Korean Journal of Environmental Biology

Original article

https://doi.org/10.11626/KJEB.2020.38.4.567

Korean J. Environ. Biol.
38(4) : 567-577 (2020)
ISSN 1226-9999 (print)
ISSN 2287-7851 (online)

Infection of marine diatom *Coscinodiscus wailesii* (Bacillariophyceae) by the parasitic nanoflagellate *Pirsonia diadema* (Stramenopiles) from Yongho Bay in Korea

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Received: 12 November 2020
Revised: 16 November 2020
Revision accepted: 17 November 2020

Abstract: The infection of marine diatom *Coscinodiscus wailesii* by a parasitic protist from the Yongho Bay of Busan, Korea was observed during the diatom bloom events in 2017 through 2018. The morphological and molecular features suggested that the parasitic nanoflagellate *Pirsonia diadema* was responsible for the infection. During the study period, the parasite prevalence ranged from 0.3% to 3.3%, and infected *C. wailesii* cells were observed only at surface seawater temperatures ranging between 10.9 and 19.9°C, although the host population appeared at temperatures above 25°C. The parasite and host system was successfully established as cultures. Using the cultures, we determined the morphological features over the infection cycle, parasite generation time, parasite prevalence as a function of inoculum size, and zoospore infectivity and survival time. The diatom *C. wailesii* was readily infected by the parasite *P. diadema*, with a parasite prevalence reaching up to 100% and a zoospore to host inoculum ratio above 20 : 1. The survival and infectivity of the parasite zoospores decreased with age. While the zoospores could survive up to 88 hours, they quickly lost their ability to infect after 48 hours. These results could lead to a better understanding of the biology and ecology of the parasitoid infecting the giant-sized diatoms in coastal waters.

Keywords: *Coscinodiscus*, diatom, parasite, *Pirsonia*, parasite prevalence

INTRODUCTION

Marine diatoms are often dominant group in temperate to polar coastal ecosystems and play important roles as primary producers in pelagic food webs. Although many previous studies have focused on grazing of zooplankton and heterotrophic flagellates on marine phytoplankton, selective parasitism would be also considered as a biological control for phytoplankton population dynamics and species succession (Scholz *et al.* 2016). Under appropriate conditions, parasites can spread rapidly through dense host populations to cause mass mortality of host cells and lead host species succession (Grahame 1976; Frenken *et al.* 2016).

Marine diatoms are susceptible to infections by a variety of parasitic protists including cercozoans, chytrids, dinoflagellates, euglenozoa, oomycetes, plasmodiophorids, and stramenopiles (Drebes 1966; Drebes and Schnepf 1988; Kühn *et al.* 1996; Drebes and Schnepf 1998; Tillmann *et al.* 1999; Bulman *et al.* 2001). The genera *Pirsonia*/*Pseudopirsonia* belonging to Stramenopiles and Cercozoa are parasitic nanoflagellates that infect various marine centric diatoms. Six *Pirsonia* species and one *Pseudopirsonia* species have been formally described from the German Bight of North Sea (Schnepf *et al.* 1990; Kühn *et al.* 1996; Sch-

weikert and Schnepf 1997; Kühn *et al.* 2004). Recently, an undescribed *Pseudopirsonia* species infecting the diatom *Coscinodiscus wailesii* was reported from Korean coastal water (Kim *et al.* 2017). *Pirsonia*/*Pseudopirsonia* species share some morphological features of developmental infection process including formation of trophosomes and auxosomes during phagocytosis. Once the parasite flagellate attached on the host frustule, the parasite pseudopodium penetrates the diatom protoplasm. The pseudopodium inside the host cell becomes a trophosome that gradually phagocytizes the host protoplasm and transports the digested host materials to the auxosome, which is a remaining part of the flagellate outside host cell. After several divisions of the auxosome, the motile stage of the parasite (i.e. zoospores) are produced. Although the developmental process is very similar in the two genera, the morphological features of the flagellates are different from each other. While *Pseudopirsonia* species have flagella inserted submedianly or in the middle of the cell, *Pirsonia* species have subapically stretched flagella (Kühn *et al.* 1996). Later, molecular phylogenetic analyses revealed that the two genera were distantly related; *Pirsonia* species placed within Stramenopiles, but *Pseudopirsonia* fell into Cercozoan group (Kühn *et al.* 2004).

In this study, infections of the marine centric diatom *C. wailesii* by the parasitic nanoflagellate *P. diadema* were observed in the Yongho Bay of Busan, Korea during the diatom bloom events from January 2017 to January 2018. During the study period, parasite prevalence (i.e. percent of infected host cells) and host abundance with environmental factors were determined. Additionally, the parasite and host system has been successfully established in culture and development and infection process, parasite generation time, parasite prevalence as a function of inoculum size, and survival and infectivity of the parasite zoospores were determined using the culture.

MATERIALS AND METHODS

1. Sampling

Surface seawater samples were collected using a Niskin bottle at a fixed site in the Yongho Bay in Busan, Korea once or twice a week from 01 January 2017 to 22 January 2018. During the bloom event of diatom *C. wailesii* from 10 November to 13 December 2017, daily sampling was performed. Water temperature and salinity were measured in situ using a YSI instrument (YSI Inc., OH, USA). To measure Chl *a* concentration, 300 mL seawater was filtered onto GF/F filter paper (47 mm; Whatman, Middlesex, UK) and the filter was stored at −20°C until further analysis. The filtrate was placed into an acid-cleaned polyethylene bottle, and stored at −20°C for analyzing inorganic nutrients. Nitrate plus nitrite and phosphate concentrations were measured using a flow injection autoanalyzer (QuikChem 8000, Lachat, CO, USA) and calibrated using standard solutions of brine (CSK Standard Solutions; Wako Pure Chemical Industries, Osaka, Japan).

