An endophytic diatom, *Pseudogomphonema* sp. (Naviculaceae, Bacillariophyceae), lives inside the red algae *Neoabbottiella* (Halymeniaceae, Rhodophyta)

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?1 Abstract: Benthic diatoms are the most common epiphytes on the surfaces of marine organisms. Although some epiphytic diatoms cause multimillion-dollar economic losses in commercially cultivated red algal plantations every year, such as *Pyropia* (formerly *Porphyra*), little is known about endophytic or pathogenic diatoms. We found a gomphonemoid diatom species that lives and reproduces inside some marine red algae from two localities on southeast Kamchatka. Both young and old infected host algae were collected in different years (2008 and 2012) and months (July, August, November) from two localities. Numerous brown patches were observed on the surface of red algal thalli. These patches contained a single species of endophytic diatom growing densely in the medullar layer of the host red algae. Some diatom cells were observed in between the filaments composing the cortex. Numerous dividing cells were present in the colonies, indicating that diatoms divided *in hospite*. In some old host plants, abundant diatom development caused warping of the thalli. Using electron microscopy the endophytic diatom was identified as *Pseudogomphonema* sp. Phylogenetic analysis based on *rbc*L gene sequence data revealed that it had 96.6% identity with an isolate from GenBank identified as *Pseudogomphonema* cf. *kamschaticum*. This is the first report of *Pseudogomphonema* living inside macroalgae and being a potentially pathogenic organism.

Key Words: Blade-like red seaweeds, Diatoms, Endophyte, Kamchatka, *Neoabbottiella*, Pathogen

INTRODUCTION

Diatoms play the most important role in the formation of epibiota on marine organic and inorganic substrata (Ryabushko 1996, 2009). Diatoms are major primary producers and they are the most abundant group of epiphytic organisms growing on the surface of benthic macroalgae. Epiphytic diatoms can seriously affect the growth of filamentous red algae because they inhabit red algal surfaces, shade light and compete for nutrients. Diatom blooms in *Pyropia* (formerly *Porphyra*) cultivation beds cause serious economic losses costing millions of dollars; the epiphytic diatoms cause bleaching of macroalgal thalli, which lowers the quality and price of the seaweed (Manabe & Ishio 1991; Imai *et al*. 2006). However, there are few studies on the directed interaction between diatoms and red algae.

Red algae are important components of marine coastal ecosystems where they have complex biotic relationships with various marine organisms. Perennial red macroalgal thalli often become substrates for the eggs of fish, mollusks, and arthropods. In addition, the red seaweeds can become substrates for the settlement of annelid larvae, bryozoan colonies, sphaeodoridaen polychaete tubes, hydroids (*Hydrozoa*) and other invertebrates. In other cases, red algae themselves are obligate epiphytes, endophytes, and epi- or endozoids, living on the surfaces or inside different animals and plants. For example, almost all members of the orders Acrochaetiales, Stylonematales and Colaconematales are epiphytes of the brown, red and occasionally green seaweeds and hydroids (Woelkerling 1983; Harper & Saunders 2002; Klochkova *et al*. 2009a, b; Wynne & Schneider 2010). Many genera of coralline algae (e.g. *Titanoderma* Nägeli, *Fosliella* Howe, *Melobesia* Lamouroux, *Pneophyllum* Kützing etc.) are epiphytes of marine algae and sea grasses (Morton & Chamberlain 1985; Klochkova 1996; Woelkerling 1993). On the other hand, red algae contain a high portion of the parasitic and semiparasitic algae compared with other macroalgal groups (e.g. Reinsch 1888, Reinke 1889; Adey *et al*. 1974; Lee & Kurogi 1978; Kim & Cho 2010) and approximately 20 red algal genera were considered as obligate parasites of other red algae (Goff 1982).

Studies on algal diseases, associated with viral, bacterial, and fungal invasions of cultivated populations especially, are becoming more frequent, since the disease agents cause great harm to the marine algae and reduce product quantity and its quality (e.g., Gachon *et al*. 2010; Klochkova *et al*. 2012). Infections of the red algae with other epi- or endobionts have always been a subject of special attention, especially in the commercially cultivated alga *Pyropia* that are infected by various pathogens, such as chytridiomycota, oomycota, diatoms and bacteria.

Marine epiphytic diatoms are common in Kamchatka, especially in the regions experiencing high pollution (i.e. Avacha Bay). The most abundant and diverse epiphyton is

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found on plants that have complex branched bushy thalli; however, it is also found on the blade-like red algae, for example *Constantinea* Postels & Ruprecht and *Neoabbottiella* L.P. Perestenko (Klochkova & Pisareva 2009; Pisareva & Klochkova 2013). Species of the genus *Pseudogomphonema* L.K. Medlin are often listed as epiphytes on macroalgae from the Arctic, Antarctic and cold-temperate regions (e.g. Kang *et al*. 2002; Cremer *et al*. 2003; Al-Handal & Wulff 2008; Majewska *et al*. 2013) and recently from warm-temperate regions, such as Korea (Lee *et al*. 2012), Australia and New Zealand (Harper *et al*. 2012). While working on the red algal genus *Neoabbottiella*, we collected several plants of *Neoabbottiella araneosa* (L.P. Perestenko) S.C. Lindstrom and *N. decipiens* N.G. Klochkova & N.A. Pisareva from southeast Kamchatka with abundant endophytic diatoms living and reproducing inside these plants. The endophyte was identified as *Pseudogomphonema* sp., providing the first report of the member of this diatom genus living inside the macroalgae and being potentially pathogenic.

MATERIAL AND METHODS

The investigated plants of *Neoabbottiella* spp. containing diatoms are listed in Table 1. Field material was kept in IMR medium (Klochkova *et al*. 2006) and incubated at 10°C under 30 μmol photons m^{-2} s^{-1} (16:8 light:dark regime). Voucher specimens were deposited in Kamchatka State Technical University and Kongju National University, and they are available from the authors upon request. Cross-sections of the blades containing diatoms were photographed with an Olympus DP50 digital camera affixed to an Olympus BX50 microscope (Olympus Corp., Tokyo, Japan).

