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Morphology, 18S rDNA sequence and *rbc*L phylogeny of *Navicula veneta* (Bacillariophyceae) from thermal muds in Italy

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Abstract – A new culture strain of *Navicula veneta* Kützing (Bacillariophyceae) was isolated from brown mats covering thermal muds in the SPA town of Abano Terme, Padova, Italy. The morphology, 18S rDNA and *rbc*L gene sequences of this strain were analysed. The results do not support an existing taxonomic proposal to lower the taxonomic rank of *Navicula veneta* to a variety of *Navicula cryptocephala* Kützing. Therefore, we consider *Navicula veneta* as a good species in its own right. We also hypothesize that the species *Navicula veneta,* as defined morphologically, contains a number of cryptic species yet to be discovered and analysed genetically.

Bacillariophyceae / morphology / *Navicula* / *rbc*L / thermal muds / ultrastructure / 18S rDNA

Résumé – **Morphologie, séquençes de l'ADNr 18S et phylogénie du *rbc*L du *Navicula veneta* (Bacillariophyceae) provenant de boues thermales en Italie.** Une nouvelle souche du *Navicula veneta* Kützing (Bacillariophyceae) a été isolée à partir de couches brunes recouvrant des boues thermales dans la ville thermale de Abano Terme, Padue, Italie. La morphologie et les séquences de l'ADNr 18S et du gène *rbc*L de cette souche ont eté étudiées. Les résultats ne s'accordent pas avec les données de la littérature, dans laquelle l'espèce *Navicula veneta* a été réduit à une variété du *Navicula cryptocephala* Kützing. Par conséquent, nous considérons le *Navicula veneta* comme une bonne espèce à tous égards. Nous formulons aussi l'hypothèse que l'espèce *Navicula veneta* définie morphologiquement contient des espèces cryptiques qui sont encore à découvrir et à analyser du point de vue génétique.

ADNr 18S / Bacillariophyceae / boues thermales / morphologie / *Navicula* / *rbc*L / ultrastructure

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INTRODUCTION

The Euganean thermal springs in Abano Terme (Italy) are an important SPA station in Europe which has long been known for various therapeutic effects of the thermal water and muds. The neutral geothermal water, flowing at about 80°C, is used in many hotels for various kinds of complementary therapies and for the so-called mud "maturation" process. For that purpose, the thermal mud is collected from the bottom of Arquà Petrarca and Lispida thermal lakes (Padova, Italy), and subsequently incubated for at least 60 days in tanks and constantly covered with flowing thermal water with temperatures ranging from 40 to 60°C.

The thermal muds harbour microbial communities which are known to be responsible for the production of various compounds during the mud maturation process (Galzigna *et al.*, 1996; Galzigna & Bellometti, 1999). These communities are composed mainly of cyanoprokaryotes (Ceschi Berrini *et al.*, 2004; Moro *et al.*, 2007a,b), which are known to be more thermo-tolerant than eukaryotes (Rothschild & Mancinelli, 2001). Additionally, diatoms may also be present but their occurrence and diversity are strongly limited by the temperature of the water flowing into the mud maturation tanks. Although some diatom species have been found in other hot springs at temperatures higher than 50°C (Kaštovský & Komárek, 2001; Bonny & Jones, 2003) and as high as 65°C (Owen *et al.*, 2008), in the Euganean thermal springs diatoms are absent at temperatures higher than 50°C, while at 40-45°C they are represented by only 3 or 4 species, as reported for some other hot springs (Stockner, 1967; Owen *et al.*, 2008).

In 2006 we collected and isolated from the surface of thermal muds of an Euganean SPA a diatom which we have conclusively identified as *Navicula veneta* Kützing. Our morphological and molecular sequence analyses of the isolate are reported here owing to their taxonomic and phylogenetic interest, and the fact that they lend support to the idea that *Navicula veneta* ought to be considered as a good species in its own right.

MATERIALS AND METHODS

Sampling and cultivation

The diatom strain examined here (ETS 07) was isolated in December 2006 from brown mats covering thermal muds at the Hotel Adriatico, Abano Terme, Padova, Italy, by means of single-cell isolation using a micropipette. The temperature of the thermal spring water flowing into the mud tank during the sampling was 43°C and pH 6.8-7. Cultivation was in f/2 medium (Guillard & Ryther, 1962; Guillard, 1975) in a growth chamber at 30°C, with a photon flux rate of 35 µmol photons m^{-2} s^{-1} and a 12:12 dark:light cycle.

