JOURNAL OF PLANKTON RESEARCH | VOLUME 34 | NUMBER 5 | PAGES 416–431 | 2012

Functional diversity in cryptic species of *Chaetoceros socialis* Lauder (Bacillariophyceae)

MARIA DEGERLUND[1]*, SIV HUSEBY[1,2], ADRIANA ZINGONE[3], DIANA SARNO[4] AND BJARNE LANDFALD[5]

[1]DEPARTMENT OF ARCTIC AND MARINE BIOLOGY, UNIVERSITY OF TROMSØ, 9037 TROMSØ, NORWAY, [2]MABCENT-SFI, UNIVERSITY OF TROMSØ, 9037 TROMSØ, NORWAY, [3]LABORATORY OF ECOLOGY AND EVOLUTION OF PLANKTON, STAZIONE ZOOLOGICA ANTON DOHRN, VILLA COMUNALE, 80121 NAPOLI, ITALY, [4]TAXONOMY AND IDENTIFICATION OF MARINE PHYTOPLANKTON, STAZIONE ZOOLOGICA ANTON DOHRN, VILLA COMUNALE, 80121 NAPOLI, ITALY AND [5]NORWEGIAN COLLEGE OF FISHERY SCIENCE, UNIVERSITY OF TROMSØ, 9037 TROMSØ, NORWAY

*CORRESPONDING AUTHOR: maria.degerlund@uit.no

Received August 9, 2011; resubmitted on December 16, 2011; accepted in principle January 17, 2012

Corresponding editor: Zoe Finkel

The cosmopolitan distribution of the marine planktonic diatom *Chaetoceros socialis* Lauder is generally interpreted as a case of wide ecological plasticity. However, cryptic diversity has recently been observed within this taxon and this genetic diversity could extend to phenotypic differences between allopatric populations. Monoclonal strains of *C. socialis* isolated from NE Atlantic/Arctic and Tyrrhenian Sea waters were cultivated at 2.5, 8 and 13°C, and analysed for morphological, phylogenetic (LSU rRNA gene) and physiological (growth rate, photosynthetic yield) traits. The molecular analysis indicated an unequivocal divergence between the southern and the northern strains. Clear differences in spore morphology between the two groups were also observed, despite their morphological similarity in vegetative form. The physiological data demonstrated a functional partition between the northern and southern strains. The northern strains showed significantly higher growth rates than the southern ones at the lower temperatures, and vice versa at 13°C. Differences between the groups were also observed in the photosynthetic yields. These results reveal the expression of allopatric speciation in several phenotypic characters, providing a functional dimension to the cryptic, or rather pseudo-cryptic, diversity observed within *C. socialis*. We believe that such an integrated approach is useful for robust taxonomic and functional delimitations of presumed cosmopolitan microorganisms.

KEYWORDS: functional diversity; cryptic diversity; cosmopolitan; species concept; diatoms

INTRODUCTION

Cosmopolitan marine phytoplankton species occurring over wide geographical ranges often show considerable ecological plasticity (Smayda, 1958; Hasle, 1976). Random dispersal of abundant species in concert with low speciation rates may also contribute to the seemingly ubiquitous distribution of many species (e.g. Finlay, 2002; Finlay *et al.*, 2004). The identification of a species and its geographical distribution is, however, tightly linked to the applied species concept and the criteria used for delineating the species in question. Evolution is assumed to be continuous and not all characters will reflect the on-going differentiation processes at a similar pace (de Queiroz, 2007). Different sets of criteria will therefore yield different species delineations and the taxonomic concepts in use are highly variable (e.g.

doi:10.1093/plankt/fbs004, available online at www.plankt.oxfordjournals.org. Advance Access publication February 20, 2012

Mann, 1999). It appears that intraspecific genetic variability is much higher than formerly assumed for marine diatoms (Kooistra *et al.*, 2007) and other phytoplankton groups (Montresor *et al.*, 2003; Sàez *et al.*, 2003), even among populations living in sympatry (Rynearson and Armbrust, 2004; Amato *et al.*, 2007). The emergence of a phylogenetic species concept has revealed unexpected diversity (e.g. Sarno *et al.*, 2005, 2007), with consequences also for our knowledge of species distribution and biogeography (Kooistra *et al.*, 2008). The disclosure of cryptic diversity, i.e. genetic differentiation contrasted with morphological similarity, in several phytoplankton genera thus challenges the validity of the traditional morphological species concept as well as the existence of ubiquitous (or cosmopolitan) species.

The ecological plasticity observed in so-called morphospecies may turn out to be physiological diversity expressed by a collection of phylogenetically distinct phytoplankton species (e.g. Balzano *et al.*, 2011). Though useful for species delimitation, phylogenetic data, as such, do not reflect the phenotypic properties of the organism. Sometimes, functional approaches, such as metabolic or trancriptomic profiling studies, can elucidate phenotypic variability for the same species in different environments, not evident in genomic sequence data (Rosselló-Mora *et al.*, 2008; Maumus *et al.*, 2009). Functional diversity arises as a consequence of genetic variability within a taxon, environmental effects on gene expression, and ecological interactions among taxa (Zak *et al.*, 1994). However, the degree to which genetic diversity is translated into taxonomic diversity is not fully understood, and even less is known about the manner in which genetic diversity affects functional diversity or ecosystem adaptability. Integrated approaches can therefore be useful when exploring the extent of phenotypic variability and allopatric differentiation expressed in so-called cosmopolitan species.

Chaetoceros socialis Lauder is a marine planktonic diatom commonly found from the cold waters of the Arctic to the much warmer waters of the Mediterranean Sea or the Gulf of California (Hasle and Syvertsen, 1997). The species was originally described by Lauder (Lauder, 1864) on material from waters close to Hong Kong (South China Sea). Further information on the smooth spores formed inside vegetative cells was provided by Cleve (Cleve, 1896) and Gran (Gran, 1897). *Chaetoceros radians* Schütt was formerly regarded as a separate species and was distinguished from *C. socialis* merely on the basis of its spiny resting spores. *Chaetoceros radians* was later reduced in rank to a form of *C. socialis* by Proschkina-Lavrenko (Proschkina-Lavrenko, 1953), who judged the separation of the two taxa not supported by the variability in spore morphology. Cryptic and pseudo-cryptic diversity within the genus *Chaetoceros* was recently described by Kooistra *et al.* (Kooistra *et al.*, 2010), who also found indications of cryptic diversity in *C. socialis* between strains from the Gulf of Naples (Tyrrhenian Sea) and a strain isolated from the Strait of Georgia (North Pacific Ocean). The wide ecological plasticity expressed by this species may therefore be the consequence of intraspecific variability in allopatric populations, so far confirmed only for genetic data, i.e. the large-subunit ribosomal RNA (LSU rRNA) gene. Information on the ecology of *C. socialis* is available from numerous field surveys (e.g. Bode and Varela, 1998; Booth *et al.*, 2002; Rines *et al.*, 2002; Tomaru *et al.*, 2009), while some experimental studies have been performed on strains from different geographical areas, e.g. Tokyo Bay (Kudo, 2003), Sea of Japan (Shevchenko *et al.*, 2008), Tyrrhenian Sea (Dimier *et al.*, 2007) and the Arctic Ocean (Leu *et al.*, 2007). To increase the value of such ecological and physiological studies, we need knowledge of whether the genetic variation in *C. socialis* is reflected in phenotypic diversity. Do we see differentiation in phenotypic characters other than morphological ones? And, most important, does the genetic differentiation observed for cryptic species have any measurable functional dimension?

The present study addresses the phenotypic and genotypic variability of cryptic species of *C. socialis*. By examining monoclonal strains of *C. socialis* from the northern and southern sections of its distribution area, we provide a confirmation of the molecular and morphological diversity indicated in previous studies. A comprehensive view of the diversity is given using an integrated approach that combines phylogenetic, morphometric and physiological tools.

METHOD

Strain cultivation

The study was performed with monoclonal strains of *C. socialis* isolated from NE Atlantic/Arctic and Mediterranean (Gulf of Naples, Tyrrhenian Sea) waters, hereafter referred to as 'northern' and 'southern' strains (Table I). The southern strains (SZN-B382–SZN-B475) were all isolated from seawater samples, whereas all but two (AMB-66, AMB-80) of the northern strains derived from the germination of spores contained in sediment samples. The northern strains were isolated in the spring, as this is the time when *C. socialis* is particularly abundant in this region (Eilertsen *et al.*, 1981; Degerlund and Eilertsen, 2010). In the Gulf of Naples,

Table I: Information on strains of C. socialis *used in Experiments I (8°C), II (2.5°C) and III (13°C)*

Species	Strain code	Collection site[a]	Position	Isolation	Exp.	Accession no.
Chaetoceros socialis Lauder	AMB-33.2	Fin		25.1.2007	III	HE573571
	AMB-41	Fin		25.1.2007	I, II	HE573572
	AMB-66	BaS	77.8°N, 12.3°E	27.6.2007	I	HE573573
	AMB-80	BaS	77.8°N, 12.3°E	27.6.2007	II, III	HE573574
	AMB-92	Kra	69.4°N, 19.0°E	26.2.2009	I, III	HE573575
	AMB-93	Mal	69.4°N, 18.4°E	24.2.2009	I	HE573576
	AMB-94	Mal	69.4°N, 18.4°E	24.2.2009	II	HE573577
	AMB-95[b]	Mal	69.4°N, 18.4°E	24.2.2009		HE573578
	AMB-96	Mal	69.4°N, 18.4°E	24.2.2009	III	HE573579
	AMB-97	Kra	69.4°N, 19.0°E	26.2.2009	II	HE573580
	SZN-B382	GoN	40.8°N, 14.3°E	23.9.2008	II, III	HE573581
	SZN-B386	GoN	40.8°N, 14.3°E	14.10.2008	I, III	HE573582
	SZN-B388	GoN	40.8°N, 14.3°E	21.10.2008	I	HE573583
	SZN-B390	GoN	40.8°N, 14.3°E	6.11.2008	I, II, III	HE573584
	SZN-B392	GoN	40.8°N, 14.3°E	12.11.2008	I, III	HE573585
	SZN-B459	GoN	40.8°N, 14.3°E	6.5.2008	I, II, III	HE573586
	SZN-B460	GoN	40.8°N, 14.3°E	6.5.2008	I, III	
	SZN-B470	GoN	40.8°N, 14.3°E	29.4.2008	I, III	HE573587
	SZN-B473	GoN	40.8°N, 14.3°E	29.4.2008	I	HE573588
	SZN-B475	GoN	40.8°N, 14.3°E	29.4.2008	II, III	HE573589
	SZN-B423[c]	GoN	40.8°N, 14.3°E			EF423468
C. costatus Pavillard	DH18	GoN	40.8°N, 14.3°E			EF423473
	NAOS17	GoP				EF423474
	SZN-B404	GoN	40.8°N, 14.3°E			GU911463
C. compressus Lauder	SZN-B402[d]	GoN	40.8°N, 14.3°E			GU911462
	DH22[d]	GoN	40.8°N, 14.3°E			EF423429
C. debilis Cleve	CCMP1578	Nar	41.6°N, 71.4°W			EF423430
	Hel2	Hel				EF423482
	SEED-2	Pac	48.5°N, 165°E			EF423483
C. brevis Schütt	CCMP163	SO	61.3°N, 54.7°W			EF423469
Bacteriastrum hyalinum Lauder	DH13	GoN	40.8°N, 14.3°E			EF423442

