DE GRUYTER Botanica Marina 2023; aop

Research Article

Andréa de Oliveira da Rocha Franco*, Matt Peter Ashworth, Debbie Du Preez, Eileen Campbell, Rodrigo Maggioni, Schonna R. Manning and Clarisse Odebrecht

Surf-diatoms from sandy beaches on the Eastern Coast of South America (Brazil): the identification of *Anaulus* Ehrenberg and *Asterionellopsis* Round

https://doi.org/10.1515/bot-2022-0067
Received October 21, 2022; accepted August 1, 2023;

Abstract: "Surf diatoms" can form high biomass in the surf zone of sandy beaches around the world, *Asterionellopsis* and *Anaulus* being the main genera of this group in Brazil. *Asterionellopsis glacialis* was considered a cosmopolitan species, and taxonomic studies using molecular and morphological tools showed that *A. glacialis* is a complex with cryptic and semicryptic species. So, it would be plausible to suppose that *Anaulus australis*, another surf zone patch-forming diatom with wide latitudinal occurrence could also be part of a species complex. We collected and identified *Anaulus* and *Asterionellopsis* strains from tropical, subtropical and warm temperate sandy beaches on the east coast of South America (Brazil) based on genetic divergence, phylogeny, single-locus automated species delimitation methods (both genera), and frustule ultrastructure (*Anaulus*). *Anaulus* and *Asterionellopsis* showed contrasting diversity patterns and spatial distribution: a single species of *Anaulus australis* was registered in tropical and subtropical beaches, while at least three species of *Asterionellopsis*: *A. tropicalis*, *A. thurstonii* and *A. guyunusae* were observed at different latitudes, indicating that *Asterionellopsis* species have distinct ecological requirements. *Asterionellopsis thurstonii* was previously reported in Europe and it is documented here for the first time in the Southern Hemisphere. The different diversity patterns between these two surf diatom genera suggest that they are likely to have distinct ages, and dispersion and/or speciation processes.

Keywords: *Anaulus*; cryptic species; diversity; phylogeny; species distribution

1 Introduction

Traditionally, diatom species are identified based on the morphology of their frustules (silicified cell wall) by light microscopy and later by electron microscopy. However, the use of molecular data enables us to examine characters which might indicate reproductive isolation before such isolation is reflected and becomes morphologically evident by microscopy (Mann et al. 2010). This "revolutionary" emergence of molecular taxonomy has cast new light on cryptic, semicryptic, and pseudocryptic diatom species, which demonstrated reproductive isolation with little to no obvious morphological variation (Amato et al. 2007; Evans et al. 2007; Mann et al. 2008; Poulìcková et al. 2010; Quijano-Scheggia et al. 2009).

Taxonomic information on South American diatoms based on molecular analysis are scarce, with the exception of some studies with *Asterionellopsis* (Brazil: Franco et al. 2016; Uruguay: Kaczmarska et al. 2014), *Chaetoceros* (Chile: Gaonkar et al. 2017), *Gedaniella* (Chile: Li et al. 2018), *Navicula* (Chile: Seddon et al. 2011), *Nitzschia* (Brazil and Paraguay: Trobajo et al. 2009, 2010), *Pinnularia* (Chile: Pinseel et al. 2017), and studies on the use of barcode markers to identify species of *Achnanthidium*, *Eolimna*, *Nitzschia* and *Sellaphora* (Ecuador: Ballesteros et al. 2020) and *Chaetoceros* (Chile: Gaonkar et al. 2018).

The so-called "surf diatoms" are a small group of taxonomically unrelated species that can form patches of up to 10^9 cells L^{-1} in the surf zones of several sandy beaches around the world, particularly between 46°55′N and 42°10′S (Campbell

---

***Corresponding author: Andrea de Oliveira da Rocha Franco**, Institute of Oceanography, Federal University of Rio Grande – FURG, Av. Italia, km 8, CEP 96203-900, Rio Grande, Brazil, E-mail: andrea.ecomarine@gmail.com
Matt Peter Ashworth, Department of Molecular Biosciences, UTEX Culture Collection of Algae, University of Texas at Austin, Austin, TX 78712, USA
Debbie Du Preez and Eileen Campbell, Department of Botany, Nelson Mandela University, Port Elizabeth 6031, South Africa
Rodrigo Maggioni, Institute of Marine Sciences – LABOMAR, Federal University of Ceará – UFC, Av. da Abolição, 3207, CEP 60165-081, Fortaleza, Brazil
Schonna R. Manning, Department of Molecular Biosciences, UTEX Culture Collection of Algae, University of Texas at Austin, Austin, TX 78712, USA; and Department of Biological Sciences, Institute of Environment, Florida International University, North Miami, FL 33181, USA
Clarisse Odebrecht, Institute of Oceanography, Federal University of Rio Grande – FURG, Av. Italia, km 8, CEP 96203-900, Rio Grande, Brazil, E-mail: claodebrecht@gmail.com. https://orcid.org/0000-0001-7159-4713

1996; Odebrecht et al. 2014). The main surf diatoms forming patches in Brazilian sandy beaches are *Asterionellopsis glacialis* (Castracane) F.E. Round complex (Bacillariophyceae) and *Anaulus australis* G. Drebes *et* D. Schulz (Mediophyceae) (Odebrecht et al. 2014), while *Aulacodiscus kittonii* Arnott *ex* Ralfs (Coscinodiscophyceae) was found on only one beach, where it was not dominant (Franco et al. 2018; Odebrecht et al. 2014).

Until recently, most surf diatom species were considered to have wide latitudinal range (Campbell 1996; Odebrecht et al. 2014). However, in taxonomic studies of genetic diversity, the ribosomal internal transcribed spacer (*ITS*) secondary structure and frustule ultrastructure of morphologically semicryptic and cryptic species of the *Asterionellopsis glacialis* complex, five species were identified (Franco et al. 2016; Kaczmarska et al. 2014) whose geographical distribution is not yet fully established. We currently know that at least *Asterionellopsis lenisilicea* L. Mather and *Asterionellopsis maritima* F. Muise may co-occur on the eastern coast of Canada (Kaczmarska et al. 2014) and the former also occurs in South Africa (Du Preez 2017). Until the present study, *Asterionellopsis thurstonii* J.M. Ehrman had been registered only in Europe (Kaczmarska et al. 2014). Other species such as *Asterionellopsis tropicalis* A.O.R. Franco and *Asterionellopsis guyunusae* I.A. Luddington are apparently associated with specific environmental conditions, the first occurring in tropical latitudes (3°S) and the latter in subtropical/temperate (32°S) Brazilian sandy beaches (Franco et al. 2016). However, there is no information on *Asterionellopsis* species occurring at mid-latitudes on the Brazilian coast (3° > 32°). Due to the wide geographical distribution, potential for diversification and high local abundance (making isolation of monoclonal cells easier), the surf diatoms are a potential model for the study of speciation patterns, gene flow, and dispersal of free microorganisms in the marine environment.

Molecular taxonomy also presents limitations, as taxa with available information, such as sequenced genetic markers, are still scarce relative to their diversity, particularly for the class Mediophyceae, including *Anaulus*. Most of the molecular information available on diatoms of the class Mediophyceae was obtained from extensive phylogenetic studies of Bacillariophyta as a whole (Medlin and Kaczmarska 2004; Theriot et al. 2010, 2015) or specific orders and families (Ashworth et al. 2013; Dąbek et al. 2019; Kaczmarska et al. 2005). While phylogenetic relationships between genera and families allowed the detection of polyphyletic genera and the recognition of new genera (Ashworth et al. 2013; Sims et al. 2018; Witkowski et al. 2020), some markers used in these studies have low variability and are of little use in the delineation of species. Therefore, it would be important to expand the molecular information on genetic markers used for species delimitation in the diatom class Mediophyceae. Indeed, some studies have utilized both morphological and molecular analyses to identify species of this class, corroborating the use of molecular tools in revealing cryptic and pseudocryptic species within *Skeletonema* (Kooistra et al. 2008; Sarno et al. 2005; Zingone et al. 2005) and the *Chaetoceros socialis* (H.S. Lauder) complex (Chamnansinp et al. 2013; Gaonkar et al. 2017), as well as describing different species of *Leptocylindrus* and erecting an allied new genus (Nanjappa et al. 2013).

The surf diatom *Anaulus australis*, Mediophyceae, was described from diatom accumulations in South Africa from a population previously identified as *Anaulus birostratus* (Grunow) Grunow (Drebes and Schulz 1989). *Anaulus australis* has been attributed a wide distribution, being responsible for forming accumulations in contrasting environmental conditions: from the tropics to subtropics, on sandy beaches of South America, South Africa, and Oceania (3°S–34°S; Odebrecht et al. 2014). Up to now, the taxonomy of *Anaulus australis* has been based on morphology only (Drebes and Schulz 1989) and we may suppose this global distribution could actually harbor a species complex, similar to *Asterionellopsis glacialis sensu lato*. Recently, the genus *Anaulus* was revised, with morphological features and molecular data supporting the transfer of some species of *Anaulus* to two new genera, *Ceratanaulus* and *Ambo* (Witkowski et al. 2020). Although *Anaulus* has fewer described species than some other mediophycean genera, there are still many questions about its taxonomy and systematics, mainly due to the lack of frustule ultrastructure and genetic information about the type species of the genus, *Anaulus scalaris* (Witkowski et al. 2020).

Our goal in this study is to identify the species of *Anaulus* and *Asterionellopsis* occurring in tropical and subtropical sandy beaches on the eastern coast of South America (Brazil). Species of *Anaulus* are identified for the first time here based on combined morphological and molecular analyses. *Asterionellopsis* species are identified based solely on molecular analysis. Previous studies have shown that morphological differentiation lags behind the genetic divergence in this genus and that molecular approaches are better suited to identify the cryptic and semicryptic species (Franco et al. 2016; Kaczmarska et al. 2014). The molecular analyses used for both genera included genetic divergence, phylogeny, Automatic Barcode Gap Discovery (ABGD) for primary species delimitation, Assemble Species by Automatic Partitioning (ASAP) and the Poisson Tree Processes (PTP).

