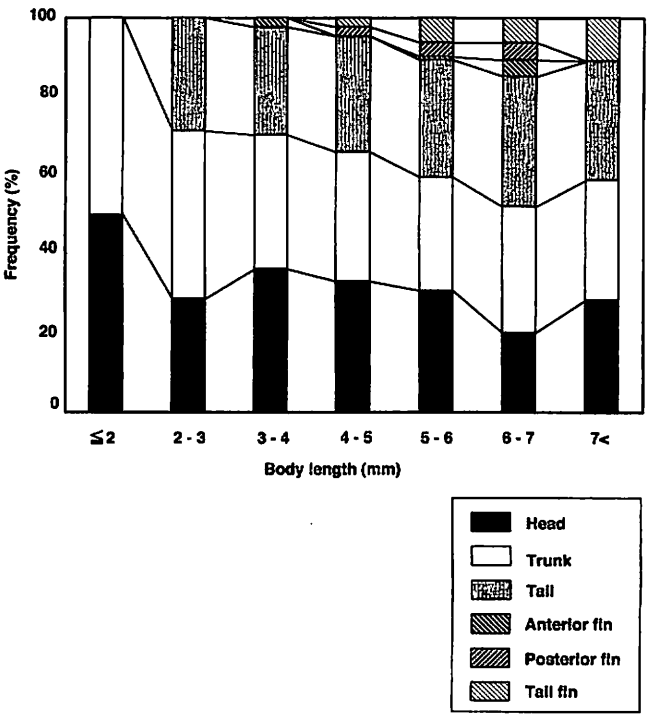

Fig. 2. Distribution of *Oodinium inlandicum* sp. nov. on body of host *Sagitta crassa*. Number of individuals examined is same as in Fig. 1.

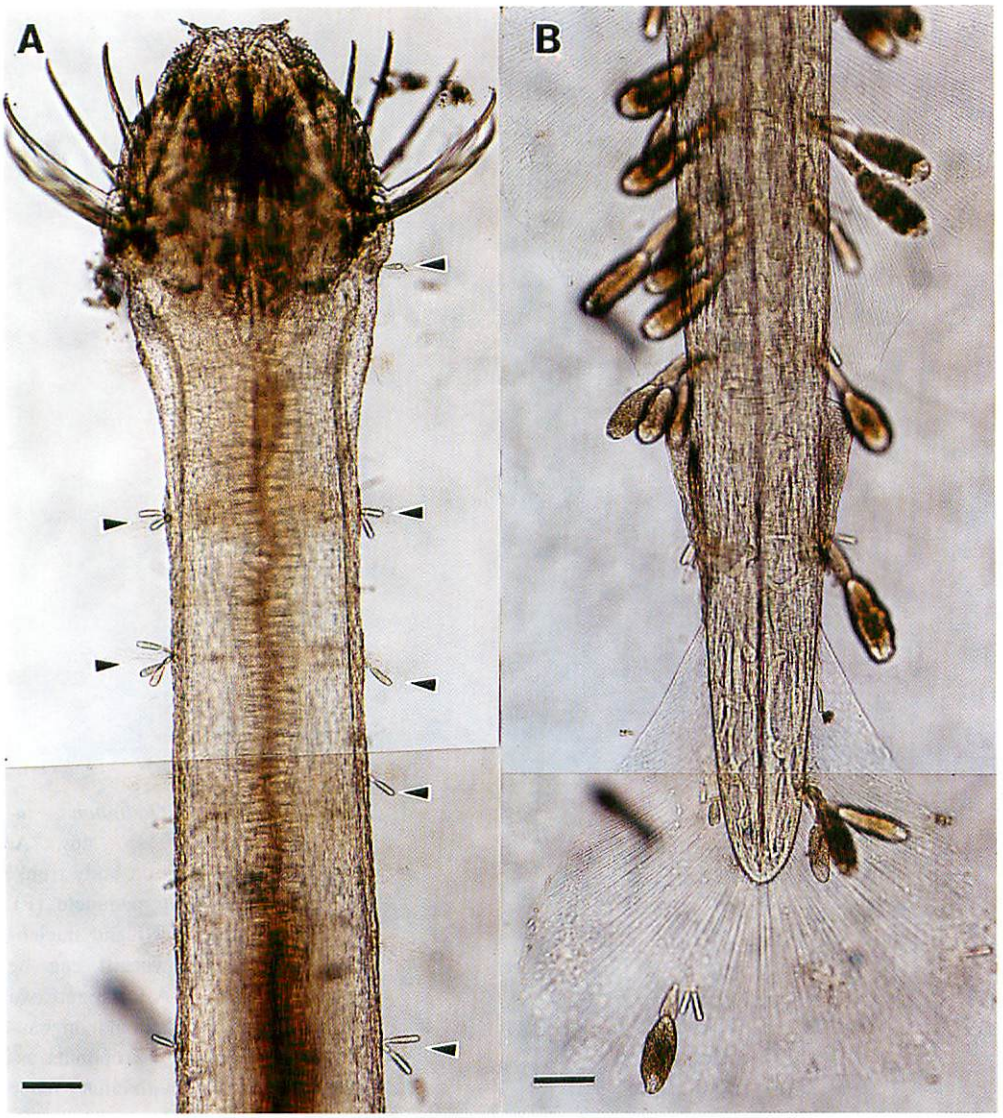

Fig. 3. Chaetognatha, *Sagitta crassa* heavily infected by *Oodinium inlandicum* sp. nov. **A**. Anterior part of *Sagitta crassa*. Arrowheads indicate *Oodinium* trophonts. **B**. Posterior part, infected by larger dinoflagellates. Scale bar = 100 μm.

nokaryotic, oval; thecal plates present; no chloroplasts and eyespot present.