Other Chaetocerotaceae species listed were included in the phylogenetic analysis.
[a]Collection sites: Fin, Finnmark coast, NE Atlantic; BaS, NW Barents Sea; Kra, Krabbenes, Troms coast, NE Atlantic; Mal, Malangen fjord, Troms coast, NE Atlantic; GoN, long-term ecological research station MareChiara in the Gulf of Naples, Tyrrhenian Sea; GoP, Gulf of Panama; Nar, Narragansett Bay; Hel, Helgoland; Pac, Pacific Ocean, NW; SO, Southern Ocean.
[b]AMB95 was not used in the experiments, but was sequenced as an additional northern strain.
[c]B423 is genetically identical to the other southern strains and was only used for morphological analyses and pictures.
[d]SZN-B402 and DH22 are referred to as *C. compressus* in GenBank, but listed with the synonym name *C. contortus* in the reference publication of Kooistra *et al.* (Kooistra *et al.*, 2010). According to Guiry (Guiry, 2011) in the World Register of Marine Species, the synonym *C. compressus* is the accepted version per today.

C. socialis occurs over multiple seasons (Ribera D'Alcalà *et al.*, 2004; Zingone *et al.*, 2010), and therefore, strains from the spring as well as the autumn were included (Table I). The monoclonal cultures of each strain were started from single cells or colonies transferred by micropipettes from cultivation plate chambers to plastic 40 ml Nunc cell cultivation flasks or 20 ml Duran glass tubes under an inverted microscope. The monoclonal cultures were cultivated in pasteurized f/2 or f/10 growth medium (Guillard and Ryther, 1962) with additional silicate [12.3 μmol $Si(OH)_4$ L^{-1}] in temperature- and irradiance-controlled rooms. Cultivation conditions for the stock cultures aimed at simulating ambient environmental conditions of their respective geographical regions, starting out with low temperatures (2–6°C) and long photoperiods (14 h) for the northern strains, and higher temperatures (18–23°C) and shorter photoperiods (12 h) for the southern strains. Light was provided by Osram L 58W/954 Daylight tubes at a scalar irradiance of ca. 20 μmol quanta m^{-2} s^{-1} (QSL-100 scalar irradiance meter, Biospherical Instruments Inc.).

Experimental set-up

A strain was considered unique when isolated from different locations or at variable dates from the same location (Table I). In order to include as many strains as possible in each experiment, several clones of each unique strain were maintained throughout the adaptation process. Clones originating from the same water sample (e.g. AMB-66 and AMB-80, Table I) were treated as identical, and during set-up of experiments,

each unique strain was generally represented by one clone only (Table I).

Three series of experiments were run on altogether 19 strains of *C. socialis* at three different temperatures: 2.5, 8 and 13°C (Table I). Cultivation temperatures were chosen according to normal ranges in each geographical area, with the aim that both groups of strains would also be exposed to temperatures outside their ordinary range. Annual sea surface temperatures range between 12.4 and 28.5°C in the Gulf of Naples (Zingone *et al.*, 2010), whereas NE Atlantic/Barents Sea waters vary between −1.8 and +9.4°C (Eilertsen and Skarðhamar, 2006; Degerlund and Eilertsen, 2010). Prior to each experiment, strains were gradually adapted to the desired cultivation temperature and kept there for at least 2 weeks before start-up. We were unable to adapt the northern strains to temperatures higher than 13°C, while few of the southern strains were able to grow at 2.5°C, and the limits for the temperatures were chosen accordingly. It should be pointed out that 13°C is indeed in the lower extreme of the normal temperature range of the southern strains, as *C. socialis* experiences far higher temperatures during summer and autumn in the Mediterranean Sea (Ribera D'Alcalà *et al.*, 2004). However, *C. socialis* is frequently abundant during late winter and early spring when water temperatures in the Gulf of Naples are low (Zingone *et al.*, 2010). Strains were cultivated in 600 ml Nunc cultivation flasks in f/10 nutrient-enriched medium, at scalar irradiance 30 μmol quanta m^{-2} s^{-1} and 12:12 (Exp. III) or 14:10 (Exp. I and II) L:D photoperiods. All other factors considered to affect phytoplankton growth (e.g. nutrients, irradiance) remained the same. In all three experiments, each strain was cultivated in three replicate units distributed randomly in front of the light source. From start concentrations of ca. 1 μg chlorophyll *a* L^{-1}, each experimental run was continued until the strain had reached a minimum of a 30-fold increase in biomass, measured as chlorophyll *a* concentration. All strains were in exponential growth phase when cultivation was terminated and final chlorophyll *a* concentrations were in the range 30−40 μg L^{-1} (Exp. I and II) or 100 μg L^{-1} (Exp. III). During the entire experimental period, the cultivation units were stirred and randomized daily. Samples for morphological, physiological and molecular analyses were taken at start and/or stop of each run. Physiological sampling for monitoring purposes (PAM fluorescence and chlorophyll *a* filtrations) was conducted regularly, i.e. weekly or more often, during the experiments.

Morphology

Samples for morphological analysis were collected in 100 ml glass bottles after each experimental run and preserved with hexamine-buffered formaldehyde to a final concentration of 4%. Observations of general morphological characters and morphometric measurements were performed in Tromsø with inverted light microscopes (LM) (Leica Leitz DM IL or Leica DMIL, 400×) or in compound microscopes (Leica DM 2500, 1000×) equipped with phase contrast and bright field optics. LM photographs were taken with a Leica DFC 320 microscope camera, controlled with Leica firecam v 3.0 software (Leica Microsystems Ltd.). In Naples, LM observations were made with a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany) equipped with Nomarski differential interference contrast, phase contrast and bright field optics. Micrographs were obtained with a Zeiss Axiocam digital camera. For electron microscopy observations (EM), cultures were treated with acids (1:1:4, sample: HNO_3:H_2SO_4), boiled for a few seconds and then washed with distilled water, in order to remove organic matter. For scanning electron microscopy (SEM), the acid-cleaned material was mounted on stubs, sputter-coated with gold or platinum and examined using a SEM JEOL JSM-6500F (JEOL-USA Inc., Peabody, MA, USA). For transmission electron microscopy (TEM), the same material was mounted on Formvar-coated grids and observed using a TEM LEO 912AB. Spores were observed from two northern strains used in the experiments (strain AMB-33.2 and AMB-94) and from a strain genetically identical to the southern material (strain SZN-B423, Table I). The morphology of the spores was described following the terminology of Ishii *et al.* (Ishii *et al.*, 2011).

In addition to species-specific characteristics, morphological observations included the number of cells per colony and the percentage of empty frustules and spores in each strain. Morphometric data such as length of apical axis (cell valve diameter or AA), pervalvar axis (cell valve length or PA), transapical axis (shorter diameter of the elliptic cell valve) and apertures (space between two cells in a colony) were only obtained from the first experimental run at 8°C. The number of cells measured was ≥90 per strain. The biovolume of the measured cells was calculated according to Olenina *et al.* (Olenina *et al.*, 2006), with the correction for a transapical axis considerably larger than half of the apical axis, i.e. estimated to ca. 3/4 of AA in our *C. socialis* strains (Fig. 1C and J; Table II). Carbon content was calculated using the formula recommended for diatoms by Menden-Deuer and Lessard (Menden-Deuer and Lessard, 2000).

Phylogenetic analysis

Cells from at least 150 ml dense culture in exponential growth were collected for molecular analysis by filtration