2 Materials and methods

2.1 Isolation and maintenance of strains

Anaulus and *Asterionellopsis* cells were isolated from surface water collected from the surf zone of tropical and subtropical beaches of

Brazil (Table 1; 3°S–26°S). Three strains of *Anaulus* (ANAAUS 1, ANAAUS 2, ANAAUS 3) were isolated from diatom accumulations at Futuro Beach, Ceará (3°41′S, 38°27′W). Two further strains of *Anaulus* (ANAAUS 4, ANAAUS 5) and two strains of *Asterionellopsis* (ASTTHU 1, ASTTHU 2) were obtained at Navegantes Beach, Santa Catarina (26°50′S, 8°38′W). Another strain (ASTTHU 3) was obtained at Pontal do Sul Beach, Paraná (25°35′S, 48°21′W). In addition, a strain of *Asterionellopsis* (BMAK9) from Baia do Trapandé, Cananéia, São Paulo (25°4′S, 47°55′W) was provided by the Aidar and Kutner Bank of Microorganisms of the Oceanographic Institute of the University of São Paulo, totaling five strains of *Anaulus* and four strains of *Asterionellopsis*.

Table 1: Strains and field samples sequenced in the present study: species name, name of strain or sample type, geographic location, date of collection of samples used to extract DNA or isolate strains, and molecular marker sequences and GenBank access numbers.

Species	Origin of DNA: strain (specimen voucher) or sample	Location and date of collection	Molecular marker analyzed (GenBank access number)
Anaulus australis G. Drebes *et* D. Schulz	ANAAUS 1	Brazil, Ceará, Futuro Beach, 3°41′S, 38°27′ W, Mar 2014	*rbc*L (MK817338); *ITS* (MN221455); *cox*1 (MK820689)
Anaulus australis	ANAAUS 2		*rbc*L (MK8173389); *ITS* (MN221456); *cox*1 (MK820690)
Anaulus australis	ANAAUS 3	Brazil, Ceará, Futuro Beach, 3°41′S, 38°27′ W, Mar 2016	*rbc*L (MK820692); *ITS* (MK828680); *cox*1 (MK820688)
Anaulus australis	ANAAUS 4	Brazil, Santa Catarina, Navegantes Beach, 26°50′S, 48°38′W, Dec 2017	*rbc*L (MK820693) *ITS* (MK828681);
Anaulus australis	ANAAUS 5		*rbc*L (MK820694); *cox*1 (MK820691)
Anaulus australis	Field sample	South Africa, Western Cape, Muizenburg, 34°05′S, 18°29′E, June 2016	*rbc*L (MK981943)[a]; *ITS* (KU146870)
Anaulus australis	Field sample	South Africa, Western Cape, Macassar, 34° 04′S, 18°44′E, June 2016	*rbc*L (MK981943)[a]; *ITS* (KU146871)
Anaulus australis	Field sample	South Africa, Western Cape, Wilderness, 34°00′S, 22°36′E, June 2016	*rbc*L (MK981943)[a]; *ITS* (KU146872)
Anaulus australis	Field sample	South Africa, Eastern Cape, Sundays River Beach, 33°42′S, 25°55′E, June 2016	*rbc*L (MK981943)[a]; *ITS* (KU146869); *cox*1 (MN047458)
Anaulus australis	Field sample	South Africa, Eastern Cape, Cintsa, 32°49′S, 28°07′E, June 2016	*rbc*L (MK981943)[a]; *ITS* (KU146873)
Asterionellopsis thurstonii I.A. Luddington	ASTTHU 1	Brazil, Santa Catarina, Navegantes Beach, 26°50′S, 48°38′W, Mar 2016	*cox*1 (MK820684)
Asterionellopsis thurstonii	ASTTHU 2	Brazil, Santa Catarina, Navegantes Beach, 26°50′S, 48°38′W, Nov 2016	*cox*1 (MK820685)
Asterionellopsis thurstonii	ASTTHU 3	Brazil, Paraná, Pontal do Sul Beach, 25°35′ S, 48°21′W, Mar 2016	*cox*1 (MK820683)
Asterionellopsis thurstonii	BMAK9	Brazil, São Paulo, Trapandé Beach, 25°4′S, 47°55′W, Sep 1992	*cox*1 (MK820686)
Asterionellopsis guyunusae J.M. Ehrman	Field sample	Brazil, Rio Grande do Sul, Torres Beach 29° 25′S, 49°47′W, Oct 2016	*cox*1 (MK820687)
Eunotogramma lunatum M.P. Ashworth	ECT3886-Eunoto HK294	USA, North Carolina Bald Head Island, June 2010	*rbc*L (JN975259.1) *ITS* (ON972512)
Eunotogramma lunatum	AP8Eunoto (HK451)	USA, Florida, Nov 2011	*rbc*L (MK817355) *ITS* (ON972513)
Cerataulina pelagica (Cleve) Hendey	ECT3845 (HK230)	USA, Texas, Corpus Christi Bay June 2009	*rbc*L (HQ912533.1)
Cerataulina pelagica	ECT3836Cpel (HK232)	USA, California, Long Beach, June 2009	*rbc*L (KC309559.1); *ITS* (MK828682)
Cerataulina pelagica	ECT3884Cpel (HK596)	USA, California Monterey Bay, June 2010	*rbc*L (MK817344); *ITS* (MK828683)
Cerataulina pelagica	UTKSA0011	Saudi Arabia, Red Sea, Jan 2013	*rbc*L (MK817345)
Cerataulina pelagica	16VIII13-1Cpel (HK595)	USA, Maine, Bar Harbor, Aug 2013	*rbc*L (MK817346)
Lithodesmium intricatum Ehrenberg	ECT3836Litho (HK231)	USA, California, Long Beach, June 2009	*rbc*L (MK817347); *cox*1 (MK817331)
Lithodesmium intricatum	ECT3850Litho (HK253)	USA, Hawaii, Jul 2009	*rbc*L (MK817348); *cox*1 (MK817332)
Lithodesmium intricatum	25VI12-1ALitho (HK586)	USA, Bar Harbor, Maine, Aug 2013	*rbc*L (MK81749); *cox*1 (MK817335)
Lithodesmium intricatum	Azo12Litho-5 (HK593)	Portugal, Azores, Nov 2014	*rbc*L (MK817350); *cox*1 (MK817333)

Table 1: (continued)

Species	Origin of DNA: strain (specimen voucher) or sample	Location and date of collection	Molecular marker analyzed (GenBank access number)
Lithodesmium intricatum	Azo12Litho-J2 (HK594)	Portugal, Azores, Nov 2014	rbcL (MK817351); cox1 (MK817334)
Neobrightwellia alternans (Bailey) M.P. Ashworth *et* P.A. Sims	ECT3886Balt (HK292)	USA, North Carolina Bald Head Island, June 2010	*rbc*L (JX401246.1); *ITS* (MK828686)
Neobrightwellia alternans	AJA028-28 (HK592)	USA, Georgia, Jekyll Island, June 2012	*rbc*L (MK817352); *ITS* (MK828687); *cox*1 (MK817336)
Neobrightwellia alternans	25VI12-1Abalt (HK542)	USA, South Carolina, Hunting Island, June 2012	*rbc*L (MK817353); *ITS* (MK828684); *cox*1 (MK817337)
Neobrightwellia alternans	AP8Balt (HK591)	USA, Florida, Hollywood, Nov 2011	*rbc*L (MN167110); *ITS* (MN221457); *cox*1 (MN167109)
Neobrightwellia alternans	Azo12Balt5 (HK541)	Portugal, Azores, Nov 2014	*rbc*L (MK817354); *ITS* (MK828685)
Pseudictyota dubia (Brightwell) P.A. Sims *et* D.M. Williams	26vi08-1Gtdub (HK199)	USA, Guam, Taeleyag Beach, June 2008	*rbc*L (MK817340)
Pseudictyota dubia	ECT3838Tdub (HK254)	USA, California, Long Beach, June 2009	*rbc*L (KC309592.1)
Pseudictyota dubia	ECT3888Tdub HK342	Spain: San Sebastian, Canary Islands, May 2010	*rbc*L (KC309593.1)
Pseudictyota dubia	Tokiane3OdonE3 (HK597)	Madagascar, Nov 2014	*rbc*L (MK817341)
Pseudodictyota dubia	UTKSA0088	Saudi Arabia, Red Sea, Jan 2013	*rbc*L (MK817342)
Pseudodictyota dubia	NateSite1Tdub2 (HK598)	USA, Hawai, Kona, Jan 2012	*rbc*L (MK817343)

[a] Sequence deposited in GenBank as representative of *Anaulus australis* from South Africa.

The strains were isolated using micropipettes and/or serial dilution (Andersen 2005), and maintained in f/2 culture medium (Guillard 1975) in incubators (FANEM, São Paulo, Brazil) with controlled temperature (20–23 °C), salinity (28–30), and photoperiod (12:12 h light:dark, irradiance 190 μmol m^{-2} s^{-1}). All strains are maintained at the Marine Microalgae Collection of the Institute of Oceanography, Federal University of Rio Grande (IO-FURG), Brazil.

2.2 Morphological analysis of *Anaulus*

Morphological observations were carried out on *Anaulus* cultures and three samples of diatom accumulations from Futuro Beach that were fixed in neutral formaldehyde solution (4 %). These diatom accumulation samples were collected in 2017 on March 31 and November 9 and 11 (Supplementary Table S1). Ultrastructural details of cingular bands of *Anaulus australis* were obtained from cells sampled on 8 September 2017 from the surf zone of Sundays River Beach, South Africa, the type locality of *A. australis*; this information is not provided in the original species description (Drebes and Schulz 1989).

Samples were washed with distilled water six times by centrifugation (3600*g*, 5 min) to remove fixatives and residues from the culture medium. Two frustule cleaning protocols were used on the pellets, according to the amount of organic matter and mucilage: (1) In samples with little organic matter, the pellet was suspended in a solution of hydrogen peroxide (approx. 23 %) and kept in a water bath with boiling water until the removal of all organic matter (Battarbee 1986); (2) In samples with more organic matter and mucilage, the pellet was suspended in a saturated solution of potassium permanganate (0.5 M) and kept at 50 °C for 12 h. Afterwards, hydrochloric acid was added, and the samples were heated in a water bath until the pellet lost coloration. Thereafter, the reagents used in the oxidation of the organic material were removed by successive centrifugation and washing with distilled water (Simonsen 1974). A drop of the cleaned and concentrated frustules was placed on a cover slip attached to an electron microscope stub with a carbon adhesive and oven dried at 50 °C. The dried samples were coated with gold by vacuum (Denton Vacuum Desk V) and examined using an SEM (JEOL JSM – 6610LV, Akshima, Japan) operated at 25–30 kV and 9 mm working distance at the Electronic Microscopy Center of the Federal University of Rio Grande (CEME-SUL). At least seven frustules in valve view or girdle view per sample were imaged.