Holotype: An embedded specimen in epoxy resin (mounted on a slide glass) has been deposited in the herbarium of the Graduate School of Science, Hokkaido University as SAP 089327. Isotype: SAP 089328.

Type locality

Takehara (34°19′N, 132°55′E), Hiroshima Prefecture, western Japan (Seto Inland Sea).

Etymology

The epithet refers to the type locality, viz. the Seto Inland Sea. The new dinoflagellate has hitherto been known only in this inland sea region.

Trends of infection

Infestations of *Oodinium inlandicum* were apparent in collections of *Sagitta crassa* made during September and October 1999 and again in October 2000. The occurrence of this parasitic dinoflagellate seems to have been restricted to warm water seasons, i.e. water temperature between 18–26°C, although *Sagitta crassa* persisted year-round in the Seto Inland Sea. It is interesting to note that a coexisting species of chaetognath, *Sagitta enflata* Grassi, at all times was found to be unaffected by the new dinoflagellate. Larger specimens (more than 6 mm long) of *S. crassa* tended to be infected more heavily by the dinoflagellate than small ones (Fig. 1). Ninety eight percent of chaetognaths larger than 6 mm were infected by the dinoflagellate, while less than 15% of specimens smaller than 2–3 mm long were infected (Fig. 1). Attachment sites of the parasite on the body of *S. crassa* are shown in Fig. 2 according to host size. Trophonts of *O. inlandicum* preferentially at-

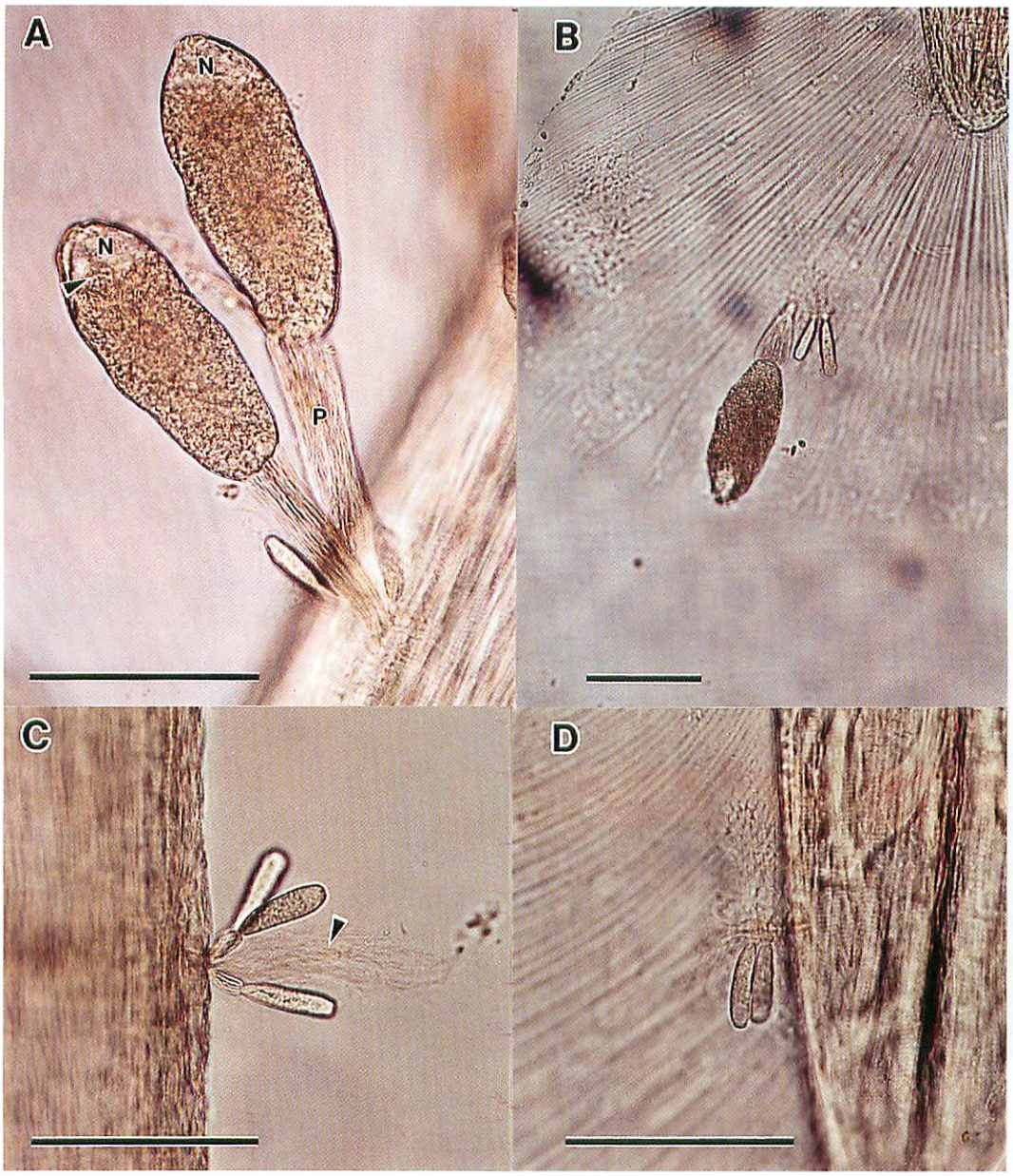

Fig. 4. *Oodinium inlandicum* sp. nov. **A.** Trophonts on body trunk. Note long peduncle (P). Nucleus (N) and nucleolus (arrowhead) can be seen. **B.** A large and two young trophonts on caudal fin. **C.** Trophonts associated with ciliary fence receptor (arrowhead). **D.** Two young trophonts on caudal fin. Scale bar= 100 μm

tached to the body (head, trunk and tail) of *S. crassa*, but were also less frequently observed on the fins (Figs 3, 4). The head, trunk and tail of the host were almost equally utilized as attachment sites except in small (less than 2 mm) chaetognaths. The anterior, posterior and tail fins were less frequently (2–15%) colonised by the parasite. The trophonts were often found to associate with the ciliary fence receptors on the body of *S. crassa* (Fig. 4C).

