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First Record of an Ectoparasitic Dinoflagellate, *Oodinium inlandicum* (Dinophyta) Infecting a Chaetognath, *Sagitta crassa* from the Korean Coasts

Takeo Horiguchi*, Ai Harada, Susumu Ohtsuka[1], Ho Young Soh[2] and Yang Ho Yoon[3]

Division of Biological Sciences, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan,
[1]Takehara Marine Science Station, Setouchi Field Science Center, Graduate School of Biosphere Science, Hiroshima University, 5-8-1 Minato-machi, Takehara, Hiroshima 725-0024, Japan,
[2]Division of Aqua-Life Science, Yosu National University, San 96-1, Dundeok-dong, Yeosu 550-749, Korea and
[3]Division of Ocean System, Yosu National University, San 96-1, Dundeok-dong, Yeosu 550-749, Korea

An ectoparasitic dinoflagellate infesting planktonic chaetognath, *Sagitta crassa* Tokioka was found, for the first time, from Korean coasts. In order to identify the species, we investigated detailed morphology of the dinoflagellate using Nomarski interferance optics as well as epifluorescent microscopes. The parasitic dinoflagellate consists of an oval to rod-shaped cell with a peduncle, by which the organism attaches to the host. The cell is covered with polygonal thecal plates. The nucleus displays two different shapes according to cell cycle stages: in young trophont the nucleus is elongated and shows typical dinoflagellate nucleus (dinokaryon), while in matured trophont, the nucleus is dome-shaped and non-dinokaryotic. The peduncle is variable in length and is ornamented with the longitudinal striations. All these characteristics point to identity that the ectoparasitic dinoflagellate infecting *Sagitta crassa* in Korean coasts is *Oodinium inlandicum* Horiguchi et Ohtsuka, originally described from the Seto Inland Sea of Japan. Relationship between prevalence and host sizes differed from those in Japan.

Key Words: chaetognath, Korea, *Oodinium inlandicum*, parasitic dinoflagellate, *Sagitta crassa*

INTRODUCTION

The dinoflagellates contain both photosynthetic and heterotrophic representatives. Among the latter, some are known to parasitize various marine organisms (e.g. Chatton 1920; Cachon and Cachon 1987; Shields 1994; Coats 1999). These parasitic forms can have a great impact on health and reproductive behavior of the infected host (e.g. Ianora *et al.* 1990; Kimmerer and McKinnon 1990; Coats *et al.* 1994) and therefore, understanding of the taxonomy and ecology of the parasitic dinoflagellates are of paramount importance. However few studies on these parasites have been carried out in Asian regions except for some recent papers (Ohtsuka *et al.* 2000; Horiguchi and Ohtsuka 2001; Park *et al.* 2004).

During the course of our parasitological studies on marine zooplankton on the Korean coasts, we have encountered a parasitic dinoflagellate infecting the pelagic cheatgnath *Sagitta crassa* Tokioka. In order to understand the biology of the parasite, it is of importance to accurately identify the parasitic organism. The aim of this paper is to identify this parasitic organism based on morphological investigations.

*Corresponding author (horig@sci.hokudai.ac.jp)