One hundred and seventeen *Anaulus* cells (frustules in valve view or girdle view) from Brazilian beaches were imaged by scanning electron microscopy (SEM): 26 cells from three diatom accumulation events in the Futuro Beach surf zone and 91 cells from cultures. Ten cleaned cingulum bands from the Sundays River Beach, South Africa specimens were imaged by SEM, and the number of areolae (per 10 μm) and rows of areolae (per girdle band) were measured and found to fit the values described for *A. australis*.

Morphological measurements were made on the scanning electron micrographs using AxioVision Zeiss software (Oberkochen, Germany). The apical, transapical, and pervalvar axes, the number of areolae (areola density) per stria in 10 μm in the central and apical regions of the valve, the number of areolar rows in each cingulum band, and the number of areolae per row in 10 μm in the cingulum band were measured, when possible. The terminology used follows Round et al. (1990) and Williams (1985).

2.3 Molecular analyses

2.3.1 DNA extraction, amplification, and sequencing: DNA from cultured strains of *Anaulus* and *Asterionellopsis* and field-collected diatom accumulations from Brazil (Table 1) was extracted from centrifuged cell pellets using the DNeasy® Plant Mini Commercial Kit (QIAGEN, Hilden, Germany). Prior to DNA extraction for field-collected diatom accumulations from Brazil (containing *Asterionellopsis*), the cell pellets were washed with sodium thiosulphate solution (0.25 M) to neutralize and remove the Lugol's (1 %) reagent from fixed samples (modified from Auinger et al. 2008). DNA of *Anaulus australis* from accumulations in the surf zone from South Africa was obtained from samples preserved in ethanol and stored at 4 °C. These samples were washed with distilled water twice, centrifuged, and the pellets were used directly in PCR analysis without DNA extraction (Lang and Kaczmarska 2011).

In order to increase knowledge of genetic divergence in Mediophyceaean species and populations for comparison with *Anaulus* and to obtain a root for our phylogenetic trees, sequences from *Eunotogramma lunatum*, *Cerataulina pelagica*, *Lithodesmium intricatum*, *Neobrightwellia alternans*, and *Pseudictyota dubia* UTEX cultured collection strains were provided. These Mediophycean species were chosen because the strains of each taxon have a wide geographical distribution and are relatively close phylogenetically to *Anaulus* within the Mediophyceae. The strains of *Eunotogramma lunatum* were those used in the original species description using morphological and molecular data (Witkowski et al. 2020), while the other 21 strains of 4 Mediophyceae species (Table 1) were previously identified as morphologically-conspecific by SEM. Fresh samples from the strains were centrifuged and the pellets were stored at −80 °C. DNA was extracted using the DNeasy® Plant Mini kit, as described above.

The PCR for the amplification of each gene marker was performed using specific primers and PCR conditions (Table 2). Three marker fragments were amplified for the molecular characterization of *Anaulus* and the additional Mediophycean species: (1) cytochrome *c* oxidase subunit 1 (*cox*1) from the mitochondrial genome; (2) ribulose 1,5-bisphosphate carboxylase/oxygenase, large subunit (*rbc*L) from the chloroplast genome; and (3) the ribosomal *ITS* region, covering *ITS*1 + *5.8S* + *ITS*2, from the nucleus. For the molecular characterization of *Asterionellopsis*, we used the *cox*1 fragment only. Previous studies had shown that reliable identification can be achieved without using all three gene markers (Franco et al. 2016; Kaczmarska et al. 2014). Each marker alone would be able to answer the taxonomic question, but *cox*1 arguably allows for easier and more reliable identification, due to the higher genetic divergence between species observed in this marker (Franco et al. 2016; Kaczmarska et al. 2014).

The PCR products were purified with commercial kits (Illustra GFX PCR and Gel Band Purification Kit or ExoSAP master mix) and were sequenced in both directions using Big Dye Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Carlsbad, California, USA), with the primers described in Table 2. PCR products from some samples were further cloned using the kit pGEM®-T Easy Vector Systems (PROMEGA®). Plasmids were sequenced in both directions using standard primers M13 (INVITROGEN®) following the same protocol used for PCR products. Sequencing reactions were purified by centrifugation in isopropanol, and read on ABI 3500 or 3700 automated DNA sequencers (Applied Biosystems, Foster City, CA, USA).

2.3.2 Calculation of genetic divergence: Sequences were edited, aligned with the ClustalW algorithm in the MEGA-X Program, then adjusted manually when appropriate and the divergence between sequences was calculated by simple distance between pairs of sequences

Table 2: Successful amplifications: the primers and PCR condition used for each molecular marker for each genus studied.

Studied genera (type of samples)	Molecular marker	Name of primers (reference)	PCR: specific condition or reference
Anaulus (strain) *Asterionellopsis* (strain and field sample)	*cox*1	CoxF and CoxR (Iwatani et al. 2005)	Franco et al. (2016)
Lithodesmium (strain)	*cox*1	CoxF and CoxR (Iwatani et al. 2005)	Iwatani et al. (2005) modified: The elongation and binding times were lengthened to 2 min and 1 min
Neobrightwellia (strain)	*cox*1	BaltCox659F-GAGCATTGCAAGTCTGTGCC and BaltCox497R-TGCCAAGCTCTTCCTGAAGG	The same PCR condition described here and used for amplifying *cox*1 using DNA of *Lithodesmium*
Anaulus (strain)	*rbc*L	Dtrbcl2F and Dtrbcl2R (Lang and Kaczmarska 2011; MacGillivary and Kaczmarska 2011)	The second step of Lang and Kaczmarska (2011)
Anaulus (field sample)	*rbc*L	DtrbcL2F and DtrbcL1R (Lang and Kaczmarska 2011); Second PCR: DtrbcL3F and DtrbcL3R (Lang and Kaczmarska 2011)	Lang and Kaczmarska (2011)
Eunotogramma, Cerataulina, Lithodesmium, Neobrightwellia, Pseudictyota, (strain)	*rbc*L	rbcL44+ and rbcL1444− (Theriot et al. 2015)	Denaturation at 94 °C (3.5 min), followed by 35 cycles of denaturation (94 °C, 30 s), annealing (48 °C, 60 s), extension (72 °C, 120 s), with a final extension at 72 °C for 15 min
Anaulus (strain)	*ITS* region	SR12cF and 25F1R (Takano and Horiguchi 2006)	The second step of Lang and Kaczmarska (2011)
Anaulus (field sample)	*ITS* region	Ns7m and LR1850 (Lang and Kaczmarska 2011); second PCR: SR12cF and 25F1R (Takano and Horiguchi 2006)	Lang and Kaczmarska (2011)
Cerataulina and *Neobrightwellia* (strain)	*ITS* region	dITS3-ACAACTTTCAGCAATGGATGTC and ITS4-TCCTCCGCTTATTGATATGC	The same PCR condition described here and used for amplifying *rbc*L using DNA of these genera, except for warmed annealing: 50 °C for 60 s

(uncorrected *p*-distance) in the MEGA-X Program (Kumar et al. 2018). The uncorrected *p*-distance is adequate for comparing sequences of close or correlated species (Nei and Kumar 2000) and a standard measurement for DNA sequence divergence of diatoms (MacGillivary and Kaczmarska 2011; Moniz and Kaczmarska 2009, 2010). The following alignments were used for the calculation of genetic divergence:

(1) *cox*1 from all *Asterionellopsis* sequences obtained in the present study, with at least one sequence of each species downloaded from GenBank; the same sequences were used to build the tree.
(2) Each marker (*cox*1, *rbc*L and *ITS*1 + *5.8S* + *ITS*2) was aligned separately with all strains of Mediophyceae sequenced (Table 1). The fragment for *rbc*L is approximately 540 bp corresponding to the barcode region suggested by MacGillivary and Kaczmarska (2011). The *cox*1 is approximately 431 bp, corresponding to the region near the 5′ end (Moniz and Kaczmarska 2009) and *5.8S* + *ITS*2 corresponds to the fragment that begins at the start codon of *5.8*S and ends in the conserved motif on helix III of *ITS*2 (Moniz and Kaczmarska 2009).

2.3.3 Phylogenetic trees: The concatenated phylogenetic trees were constructed using *rbc*L + partial *5.8S* + partial *ITS*2 (872 bp) with four Brazilian *Anaulus*, five South African *Anaulus australis* plus two *Eunotogramma lunatum* sequences, the latter taxa as an outgroup, and phylogenetically close to *Anaulus* (Witkowski et al. 2020). The *cox*1 *Anaulus* tree (436 bp) was built with four Brazilian *Anaulus*, one South African *Anaulus australis* and five *Lithodesmium intricatum* sequences; the latter is the closest relative to *A. australis* with sequenced *cox*1 markers (Witkowski et al. 2020). The *cox*1 *Asterionellopsis* tree was constructed with five sequences obtained in this study (Table 1), plus 13 sequences of *Asterionellopsis* strains from GenBank.

The phylogenetic trees were constructed with a best-fit model for each data matrix that was estimated using the Akaike Information Criterion (Akaike 1974) by the software JModeltest 2 (Darriba et al. 2012). The Bayesian analysis (Markov Chain Monte Carlo method) was performed using the software MrBayes 3.2.3 (Ronquist and Huelsenbeck 2003). This analysis was conducted in two independent runs, four chains of one million generations each, with one topology sampled each 100 generations and a burn-in of 25 % of the generations. The convergence between independent runs, the burn-in of generations, and the analysis performance were validated with the likelihood plots for each run and the effective sample size (>200) using the software Tracer 1.7 (Rambaut et al. 2018).

2.3.4 Molecular species delimitation models: Three automated molecular species delimitation models were used to evaluate if the Brazilian *Anaulus* and South African *Anaulus australis* strains are conspecific. These models were similarly used to evaluate species delimitation in the *Asterionellopsis glacialis* complex. The alignments composed by all sequences used to build the phylogenetic trees were used on ABGD and ASAP. To avoid underestimation of species by ABGD analyses, the studied clades utilize at least three sequences (Puillandre et al. 2012) and included sequences of at least one species known to be closely related to the studied taxon (Franco et al. 2023). The ABGD and ASAP models used simple distance (*p*-distance) to calculate the pairwise distance in the webserver available at https://bioinfo.mnhn.fr/abi/public/abgd/and https://bioinfo.mnhn.fr/abi/public/asap/for ABGD and ASAP, respectively (Puillandre et al. 2012, 2021). We adopted the result with the highest number of partitions for ABGD analysis (Franco et al. 2023; Puillandre et al. 2012). For ASAP analysis we used those with the lowest ASAP score and *p*-value according to Puillandre et al. (2021).

