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PROTOZOOLOGICA

Morphological, Developmental, and Ecological Characteristics of the Suctorian Ciliate *Ephelota gigantea* (Ciliophora, Phyllopharyngea, Ephelotidae) Found on Cultured Wakame Seaweed in Northeastern Japan

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Abstract. Wakame seaweed is an important aquatic resource in Iwate Prefecture. However, a suctorian *Ephelota gigantea* sometimes causes great damage to wakame culture. Since little is known about the biological characteristics of *E. gigantea*, its detailed morphology and temporal change of biological characteristics during the 2010 culture season were investigated. Scanning electron microscope observations showed that *E. gigantea* had different striation patterns on the stalk; there was a swell made of cement by which the stalk was attached to wakame firmly; and the buds had cilia arranged in concentric circles about a ring in the center of the ventral side. A suctorian parasite was found to infect *E. gigantea*, and the infection seemed to have decreased drastically the attached density of *E. gigantea* on wakame. Cell size of parasite-infected *E. gigantea* individuals was larger than that of uninfected individuals, probably because larger *E. gigantea* has larger surface area for attachment of the parasite. Cyst formation or conjugating individuals were not observed.

Key words: *Ephelota gigantea*, wakame seaweed, parasite, suctorian.

INTRODUCTION

Suctorians belonging to the genus *Ephelota* are found worldwide in marine habitats. They are characterized by the absence of a lorica and by the presence

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of two types of tentacles (suctorian and prehensile), as well as a stalk and a ramous macronucleus (Kahl 1934, Guilcher 1951, Jankowski 1967). About a dozen species of *Ephelota* associated with aquatic plants and animals, including algae, bryozoans, hydrozoans, and crustaceans have been described (Sawyer *et al*. 1976). However, life cycle of *Ephelota* is poorly known. Kobayashi *et al*. (2011) was the first to describe the growth of *Ephelota gigantea* settled on plastic nets placed on wakame long-line culture along northeastern coast of

Japan. Tazioli and Di Camillo (2013) reported the life cycle of *Ephelota gemmipara* settled on the hydroid *Eudendrium racemosum* in the Adriatic Sea throughout the year.

Wakame (*Undaria pinnatifida*) is an important aquatic resource commercially exploited in Iwate Prefecture, northeastern Japan. The life cycle of wakame is annual and consists of microscopic and macroscopic stages. A macroscopic stage (the sporophyte) releases microscopic zoospores in early July in coastal area of Iwate Prefecture. Once all the zoospores are released, it dies. Zoospores settle, germinate and grow into microscopic gametophytes. Wakame culture begins with seedling collection in late June–early August, and grown-up seedlings are transported to culture field facing the open ocean in November. Wakame grows in winter into harvestable size (1–2 m) and harvesting lasts from March to May. However, *Ephelota* suctorians sometimes cause great damage to cultured wakame, because damaged *Ephelota* cells release an unpleasant odor and the stalks of *Ephelota* remain on wakame fronds even after boiling, which greatly diminishes the commercial value of wakame.

Suctorians that attach to wakame in Iwate Prefecture were identified as *Ephelota gigantea* and *E. gemmipara* (Ryuzo Yagiu pers. comm.), of which the former species cause most of the damage. However, since *E. gigantea* was described in 1920s by Noble (1929), there was no other references to this species until those of Fernadez-Leborans *et al*. (2005), who reported it as an epibiont on the parasitic copepod, *Lepeophtheirus salmonis*. Recently, the growth rate of *E. gigantea* was estimated for the first time through a short-term in situ experiment by Kobayashi *et al*. (2011), but further detail of its biological characteristics remain unknown. During the 2010 culture season of wakame in Fudai, Iwate Prefecture, two *Ephelota* species were found to have attached to wakame fronds: *E. gigantea* from March to June and *E. gemmipara* from May to June. It is not known from where *E. gigantea* comes to the culture field of wakame. One hypothesis is that cold-water euphausiid, *Euphausia pacifica*, brings this suctorian with cold Oyashio Current from north, since *Ephelota* sp. is known to attach to this euphausiid species (Endo *et al*. 1985, Fernandez-Leborans 2011).

The organisms infecting *E. gigantea* were discovered for the first time, when observing biological characteristics of this suctorian in the 2010 culture season. This organism seems to feed *E. gigantea* using a long tentacle.

The goal of this study is to: a) explore the fine structures of *E. gigantea*, which was not well-known, by using scanning electron microscopy (SEM); b) investigate the temporal change in the biological characteristics of *E. gigantea* during wakame culture in the 2010 season; and c) examine if the same *Ephelota* species attach to wakame and the euphausiid *Euphausia pacifica*.