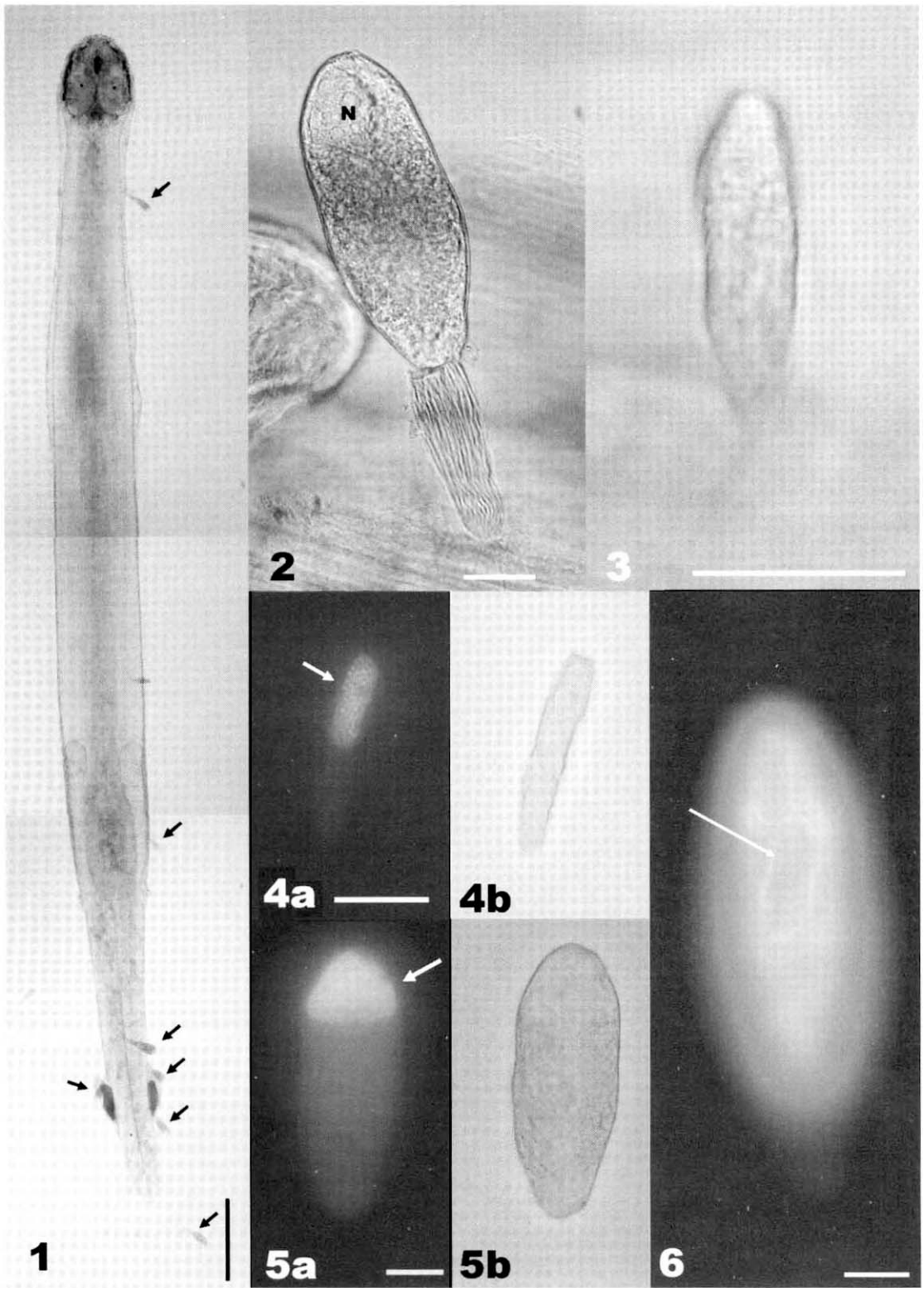
MATERIALS AND METHODS

Sampling

Zooplankters were collected from Mansungri Beach, Yeosu, Korea at night on September 5, 2003. A small conical plankton net (30 cm mouth diameter, 0.1 mm mesh size) was horizontally towed on the surface several times. Soon after collection, samples were fixed with 10% neutralized formalin/sea water. All chaetognaths were picked up with a pair of forceps under a binocular.

Light microscopy

Chaetognaths infected by the parasites were preserved in 10% formalin/sea-water and observed under BX-50 light microscope equipped with Nomarski interference optics (Olympus, Tokyo, Japan). For observation of the



Figs 1-6. *Oodinium inlandicum* Horiguchi et Ohtsuka infecting *Sagitta crassa* Tokioka. **Fig. 1.** *Sagitta crassa* infected by *O. inlandicum* (arrows). **Fig. 2.** Close-up of matured trophont. The nucleus (N) is located in upper part of the cell and the distinct peduncle with longitudinal straiations is visible. **Fig. 3.** Close-up of a young trophont. **Fig. 4a.** DAPI-stained young trophont viewed under the fluorescent microscope. The nucleus (white arrow) is elongated and contains many small dots (chromosomes). **Fig. 4b.** The same cell under the bright field optics. **Fig. 5a.** DAPI-stained matured trophont, showing dome-shaped nucleus (white arrow). **Fig. 5b.** The same cell under the bright field optics. **Fig. 6.** Trophont stage, stained by Fluorescent Brightener 28 and viewed under fluorescent microscope, showing sutures (white arrow) of thecal plates. Scale bars: Fig. 1 = 100 μm, Figs 2-6 = 25 μm.