Light microscopy

Trophonts are oval to rod-shaped, 30–150 μm in length with a distinct absorption apparatus (peduncle) (Figs 3, 4). The size of the peduncle is extremely variable and is 6 to 500 μm in length. The peduncle length of large cells (longer than 130 μm) varies greatly, from 72 to 493 μm (Fig. 5). The significance of a long peduncle is, however, as yet unknown. Mature trophonts are tinged yellow-brown and are uniformly granular, except for the upper extremity where a nucleus is located (Figs 3, 4). This nuclear region is hemispherical and appears to be more or less transparent. In younger trophonts, the color of the cell is paler and the distinction between the nuclear region and the remainder of the cell is less evident. In the mature trophont, the nucleus is non-dinokaryotic and no condensed chromosomes can be seen (Fig. 4A). A nucleolus-like spherical structure can be seen in the nucleus (Fig. 4A). The nuclear region occupies about 1/5–1/4 of the total cell length. Immature trophonts possess a distinct, elongated nucleus, which occupies almost half of the cell length and it exhibits a typical dinokaryotic condition. Numerous granular chromosomes can be seen when cells are stained with DAPI (Fig. 6). No

other organelles are visible in either mature or immature trophonts with the light microscope. The cell covering of the trophonts is composed of thecal plates, which are clearly visible when they are stained with fluorescent dye (Fig. 7). The thecae consist of four equatorial series of thecal plates. However, assignment of these plates into conventional Kofoid's tabulation system (Fensome et al. 1993) is difficult, due to a lack of conventional reference points for the recognition of plate series. The absorption apparatus is referred to as the peduncle (McLean & Nielsen 1989), although whether it has truly originated from the peduncle of a motile cell or not remains unclear at present. The shape of the peduncle varies from bulbous to rod-shaped (Figs 3, 4), is almost transparent and its surface is heavily ornamented by many longitudinal striations (Fig. 4A). The tip of the peduncle is embedded in the host tissue, but penetration seems superficial. Although the trophonts are firmly attached to the host tissue by the peduncle, the connection between the cell body and the peduncle is rather loose and the cells are readily detached from the peduncle during routine handling of the specimens.

Transmission electron microscopy

In the mature trophont, the cytoplasm consists of two parts, a central endoplasm rich in cytosol and a highly membranous exoplasm.(Fig. 8A). The complex membrane system in the exoplasm is composed of a folded membrane layer each consisting of three membranes (Fig. 9B). These membranes are probably derived from an invagination of the cytoplasmic membranes (Fig. 9D). The narrow cytoplasmic region between the membranes in the exoplasm is chiefly occupied by mitochondria (Fig. 9B). The nucleus is hemispherical and located in the upper extremity of the trophont (Figs 8, 9A). The nucleoplasm is uniform and no electron dense structures have been observed. Trichocysts, typical of dinoflagellates, have been observed both in the upper and lower extremities of the cell, but more trichocysts are found near the nucleus (Fig. 9A). Spherical lipid granules are present in the cytoplasm (Figs. 9B, C), but no starch-like grains and no chloroplasts have been observed. In the young trophont (Fig. 8B), the upper half of the cytoplasm is almost fully occupied by the elongated nucleus. The nucleus is typically dinokaryotic and condensed chromosomes and profiles of a nucleolus are visible. The lower half of the cell is composed of the same endoplasm and exoplasm as in the mature trophont, but the membranous nature in the exoplasm of the young trophont is less prominent compared to that of the highly membranous exoplasm in the matured individuals. The amphiesma or cell covering of the trophont consists of a plasma membrane, an outer plate membrane, followed by the thecal plates and cytoplasmic membranes of unknown number (Fig. 9C). No pellicular layer or uniform cell wall layer has been detected beneath the thecal plates, nor was any ornamentation found on the surface of the thecal plates. The pusule (Fig. 10B) is located near the peduncle and is typical of dinoflagellates (Dodge 1972). It consists of a chamber and surrounding pusular vesicles. The chamber opens to the outside via a pore from which the peduncle also emerges.