Microscopy

Observations on living cells were made using a Leica DM5000 light microscope (LM) fitted with a digital image capture system. Cell viability was ascertained based on the presence of chlorophyll red autofluorescence under UV light excitation (Fry, 1988), using a Zeiss Axioscop epifluorescence microscope

fitted with a BP 365/12 excitation filter, an FT 395 chromatic beam splitter and an LP 397 barrier filter. For scanning electron microscopy (SEM), a culture sample was fixed for 2 h in 6% glutaraldehyde in 0.1 M cacodylate buffer, pH 6.9, post-fixed for 2 h in 1% OsO_4 in the same buffer, dehydrated in a graded EtOH, critical-point dried, sputter-coated with gold, and examined with a Cambridge Stereoscan 260 SEM operating at 25 kV.

Molecular analyses

DNA was extracted from a pellet of cultured diatoms based on the modified CTAB method (Doyle & Doyle, 1987). The 18S rDNA gene sequence was amplified by polymerase chain reaction (PCR) using primers Dia18SF 5'-AGTAGTCATACGCTCGTCT-3' and Dia18SR 5'- AAGGTTTAGACAAGTT CTCG-3', chosen based on the alignment of 18S rDNA gene sequences of some *Navicula* species. The *rbc*L gene sequence was amplified by using primers and conditions reported in Bruder & Medlin (2007). PCR products were visualized by ethidium bromide staining after electrophoresis in a 1% agarose gel, purified with the ExoSAP-IT kit (Amersham Biosciences) and sequenced directly. Sequencing was carried out at the BMR-Genomics Sequencing Service, Universiy of Padova, using automated DNA sequencers. Sequencing was performed on both strands to ensure accuracy of the results. The final consensus sequence was assembled using the SeqMan II programme from the Lasergene software package (DNAStar)). The identity of new sequences was checked using the BLAST programme (Altschul *et al.*, 1990) operating at NCBI (http://www.ncbi.nlm.nih.gov). The *rbc*L gene sequence was translated into the corresponding protein to control for correctness of the reading frame.

DDBJ/GenBank/EBI Data Bank accession numbers of the other *rbc*L sequences included in this paper and their corresponding diatom species are listed in Table 1. Nucleotide and aminoacid pair-wise comparisons between *rbc*L sequences were performed using the MEGA4 programme (Tamura *et al.*, 2007). A *rbc*L dataset including almost all sequences of naviculoid diatoms belonging to the section Lineolatae (Bruder & Medlin, 2007), was created to assess the phylogenetic position of the Euganean isolate. *Navicula phyllepta* strain CCY0227 (EU938318) was excluded from the analyses because of the shortness of its *rbc*L sequence. The alignment was 657 positions long and is available upon on request. A phylogenetic tree was inferred based on the maximum likelihood (ML) method using the PHYML 2.4.4 programme (Guindon & Gascuel, 2003) and applying the GTR+I+G evolutionary model (Lanave *et al.*, 1984). Non-parametric bootstrap (Felsenstein, 1985) was performed to test the robustness of the tree topology (1000 replicates).

The 18S rDNA and *rbc*L gene sequences of strain ETS 07 have been deposited in DDBJ/GenBankTM/EBI Data Bank with accession numbers FN398345 and FN392686, respectively.

RESULTS

Morphology

By LM cells of the Euganean strain were solitary, with linear-lanceolate valves bearing broadly sub-rostrate apices (Fig. 1). Valves were 18.5-23 μm long, 5-6 μm wide and with 13-19 striae in 10 μm. Cells had two plate-like chloroplasts

Tab. 1. List of taxa considered in this paper and accession numbers of the *rbc*L sequences. The investigated species is given in bold. (§ strains having identical *rbc*L sequences).