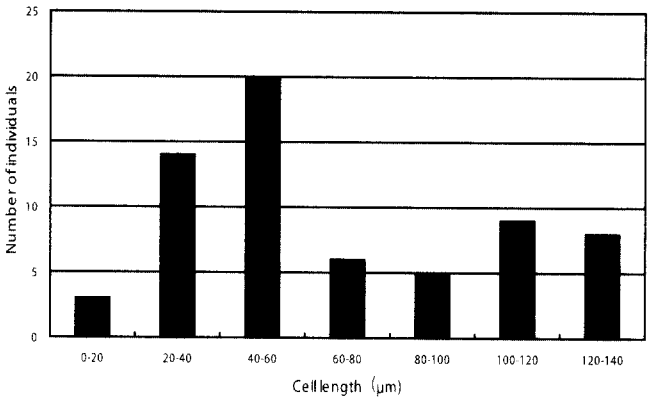


Fig. 7. Distribution of cell length (without peduncle) of trophonts of *Oodinium inlandicum* from the sample collected on 5 September, 2003.

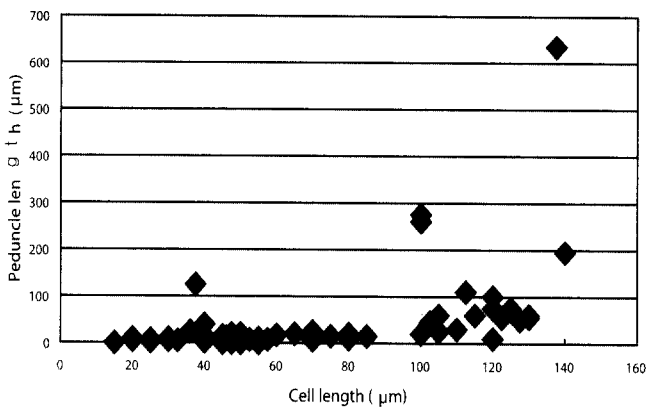


Fig. 8. Length of peduncle relative to the cell length of *Oodinium inlandicum*.

thecal plates, detached parasites were stained with 1% Fluorescent Brightener 28 (Sigma) and observed with a Optiphoto-2 microscope (Nikon, Tokyo, Japan), equipped with a Nikon EFD2 epifluorescent 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 observed 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 >500 randomly selected individuals from a sample collected on 5 September 2003. The ratio of infected to non-infected hosts was estimated, and the distribution of the ratio relative to the body size of the host was investigated.

RESULTS

Light microscopy

Trophonts were oval to rod-shaped (sausage-shaped), 15-140 μm in length (Figs 1-6). The distribution of cell length of trophont stage was shown in Fig. 7. The most frequently encountered individuals were those measuring between 20-60 μm, while the larger cells over 100 μm were also observed. The length of absorption apparatus or peduncle was extremely variable from specimen to specimen, measuring almost nothing to 635 μm. The peduncle length relative to cell length was plotted (Fig. 8). The large matured trophont was oval in shape and most of the body was filled with granular cytoplasm (Fig. 2). The nucleus was located in the distal end of the cell and only this part looked almost transparent (Fig. 2). The dome-shaped nucleus situated in the extremity of the cell was clearly stained by the DAPI and could be observed by the fluorescent microscope (Fig. 5). The nucleus looked uniform and no chromosomes could be observed in it. The young trophonts were smaller and thinner than the matured trophonts and the nucleus was elongated, located upper half of the cell body (Figs 3, 4). The nucleus in the young trophont showed granular appearance (chromosomes), which was typical for dinoflagellate nucleus (Fig. 4). The peduncle extended from the proximal end of the cell and penetrated into the host body at the distal end of the structure. The diameter of the peduncle was about half of the cell width and the length was extremely variable as mentioned. The surface of the peduncle was marked by the characteristic longitudinal striations (Fig. 2). The trophonts were covered with thecal plates. The thecal plates were thin and no ornamentations could be seen (Fig. 6). The thecal plate arrangement has not been elucidated, yet.