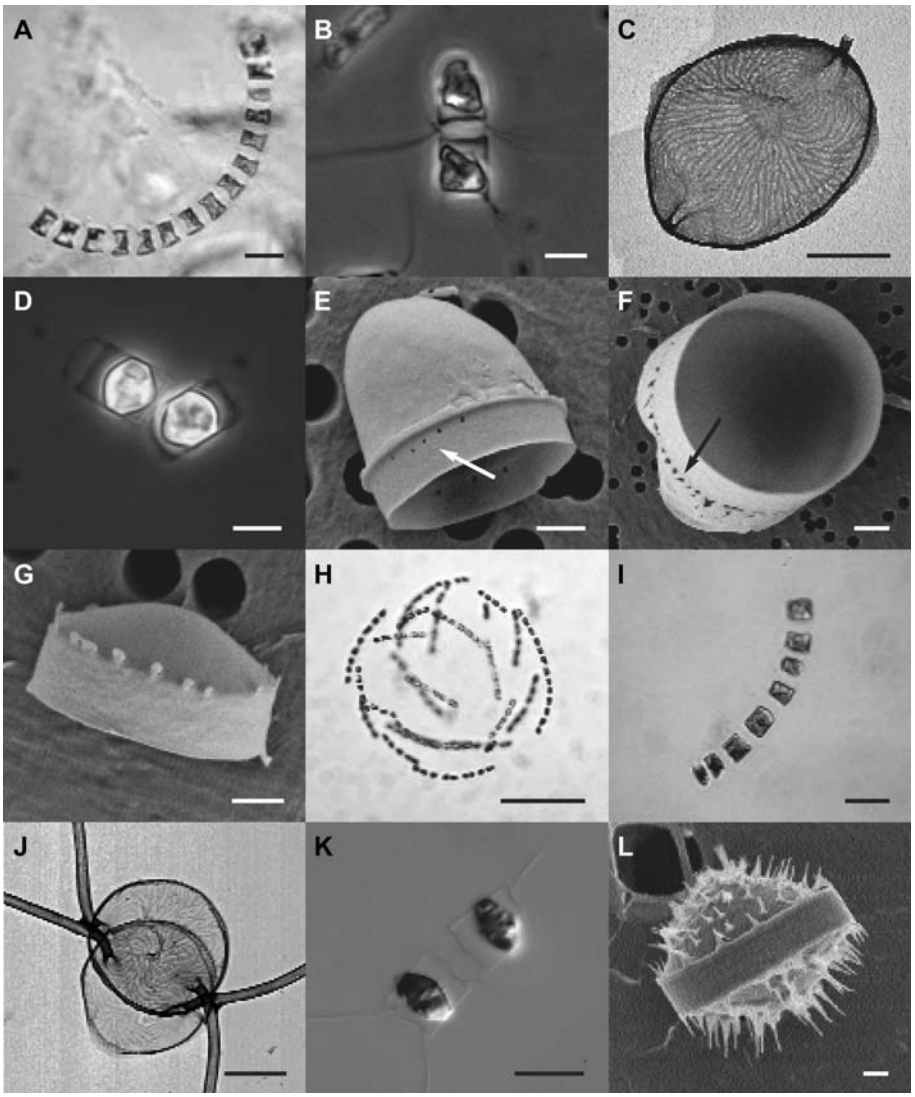

Fig. 1. Morphology in LM, TEM and SEM of northern (**A**–**G**) and southern (**H**–**L**) material of *C. socialis*. (A) Curved chain in broad girdle view, strain AMB-93, LM. Scale bar: 10 μm. (B) Two cells in colony, strain AMB-94, LM. Scale bar: 5 μm. (C) Valve with broken setae, strain AMB-33.2, TEM. Scale bar: 2 μm. (D) Two spores inside vegetative cell frustules, strain AMB-94, LM. Scale bar: 5 μm. (E): Secondary valve of a spore, strain AMB-33.2, SEM. Note the row of puncta (arrow) on the advalvar margin of the mantle. Scale bar: 1 μm. (F) Primary valve of a spore in the internal view, strain AMB-33.2, SEM. Note the sheath (collar) perforated by fissures (arrow). Scale bar: 1 μm. (G) Primary valve of a spore with marginal knobs, strain AMB-33.2, SEM. Scale bar: 1 μm. (H) A spherical colony from a spring strain during the isolation process in Naples, not named at the time, LM. Scale bar: 100 μm. (I) Curved chain in broad girdle view, strain SZN-B460, LM. Scale bar: 10 μm. (J) Two valves, natural material from the Gulf of Naples, TEM. Scale bar: 2 μm. (K) Two cells containing spores, strain SZN-B423, LM. Scale bar: 10 μm. (L) Complete spiny spore, strain SZN-B423, SEM. Scale bar: 2 μm.

onto 1.0 μm pore-size polycarbonate membrane filters (Nucleopore®) supported by GF/C filters pre-heated to 450°C for 8 h. Filters were frozen in liquid N_2 and stored at −78°C. Total DNA was extracted from the filters using a DNeasy® Blood and Tissue Extraction kit (Qiagen, Hilden, Germany) which has proven efficient for extraction of DNA from algal material (Simonelli *et al.*, 2009). The hypervariable D1–D4 region of the nuclear LSU rRNA gene was amplified with the forward primer D1R (5′-ACCC GCTGAATTTAAGCATA-3′) and the reverse primer D3Ca (5′-ACGAACGATTTGC ACGTCAG-3′) (Leaners *et al.*, 1989; Scholin *et al.*, 1994). The polymerase chain reaction (PCR) was performed with an Applied Biosystems 2720 Thermal Cycler using a ReddyMix PCR Master Mix (Thermo Fisher Scientific, Epson, Surrey, UK) with an additional 1.5 mM $MgCl_2$. Thirty-five cycles (45 s at 94°C, 90 s at 55°C, 90 s at 72°C) were run, with an initial step of

Table II: Descriptive and non-parametric statistics for morphological data on northern (N) and southern (S) strains of C. socialis *cultivated at 8°C*

		N	S	*P* (N *vs.* S)
Apical axis (μm)	Min–max	1–10	2–10	
	Median; quartile range	3.75; 2.5	3.0; 1.75	<0.001
	Mean ± SD	4.22 ± 1.79	3.21 ± 1.41	
	n	694	1028	
Pervalvar axis (μm)	Min–max	3.75–15	3.75–19	
	Median; quartile range	7.50; 3.0	8.0; 3.0	<0.001
	Mean ± SD	7.13 ± 2.14	7.94 ± 2.08	
	n	688	1024	
Transapical axis (μm)	Min–max	2.5–8	2–7	
	Median; quartile range	4.0; 1.6	4.0; 2.0	0.016[a]
	Mean ± SD	4.82 ± 1.42	3.91 ± 1.06	
	n	24	79	
Apertures (μm)	Min–max	1–5	1–6.25	
	Median; quartile range	2.50; 0.5	3.0; 1.75	<0.001
	Mean ± SD	2.5 ± 0.8	2.90 ± 1.0	
	n	559	384	
Cells per colony[b]	Min–max	2–13	2–10	
	Median; quartile range	2.0; 1.0	2.0; 1.0	<0.001
	Mean ± SD	2.93 ± 1.48	2.51 ± 0.89	
	n	2133	2885	
Biovolume (μm^3)	Min–max	4.71–442	9.4–451	
	Median; quartile range	62.1; 78.2	37.1; 34.8	<0.001
	Mean ± SD	79.4 ± 62.7	54.2 ± 57.7	
	n	688	1024	
Carbon content (pg $cell^{-1}$)	Min–max	1.01–40.2	1.78–40.9	
	Median; quartile range	8.20; 8.26	5.40; 4.05	<0.001
	Mean ± SD	9.60 ± 6.06	6.96 ± 5.47	
	n	688	1024	

[a]*P*-value from the Kruskal–Wallis test on data from three groups: northern strains, southern autumn, and southern spring strains, where the differences in transapical axis size were significant only between northern and southern autumn strains.
[b]Data on cells per colony are from all three experiments.

180 s at 94°C and a final one of 420 s at 72°C, after which the samples were kept at 4°C. Correctly sized PCR products were confirmed by agarose gel electrophoresis (1% Sea Kem LE Agarose gel with ethidium bromide as stain) and purified by the QIAquick® PCR purification kit (Qiagen). The amplified partial genes were sequenced from both ends by employing the same primers as in the PCR reaction. The sequencing reaction was performed according to the BigDye® v3.1 Sequencing Protocol (PE Biosystems, Foster City, CA, USA), followed by a fragment separation on an Applied Biosystems 3130 × l Genetic Analyzer.

End-trimmed and quality-checked partial LSU rRNA gene sequences obtained in the present study were uploaded to the European Nucleotide Archive (ENA; www.ebi.ac.uk/ena) with accession numbers HE573571–HE573589 (Table I). A sequence alignment including the 19 sequences from this study, 3 from *C. socialis* and 10 from related (Chaetocerotaceae) species, downloaded from GenBank (www.ncbi.nlm.nih.gov), were aligned by the Clustal W algorithm (Higgins *et al.*, 1994), with default settings as implemented in the MEGA 5.0 program package (Tamura *et al.*, 2011). Phylogenetic inferences, based on 671 unambiguously aligned positions, gaps not included, were obtained by employing the Bayesian analysis program MrBayes 3.1 (Ronquist and Huelsenbeck, 2003) and by the MEGA 5.0 implementation of maximum likelihood (ML) analysis. The Bayesian analysis was done under the presumption of a general time-reversible model with invariable sites and gamma distribution of rates across sites (GTR + I + G). Otherwise, default single-nucleotide prior settings were employed. Two independently seeded chains, 1 000 000 generations each, were run to secure average standard deviation of split frequencies <0.01. In the ML analysis, the Tamura-Nei substitution model, gamma rate distribution across sites + invariant sites was implemented. Bootstrap values were calculated from 1000 re-samplings.

Physiology

Growth rates [μ or doublings $day^{-1} = \log_2 (t_2/t_1)/t$] of the strains were calculated on the basis of both cell abundance and chlorophyll *a* concentrations which were measured at the start (t_1) and end (t_2) of each experiment. The duration of experiments (t) varied in the range of 9–27 days for all the experiments, since the

purpose was to reach a certain increase in biomass level in every cultivation unit. Cell abundance was assessed on 5 ml samples preserved with acid Lugol's solution (250 μL sample mL^{-1}) and kept refrigerated until analysis. Cell counts were performed with inverted microscopes on 2 ml Nunclon 4-well cell cultivation chambers, allowing samples to settle for at least 2 h prior to the analysis. At least 100 cells per sample were counted. Chlorophyll *a* concentrations ($\mu g\ L^{-1}$) were measured from algal material filtrated on GF/C filters and extracted in methanol for 24 h (Holm-Hansen and Riemann, 1978), using a Turner Designs TD-700 fluorometer (Exp. II and III) or a Turner Designs 10-AU fluorometer (Exp. I). Filters from Experiment II were kept frozen (−20°C) for 2 months prior to extraction and subsequent analysis. Photosynthetic efficiency or maximum quantum yield in Photo System II, Φ_{PSII}, was measured using a Water-PAM (Pulse Amplitude Modulated) fluorometer (Water-ED/B, Heinz Waltz GmbH). Three minutes of dark adaptation (5 μs measuring light pulses applied at a frequency of 18 Hz) was applied and a saturation light pulse peaking at 660 nm was modulated at a frequency of 20 kHz. Φ_{PSII} was calculated as $\Phi_{PSII} = (F_m - F_0)/F_m$, where F_m is the maximum fluorescence from the saturating light pulse in dark-acclimated cells and F_0 the initial fluorescence (from the measuring light) in dark-acclimated cells.

Statistical analysis

Descriptive statistics and the Shapiro–Wilk *W*-tests for normality were performed on all morphometric and physiological data in the STATISTICA 7.1 software package. Non-parametric data were compared using the Kruskal–Wallis analysis of variance (ANOVA) for data from multiple independent groups (northern, southern autumn and southern spring strains). As autumn and spring strains were indivisible, i.e. multiple comparisons of *P*-values = 1.00, these two subgroups were merged into one and compared with the northern group by a Mann–Whitney *U*-test on data from two independent groups (northern and southern). Non-parametric tests were run both on raw data and on mean values of the three replicate units. Within-group or intra-strain variability was also tested with the Kruskal–Wallis ANOVA.