MATERIALS AND METHODS

Samples were collected from long-line wakame culture in coastal area of Fudai (40.01N, 141.91E) Iwate Prefecture, 8 times from March to June 2010 (Fig. 1, Table 1). Collected samples were fixed with 5% formalin, 95% ethanol or 10% Bouin solution for general observation, genetic analysis and cytological observation, respectively. Wakame samples collected in coastal waters of Okirai (39.08N, 141.85E) in 2009 and Taro (39.72N, 141.91E) in 2010 were fixed with 95% ethanol for genetic analysis.

Scanning electron microscope preparation

E. gigantea that was collected from Fudai and fixed with formalin was used to observe fine structure of *E. gigantea*. Wakame was cut into 1 cm^2 pieces and rinsed three times in filter-sterilized seawater and dehydrated in graded series of ethanol: 50% for 30 minutes; 70%, 80%, 90% and 95% for 10 minutes each; and three times 100% for 10 minutes each. After dehydration, the samples were transferred to t-butyl alcohol and were freeze-dried. Dried filters containing the cells were mounted on a stub using carbon conductive tape and observed with a scanning electron microscope (Hitachi S-4200).

DNA extraction and analysis

Genomic DNA was extracted from *E. gigantea* that attached to wakame collected from Okirai in 2009, Fudai and Taro in 2010 and from *Ephelota* sp. that attached to the euphausiid *Euphausia pacifica* collected off Kamaishi Iwate Prefecture (39°17N, 141°19E) in March 2010. Cells were washed by several transfers in filtered (pore size 0.2 μm) seawater and distilled water to remove as much extraneous matter as possible. PCR tubes (0.2 mL) each containing 50 μl of 10% Chelex® suspension (Bio-Rad Laboratories Inc., Richmond, CA) and single cells of *Ephelota* spp. were heated at 95°C for 20 min to extract genomic DNA according to Richlen and Barber (2005). Extracted DNAs were used as templates to amplify the target regions. All PCRs were performed on a thermal cycler in a reaction mixture (25.0 μl) containing 1.0 μl of template DNA, 0.2 mM of each deoxynucleoside triphosphate (dNTP), 1 × PCR buffer, 2.0 mM MgSO$_4$, 0.4 unit of KOD-Plus-ver. 2 DNA polymerase (Toyobo, Osaka, Japan; with intensive 3'→5' exonuclease activity), and 0.2 μM of each primer. For amplifying the nuclear 18S rRNA gene of the *Ephelota* spp., three primer pairs (18S-1F1/18S-1R632, 18S-2F576/18S-2R1209, and 18S-3F1129/18S-R1772) were used (Nishitani *et al*. 2012). The PCR cycling conditions were as follows: initial denaturation at 94°C for 2 min and 38 cycles at 94°C for 15 s, 54°C for 30 s, and 68°C for 45 s. The results of PCR amplification were confirmed on 1.5% agarose gels by using ethidium
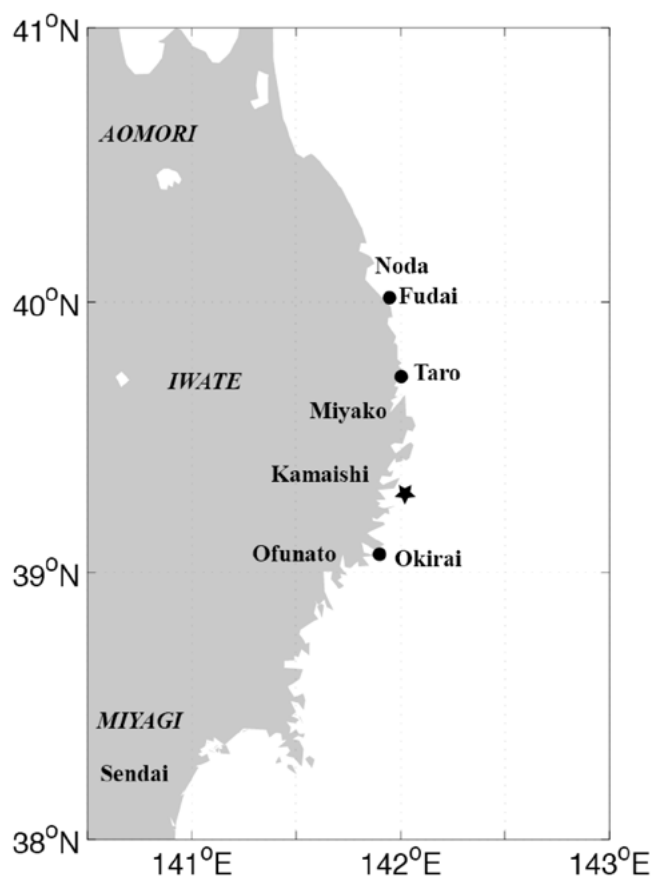
Fig. 1. Map showing the northeastern part of Japan. Places where wakame samples (●) and krill sample (★) were collected are also shown.

Table 1. Sampling date and locations of wakame seaweed and krill in this study. *Ephelota* species attached to wakame seaweed and krill were analyzed for rDNA and biological characteristics.