The proximal part of the peduncle inside the trophont is

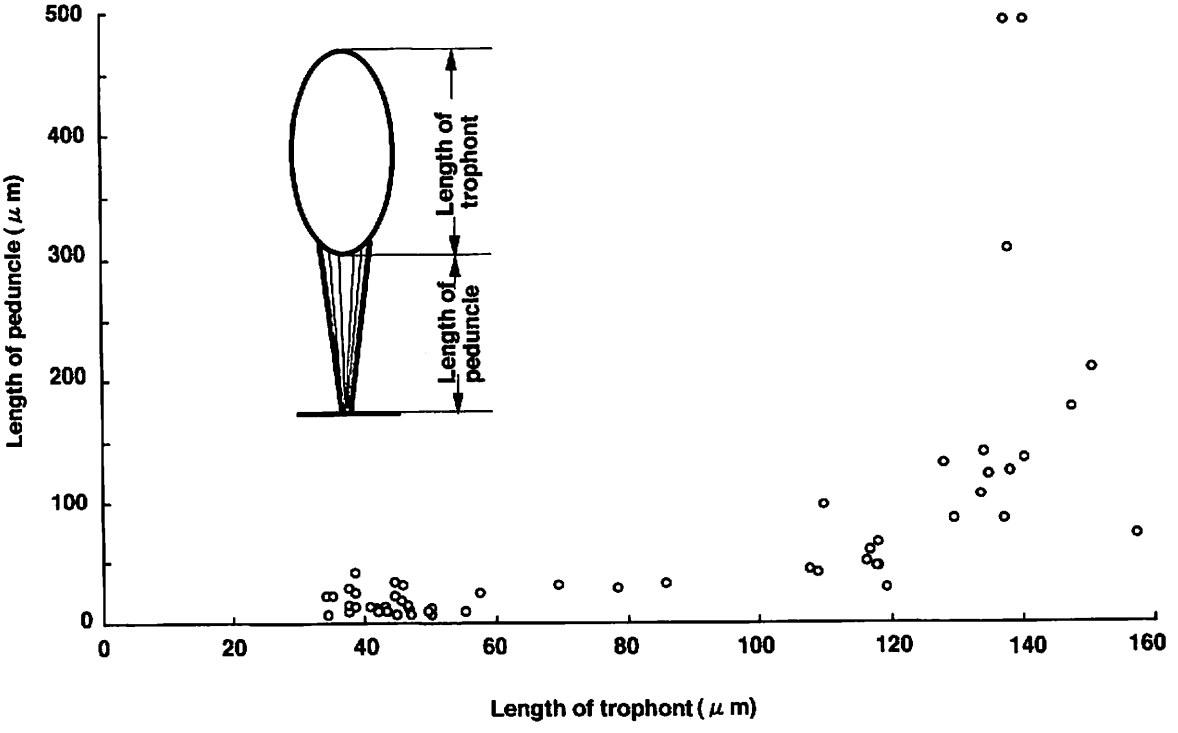

Fig. 5. *Oodinium inlandicum* sp. nov. Relationship between cell length and length of peduncle.

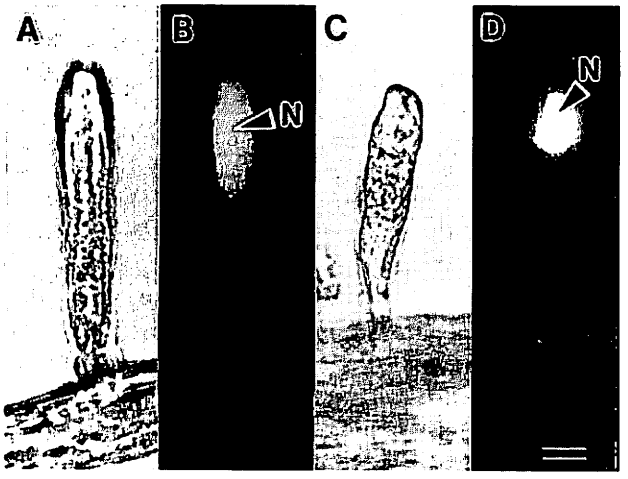

Fig. 6. *Oodinium inlandicum* sp. nov. Nature of nucleus (N) at different life cycle stages. **A.** Bright field light micrograph of immature trophont. **B.** DAPI stained cell (same as A) showing numerous granular chromosomes typical of dinokaryotic nucleus. **C.** Bright field light micrograph of mature trophont. **D.** DAPI stained cell (same as C), showing non-dinokaryotic nature of nucleus. Scale bar=10 μm.

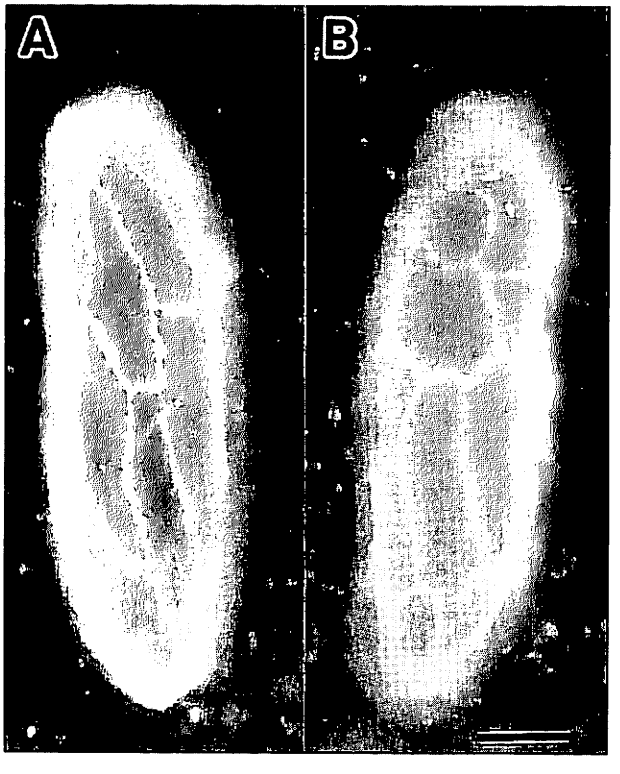

Fig. 7. *Oodinium inlandicum* sp. nov. Thecal plate arrangement is revealed by fluorescent dye, Fluorescent Brightener 28. **A**, **B** show different views. Scale bar=2 μm.