Diatom cells were extracted from the tissues of *Neoabbottiella* and organic materials were removed by a modified acid method (Lee & Lee 2012). Specimens were placed on cleaned aluminum stubs, sputter-coated with gold–palladium and viewed under a JSM-5600LV (JEOL Ltd, Tokyo, Japan) microscope operated at 15 kV.

Brown patches on *Neoabbottiella* thalli containing diatoms were excised and genomic DNA was extracted. Materials were transferred to 2.0 ml cryotubes and frozen in liquid nitrogen. The tubes were shaken at 5000 revolutions min^{-1} for 2 min with a cell homogenization machine. DNA was extracted using hexadecyltrimethylammonium bromide method (Doyle & Doyle 1987). The *rbc*L gene of the diatom was amplified and sequenced using the following primers: DP*rbc*L1 (AAG GAG AAA THA ATG TCT; Jones *et al*. 2005), *rbc*L527F1 (AAA ACA TTC CAA GGT CCT GCT; Alverson *et al*. 2007), *rbc*L1255R1 (TTG GTG CAT TTG ACC ACA GT; Alverson *et al*. 2007), *rbc*L587R1 (GTC TAA ACC ACC TTT TAA MCC TTC; Alverson *et al*. 2007). *TaKaRa Ex Taq*[TM], 10X *Ex Taq*[TM] buffer (Mg^{2+} free), 25 mM $MgCl_2$, and deoxynucleotide triphosphate mixture (Takara Biomedicals, Otsu, Japan) were used as a DNA polymerase for all polymerase chain reaction (PCR)

Table 1. Specimens of *Neoabbottiella* contaminated with endophytic *Pseudogomphonema* sp. and used in this study, their collection site and date.

Species	Type of collected plant & voucher no.	Collection site	Collection date (collector)	Notes
Neoabbottiella araneosa	3 female plants bearing cystocarps and carpospores (KamGTU_NA0001–0003) 1 juvenile blade, sterile (KamGTU_NA0004)	Starichkov Island (Avachinsky Inlet, Kamchatka, 52°46′38″N, 158°36′55″E); depth 18 m, boulders	19 Aug. 2008 (N. Sanamyan)	in female plants, diatoms were localized inside the cystocarps and between the carpospores; in juvenile blade, abundant diatoms were scattered throughout the whole blade and were mostly found in the cortical layer
Neoabbottiella decipiens	1 large perennial blade (KamGTU_ND0001)	Starichkov Island (Avachinsky Inlet, Kamchatka, 52°46′38″N, 158°36′55″E); depth 15–16 m, boulders	7 Nov. 2008 (A. Ryabez)	abundant diatoms were scattered throughout the whole blade in the medullar layer
Neoabbottiella decipiens	1 large perennial blade, sterile (KamGTU_ND0002)	Starichkov Island (Avachinsky Inlet, Kamchatka, 52°46′38″N, 158°36′55″E); depth 15 m, boulders	17 Jul. 2012 (A. Ryabetz)	same as above
Neoabbottiella decipiens	1 large perennial blade, sterile (KamGTU_ND0003)	Cape Razdelnij (Avacha Bay, Kamchatka, 52°37′00″N, 158°25′00″E); depths 6–7 m, boulders, seawater temperature 7–8°C	17 Jul. 2012 (A. Ryabetz)	same as above

amplifications in this study. The following PCR program was used: initial denaturation at 95℃ for 2 min was followed by 35 cycles of denaturation at 95℃ for 30 s, annealing at 48℃ for 40 s, extension at 72℃ for 1.5 min and then a final extension at 72℃ for 10 min. PCR products were checked on an agarose gel for length, concentration and purity. Nucleotide sequences were aligned using the program Geneious ver. 6.1.6 (Biomatters, Auckland, New Zealand). Our sequence (1125 base pairs) and 16 sequences retrieved from GenBank were individually added to the alignment and manually refined. Phylogeny was reconstructed using maximum likelihood analyses. HKY85 was used as a substitution model. Bootstrap values were obtained from 100 replications. *Cyclotella choctawhatcheeana* (AM778962) was used as an outgroup species following Bruder and Medlin (2008). The new sequence has been deposited in GenBank under accession number KF895387.

RESULTS

Abundant diatom colonies were found inside two red algae, *N. araneosa* and *N. decipiens*, collected in different years (2008 and 2012) and months (July, August, November) from two localities on southeast Kamchatka (Table 1). The host plants grew at depths of 6–18 m. The infected host thalli were easily seen as they had numerous darker brownish spots than the surrounding tissues (Fig. 1). In some old host plants, abundant development of these endophytes caused warping of the thalli (Fig. 2).

Observation of the cross-sectioned infected blades revealed that the endophytic organism was a gomphonemoid diatom (Figs 3–9), identified as *Pseudogomphonema* sp. with scanning electron microscopy (Figs 10–17) and *rbc*L gene sequencing data (Fig. 18). Diatom colonies were numerous, unialgal and very dense, scattered throughout the whole host blade in its medullar layer (Figs 3, 4). Each colony contained from several dozens to several hundred cells. Numerous dividing cells were present in the colonies (Fig. 6), indicating that diatoms were dividing while inside the red algal host. In some cases, diatoms were seen in between the filaments composing the cortex (Figs 7, 8). When diatom cells were present in the cortex, they were always inserted perpendicularly with their foot pole facing the host's medullar layer (Fig. 9). Except for occasional cells, the outer surface of infected *Neoabbottiella* was not covered with other epiphytic diatoms (Fig. 7).

Morphological details of our *Pseudogomphonema* strain and its comparison with other taxonomically accepted *Pseudogomphonema* species is given in Table 2. The size of cells was 9.3–19.7 μm long and 3.2–4.4 μm wide, i.e., they were smaller than other recorded planktonic or epiphytic *Pseudogomphonema* spp. except for *P. plinskii* A. Witkowski, D. Metzeltin & Lange-Bertalot (see Table 2). However, the striation pattern was different from *P. plinskii*, as our strain had 13–20 striae in 10 μm (Figs 10–12).