2. Parasite prevalence in the field

During microscopic examination, the diatom *Coscinodiscus* species were frequently observed to have the outward attachments by parasite protists. The morphological features of the parasite development were tracked using live materials and what types of the parasites infected the diatoms was discriminated based on previous descriptions for the diatom parasites (Kühn *et al.* 1996, 2004; Kim *et al.* 2017). The parasite prevalence (percent of infected host cells; %) were determined by examination of more than 30 host cells from live samples at each sampling date.

3. Parasite and host culture system preparation

The diatom *C. wailesii* cells infected by the parasitic nanoflagellate *P. diadema* were isolated using a drawn glass Pasteur pipet from net-tow sample taken on 28 November 2017. The isolates of infected *C. wailesii* were gently washed five to six times with sterile filtered seawater and then placed into each well of six-well plate. Ten of uninfected *C. wailesii* cells were added into the well containing the infected isolate. A total of five *Pirsonia* strains were successfully established in cultures. Uninfected *C. wailesii* cells were also isolated as described above and established as host stock cultures. Parasite cultures were propagated by sequentially transferring aliquots of infected host stock culture into uninfected host stock cultures grown in salinity 30 f/2 medium twice a week. Parasite and host cultures have been maintained at 20°C, with a 14 : 10 light : dark cycle of cool-white fluorescent light at 150 μmol photons m^{-2} s^{-1}.

4. Light microscopy

Live or lugol-fixed specimens were observed using an in-

verted microscope (Axio Vert A1, Carl Zeiss Inc., Oberkochen, Germany) equipped with differential interference contrast and phase contrast optics. Light micrographs were taken at 400× to 1000× magnification using an AxioCam HRc (Carl Zeiss Inc.) and video-recorded using a Full HD mini box camera (MediCAM-X, Comart System, Seoul, Korea) photomicrographic system coupled to the microscope.

5. Scanning electron microscopy

Cultures of infected host cells and parasite zoospores were fixed in glutaraldehyde (2% final concentration) with 0.2 M cacodylate buffer (pH 7.4) for 1 h at 4°C. The sample was then filtered onto 0.8 μm pore sized isopore membrane filter (Whatman, Florham Park, USA), washed in distilled water for 1 h, dehydrated in a graded ethanol series (30, 50, 70, 80, 90 and 99%) for 15 min at each step, and then rinsed three times in absolute ethanol for each 15 min. The filter was critical point dried in liquid CO_2 using an HCP-2 (Hitachi, Tokyo, Japan) and then sputter-coated with platinum for 3 min and examined using a Hitachi FE-SEM (model S-4700; Hitachi) operating at 15 kV.

6. DNA extraction, PCR, sequencing

About 200 cells of parasite nanoflagellates detached from the host cells at the late stage of infection were collected using a capillary pipet, washed several time with sterile filtered seawater, placed into a PCR tube, and then pelleted by centrifugation. A total six strains of parasite nanoflagellate were pelleted as described above. Total genomic DNA was extracted from the pellet according to a chelex extraction method (Kim and Park 2014). The purity and quantity of the DNA extract was checked with a NanoDrop ND-1000 system (Thermo Fisher Scientific, DE, USA). Small subunit ribosomal DNA (SSU rDNA) region of the parasite nanoflagellate was amplified using primer sets of Euk328f and Euk329r (Moon-van der Staay *et al.* 2000). PCRs were performed in a total of 20 μL of reaction solution containing 2 μL of DNA as a template using an AccuPower PCR premix kit (Bioneer, Daejeon, Korea). The reactions were conducted using a C1000 Touch Thermal Cycler (Bio-Rad, Hercules, CA, USA) with the following conditions: initial denaturing step at 95°C for 3 min followed by 39 cycles (95°C for 45 s, 53°C for 45 s, and 72°C for 1 min 30 s), with a final extension at 72°C for 7 min and stored at 8°C. Amplified products were visualized on EcoDye (SolGent Co., Daejeon, Korea) stained 1% agarose gels, purified with a PCR purification kit (Bioneer, Daejeon, Korea) and gel extraction kit (Bioneer, Daejeon, Korea) according to the manufacturer's manual. The purified PCR products were sequenced with PCR primers, Euk328f and Euk329r, and sequencing primers, SR4 and SR5 (Yamaguchi and Horiguchi 2005) using a Big-Dye Terminator v3.0 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and an ABI model 3730 sequencer (Applied Biosystems) at Macrogen Corp. (Seoul, Korea). ContigExpress (Vector NTI ver. 10.1; Invitrogen, Grand Island, NY, USA) was used to edit out low quality regions and assemble the sequence reads. The assembled sequences were verified by comparison using BLASTN search in the NCBI database and deposited in GenBank (MW255637).

7. Alignments and phylogenetic analyses

Sequences were primarily aligned using CLUSTALX 1.83 (Larkin *et al.* 2007) and then refined manually using Mesquite 3.61 (Maddison and Maddison 2019). Unambiguously aligned positions were applied for phylogenetic analyses. Alignment of 18S ribosomal DNA sequences contained 47 taxa in stramenopiles as ingroup and two taxa of glaucophytes as outgroup. Maximum Likelihood (ML) analysis was performed using RAxML 8.0.0 (Stamatakis 2014) with the general time-reversible model with gamma correction and 2,000 replicates. Bayesian analysis used MrBayes 3.1.1 (Ronquist *et al.* 2012) running four simultaneous Monte Carlo Markov Chains for 2,000,000 generations and sampling every 100 generations, following a prior burn-in of 100,000 generations (1,000 sampled trees were discarded).

