www.genaqua.org
ISSN 2459-1831
Genetics of Aquatic Organisms 2: 49-52 (2018) DOI: 10.4194/2459-1831-v2_2_04

PROOF RESEARCH PAPER

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

Özgür Baytut[1,*], Fatih Gümüş[2], Elif Ersanlı[2]

[1] University of Ondokuz Mayıs, Faculty of Arts and sciences, Department of Biology, Samsun, Turkey
[2] University of Sinop, Faculty of Arts and sciences, Department of Biology, Sinop, Turkey

* Corresponding Author: Tel.: +90.362.3121919
E-mail: obaytut@gmail.com

Received 9 October 2018
Accepted 19 December 2018

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 raphid and araphid groups (Medlin, 1997a). Diatom systematics (Bacillariophyta) have recently been composed of four main classes; Bacilariophyceae, Classis Incertae Setis, Coscinodiscophyceae and Mediophyceae (Guiry & Guiry, 2018) although most common classification was consisting of three subgroups; Bacillariophycidae, Coscinodiscophycidae and Fragilariophycidae (Round *et al*., 1990).

Pennate diatoms are often defined as naviculoid diatoms that are symmetrical about three planes with a central non-fibulate raphe system on each valve. Those naviculoids are mainly part of benthic microalgal 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 ddH_2O. 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 lysis reagent and 1 µL of 1:100 diluted proteinase K) was used to rupture the dinoflagellate cells. Lysates were stored at –20°C until needed. Amplifications of the *rbc*L region of chloroplast rDNA were performed from the crude lysates directly with primers rbcL *rbc*L-68F (Draisma *et al*., 2001) and *rbc*L-708R (Bittner *et al*., 2008). For amplifications, 15 µL of PCR master mix composed of 1 mM dNTP mix, 1.5 mM $MgCl_2$, 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 process: 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 & Gascuel, 2003; Posada, 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 affilities of the sequences. After eliminating haplotypes and unrelevant 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 *Rhoiconeis pagoensis* (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* Hagelstein 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 stauros 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.

	Description	Max score	Total score	Query cover	E value	Ident	Accession
1	*Haslea howeana* isolate KSA0102 rbcL gene	776	776	99%	0.0	91%	KX981821.1
2	*Haslea* sp. strain GU52X-1 Has-ED-22 rbcL gene	765	765	99%	0.0	90%	MH064095.1
3	*Navicula ramosissima* strain TA316 rbcL gene	765	765	99%	0.0	90%	KY320301.1
4	*Navicula* sp. KEL-2015 clone JAR45_ rbcL gene	765	765	99%	0.0	90%	KM999105.1
5	*Seminavis* sp. strain TA305 rbcL gene	763	763	99%	0.0	90%	KY320335.1
6	*Entomoneis paludosa* strain TA208 rbcL gene	760	760	99%	0.0	90%	KY320278.1
7	*Navicula viridula* var. *rostellata* isolate TCC502 rbcL gene	760	760	99%	0.0	90%	KT072916.1

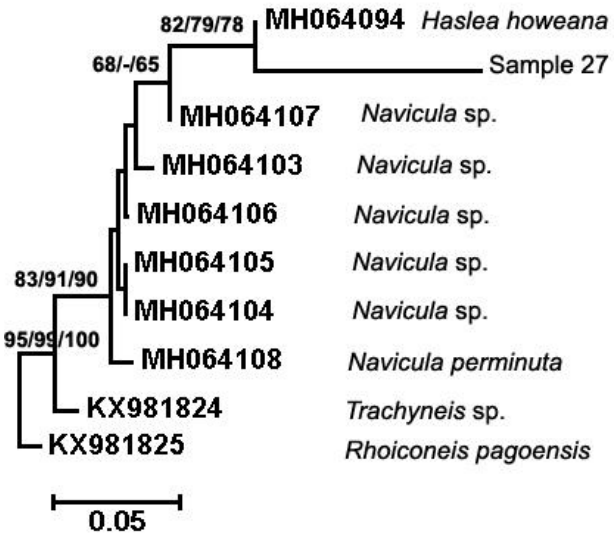

Figure 1. The evolutionary phylogram was inferred using the partial rbcL gene region of cpDNA. The bootsrap supports (above 50%) of NJ/MP/ML algorithms were respectively shown at the nods of the phylogram. T92+G model was used according to the results of J model test. The gamma parameter was set to 0.05. There were a total of 583 positions in the final dataset. KX981825[1], MH064135[2], KX981824[1], MH064103[2], MH064094[2], MH064106[2], MH064108[2], MH064107[2], MH064105[2], MH064104[2]. Literature for haplotypes obtained from GenBank as follows: [1] Ashworth et al., 2016, [2]Sabir et al., 2018.