conical in shape, and it is this from which the main part of the peduncle develops (Fig. 10A). The cytoplasm of this part consists of both electron lucent and electron dense materials. This conical part does not extend far into the cyto-

Fig. 8. *Oodinium inlandicum* sp. nov. Transmission electron micrographs. **A.** Longitudinal section through matured trophont. N: nucleus, P: peduncle. **B.** Longitudinal section through immature trophont. Note that nucleus (N) shows typical dinokaryotic nature. P: peduncle. Scale bar=10 μm.

plasm and no constriction as can be seen in other members of *Oodinium* is apparent here. It superficially resembles the nucleoplasm of ordinary eukaryotic interphase nuclei, but observations with DAPI revealed the absence of DNA in this region (Fig. 6). The peduncle emerges from a pore, which is lined by the thecal plates (Fig. 10B). The pore is not bordered by an electron dense ring as in other *Oodinium* species. The bulk of the peduncle (outside the trophont) is composed of highly reticulate cytoplasm, giving it a sponge-like appearance (Fig. 10). In the section, several cytoplasmic "islands" are connected to each other by a network of thread-like (reticulate) cytoplasm. The "islands" also exhibit the electron lucent and electron dense nature, but no identifiable structures are present. In some cases, the dinoflagellate attacked the site of ciliary fence receptors, the sensory organ. The tip of the peduncle is entangled with the ciliary hairs (Fig. 10C). The peduncle penetrates the host tissue, and the tip widens and branches in a finger-like fashion (Fig. 10D). The cytoplasm here contains fibrous materials, but no membranous invaginations or other special structures have been observed (Fig. 10D). Penetration is rather superficial and mechanical damage caused by the dinoflagellate does not appear extensive.

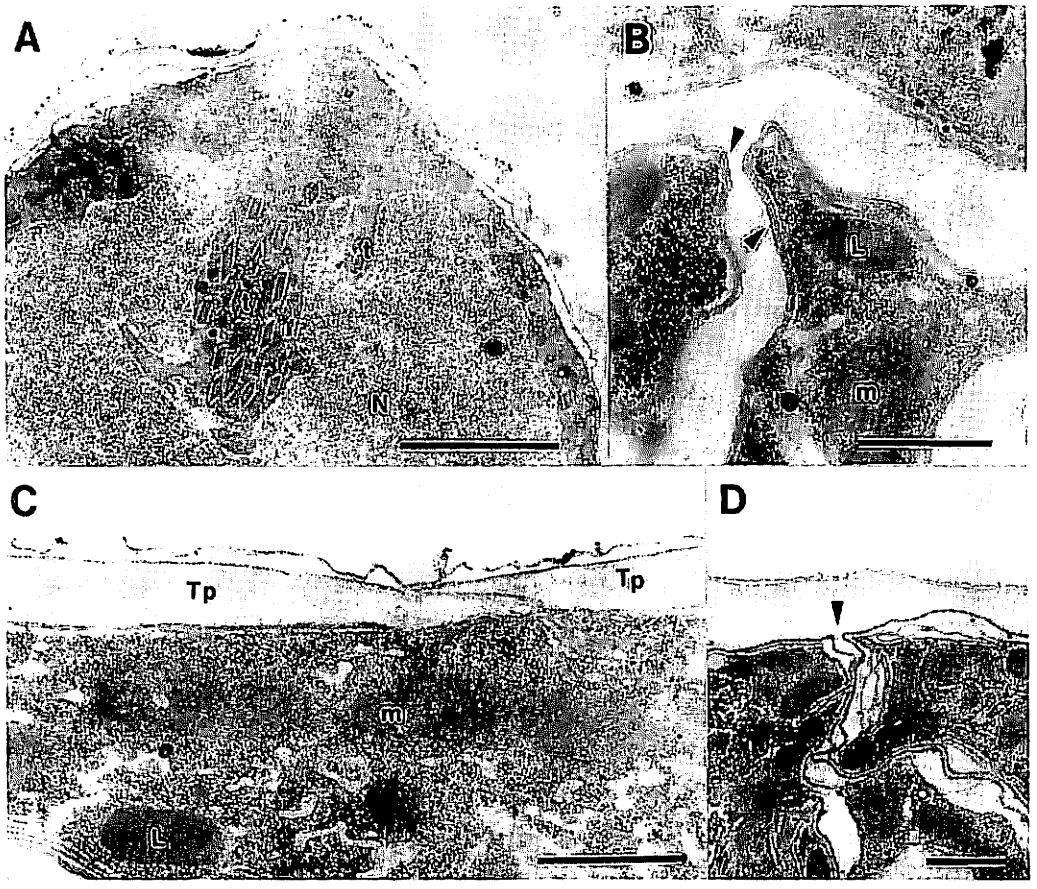

Fig. 9. *Oodinium inlandicum* sp. nov. Transmission electron micrographs. **A.** Close-up photograph of upper part of the cell, showing part of non-dinokaryotic nucleus (N) and groups of trichocysts (t). **B.** Close-up of exoplasm, showing triple membrane nature (arrowheads) of invaginated membrane system. L: lipid grain, m: mitochondrion. **C.** Section through thecal plates (Tp). Suture of two plates can be seen. L: lipid grain, m: mitochondrion. **D.** Portion of exoplasm, showing the point where one of the membrane invaginations commences (arrowhead). A: Scale bar=10 μm; B–D: Scale bar=1 μm.