8. Parasite prevalence as a function of inoculum size

To determine parasite prevalence as a function of inoculum size, triplicate 24 well plates containing host cells were inoculated with zoospores and incubated for 12 h under growth culture conditions as described above. Incubation time was shorter than the time needed for the parasite to reach maturity. Inoculum size for each set of triplicate wells was adjusted to achieve zoospore:host ratios of 1:1, 2:1, 5:1, 10:1, 20:1, 50:1, and 100:1. After incubation, samples were examined under inverted microscopy (Axio Vert A1, Carl Zeiss Inc., Oberkochen, Germany). Parasite prevalence, i.e. percent of infected cells, was determined

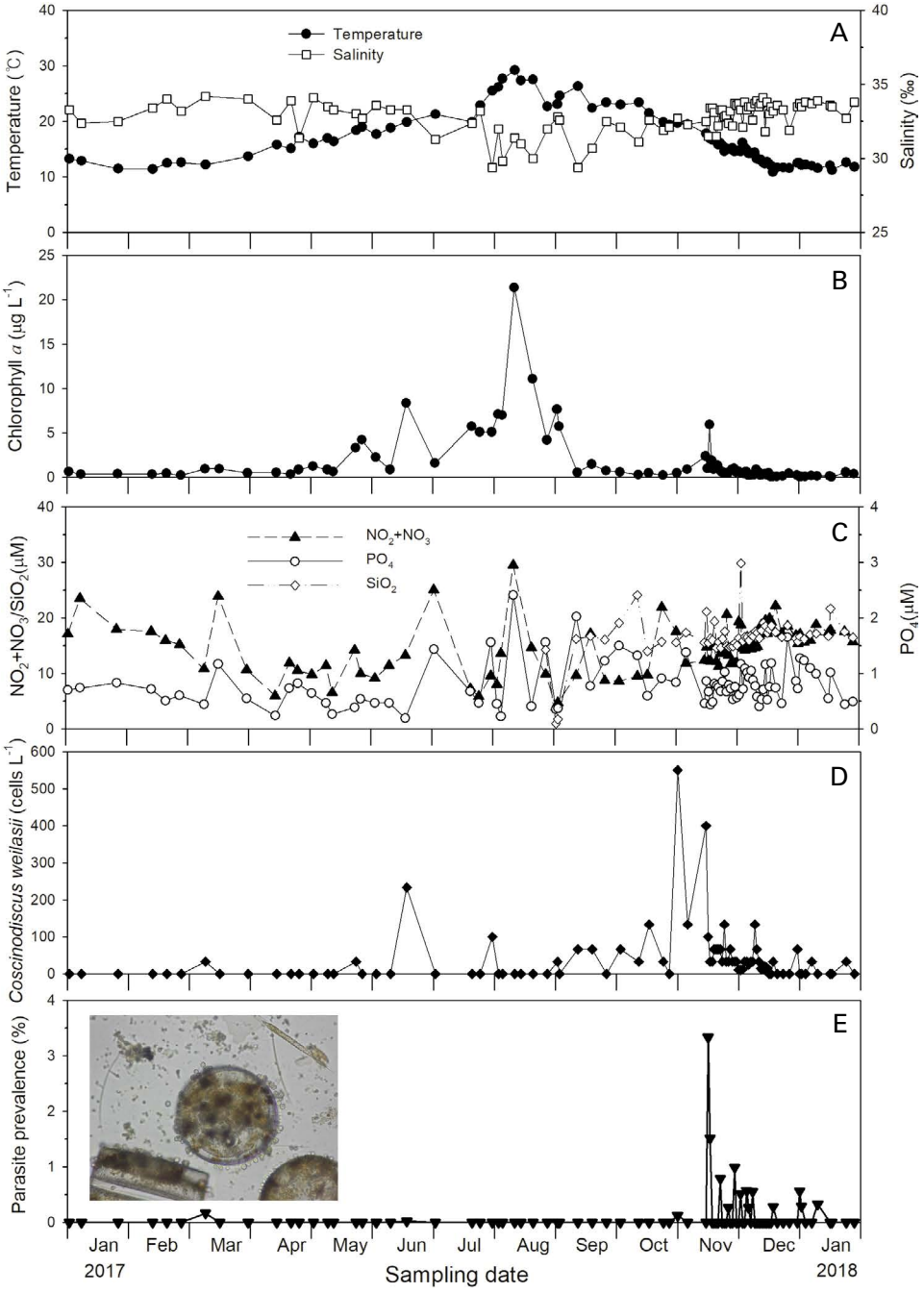

Fig. 1. Temporal variations in (A) surface seawater temperature and salinity, (B) chlorophyll *a* concentration (C) dissolved inorganic nutrients, (D) host abundance, and (E) parasite prevalence during the study period. Insert: *Coscinodiscus wailesii* infected by *Pirsonia diadema* in a field sample.

by scoring at least 30 host cells per sample as infected or uninfected. Data were fitted to a single two-parameter exponential rise to maximum. The equation for the curve fit was $y = a \times (1 - e^{-bx})$, where a is the maximum incubation level (I_{max}) and b is α / I_{max} (Coats and Park 2002). Alpha (α) indicates the the initial linear slope of the fitted curve and reflects the potential of zoospores to infect host cells. Estimate for α was derived as $I_{max} \times b$.