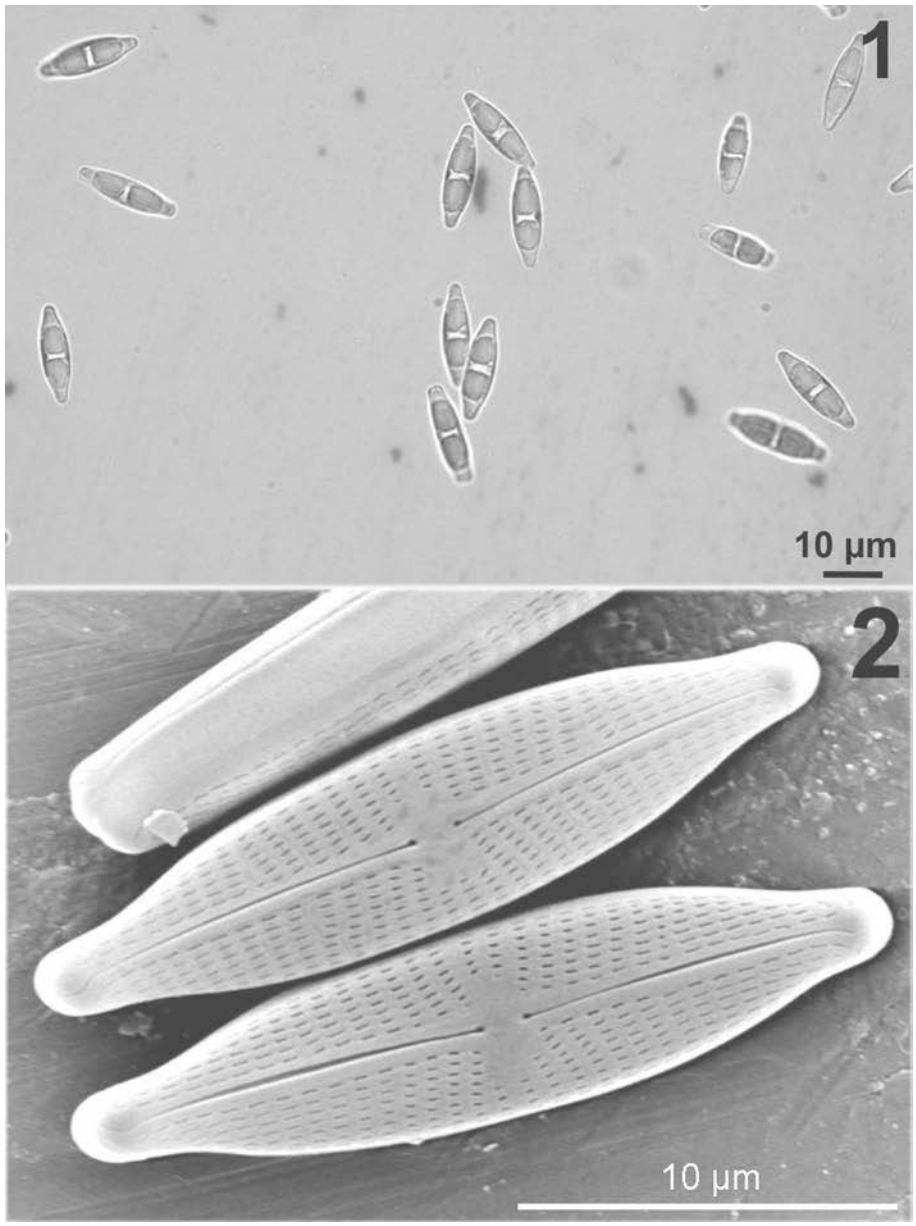
Taxon	*Strain Designation*	*rbcL accension number*
Adlafia brockmanni (Hustedt) Bruder	AT-111Gel110	AM710487
Amphora pediculus (Kützing) Grunow	AT-117.11	AM710426
Lyrella atlantica (Schmidt) Mann	E3467	AY571747
Navicula capitatoradiata Germain	AT-212Gel07	AM710479
Navicula cari Ehrenberg	AT-82.04c	AM710457
Navicula cryptocephala Kützing §	AT-176Gel05	AM710463
Navicula cryptotenella Lange-Bertalot	AT-202Gel03	AM710496
Navicula cf *duerrenbergiana* Hustedt	E3458	AY571749
Navicula gregaria Donkin	AT-117Gel05	AM710440
Navicula radiosa Kützing	AT-205.02b	AM710501
Navicula reinhardtii Grunow	AT-124.15	AM710442
Navicula salinicola Hustedt	CCMP1730	AY604699
Navicula tripunctata (O.F. Müller) Bory	AT-202.01	AM710495
Navicula veneta Kützing	AT-117Gel20b	AM710441
***Navicula veneta* Kützing**	**ETS-07**	**FN392686**
Navicula sp.	AT-145.08	AM710474
Navicula sp.	AT-201Gel01	AM710466
Navicula sp. §	NAV321TM	EF143288
Navicula sp.	ArM0002	EU090047

along each side of the girdle, a central cytoplasmic bridge and two spherical volutin granules (Fig. 1). The red autofluorescence of chlorophyll confirmed that cells were viable. SEM revealed that valves were characterized by lineolate striae, as is typical in the genus *Navicula,* with weakly radiate striae in the centre of the valves (Fig. 2). The central area of the valves was widened, reflecting the presence of 2-3 shorter striae in that region (Fig. 3). The central endings of the external raphe fissures were slightly expanded, while the polar endings were hook-shaped over the valve apices (Fig. 4).