Discussion

Taxonomy

The possession of both non-dinokaryotic and dinokaryotic nuclei within the life cycle of an ovoid ectoparasite with a well developed peduncle support the placement of this taxon in the family Oodiniaceae of the order Blastodiniales (Fensome et al. 1993). In fact, the dinoflagellate described in this paper shows similarity with the members of the genus *Oodinium* and its allied genera, such as *Amyloodinium*, *Crepidoodinium* and *Piscinoodinium*. However, this species is clearly distinguishable from members of the genera *Crepidoodinium* and *Piscinoodinium* which are characterized by the possession of chloroplasts and the lack of thecal plates (Lom 1981). According to Brown & Hovasse (1946), *Amyloodinium* can be distinguished from *Oodinium* by the possession of rhizoid- and root-like processes for attachment and by the production of starch grains. The genus *Oodinium*, on the other hand, is characterized by the possession of a disk, rather than rhizoids, for attachment and by the lack of starch grains. The present dinoflagellate does not produce rhizoids or root-like projections for attachment and starch-like granules are absent. Although attachment is not achieved by the production of a disk, other features, such as the gross morphology, the presence of a fibrillar peduncle and the absence of starch, support the placement of this dinoflagellate in the genus *Oodinium* rather than in *Amyloodinium*. The presence of thecal plates in the type species of the genus, *O. pouchetii* (Hovasse 1935), and other species of *Oodinium* (Cachon & Cachon 1971) further supports the assignment of the present new species to this genus.

The genus *Apodinium* in the family Apodiniaceae also possesses somewhat similar organization to the members of the Oodiniaceae. However, the former family is distinguished from the latter by the mode of the life cycle. In *Apodinium*, cell division starts while the parasite is attached to the host, whereas in the Oodiniaceae, cell division only takes place after the parasite becomes detached from the host (Loeblich III 1982). Although we were not able to observe the complete life cycle of *Oodinium inlandicum*, we have never observed cell division in the trophonts attached to the host regardless of the size of the parasite. This indicates that *O. inlandicum* does not have the *Apodinium*-type of life cycle and thus the placement of our species in the genus *Oodinium* is justified.

Five species of *Oodinium* are currently recognized (Cachon & Cachon 1987, McLean & Nielsen 1989): *Oodinium pouchetii* (Lemmermann) Chatton, *O. fritillariae* Chatton, *O. acanthometrae* J. Cachon, *O. dogieli* J. et M. Cachon and *O. jordani* McLean et Nielsen. Table 1 summarizes the morphological characteristics of these species, with the exception of *O. acanthometrae*, a parasite of acantharians in the Mediterranean Sea, whose morphological details are

