Feeding behavior, spatial distribution and phylogenetic affinities of the heterotrophic dinoflagellate *Oxyphysis oxytoxoides*

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ABSTRACT: We documented the abundance of the heterotrophic dinoflagellate *Oxyphysis oxytoxoides* Kofoid along the Korean coast, cultured it (food source: the marine ciliate *Myrionecta rubra* Jankowski [= *Mesodinium rubrum* Lohmann]), sequenced its nuclear-encoded large-subunit (LSU) rDNA, and resolved its phylogenetic relationship within the Dinophysiales. Abundance was low, usually $<9.7 \times 10^3$ cells l^{-1}, in samples from 2005 and 2006. It co-occurred with the mixotrophic dinoflagellate *Dinophysis acuminata*, possibly indicating a common pool of prey. Growth on *M. rubra* reached a density of 38×10^5 cells l^{-1}. Observations on live cells from our established cultures revealed that *O. oxytoxoides* uses a peduncle to suck up the cell contents of *M. rubra*, the number of food vacuoles increasing as feeding proceeds. No regional variability was found in partial LSU rDNA sequences (domains D1–D2) of our strains, despite different sampling sites and dates. Phylogenetic analysis, based on LSU rDNA, showed that while *Amphisolenia* forms an early divergent basal group in the Dinophysiales, the other dinophysoids form 2 separate groups: (1) *Oxyphysis* and *Phalacroma*, and (2) *Histioneis*, *Ornithocercus*, *Citharistes* and *Dinophysis*. The presence of a peduncle in several genera, and the widespread distribution of pedunculate dinophysoids, indicate that these species may be fundamentally heterotrophic with a common feeding mechanism. Our results shed light on the feeding ecology and phylogenetic position of *O. oxytoxoides*.

KEY WORDS: Dinophysiales · *Myrionecta rubra* · *Oxyphysis oxytoxoides* · Peduncle feeding · Phylogeny

INTRODUCTION

The order Dinophysiales (Dinophyceae) is a group of thecate dinoflagellates exhibiting a highly diverse morphology and trophic mode. This order consists of 3 families — Amphisoleniaceae, Dinophysiaceae and Oxyphysiaceae — which contain 12 genera and 282 species (Fensome et al. 1993, Steidinger & Tangen 1996, Gómez 2005). While the genus *Dinophysis* includes 104 species, which are more than in any of the other genera, the genera *Metaphalacroma* and *Oxyphysis* each contain only 1 species (Gómez 2005). Although species belonging to the Dinophysiales show worldwide distribution in the marine environment,

some genera show a restricted distribution pattern; for example, genera such as *Ornithocercus* and *Histioneis* are confined to tropical waters, and *Oxyphysis* is restricted to the North Pacific (Foissner 2006, Taylor et al. 2008).

The genus *Oxyphysis* was usually included in the Amphisoleniaceae (e.g. Schiller 1933, Loeblich 1982) until Sournia (1984) separated it into its own family. The family Oxyphysiaceae is characterized by the location of the ventral pore at the apex, unlike the families Amphisoleniaceae and Dinophysiaceae in which the pore is situated ventrally (Fensome et al. 1993). The family currently contains *O. oxytoxoides* only (as a type species), which is a laterally compressed, moder-

ately elongate, and thecate heterotrophic species (Kofoid 1926). Our knowledge of the ecophysiology, life cycle and molecular phylogeny of *O. oxytoxoides* is limited, although its feeding mechanism and behavior have been reported (Inoue et al. 1993).

Intergeneric relationships within the Dinophysiales have received much attention over the past ~80 yr. Due to its structural similarities with the genus *Oxytoxum*, Kofoid (1926) considered the genus *Oxyphysis* as a possible link between the orders Dinophysiales and Peridiniales. Kofoid & Skogsberg (1928) proposed, for the first time, a hypothetical evolutionary radiation for the dinophysoids, based on information about structural resemblance and degree of specialization in thecal morphology. Abé (1967) considered that the elongate genera *Amphisolenia* and *Triposolenia* arose from the genus *Oxyphysis*. Taylor (1980) proposed that genera such as *Metaphalacroma*, *Pseudophalacroma*, *Oxyphysis*, *Proheteroschisma* and *Heteroschisma* exhibit morphological experimentation of the apical plate region and represent several short lines rather than a continuing evolutionary sequence. Very recently, Jensen & Daugbjerg (2009) examined the phylogenetic relationships among 6 genera (*Amphisolenia*, *Citharistes*, *Dinophysis*, *Histioneis*, *Ornithocercus* and *Phalacroma*) based on LSU rDNA sequences and — based on compiled information from Kofoid & Skogsberg (1928), Tai & Skogsberg (1934), Taylor (1980) and Hallegraeff & Lucas (1988) — hypothesized that *Oxyphysis* radiated from *Metaphalacroma*. Handy et al. (2009) addressed relationships among 4 genera (*Dinophysis*, *Histioneis*, *Ornithocercus* and *Phalacroma*) in the Dinophysiaceae based on rDNA sequences. None of these studies, however, included *O. oxytoxoides* in the phylogenetic analysis to resolve intergeneric relationships among genera in the order Dinophysiales.