The PTP model used as input trees that comprised distinct haplotypes only. The identical sequences were removed, leaving only one representative sequence per haplotype in the alignments before building the trees by Bayesian analyses. These trees were constructed using the same methods cited above (see Section 2.3.3). In order to obtain reliable results, the trees used in the PTP analysis included sequences of at least one species known to be closely related to the studied taxon, as this is required for reliable species delimitation (Franco et al. 2023). The PTP analysis was performed on the Bayesian implementation in the PTP web server (https://species.h-its.org/) (Zhang et al. 2013), using the maximum number of Markov Chain Monte Carlo (MCMC) generations (500,000), a 0.25 burn-in and default sampled trees. The convergence of MCMC chains was verified using likelihood plots, following the recommendations of the PTP web server (Zhang et al. 2013).

3 Results

3.1 Molecular analyses

The comparison of the *Anaulus* strains isolated within each geographic location (Brazil and South Africa) resulted in 0 % genetic divergence for all genetic markers (Table 3). In addition, the genetic divergence between the *Anaulus* samples from Brazil versus South Africa (*rbc*L: 1 %, *5.8S* + *ITS*2*:* 1 %, *cox*1: 3 %) was within the range of intraspecific variation estimated for the other Mediophycean species, *Eunotogramma lunatum*, *Cerataulina pelagica*, *Lithodesmium intricatum*, *Neobrightwellia alternans*, and *Pseudictyota dubia* (Table 3). The *Anaulus* sequences from Brazil and South Africa formed a clade with high support (posterior probability, PP = 1) in the phylogenetic tree with two concatenated genetic markers (*rbc*L + partial *5.8S* + partial *ITS*2) (Figure 1) and the single locus tree of *cox*1 (Supplementary Figure S1).

Regarding the automated molecular species delimitation methods, all *Anaulus* sequences were considered to be conspecific for: the *rbc*L + partial *5.8S* + partial *ITS*2 (872 bp) alignment (analyzed by ABGD), the haplotype tree (analyzed by PTP; Figure 1), and the *cox*1 (436 bp) alignment (analyzed by ASAP) and the haplotype tree, analyzed by PTP (Figure S1). However, ASAP indicated each clade as a different species in *rbc*L + partial *5.8S* + partial *ITS*2 alignment (Figure 1). The concatenated tree (Figure 1) and alignment did not include *cox*1, because of the lack of multiple sequences from South African *Anaulus* or any sequences from *E. lunatum* (best outgroup), owing to amplification difficulties. Since the ABGD requires at least 2–3 sequences per studied clade (in this case: Brazilian *Anaulus* vs South African *Anaulus australis*), we could not use this marker in the analysis. In addition, we were not able to amplify all three markers simultaneously from the same Brazilian subtropical *Anaulus* strain, so that this population is not represented in a concatenated tree of three markers.

Table 3: Genetic divergence of diatoms of the Mediophyceae estimated for three barcode genes. *N*, number of pair-wise comparisons.

Taxon	*N*	*rbc*L (540 bp)	*N*	*5.8S* + *ITS*2 (317 bp)	*N*	*cox*1 (436 bp)
Eunotogramma lunatum	1	0 % (±0)	–	–	–	–
Cerataulina pelagica	10	0–1% (1 % ± 0.3)	1	1 % (±0)	–	–
Lithodesmium intricatum	10	0–1% (1 % ± 0.3)	–	–	10	0–5% (3 % ± 2)
Neobrightwellia alternans	10	0 % (±0)	10	0 % (±0)	3	0 % (±0)
Pseudictyota dubium	15	0 % (±0)	–	–	–	–
Mediophyceae/Intraspecific divergence	46/(5 spp.)	0–1% (0.3 % ± 0.4)	11/(3 spp.)	0–1% (0.1 % ± 0.2)	13/(2 spp.)	0–5% (3 % ± 2)
Mediophyceae/Interspecific divergence	207/(5 spp.)	7–11 % (9 % ± 1)	10/(3 spp.)	28–29 % (28 % ± 0.4)	15/(2 spp.)	15–16 % (16 % ± 0.3)
Anaulus australis (South Africa) vs. *Anaulus australis* (South Africa)	10	0 % (±0)	10	0 % (±0)	–	–
Anaulus sp. (Brazil) vs. *Anaulus* sp. (Brazil)	10	0 % (±0)	1	0 % (±0)	6	0 % (±0.1)
Anaulus australis (South Africa) vs. *Anaulus* sp. (Brazil)	25	1 % (±0.1)	10	1 % (±0)	4	3 % (±0)

N, number of pair-wise comparisons.

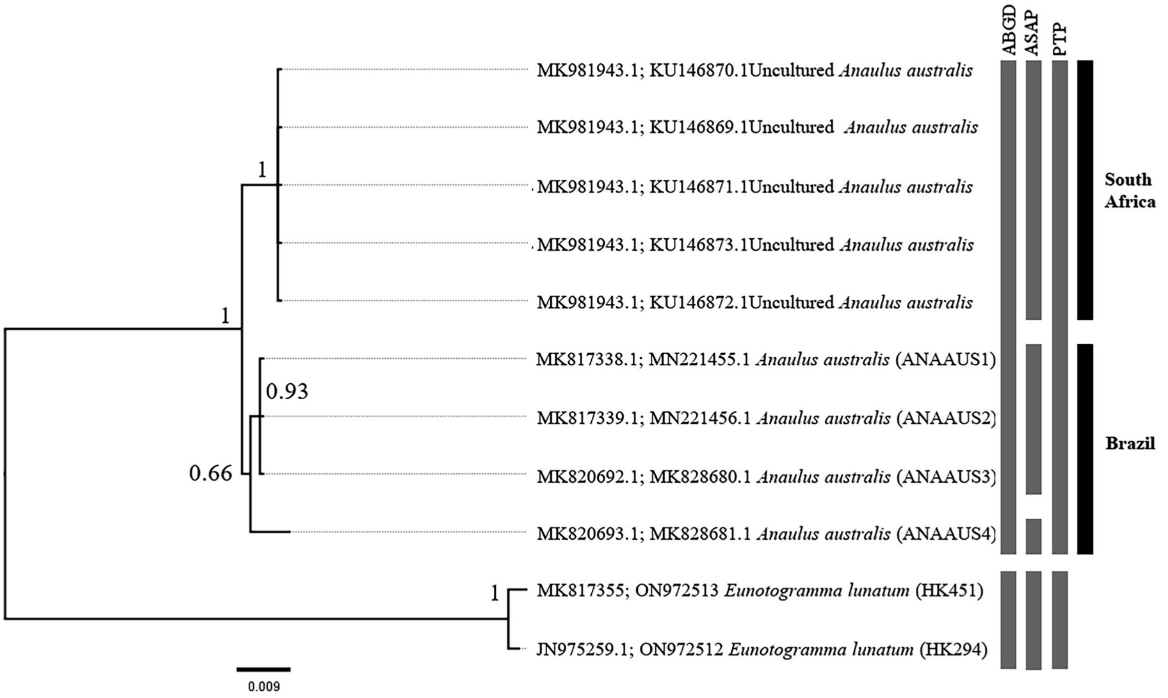

Figure 1: Bayesian phylogenetic tree of *Anaulus* built with three concatenated genes, *rbc*L + partial 5.8S + partial *ITS*2 (872 bp) and posterior probability. The results of species delimitation using Automatic Barcode Gap Discovery (ABGD) for primary species delimitation, Assemble Species by Automatic Partitioning (ASAP) and Poisson Tree Processes (PTP) models are indicated by gray bars, and the geographical origin of *Anaulus* sequences by black bars.

Within the *Anaulus* clade, the South African *Anaulus australis* (32°S–34°S) formed an internal clade with high support (PP = 1) indicating that these diatoms belong to a population of *Anaulus australis* distinct from those from Brazil (3°S–26°S) (Figure 1). The two internal clades formed by Brazilian *Anaulus autralis* were supported with PP values of between 0.65 and 0.93 (Figure 1).

For the *Asterionellopsis* strains, the sequences from Navegantes (ASTTHU 1, 2), Pontal do Sul (ASTTHU 3), and Cananéia (BMAK9) formed a clade with high support (PP = 1) with *Asterionellopsis thurstonii* in the *cox*1 tree (Figure 2). The genetic divergence among these strains of *A. thurstonii* was 0–1%, while the genetic divergence between these strains and other species was much higher (>10 %; Table 4). Therefore, we suggest that these strains isolated from Brazil correspond to *A. thurstonii* (Figures 3 and 4A).

The sequence obtained from a field sample at Torres Beach formed a clade with high support (PP = 1) with