Phylogenetic analysis using *rbc*L gene sequencing data revealed that our endophytic diatom had closest affinity (96.6% identity) with an isolate identified as *Pseudogomphonema* cf. *kamschaticum* (Grunow) L.K. Medlin (AY571748,

collected from Scotland). These two isolates formed a distinct subclade supported by high bootstrap values. The closest affinity was with species from the genus *Navicula* Bory de Saint-Vincent.

To determine whether endophytic *Pseudogomphonema* sp. that grew inside *Neoabbottiella* was capable of survival outside the host plant, infected blades of *Neoabbottiella* were sliced into thin sections, allowing diatoms to escape from the wounded edges. Since the wounded edges could not regenerate, the host tissue deteriorated after several days. The majority of endophytic diatom cells did not release from the host tissue. Some cells formed colonies on the bottom of Petri dishes; however, they could not be maintained outside the host tissue for a prolonged period of time and could not be isolated into a unialgal culture. The free-living diatom cells developed numerous oil droplets and the chloroplasts became pale after 3 weeks and afterward the cells died. The host blades did not grow in the laboratory culture; thus a stable host–parasite dual culture was not established.

DISCUSSION

This study reports a hitherto undescribed property of the gomphonemoid diatom *Pseudogomphonema* to live inside some marine macroalgae. Diatoms have been reported as symbionts inside dinoflagellates, foraminifera (Björn & Cronberg 2009) and sponges (Bavestrello *et al.* 2000) and as endophytes in the red alga *Coelarthrum opuntia* (Endlicher) Brgesen (Okamoto *et al.* 2003). Diatom symbionts are beneficial to the foraminifera because they supply photosynthetic products to the host. In addition, the diatoms help the host's calcification because the photosynthetic fixation of carbon dioxide raises the pH around the foraminifera (Erez 2003). A small group of dinoflagellates in the order Peridinales contain the 'dinotoms' (i.e. obligate tertiary diatom endosymbiont) that have been shown to originate from three different diatom lineages, one pennate lineage (Chesnick *et al.* 1997; McEwan & Keeling 2004; Imanian & Keeling 2007; Pienaar *et al.* 2007) and two centric lineages (Horiguchi & Takano 2006; Takano *et al.* 2008).

Coelarthrum opuntia is so far the only reported case of endophytic relationships among red macroalgae and diatoms. The endophytic pennate diatom *Gyrosigma coelophilum* Okamoto & Nagumo formed dense unialgal colonies in the mucilage that filled the internodes of this alga. This *Coelarthrum*-infecting diatom was described as a new species due to its unusual habitat and general generic morphology (Okamoto *et al.* 2003). Unfortunately, the taxon was not sequenced (National Center for Biotechnology Information 2013) and it is not possible to elucidate its relationships to the other members of the genus.