The objectives of this study were (1) to document the spatial distribution of the heterotrophic dinoflagellate *Oxyphysis oxytoxoides* along the Korean coasts, (2) to establish *O. oxytoxoides* in culture with the ciliate prey *Myrionecta rubra*, (3) to sequence the nuclear-encoded LSU rDNA of our Korean strains of *O. oxytoxoides* (and to check for any regional variations), and (4) to resolve the phylogenetic relationship of *O. oxytoxoides* within the order Dinophysiales.

MATERIALS AND METHODS

Sampling. Samples were taken from 15 sites along the Korean coast (Fig. 1) during the following periods: 26 to 30 July, 24 to 27 August and 23 to 26 September 2005, and 11 to 14 May 2006. During each of the sampling periods, samples were collected from all of the 15 sites. During each sampling, surface water samples
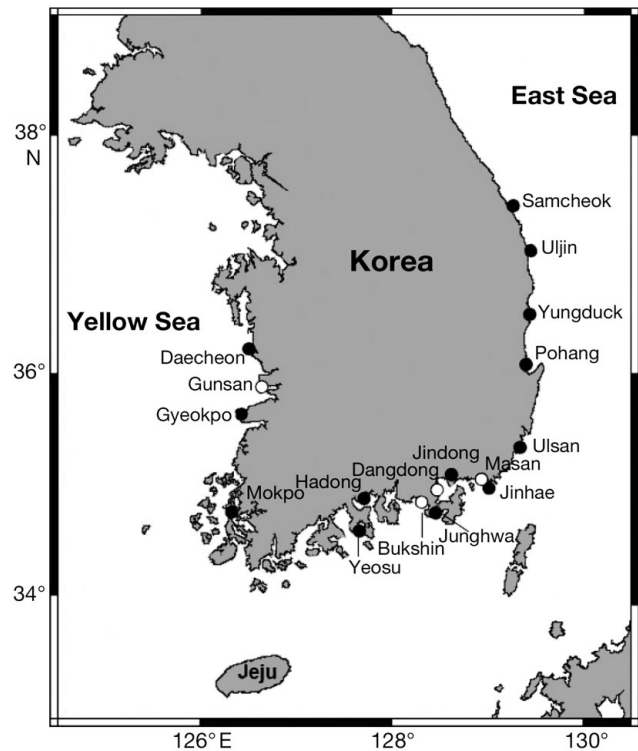

Fig. 1. Sampling sites along the Korean coasts. Open circles indicate sites where samples for single-cell PCR were also collected

(300 ml) were collected using a bucket and then immediately fixed with acidic Lugol's solution (2 % final conc.).

Cell isolation and cultures. Seawater samples for establishing cultures, and for carrying out single-cell polymerase chain reaction (PCR), were collected using a 20 μm plankton net from 4 sites in September and December 2009 (Fig. 1, Table 1). *Oxyphysis oxytoxoides* cells (1 to 6 cells per sampling site) were isolated from the concentrated plankton samples using drawn Pasteur glass pipets under an inverted microscope (Olympus model IX51) or a stereomicroscope (Olympus model SZX7). The isolated single cells were used for either setting up cultures or as templates for single-cell PCR to determine a partial LSU rDNA sequence (see 'DNA extraction, PCR amplification and DNA sequencing' below). To establish cultures, individual cells were first washed at least 5 times in GF/F-filtered, autoclaved seawater and then transferred into 6-well Nunc plates (Nunclon™) containing 3 ml of f/2-Si medium with salinity 30 (+5 % v/v soil extract). Then, the ciliate *Myrionecta rubra* strain MR-MAL01, which was grown using the cryptophyte strain CR-LOHA-BE01 as prey, was provided at a concentration of about 1000 cells ml^{-1} as a food source for *O. oxytoxoides*. All cultures used in this study were maintained at 20°C on

Table 1. Summary of position of sampling sites, sampling dates, salinity, water temperature, and numbers of *Oxyphysis oxytoxoides* cells isolated for single-cell PCR along the Korean coasts. nd: not determined

Sampling sites	Latitude (°N)	Longitude (°E)	Sampling dates (2009)	Salinity (psu)	Temperature (°C)	No. of cells isolated
Gunsan	35° 56′ 16″	126° 31′ 45″	Sep 11	31.0	25.5	1
Bukshin	34° 51′ 34″	128° 25′ 08″	Sep 19	28.2	25.6	5
Dangdong	34° 59′ 21″	128° 24′ 29″	Sep 11	29.8	26.3	3
Masan	35° 12′ 04″	128° 34′ 42″	Sep 11	27.7	24.7	6
			Sep 19	30.6	24.0	3
			Dec 28	31.6	nd	1

a 14 h light:10 h dark cycle, with cool-white fluorescent lamps providing 50 µmol photons m^{-2} s^{-1}.