RESULTS

Morphological and morphometric traits

No differentiation in vegetative morphology was apparent between the northern and southern strains of *C. socialis* (Fig. 1). In both groups of strains, vegetative cells have one large chloroplast and one straight seta, longer than the other three, converging to a common point on one side of the colony, thus forming chains curved in broad girdle view (Fig. 1A and I). The colonial apertures are hexagonal (Fig. 1B and K), as the setae emerge internally from the cell corners and merge with those of the sibling cell beyond the lateral margin of the colony. Under TEM, valves show a pattern of delicate costae which do not differ significantly between the two groups of strains (Fig. 1C and J). In natural samples and early cultivation phases, spherical, second-order colonies are at times present, with several different first-order colonies connected in the centre of the colony through the tips of the long setae (Fig. 1H).

However, differentiation in spore morphology was observed between the two geographical groups (Fig. 1). In both northern and southern strains, spores are highly silicified and heterovalvate, with dome-shaped valves of different curvature (Fig. 1D–G, K and L). On the advalvar margin of the secondary valve mantle, a single row of puncta is present (Fig. 1E, not shown for southern strains), which is covered by the mantle of the primary valve. In northern strains, spore valves are deprived of spines (Fig. 1D–G) and can bear a silica sheath (collar). The sheath can be perforated by scattered holes or fissures (Fig. 1F), or can be notably reduced or absent within the same strain, at times being replaced by crests (not shown) or knobs (Fig. 1G). In southern strains, both spore valves lack a sheath and are covered with spines over the whole surface (Fig. 1L), with palisade spines often present at the margin. Spines are hardly visible in LM (Fig 1K) and can vary in length also between the valves of the same spore.

When analysed statistically, morphometric data from northern and southern strains used in the experiment at 8°C showed significant differences in the size of the cell valves and the distance between colonial cells (apertures) (Table II). In the southern group of strains, the apical axis was smaller, whereas the pervalvar axis and the apertures were larger (Table II; $P < 0.001$). The transapical axis also differed in size, but significantly only between the northern strains and the southern autumn (SZN-B386–B392) strains (Table II; $P = 0.016$). Numbers of cells per colony were statistically different for the two groups in all three experiments; the northern strains on average having 0.4 more cells per chain than the southern ones ($P < 0.001$). Percentages of empty frustules and spores were generally low in all three experiments (not shown). Morphometric differences were reflected in the calculated biovolume and carbon content (from experiment at 8°C) of the cells, the southern strains being slightly

smaller in volume and hence with smaller amounts of carbon per cell than the northern ones (Table II, $P < 0.001$).

Phylogenetic position

Phylogenetic inferences based on the partial LSU rRNA gene by the Bayesian (Fig. 2) and ML approaches produced identical tree topologies, with similar branch lengths and levels of support for the individual bipartitions. The same tree topology was also confirmed with the neighbour-joining algorithm (not shown). The analyses showed an unambiguous divergence between the southern and the northern strains of *C. socialis* by placing the geographically distinct groups of the species into confidently separated clades. On the other hand, the *C. socialis* strains jointly constituted a statistically robust monophyletic group relative to the other Chaetocerotaceae species included in the analysis (Fig. 2). The estimated mean pairwise evolutionary distance between the two *C. socialis* clades was 0.046 (corresponding to 29 different nucleotide positions, on average, in the 671 bp alignment), while the corresponding mean distance between all *C. socialis* strains and the representatives of their closest relative, *C. costatus* Pavillard, was 0.096 (60 different nucleotides, on average). The strain CCMP172 [GenBank, accession no. EF423466 submitted as a representative of *C. debilis*, but identified as *C. socialis* by Kooistra *et al.*, 2010, and correctly named in the CCMP collection] proved a sister taxon to the NE Atlantic/Arctic clade of strains. The LSU rRNA sequence data of the strains from the Gulf of Naples did not show differences between the spring (SZN-B459–B475) and autumn (SZN-B382–B392) isolates, and neither did sequence data on the hypervariable ITS gene region analysed for these two seasonal groups in Naples (A.Z., unpublished data).

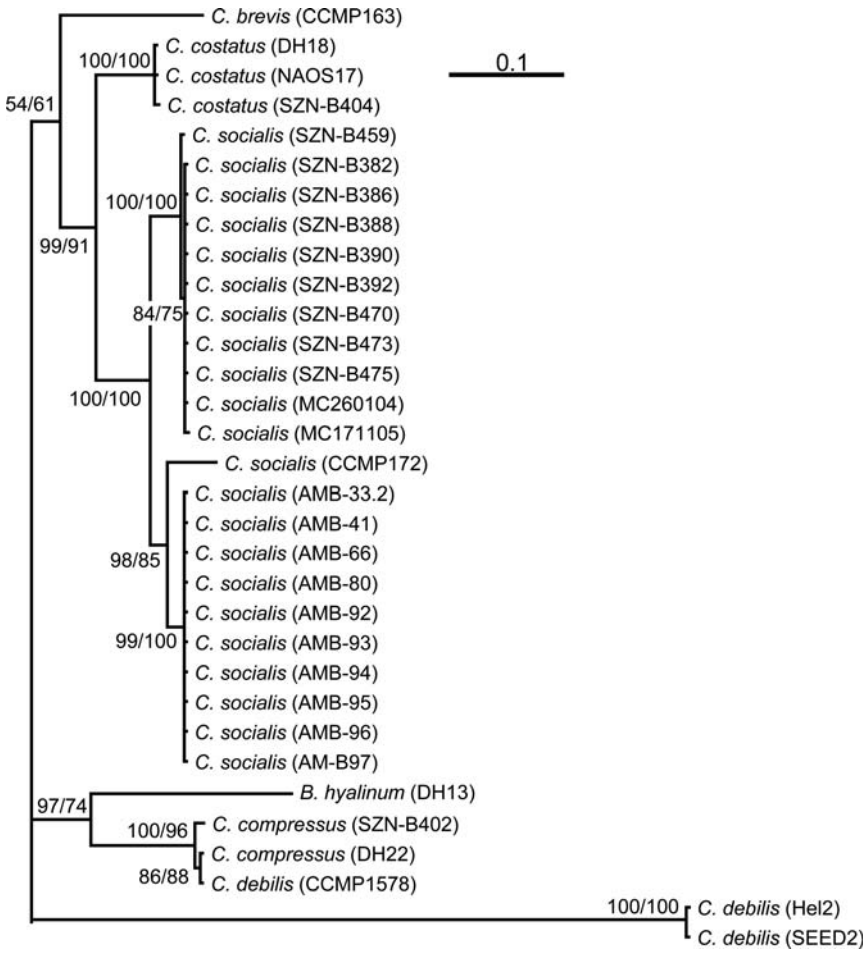

Fig. 2. Bayesian consensus tree, based on 671 unambiguously aligned positions of the D1–D4 region of the LSU rRNA gene. Partitions with posterior probabilities <50% are kept unresolved. Internal labels show posterior probabilities (%) ahead of slash (/) and % bootstrap values for ML tree of identical topology behind slash.

Growth rate and photosynthetic efficiency

Growth rates (as doublings day^{-1}, based on cell abundance and chlorophyll *a*) were significantly different between northern and southern strains of *C. socialis* at all three cultivation temperatures (Fig. 3, Table III). The northern strains showed higher growth at the two lowest temperatures ($P < 0.001$), while the southern strains had higher growth rates at 13°C ($P < 0.001$; Fig. 3, Table III). There was no significant difference in growth rate between the spring (SZN-B459–B475) and autumn (SZN-B382–B392) strains from the Gulf of Naples (not shown). Within the southern group of strains, a maximum for chlorophyll *a*-based growth rate was observed at 13°C, while growth rates at the two lower temperatures were significantly lower ($P \leq 0.01$). The growth rates of the northern strains did not show significant differences among temperatures, being only slightly higher at 8 than at 2.5 and 13°C when calculated from cell abundance values. Differences in growth rates calculated on the basis of abundance and chlorophyll *a* measurements were largely related to the higher reproducibility of the latter (not shown) and to the difference in the nature of these two parameters (abundance *vs.* biomass proxies). Chlorophyll-based growth rates could also be affected by the change in chlorophyll content per cell during the time period of growth rate measurement. This change was significant in the southern group of strains at 8°C.

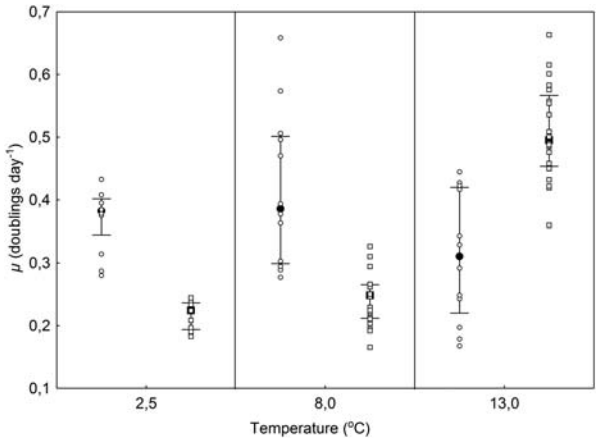

Fig. 3. Growth rate (doublings day^{-1}, abundance based) of *C. socialis* cultivated at 2.5, 8, and 13°C. Medians displayed with filled circles (northern strains) and squares (southern strains). Raw data are displayed with open circles (northern strains) and open squares (southern strains). Whiskers display 25th and 75th percentiles.