The valve margin was characterized by a distinct peripheral row of pores that appeared V-shaped near the apices (Fig. 4).

Molecular analyses

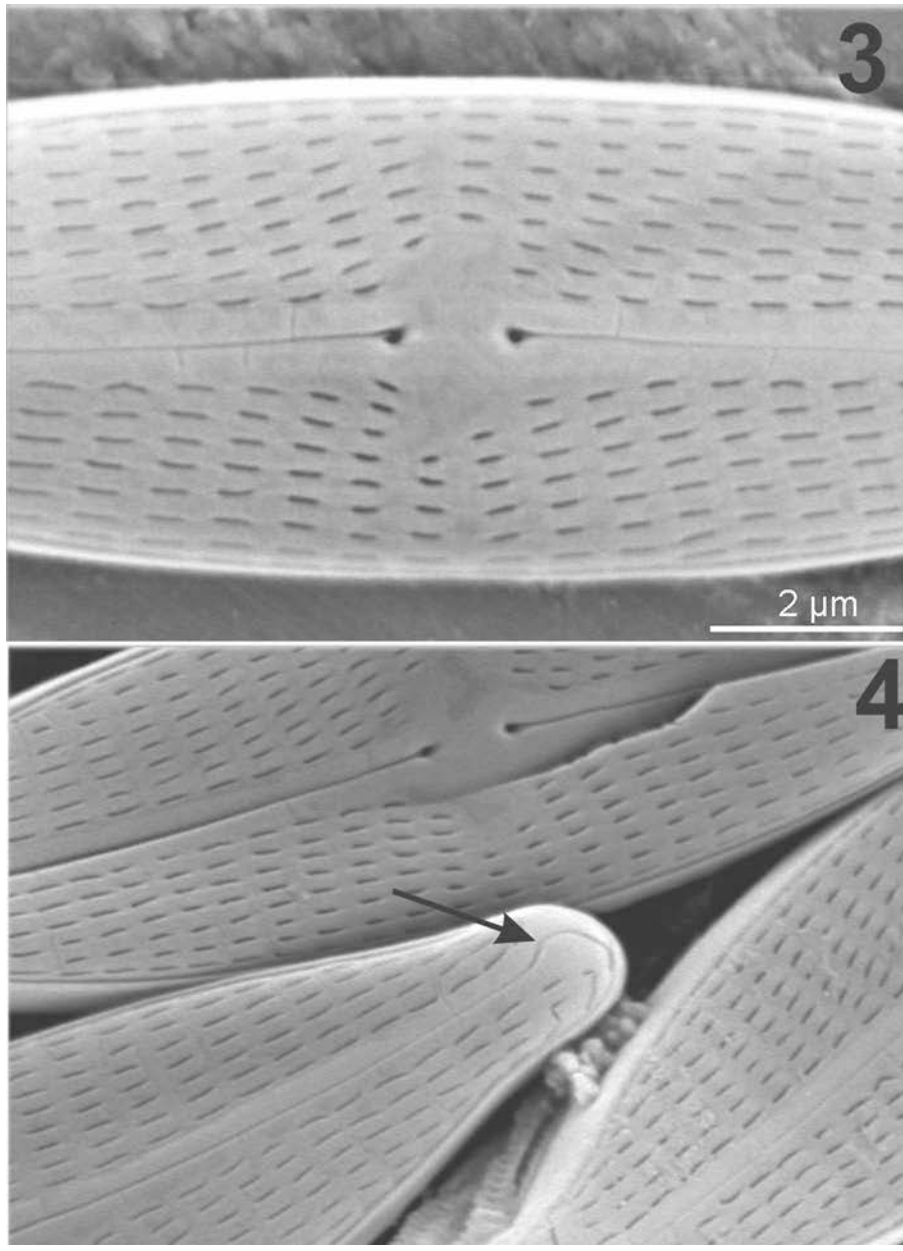
The 18S rDNA sequence of strain ETS 07 was 1640 bases long. The ETS 07 sequence shared the highest level of similarity with the GenBank-deposited sequences of strains NCB (reported as *Navicula cryptocephala* var.



Figs. 1-2. Light and scanning electron micrographs of the Euganean strain *Navicula veneta* ETS 07.

veneta: Beszteri *et al*., 2001), and AT-117Gel20b, AT-110Gel19 and AT-108Gel01, identified as *Navicula veneta* (Bruder & Medlin, 2007). The ETS 07 sequence differed only in 2 bases (data not shown).

