Molecular phylogeny of a thycoplanktonic naviculoid diatom, *Haslea howeana*, from the Black Sea: a new record for the Turkish Algal Flora

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Abstract

The aim of this study was to identify diatoms from the Southern Black Sea. Water samples were collected using a plankton net with 22 μm pores. Diatom cells were investigated under a light microscope and were then isolated for the single-cell PCR procedure. Phylogenetic inference showed that the isolates collected from the study area were affiliated with a naviculoid diatom *Haslea howeana*. We have reported the first molecular data concerning these species from the Black Sea and have contributed a new species record for the Turkish Algal Flora.

Keywords: Naviculoid diatoms, phylogeny, PCR, haslea, Sinop.

Introduction

Diatoms are siliceous algae, forming the one of the most important group of benthic and planktonic community in aquatic environments. Their cells, being encased in a rigid siliceous box, composed of two valves and a series of girdle bands (Cox & Williams, 2006). The classification of diatoms has still been primarily based on light and ultrastructural microscopy of the preserved siliceous parts, “frustules” (Beszteri et al., 2001).

Diatoms were traditionally classified into two major groups; centric and pennate forms. Centric diatoms have radial symmetry whereas the pennates are symmetrical along the elongated transapical axis (Medlin & Kaczmarska, 2004). Recent studies have however shown centric diatoms to be paraphyletic (Cox & Williams, 2006). When the molecular phylogenetic investigations become available, molecular data contradicted the traditional classification, suggesting an early divergence of diatoms into centrics and pennates (Kociolek & Stoermer, 1989) and also that of the pennates into raphe and araphid groups (Medlin, 1997a). Diatom systematics (Bacillariophyta) have recently been composed of four main classes; Bacillariophyceae, Classis Incertae Setis, Coscinodiscophyceae and Mediophyceae (Guiry & Guiry, 2018) although most common classification was consisting of three subgroups; Bacillariophyceidae, Coscinodiscophyceidae and Fragilariophyceidae (Round et al., 1990).

Pennate diatoms are often defined as naviculoid diatoms that are symmetrical about three planes with a central non-fibulately raphe system on each valve. Those naviculoids are mainly part of benthic microagal community. However, many benthic diatoms are commonly found in plankton samples but they are typically regarded as passively entrained into the plankton environment, being called tychoplankton; tycho-from the Greek is meaning random, casual or accidental (Sabir et al., 2018). Tychoplanktonic diatoms can contribute a majority component to planktonic primary productivity, suggesting that they remain physiologically active in the plankton (Shaffer & Sullivan, 1988). We observed a thycoplanktonic naviculoid diatom in plankton samples of the Sinop Peninsula and investigated the molecular phylogeny to study cryptic diversity of the marine algal community the southern Black Sea.

Materials and Methods

Water samples were collected horizontally using a plankton net with a pore size of 22 μm from İnceburun region, Sinop coasts. The samples were transferred to the laboratory in Plexiglas bottles. Single diatom cells were visualized under an inverted microscope for identification purposes. Morphometric
observations were made before the live cells were isolated using a drawn Pasteur glass pipette. Then each cell was sequentially rinsed with sterile seawater and transferred to a PCR tube with 1 μL of ddH2O. The PCR tubes were incubated at 95°C for 5 min and stored at −20°C until needed.

Genomic DNA isolation was performed using a DirectPCR lysis reagent (Viagen, USA) according to the manufacturer’s protocol with the following modifications: 4 μL of lysis reagent mix (3 μL of 1:10 diluted DirectPCR reagent mix and 1 μL of 1:100 diluted protease K) was used to rupture the dinoflagellate cells. Lysates were stored at −20°C until needed. Amplifications of the rbcl region of chloroplast rDNA were performed from the crude lysates directly with primers rbcl rbcl-L68F (Draisma et al., 2001) and rbclL708R (Bittner et al., 2008). For amplifications, 15 μL of PCR master mix composed of 1 mM dNTP mix, 1.5 mM MgCl2, 0.4 pmol of each primer (in final concentration), 0.5 U of Taq DNA polymerase (Promega Corp.), and 1X PCR buffer were added to the PCR tubes containing approximately 5 μL of the crude lysate. An MWG-Biotech thermal cycler was used for amplifications with the following program: initial denaturation at 95°C for 5 min followed by 40 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 90 s. Final extension was at 72°C for 10 min. The PCR products were electrophoresed on 1% agarose gel (Amresco, Solon, OH, USA) prepared in 1X TBE (Tris-Borate-EDTA) buffer and were visualized after staining with ethidium bromide. Nucleotide sequencing was performed directly from the purified PCR products with the same primers used for the amplifications. PCR product purification and nucleotide sequencing were made commercially by Macrogen Inc. (Korea). The assemblage of the sequencings from both strands were made with BioEdit (Hall, 1999). ClustalX (Thompson et al., 1997) was used to generate multiple nucleotide sequence alignments. To determine the best fitting evolutionary model for our data sets we performed Akaike information criterion (AIC) and Bayesian information criterion (BIC) tests with the software package jModelTest v. 0.1 (Guindon, 2008). Neighbor joining (NJ), maximum parsimony (MP), and Maximum Likelihood (ML) were employed to evaluate phylogenetic relationships among isolates using the software PAUP* v. 4.0ba164 (Swofford, 1998). MP analyses were performed with the heuristic search approach using the TBR swapping algorithm (10 random repetitions). To determine the reliability of the phylogenetic trees, the bootstrap test was conducted with 10,000 and 1000 pseudoreplicates for NJ and MP trees, respectively.