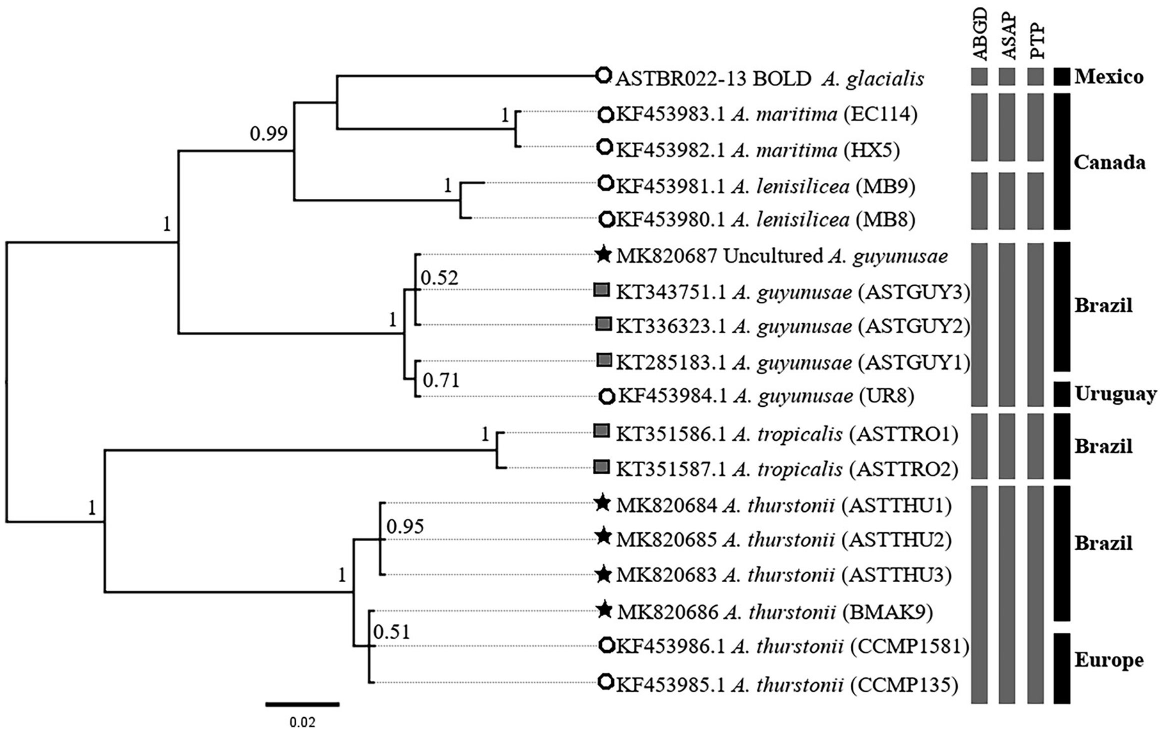

Figure 2: Bayesian phylogenetic tree of *Asterionellopsis* built with *cox*1 (423 bp) and posterior probability. The results of species delimitation using Automatic Barcode Gap Discovery (ABGD) for primary species delimitation, Assemble Species by Automatic Partitioning (ASAP) and Poisson Tree Processes (PTP) models are indicated by gray bars, and the geographical origin of *Asterionellopsis* sequences by black bars. The sequences of *Asterionellopsis* from Kaczmarska et al. (2014), Franco et al. (2016) and the present work are shown by open circles, gray squares and black stars, respectively.

Table 4: Genetic divergence between strains and field samples of *Asterionellopsis* isolated from the Brazilian coast and known species of *Asterionellopsis glacialis* complex.

	A. thurstonii	*A. guyunusae*	*A. tropicalis*	*A. glacialis*	*A. maritima*	*A. lenisilicea*
Asterionellopsis, Cananeia, São Paulo	0 %[a]	13 %	13 %	14 %	13 %	12–13 %
Asterionellopsis, Pontal do Sul, Paraná	1 %[a]	13 %	13 %	14 %	14 %	13 %
Asterionellopsis, Navegantes Beach, Santa Catarina (spring)	1 %[a]	13 %	13 %	14 %	14 %	13 %
Asterionellopsis, Navegantes Beach, Santa Catarina (winter)	1 %[a]	13 %	13 %	14 %	14 %	13 %
Asterionellopsis, Torres Beach, Rio Grande do Sul	13 %	0 %[a]	15 %	12 %	11 %	10–11 %

[a] Low genetic divergence that enables the identification of *Asterionellopsis* as the same species.

Asterionellopsis guyunusae (Figure 2) and the genetic divergence between this sequence and strains of *A. guyunusae* was 0 % (Table 4). Comparing this sequence with other species of the genus resulted in a much higher genetic divergence (10–15 %; Table 4). Therefore, we identified the diatoms of this accumulation as *A. guyunusae*. The ABGD, ASAP and PTP models were congruent, delimiting the six known *Asterionellospsis* species in the *cox*1 tree (Figure 2).

The clades of *A. thurstonii* and *A. guyunusae* also showed an internal structure that can be associated with populations, when they have high support (Figure 2). Some of the *A. thurstonii* from Brazil (ASTTHU 1, ASTTHU 2, ASTTHU 3) formed a clade with high support (PP = 0.95), while *A. thurstonii* from Europe and Cananeia (BMAK9) belong to a distinct clade that was not well resolved, due to low support (PP = 0.51; Figure 2). The *A. guyunusae* strains from the relatively close beaches of Torres (29°S), Cassino (32°S), and Uruguay (34°S) formed two internal clades with low support (PP = 0.52 and 0.71; Figures 2, 3, 4B). Thus, there are three *Asterionellopsis* species in Brazilian waters (Figures 2, 3 and 4A–C), based on the sequences produced by the present study and previous data available in the GenBank.

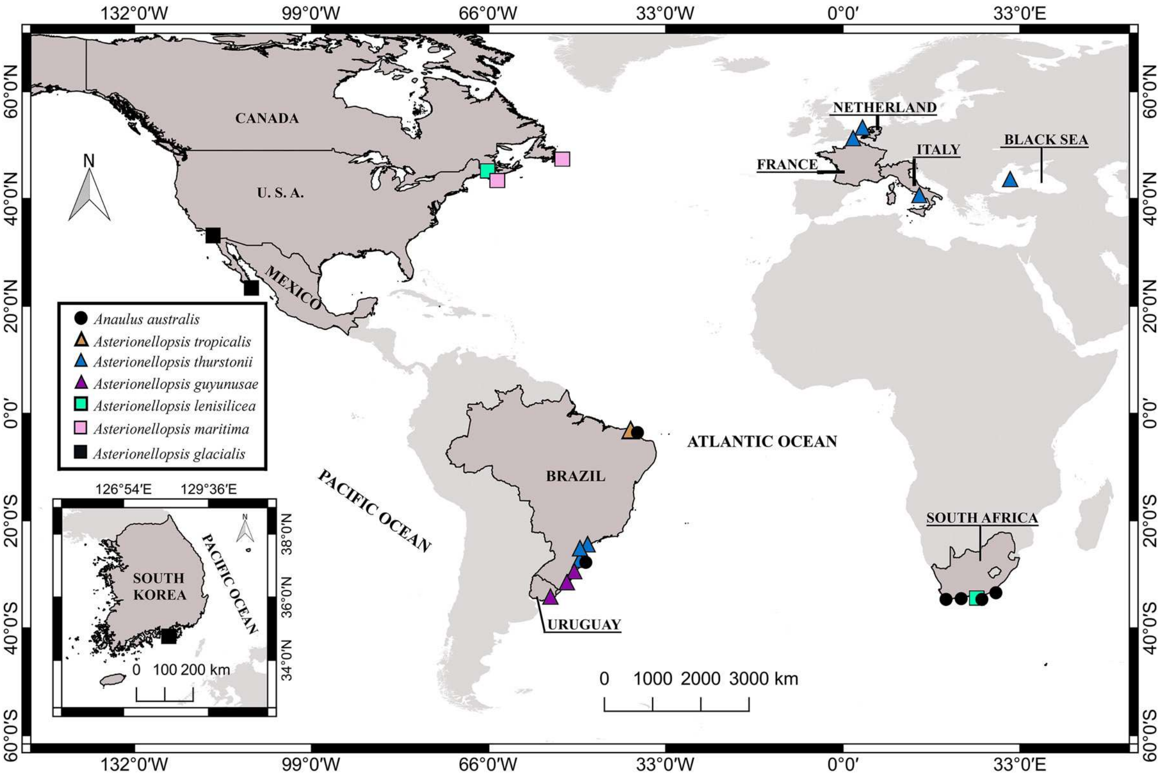

Figure 3: Global distribution of *Anaulus* and *Asterionellopsis* species identified by molecular tools, based on the results of the present work, Du Preez (2017), Franco et al. (2016), Kaczmarska et al. (2014), Montuori et al. (2023) and Daniele De Luca (personal communication).

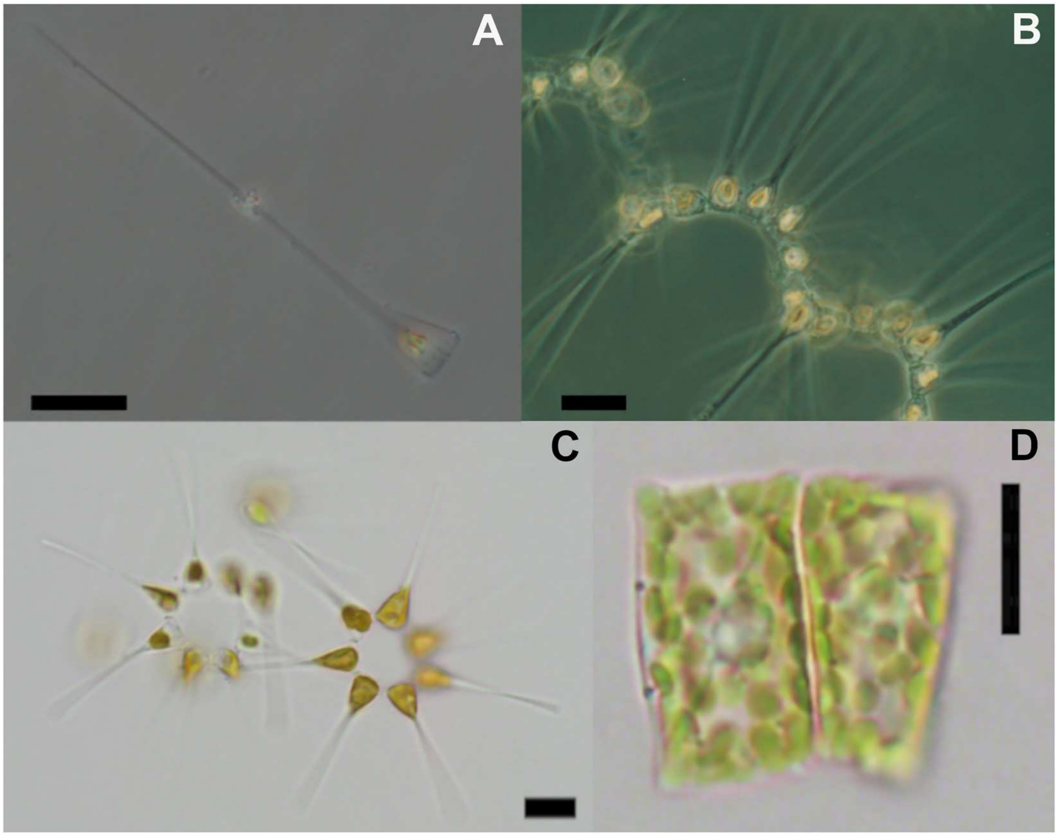

Figure 4: Light microscopy images of live cells of *Asterionellopsis* species and *Anaulus australis*. (A) *Asterionellopsis thurstonii* in girdle view from field samples collected at Navegantes Beach. Typical colonies of (B) *Asterionellopsis guyunusae* from field samples collected at Cassino Beach and (C) *Asterionellopsis tropicalis* (ASTTRO2 – strain) collected at Futuro Beach. (D) Typical small chain in girdle view of two live *Anaulus australis* cells. Scale bars = 20 μm.