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 ultrastructual 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 inwhich *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

Ashworth, M.P., Lobban, C.S., Witkowski, A., Theriot, E.C., Sabir, M.J., Baeshen, M.N., Hajarah, N.H., Baeshen, N.A., Sabir, J.S. & Jansen, R.K. 2016. Molecular and Morphological Investigations of the Stauros-bearing, Raphid Pennate Diatoms (Bacillariophyceae): Craspedostauros E.J. Cox, and Staurotropis T.B.B. Paddock, and their Relationship to the Rest of the Mastogloiales. Protist 168:(1)48-70

Beszteri, B., Vaacs, E., Makk, J., Kovacs, G., Marialigeti, K., Kiss, K.T. 2001. Phylogeny of six naviculoid diatoms based on 18S rDNA sequences. *International Journal of Systematic and Evolutionary Microbiology* (2001), 51, 1581–1586.

Bittner, L., Payri, C.E., Couloux, A., Cruaud, C., de Reviers, B., & Rousseau, F. 2008. Molecular phylogeny of the Dictyotales and their position within the Phaeophyceae, based on nuclear, plastid and mitochondrial DNA sequence data. Molecular Phylogenetic and Evolution, 49: 211–216.

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

Draisma, S.G.A., van Reine, P., Stam, W.T., & Olsen, J.L., 2001. A reassessment of phylogenetic relationships within the phaeophyceae based on RUBISCO large subunit and ribosomal DNA sequences, *Journal of Phycology*, 37:586-603

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/ NT. Nucleic Acids 41: 95-98.

Guindon, S., Gascuel, O. 2003. A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52: 696-704.

Guiry, M.D., & Guiry, G.M. 2018. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 05 December 2018

Kociolek, J.P., Stoermer, E.F. 1989. Chromosome numbers in diatoms: a review. *Diatom Res* 4, 47-54.

Li, Y., Chen, X., Sun, Z., & Xu, K. 2017. Taxonomy and molecular phylogeny of three marine benthic species of *Haslea* (Bacillariophyceae), with transfer of two species to *Navicula*. Diatom Research, 32:4, 451-463, DOI: 10.1080/0269249X.2017.1401008.

Medlin, L.K., Kooistra, W.H.C.F., Gersonde, R., Sims, P.A., & Wellbrock, U. (1997). Is the origin of the diatoms related to the end-Permian mass extinction? Nova Hedwigia 65, 1-11.

Medlin, L.K., & Kaczmarska, I. 2004. Evolution of the diatoms: V. Morphological and cytological support for the major clades and a taxonomic revision. Phycologia 43, 245-270.

Posada, D. 2008. jModel test: phylogenetic model averaging. Mol Biol Evol 25: 1253-1256.

Round, F.E., Crawford, R.M., & Mann, D.G. 1990. The diatoms: biology and morphology of the genera. Cambridge University Press: London, UK. ISBN 0-521-363187. 747 pp.

Sabir, J.S., Theriot, E.C., Manning, S.R., Al-Malki, A.L., Khiyami, M.A., Al-Ghamdi, A.K., ... & Jansen, R.K. (2018). Phylogenetic analysis and a review of the history of the accidental phytoplankter, *Phaeodactylum tricornutum* Bohlin (Bacillariophyta). *PloS one*, *13*(6), e0196744. https://doi.org/10.1371/journal.pone.0196744

Shaffer, G.P., & Sullivan, M.J. 1988. Water column productivity attributable to displaced benthic diatoms in wellmixed shallow estuaries. J Phycol.; 24(2):132±40. https://doi.org/10.1111/j.1529-8817.1988.tb04226.x

Simonsen, R. 1974. The diatom plankton of the Indian Ocean Expedition of R/V Meteor 1964-5. *"Meteor" Forschungsergebnisse, Reihe D, Biologie* 19: 1–107.

Swofford, D.L. 1998. PAUP*: phylogenetic analysis using parsimony (*and other methods). Version 4 beta 10. Sunderland, MA, USA: Sinauer Associates.

Sterrenburg, F.A.S., Tiffany, M.A., Hinz, F., Herwig, W.E., & Hargraves, P.E. 2015b. Seven new species expand the morphological spectrum of *Haslea*. A comparison with *Gyrosigma* and *Pleurosigma* (Bacillariophyta). *Phytotaxa* 207: 143–162.

Stevenson, R.J., & Peterson, C.G. 1989. Variation in benthic diatom (Bacillariophyceae) immigration with habitat characteristics and cell morphology. J Phycol.; *25*(1):120-9.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., & Higgins, D.G. 1997. The ClustalX-Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res *25*: 4876-4882.