Cell enumeration and microscopy. For enumeration of heterotrophic *Oxyphysis oxytoxoides* and mixotrophic *Dinophysis acuminata*, Lugol's-fixed samples were first concentrated 6-fold to 50 ml; 1 ml of the concentrated samples was then loaded into a SedgeWick Rafter chamber and whole area of the chamber was scanned.

Live observations of the feeding process were made in a 6-well plate using an Olympus IX51 microscope at 400× magnification and recorded with a Sony Progressive 3CCD color video camera attached to a digital image time-lapse recording system (TCS Korea). Video sequences were frame-grabbed, and individual frames were exported in BMP format. Bright-field and epifluorescence micrographs of live cells or Lugol's-fixed cells were taken at 1000× magnification using a digital camera (PowerShot G5) coupled to the Olympus BX51 microscope equipped with differential interference contrast and fluorescence cube (U-MWB2, 450 to 480 nm excitation, 500 nm emission).

DNA extraction, PCR amplification and DNA sequencing. Single-cell PCR technique was used to obtain partial LSU rDNA sequences. Individual *Oxyphysis oxytoxoides* cells isolated from each sampling site (Table 1) were put into 10 µl of Tris-EDTA (TE) buffer (pH 8.0). The cells were then heated to 95°C for 10 min to achieve cell lysis. One microliter of each heat-treated specimen was used as a template to amplify the LSU rDNA (D1–D3 region). Amplification of LSU rDNA was performed using a primer set, D1R (5′-ACC CGC TGA ATT TAA GCA TA-3′) and D3B (5′-TCG GAG GGA ACC AGC TAC TA-3′) (Hansen et al. 2000). The PCR reaction mixture (25 µl) consisted of 1 µl of template DNA, 0.5 µl of a 10 mM solution of each dNTP, 1 µl of 10 mM primers, 2.5 µl of 10× *Taq* buffer (Diastar^{TM}), and 0.6 unit of *Taq* DNA polymerase (Diastar^{TM}). The PCR reaction was performed using MyGenie^{TM} 96 Gradient Thermal Block (Bioneer) according to the following protocol: an initial denaturation step at 94°C for 4 min, 35 cycles at 94°C for 1 min, 43°C for 50 s and 72°C for 1.5 min; we used a post-extension step of

72°C for 5 min. The size of the PCR products was ~1 kb when analyzed by electrophoresis in 1.5% agarose gels and visualized with ethidium bromide. The amplified products were directly sequenced using the same primer set for PCR, D1R and D3B. Sequencing was performed with an Applied Biosystems automated sequencer (ABI 3730xl) at Macrogen Corp, Seoul, Korea. The LSU rDNA sequences have been deposited in GenBank under accession numbers HM563682 and HQ131916.

Phylogenetic analysis. The LSU rDNA sequences were compared to the sequences of closely related taxa within the Dinophysiales obtained from the GenBank database using a BLAST search. *Prorocentrum micans* and *P. minimum* were used as an outgroup, since prorocentroids and dinophysoids not only appear to be derived from peridinioids (Taylor 2004), but also share several unique morphological characters, including the sagittal suture and the megacystic growth zone (Fensome et al. 1993). Sequences including 59 in-group taxa and the 2 outgroup taxa were aligned using ClustalX v.1.83 (Thompson et al. 1997) and manually adjusted using MacClade v.4.08 (Maddison & Maddison 2002). The alignment was trimmed to 747 positions (D1–D2 region) for phylogenetic analysis. Phylogenetic trees were inferred using maximum likelihood (ML) and Baysian inference. Modeltest v.3.7 (Posada & Crandall 1998) was used to select the most appropriate model of substitution for the ML method, run with PAUP* v.4b10 (Swofford 2003). The GTR + I + Γ (–lnL = 8575.6182, I = 0.1440, G = 0.7642) model was selected as the best-fit model for the dataset. ML analysis was performed using RAxML (Stamatakis 2006) with the rapid bootstrapping option and 1000 replicates. Baysian analysis was run with MrBayes v.3.1.2 (Ronquist & Huelsenbeck 2003). Posterior probability was determined in 2 runs, each with 4 Markov Chain Monte Carlo (MCMC) processes for 10 million generations, sampled every 100 generations, and followed a burn-in of 1 million generations. The MCMC output files were checked for stationarity with Tracer v.1.5 (http://tree.bio.ed.ac.uk/software/tracer/).