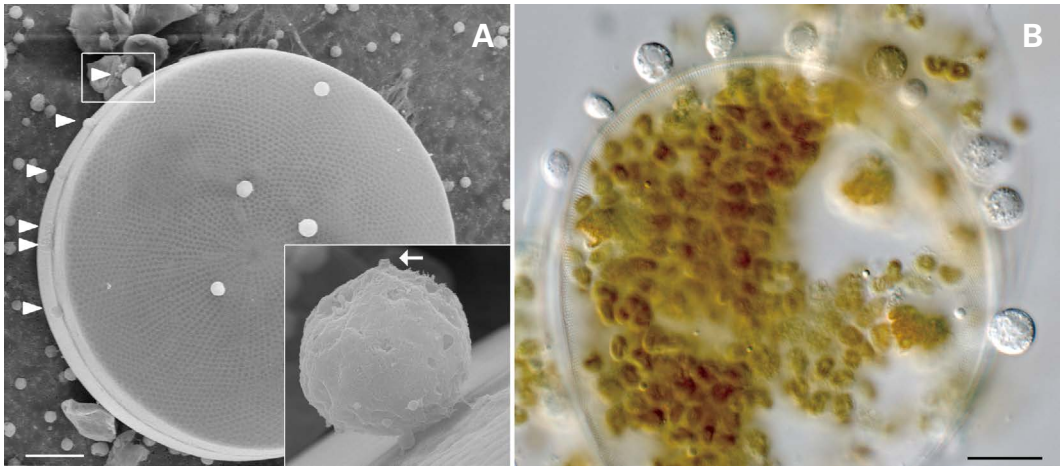

Fig. 2. *Pirsonia diadema* in *Coscinodiscus wailesii*. (A) Primary auxosomes attached to the margin of the diatom valve ring. Insert: Retraction of the parasite flagellum (B) appearance of the diadem by heavy infections. Arrow heads indicate auxosomes. Scale bars represent 20 μm.

9. Survival and infectivity of parasite zoospores

Fifty mL volumes of newly detached mature zoospores were harvested using a sterile capillary pipet from the infected host *C. wailesii* cells and distributed into triplicate 20 mL vials. The vials were held under the same culture condition as described above and subsamples were taken from each vial over time to estimate zoospore survival and infectivity. At each sampling period, 1 mL volume of zoospores were fixed with Lugol's solution and examined for zoospore abundance using a Palmer-Maloney counting chamber at 400x magnification using a bright-field microscope (Axioscope, Carl Zeiss Inc., Germany).

To determine the ability of zoospores to infect host cells with aging, 1 mL subsamples from each of the triplicate 50 mL volumes of zoospores were distributed into a 24-well plate containing host cells (10 cells per well) at each sampling period (8 h interval). Ratio of zoospore : host of 37 : 1 were applied for the initial sampling time (T0) and ratios for subsequent sampling times varied with survival of zoospores. Each well was incubated for 12 h and determined parasite prevalence under a bright-field microscopy.

RESULTS

1. Environmental condition

Surface seawater temperature ranged from 11.2°C (on 11 January 2018) to 29.2°C (on 8 August 2017) during the study period, showing a typical seasonal variation in temperate coastal waters (Fig. 1A). Salinity ranged from 29.4 to 34.2 and relatively lower salinity was shown between July and August due to frequent rainfalls (Fig. 1A). Chlorophyll *a* concentrations ranged from 0.06 to 21.37 μg L^{-1}, with several peaks observed between May and November and the highest peak on 8 August, 2017 (Fig. 1B). Concentrations of inorganic nutrients remained high throughout the study period, with nitrate plus nitrite ($NO_3 + NO_2$) ranging from 5.9 to 29.5 μM (14.5 ± 0.50; mean ± SE, $n = 87$), phosphate (PO_4) ranging from 0.2 to 2.4 μM (0.8 ± 0.04; mean ± SE, $n = 87$), and silicate (SiO_2) ranging from 1.0 to 29.8 μM (16.5 ± 0.49; mean ± SE, $n = 59$), respectively (Fig. 1C).

2. Dynamics of host abundance and parasite prevalence

The centric diatom *C. wailesii* cells occurred throughout the study period (Fig. 1D). The highest cell abundance (up to 550 cells L^{-1}) was recorded on 27 October and then decreased sharply to 133 cells L^{-1} on 1 November. Subsequent peak of *C. wailesii* cell abundance was observed on 10 November with the cell abundance of 400 cells L^{-1} and gradually declined thereafter. During the diatom bloom events, infections of *C. wailesii* by the parasitic nanoflagellate *P. diadema* were observed with the parasite prevalence ranging from 0.13 to 3.33% (Fig. 1E). The highest parasite prevalence was observed on 12 November, just the day after the second peak of the host abundance (11 November). Parasite infections were not detected between June and September although relatively high host abundances were recorded during that period (Fig. 1E). In addition,