Phylogenetic relationships within *Pseudogomphonema* have not been resolved to date. Of six described species, only three members of the genus have been sequenced (AY571748, AF525663, AJ535152); however, even for those three sequences deposited in GenBank (National Center for Biotechnology Information 2013) the species names were not identified precisely (i.e., identified either as cf. or sp.). The strain from Scotland identified as *P.* cf. *kamtschaticum*
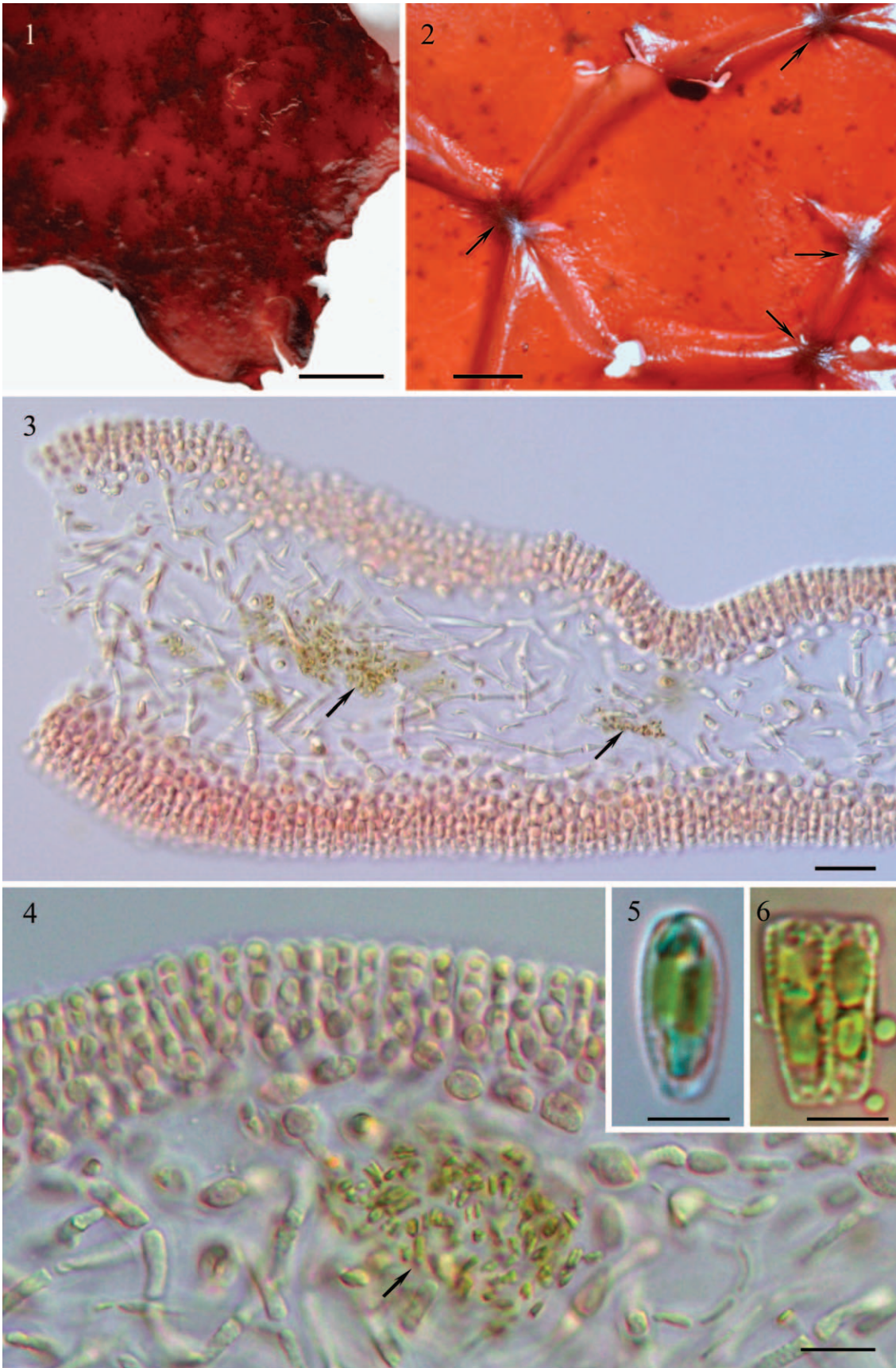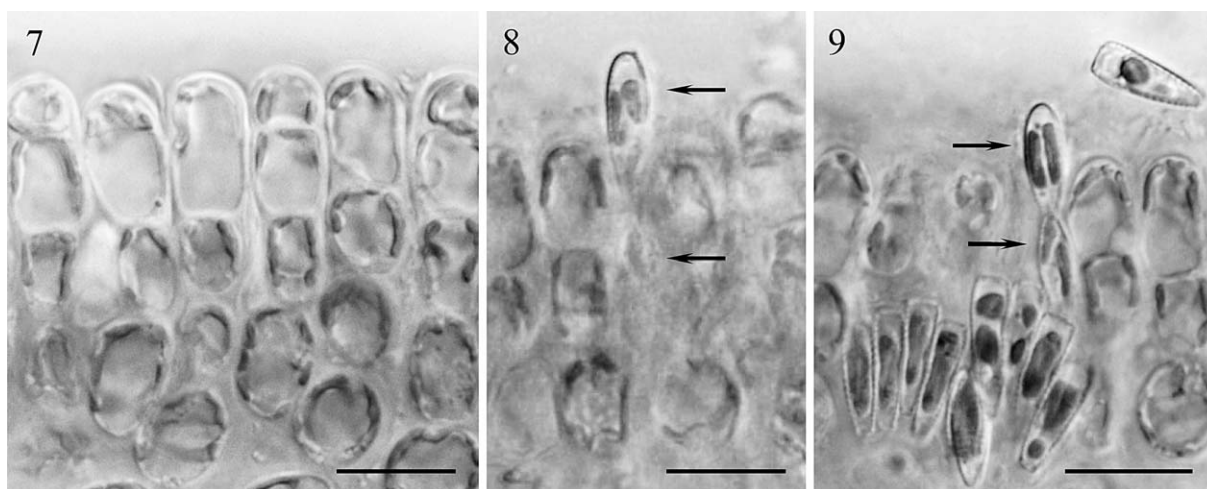
Figs 7–9. Endophytic diatom, *Pseudogomphonema* sp., embedded in the cortex of *Neoabbottiella decipiens*. Scale bars = 10 μm.
    Fig. 7. Structure of the cortex in *Neoabbottiella*. Cortical filaments are parallel and not covered on the top with cuticle.
    Fig. 8. Two diatom cells (arrows) inserted in the cortex of *Neoabbottiella*.
    Fig. 9. Many diatoms localized beneath the cortex and two cells inserted in the cortex (arrows).

(AY571748) was collected far from the type locality of this taxon, viz., Kamchatka peninsula; therefore, it should not serve as a type sequence for *P. kamtschaticum*. We refer to our organism as *Pseudogomphonema* sp. because it may be a new species. For example, our endophytic strain was very small when compared with other planktonic or epiphytic *Pseudogomphonema*.

A gliding movement of the diatoms may explain their entry into *Neoabbottiella* tissue. In *Neoabbottiella* species, cortex is formed with aligned parallel filaments composed of several cells with thickened mucous walls and without a cuticle covering the entire blade. Therefore, the cortex is semipermeable and, perhaps, easier for the diatom cells to enter the blade if they attach between the cortical filaments and push themselves inside the host tissue by a gliding locomotion. Other epiphytic diatoms exist in the same locality in Kamchatka but none has been found inside *Neoabbottiella*.

Two questions are: Why does *Pseudogomphonema* get inside the *Neoabbottiella* tissue? How does *Pseudogomphonema* benefit from the coexistence with *Neoabbottiella*? There is no direct evidence that these diatoms are parasitic on *Neoabbottiella*; on the other hand, there are arguments that they are potentially pathogenic organisms because their abundant growth caused morphological changes in the hosts (i.e. warping of the thalli). Despite warped thalli, death and cessation of growth did not occur in the infected plants, and they developed cystocarps and carpospores. In case of

foraminifera, endosymbiotic diatoms living inside the cytoplasm of the hosts are protected by the foraminiferal shell (test) and are possibly exposed to higher levels of inorganic nutrients compared with free-living algae (Lee & Hallock 1987); however, they do not form frustules *in hospite* (Lee 2006). Our strain divided *in hospite* as we found many dividing cells inside the host tissue. The nutritional aspects of our endophytic diatom are currently unknown. In case of the endophytic diatom *G. coelophilum*, more than half the light that it could potentially exploit was absorbed by its host alga, so it was suspected that the diatom was partially heterotrophic (Okamoto *et al.* 2003). Our host algae grow at depths of 6–18 m, where it can be dark during daytime or the light is very dim, and also thick, pigmented host's cortices reduce the light transmittance. In its growth locality, increased turbidity due to terrigenous runoff and soil erosion during snow melting occurs annually, which also contributes to the reduction of light transmission at the depths. Therefore, it is possible that our diatom may have a mixotrophic nature.