RESULTS

Abundance of *Oxyphysis oxytoxoides* in Korean coastal waters

The abundance of *Oxyphysis oxytoxoides* ranged from undetectable levels to 9.7×10^3 cells l^{-1} during 4 sampling periods (Fig. 2A). Except for August 2005, when *O. oxytoxoides* was not observed across all sampling sites, the highest cell concentrations were always found at Masan. During the same period, the abundance of *Dinophysis acuminata* ranged from undetectable levels to 11.5×10^3 cells l^{-1} (Fig. 2B). The highest cell concentrations of *D. acuminata* were also found at Masan.

Cultivation of *Oxyphysis oxytoxoides*

When grown in a salinty 30 f/2-Si medium at 20°C in a 14 h light:10 h dark cycle (50 µmol photons m^{-2} s^{-1}) and supplied with the marine ciliate *Myrionecta rubra*

as prey, *Oxyphysis oxytoxoides* grew well (Fig. 3A). We managed to maintain the culture under these growth conditions for ~5 mo, after which, unfortunately, we lost the culture due to an accident. During cultivation, the highest cell density was 38×10^5 cells l^{-1}. No sustained growth was observed in the absence of the ciliate prey.

Feeding

Observations of live cells under the microscope, using cells from our established cultures, revealed that the nutritional mode of *Oxyphysis oxytoxoides* was phagotrophy by myzocytosis (Fig. 3B,C). The ciliate *Myrionecta rubra*, with pronounced jumping behavior, was captured upon mechanical contact. After making physical contact, *O. oxytoxoides* pierced *M. rubra* with a peduncle (Fig. 3B, see video available as supplementary material online at www.int-res.com/articles/suppl/a062p279_supp/). Once the ciliate had been trapped, the dinoflagellate swam around towing the attached ciliate. At that time, *O. oxytoxoides* started gradually to consume the ciliate. During the early stage of feeding (i.e. capturing of prey and swimming), most cilia were shed from the ciliate's body (Fig. 3C). During feeding, *O. oxytoxoides* sucked out the contents of the prey using a peduncle that extended from the flagellar pore. As the feeding process proceeded, the number of food vacuoles increased within the *O. oxytoxoides* cell (Fig. 4). Epifluorescence microscopy revealed that the food vacuoles within *O. oxytoxoides* emitted bright yellow-orange fluorescence under blue-light excitation (Fig. 4B,C), typical of cryptophycean phycobilin (phycoerythrin).

LSU rDNA sequences

The partial LSU rDNA (domains D1–D3; 917 nucleotides) of *Oxyphysis oxytoxoides* was successfully amplified from a total of 19 individual cells. All but 1 of the sequences was identical, in spite of 4 different sampling sites and dates. One exception was found for a cell isolated from Gunsan coastal water, which differed from the other isolates by only 1 nucleotide. The sequences from our isolates were also identical to the *O. oxytoxoides* sequence (accession no. EF613359) deposited in the GenBank database (Kim & Kim 2007).
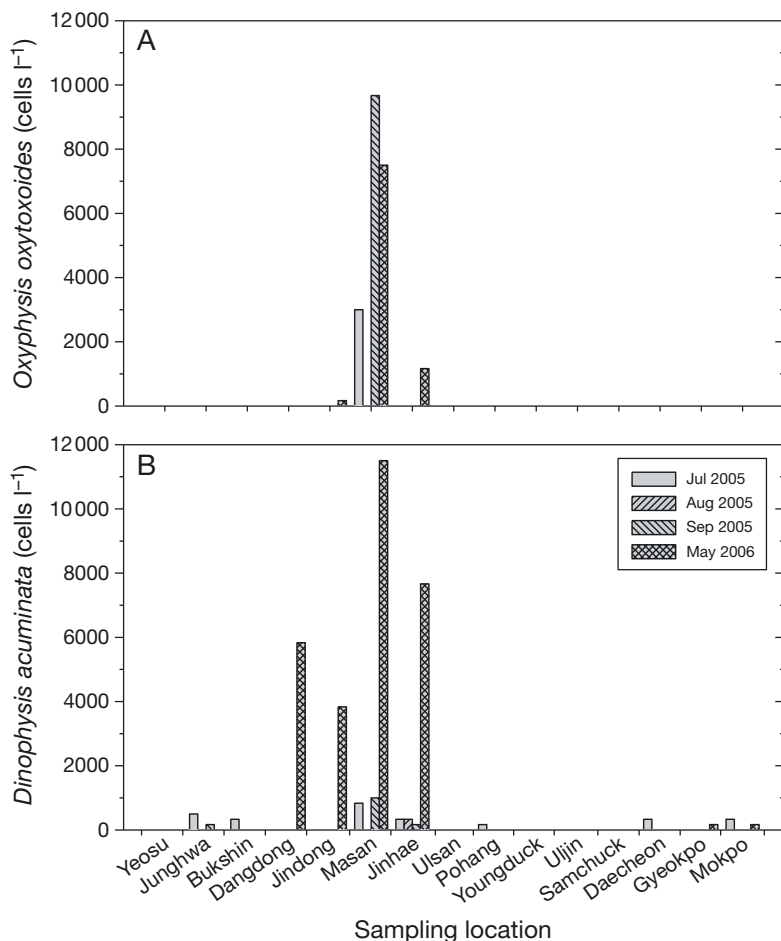