The *rbc*L sequence of strain ETS 07 was 657 bases long, *i.e. ca* 50% of the gene length.The homologous counterparts of this sequence were obtained



Figs 3-4. Scanning electron micrographs of *Navicula veneta* ETS 07. **3**. The higher magnification shows the central area of the valve characterized by two or three shorter striae. **4**. Note the polar raphe fissures hooked over the valve apex (arrow).

from GenBank using the BLAST program. All the sequences that showed the highest levels of similarity with the ETS 07 *rbc*L gene belonged to species of the genus *Navicula.* The ETS 07 *rbc*L sequence was clearly different from all *rbc*L sequences currently known from described and undescribed taxa of *Navicula* which are available on the NCBI web server.

Pair-wise comparisons with selected *Navicula* species (Table 2) showed that the ETS 07 *rbc*L sequence of shared the highest level of similarity with the *rbc*L sequences of strains AT-117Gel20b, AT-110Gel19 and AT-108Gel01 (all of which are virtually identical to one another), which have been identified as *Navicula veneta* (Bruder & Medlin, 2007). They showed 18 base differences, corresponding to a genetic divergence of 2.74%, mirrored by 3 different aminoacids in the corresponding protein sequences.

A pair-wise comparison was also carried out with *Navicula cryptocephala* strain AT-176Gel05 (Table 2) because *Navicula veneta* has been considered as a variety of *N. cryptocephala* Kützing (Cox, 1995; Ettl & Gärtner 1995). This comparison identified 41 nucleotide differences mirrored by 3 distinct amino acids. Moreover, the pair-wise comparison between strain ETS 07 and *Navicula phyllepta* strain CCY0227, performed on 304 bases, revealed that these two taxa were clearly distinct (21 different bases).

Tab. 2. Pair-wise comparisons among nucleotides *rbc*L sequences of *Navicula veneta* ETS-07 versus other strains belonging to the *Navicula* genus.

Pair-wise comparison	*N°of differences*
Navicula ETS 07 vs *Navicula veneta* AT-117Gel20b	18
Navicula ETS 07 vs *Navicula tripunctata* AT-202.01	43
Navicula ETS 07 vs *Navicula* sp. ArM0002	43
Navicula ETS 07 vs *Navicula* sp. AT-201Gel01	58
Navicula ETS 07 vs *Navicula* sp. AT14508	37
Navicula ETS 07 vs *Navicula salinicola* CCMP1730	51
Navicula ETS 07 vs *Navicula reinhardtii* AT-124.15	38
Navicula ETS 07 vs *Navicula radiosa* AT-205 02b	39
Navicula ETS 07 vs *Navicula gregaria* AT-117Gel05	37
Navicula ETS 07 vs *Navicula* cf. *duerrenbergiana* E3458	41
Navicula ETS 07 vs *Navicula cryptotenella* AT-202Gel03	49
Navicula ETS 07 vs *Navicula cryptocephala* AT-176Gel05	41
Navicula ETS 07 vs *Navicula cari* AT-82.04c	35
Navicula ETS 07 vs *Navicula capitatoradiata* AT-212Gel07	38

To better define the position of strain ETS 07 within the genus *Navicula*, we carried out an *rbc*L gene molecular phylogenetic analysis based on maximum likelihood (ML). A dataset including all available *rbc*L sequences obtained from a group of 15 naviculoid diatoms belonging to the section Lineolatae plus three

outgroup sequences belonging to the genera *Lyrella*, *Adlafia* and *Amphora* was created. Taxa that presented identical *rbc*L sequences from a preliminary pair-wise comparison are reported once. The *rbc*L multiple alignment used in the phylogenetic analysis was 657 positions long. The ML (-Ln=2624.106157) tree obtained from the *rbc*L data set is shown in Fig. 5. Strain ETS 07 was a sister taxon to strain AT-117Gel20b, reported as *Navicula veneta*, (bootstrap value 95%). Strain AT-117Gel20b and *Navicula cryptocephala* were not strictly related and were placed far apart in the phylogenetic tree. The species *Navicula salinicola* appeared to be the most basal *Navicula* taxon in this analysis.