Intimate associations among algae are quite common in nature, and numerous species of small filamentous green, brown, and red algae have been reported as endophytes of various macroalgal hosts (Correa 1994). However, information about endophytic diatoms in the macroalgal hosts is very limited. Endophytic diatoms may be more common than what is known to date. It is hard to believe that those endophytic diatoms lock themselves inside red algae without

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Figs 1–6. *Neoabbottiella* infected with *Pseudogomphonema*.
    Fig. 1. Enlarged part of the infected blade. Darker areas are places infected with diatoms. Scale bar = 1 cm.
    Fig. 2. Thallus warping (arrows) caused by abundant diatom growth. Scale bar = 1 cm.
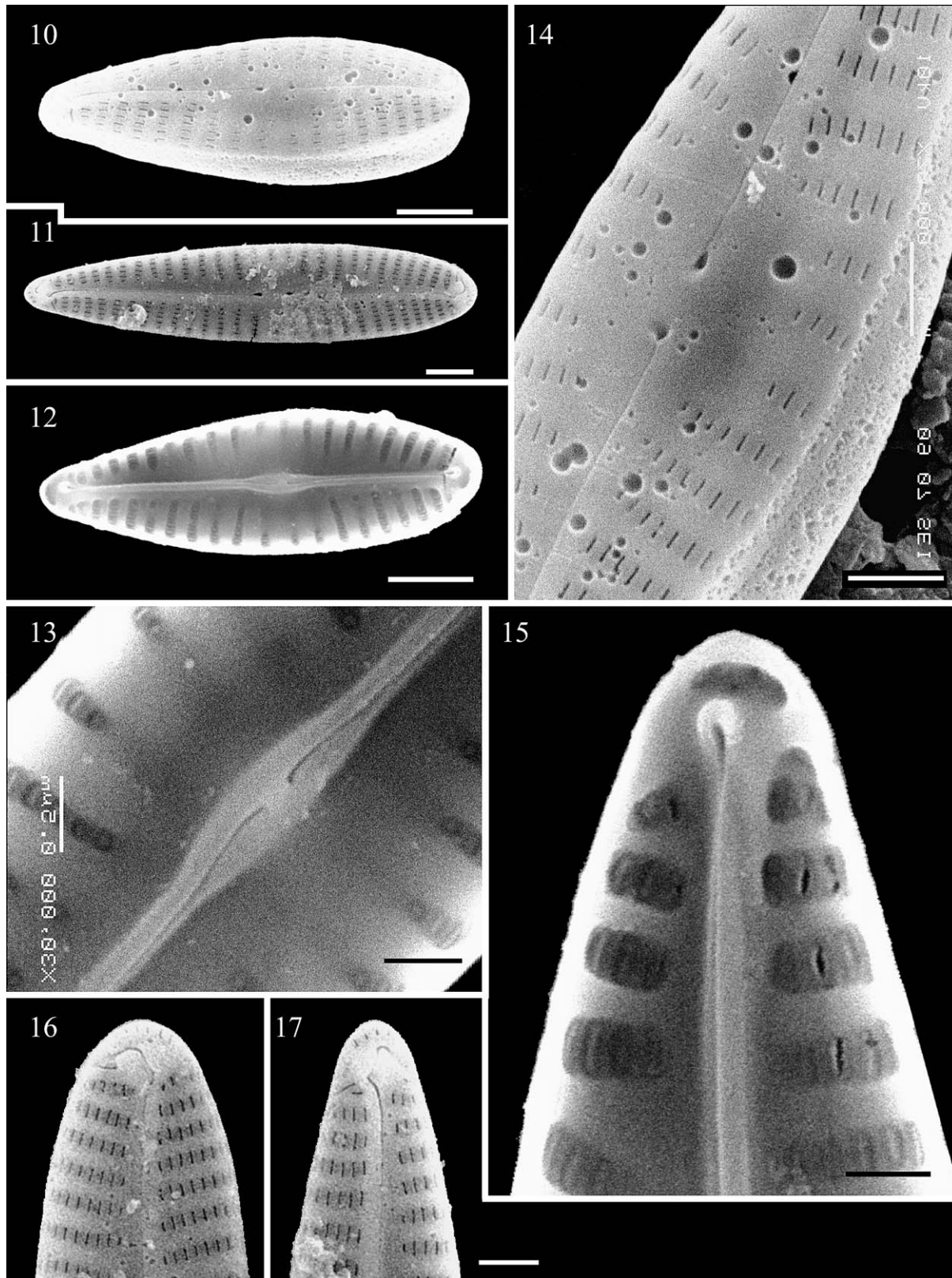   **Fig. 3.** Cross-section of an infected blade showing diatom colonies (arrows). Scale bar = 50 μm.
    Fig. 4. Enlarged view of a cross-sectioned blade showing diatom colonies (arrow). Scale bar = 20 μm.
    Fig. 5. *Pseudogomphonema* sp. in valve view. Scale bar = 5 μm.
   **Fig. 6.** *Pseudogomphonema* sp. in lateral view showing cell division. Scale bar = 5 μm.