The differences observed between groups of strains were also observed in the PAM-estimated maximum quantum yields measured at the start and stop of experiments (Fig. 4, Table III). At 8°C, Φ_{PSII} was higher in the southern strains, even though growth was slower compared with the northern strains at this temperature (Table III). Maximum quantum yields for the northern strains were at their highest at 2.5°C, whereas the southern strains had maximum yields at 13°C. Results at 8°C were intermediate for both groups. Physiological differences between the two groups were statistically significant ($P < 0.05$) also when tested on mean values of the three replicate cultivation units, i.e. considering the intra-strain variability before comparing the geographical groups (not shown). Cultivation unit 94C (Exp. II at 2.5°C) was identified as an outlier on the basis of the physiological monitoring performed

Table III: Descriptive and non-parametric statistics for physiological data on northern (N) and southern (S) strains of C. socialis *cultivated at 2.5, 8 and 13°C*

T (°C)	2.5			8			13		
Group	N	S	*P*-value	N	S	*P*-value	N	S	*P*-value
μ_abund									
Median	0.382	0.224	0.000032	0.386	0.248	0.000027	0.310	0.495	0.000008
Mean ± SD	0.38 ± 0.06	0.22 ± 0.02		0.42 ± 0.12	0.25 ± 0.05		0.31 ± 0.10	0.51 ± 0.07	
n	12	12		12	24		12	24	
μ_chl									
Median	0.543	0.272	0.000532	0.502	0.413	0.000223	0.489	0.709	0.000005
Mean ± SD	0.53 ± 0.10	0.274 ± 0.01		0.49 ± 0.06	0.41 ± 0.03		0.47 ± 0.13	0.70 ± 0.05	
n	12	12		12	24		12	24	
Φ_{PSII_start}									
Median	0.621	0.597	0.009375	0.618	0.708	0.000019	0.598	0.726	0.000001
Mean ± SD	0.62 ± 0.02	0.59 ± 0.03		0.62 ± 0.05	0.70 ± 0.03		0.60 ± 0.03	0.72 ± 0.03	
n	12	12		12	24		12	24	
Φ_{PSII_stop}									
Median	0.677	0.626	0.000123	0.675	0.714	0.000001	0.641	0.737	0.000001
Mean ± SD	0.67 ± 0.02	0.63 ± 0.01		0.67 ± 0.01	0.72 ± 0.01		0.64 ± 0.02	0.74 ± 0.01	
n	12	12		12	24		12	24	

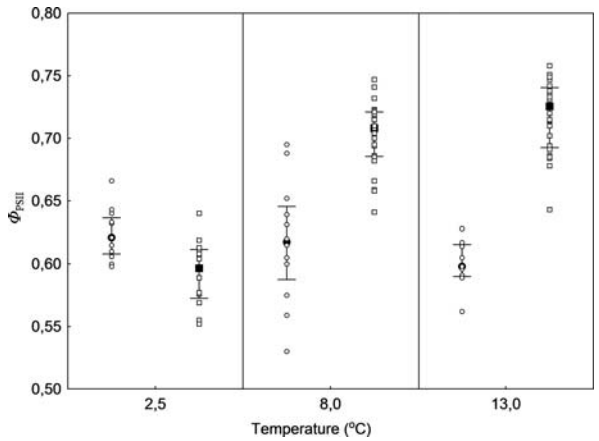

Fig. 4. Photosynthetic efficiency or maximum PAM estimated quantum yield (Φ_{PSII}) of *C. socialis* measured at the start of experiments run at 2.5, 8, and 13°C. Medians displayed with filled circles (northern strains) and squares (southern strains). Raw data are displayed with open circles (northern strains) and open squares (southern strains). Whiskers display 25th and 75th percentiles.

during the experiment (not included in calculations of intra-strain mean values, but included in the statistical analyses on all units).

DISCUSSION

Cryptic diversity in *C. socialis*

The northern (NE Atlantic/Arctic) and the southern (Tyrrhenian Sea) strains of *C. socialis* used in the present study formed two separate phylogenetic groups in the analysis of partial sequences of the LSU rRNA gene. This result consolidates previous indications of cryptic diversity in *C. socialis* (Kooistra *et al.*, 2010) based on the phylogenetic difference between *C. socialis* strain CCMP172 from the Strait of Georgia (North Pacific Ocean) and strains collected in the Gulf of Naples. In addition, the present phylogenetic analysis affiliated strain CCMP172 to our northern group of strains, indicating that the *C. socialis* strain from the North Pacific is more closely related to the NE Atlantic and Arctic strains than to the southern strains of *C. socialis*. Only three additional partial LSU rRNA gene sequences of *C. socialis* were available in public databases, restricting the possibility to compare sequence data from diverse geographical regions. An extensive comparison across the whole distribution range of *C. socialis* would be necessary for a proper evaluation of the phylogeographic status of the species. Such a comparison should preferentially also include the Internal Transcribed Spacer 2 and the 5.8S regions of the rRNA gene complex. Moniz and Kaczmarska (Moniz and Kaczmarska, 2010) have provided strong arguments for the ITS-2 region (combined with the 5.8S gene) as the preferred candidate for DNA barcoding of diatoms. Furthermore, Coleman (Coleman, 2009) has demonstrated the presence of compensatory base substitutions in the highly conserved secondary structure of helix III of the ITS-2 domain as an unequivocal indicator of reproductive isolation in a wide range of Eukarya.

Though appearing similar in vegetative morphology, *C. socialis* strains from the two geographical regions showed clear differences in spore morphology when examined with LM and SEM. These differences were consistent with observations on field samples from both regions collected over several years (data not shown). Thus, the limitation of having examined only a few strains from each geographical region in the present study is moderated by earlier reports on *C. socialis* spores confirming the same pattern. Northern *C. socialis* spores were smooth, matching the descriptions of *C. socialis* given by Cleve (Cleve, 1896) and Gran (Gran, 1897) and observations of the form *C. socialis* f. *socialis* Proschkina-Lavrenko (Proschkina-Lavrenko, 1963) from waters close to Trondheim, Norway (e.g. Figs 36–38 in Evensen and Hasle, 1975; Figs 48–50 in Hargraves, 1979). In contrast, spores observed in cultured and natural material from the Gulf of Naples had spines on their surface, confirming previous observations from the same area (Kooistra *et al.*, 2010). These spores were similar to those described for *C. radians* Schütt (Schütt, 1895), later transferred to *C. socialis* f. *radians* by Proschkina-Lavrenko (Proschkina-Lavrenko, 1953, 1963). Morphometric features of the northern and southern groups of *C. socialis* also differed when both were cultivated at 8°C. Though within reported ranges for cell diameters (apical axis) of *C. socialis* (apical axis 2–14 μm in Hasle and Syvertsen, 1997), population medians (and means) of several morphometric features were significantly different between the northern and southern groups (Table II). Are these observations then an expression of evolutionarily established phenotypic differences between the two groups of strains and are the observed morphometric differences important for species delimitation in *C. socialis*?

In diatoms, a reduction in cell diameter is the inevitable result of each mitotic cell division until the maximum size of the cell is restored during sexual reproduction. Cell size is therefore highly variable (Armbrust and Chisholm, 1992; Jewson, 1992). We do not know if all size or age classes were adequately represented in the random selections of measured cells in our study, thereby keeping the nature of the diatom life cycle from interfering with the morphometric results obtained (e.g. Mann, 1988). Based on microscopic

inspection of our strains, no signs of sexual stage formation were visible, and the numbers of measured cells were relatively high (90–150 strain^{-1}; numbers included three replicate cultures of each strain). However, we lack morphometric data from the two other experimental temperatures employed in the present study, and therefore refrain from assigning the differences observed at one temperature any taxonomic significance. Nevertheless, the observed differences in spore morphology coupled with the considerable phylogenetic distance indicate that the northern and the southern groups of *C. socialis* strains belong to two distinct allopatric taxa. Rather than cryptic species, the two taxa should be defined as pseudo-cryptic (Alverson, 2008), considering that spore morphology could be a discriminative character among taxa hitherto lumped together under the name *C. socialis*. Four different types of *C. socialis* spores have been observed by Hargraves (Hargraves, 1979), suggesting the possible existence of more than two pseudo-cryptic species within this taxon.

According to the current taxonomic status of *C. socialis*, the southern strains examined in this study should be attributed to *C. socialis* f. *radians*. In the light of our results, however, the reduction in rank of *C. radians* Schütt to a form of *C. socialis* (Proschkina-Lavrenko, 1953) is questionable. In fact, this taxonomic change was motivated by the lack of good illustrations and sound evidence of smooth spores in *C. socialis* (Proschkina-Lavrenko, 1953). Proschkina-Lavrenko (1953) also acknowledged the fact that misidentifications of spores were common using microscopy (LM) where spiny spores may appear smooth at an early stage of their development. In a study of material from the Black Sea (Proschkina-Lavrenko, 1953) and the Sea of Azov (Proschkina-Lavrenko, 1963), identical spore morphology was observed in spring and autumn populations of what was first interpreted as the allochronic species *C. radians* Schütt and *C. socialis* Lauder, respectively. Due to the absence of smooth spores in the autumn population, the author concluded that the two species were actually one, but, following the taxonomic nomenclature rules, attributed the two seasonal forms to *C. socialis* instead of *C. radians*, subsequently naming them *C. socialis* f. *socialis* and *C. socialis* f. *radians* Proschkina-Lavrenko (Proschkina-Lavrenko, 1963). However, in both seasonal populations from the Black and Azov Seas, the spores were spiny (Proschkina-Lavrenko, 1953, 1963) and very similar to the spores of *C. radians* Schütt. The latter taxon (as *C. socialis* f. *radians*) appears highly ecologically variable (Proschkina-Lavrenko, 1953, 1963; Jensen and Moestrup, 1998; Shevchenko *et al.*, 2008), which is also manifested by *C. socialis* f. *radians* occurring during multiple seasons in the Gulf of Naples (Ribera D'Alcalà *et al.*, 2004; Zingone *et al.*, 2010). The southern strains used in our study were also collected and isolated in different periods of the year, but all showed similar morphological and phylogenetic characters (Fig. 2). In other words, populations found in different seasons need not be considered taxonomically different at any taxonomic level solely on the basis of their seasonality. We can conclude that both seasonal populations studied by Proschkina-Lavrenko (Proschkina-Lavrenko, 1953, 1963) probably belonged to *C. radians*, whereas no *C. socialis* f. *socialis* (nor *C. socialis* Lauder) was observed in that material. We believe that *C. radians* Schütt should be reinstated at the species level, but we refrain from introducing this change due to a lack of a thorough phylogeographical investigation that can resolve the taxonomic position of the possibly numerous pseudo-cryptic species now referred to as *C. socialis*.

Functional diversity in *C. socialis*

The physiological data on *C. socialis* showed clear differentiation between the northern and the southern groups of strains at 2.5, 8 and 13°C. The two groups showed significantly different growth rates and PAM-estimated maximum quantum yields (Figs 3 and 4, Table III), implying that they are physiologically different. This functional diversity is also supported by metabolite profile data from the same two groups of strains (S.H., unpublished data).