Fig. 2. *Oxyphysis oxytoxoides* and *Dinophysis acuminata*. Abundance of (A) *O. oxytoxoides* and (B) *D. acuminata* at each of the sampling sites during July, August and September 2005 and May 2006
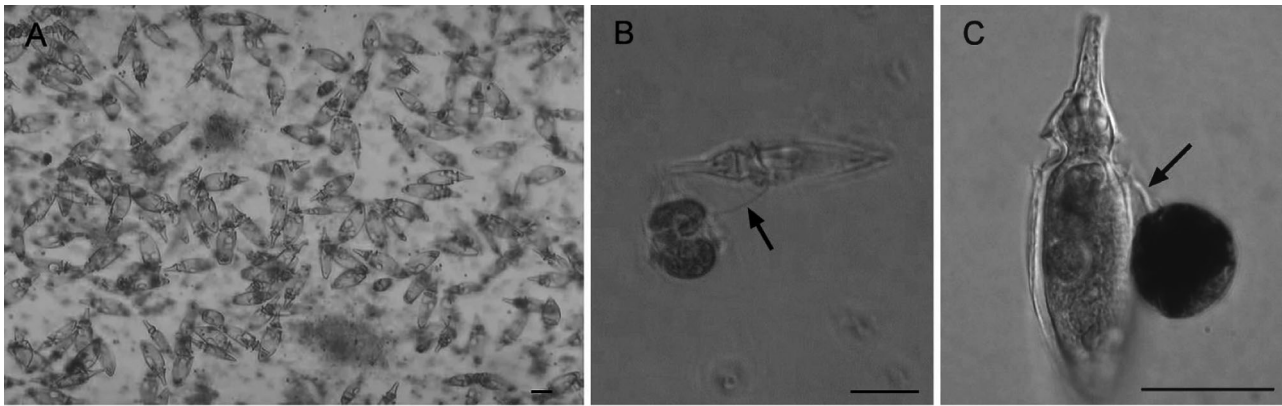
Fig. 3. *Oxyphysis oxytoxoides.* Live cells of (A) a culture grown on the ciliate *Myrionecta rubra* as prey during this study, (B) *O. oxytoxoides* with a recently captured *M. rubra* prey organism, and (C) peduncle-feeding (myzocytosis) on *M. rubra*. Arrows indicate a peduncle extending from the flagellar pore. Scale bars = 20 µm

Phylogenetic analyses

The phylogenetic analyses of 59 in-group taxa of Dinophysiales and 2 outgroup taxa were performed on the basis of partial LSU rDNA, including the D1–D2 domains (747 bp). The optimal tree yielded by Bayesian analysis is shown in Fig. 5. The tree topologies of most major groups were largely congruent with the maximum likelihood (ML) tree, although discrepancies were found in a few branches.

The *Amphisolenia* species formed a strongly supported basal clade (Clade A) with a 100% ML bootstrap value and a posterior probability (PP) of 1. The remaining dinophysoids divided into 2 highly supported groups labeled Clade B and Clade C, respectively. Clade B was strongly supported by an ML bootstrap value of 98% and PP of 1 and included *Oxyphysis oxytoxoides* and *Phalacroma* species, except for *P. apicatum* and *P.* cfr. *argus*. *O. oxytoxoides* clustered with *P. acutum* and the type species *P. porodictyum*, but the relationship was not well supported (an ML bootstrap value of 50% and PP of 0.53). Clade C formed by *Histioneis*, *Ornithocercus*, *Citharistes*, *Dinophysis* and 2 *Phalacroma* species (*P. apicatum* and *P.* cfr. *argus*) was moderately supported by a PP of 1 and an ML bootstrap value of 83%, but the relationship between the genera was not well resolved. Interestingly, *P. apicatum* and *P.* cf. *argus* were distantly related to the other *Phalacroma* species in Clade B. It is unclear whether the genus *Dinophysis* is monophyletic, as species belonging to the genus formed 4 lineages within Clade C.
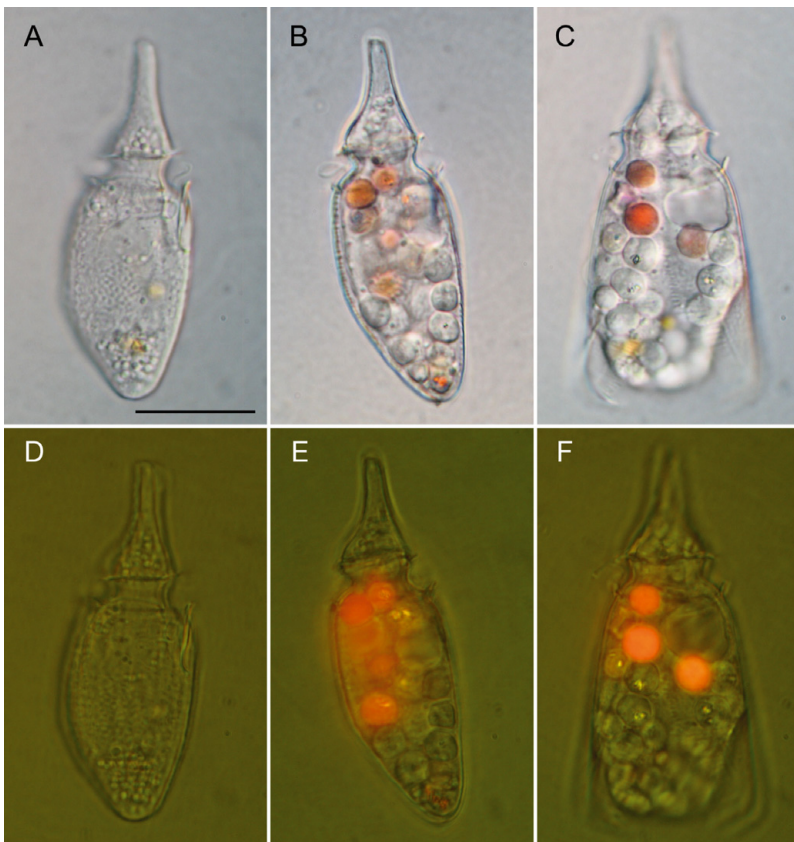