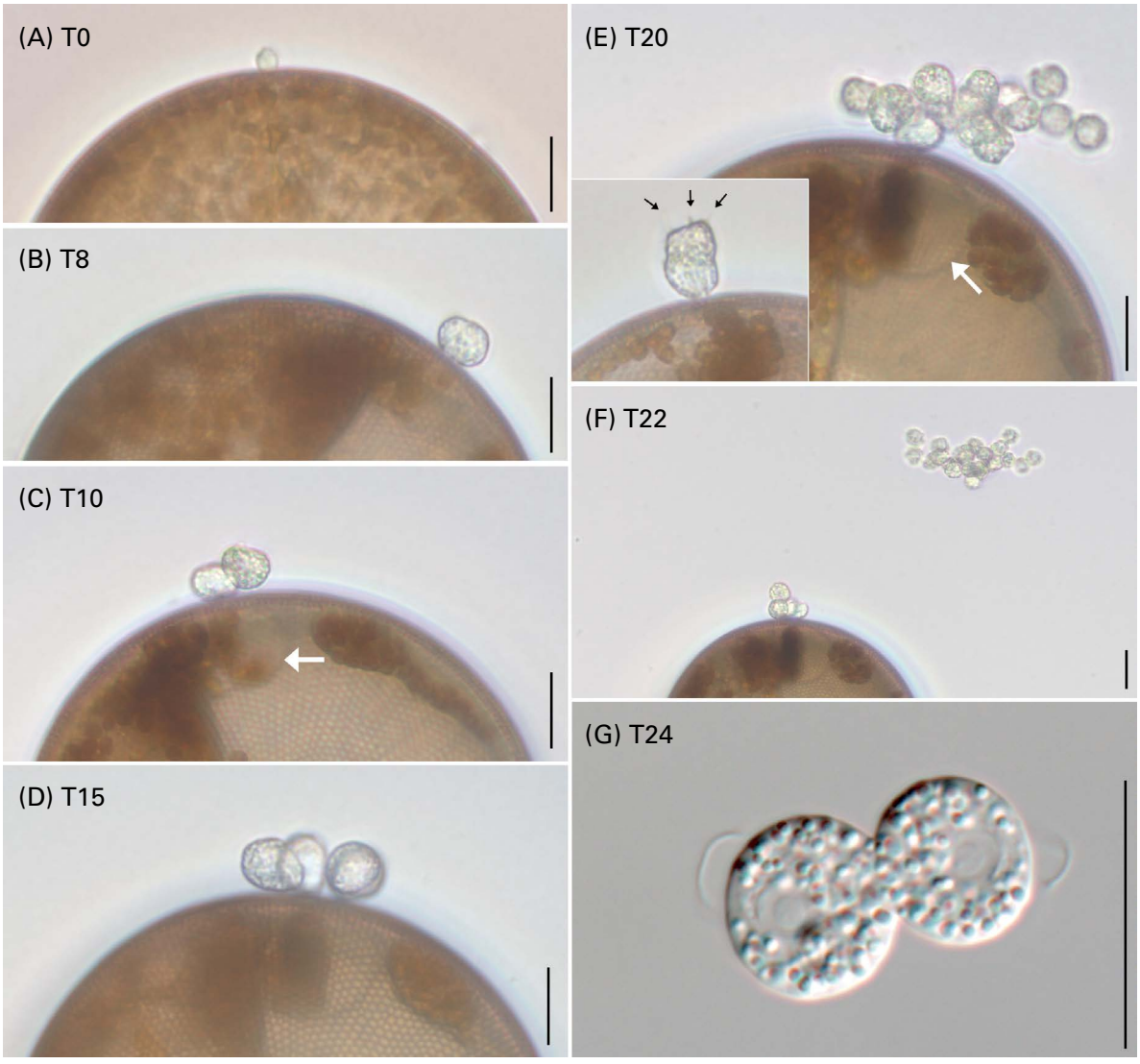

Fig. 3. Time series of the infection development process. (A) Attachment of the flagellate on the diatom frustule and retraction of the flagella. (B) Auxosome growing with phagocytosis. (C) First division of the primary auxosome. (D) Group of 4 secondary auxosomes. (E) Large group of secondary auxosomes. Insert: FMC with short flagella. (F) Group of secondary auxosomes detached from the host cell. (G) Dividing FMC. White and black arrows indicate trophosomes and short flagella of FMC, respectively. Scale bars represent 20 μm.

infections by the parasite *P. diadema* were observed only on *C. wailesii* although various diatom species co-occurred during the period (Fig. 1E).

3. Parasite morphology and development

Infection of the diatom *C. wailesii* initiated with the attachment of the parasite *P. diadema* zoospore and mostly observed at the margin of the diatom valve ring and additionally on the valve face, where rimoportulae of *C. wailesii* located in (Fig. 2). When heavily infected, almost every rimoportula of the diatom was occupied by the parasite, which provided the appearance of diadem (Fig. 2B). Once the motile parasite flagellate attached to the host, its flagella retracted and disappeared soon (Fig. 2A; insert). Time-series developmental process of the parasite after attachment on the host cell was shown in Figure 3. The attached flagellate invaded into the host's frustule using a pseudopodium, which became a trophosome inside the diatom protoplast and the flagellate remain outside of the host cell, which be-

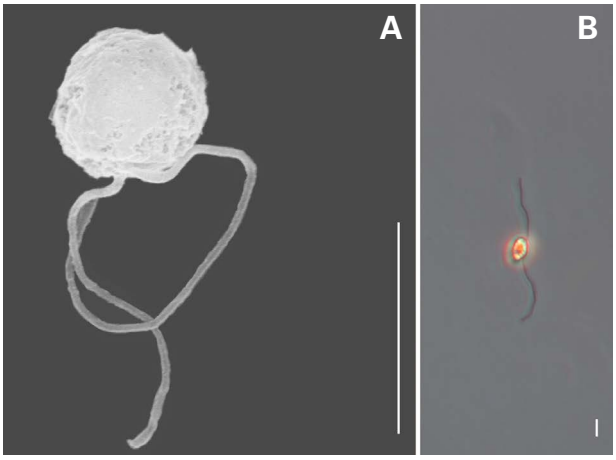

Fig. 4. Zoospore morphology of *Pirsonia diadema*. (A) Scanning electron micrograph. (B) Light micrograph with phase contrast. Scale bars represent 5 μm.