Figs 10–17. Scanning electron microscopy (SEM) of *Pseudogomphonema* sp. extracted from the tissues of *Neoabbottiella*.
  Figs 10–11. External valve view. Scale bars = 2 μm.
  Fig. 12. Internal valve view. Scale bar = 2 μm.
  Fig. 13. Detail of internal valve center. Scale bar = 500 nm.
  Fig. 14. Detail of external valve center. Scale bar = 1 μm.
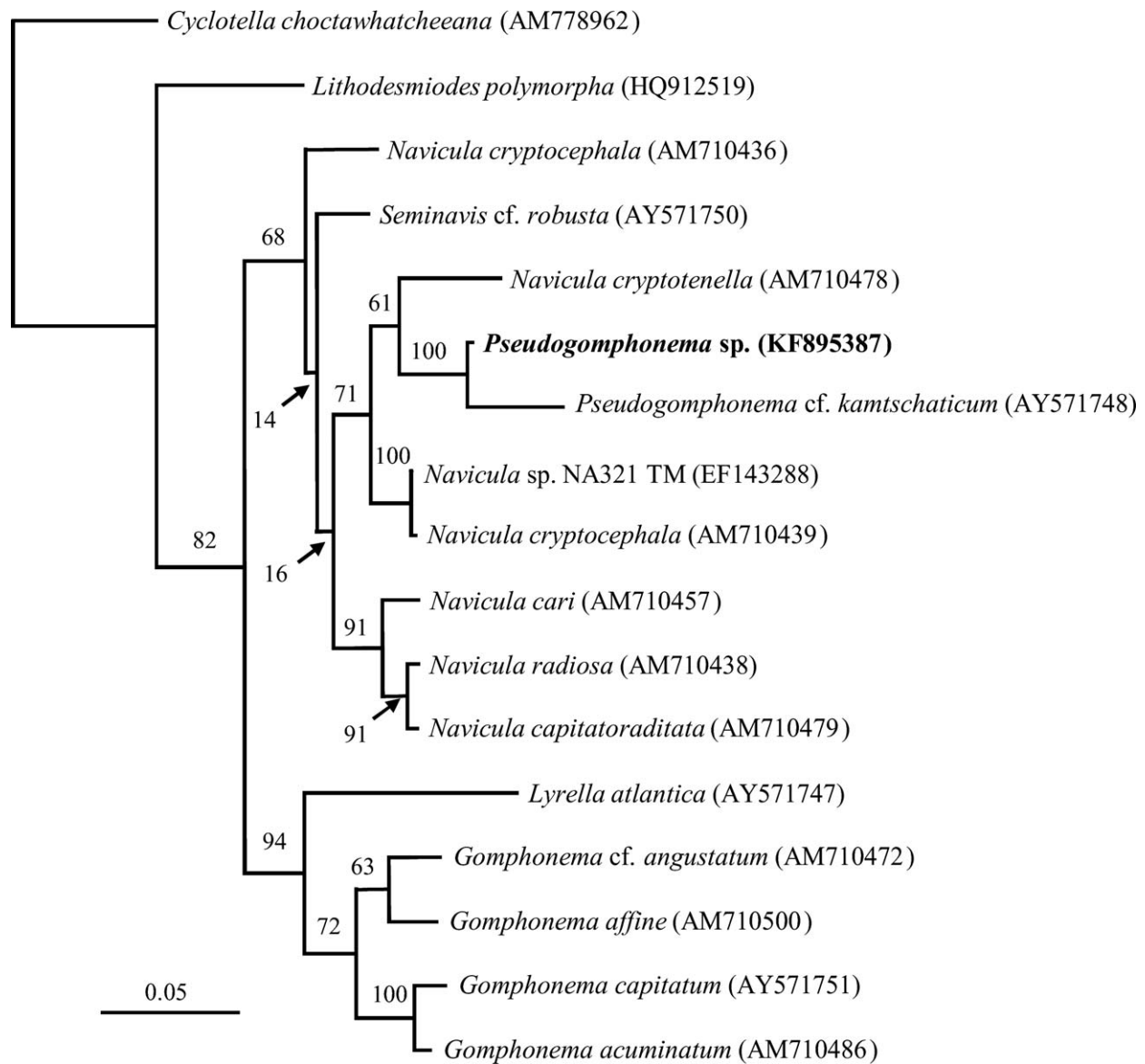
Fig. 18. Maximum likelihood tree of *rbc*L data of endophytic *Pseudogomphonema* sp. Labels on branches are bootstrap proportions. Scale bar = number of nucleotide substitutions per site.

any ecological benefits. It seems that there are some species-specific relationships in the entry of *Pseudogomphonema* into *Neoabbottiella*. The underlying reasons of such associations are currently unknown and need further investigation.

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Fig. 15. Detail of foot pole, showing lateral opening of raphe and small depression/chamber above the helictoglossum (internal valve). Scale bar = 500 nm.
Fig. 16. Detail of the external valve head pole. Scale bar = 1 μm.
Fig. 17. Detail of the external valve foot pole. Scale bar = 1 μm.

Table 2. Comparison of morphological characters for taxonomically accepted *Pseudogomphonema* species and our isolate.

Taxon[1]	Cell dimensions, μm		Cell shape	Striation pattern	Presence of chamber	Distribution	Ecology
	Long	Wide					
Pseudogomphonema arcticum (Grunow) Medlin	20–45	8–10	valves linear-lanceolate, with slightly rostate head poles and acutely rounded foot poles	15–18 in 10 μm, radiate	not well developed	Arctic	epiphyte on *Melosira* from ice samples in the Arctic
P. groenlandicum (Østrup) Medlin	up to 108	12	valves linear with rounded apices	7–8 in 10 μm on the primary side 10–12 on the other side, radiate throughout	at both poles (slightly larger at head pole)	Arctic	present on pack ice and as an epiphyte
P. kamtschaticum (Grunow) Medlin	18–70	4–11	valves linear-lanceolate, with rounded apices	16–23 in 10 μm, radiate throughout (Medlin & Round 1986); 10–14 in 10 μm (Medlin & Priddle 1990)	at foot pole	cold temperate seas: Kamchatka, California Arctic, Antarctica (Bouvet Island, Windmill Islands, Terra Nova Bay, Ross Sea) Warm temperate seas: Korea, Australia, New Zealand	epiphyte on macroalgae and on *Melosira* from ice samples in the Arctic
P. monicae Witkowski, Metzeltin & Lange-Bertalot	14–38	5–7	valves linear-elliptical	20–28 in 10 μm	at foot pole (?)	Bären-Insel (Svalbard), Wattenmeer-Sediment	associated with sea sediments
P. plinskii Witkowski, Metzeltin & Lange-Bertalot	8.5–11	3–3.5	valves linear-lanceolate, with acute apices	20–26 in 10 μm	N/A[2]	Bären-Insel (Svalbard), Wattenmeer-Schlick	associated with sea mud
P. septentrionale (Østrup) Medlin	22–30	5–7	valves linear-lanceolate, with rounded apices	10 in 10 μm on the primary side, 12 on the other side	not well developed, may be only recessed into the valve surface	restricted to polar or cold temperature seas	often associated with sea ice
Pseudogomphonema sp. – endophytic[3]	9.3–19.7	3.2–4.4	valves linear-lanceolate, with rounded apices	13–20 in 10 μm	not well developed at both poles	cold temperate seas: Southeast Kamchatka	endophytic (host algae: *Neoabbottiella*)

[1] Data summarized from Medlin & Round (1986), Medlin & Priddle (1990), Metzeltin & Witkowski (1996), Lee *et al.* (2012) and Harper *et al.* (2012).
[2] N/A – not specified in the reference.
[3] Newly collected data from this study.