3.2 Morphology of *Anaulus australis*

The cells of the Brazilian *Anaulus* contain many discoid chloroplasts (Figure 4D). The frustules are elliptical and slightly dorsoventral in valve view (Figures 5A–B), but the cells are more often seen in girdle view (Figures 4D, 5C and 6A) by light microscopy (Figure 4D) as rectangular cells with slight elevations at the apices (Figures 4D and 6A). The valves have a rimoportula located eccentrically between two primary internal costae in each valve (Figures B and BCFigures 5B 6B–CB B–CFigures 5B and 6B–C). There is a delicate areolation that radiates from the rimoportula (Figures 5A,B and 6B) and forms parallel rows as they reach the valve mantle (Figures 5C and 6A). This central areolation is interrupted by two hyaline lines (Figure 6B) that do not extend to the edges of the valve mantle (Figures 5C and 6A). These two hyaline lines are located just above the costae that cross the entire valve face and end in the mantle (Figures 5A and B, seen as hyaline lines outside the valve, and primary costae inside the valve). The girdle bands are open and show striae of pores, ligula and antiligula (Figures 6A, D). These characters correspond to the original description of *Anaulus australis* in samples from Algoa Bay, Sundays River Beach, South Africa.

All measurements of *Anaulus* from Futuro Beach samples showed values within the range of the original species description (Table 5). The frustules of the three tropical strains and two subtropical strains showed few differences compared with *Anaulus australis* holotype.

The cells from the two strains from Navegantes Beach (subtropical strains: ANAAUS 4, 5) were larger in their apical and transapical axes than in field samples and in the strains isolated from Futuro Beach (tropical region); however, these values were within the range of the original species description (Table 5). The pervalvar axis of the cells from Brazilian tropical strains was smaller (6.5–19 µm) than those observed in South African cells (12–24 µm; Table 5).

The areola density (number of areolae per stria in 10 µm) in the apical region (between the apex and the costa – Figures 5A and B) was similar to the areola density in the central region (between the two costae), indicating a homogeneous distribution of areolae on the valve face. In contrast, the areolar density on the valve in the central region of *A. australis* is the most variable morphological character in culture, as some cells of strains isolated from Futuro Beach (ANAAUS 1, 2, 3; 22–35) and Navegantes Beach

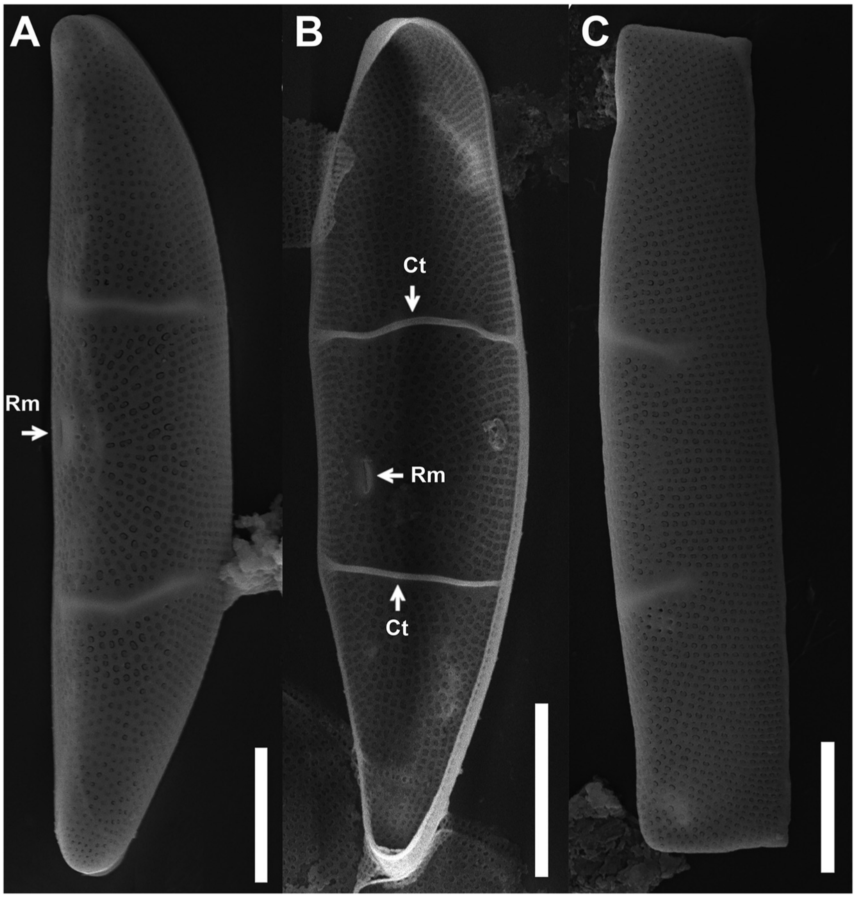

Figure 5: Scanning electron micrographs of Brazilian strains of *Anaulus australis* and from diatom accumulations collected during environmental plankton sampling at Futuro Beach. (A) External view of valve face showing radial areolation around opening of rimoportula (Rm) and two hyaline regions just above costae, which are located inside the cell. (B) Internal view of valve face showing position and form of two primary internal costae (Ct) and internal opening of the rimoportula (Rm). (C) Lateral view of valve mantle, showing parallel areolation and hyaline bands that extend to a part of the valve mantle. Scale bars = 5 µm.

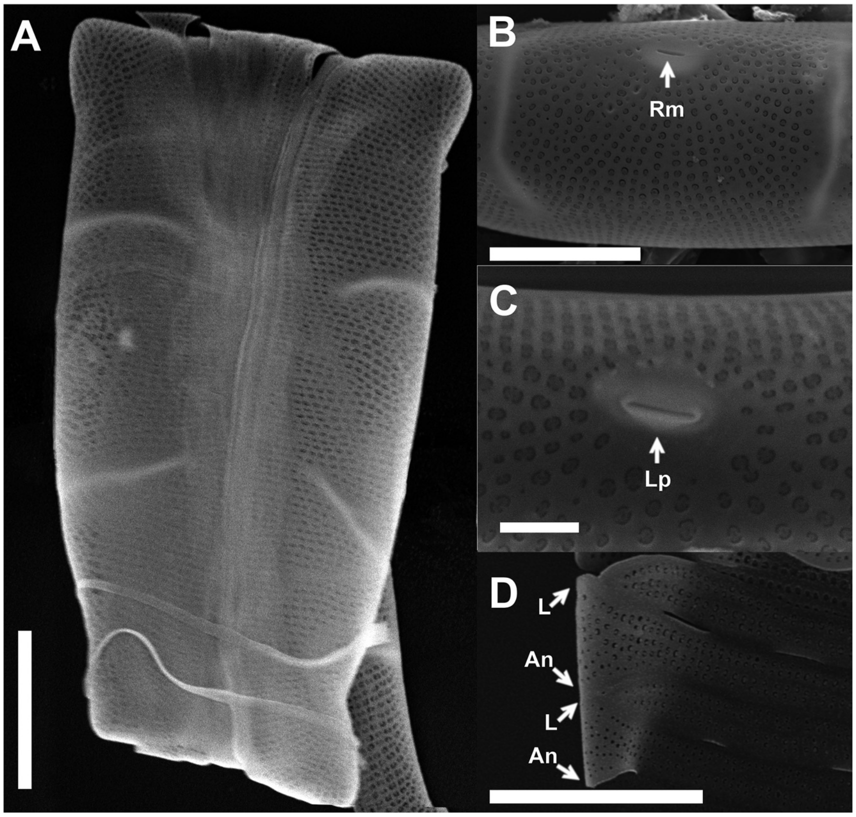

Figure 6: Scanning electron micrographs of Brazilian strains of *Anaulus australis* and from diatom accumulations collected during environmental plankton sampling at Futuro Beach. (A) Lateral view of frustule showing the cingulum connecting the two valves. (B) Details of valve central region, showing external slit opening of rimoportula (Rm). (C) Details of internal opening of rimoportula, a labiate process (Lp). (D) Details of girdle bands which are open and have striae of pores, ligula (L) and antiligula (An). Scale bars (A, B) = 5 μm; (C) = 1 μm; (D) = 5 μm.

Table 5: Morphological characters of *Anaulus australis* from Brazilian and South African field samples and from Brazilian tropical and subtropical strains. Minimum (Min), maximum (Max) and number of measurements (*N*) are provided.

Anaulus australis	South Africa, subtropical field samples, Sundays River Beach	Brazil, tropical field samples, Futuro Beach	Brazil, tropical strains	Brazil, subtropical strains
	Min–Max (*N*)	Min–Max (*N*)	Min–Max (*N*)	Min–Max (*N*)
Apical axis (μm)	10–52^{a}	18.8–27.9 (19)	18.8–36.2 (59)	30.0–37.4 (21)
Transapical axis (μm)	3–9^{a}	4.1–7.4 (17)	4.6–7.1 (29)	6.7–7.9 (10)
Pervalvar axis (μm)	12–24^{a}	–	6.5–19.0 (15)	–
Number of areolae per stria in 10 μm on the valvar central region	25–35^{a}	26–35 (24)	22–35 (37)	21–32 (14)
Number of areolae per stria in 10 μm on the valvar apex region	–	27–35 (20)	26–40 (34)	21–30 (13)
Number of areolae lines (rows of pores) on the cingular band	2–6^{1}	2–4	2–4	2–4
Number of areolae per stria in 10 μm on the row of cingular band	43–54 (10)^{b}	47–51 (6)	45–54 (24)	37–40 (3)

[a] Original species description (Drebes and Schulz 1989). [b] Measurements of cells from the holotype region: Sundays River Beach – Algoa Bay (present study).

(ANAAUS 4, 5; 21–32) had a smaller number than either the species description (25–35) or field samples from Futuro Beach (26–35; Table 5).

In the Brazilian *Anaulus* (strains and field samples), we observed 2–4 rows of pores in the cingular bands (Figure 6D), within the range of the original species description (2–6).

In both tropical field samples (Futuro Beach) and tropical strains (ANAAUS 1, 2, 3), the areola density per row in 10 μm in the cingulum band was within the range of South African specimens (Table 5). However, the subtropical strains (ANAAUS 4, 5) had a lower areola density in the cingulum band (37–40) than South African field samples (43–54; Table 5).