	Date	Location	Analysis
Wakame	June 2009	Off Okirai	rDNA
	June 2010	Off Taro	rDNA
	March–June 2010	Off Fudai	rDNA, biological characteristics
Krill	March 2010	Off Kamaishi	rDNA

measurements were made only on *E. gigantea* fixed with formalin, because the shrinkage may differ among preservatives.

In this study we found parasites to be attached to the proximal periphery of the stalk of *E. gigantea*. We observed the behavior and development of the parasites.

Statistical analysis

We used multiple comparison of ANOVA for the difference of means of body size of *E. gigantea* among sampling dates and ANOVA for the difference of those between budding and non-budding individuals and between infected and uninfected individuals of *E. gigantea* using MATLAB R2014a (The MathWorks Inc. 2014).
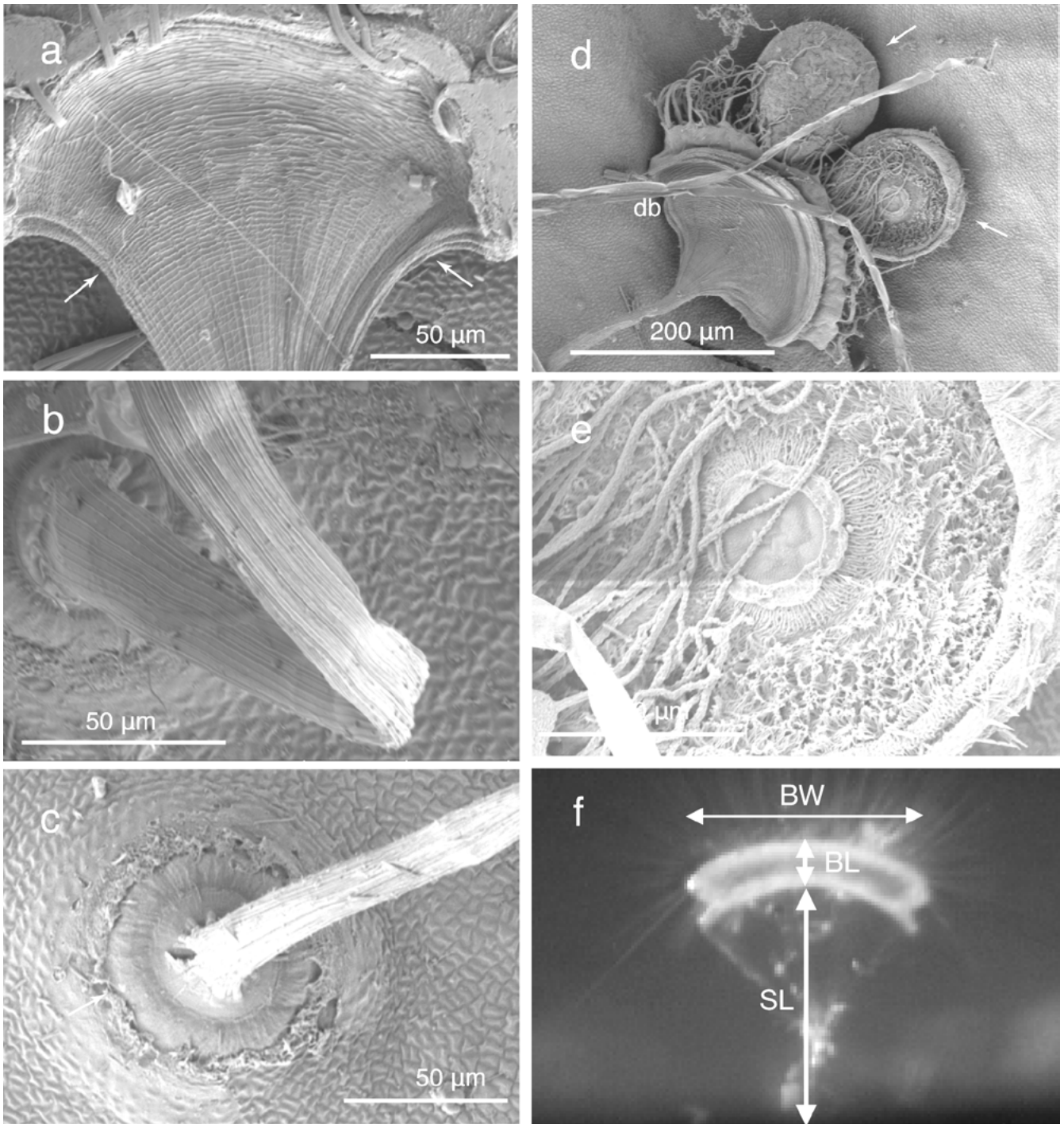
RESULTS

Identification of *Ephelota* species

Two *Ephelota* species occurred in the present study. The larger one has been known as a major problem to wakame culture in Iwate Prefecture and was identified as *E. gigantea*. This is an astonishingly large *Ephelota* species with laterally compressed body supported by a stalk which is spread into a funnel-shaped structure at its proximal end (Noble 1929). The smaller species was identified as *E. gemmipara*: the body is pyriform, with body length of 50–140 μm, body width of 50–450 μm, and stalk length of 400–1800 μm. Stalk is conical and widest at its junction with the body, gradually narrowing toward distal end.