DISCUSSION

Our investigation shows that the correct generic assignment of strain ETS 07 is within the genus *Navicula*. This strain has the characteristic morphological features of naviculoid diatoms, i.e. lineolate striae and the presence of weakly radiate striae in the centre of the valves. The 18S rDNA sequence of strain ETS 07 also confirms a placement within *Navicula.*

At the species level, morphometric, morphological and ultrastructural features of strain ETS 07 such as raphe morphology and the size and arrangement of striae coincide with those of *Navicula veneta* Kützing, confirming that our strain is correctly identified as belonging to that species. Although there still exist some uncertanties in relation to the type material of *Navicula veneta* (Cox, 1991), our conclusion on the specific identity of strain ETS 07 is based on a comparison with morphological data reported by Cox (1995) and the lectotype fixed therein for this species. Our identification is further supported by the geographic proximity between the collecting site of this strain and the type locality of *Navicula veneta*, i.e. a brackish pool in a Venetian botanical garden (Kützing, 1844).

Taxonomically, Cox (1995) considered *Navicula veneta* to be a simple variety of *Navicula cryptocephala (*Cox, 1995) rather than a species in its own right. By contrast, we maintain that the 41 base differences which emerged in our pair-wise comparison between the *rbc*L sequences of strain ETS 07 and that of *Navicula cryptocephala* strain AT-176Gel05 do not support this view. Consequently, based on our findings we consider *Navicula veneta* to be a good species whose reduction to a variety of *Navicula cryptocephala* is unsupported by the available evidence, and therefore untenable.

Our pair-wise comparisons of the *rbc*L sequences support the use of *rbc*L as a useful genetic marker for the recognition of *Navicula* species which are difficult to distinguish morphologically. This is also in agreement with the lack of intraspecific variability in *rbc*L gene sequences which has been found in species of heterokont pelagophytes (Bailey & Andersen, 1999) and xanthophytes (Negrisolo *et al.,* 2004).

Since the time of its first collection and description by Kützing (1844), *Navicula veneta* has been reported from different kinds of habitats (Lange-Bertalot 1979; Ettl & Gärtner 1995), suggesting that from a morphological point of view it might be a cosmopolitan species. Dell'Uomo (1986) recorded it in thermal sulphureous waters at Triponzio (Val Valnerina, Perugia, Italy). It was also found in a river sample at Lesum, Germany (Bruder & Medlin, 2007) and in the Laskó stream, Hungary (Beszteri *et al*., 2001).