REFERENCES

ADEY W.H., MASAKI T. & AKIOKA H. 1974. *Ezo epiyessoense*, a new parasitic genus and species of Corallinaceae (Rhodophyta, Cryptonemiales). *Phycologia* 13: 329–344.

AL-HANDAL A.Y. & WULFF A. 2008. Marine benthic diatoms from Potter Cove, King George Island, Antarctica. *Botanica Marina* 51: 51–68.

ALVERSON A.J., JANSEN R.K. & THERIOT E.C. 2007. Bridging the rubicon: phylogenetic analysis reveals repeated colonizations of marine and fresh waters by thalassiosiroid diatoms. *Molecular Phylogenetics and Evolution* 45: 193–210.

BAVESTRELLO G., ARILLO A., CALCINAI B., CATTANEO-VIETTI R., CERRANO C., GAINO E., PENNA A. & SARA M. 2000. Parasitic diatoms inside Antarctic sponges. *Biological Bulletin* 198: 29–33.

BJÖRN L.O. & CRONBERG G. 2009. Diatoms: their strange evolution and remarkable properties. *Acta Biologica Slovenica* 52: 33–40.

BRUDER K. & MEDLIN L.K. 2008. Morphological and molecular investigations of naviculoid diatoms. III. *Hippodonta* and *Navicula* S.S. *Diatom Research* 23: 331–347.

CHESNICK J.M., KOOISTRA W.H.C., WELLBROCK U. & MEDLIN L.K. 1997. Ribosomal RNA analysis indicates a benthic pennate diatom ancestry for the endosymbionts of the dinoflagellates *Peridinium foliaceum* and *Peridinium balticum* (Pyrrophyta). *Journal of Eukaryotic Microbiology* 44: 314–320.

CORREA J.A. 1994. Infections by pigmented algal endophytes: misuse of concepts and terminology. *Revista Chilena de Historia Natural* 67: 4–8.

CREMER H., ROBERTS D., MCMINN A., GORE D. & MELLES M. 2003. The holocene diatom flora of marine bays in the Windmill Islands, East Antarctica. *Botanica Marina* 46: 82–106.

DOYLE J.J. & DOYLE J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

EREZ J. 2003. The source of ions for biomineralization in foraminifera and their implications for paleoceanographic proxies. *Reviews in Mineralogy and Geochemistry* 54: 115–149.

GACHON C.M.M., SIME-NGANDO T., STRITTMATTER M., CHAMBOUVET A. & KIM G.H. 2010. Algal diseases: spotlight on a black box. *Trends in Plant Science* 15: 633–640.

GOFF L.J. 1982. The biology of parasitic red algae. *Protoplasma* 89: 189–195.

HARPER J.T. & SAUNDERS G.W. 2002. A re-classification of the Acrochaetiales based on molecular and morphological data, and establishment of the Colaconematales, ord. nov. *British Phycological Journal* 37: 463–475.

HARPER M.A., CASSIE COOPER V., CHANG F.H., NELSON W.A. & BROADY P.A. 2012. Phylum Ochrophyta: brown and golden-brown algae, diatoms, silicioflagellates, and kin. In: *New Zealand inventory of biodiversity,* vol. 3. *Kingdoms Bacteria, Protozoa, Chromista, Plantae, Fungi* (Ed. by D.P. Gordon), pp. 114–163. Canterbury University Press, Christchurch, New Zealand.

HORIGUCHI T. & TAKANO Y. 2006. Serial replacement of a diatom endosymbiont in the marine dinoflagellate *Peridinium quinquecorne* (Peridinales, Dinophyceae). *Phycological Research* 54: 193–200.

IMAI I., YAMAGUCHI M. & HORI Y. 2006. Eutrophication and occurrences of harmful algal blooms in the Seto Inland Sea, Japan. *Plankton & Benthos Research* 1: 71–84.

IMANIAN B. & KEELING P.J. 2007. The dinoflagellates *Durinskia baltica* and *Kryptoperidinium foliaceum* retain functionally overlapping. *BMC Evolutionary Biology* 7: 172.

JONES H.M., SIMPSON G.E., STICKLE A.J. & MANN D.G. 2005. Life history and systematics of Petroneis (Bacillariophyta), with special reference to British waters. *European Journal of Phycology* 40: 61–87.

KANG J.-S., KANG S.-H., LEE J.-H. & LEE S. 2002. Seasonal variation of microalgal assemblages at a fixed station in King George Island, Antarctica, 1996. *Marine Ecology Progress Series* 229: 19–32.

KIM M.S. & CHO G.Y. 2010. A new red algal parasite, *Symphyocolax koreana* gen. et sp. nov. (Rhodomelaceae, Ceramiales), from Korea. *Algae* 25: 103–113.

KLOCHKOVA N.G. 1996. *Flora vodoroslej-makrofitov Tatarskogo proliva i osobennosti ee formirovaniya [The marine algae flora of Tatars Strait (Sea of Japan) and history of its formation].* Dalnauka, Vladivostok, Russia. 289 pp. [in Russian].

KLOCHKOVA N.G. & PISAREVA N.A. 2009. The genus *Constantinea* (Rhodophyta: Gigartinales) in the Far-Eastern seas of Russia. *Russian Journal of Marine Biology* 35: 206–214.

KLOCHKOVA N.G., KOROLYOVA T.N. & KUSIDI A.E. 2009a. *Marine algae of Kamchatka and surrounding areas,* vol. 1. KamchatNIRO Press, Petropavlovsk-Kamchatsky, Russia. 216 pp. [in Russian].

KLOCHKOVA N.G., KOROLYOVA T.N. & KUSIDI A.E. 2009b. *Marine algae of Kamchatka and surrounding areas,* vol. 2. KamchatNIRO Press, Petropavlovsk-Kamchatsky, Russia. 300 pp. [in Russian].