Allometric relationships have frequently been proposed for several groups of organisms and observations of size-dependent maximum growth rates in diatoms (e.g. Sarthou *et al.*, 2005) predict a higher growth rate for smaller cells. In contrast, growth rates in diatoms have been found to be higher in cells recently formed by sexual reproduction, i.e. in cells larger than the cells from which they originated (Costello and Chisholm, 1981). Maximum growth rates at intermediate cell size are also reported in diatoms (Amato *et al.*, 2005; D'Alelio *et al.*, 2009) which may relate to the size window for spermatogenesis in a strain (von Dassow *et al.*, 2006). In other words, the life cycle of a diatom is expected to interfere with its physiological performance, but the quality and extent of this interference appear highly variable. Observations of cell size in relation to growth phase in *C. socialis* f. *radians* showed a rather constant cell size during exponential phase, whereas it changed considerably during stationary phase (Shevchenko *et al.*, 2008). In our experiments on *C. socialis*, the larger northern cells had higher growth rates than the smaller southern ones at 8°C. We cannot draw definite conclusions on the life cycle phase of the strains

used in this experiment, but the differences in cell size (apical axis) did not show any relationship with the observed differences in growth rate between the northern and southern groups of strains at 8°C (not shown). We believe the adaptive strategy of the species in its response to different environmental conditions was more important for physiological performance than the morphometric features of the cell (e.g. Kagami and Urabe, 2001).

High adaptability to changing light conditions has been observed for photosynthetic activity of *C. socialis* from the Tyrrhenian Sea (Dimier *et al.*, 2007). At 8°C, PAM-estimated quantum yields were significantly higher in the southern group of strains than in the northern group, despite the higher growth rates of the latter at this temperature (Table III, Figs 3 and 4). This mismatch was also reflected in increased chlorophyll content per cell in the southern strains (not shown), possibly interfering with our calculations of abundance-based growth rates at this temperature. High photosynthetic activity or carbon production in concert with low growth rate has been observed in phytoplankton as a response to low temperature (McBride *et al.*, 2009) or nutrient limitation (Thingstad *et al.*, 2008). This response was, however, not observed in the southern strains at 2.5°C, a temperature even further beyond the range normally experienced by southern *C. socialis* in its natural habitat (Ribera D'Alcalà *et al.*, 2004; Zingone *et al.*, 2010). Perhaps some other factors than temperature limited the production of southern *C. socialis* at 8°C. However, this lack of consistency in our results does not undermine our overall conclusion on functional diversity between the two geographical groups, as both chlorophyll-based and abundance-based growth rate differences between the two groups were significant at 8°C, despite the small numerical differences of the former, and the possibly biased estimations of the latter (Table III).

Our data strongly indicate that the two groups of *C. socialis* strains are functionally different, reflecting distinct adaptive strategies. The observed differences may well be species-specific, but can also be an expression of intraspecific variation. Species-specific growth rates are difficult to establish (Gilstad and Sakshaug, 1990) and the variation among clones may be high (Brand, 1981; Wood and Leatham, 1992). Also, the different adaptive strategies of so-called ecological groups are not always evident (Gilstad and Sakshaug, 1990), as the within-group variation tends to overshadow the variation between ecological groups. The theoretical ecological niche of a species may often be far greater than required in the habitat from which a particular strain is isolated (Fenchel, 2005). This is often clearly demonstrated by the cases where ranges of optimal growth under laboratory conditions do not match those at which a species grows in nature (e.g. Baker *et al.*, 2009). Data on functional diversity in cryptic or pseudo-cryptic phytoplankton species are, however, relatively scarce. Physiological differences in terms of growth rates among different populations of the diatom *Ditylum brightwellii* (West) Grunow have been reported to occur in concert with differentiation in genetic structure (Rynearson and Armbrust, 2004), genome size as well as cell size (Koester *et al.*, 2010). Phenotypic (morphology, growth rate) and genotypic heterogeneity has been observed in *Skeletonema marinoi* Sarno et Zingone (Ellegaard *et al.*, 2008; Saravanan and Godhe, 2010; Balzano *et al.*, 2011), a pseudo-cryptic diatom which is also reported as highly variable in terms of intracellular metabolites (Vidoudez and Pohnert, 2011). Ecological diversity has been reported for genetically distinct *Pseudo-nitzschia* species (Orsini *et al.*, 2004; McDonald *et al.*, 2007) and also among morphs of the same species (Cerino *et al.*, 2005) occupying different seasonal niches. High levels of functional diversity have also been observed in concert with high genetic differentiation, e.g. in the cryptic dinoflagellate *Oxhyrris marina* Dujardin, but there is not always an obvious correlation between these levels of diversity (e.g. Lowe *et al.*, 2005, 2011). Cases where the biological species concept, i.e. the reproductive isolation of cryptic species, has been tested are more straightforward (Amato *et al.*, 2007). This method, however, is difficult to apply to homothallic diatoms, such as Chaetocerotaceae (Chepurnov *et al.*, 2004; Assmy *et al.*, 2008). Secondary structures of ITS transcripts have proven indicative of reproductive isolation in eukaryotes (Coleman, 2009), and perhaps this could be an approach to resolve the speciation processes acting on the *C. socialis* complex.

Towards a functional species concept

In order to describe the geographical distribution of a species, a precise identification is crucial. The species concept and the methodology applied will naturally affect the outcome of the identification, the precision of which may be of variable importance to different fields of biological research. In a strict ecological context, it is evident that physiology is closely tied to species. Morphological plasticity, in particular variability in species-specific characters, makes the use of morphological species concepts difficult (Sharma and Rai, 2011). Such variability is often connected to environmental factors, as in the case of the closely related diatoms *Thalassiosira gravida* Cleve and *Thalassiosira rotula* Meunier, where species-specific differences in girdle band morphology seem to be dependent on

temperature and nutrient conditions (Syvertsen, 1977; Sar *et al.*, 2011). Morphological variability in diatoms may also relate to life cycle characteristics (e.g. D'Alelio *et al.*, 2010). In pseudo-cryptic species that are phylogenetically different but show only subtle morphological differentiation, morphological plasticity may be particularly problematic, as in *Skeletonema dohrnii* Sarno et Kooistra and *S. marinoi* Sarno et Zingone where the ultrastructural species-specific features of the girdle bands sometimes overlap (Ellegaard *et al.*, 2008). The morphological species concept also forms the foundation for the hypothesis on low diversity and ubiquitous distribution of eukaryotic microorganisms (e.g. Finlay, 2002; Finlay *et al.*, 2004; Fenchel, 2005). The development of molecular techniques and a phylogenetic species concept with the concomitant detection of cryptic and pseudo-cryptic diversity (i.e. increased diversity) have clearly challenged the cosmopolitan species hypothesis in microalgae.

The broad ecological tolerance exhibited by morphologically determined species within phylogenetically diverse species complexes is not necessarily possessed by the individual taxa comprising the complex, as exemplified by the *Sellaphora* (Poulíčková *et al.*, 2008; Evans *et al.*, 2009) and *Skeletonema* complexes (Sarno *et al.*, 2007; Kooistra *et al.*, 2008). Cryptic or pseudo-cryptic species within such species complexes may of course, in their turn, be cosmopolitans or have wide geographical distributions. This applies to certain members of the pseudo-cryptic *Navicula cryptocephala* complex (Poulíčková *et al.*, 2010) or some of the pseudo-cryptic *Pseudo-nitzschia* species (Hasle, 2002). Examples of limited dispersal and possibilities for allopatric (Evans *et al.*, 2009) and even sympatric speciation (Amato *et al.*, 2007) are found in diatoms, often in concert with demonstrations of functional diversity in pseudo-cryptic species (Rynearson and Armbrust, 2004; Koester *et al.*, 2010). The present study also contradicts the assumed cosmopolitan distribution (e.g. Hasle and Syvertsen, 1997) of *C. socialis*. More likely, allopatric speciation has given rise to at least two pseudo-cryptic species of *C. socialis*, and a thorough biogeographic investigation is needed to clarify the taxonomy and the ecological preferences of all the members of this species complex.

Although providing a lot of information on the biology of the species, traditional morphological species descriptions have proven insufficient in delineating marine microbial species (e.g. Sarno *et al.*, 2005, 2007). A combined approach applying a phylo-phenetic species concept that considers morphological as well as genetic species data seems more appropriate (McManus and Katz, 2009). The fundamental value of a species concept should be to provide information on the phenotypic properties of the organism (e.g. Fenchel, 2005), and from an ecological point of view, an integrated approach is needed where genetic information is combined with several aspects of the phenotype. This would be particularly useful when studying cryptic species, possibly supplementing the genetic differentiation with a functional dimension which is not necessarily reflected in morphological characters. The recognition of cryptic species in ecological studies may perhaps also be facilitated through a functional approach, thereby improving the value of such work significantly. Attempts in this direction have been made in studies where functional diversity has been described in terms of so-called ecotypes of eukaryotes, e.g. in *Ostreococcus*, Mamiellophyceae (Rodríguez *et al.*, 2005). Integration of genetic and ecological information has also been suggested to define bacterial species (Fraser *et al.*, 2009 and references therein). There is, however, no straightforward single method to achieve a functional species concept, and such an integrated approach would probably need the combined resources of several biological fields. The call for a so-called integrative taxonomy has been made by others (Dayrat, 2005; Will *et al.*, 2005; Valdecasas *et al.*, 2008; Schlick-Steiner *et al.*, 2010), and if a universal definition of the term could be agreed upon (Yeates *et al.*, 2010) and followed up in future taxonomical studies, this seems a promising approach towards a functional species concept.

CONCLUSION

The functional and morphological diversity observed in the present study among genetically distinct strains of different provenance raises the need for a revision of the taxonomy of *C. socialis*. We argue that the monophyletic genetic differentiation observed between northern and southern strains, reflected in several phenotypic characters, indicates the existence of at least two allopatric species in *C. socialis*. A broad study of the *C. socialis* complex is needed to resolve the number of cryptic or pseudo-cryptic species hidden therein. Investigations of more strains from a wider geographical area are necessary for a proper revision of the biogeography of this species. Integrated approaches, combining genetic, morphological and functional traits, are suggested for future taxonomical studies.

ACKNOWLEDGEMENTS

Technical assistance provided by Gunilla K. Eriksen, Richard A. Ingebrigtsen (University of Tromsø), Gandi Forlani, Carmen Minucci, Gennaro Iamunno and

Franco Iamunno (Stazione Zoologica Anton Dohrn, Naples) is greatly acknowledged. Nataliya Byelyey (University Library of Tromsø) and Svetlana Jakobsen (Russisk språkkompetanse, Tromsø) are acknowledged for translations from Russian. Comments by Hans Christian Eilertsen are highly appreciated.

FUNDING

This work was supported by the University of Tromsø, Norway (M.D., B.L.), the MabCent-SFI Centre for marine bioactives and drug discovery, University of Tromsø, Norway (S.H.), and the Stazione Zoologica Anton Dohrn, Naples, Italy (A.Z., D.S.).

REFERENCES

Alverson, A. J. (2008) Molecular systematics and the diatom species. *Protist*, **159**, 339–353.