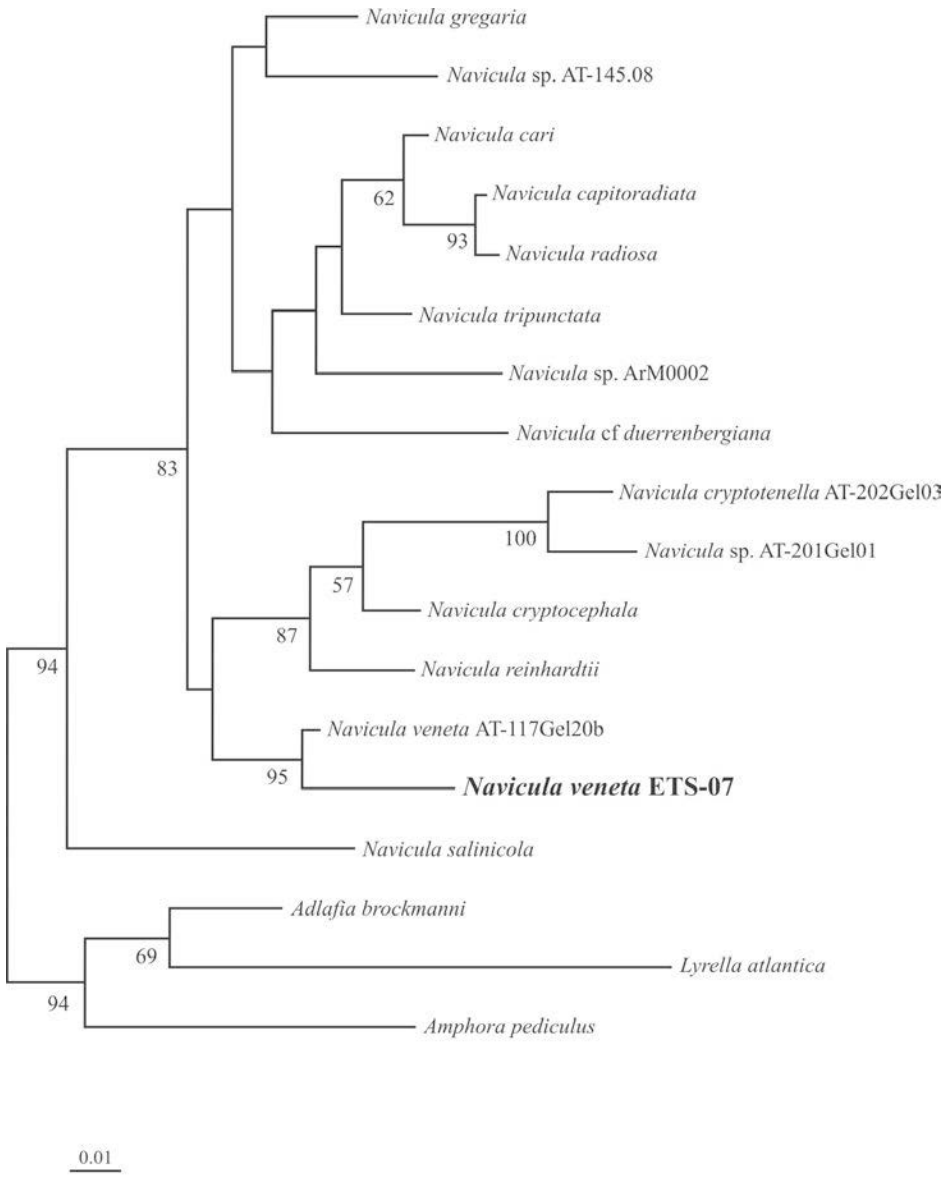


Fig. 5. Phylogenetic tree based on the *rbc*L gene sequences, reconstructed using the maximum-likelihood analysis of evolutionary distances determined by the GTR+I+G model. Numbers on branches indicate bootstrap values greater than 50%. Bar represents 0.01 nucleotide substitutions per site.

In addition to postulating a cosmopolitan geographic distribution, we also hypothesize that in *Navicula veneta* (as defined morphologically) there exist several genetically distinct entities (cryptic species), analogous to other diatoms (Kooistra *et al*., 2008; Evans *et al*., 2007; Poulíčková *et al*., 2010). Further analyses using more informative molecular markers (such as *cox*1 or ITS) will establish if this hypothesis is correct. The use of *cox*1 sequences is useful to distinguish cryptic species because the level of nucleotide divergence is usually greater than that of *rbc*L (Evans *et al.* (2007), and ITS secondary structure is also a powerful tool to analyze inter-taxa relationships, from subspecies to orders (Coleman, 2003). Unfortunately, no strains of *Navicula veneta* exist any longer in algal culture collections, and therefore no genetic comparisons using these markers could be carried out in our study to investigate the extent of molecular diversity within this species. However, *cox1* and ITS analyses might become possible in the future if newly isolated strains of *Navicula veneta* from different geographic locations will become available.

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REFERENCES

ALTSCHUL S. F., GISH W., MILLER W., MYERS E. W. & LIPMAN D. J., 1990 — Basic local alignment search tool. *Journal of molecular biology* 215: 403-410.

BAILEY J.C. & ANDERSEN R.C., 1999 — Analysis of clonal cultures of the brown tide algae *Aureococcus* and *Aureoumbra* (Pelagophyceae) using 18S rRNA, *rbc*L and RUBISCO spacers sequences. *Journal of phycology* 35: 570-574.

BESZTERI B., ÁCS E., MAKK J., KOVÁCS G., MÁRIALIGETI K. & KISS K.T., 2001 — Phylogeny of six naviculoid diatoms based on 18S rDNA sequences. *International journal of systematic and evolutionary microbiology* 51: 1581-1586.

BONNY S. & JONES B., 2003 — Relict tufa at Miette Hot Springs, Jasper National Park, Alberta, Canada *Canadian journal of earth sciences* 40: 1459-1481.

BRUDER K. & MEDLIN L.K., 2007 — Molecular assessment of phylogenetic relationships in selected species/genera in the naviculoid diatoms (Bacillariophyta). I. The genus *Placoneis*. *Nova Hedwigia* 85: 331-352.

CESCHI-BERRINI C., DE APPOLONIA F., DALLA VALLE L., KOMÁREK J. & ANDREOLI C., 2004 — Morphological and molecular characterization of a thermophilic cyanobacterium (Oscillatoriales) from Euganean Thermal Springs (Padua, Italy). *Algological studies* 113: 73-85.

COLEMAN, A.W., 2003 — ITS2 is a double-edged tool for eukaryote evolutionary comparisons. *Trends in genetics* 19: 370-375.

COX E.J., 1995 — Studies on the diatom genus *Navicula* Bory. VII. The identify and typification of *Navicula gregaria* Donkin, *N. cryptocephala* Kütz. and related taxa. *Diatom research* 10: 91-111.

DELL'UOMO A., 1986 — Diatoms and other algae from the thermal-sulphur springs of Triponzo (Central Italy). *Archiv für Hydrobiologie – Algological studies* 42: 79-91.