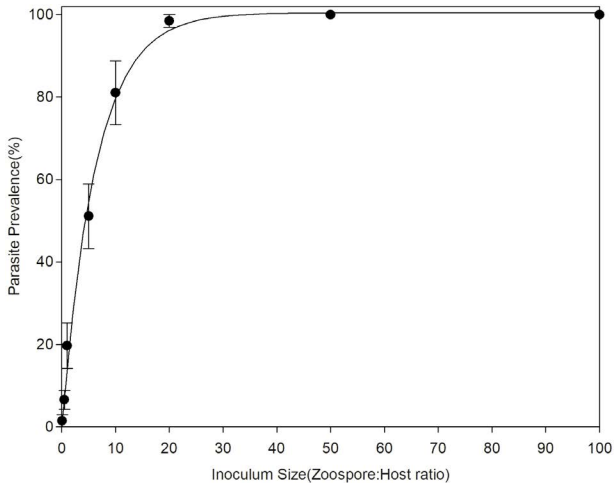

Fig. 5. Parasite prevalence as a function of inoculum size for *Pirsonia diadema* infecting *Coscinodiscus wailesii*. Data are shown as the mean ± SE.

came an auxosome (Fig. 3). The primary auxosome of the parasite was apple-shaped with 9.4 ± 0.2 μm in length and 10.2 ± 0.4 μm in width (mean ± SE, $n = 30$) (Fig. 3B). First division of the primary auxosome occurred longitudinally after 9.7 ± 0.5 h (mean ± SE, $n = 30$) from the initial attachment (Fig. 3C). Subsequent auxosome divisions generally occurred transversely (Fig. 3D). The resultant secondary auxosomes and the FMCs (Flagellate Mother Cells) with short flagella were observed after 20 h (Fig. 3E; insert). Mature motile flagellates were observed after 22 h and total generation time was estimated to be 20.3 ± 2.0 h (mean ± SE, $n = 12$). The auxosomes and zoospores contained colorless granules (Fig. 3). Newly formed flagellates had ovoid shape and were measured as 6.5–9.9 μm (on average 7.6 μm) 3.5–7.7 μm (on average 5.6 μm), with two flagella of 9.4–22.8 μm (on average 16.9 μm) × 13.4–28.5 μm (mean 22.6 μm) (Fig. 4).

4. Parasite prevalence as a function of inoculum size

Parasite prevalence of *P. diadema* showed an exponential increase as a function of zoospore inoculum size (Fig. 5). Estimates for maximum infection levels (I_{max}) and initial slope of the fitted curve (a) were $I_{max} = 100$ and $a = 15.86$ ($r^2 = 0.997$; $p < 0.0001$), respectively. The parasite prevalence increased sharply to near the maximum level by zoospore to host ratio of 20 to 1. Inoculations above a 20 : 1 ratio typically provided 100% infection of the host *C. wailesii* cells (Fig. 5).

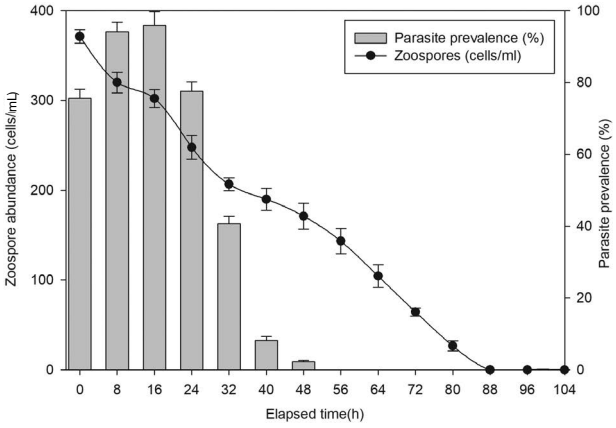

Fig. 6. Survival and infectivity of *Pirsonia diadema* zoospore with age. Error bars indicate standard errors.

5. Zoospore survival and infectivity

Newly formed (less than 3 h) zoospores from infected host cultures were applied to determine the survival and infectivity of the parasite. From the onset of the experiment, zoospore abundance gradually decreased with elapsed time and cells were not detected after 88 h (Fig. 6). The ability of zoospores to initiate infections (i.e. attachment of zoospore on host's frustule) decreased with age (Fig. 6). The infection rate of the surviving zoospores increased to 95.9 ± 3.9% over the following 16 h, and then rapidly declined until 48 h. After 48 h, infections of the host cells by the parasite zoospores were not observed any more although the ratio of