E. gigantea appeared in March–June earlier than *E. gemmipara*, which occurred from May 24 onwards. This order of occurrence was consistent with the previous report (Iwate Prefecture Aquaculture Center 1981).

Fine structure of *Ephelota gigantea*

SEM observations revealed that *E. gigantea* has different striation patterns on the stalk: only longitudinal striations at the cylindrical part, longitudinal and

staining. The PCR products were sequenced directly with an automated DNA sequencer (ABI 3100; Applied Biosystems). The forward and reverse complementary sequences were combined, and the three nucleotide sequences obtained with the three primer pairs were aligned using Genetyx software (Genetyx Corp., Tokyo, Japan). Partial sequences of the nuclear 18S rRNA gene were aligned with the sequences of other related species obtained from GenBank by using the CLUSTAL W algorithm (Thompson *et al.* 1994).

Temporal change of biological characteristics of *E. gigantea*

The density of *E. gigantea* attached per 1 cm² of wakame frond collected from Fudai was determined under a stereo microscope (Leica WILDMZ8). Budding rate (percentage of *E. gigantea* with any number of buds) and infection rate (percentage of *E. gigantea* that was infected by parasites) were determined under the stereo microscope. These rates were examined on *E. gigantea* fixed with any preservatives mentioned above.

Stalk length, body length and body width of 100 individuals of *E. gigantea* were measured for each sampling date (Fig. 2f). These

Figs 2a–f. Scanning electron micrographs (a–e) and measured body parts (f) of *Ephelota gigantea*. **a** – upper part of the stalk. Only cross striation near the cell body while longitudinal and cross striations below arrows; **b** – lower part of the stalk with only longitudinal striation; **c** – swelled part where *E. gigantea* is attached to wakame (arrow); **d** – *E. gigantea* with two buds (arrows); **e** – ventral side of a bud. Scopula (arrow) is visible; **f** – measured part of *E. gigantea* cell. db – debri, BL – body length, BW – body width, SL – stalk length.

cross striations where the cylinder swells to become fan-shaped, and only cross striations near the cell body (Figs 2a, b). There was a swelling of cement where the distal end of the stalk is attached to wakame and the diameter of the swelled part was 3.4 times wider than the stalk diameter (Fig. 2c). The last characteristic enables *E. gigantea* to attach to wakame firmly.

The buds have cilia arranged in concentric circles around a central scopula in the center of the ventral side of the body (Figs 2d, e). The released swarmers were ovoid with long axis of 159.1 ± 4.6 μm and short axis of 117.3 ± 3.2 μm (n = 20).

rDNA analysis

E. gigantea that attached to wakame collected from three different places from Iwate Prefecture showed exactly the same rDNA sequence (GenBank, Accession number: AB979870). rDNA sequence of *E. gigantea* differed from that of eight individuals of *Ephelota* species that attached to the euphausiid *Euphausia pacifica* collected off Kamaishi, Iwate Prefecture in March 2010. *Ephelota* sp. (Accession number: AB979871) that attached to *E. pacifica* was smaller than *E. gigantea* with body length of 42.1–89.5 μm and body width of 19.6–105.3 μm and showing multiple budding. Our results of the gene sequencing showed that 103 out of 1635 base pairs were different (including insertions/deletions of 8 base pairs). Therefore, a combination of morphological, developmental and genetic differences, in addition to the difference in host indicates that they are different species.

Temporal change of biological characteristics of *Ephelota gigantea*

Attached density of *E. gigantea* was largest, 453.2 ind cm⁻², on 17 March, but it decreased to less than 7 ind cm⁻² in early May and kept low values in the rest of the study period (Fig. 3a). Budding individuals were observed on 17 March, 6 April and 12 and 24 May (Fig. 3c). Budding rate was highest on 12 May (7%) and never attained more than 10% in other periods.

The cell dimensions of *E. gigantea* were as follows: stalk length ranged from 280.0 to 2162.5 μm, body length from 30.0 to 190.0 μm, and body width from 60.0 to 740.0 μm. The mean stalk length was longest in March, 945.5 μm, but became shorter, about 600 μm from May onwards (Fig. 4). The stalk length of 17 March differed from other dates, and that between 4 May and 12 May differed significantly, but those of other dates did not differ significantly (multiple com-

parison of ANOVA). Body length did not look different markedly, but increased gradually during the study period. The body lengths of 17 March and 4 May, 12 and 18 May and 9 June differed significantly with the value of the last date being the largest. The mean body width was short at first, but increased steadily until mid May and became shorter again in June. The body widths of any sampling dates from 17 March to 18 May differed significantly, but that of 9 June did not differ from that of 12 May.