Amato, A., Kooistra, W. H. C. F., Levialdi Ghiron, J. H. *et al.* (2007) Reproductive isolation among sympatric cryptic species in marine diatoms. *Protist*, **158**, 193–207.

Amato, A., Orsini, L., D'Alelio, D. *et al.* (2005) Life cycle, size reduction patterns, and ultrastructure of the pennate planktonic diatom *Pseudo-nitzschia delicatissima* (Bacillariophyceae). *J. Phycol.*, **41**, 542–556.

Armbrust, E. V. and Chisholm, S. W. (1992) Patterns of cell size change in a marine centric diatom: variability evolving from clonal isolates. *J. Phycol.*, **28**, 146–156.

Assmy, P., Hernández-Becerril, D. U. and Montresor, M. (2008) Morphological variability and life cycle traits of the type species of the diatom genus Chaetoceros, *C. dichaeta*. *J. Phycol.*, **44**, 152–163.

Baker, J. W., James, P., Grover, J. P. *et al.* (2009) Growth at the edge of the niche: an experimental study of the harmful alga *Prymnesium parvum*. *Limnol. Oceanogr.*, **54**, 1679–1687.

Balzano, S., Sarno, D. and Kooistra, W. H. C. F. (2011) Effects of salinity on the growth rate and morphology of ten *Skeletonema* strains. *J. Plankton Res.*, **33**, 937–945.

Bode, A. and Varela, M. (1998) Primary production and phytoplankton in three Galician Rias Altas (NW Spain): seasonal and spatial variability. *Sci. Mar.*, **62**, 319–330.

Booth, B. C., Larouche, P., Bélanger, S. *et al.* (2002) Dynamics of *Chaetoceros socialis* blooms in the North Water. *Deep-Sea Res. Part II*, **49**, 5003–5025.

Brand, L. E. (1981) Genetic variability in reproduction rates in marine phytoplankton populations. *Evolution*, **35**, 1117–1127.

Cerino, F., Orsini, L., Sarno, D. *et al.* (2005) The alternation of different morphotypes in the seasonal cycle of the toxic diatom *Pseudo-nitzschia galaxiae*. *Harmful Algae*, **4**, 33–48.

Chepurnov, V. A., Mann, D. G., Sabbe, K. *et al.* (2004) Experimental studies on sexual reproduction in diatoms. *Internat. Rev. Cytol.*, **237**, 91–154.

Cleve, P. T. (1896) Diatoms from Baffins Bay and Davis Strait. *Bihang till K. Svenska Vet.-Akad. Handlingar*, **22**, 1–22.

Coleman, A. (2009) Is there a molecular key to the level of 'biological species' in eukaryotes? A DNA guide. *Mol. Phylogenet. Evol.*, **50**, 197–203.

Costello, J. C. and Chisholm, S. W. (1981) The influence of cell size on the growth rate of *Thalassiosira weissflogii*. *J. Plankton Res.*, **3**, 415–419.

D'Alelio, D., Amato, A., Luedeking, A. *et al.* (2009) Sexual and vegetative phases in the planktonic diatom *Pseudo-nitzschia multistriata*. *Harmful Algae*, **8**, 225–232.

D'Alelio, D., Ribera d'Alcalà, M., Dubroca, L. *et al.* (2010) The time for sex: a biennial life cycle in a marine planktonic diatom. *Limnol. Oceanogr.*, **55**, 106–114.

Dayrat, B. (2005) Towards integrative taxonomy. *Biol. J. Linn. Soc.*, **85**, 407–415.

Degerlund, M. and Eilertsen, H. C. (2010) Main species characteristics of phytoplankton spring blooms in NE Atlantic and Arctic waters (68-80°N). *Est. Coasts*, **33**, 242–269.

de Queiroz, K. (2007) Species concepts and species delimitation. *Syst. Biol.*, **56**, 879–886.

Dimier, C., Corato, F., Tramontano, F. *et al.* (2007) Photoprotection and xanthophyll-cycle activity in three marine diatoms. *J. Phycol.*, **43**, 937–947.

Eilertsen, H. C., Schei, B. and Taasen, J. P. (1981) Investigations on the plankton community of Balsfjorden, northern Norway. The phytoplankton 1976–1978. Abundance, species composition, and succession. *Sarsia*, **66**, 129–141.

Eilertsen, H. C. and Skarðhamar, J. (2006) Temperatures of north Norwegian fjords and coastal waters: variability, significance of local processes and air-sea heat exchange. *Est. Coast. Shelf Sci.*, **67**, 530–538.

Ellegaard, M., Godhe, A., Härnström, K. *et al.* (2008) The species concept in a marine diatom: LSU rDNA-based phylogenetic differentiation in *Skeletonema marinoi/dohrnii* (Bacillariophyceae) is not reflected in morphology. *Phycologia*, **47**, 156–167.

Evans, K. M., Chepurnov, V. A., Sluiman, H. J. *et al.* (2009) Highly differentiated populations of the freshwater diatom *Sellaphora capitata* suggest limited dispersal and opportunities for allopatric speciation. *Protist*, **160**, 386–396.

Evensen, D. L. and Hasle, G. R. (1975) The morphology of some *Chaetoceros* (Bacillariophyceae) species as seen in the electron microscope. *Nova Hedwigia Beih.*, **53**, 153–174.

Fenchel, T. (2005) Cosmopolitan microbes and their cryptic species. *Aquat. Microb. Ecol.*, **41**, 49–54.

Finlay, B. J. (2002) Global dispersal of free-living microbial eukaryote species. *Science*, **296**, 1061–1063.

Finlay, B. J., Esteban, G. F. and Fenchel, T. (2004) Protist diversity is different? *Protist*, **155**, 15–22.

Fraser, C., Alm, E. J., Polz, M. F. *et al.* (2009) The bacterial species challenge: making sense of genetic and ecological diversity. *Science*, **323**, 741–746.

Gilstad, M. and Sakshaug, E. (1990) Growth rates of ten diatoms species from the Barents Sea at different irradiances and day lengths. *Mar. Ecol. Prog. Ser.*, **64**, 169–173.

Gran, H. H. (1897) Botany. *Protophyta: Diatomaceae, Silicoflagellata and Cilioflagellata. The Norwegian North-Atlantic Expedition 1876–1878*. Grøndahl & Søn, Christiania, pp. 36.

Guillard, R. R. L. and Ryther, J. H. (1962) Studies of marine plankton diatoms. I. *Cyclotella* nana Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.*, **8**, 229–239.

Guiry, M. D. (2011) *Chaetoceros compressus*. In: Guiry, M. D. and Guiry, G. M. (eds), (2011). *AlgaeBase*. World-wide Electronic Publication, National University of Ireland, Galway. Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=149129 (16 December 2011).

Hargraves, P. E. (1979) Studies on marine plankton diatoms IV. Morphology of *Chaetoceros* resting spores. *Nova Hedwigia Beih.*, **64**, 99–120.

Hasle, G. R. (1976) The biogeography of some marine planktonic diatoms. *Deep-Sea Res.*, **23**, 319–338.

Hasle, G. R. (2002) Are most domoic acid-producing species of the diatom genus *Pseudo-nitzschia* cosmopolites? *Harmful Algae*, **1**, 137–146.

Hasle, G. R. and Syvertsen, E. E. (1997) Marine diatoms. In Tomas, C. R. (ed.) *Identifying Marine Phytoplankton*. Academic Press, San Diego, pp. 5–385.

Higgins, D., Thompson, J., Gibson, T. *et al*. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**, 4673–4680.

Holm-Hansen, O. and Riemann, B. (1978) Chlorophyll *a* determination: improvements in methodology. *Oikos*, **30**, 438–447.

Ishii, K.-I., Iwataki, M., Matsuoka, K. *et al*. (2011) Proposal of identification criteria for resting spores of *Chaetoceros* species (Bacillariophyceae) from a temperate coastal sea. *Phycologia*, **50**, 351–362.

Jensen, K. G. and Moestrup, Ø. (1998) The genus *Chaetoceros* (Bacillariophyceae) in inner Danish coastal waters. *Opera Bot.*, **133**, 1–68.

Jewson, D. H. (1992) Size reduction, reproductive strategy and the life cycle of a centric diatom. *Philos. Trans. R. Soc. Lond. B*, **336**, 191–213.

Kagami, M. and Urabe, J. (2001) Phytoplankton growth rate as a function of cell size: an experimental test in Lake Biwa. *Limnology*, **2**, 111–117.

Koester, J. A., Swalwell, J. E., von Dassow, P. *et al*. (2010) Genome size differentiates co-occurring populations of the planktonic diatom *Ditylum brightwellii* (Bacillariophyta). *BMC Evol. Biol.*, **10**, 1. Doi:10.1186/1471-2148-10-1.

Kooistra, W. H. C. F., Gersonde, R., Medlin, L. K. *et al*. (2007) The origin and evolution of the diatoms: their adaptation to a planktonic existence. In Falkowski, P. G. and Knoll, A. H. (eds), *Evolution of Primary Producers in the Sea*. Elsevier Academic Press, Burlington, pp. 207–249.

Kooistra, W. H. C. F., Sarno, D., Balzano, S. *et al*. (2008) Global diversity and biogeography of *Skeletonema* species (Bacillariophyta). *Protist*, **159**, 177–193.

Kooistra, W. H. C. F., Sarno, D., Hernández-Becerril, D. U. *et al*. (2010) Comparative molecular and morphological phylogenetic analyses of taxa in the Chaetocerotaceae (Bacillariophyta). *Phycologia*, **49**, 471–500.

Kudo, I. (2003) Change in the uptake and cellular Si:N ratio in diatoms responding to the ambient Si:N ratio and growth phase. *Mar. Biol.*, **143**, 39–46.

Lauder, H. S. (1864) Remarks on the marine diatomaceae found at Hong Kong, with descriptions of new species. *Trans. R. Microsc. Soc. Lond., New Series*, **12**, 75–79.

Leaners, G., Maroteaux, L., Michot, B. *et al*. (1989) Dinoflagellates in evolution. A molecular phylogenetic analysis of large subunit ribosomal RNA. *J. Mol. Evol.*, **29**, 40–51.

Leu, E., Falk-Petersen, S. and Hessen, D. O. (2007) Ultraviolet radiation affects growth but not food quality of arctic diatoms. *Limnol. Oceanogr.*, **52**, 787–797.

Lowe, C. D., Day, A., Kemp, S. J. *et al*. (2005) There are high levels of functional and genetic diversity in *Oxyrrhis marina*. *J. Eukaryot. Microbiol.*, **52**, 250–257.