Fig. 4. *Oxyphysis oxytoxoides.* (A–C) Light and (D–F) epifluorescence micrographs of live cells (A) with no food vacuoles, and (B,C) with several food vacuoles within the cell. Note that some food vacuoles are filled with orange fluorescent plastids derived from the ciliate prey *Myrionecta rubra*. Scale bar = 20 µm. The scale in (A) applies to all panels
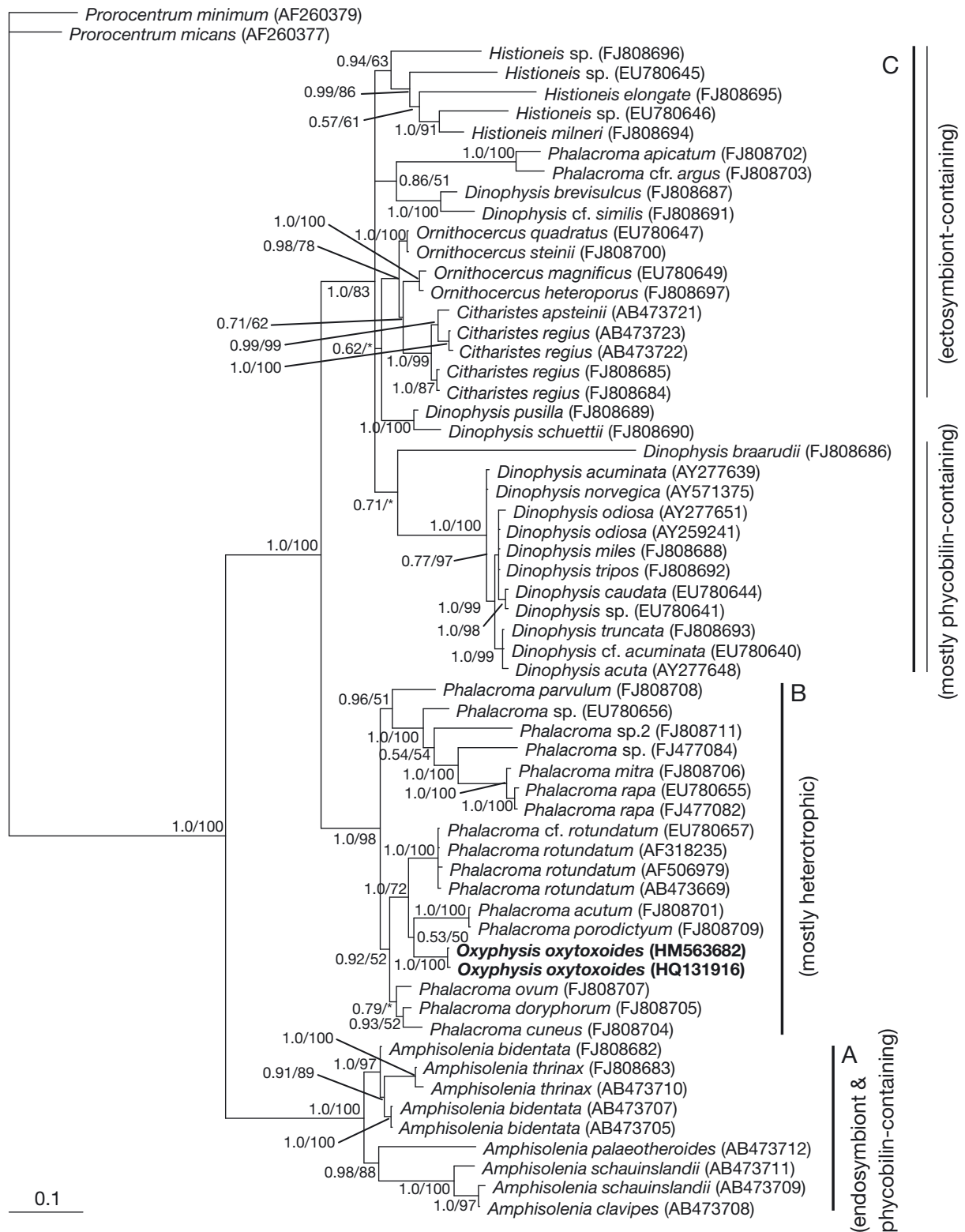
Fig. 5. Phylogenetic tree of 59 in-group taxa of Dinophysiales species and 2 outgroup taxa of prorocentroids inferred from Bayesian analysis based on large-subunit (LSU) rDNA (domains D1–D2) sequences (747 bases). LSU rDNA sequences from *Oxyphysis oxytoxoides* determined in this study are in **bold**. Supports over nodes are indicated as Bayesian posterior probabilities/ML bootstraps (100 replicates). *: Bootstrap values of <50%. GenBank accession numbers are shown in parentheses