Modal length for body length and width appeared to increase from 17 March to 12 May, and body length kept similar values afterwards and body width decreased on 9 June (Fig. 5). On the other hand, stalk length showed largest size range on 17 March, and modal length did not show steady increase.

As buds grow, contents in the mother cell are expected to be transported to buds, so body length of budding individuals might be shorter than non-budding ones. Therefore, cell size was compared between *E. gigantea* with one or more buds and those without buds. Size ranges of the former cells were within the ranges of the latter cells. There was, however, no significant difference in mean body length between budding and non-budding individuals (F = 3.860, n = 499, P = 0.672). The mean body width of budding individuals (343 μm) was not significantly different from that of non-budding individuals (308.0 μm) (F = 3.860, n = 499, P = 0.433).

The minimum cell size of budding individuals was 120 μm and 60 μm for body width and body length, respectively, suggesting that *E. gigantea* can start budding when body size reaches this size.

Cell size of parasite-infected *E. gigantea* individuals and uninfected individuals were compared to investigate size selection by the parasitic organisms or the influence of parasitism. There was a significant difference in body length between infected individuals (x = 105.1 μm) and uninfected individuals (x = 94.1 μm) (ANOVA, F = 3.865, n = 399, P < 0.001). Body width showed a significant difference between infected individuals (x = 395.3 μm) and uninfected individuals (x = 314.7 μm) (F = 3.865, n = 399, P < 0.001).

Cyst formation or conjugating individuals were not observed in this study.

In this study we observed *E. gigantea* to be infected with parasites. Further examination of one individual that was heavily infected with parasites showed that the cell content of *E. gigantea* decreased appreciably in 18 hours, which indicated that the parasites were eating *E. gigantea* using its long tentacle (Fig. 6). The maxi-
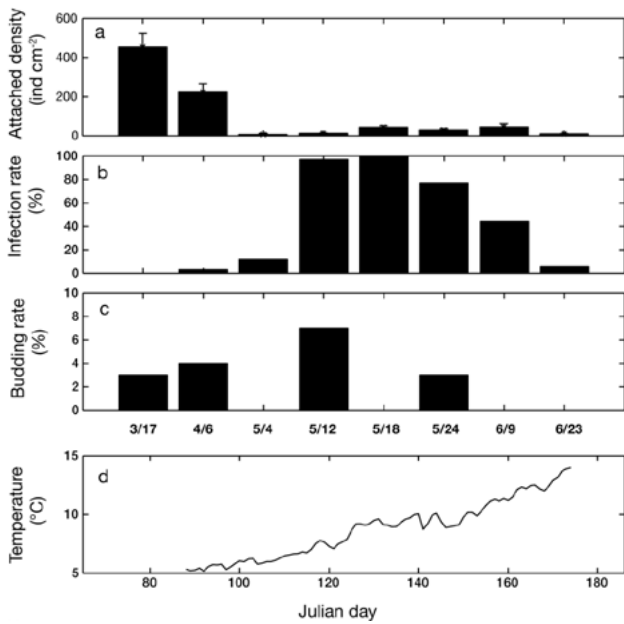
Figs 3a–d. Temporal change in biological characteristics of *Ephelota gigantea*. **a** – attached density. Vertical bars represent ± 1 SE; **b** – percentage of cells infected by the parasite; **c** – percentage of budding cells; **d** – seasonal change of surface water temperature in the coastal area of Noda, Iwate Prefecture from late March to late June, 2010.
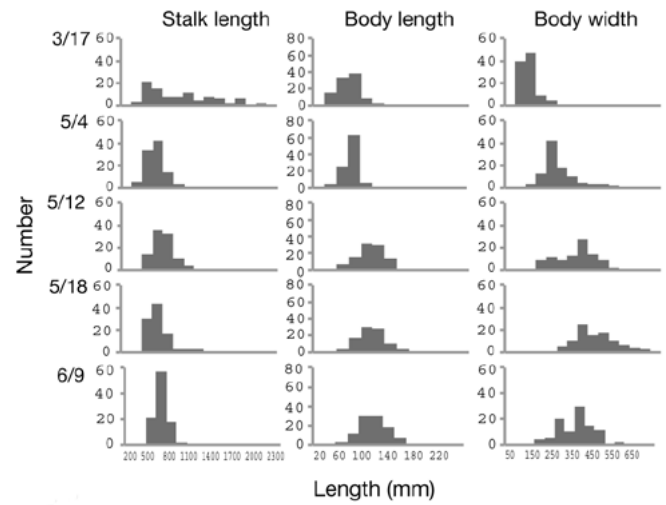