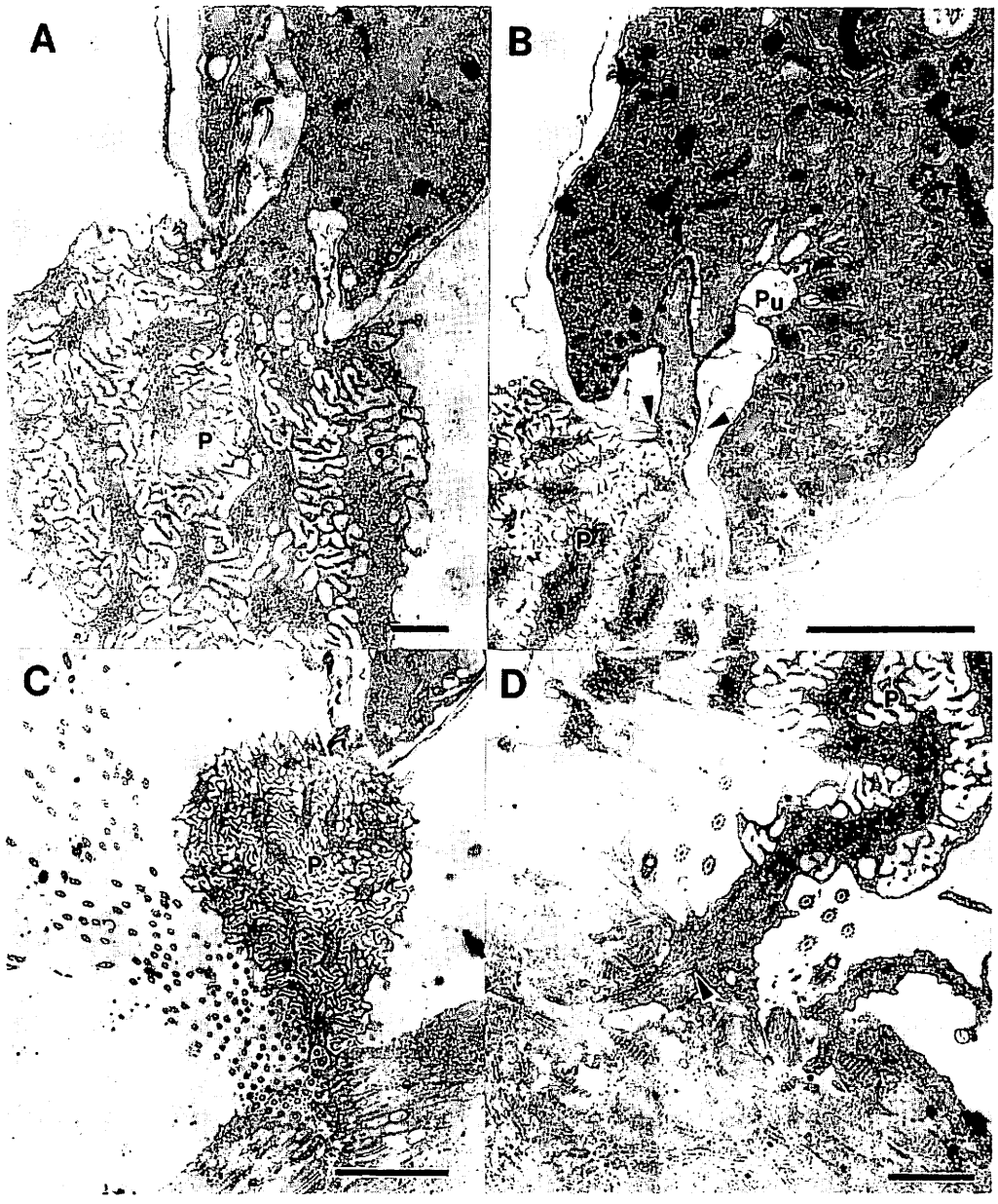

Fig. 10. *Oodinium inlandicum* sp. nov. Detail of peduncle (P). **A.** Close-up photograph of junction between cytoplasm and peduncle. **B.** Similar section as A, but pusule (Pu) is clearly visible. Arrowheads indicate terminal points of thecal plates which border a pore from which peduncle emerges. **C.** Infection point of peduncle. Many dot-like structures near peduncle are cross sections of axonemes of the ciliary fence receptor. **D.** Close-up of infection site. Tip of the peduncle branches off like fingers. Cytoplasm of this portion contains fibrous materials (arrowhead). A, D: Scale bar=1 μm. B, C: Scale bar= 5 μm.

poorly known (Cachon 1964; Cachon & Cachon 1971), and *Oodinium inlandicum* is clearly distinguishable from all of them. Of the species listed, *O. jordani* is most like *O. inlandicum* both with regard to gross morphology and to their hosts, which are different species of the chaetognath, genus *Sagitta*. A species similar to *O. jordani*, and almost from the same locality, was reported to parasitize five species of ctenophores (Ctenophora) and a hydromedusa (Cnidaria), but an accurate identification of this species has not been made (Mills & McLean 1991). *Oodinium inlandicum*, however, is different from *O. jordani* in several respects (McLean & Niellsen 1989) (see also Table 1): (1) The position and shape of the nucleus are markedly different: The nucleus of *O. jordani* is ovoid and almost central in the cell, whereas it is hemispherical and in the upper extremity of the cell in *O. inlandicum*. (2) The ultrastructure of the peduncle is also different: In *O. jordani*, the peduncle penetrates the host tissue and extends laterally, over 80 μm from the point of penetration, causing extensive damage to the host tissue. The terminal surface of the peduncle consists of many vase-like structures formed by an invagination of the double membrane system. The extent of penetration by *O. inlandicum* is not so extreme and damage to the host tissue seems to be minimal. There are no vase-like membrane invaginations at the terminal surface of the peduncle. (3) The parts of the host body that the dinoflagellate attacks are also different: *O. jordani* mainly parasitizes the fins, while *O. inlandicum* attacks any part of the host body, but less frequently the fins. (4) The geographical distribution of *O. jordani* appears restricted to northwest Washington, U.S.A. (northeast Pacific), while *O. inlandicum* appears restricted to the coastal waters of the Seto Inland Sea, Japan (north-

Table 1. Comparisons of morphology of trophonts of *Oodinium* spp.

	O. pouchetii	*O. fritillariae*	*O. dogieli*	*O. jordani*	*O. inlandicum*
Geographical Distribution	Mediterranean Sea	Mediterranean Sea	Mediterranean Sea	Off San Juan Is., Washington, USA	Seto Inland Sea, Japan
Host	Appendicularia	Appendicularia	Annelida	Chaetognatha	Chaetognatha
Cell					
Maximum size (length)	up to 180 μm	up to 130 μm	N.D.	up to 394 μm	up to150 μm
Cell shape	baloon-shaped	ellipsoidl	obovoidal	oval	oval to rod-shaped
Shape of nucleus	spherical to hemispherical	hemispherical	oval	oval	hemispherical
Position of nucleus	central to upper part	central	central	central	upper extremity
Thecal plates	present	present	present	present	present
Pusule	present	present	present	present	present
Peduncle					
Shape of attachemnt site	disk	disk	disk	disk	finger-like projections
Osmiophilic ring	present	present	present	present	absent
Constriction(s) in the middle of peduncle	present	present	present	present	absent
Membrane invaginations at terminal surface	present	present	present	present	absent
Reference	Chatton 1912, Hovasse 1935, Cachon & Cachon 1971	Chatton 1912, Cachon & Cachon 1971	Dogiel 1910, Chatton 1920, Cachon & Cachon 1971	McLean & Nielsen 1989	This paper

N.D. = no data.

west Pacific). *Oodinium dogieli*, an ectoparasite of Mediterranean annelids, is rather similar to *O. jordani* and thus has some resemblance to *O. inlandicum*. Once again, however, it is distinguished from the latter by the position and shape of the nucleus and by the shape of the peduncle, especially the portion retained inside the parasite (Dogiel 1910; Chatton 1920). The type of host organisms (annelid vs. chaetognath) and geographical distribution (Mediterranean Sea vs. northwestern Pacific) are also different.

Oodinium pouchetii and *O. fritilariale* are easily distinguished from *O. inlandicum* by their gross morphology, the shape of the peduncle, the position and shape of the nucleus, the host type and their geographical distribution (Table 1) (Chatton 1912, 1920; Hovasse 1935; Cachon & Cachon 1971). In conclusion, the present new parasitic dinoflagellate from Japan is clearly distinguishable from the known species of *Oodinium* and it is therefore appropriate to establish a new species, *Oodinium inlandicum* Horiguchi et Ohtsuka sp. nov.