4 Discussion

4.1 *Anaulus* (Mediophyceae)

Morphological and molecular analyses corroborate the identification of *Anaulus* cells sampled in the surf-zone of tropical (3°S; Futuro Beach) and subtropical (26°S; Navegantes Beach) beaches as *Anaulus australis*, a species originally described from subtropical beaches in South Africa (Drebes and Schulz 1989). The low genetic divergence among the *Anaulus* strains from Brazil and South Africa corresponded to the range of intraspecific values found in other Mediophycean species analyzed in the present and previous studies (*cox1* 2–4% – Moniz and Kaczmarska 2009; *5.8S* + *ITS1* 0–11 %, mean 2 % – Moniz and Kaczmarska 2010; *rbcL* ≤ 2 % – MacGillivray and Kaczmarska 2011). In addition, all phylogenetic trees exhibited the formation of a clade with high support (PP = 1), including the Brazilian and South African *Anaulus*. The internal clades in *Anaulus* were characterized by short branches and high posterior probability in the South African clade, precluding a distinct population difference between Brazil and South Africa. In addition, most species identification models agreed that all sequences of *Anaulus* belong to the same species.

The morphological measurements of cells from field samples collected in the tropical Futuro Beach were all within the range of the species description (Drebes and Schulz 1989). However, the *Anaulus* strains isolated from the same beach had some cells with smaller size of the pervalvar axis and a lower areolar density per stria on the valve than the species description (Drebes and Schulz 1989). Since the pervalvar axis is associated with the addition of bands during cell growth (Round et al. 1990), these lower values may be associated with newly divided cells in culture. The lower areolar density per stria on the valve in some cells can be interpreted as an artifact of the culture conditions, since it did not occur in frustules from field samples. Almost all measurements of the frustules of the subtropical strains were within the range of the species description, except for the one lower number of areolae per row in 10 μm on the girdle band. Unfortunately, we do not have field samples from Navegantes Beach to verify if this was also true for field-collected cells. However, slight morphological differences between strains and field-collected cells are common in diatoms due to culture artifacts (Cox 2014).

Accumulations of cells identified by morphology as *Anaulus australis* were also observed in the surf zones of Australia and New Zealand (see references in Campbell 1996; Odebrecht et al. 2014), thus showing a wide geographical distribution in the Southern Hemisphere. However, further studies using molecular tools and ultrastructure are needed to confirm the identification of this species/population in Oceania. In addition to our study sites in Brazil, *A. australis* was registered in Bahia State (14°30′S–15°05′S; Tedesco et al. 2017), São Paulo State (24°S–25°S; Villac and Noronha 2008) and at Gi Beach, Santa Catarina State (28°28′S), its highest latitudinal occurrence in South America (Dutra and Garcia 2016). These environments are measurably different, as the temperature of Futuro Beach is stable all year (3°S; 27–30 °C, salinity 33–40; Franco et al. 2018) compared to Navegantes Beach (26°S; 17–29 °C, salinity 27–36; Macedo 2007) and also the South African coast (15–27 °C; Knox 2000). A connection between these continents can occur through ocean circulation, such as the anticyclonic Agulhas rings or eddies formed on the west coast of South Africa, which transport plankton to the South Atlantic subtropical basin (Villar et al. 2015). Interestingly at Cassino Beach, located at a higher latitude in Brazil, no *Anaulus* species were ever registered during a 26-year study (1994–2019) of phytoplankton samples analyzed monthly by light microscopy, a study carried out in the framework of the Long-Term Ecological Research in the Patos Lagoon estuary and adjacent coast (Odebrecht, personal communication). Compared to the other beaches studied, lower water temperatures during austral winter (usual range 10–15 °C) but also in summer (usually around 25 °C) were observed at Cassino Beach (32°S; Odebrecht et al. 2010). Therefore, there seems to be some sort of ecological barrier, perhaps temperature, that restricts the occurrence of *Anaulus* further south on the east coast of South America. Considering that *Anaulus* is present, and often dominant, in South African beaches located at higher latitudes and similar temperature ranges as found in Cassino Beach, other factors could also be important as phytoplankton dynamics at Cassino Beach are driven by diverse physical and chemical factors.

4.2 *Asterionellopsis glacialis* complex (Bacillariophyceae)

The taxonomy of *Asterionellopsis* species is better resolved (Du Preez 2017; Franco et al. 2016; Kaczmarska et al. 2014) than that of *Anaulus* (Witkowski et al. 2020). Previous studies have shown that morphological differentiation in the genus *Asterionellopsis* lags behind the genetic divergence (e.g. Franco et al. 2016; Kaczmarska et al. 2014) leading to morphologically cryptic or semicryptic species, in which the morphological measurements verified by light microscopy and SEM overlap with at least one other species (Franco et al. 2016; Kaczmarska et al. 2014). These species show clear molecular differentiation among themselves (Franco et al. 2016; Kaczmarska et al. 2014) and, for this reason, a reliable identification of *Asterionellopsis* strains at species level requires molecular approaches.

The *cox*1, *ITS* region and/or *rbc*L markers have the taxonomic resolution to define all *Asterionellopsis* species and produce the same identification results (Franco et al. 2016; Kaczmarska et al. 2014). On molecular phylogenetic analysis of *Asterionellopsis*, each species forms a distinct clade with high support using either *cox*1, *ITS* region and/or *rbc*L markers, in singe-locus or multi-locus trees (Franco et al. 2016; Kaczmarska et al. 2014). Due to the molecular differences between species, interspecific divergences are greater in *cox*1 (9–16 %) than in *rbc*L (2–6%) and *5.8S* + *ITS*2 (2–14 %) (Franco et al. 2016; Kaczmarska et al. 2014). Considering its higher genetic divergence, *cox*1 is less likely to produce misidentifications than *rbc*L and *5.8S* + *ITS*2. For these reasons, the cox1 data set was chosen as the best depiction of the taxonomic analysis outcome for *Asterionellopsis.*

The *Asterionellopsis* sequences in the present study were robustly and congruently identified by all molecular tools. Two species of the *Asterionellopsis glacialis* complex, *A. tropicalis* and *A. guyunusae,* have previously been recorded in Brazilian sandy beaches forming accumulations in tropical (3°S; Futuro Beach) and subtropical (32°S; Cassino Beach; 33°S; Chuy Beach) regions (Franco et al. 2016; Odebrecht et al. 2014). The known distribution of *A. guyunusae* is now expanded towards Torres Beach (29°S), which is the lowest latitude record for this species. We recorded *A. thurstonii* in the Southern Hemisphere for the first time: the *Asterionellopsis* sequences from beaches located at intermediate latitudes (25°S–26°S) in the states of São Paulo, Paraná, and Santa Catarina showed very low divergence and formed a clade with *A. thurstonii* that, until now, was found only in Europe (Kaczmarska et al. 2014; Montuori et al. 2023). All three *Asterionellopsis* species identified in Brazilian sandy beaches have at least one strain isolated from the surf zone where diatom accumulations were reported (Odebrecht et al. 2014: Futuro Beach, Navegantes Beach, Cassino Beach). However, only *A. tropicalis* and *A. guyunusae* were directly isolated from diatom accumulations (Franco et al. 2016). Therefore, it remains a question if *A. thurstonii* also forms surf zone patches. This species has been recorded in other neritic/coastal habitats elsewhere: strains of *A. thurstonii* were isolated from the Black Sea (Kaczmarska et al. 2014); the English Channel – waters off Dunkirk, France; and from the Gulf of Naples, Mediterranean Sea, at the Long-term Ecological Research station MareChiara (Montuori et al. 2023). By the same token, we cannot discard the hypothesis that *A. tropicalis* and *A. guyunusae,* species described from surf zone samples, also inhabit other neritic habitats.

The *Asterionellopsis glacialis sensu lato* complex is an important neritic taxon (Abreu et al. 1995; Al-Kandari et al. 2009; Hoppenrath et al. 2009), which includes different species that together show a wide environmental distribution. In South America, the latitudinal distribution of three species suggests that water temperature could be an influencing factor, but specific studies should be conducted to test this hypothesis. Seasonal variation could also influence species distribution, mainly in subtropical and warm temperate beaches where seasonal water temperature changes are observed. Strains of *A. glacialis sensu lato* isolated from different Brazilian beaches have distinct fatty acid compositions and appear to have different salinity preferences: salinity 21 for a strain isolated from Navegantes Beach (26°S) and salinity 28 for a strain isolated from Rincão Beach (28°S; Rörig et al. 2017). The reliable identification of *Asterionellopsis* species may be an important key to understanding the ecological and physiological processes in surf zones.

4.3 Diversity patterns of *Anaulus* and *Asterionellopsis*

The *Anaulus australis* and *Asterionellopsis glacialis* complexes co-occur and alternate in dominance in most accumulations observed in Brazilian tropical and subtropical sandy beaches (Odebrecht et al. 2014). However, only one species of *Anaulus* was identified on the Southwest Atlantic coast, whereas three species of the *Asterionellopsis glacialis* complex (*A. tropicalis*, *A. thurstonii*, *A. guyunusae*) were recorded for the same region, with *A. thurstonii* being recorded here for the first time in the Southern Hemisphere. *Anaulus* appears to be restricted to surf zones and *Asterionellopsis* species also occur in other neritic habitats. We may suppose that these taxa are under similar environmental selective pressures in surf zones and should overlap in their ecological niches. Diversity patterns may indicate evolutionary and speciation processes (Schluter and Pennell 2017) and the higher number of *Asterionellopsis* than of *Anaulus* species in the same area and habitat may be related to distinct rates of speciation and gene flow, dispersion or age differences between the genera.

The geographical distribution and presence of internal structure in clades of *Anaulus australis* and *Asterionellopsis* species agree with the proposed “intermediate dispersal hypothesis” (Mann and Vanormelingen 2013). According to this hypothesis, long-distance dispersal can occur and be effective enough for some diatom species to establish over large geographic areas, but gene flow is not powerful enough to prevent population differentiation, as observed in the distribution of *A. thurstonii* (Brazil vs. Europe), *Anaulus australis* (Brazil vs. South Africa) and *A. lenisilicea* (Canada vs. South Africa), the latter based on data from Du Preez (2017) and Kaczmarska et al. (2014). *Asterionellopsis glacialis* also shows a wide distribution (USA vs. Mexico vs. South Korea), but no distinct phylogenetic clades to indicate population variations appear in trees produced by Du Preez (2017) and Kaczmarska et al. (2014).