Fig. 5. Histograms of stalk length, body length, and body width of *Ephelota gigantea* preserved in formalin.
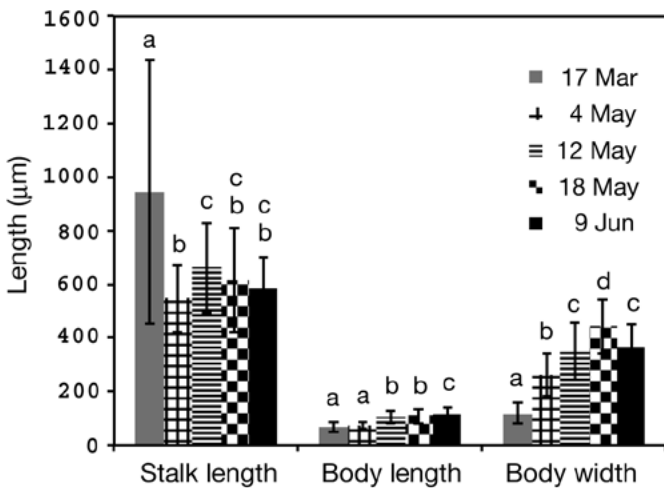

Fig. 4. Morphometric characteristics of *Ephelota gigantea* collected from cultured wakame in the coastal area of Fudai in 2010 (n = 100). Vertical bars represent ± 1 SD. Different letters above the columns indicate significant differences for each body part measured.

mum number of the parasite was up to 30–40 per *E. gigantea* individual. Parasites on *E. gigantea* appeared on 6 April for the first time and infection rate increased rapidly from 4 May (12%) to 12 May (97%), attained the maximum value (100%) on 18 May, and decreased to 6% by 23 June (Fig. 3b).

As parasitic suctorians that infect *Ephelota* species, three species *Tachyblaston ephelotensis*, *Acinetopsis rara* and *Enigmocoma acinetarum* are known to feed on *E. gemmipara* (Grell 1973, Tazioli and Di Camillo 2013). Cell morphology and 18S rDNA analysis suggest, however, that this parasite is a new suctorian species and it will be addressed in a future study.

DISCUSSION

Species identification and cell structure of *Ephelota gigantea*

The stalk was long and cell size, especially body width of *E. gigantea* was small in mid March. Body width was 4 times smaller than that collected in mid May. However, more than 10 times difference is reported for body width of *E. gemmipara* (Tazioli and Di Camillo 2013). Therefore, we believe that *Ephelota* species collected in mid March is also *E. gigantea*.

As for the striation pattern of the stalk, Noble (1929) described briefly that the stalk of *E. gigantea* is both longitudinally and cross-striated. However, our

SEM observation revealed that stalk striation changed from longitudinal one at the cylindrical part to both longitudinal and cross ones at the swelled part and finally to cross one only near the cell body. Chen *et al*. (2008) showed that the stalk surface of *E. gemmipara* is highly variable in appearance and identified four types, and suggested these types are population specific. Therefore, the striation pattern observed in the present study should be examined if it is conservative at species level in the future study. SEM observation also showed that the stalk firmly attached to wakame frond by cement and, therefore, not readily detach from wakame frond.

The buds have cilia arranged in concentric circles. Swarmers of *E. gigantea* do not swim but crawl (Noble 1929, Kobayashi *et al*. 2011), so they use cilia of their ventral side when they crawl. In this sense, the swarmer of this species is not a typical ciliate swarmer which is defined as the detached, free-swimming stage arising from sessile adult forms (Lee *et al*. 1985).

rDNA analysis suggests that *Ephelota* species that attached to wakame, namely *E. gigantea*, is different from *Ephelota* species that attached to the euphausiid *Euphausia pacifica*. Therefore, the hypothesis that *Euphausia pacifica* bring *Ephelota* to wakame culture field when dense population of this euphausiid species come close to the coastal area in spring proved to be false. Fernandez-Leborans (2011) identified *Ephelota* species that attached to *Euphausia pacifica* collected from coastal waters of Iwate Prefecture, Japan as *Ephelota plana*. Body size of *Epholota* sp. that attached to *Euphausia pacifica* in our study was within the range reported by Fernandez-Leborans (2011), which suggests that this species is also *E. plana*, although there was no concave cavity in the apical region of the ciliate body as shown in *E. plana* that attached to two decapod species from Scotland (Fernadez-Leborans and Gabilondo 2005).

The temporal change of biological characteristics of *E. gigantea* and attached parasite

Fujiwara and Nakano (2009) showed experimentally that survival rate of *E. gigantea* decreased significantly and reproduction rarely occurred at 13°C or higher. However, when the density of *E. gigantea* decreased abruptly in April–May in the present study, the water temperature of coastal areas of Noda which is close to Fudai from April to early May in 2010, was below 10°C (Fig. 3d). Hence, drastic density decrease of *E. gigantea* in this study might be caused by factors other than water temperature, probably parasitic organisms that infected *E. gigantea*. This parasite seems to be able to reproduce very rapidly and reduced drastically the attachment of *E. gigantea* to wakame.