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

ETTL H. & GÄRTNER G., 1995 — *Syllabus der Boden, Luft und Flechtenalgen*. Stuttgart, G. Fischer, 721 p.

EVANS K.M., WORTLEY A.H. & MANN D.G., 2007 – An assessment of potential diatom "barcode" genes (*cox*1, *rbc*L, 18S and ITS rDNA) and their effectiveness in determining relationships in *Sellaphora* (Bacillariophyceae). *Protist* 158: 349-364.

FELSENSTEIN J., 1985 – Confidence limits on phylogenies: an approach using bootstrap. *Evolution* 39: 783-791.

FRY S.C., 1988 — *The growing plant cell wall: chemical and metabolic analysis*. Harlow, Longman Scientific and Technical, 333 p.
GALZIGNA L. & BELLOMETTI S., 1999 — La maturation de la boue thermale et sa mesure. Première partie. *Presse termale et climatique* 1: 23-26.
GALZIGNA L., MORETTO C. & LALLI A., 1996 — Physical and biochemical change of thermal mud after maturation. *Biomed & pharmacother* 50: 306-308.
GUILLARD R.R.L. & RYTHER J.H., 1962 – Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Canadian journal of microbiology* 8: 229-239.
GUILLARD R.R.L., 1975 – *Culture of phytoplankton for feeding marine invertebrates*. *In*: Smith W.L. & Chanley M.H. (eds), *Culture of marine invertebrates animals*. New York, Plenum Press, pp. 26-60.
GUINDON S. & GASCUEL O., 2003 — A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic biology* 52: 696-704.
KAŠTOVSKÝ J. & KOMÁREK J., 2001 — Phototrophic microvegetation of thermal springs in Karlovy Vary, Czech Republic. *Nova Hedwigia, Beiheft* 123: 107-120.
KOOISTRA W.H.C.F., SARNO D., BALZANO S., HAIFENG G., ANDERSEN R.A. & ZINGONE A., 2008 – Global diversity and biogeography of *Skeletonema* species (Bacillariophyta). *Protist* 159: 177-193.
KÜTZING F.T., 1844 – *Die kieselschaligen bacillarien oder diatomeen*. Köhne, Nordhausen, 152 p.
LANAVE C., PREPARATA G., SACCONE C. & SERIO G., 1984 — A new method for calculating evolutionary substitution rates. *Journal of molecular evolution* 20: 86-93.
LANGE-BERTALOT H., 1979 – Toleranzgrenzen und populationsdynamik bentischer diatomeen bei unterschiedlich starker abwasserbelastung. *Algological studies* 23: 184-219.
MORO I., DI BELLA M., RASCIO N., LA ROCCA N. & ANDREOLI C., 2007b — *Conferva duplisecta* Pollini: rediscovery in the Euganean Thermal Springs (Italy) and new assignment to the *Oscillatoria* genus. *Caryologia* 60: 133-136.
MORO I., RASCIO N., LA ROCCA N., DI BELLA M. & ANDREOLI C., 2007a — *Cyanobacterium aponinum*, a new Cyanoprokaryote from the microbial mat of Euganean thermal springs (Padua, Italy). *Algological studies* 123: 1-15.
NEGRISOLO E., MAISTRO S., MORO I., INCARBONE M., DALLA VALLE L., BROADY P.A. & ANDREOLI C., 2004 — Morphological convergence characterizes the evolution of Xanthophyceae (Heterokontophyta): evidence from nuclear SSU rDNA and plastidial *rbc*L genes. *Molecular phylogenetics and evolution* 33: 156-170.
OWEN R.B., RENAUT R.W. & JONES B., 2008 – Geothermal diatoms : a comparative study of floras in hot spring systems of Iceland, New Zealand, and Kenya. *Hydrobiologia* 610: 175-192.
POULÍČKOVÁ A., VESELÁ J., NEUSTUPA J. & ŠKALOUD P., 2010 – Pseudocryptic diversity versus cosmopolitanism in diatoms: a case study on *Navicula cryptocephala* Kütz. (Bacillariophyceae) and morphologically similar taxa. *Protist* doi: 10.1016/ j.protis.2009.12.003.
ROTHSCHILD L.J. & MANCINELLI R.L., 2001 — Life in extreme environments. *Nature*, 409: 1092-1101.
STOCKNER J.G., 1967 — Observations of thermophilic algal communities in Mount Rainer and Yellowstone National Parks. *Limnology and oceanography* 12: 13-17.
TAMURA K., DUDLEY J., NEI M. & KUMAR S., 2007 — MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular biology and evolution* 24: 1596-1599.