Infection prevalence and body size of the host

The parasitic dinoflagellates have been found infesting planktonic chaetognath, *Sagitta crassa* (Fig. 1). The parasites could be found anywhere of the host body surface (Fig. 1). We have investigated 557 individuals of *S. crassa*. Of these, only 28 was found to be infested by the dinoflagellate. The infection ratio, therefore, was rather low (5%) in our particular sample. We have also investigated the relationship between the body size of *S. crassa* and infection ratio (Fig. 9). No clear tendency has been noted. Even very small individuals less than 1.5 mm were infected, while the individual nearly 5 mm were also infested (Fig. 9). No infection was noted on the individuals over 5 mm from our sample.

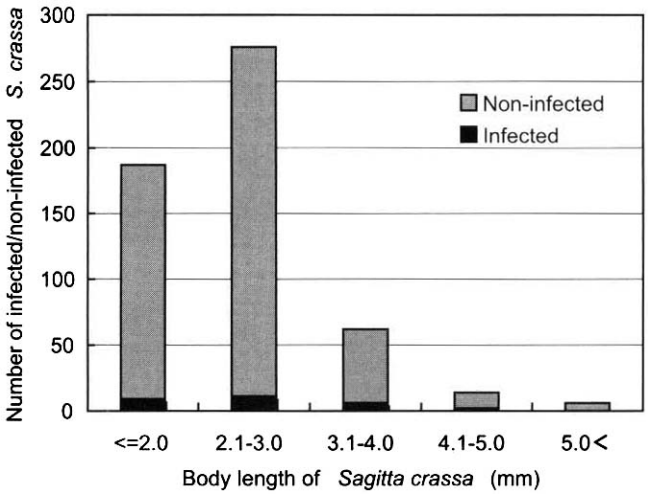


Fig. 9. Number of infected and non-infected individuals of *Sagitta crassa* by *Oodinium inlandicum* relative to body size of the host.

Table 1. Comparison of *Oodinium inlandicum* with the parasitic dinoflagellate infecting a cheatgnath, *Sagitta crassa* from Korean waters

	Oodinium inlandicum from Seto Inland Sea, Japan	Parasitic dinoflagellate from Yeosu, Korea
Host	*Sagitta crassa*	*Sagitta crassa*
Cell shape	Oval to rod-shaped	Oval to rod-shaped
Cell size	30-150 μm	15-140 μm
Peduncle length	6-500 μm	0-635 μm
Nucleus in young trophont	Elongated, dinokaryon	Elongated, dinokaryon
Nucleus in mature trophont	Dome-shaped, non-dinokaryotic	Dome-shaped, non-dinokaryotic
Source	Horiguchi and Ohtsuka 2001	present study

DISCUSSION

Identity

The ectoparasite infecting *Sagitta crassa* from Korean coastal waters possesses two different types of nuclear morphology according to cell cycle stages, i.e. in young trophont, the nucleus is elongated and shows typical dinoflagellate nucleus (dinokaryon), while in matured trophont, the nucleus is dome-shaped and non-dinokaryotic. This nuclear dimorphism suggests that the parasite is a member of parasitic dinoflagellate order, the Blastodiniales (Fensome *et al.* 1993). The morphological characteristics of the parasite from Korea clearly indicate affinity to the genus *Oodinium*, especially to *Oodinium inlandicum* Horiguchi et Ohtsuka. *O. inlandicum* was recently described from Seto Inland Sea, Japan (Horiguchi and Ohtsuka 2001). Table 1 shows comparisons between *O. inlandicum* from Japan and the ectoparasite from Korea. Both share the host species and other cytological features, such as nature of nucleus and possession of thecal plates. Size of cells and peduncles are in the same range. Therefore, we concluded that the ectoparasite infesting a cheatognath *Sagitta crassa* in Korean water is indeed *Oodinium inlandicum*. It is the first record of this species for Korean coastal waters.

Host-parasite relationships

The main host of *Oodinium inlandicum* seems to be the coastal chaetognath *Sagitta crassa*, including several seasonal forms, that is endemic to East Asia (Tokioka 1940; Hirota 1961; Park 1970) on the basis of our previous studies (Horiguchi and Ohtsuka 2001; Ohtsuka unpublished data) and the present study. No parasite has attached to a co-occurring predominant chaetognath *S. enflata* Grassi in the Seto Inland Sea of Japan (Horiguchi and Ohtsuka 2001). Although *S. crassa* is found on the coast of East Asia throughout the year (Tokioka 1940; Hirota 1961, 1979; Park 1970), the occurrence of trophonts of the parasite on the host appears to be restricted to the warm water seasons (Horiguchi and Ohtsuka 2001; Soh unpublished data). Interestingly no trophont has been found on the host since a typhoon hit Korea on 14 September 2003, although it was not uncommon at the collection time (September 2003).