Results and Discussion

As a result of morphological observations an sample 27 were identified as naviculoid cell and it was processed on single cell PCR, being described above. After sequencings, we first conducted a BLAST (GENBANK, NCBI site) search process in order to reveal maximum identities of base pairs of the recorded sequences (Table 1). Consequently, the best match with our sample 27 was the Haslea howeana (isolate KSA0102)

An initial NJ phylogram was derived from a bigger DNA data set including sequences of BLAST result and related literature to reveal phylogenetic affinities of the sequences. After eliminating haplotypes and unrelated taxa, the final phylogenetic research was however, conducted with much less data (with 10 DNA sequences) in order to increase phylogenetical resolution.

The NJ phylogram was drawn with 583 bp length partial rbcl gene sequences and the evolutionary algorithm was conducted according to T92+G (G=0.05) evolutionary model (Figure 1). Accordingly, sequence of Trachneis sp. (KX981824) was branched off as sister to outgroup Rhoicoseis pagonensis (KX981825) with bootstrap support (95/99/100). Two naviculoid lineages was divided as sister to sequence of Navicula perminuta (MH064108). The monophyletic group included the Navicula and Haslea sequences and the sample 27 was related to the strain Haslea howeana with strong bootstrap supports (82/79/78). The strong relation between the Haslea strain and sample 27 were formed at all topologies from ML, MP and NJ analyses.

The species, H. howeana, is the synonym of Navicula howeana Haegelein which was described as one of naviculoid diatoms in 1939 (Round et al., 1990). The genus Haslea Simonsen, formerly belonged to the genus Navicula. Simonsen (1974) erected the genus Haslea, which is characterized by a spindle-shaped staurios bearing valve outline, transverse parallel striae

Table 1. Blast Search results of the NCBI. Accession numbers are shown with lowest e values and Max scores above 760.

<table>
<thead>
<tr>
<th>Description</th>
<th>Max score</th>
<th>Total score</th>
<th>Query cover</th>
<th>E value</th>
<th>Ident</th>
<th>Accession</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Haslea howeana isolate KSA0102 rbcl gene</td>
<td>776</td>
<td>776</td>
<td>99%</td>
<td>0.0</td>
<td>91%</td>
<td>KX981821.1</td>
</tr>
<tr>
<td>2 Haslea sp. strain GU52X-1 Has-ED-22 rbcl gene</td>
<td>765</td>
<td>765</td>
<td>99%</td>
<td>0.0</td>
<td>90%</td>
<td>MH064095.1</td>
</tr>
<tr>
<td>3 Navicula ramossissima strain TA316 rbcl gene</td>
<td>765</td>
<td>765</td>
<td>99%</td>
<td>0.0</td>
<td>90%</td>
<td>KY320301.1</td>
</tr>
<tr>
<td>4 Navicula sp. KEL-2015 clone JAR45 , rbcl gene</td>
<td>765</td>
<td>765</td>
<td>99%</td>
<td>0.0</td>
<td>90%</td>
<td>KM999105.1</td>
</tr>
<tr>
<td>5 Seminavis sp. strain TA305 rbcl gene</td>
<td>763</td>
<td>763</td>
<td>99%</td>
<td>0.0</td>
<td>90%</td>
<td>KY320335.1</td>
</tr>
<tr>
<td>6 Entomoneis paludosus strain TA208 rbcl gene</td>
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<td>760</td>
<td>99%</td>
<td>0.0</td>
<td>90%</td>
<td>KY320278.1</td>
</tr>
<tr>
<td>7 Navicula viridula var. rostellata isolate TCC502 rbcl gene</td>
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<td>760</td>
<td>99%</td>
<td>0.0</td>
<td>90%</td>
<td>KT072916.1</td>
</tr>
</tbody>
</table>
crossed perpendicularly by longitudinal striae and covered by longitudinal strips that are continuous over the external valve face. Sterrenburg et al. (2015) recently denoted that all species of Haslea have a ‘sandwich-type’ valve structure. With this implication, the genus became morphologically very highly diverse group, including 37 species. Li et al., (2017) as a result of molecular phylogeny and ultrastructural study, however, stated that some of these belong in Navicula genus and the real synapomorphic character of the true Haslea species is “sandwich” structure. At the final context, the genus Haslea needs more ultrastructural and molecular data. It is also considered that naviculoid diatoms should not be considered as a monophyletic group in which Haslea and a series of diatom genus such as Craticula, Fallacia etc. were emended. (Bestzeri et al., 2001).

Tychoplanktonic diatoms can contribute a majority component to planktonic primary productivity (Schaffer & Sullivan, 1988), suggesting that they remain physiologically active in the plankton. At the very least recruitment of benthic diatoms into the plankton is important in their dispersal, which can be important in their evolutionary success (Stevenson & Peterson, 1989)

Conclusions

We contributed a new record for the Turkish Algal Flora. The molecular data should be more common within these taxonomical for investigation of cryptic flora of Turkey. Because less data is recently present in molecular records in this study area.

References


E. Cox, & Williams, D.M. 2006 Systematics of naviculoid diatoms (Bacillariophyta): A preliminary analysis of protoplast and frustule characters for family and order level classification. Systematics and Biodiversity, 4:4, 385-399, DOI: 10.1017/S1477200006001940