Noble (1929) reported that the number of buds that individual *Ephelota gigantea* produces is from one to six, but the number of buds was only one or two in this study. In the laboratory, it took about one day from start budding to the release of buds as swarmers in *E. gigantea* (Kobayashi *et al*. 2011). *E. gigantea* samples used in this study were transported from Fudai, Iwate Prefecture to Sendai, Miyagi Prefecture and it took about one day. Therefore, buds may have been released during transportation and the number of buds may be underestimated. It is also probable that body length did not differ significantly between budding and non-budding individuals because "non-budding" individuals had released swarmers during the transportation.

Cell size of parasite-infected *E. gigantea* was significantly larger than that of uninfected *E. gigantea*, probably because larger *E. gigantea* has larger surface area for attachment of the parasite.

Kobayashi *et al*. (2011) made an in situ growth experiment of *E. gigantea* and found that stalk elongation stopped after the 4th day of settlement, but body length and body width kept growing during the experiment of seven days. So the possibility was suggested that the body length and body width can be used as an indicator of their growth. In the present study, a significant positive correlation was also found ($r = 0.6235$, $n = 500$, $P < 0.05$) between body length and body width over larger cell size range than reported by Kobayashi *et al*. (2011) (Fig. 7).

There seems to be an apparent cell growth during two months from 17 March to 18 May from the histograms (Fig. 5), although individual *E. gigantea* settled on wakame frond on different date. However, considering the growth rates of body length (19 μm d^{-1}) and body width (30 μm d^{-1}) by Kobayashi *et al*. (2011), the highest growth rates calculated from the histogram in the present study, namely, 20 μm between 4 May and 12 May in body length (2.5 μm d^{-1}) and 150 μm during the same period in body width (18.8 μm d^{-1}), are too low. Therefore, this seeming growth is not real, but their cell size may reflect the environmental conditions such as food availability in the culture field at the time of their settlement. The growth conditions may have been better during the period from 12 May to 9 June than in the earlier period. Tazioli and Di Camillo (2014) also reported that there is an active growth period for
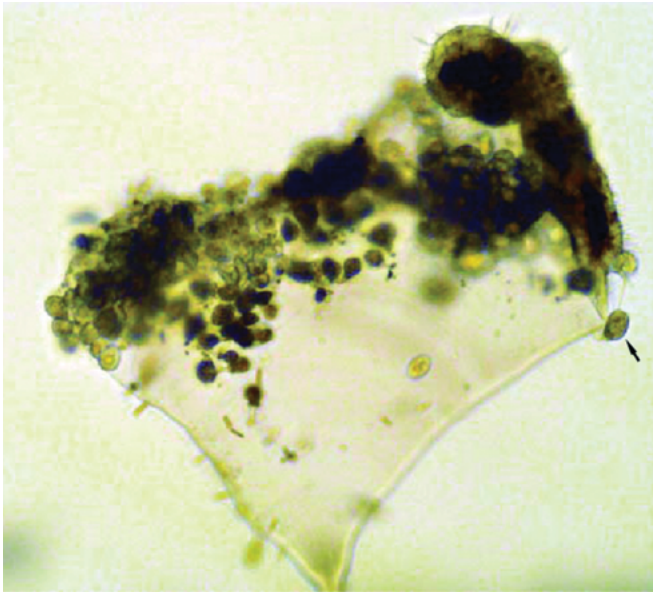
Fig. 6. Heavily infected *Ephelota gigantea*. Arrow shows a parasite with a stalk and a tentacle.
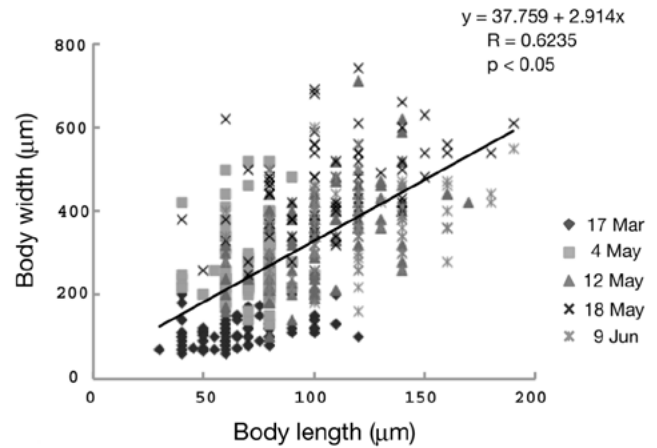

Fig. 7. Relationship between body width and body length of all individuals of *Ephelota gigantea* measured on formalin-preserved samples at each sampling

Ephelota gemmipara in the Adriatic Sea based on their 16-month survey.