KLOCHKOVA T.A., KANG S.H., CHO G.Y., PUESCHEL C.M., WEST J.A. & KIM G.H. 2006. Biology of a terrestrial green alga, *Chlorococcum* sp. (Chlorococcales, Chlorophyta), collected from the Miruksazi stupa in Korea. *Phycologia* 45: 349–358.

KLOCHKOVA T.A., SHIM J.B., HWANG M.S. & KIM G.H. 2012. Host–parasite interactions and host species susceptibility of marine fungal parasite, *Olpidiopsis* sp., from Korea that infects red algae. *Journal of Applied Phycology* 24: 135–144.

LEE I.K. & KUROGI M. 1978. *Neohalosacciocolax aleutica* gen. et sp. nov. (Rhodophyta), parasitic on *Halosaccion minjaii* I. K. Lee from the north Pacific. *British Phycological Journal* 13: 131–139.

LEE J.J. 2006. Algal symbiosis in larger foraminifera. *Symbiosis* 42: 63–75.

LEE J.J. & HALLOCK P. 1987. Algal symbiosis as the driving force in the evolution of larger foraminifera. *Annals of New York Academy of Science* 503: 330–347.

LEE J.M. & LEE J.H. 2012. Morphological study of the genus *Eucampia* (Bacillariophyceae) in Korean coastal waters. *Algae* 27: 235–247.

LEE S.D., PARK J.S. & LEE J.H. 2012. New record of diatom species in Korean coastal waters. *Korean Journal of Environmental Biology* 30: 245–271.

MAJEWSKA R., GAMBI M.C., TOTTI C.M. & DE STEFANO A. 2013. Epiphytic diatom communities of Terra Nova Bay, Ross Sea, Antarctica: structural analysis and relations to algal host. *Antarctic Science* 25: 501–513.

MANABE T. & ISHIO S. 1991. Bloom of *Coscinodiscus wailesii* and DO deficit of bloom water in Seto Inland Sea. *Marine Pollution Bulletin* 23: 181–184.

MCEWAN M.L. & KEELING P.J. 2004. HSP90, tubulin and actin are retained in the tertiary endosymbiont genome of *Kryptoperidinium foliaceum. Journal of Eukaryotic Microbiology* 51: 651–659.

MEDLIN L.K. & PRIDDLE J. 1990. *Polar marine diatoms.* British Antarctic Survey, Natural Environmental Council, Cambridge, UK. 214 pp.

MEDLIN L.K. & ROUND F.E. 1986. Taxonomic studies of marine gomphonemoid diatoms. *Diatom Research* 1: 205–225.

METZELTIN D. & WITKOWSKI A. 1996. Diatomeen der Bären-Insel. Süsswasser – und marine Arten. In: *Iconographia diatomologica. Annotated diatom micrographs,* vol. 4 (Ed. by H. Lange-Bertalot), pp. 3–217.

MORTON O. & CHAMBERLAIN Y.M. 1985. Records of some epiphytic coralline algae in the north-east of Ireland. *Irish Naturalists' Journal* 21: 436–440.

NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION. 2013. GenBank. http://www.ncbi.nlm.nih.gov; searched on 30 August 2013.

OKAMOTO N., NAGUMO N., TANAKA J. & INOUYE I. 2003. An endophytic diatom *Gyrosigma coelophilum* sp. nov. (Naviculales, Bacillariophyceae) lives inside the red alga *Coelarthrum opuntia* (Rhodymeniales, Rhodophyceae). *Phycologia* 42: 498–505.

PIENAAR R.N., SAKAI H. & HORIGUCHI T. 2007. Description of a new dinoflagellate with a diatom endosymbiont, *Durinskia capensis* sp. nov. (Peridiniales, Dinophyceae) from South Africa. *Journal of Plant Research* 120: 247–258.

PISAREVA N.A. & KLOCHKOVA N.G. 2013. Genus *Neoabbottiella* (Rhodophyta) from the Russian Far Eastern seas. *Russian Journal of Marine Biology* 39: 411–421.

REINKE J. 1889. *Algenflora der westlichen Ostsee deutschen Antheils. Eine systematisch-pflanzengeographische Studie.* Bericht der Kommission zur Wissenschaftlichen Untersuchung der deutschen Meere in Kiel 6: pp. 1–101, 8 figs, map.

REINSCH P.F. 1888. Species et genera nova algarum ex insula Georgia australi. *Berichte der Deutschen Botanischen Gesellschaft* 6: 144–156.

RYABUSHKO L.I. 1996. Fouling diatoms on the benthic plants of the Black Sea by Cape Omega. *Hydrobiological Journal* 32: 15–22.

RYABUSHKO L.I. 2009. *Microphytobenthos of the Black Sea*. Dissertation for scientific degree of Doctor of Biological Science, A.O. Kovalevsky Institute of Biology of the Southern Seas, National Academy of Sciences of Ukraine, Sevastopol, vol. 1. 439 pp.

TAKANO Y., HANSEN G., FUJITA D. & HORIGUCHI T. 2008. Serial replacement of diatom endosymbionts in two freshwater dinoflagellates, *Peridiniopsis* spp. (Peridiniales, Dinophyceae). *Phycologia* 47: 41–53.

WOELKERLING W.J. 1983. The *Audouinella* (*Acrochaetium-Rhodochorton*) complex (Rhodophyta): present perspectives. *Phycologia* 22: 59–92.

WOELKERLING W.J. 1993. Type collections of Corallinales (Rhodophyta) in the Foslie Herbarium (TRH). *Gunneria* 67: 1–289.

WYNNE M.J. & SCHNEIDER C.W. 2010. Addendum to the synoptic review of red algal genera. *Botanica Marina* 53: 291–299.

Queries for phya-53-03-01

1. Author: This article has been edited fro grammar, style, and usage. If no change is required in response to a question, please indicate "OK as set". <mark>Copy editor</mark>
2. Author: <mark>Table 1</mark> has been reformatted as per journal style. Copy editor