Prevalence of *O. inlandicum* was higher in larger chaetognaths than in smaller one in the Seto Inland Sea of Japan (Horiguchi and Ohtsuka 2001). In contrast such tendency was not clearly found in the present study (see Fig. 9). Infection ratio for each size group of host from the smallest to the largest ones (1-2, 2-3, 3-4, 4-5 and 5 < mm in length) were 16.2, 7.5, 19.3, 40.0, and 0%, respectively. Collections were made at the same period (September-October) at both localities. The size range of the trophonts seems to be the same in both regions, suggesting that, although free-living dinospores of *O. inlandicum* (cf. Cachon and Cachon 1987; Ianora *et al.* 1990; Kimmerer and McKinnon 1990) have never been discovered, the life cycle of the Korean parasite may have been in a manner similar to that in Japan. In contrast the host specimens larger than 5 mm were very rare in Korea in comparison with those in Japan. These differences in size classes of the host were likely to be related to the regional differences in infection

prevalence.

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REFERENCES

Cachon J. and Cachon M. 1987. Parasitic dinoflagellates. In; Taylor F.J.R. (ed.), *The Biology of Dinoflagellates.* Botanical Monograph, Vol. 21, Blackwell Sci. Publ., Oxford, p. 571-610.

Chatton E. 1920. Les Péridiniens parasites. Morphologie, reproduction, ethologie. *Arch. Zool. exp. gén.* **59:** 1-475.

Coats D.W. 1999. Parasitic life styles of marine dinoflagellates. *J. Eukaryot. Microbiol.* **46:** 402-409.

Coats D.W., Bockstahler K.R., Berg G.M. and Sniezek J.H. 1994. Dinoflagellate infections of *Favella panamensis* from two North American estuaries. *Mar. Biol.* **119:** 105-113.

Fensome R.A., Taylor F.J.R., Norris G., Sarjeant W.A.S., Wharton D.I. and Williams G.L. 1993. A classification of living and fossil dinoflagellates. *Micropaleontology, Spec. Publ. Num.* **7:** 1-351.

Hirota R. 1961. Zooplankton investigations in the Bingo-nada region of the Setonaikai (Inland Sea of Japan). *J. Sci. Hiroshima Univ., Ser. B, Div. 1.* **20:** 83-145.

Hirota R. 1979. Seasonal occurrence of zooplankton at a definite station off Mukaishima from July of 1976 to June of 1977. *Pubs Amakusa Mar. Biol. Lab., Kyushu Univ.* **5:** 9-17.

Horiguchi T. and Ohtsuka S. 2001. *Oodinium inlandicum* sp. nov. (Blastodiniales, Dinophyta), a new ectoparasitic dinoflagellate infecting a chaetognath, *Sagitta crassa. Plankton Biol. Ecol.* **48:** 85-95.

Ianora A., Scotto di Carlo B., Mazzocchi M.G. and Mascellaro P. 1990. Histomorphological changes in the reproductive condition of parasitized marine plankotnic copepods. *J. Plankton Res.* **12:** 249-258.

Kimmerer W.J. and McKinnon A.D. 1990. High mortality in a copepod population caused by a parasitic dinoflagellate. *Mar. Biol.* **107:** 449-452.

Ohtsuka S., Nagasawa K. and Gejima K. 2000. Review of parasites of marine zooplankton. *Bull. Plankton Soc. Jpn* **47:** 1-16. (in Japanese with English abstract)

Park M.G., Yih W. and Coats D.W. 2004. Parasites and phytoplankton, with special emphasis on dinoflagellate infections. *J. Eukaryot. Microbiol.* **51:** 145-155.

Park J.S. 1970. The chaetognaths of Korean waters. *Bull. Fish. Res. Dev. Agen. Korea* **6:** 1-174. (In Korean)

Shields J.D. 1994. The parasitic dinoflagellates of marine crustaceans. *Ann. Rev. Fish Dis.* **4:** 241-271.

Tokioka T. 1940. Phylum Chaetognatha. Fauna Nipponica V(2): 1-129. (In Japanese)

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