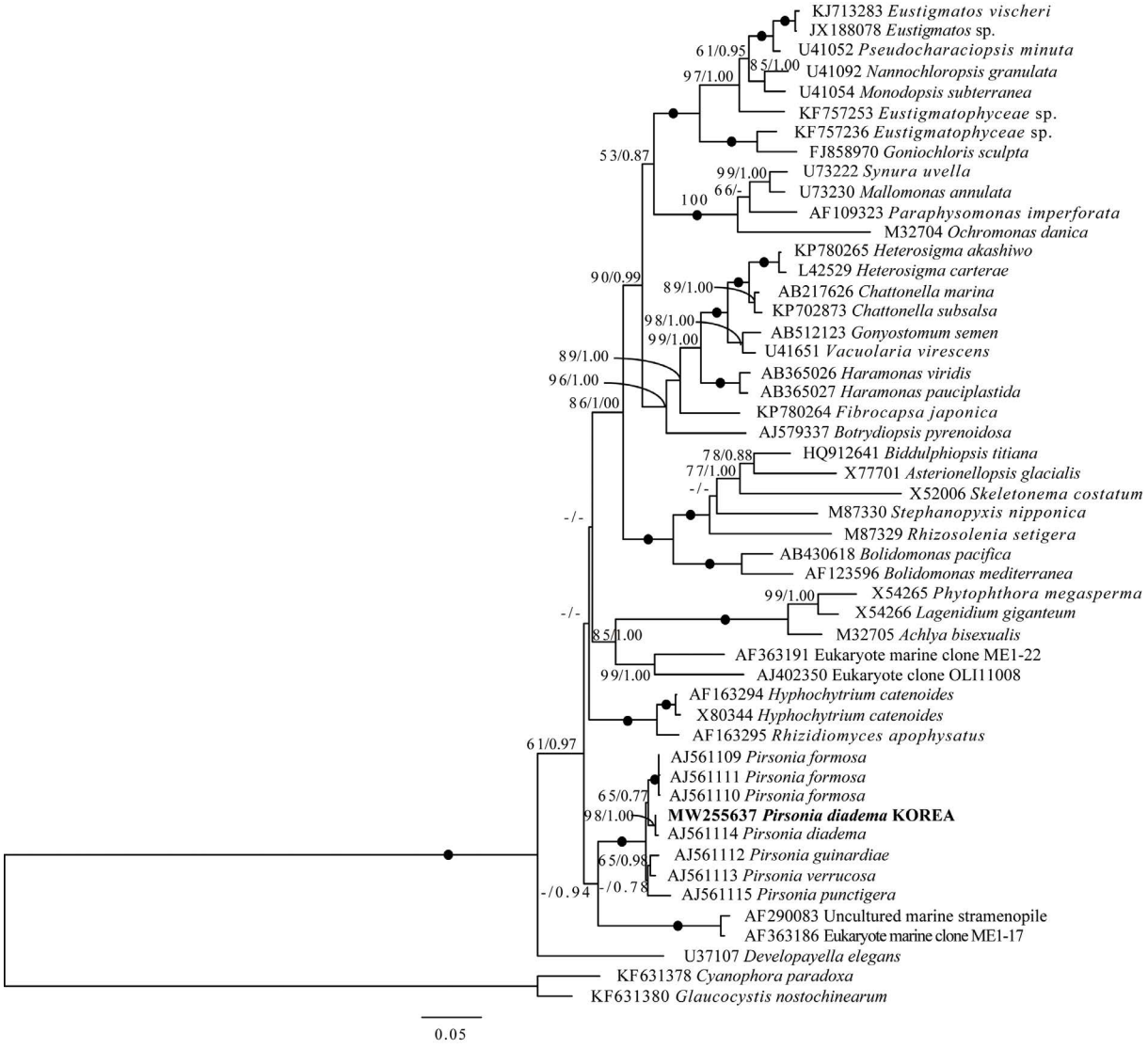

Fig. 7. Phylogenetic tree inferred from 18S rRNA gene sequences including 47 stramenopiles and two glaucophytes as outgroups. Numbers above the nodes indicate support values of ML bootstrap (left) and Bayesian posterior probabilities (right) higher than 50% and 0.7, respectively. Closed circles represent robust statistical supports (100/1.00 for ML/BP).

surviving zoospores to host was over 10 to 1 (Fig. 6).

6. Molecular phylogeny

Partial 18S rRNA gene (18S rDNA) sequences of 1,605 bp were successfully obtained from three *P. diadema* strains infecting *C. wailesii* collected from Korean coastal water in November 2017. Based on the amplified region, all sequences of the three strains showed 100% identity. BLASTN search of GenBank provided a maximum 99.69% matches (2 nucleotides difference and 3 gaps with 100% query coverage) of the 18S rDNA sequence of the Korean *P. diadema* to that of the *P. diadema* isolate P757 (AJ561114) collected from the North Sea.

Phylogenetic analyses inferred from 18S rDNA sequences revealed that the Korean *P. diadema* clustered together with other *Pirsonia* species isolated from the North Sea and formed a monophyletic group with high statistical supports of bootstrap proportion (BP)/posterior probabilities (PP) (100/1.00) (Fig. 5). In the ML tree, the *Pirsonia* species diverged into two distinct lineages, one consisting of three *P. formosa* strains and two *P. diadema* strains and the

Table 1. Pairwise distance between *Pirsonia* species based on partial 18S rRNA gene sequences (1,594 bp)

No.	Species (Accession No.)	1	2	3	4	5	6	7
1	*P. formosa* (AJ561109)	–						
2	*P. formosa* (AJ561110)	0.001	–					
3	*P. formosa* (AJ561111)	0.001	0.001	–				
4	*P. diadema* (AJ561114)	0.011	0.010	0.011	–			
5	*P. guinardiae* (AJ561112)	0.009	0.010	0.010	0.012	–		
6	*P. punctigera* (AJ561115)	0.016	0.022	0.022	0.019	0.021	–	
7	*P. verrucosa* (AJ561113)	0.008	0.008	0.008	0.010	0.006	0.018	–
8	*P. diadema* (This study)	0.009	0.010	0.010	0.001	0.011	0.018	0.008

other including *P. punctigerae* and the clade of *P. verrucosa* and *P. guinardiae*, although inner nodes for the relationships were weakly to moderately supported (Fig. 7)

Pirsonia species were very closely related each other and the pairwise distance (*p*-distance) between *Pirsonia* species ranged from 0.006 to 0.022. The 18S rRNA gene sequence of *P. diadema* was the most closely related to *P. verrucosa* (AJ561113) with 0.008 of *p*-distance and the most distantly related to *P. guinardiae* (AJ561112) with 0.018 of *p*-distance, respectively (Table 1).