Lowe, C. D., Keeling, P. J., Martin, L. E. *et al*. (2011) Who is *Oxyrrhis marina*? Morphological and phylogenetic studies on an unusual dinoflagellate. *J. Plankton Res.*, **33**, 555–567.

Mann, D. G. (1988) Why didn't Lund see sex in Asterionella? A discussion of the diatom life cycle in nature. In Round, F. E. (ed.), *Algae and the Aquatic Environment*. Biopress, Bristol, pp. 384–412.

Mann, D. G. (1999) The species concept in diatoms. *Phycologia*, **38**, 437–495.

Maumus, F., Allen, A. E., Mhiri, C. *et al*. (2009) Potential impact of stress activated retrotransposons on genome evolution in a marine diatom. *BMC Genomics*, **10**, 624. doi:10.1186/1471-2164-10-624.

McBride, B. B., Muller-Parker, G. and Jakobsen, H. H. (2009) Low thermal limit of growth rate of *Symbiodinium californium* (Dinophyta) in culture may restrict the symbiont to southern populations of its host anemones (*Anthopleura* spp.; Anthozoa, Cnidaria). *J. Phycol.*, **45**, 855–863.

McDonald, S. M., Sarno, D. and Zingone, A. (2007) Identifying *Pseudo-nitzschia* species in natural samples using genus-specific primers and clone libraries. *Harmful Algae*, **6**, 849–860.

McManus, G. B. and Katz, L. A. (2009) Molecular and morphological methods for identifying plankton: what makes a successful marriage? *J. Plankton Res.*, **31**, 1119–1129.

Menden-Deuer, S. and Lessard, E. J. (2000) Carbon to volume relationships for dinoflagellates, diatoms and other protist plankton. *Limnol. Oceanogr.*, **45**, 569–579.

Moniz, M. B. J. and Kaczmarska, I. (2010) Barcoding of diatoms: nuclear encoded ITS revisited. *Protist*, **161**, 7–34.

Montresor, M., Sgrossi, S., Procaccini, G. *et al*. (2003) Intraspecific diversity in *Scrippsiella trochoidea* (Dinophyceae): evidence for cryptic species. *Phycologia*, **42**, 56–70.

Olenina, I., Hajdu, S., Eder, L. *et al*. (2006) Biovolumes and size-classes of phytoplankton in the Baltic Sea. *HELCOM Balt. Sea Environ. Proc.*, **106**, 1–144.

Orsini, L., Procaccini, G., Sarno, D. *et al*. (2004) Multiple rDNA ITS-types within the diatom *Pseudo-nitzschia delicatissima* (Bacillariophyceae) and their relative abundances across a spring bloom in the Gulf of Naples. *Mar. Ecol. Prog. Ser.*, **271**, 87–98.

Poulíčková, A., Špačková, J., Kelly, M. G. *et al*. (2008) Ecological variation within *Sellaphora* species complexes (Bacillariophyceae): specialists or generalists? *Hydrobiologia*, **614**, 373–386.

Poulíčková, A., Veselá, J., Neustupa, J. *et al.* (2010) Pseudocryptic diversity versus cosmopolitanism in diatoms: a case study on *Navicula cryptocephala* Kütz. (Bacillariophyceae) and morphologically similar taxa. *Protist*, **161**, 353–369.

Proschkina-Lavrenko, A. I. (1953) Species *Chaetoceros* novae et curiosae Maris Nigri I. Botanicheskie materialy, Otdela sporovykhastenii. *Akad. NAUK SSSR*, **9**, 46–56.

Proschkina-Lavrenko, A. I. (1963) *Diatomovye vodorosli planktona Azovskogo Morja*. Akad. NAUK SSSR, Moskva, pp. 190.

Ribera D'Alcalà, M., Conversano, F., Corato, F. *et al.* (2004) Seasonal patterns in plankton communities in a pluriannual time series at a coastal Mediterranean site (Gulf of Naples): an attempt to discern recurrences and trends. *Sci. Mar.*, **68**, 65–83.

Rines, J. E. B., Donaghay, P. L., Dekshenieks, M. M. *et al.* (2002) Thin layers and camouflage: hidden *Pseudo-nitzschia* spp. (Bacillariophyceae) populations in a fjord in the San Juan Islands, Washington, USA. *Mar. Ecol. Prog. Ser.*, **225**, 123–137.

Rodríguez, F., Derelle, E., Guillou, L. *et al.* (2005) Ecotype diversity in the marine picoeukaryote Ostreococcus (Chlorophyta, Prasinophyceae). *Environ. Microbiol.*, **7**, 853–859.

Ronquist, F. and Huelsenbeck, J. P. (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Rosselló-Mora, R., Lucio, M., Peña, A. *et al.* (2008) Metabolic evidence for biogeographic isolation of the extremophilic bacterium *Salinibacter ruber*. *ISME J.*, **2**, 242–253.

Rynearson, T. A. and Armbrust, E. V. (2004) Genetic differentiation among populations of the planktonic marine diatom *Ditylum brightwellii* (Bacillariophyceae). *J. Phycol.*, **40**, 34–43.

Sàez, A. G., Probert, I., Geisen, M. *et al.* (2003) Pseudo-cryptic speciation in coccolithophores. *Proc. Natl Acad. Sci. USA*, **100**, 7163–7168.

Sar, E. A., Sunesen, I., Lavigne, A. S. *et al.* (2011) *Thalassiosira rotula*, a heterotypic synonym of *Thalassiosira gravida*: morphological evidence. *Diatom Res.*, **26**, 109–119.

Saravanan, V. and Godhe, A. (2010) Genetic heterogeneity and physiological variation among seasonally separated clones of *Skeletonema marinoi* (Bacillariophyceae) in the Gullmar Fjord, Sweden. *Eur. J. Phycol.*, **45**, 177–190.

Sarno, D., Kooistra, W. H. C. F., Balzano, S. *et al.* (2007) Diversity in the genus *Skeletonema* (Bacillariophyceae): III. Phylogenetic position and morphological variability of *Skeletonema costatum* and *Skeletonema grevillei*, with the description of *Skeletonema ardens* sp. nov. *J. Phycol.*, **43**, 156–170.

Sarno, D., Kooistra, W. H. C. F., Medlin, L. K. *et al.* (2005) Diversity in the genus *Skeletonema* (Bacillariophyceae): II. An assessment of the taxonomy of S. *costatum*-like species with the description of four new species. *J. Phycol.*, **41**, 151–176.

Sarthou, G., Timmermans, K. R., Blain, S. *et al.* (2005) Growth physiology and fate of diatoms in the ocean: a review. *J. Sea Res.*, **53**, 25–42.

Schlick-Steiner, B. C., Steiner, F. M., Seifert, B. *et al.* (2010) Integrative taxonomy: a multisource approach to exploring biodiversity. *Annu. Rev. Entomol.*, **55**, 421–438.

Scholin, C. A., Villac, M. C., Buck, K. R. *et al.* (1994) Ribosomal DNA sequences discriminate among toxic and non-toxic *Pseudonitzschia* species. *Nat. Toxins*, **2**, 152–165.

Schütt, F. (1895) Arten von *Chaetoceros* und *Peragallia*. Ein Beitrag zur Hochseeflora. *Berichte der deutschen botanischen Gesellschaft*, **13**, 35–48.

Sharma, N. K. and Rai, A. K. (2011) Biodiversity and biogeography of microalgae: progress and pitfalls. *Environ. Rev.*, **19**, 1–15. doi:10.1139/A10-020.

Shevchenko, O. G., Orlova, T. Y. and Aizdaicher, N. A. (2008) Development of the diatom *Chaetoceros socialis* f. *radians* (Schütt) Proschkina-Lavrenko 1963 in laboratory culture. *Russ. J. Mar. Biol.*, **34**, 224–229.

Simonelli, P., Troedsson, C., Nejstgaard, J. C. *et al.* (2009) Evaluation of DNA extraction and handling procedures for PCR-based copepod feeding studies. *J. Plankton Res.*, **31**, 1465–1474.

Smayda, T. J. (1958) Biogeographical studies of marine phytoplankton. *Oikos*, **9**, 158–191.

Syvertsen, E. E. (1977) *Thalassiosira rotula* and *T. gravida*: ecology and morphology. *Nova Hedwigia Beih.*, **54**, 99–112.

Tamura, K., Peterson, D., Peterson, N. *et al.* (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.*, **28**, 2731–2739.

Thingstad, T. F., Bellerby, R. G. J., Bratbak, G. *et al.* (2008) Counterintuitive carbon-to-nutrient coupling in an Arctic pelagic ecosystem. *Nature*, **455**, 387–391.

Tomaru, Y., Takao, Y., Suzuki, H. *et al.* (2009) Isolation and characterization of a single-stranded RNA virus infecting the bloom-forming diatom *Chaetoceros socialis*. *Appl. Environ. Microbiol*, **75**, 2375–2381.

Valdecasas, A. G., Williams, D. and Wheeler, Q. D. (2008) Integrative taxonomy then and now: a response to Dayrat (2005). *Biol. J. Linn. Soc.*, **93**, 211–216.

Vidoudez, C. and Pohnert, G. (2011) Comparative metabolomics of the diatom *Skeletonema marinoi* in different growth phases. *Metabolomics*, doi:10.1007/s11306-011-0356-6.

von Dassow, P., Chepurnov, V. A. and Armbrust, V. G. (2006) Relationships between growth rate, cell size, and induction of spermatogenesis in the centric diatom *Thalassiosira weissflogii* (Bacillariophyta). *J. Phycol.*, **42**, 887–899.

Will, K. W., Mishler, B. D. and Wheeler, Q. D. (2005) The perils of DNA barcoding and the need for integrative taxonomy. *Syst. Biol.*, **54**, 844–851.

Wood, M. A. and Leatham, T. (1992) The species concept in phytoplankton ecology. *J. Phycol.*, **28**, 723–729.

Yeates, D. K., Seago, A., Nelson, L. *et al.* (2010) Integrative or iterative taxonomy? *Syst. Entomol.* doi:10.1111/j.1365-3113.2010.00558.x.

Zak, J. C., Willig, M. R., Moorhead, D. L. *et al.* (1994) Functional diversity of microbial communities: a quantitative approach. *Soil Biol. Biochem.*, **26**, 1101–1108.

Zingone, A., Dubroca, L., Iudicone, D. *et al.* (2010) Coastal phytoplankton do not rest in winter. *Est. Coasts*, **33**, 342–361.