The stalk length of *E. gigantea* on 17 March varied very much and extremely longer stalks appeared than the other days (Fig. 5). Stalk length may be related to the attached density of *E. gigantea* on wakame frond, when densely attached *E. gigantea* may lengthen its stalk to compete for prey in the water column. Alternatively, stalk length may be related to current velocity at the time of settlement; the higher the current speed, the longer the stalk to be more flexible (Koehl 1984, Dovgal and Kochin 1997), although wakame frond itself may dampen current stress.

It is still not known the source/bearer of *E. gigantea* and how wakame fronds are infected. Further studies are needed to clarify its life history in order to know its own biology and to alleviate the damage to wakame culture.

REFERENCES

Chen X., Song W., Warren A., Al-Rasheid K. A. S., Al-Farraj S. A., Al-Quraishy S. A., Gong J. (2008) Redefinitions of two marine suctorian ciliates, *Ephelota gemmipara* (Hertwig, 1876) Butschili, 1889 and *E. crustaceorum* Haller, 1880 (Ciliophora, Suctoria), with a brief description of the asexual reproduction process in *E. gemmipara*. *Acta Protozool.* **47:** 113–124

Dovgal I. V., Kochin V. A. (1997) Fluid boundary layer as an adaptive zone for sessile protists. *J. Gen. Biol.* 58: 67–74

Endo Y., Hanamura Y., Taniguchi A. (1985) In situ observations on the surface swarm of *Euphausia pacifica* in Sendai Bay in early spring with special reference to their biological characteristics. *La mer* 23: 135–140

Fernandez-Leborans G. (2011) Epibionts on the krill (*Euphausia pacifica*) from the E coast of Japan. *Acta Zool.* **94:** 167–176

Fernandez-Leborans G., Gabilondo R. (2005) Hydrozoan and protozoan epibionts on two decapod species, *Liocarcinus depurator* (Linnaeus, 1758) and *Pilumnus hirtellus* (Linnaeus, 1761), from Scotland. *Zool. Anz.* **244:** 59–72

Fernandez-Leborans G., Freeman M., Gabilondo R., Sommerville C. (2005) Marine protozoan epibionts on the copepod *Lepeophtheirus salmonis*, parasite of the Atlantic salmon. *J. Nat. Hist.* **39:** 587–596

Fujiwara T., Nakano N. (2009) Studies on the mechanism of pest occurrence in seaweed farming (in Japanese). *Ann. Rep. Iwate Fish. Tech. Cent.* 92–97

Grell K. G. (1973) Protozoology. Springer, Berlin Heidelberg

Guilcher Y. (1951) Contribution a l'etude des cilies gemmipares, chontriches et tentaculiferes. *Ann. Sci. Nat. Zool.* **13:** 33–132

Iwate Prefecture Aquaculture Center (1981) Rep. Iwate Prefecture Aquaculture Cent. 1981.

Jankowski A. W. (1967) New genera of tentacle Infusoria (Suctoria). *Mat. V Konf. Mold. Zool.* 35–36

Kahl A. (1934) Suctoria. *Tierwelt N.-u Ostsee* **26:** 184–226

Kobayashi T., Nakano N., Muto T., Endo Y. (2011) Growth characteristics of *Ephelota gigantea*: a pest to seaweed culture along the northeastern coast of Japan. *Acta Protozool.* **50:** 339–343

Koehl M. A. R. (1984) How do benthic organisms withstand moving water? *Amer. Zool.* **24:** 57–70

Lee J. J., Hunter S. H., Bovee E. C. (1985) An Ilustrated Guide to the Protozoa. Society of Protozoologists Lawrence, Kansas, Allen Press

Lynn D. H. (2008) The ciliate protozoa. Characterization, Classification, and Guide to the Literature. 3rd edition. Springer, London

Nishitani G., Nagai S., Hayakawa S., Kosaka Y., Sakurada K., Kamiyama T., Gojobori T. (2012) Multiple plastids collected by the dinoflagellate *Dinophysis mitra* through kleptoplastidy. *Appl. Environ. Microbiol.* **78:** 813–821

Noble A. E. (1929) Two new species of the protozoan genus *Ephelota* from Monterey Bay California. *Univ. Cal. Pub. Zool.* **33:** 13–26

Richlen M. L., Barber P. H. (2005) A technique for the rapid extraction of microalgal DNA from single live and preserved cells. *Mol. Ecol. Notes* **5:** 688–691

Sawyer T. K., MacLean S. A., Ziskowski J. (1976) A report on *Ephelota* sp. (Ciliata, Suctorida) as an epibiont on the gills of decapod crustaceans. *Trans. Am. Microsc. Soc.* **95:** 712–717

Tazioli S., Di Camillo C. G. (2013) Ecological and morphological characteristics of *Ephelota gemmipara* (Ciliophora, Suctoria), epibiontic on *Eudendrium racemosum* (Cnidaria, Hydrozoa) from the Adriatic Sea. *Eur. J. Protistol.* **49:** 590–599

Thompson J. D., Higgins D. G., Gibson T. J. (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22:** 4673–4680