According to the phylogenetic analyses, Clades A and C included species containing phycobilin-pigmented endosymbionts (*Amphisolenia*) (Lucas 1991, Tarangkoon et al. 2010), cyanobacterial ectosymbionts (*Histioneis*, *Ornithocercus* and *Citharistes*) (Carpenter 2002, Tarangkoon et al. 2010), or mixotrophic species having phycobilin pigment (*Dinophysis*) (Park et al. 2006, 2008), whereas Clade B contained mostly heterotrophic species (*Oxyphysis* and *Phalacroma*) (present study; Inoue et al. 1993, Jensen & Daugbjerg 2009).

DISCUSSION

Feeding and food source

To our knowledge, this is the first report of extended cultivation of *Oxyphysis oxytoxoides* using the marine ciliate *Myrionecta rubra* as prey. The heterotrophic dinoflagellate *O. oxytoxoides* occurs usually in low numbers (less than a few cells per ml) in coastal marine environments (e.g. Nakane et al. 2008). Indeed, during the field sampling along the Korean coasts between 2005 and 2006, we observed maximum cell densities of 11.5×10^3 cells l^{-1} (Fig. 2A). Given the general low abundance of *O. oxytoxoides* in natural environments, cell densities (38×10^5 cells l^{-1}) obtained in culture established in this study are remarkable, suggesting that the mixotrophic ciliate *M. rubra* may be an appropriate prey for developing cultures of the heterotrophic dinoflagellate. More interestingly, *O. oxytoxoides* often co-occurred with the mixotrophic dinoflagellate *Dinophysis acuminata* in field samples, indicating that both dinoflagellates may be feeding on a common pool of prey (e.g. the ciliate *M. rubra*). Indeed, Park et al. (2006, 2008) and several subsequent studies (e.g. Nagai et al. 2008, Nishitani et al. 2008) showed that mixotrophic *Dinophysis* species grow well when provided with the ciliate *M. rubra* as prey, as *O. oxytoxoides* did in the present study. Given that *M. rubra* is a cosmopolitan species (Taylor et al. 1971, Lindholm 1985), our result indicates that, in addition to mixotrophic *Dinophysis* spp. and heterotrophic *O. oxytoxoides*, other dinophysoids might feed on this ciliate as a common food source either to meet nutritional demands or to acquire kleptoplastids.

In addition to *Oxyphysis oxytoxoides*, several other dinophysoids have been observed feeding on ciliates using a peduncle. For example, Hansen (1991) found that the heterotrophic species *Phalacroma rotundatum* (known as *Dinophysis rotundata*; Jensen & Daugbjerg 2009) and *Dinophysis hastata* were able to feed on the ciliate *Tiarina fusus* by myzocytosis. As in the present study, Inoue et al. (1993) also observed that *O. oxytox-oides* fed on loricate and non-loricate ciliates by the use of a peduncle. Mixotrophic *Dinophysis* spp. were also observed feeding on *Myrionecta rubra* by myzocytosis (Park et al. 2006, 2008, Nagai et al. 2008, Nishitani et al. 2008). Very recently, Tarangkoon et al. (2010) observed, with transmission electron microscopy, that *Ornithocercus magnificus* and *Ornithocercus quadratus* have a peduncle. Further, they observed that *Ornithocercus* spp. contained numerous food vacuoles with trichocyst-like remnants that most likely originated from an ingested ciliate. While myzocytosis is also common in numerous species belonging to Peridiniales (e.g. *Pfiesteria* spp.; Lewitus et al. 1999), subsequent findings of the presence of a peduncle for several genera, as well as widespread distribution of the species having a peduncle across Clades B and C within the dinophysoids, indicate that species belonging to the dinophysoids may be fundamentally heterotrophic (Schnepf & Elbrächter 1988) with a common feeding mechanism.