DISCUSSION

In the early classical perception of energy transfer in marine planktonic food web, phytoplankton production is simply transferred to larger heterotrophic zooplankton through grazing food chain and the predators are typically an order of magnitude larger than their prey (Steele 1970; Sheldon *et al.* 1977). In the late 20th century, it has become evident that the energy transfer between trophic levels in marine plankton takes place through a highly complex food web than previously thought. Heterotrophic nanoflagellates are ubiquitous small protozoans in marine ecosystems and generally considered as consumers of picoplankton, including mainly heterotrophic bacteria and cyanobacteria and transfer bacterial production to higher trophic level via microbial food web (Sherr *et al.* 1991). In addition to small-sized prey, however, heterotrophic nanoflagellates could also directly consume large-sized phytoplankton through parasitism (e.g. Kühn *et al.* 1996). Few studies have focused on the ecological impact of the parasitic protists on marine diatom populations (e.g. Tillmann *et al.* 1999).

Coscinodiscus species are large-sized centric diatoms and frequently occur in temperate coastal waters. Due to the large cell size, they are difficult to be grazed by zooplankton like copepods and the phytoplankton biomass could not be efficiently transferred to higher trophic level (Roy *et al.* 1989). In this case, infections by host specific parasites may be the most important biological factors that cause mortality of marine diatom populations and species succession. Therefore, the large diatoms could be converted into biomass of small parasites and then the parasite zoospores could be readily eaten by microzooplankton as noted in early studies (Johansson and Coats 2002).

Infections of *Coscinodiscus* have been reported to be caused several parasites such as the parasitic nanoflagellates *P. diadema* (Stramenopiles) and *Victoriniella multiformis* (incerta sedis) from the German Bight of North Sea (Kühn *et al.* 1996; Kühn 1997) and *Pseudopirsonia* sp. (Cercozoa) from Nokdong harbor of Korea (Kim *et al.* 2017) and the parasitic fungus *Lagenisma coscinodisci* (Oomycetes) from the northern North Sea (Drebes 1968; Grahame 1976; Wetsteyn and Peperzak 1991), from the Weser estuary of northern Germany (Chakravarty 1974), from the Puget Sound of Washington (Gotelli 1971), and from the Kingston harbor of Jamaica (Grahame 1976). To our knowledge, however, few investigations have determined the impact of the parasites on marine *Coscinodiscus* populations and parasite prevalence data are available only for the oomycetes *L. coscinodisci*. The highest infection rates of *L. cosinodisci* varied between 22.2 and 58.3% in *C. concinnus* and between 7.1 and 41.9% in *C. granii* from the Oosterschelde of Netherlands at the water temperature ranging from 13.2–18.7°C (Wetsteyn and Peperzak 1991). The present study did for the first time determine prevalence of the parasitic nanoflagellate *P. diadema* on the marine *Coscinodiscus* field populations, showing that the infection rates of *P. diadema* were relatively low (0.3 to 3.3%) compared to that of the oomycetes *L. coscinodisci*.

Generally, parasite epidemics could be affected by abiotic factors such as light, temperature, turbulence, and nutrient deficiency (Kühn *et al.* 1998; Kühn and Hofmann 1999). Temperature is one of the most important environmental factors. In this study, infections by *P. diadema* were observed only at the water temperature ranging between 10.9 and 19.9°C although the host populations appeared at temperature above 25°C. High temperature is known to decrease the number of zoospores produced per sporangium and infectivity of zoospores, and influence negatively on the parasite lifetime (Bruning 1991). Our results from culture experiment showed that the *P. diadema* zoospores lost infectivity within a very short time (less than 48 h), suggesting that the parasite zoospores might quickly lose their infectivity at the temperature above 25°C. Similarly, infections of marine diatoms by other parasites have been observed at the temperature below 20°C in field observations (Wetsteyn and Peperzak 1991; Kühn *et al.* 1996; Tillmann *et al.* 1999).

Turbulence, i.e. water movement, is also considered as the most important abiotic factor to affect parasite epidemics (Kuhn and Hofmann 1999). Typically, increasing turbulent mixing could increase the encounter rates between planktonic organisms, i.e. prey and predator (Rothschild and Osborn 1988). However, the contact time between nanoflagellates and diatoms will decrease with increased turbulent mixing (Kühn and Hofmann 1999). Many parasitic nanoflagellates including *P. diadema* are host specific and infect only host diatom species or related species. Under turbulent condition, the nanoflagellate *P. diadema* that infects specific sites only on the diatom frustule, i.e. rimoportulae, will have more difficulties to attach. Also, they may depend on chemical stimuli to detect their diatom host. Diatoms are known to release a variety of extracellular organic substances (Mague *et al.* 1980). Turbulence would destroy the gradients of extracellular release around the host diatom, so that the parasites will be difficult to detect their hosts. Furthermore, unlike endoparasites that invade their diatom hosts such as *L. coscinodisci* and *V. multiformis*, in the case of the ectoparasite *P. diadema*, the developing premature offspring would be more likely to be readily detached mechanically from the diatom frustule under turbulent condition. Our study area is shallow (5–7 m in depth) and the entire water column is well mixed during the diatom bloom events in spring and fall. Particularly, during autumn, this area is strongly affected by frequent typhoon and strong wind. This turbulent environmental condition may explain part of the low parasite prevalence in this study.

Lastly, unfavoured physiological conditions or stress like nutrient deficiency might also affect host susceptibility to parasite infection (Tillmann *et al.* 1999). Given that inorganic nutrients concentrations in the study area remained high during the study period, it might be that the replete nutrient condition caused such a low parasite prevalence. However, the relationship between parasite epidemics and poor nutrient condition has not been distinctly demonstrated yet in this host-parasite system and needs to be addressed in future.

ACKNOWLEDGEMENTS

This research was supported by a Research Grant of Pukyong National University 2019.

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