Ultrastracture

The ultrastructure of the genus *Oodinium* has been investigated by Cachon et al. (1970), Cachon & Cachon (1971, 1977), McLean & Niellsen (1989), but these works have concentrated only on isolated components of the cell rather than dealing with the cell as a whole.

The peduncle is the most prominent organelle in the oodinioid dinoflagellates. It is essential for both attachment and absorption but its structure differs from genus to genus (Cachon & Cachon 1987). The peduncle of *Oodinium inlandicum* is quite different from those of known species of *Oodinium*, which hitherto were reported to consist of the following basic elements; the adhesive disc, the peduncle itself which is outside of the cell, the bulbous part (either single or double bulbs) and the terminal region which spreads out toward the nucleus. On the distal surface of the adhesive disc of *O. fritillariae* are numerous finger-like membrane invaginations which are connected to tubular channels that run through the peduncle to terminate in the perinuclear region. In *O. jordani*, however, although finger-like (vase-like) invaginations exist, no similar connections have been demonstrated. In *O. inlandicum*, there are neither finger-like projections on the distal surface of the peduncle nor continuous tubular connections within the peduncle. The nutrients from the host must be directly absorbed from the contacting surface, not via the finger-like membranous invaginations. The portion of the peduncle retained within the trophont is more or less bulbous in most species of *Oodinum* and the proximal portion of the peduncle is so deep set in the cell that it nearly reaches the nucleus (Cachon & Cachon 1971, 1987). In *O. inlandicum*, however, it is conical and does not extend very far into the parasite cytoplasm.

From observations on both mature and immature trophonts, it is clear that the same basic components are present, but that drastic changes have taken place during cell maturation, particularly with regard to nucleus ultra-

structure and the organization of the cytoplasm. In the mature trophont, the cytoplasm consists of two distinct parts, the central endoplasm and the outer exoplasm. These two components probably correspond to those of the membranous cytoplasm and the granular cytoplasm of *O. jordani* (McLean & Nielsen 1989). In young trophonts, membranous exoplasm is not so evident and it is restricted to the lower half of the cell. The change in the nature of the nucleus within the life cycle of *Oodinium* has been well documented by Cachon and Cachon (1977) and it is evident that *O. inlandicum* also alternates between a non-dinokaryotic and a dinokaryotic nucleus within its life cycle. Unfortunately, only young and mature trophonts could be investigated and details of zoospore formation remain a mystery.

Ecology

The morphology and ecology of the host chaetognath, *Sagitta crassa*, have been intensively studied in the Seto Inland Sea, because the species exhibits great seasonal and morphological variations and because it is the most abundant species occurring throughout the year (Kado 1953, 1954, 1957; Hirota 1959, 1961; Kado & Hirota 1957; Murakami 1959; D. Liang & S. Uye, unpublished data). However no record of the parasitic dinoflagellate, *Oodinium*, here pre-exists this paper: It has been simply overlooked for a long time or, more likely, it has been recently introduced e.g., by ballast waters. Furthermore, Nagasawa & Marumo (1979, 1981, 1984), who studied parasites of pelagic chaetognaths in Tokyo and Suruga Bays and the East China Sea, Japan, described parasitic protozoans such as ciliates and gregarines, but never dinoflagellates. This suggests that this parasitic dinoflagellate is restricted to the Seto Inland Sea. Recently, *S. crassa*, parasitized by *O. inlandicum*, has been reported in additional sites in the central part of the Seto Inland Sea (off Mihara, November 2000 and in Fukuyama Port, December 2000; D. Liang, personal communication). The host-specificity of *O. inlandicum* seems to be extreme, because, in a total of 2454 indiv. that were examined in a recent study of pelagic chaetognaths in the Seto Inland Sea and its adjacent waters, including the Kuroshio Current, only the neritic species, *S. crassa* was found to be parasitized (Ohtsuka et al., unpublished data).

The host, *S. crassa*, was found to occur year-round over the period 1998 to 2000 in the type locality, but the parasite seems to be limited to the warm season, between June and October (Ohtsuka et al., unpublished data). The disappearance of the parasite from the chaetognath body during the cold season could be due to: (1) hibernation, in the form of resting spores on the sea bottom, induced by the cold temperature. Although the life cycle of *Oodinium* is partially known, resting spores have so far not been demonstrated in any member of the family Oodiniaceae (Cachon & Cachon 1987; Fensome et al. 1993); (2) introduction of the parasite into the central part of the Seto Inland Sea from its adjacent waters and subsequent death of the parasites after the season. This is supported by the observation that some oceanic zooplankters such as copepods, chaetognaths and marine skaters were introduced into the Seto Inland Sea mainly during October and November (Kado1957; Hirota 1961, 1979; Ohtsuka, unpublished data). However, no parasitic dinoflagellates have been reported from oceanic regions adjacent to the Seto Inland Sea (Ohtsuka et al., unpublished data); (3) the existence of alternative hosts other than *S. crassa*. Considering the highly specific nature of the host preference in the new dinoflagellate, the idea of an alternative host seems unlikely.

The pathological impacts of *O. inlandicum* on the host are as yet uncertain, but our ecological and morphological observations suggest that it is not too debilitating. A heavily infested individual of *S. crassa* (see Fig. 3) continued to feed normally on copepods and appendicularians, and its ovary seemed to be normal. In addition, the superficial penetration of the parasite peduncle into the host tissue (see Fig. 10C, D) also supports the above hypothesis.

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