From evolutionary and ecological points of view, several questions still remain open. For example, despite having a common feeding mechanism (i.e. peduncle feeding), why do mixotrophic *Dinophysis* species and heterotrophic *Oxyphysis* not treat the plastids obtained from the ciliate prey *M. rubra* in the same way? The mixotrophic *Dinophysis* species are known to feed on the ciliate prey *Myrionecta rubra* and actively retain its plastids (i.e. kleptoplastidy; Nagai et al. 2008, Park et al. 2006, 2008, Minnhagen et al. 2011), whereas the heterotrophic *Oxyphysis* feeds on the same prey but digests the plastids. Does *Dinophysis* have an ability to discern the plastids from the rest of the ingested prey? Or, does *Oxyphysis* not yet have the ability to retain the plastids from the prey? From an ecological point of view, it would be interesting to test whether *Oxyphysis* can successfully compete with *Dinophysis* for a common ciliate prey, *M. rubra*, and whether *Oxyphysis* can survive on other prey. Addressing these questions in the future will improve our knowledge of plastid evolution and will help us to better understand complex biological interactions of protistan planktonic food webs.

Phylogenetic relationships

Our phylogenetic analyses including *Oxyphysis oxytoxoides* did not greatly affect the overall topology of the Dinophysiales obtained by Jensen & Daugbjerg (2009) and Handy et al. (2009): while *Amphisolenia* formed an early divergent basal group, the remaining dinophysoids diverged into 2 separate groups, the first formed of *Oxyphysis* and *Phalacroma*, the second of *Histioneis*, *Ornithocercus*, *Citharistes* and *Dino-*

physis. Abé (1967) believed that the elongate genera *Amphisolenia* and *Triposolenia* evolved from the genus *Oxyphysis*. Taylor (1980) considered genera such as *Metaphalacroma*, *Pseudophalacroma*, *Oxyphysis*, *Proheteroschisma* and *Heteroschisma* to represent several short lines rather than a continuing evolutionary sequence as in the *Dinophysis–Ornithocercus–Citharistes–Histioneis* series. Jensen & Daugbjerg (2009) hypothesized that *Oxyphysis* radiates from *Metaphalacroma*, based on compiled information from previous studies. However, it seems unlikely that *O. oxytoxoides* diverged early from the ancestor of the dinophysoids, which was hypothesized when based only on morphology in the previous studies. Rather, our phylogenetic analyses showed that *O. oxytoxoides* shared a more recent common ancestor with *Phalacroma* species than with other dinoflagellates.

On the other hand, it is noteworthy that Clade B, formed of *Oxyphysis* and *Phalacroma* species, contained mostly heterotrophic species, except for *Phalacroma mitra*, which was recently reported to contain kleptoplastids of haptophyte origin (Koike et al. 2005), and *P. rapa*, which is known to contain chloroplasts whose nature has not yet been determined — i.e. are they true, permanent chloroplasts or kleptoplastids derived from chrysophyte or prymnesiophyte symbionts (Hallegraeff & Lucas 1988)? Aside from Clade B, the other 2 clades (A and C) are characterized by the presence of phycobilin-containing plastids or cyanobacterial endo- (or ecto-)symbionts, although eukaryotic endosymbionts within *Amphisolenia* and *Triposolenia* have also been observed (Tarangkoon et al. 2010). Taylor (2004) thought that the phycobilin-containing dinophysoids *Dinophysis*, *Amphisolenia* and *Triposolenia* seemed more closely related to each other than to more morphologically complex (i.e. more elaborate in shape and size of the cingular and sulcal lists) genera such as *Ornithocercus*, *Histioneis* and *Citharistes*. However, our phylogenetic analysis suggests that the phycobilin-containing dinophysoids are not monophyletic, which also agrees with the phylogenetic analyses made by Handy et al. (2009) and Jensen & Daugbjerg (2009).

Sinophysis is thought to be an ancestor of the dinophysoids (Taylor 1980) and is known to host numerous cyanobacterial symbionts (Escalera et al. 2010). Thus, it may be likely that species belonging to the dinophysoids, which once had symbionts, may be skillful in feeding on, or having symbiotic relationships with, phycobilin-containing microalgae (cyanobacteria and cryptophyte). This may partly explain why dinophysoids are highly specialized in either having phycobilin-pigmented plastids or hosting cyanobacterial symbionts.

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