Using a time-calibrated tree with 11 genes and 1151 taxa, Nakov et al. (2018) estimated that the diversification rate can be higher in raphid pennate diatoms than in the centric diatoms (Mediophyceae and Coscinodiscophyceae). These results support the hypothesis that centric diatoms maintain a greater gene flow among distant populations and lower diversification rates than raphid pennate diatoms. Our results suggest that araphid pennates, at least in the genus *Asterionellopsis,* also present higher diversification rates than *Anaulus* (centric diatom – Mediophyceae), since *Anaulus* and *Asterionellopsis* showed a contrasting diversity pattern from the tropical to subtropical/warm temperate eastern coast of South America (3°S to 34°S; Figure 3). However, the worldwide distribution of both genera is still poorly known and further studies, particularly in the Pacific and Indian Oceans (for both genera), and in Northern Hemisphere waters (for *Anaulus*), are necessary to advance our knowledge of gene flow and dispersal of free microorganisms in the marine environment.

5 Conclusions

We have shown that surf diatoms are a potential model for the study of speciation patterns in the marine environment. The genera *Asterionellopsis* and *Anaulus* show contrasting diversity patterns and spatial distribution in surf zones along the Brazilian coast: a single species of *Anaulus australis* was recorded in tropical and subtropical beaches, while at least three species of *Asterionellopsis*: *A. tropicalis*, *A. thurstonii* and *A. guyunusae* were observed in distinct latitudes. The phylogeny and spatial distribution of *Anaulus australis* and *Asterionellopsis thurstonii* agree with the proposed “intermediate dispersal hypothesis”. In the present study, *Asterionellopsis thurstonii* was recorded for the first time in South America. The apparent absence of *Anaulus australis* at Cassino Beach (32°S) requires further experimental physiological studies to test the role of water temperature as a potential environmental barrier restricting this species to lower latitude beaches in Brazil. Experimental studies would also help to answer the question whether the observed latitudinal distribution of *Asterionellospsis* species along the Brazilian coast is a function of water temperature and salinity ranges.

Acknowledgments: We thank the Plankton Laboratory team of LABOMAR of the Federal University of Ceará and Prof. Dr. Luiz L. Mafra Jr. of Federal University of Paraná for providing diatom samples for isolation. The Banco de Microrganismos Aidar & Kutner (BMA&K) of University of São Paulo for providing the strain from Baía do Trapandé, Cananéia, São Paulo. We would also like to thank the reviewers for their detailed analysis which improved the manuscript.

Research ethics: Not applicable.

Author contributions: The authors have accepted respon-sibility for the entire content of this manuscript and approved its submission. A.O.R. Franco: original concept, drafting and editing manuscript, culturing, acquiring molec-ular and morphological electron microscopy data, phylo-genetic and morphological analysis; M.P. Ashworth: drafting and editing manuscript, culturing, acquiring molecular data; D. Du Preez: editing manuscript, environmental sampling, acquiring molecular data; E. Campbell: editing manuscript, environmental sampling, morphological electron microscopy data; R. Maggioni: editing manuscript, acquiring molecular data, phylogenetic analysis; S.R. Manning: editing manuscript, culturing, acquiring molecular data; C. Odebrecht: original concept, drafting and editing manuscript, environmental sampling for culture.

Competing interests: The authors states no conflict of interest.

Research funding: This work was supported by Brazilian funding agencies: CNPq Proc. 203883/2017-9; the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001; Brazilian Long-Term Ecological Research: CNPq/CAPES/FAPERGS/BC – Fundo Newton/PELD nº 15/2016.

Data availability: The alignments and bayesian phylogenetic trees used are available in FigShare: https://doi.org/10.6084/m9.figshare.20372232.v2.

References

Abreu, P.C.O.V., Graneli, H.W., and Odebrecht C. (1995). Produção fitoplanctônica e bacteriana na região da pluma estuarina da Lagoa dos Patos, RS, Brasil. Atlântica 17: 35–52.

Akaike, H. (1974). A new look at the statistical model identification. IEEE Trans. Autom. Contr. 19: 716–723.

Al-Kandari, M., Al-Yamani F. and Al-Rifaie, K. (2009). *Phytoplankton atlas of Kuwait's waters*. Kuwait Institute for Scientific Research. Lucky Printing Press, Kuwait.

Amato, A., Kooistra, W.H.C.F., Ghiron, J.H.L., Mann, D.G., Pröschold, T., and Montresor, M. (2007). Reproductive isolation among sympatric cryptic species in marine diatoms. Protist 158: 193–207.

Andersen, R.A. (2005). *Algal culturing techniques*. Elsevier Academic Press, Amsterdam.

Ashworth, M., Nakov, T., and Theriot, E.C. (2013). Revisiting Ross and Sims (1971): toward a molecular phylogeny of the Biddulphiaceae and Eupodiscaceae (Bacillariophyceae). J. Phycol. 49: 1207–1222.

Auinger, B.M., Pfandl, K., and Boenigk, J. (2008). Improved methodology for identification of protists and microalgae from plankton samples preserved in Lugol's iodine solution: combining microscopic analysis with single–cell PCR. Appl. Environ. Microbiol. 74: 2505–2510.

Ballesteros, I., Castillejo, P., Haro, A.P., Montes, C.C., Heinrich, C., and Lobo, E.A. (2020). Genetic barcoding of Ecuadorian epilithic diatom species suitable as water quality bioindicators. C. R. Biol. 343: 41–52.

Battarbee, R.W. (1986). Diatom analyses. In: Berglund, B.E. (Ed.), *Handbook of Holocene palaeoecology and paleohydrology*. Wiley, Chichester, pp. 527–570.

Campbell, E.E. (1996). The global distribution of surf diatom accumulations. Rev. Chil. de Hist. Nat. 69: 495–501.

Chamnansinp, A., Li, Y., Lundholm, N., and Moestrup, Ø. (2013). Global diversity of two widespread, colony–forming diatoms of the marine plankton, *Chaetoceros socialis* (syn. C. radians) and *Chaetoceros gelidus sp. nov*. J. Phycol. 49: 1128–1141.

Cox, E. (2014). Diatom identification in the face of changing species concepts and evidence of phenotypic plasticity. J. Micropalaeontol. 33: 111–120.

Dąbek, P., Ashworth, M.P., Gorecka, E., Krzywda, M., Bornman, T.G., Sato, S., and Witkowski, A. (2019). Toward a multigene phylogeny of the Cymatosiraceae (Bacillariophyta, Mediophyceae) II: morphological and molecular insights into the taxonomy of the forgotten species *Campylosira africana* and of *Extubocellulus*, with a description of two new taxa. J. Phycol. 55: 425–441.

Darriba, D., Taboada, G.L., Doallo, R., and Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. Nat. Methods 9: 77.

Drebes, G. and Schulz, D. (1989). *Anaulus australis* sp. nov. (Centrales, Bacillariophyceae), a new marine surf zone diatom, previously assigned to *A. birostratus* (Grunow) Grunow. Bot. Mar. 32: 53–64.

Du Preez, D.P. (2017). *Phylogeny and phylogeography of dominant South African surf diatoms*, Master dissertation. Port Elizabeth, Nelson Mandela University, Available at: <https://vital.seals.ac.za/vital/access/manager/Repository/vital:30778?site_name=Global> (Accessed May 2022).

Dutra, D.B. and Garcia, M. (2016). Diatomáceas fitoplanctônicas da Praia do Gi, Laguna, Santa Catarina, Brasil. Acta Biológica Catarinense 3: 102–120.

Evans, M.K., Wortley, A.H., and Mann, G.D. (2007). An assessment of potential diatom “barcode” genes (*cox*1, *rbc*L, 18S and *ITS* rDNA) and their effectiveness in determining relationships in *Sellaphora* (Bacillariophyta). Protist 158: 349–364.

Franco, A.O.R., They, N.H., Canani, L.G.C., Maggioni, R., and Odebrecht, C. (2016). *Asterionellopsis tropicalis* (Bacillariophyceae): a new tropical species found in diatom accumulations. J. Phycol. 52: 888–895.

Franco, A.O.R., Soares, M.S., and Moreira, M.O.P. (2018). Diatom accumulations on a tropical meso-tidal beach: environmental drivers on phytoplankton biomass. Estuar. Coast. Shelf Sci. 207: 414–421.

Franco, A.O.R., Ashworth, M.P., and Odebrecht, C. (2023). Comparison between *p*-distance and single-locus species delimitation models for delineating reproductively tested strains of pennate diatoms (Bacillariophyceae) using *cox1, rbcL* and *ITS*. J. Eukaryot. Microbiol. e12986, https://doi.org/10.1111/jeu.12986.

Gaonkar, C.C., Kooistra, W.H.C.F., Lange, C.B., Montresor, M., and Sarno, D. (2017). Two new species in the Chaetoceros socialis complex (Bacillariophyta): *C. sporotruncatus* and *C. dichatoensis*, and characterization of its relatives, *C. radicans* and *C. cinctus*. J. Phycol. 53: 889–907.

Gaonkar, C.C., Piredda, R., Minucci, C., Mann, D.G., Montresor, M., Sarno, and D., and Kooistra, W.H.C.F. (2018). Annotated 18S and 28S rDNA reference sequences of taxa in the planktonic diatom family Chaetocerotaceae. PLoS One 13: e0208929.

Guillard, R.R.L. (1975). Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., and Chanley, M.H. (Eds.), *Culture of marine invertebrate animals*. Springer, New York, pp. 29–60.

Hoppenrath, M., Elbrächter, M., and Drebes, G. (2009). *Marine phytoplankton: selected microphytoplankton species from the North Sea around Helgoland and Sylt*. E. Schweizerbart'sche Verlagbuchhandlung, Stuttgart, Germany.

Iwatani, N., Murakami, S., and Suzuki, Y. (2005). A sequencing protocol of some DNA regions in nuclear, chloroplastic and mitochondrial genomes with an individual colony of *Thalassiosira nordenskioeldii* Cleve (Bacillariophyceae). Polar Biosci 18: 35–45.

Kaczmarska, I., Beaton, M., Benoit, A.C., and Medlin, L.K. (2005). Molecular phylogeny of selected member of the order Thalassiosirales (Bacillariophyta) and evolution of the Fultoportula. J. Phycol. 42: 121–138.

Kaczmarska, I., Mather, L., Luddington, I.A., Muise, F., and Ehrman, J.M. (2014). Cryptic diversity in a cosmopolitan diatom known as *Asterionellopsis glacialis* (Fragilariaceae): implications for ecology, biogeography, and taxonomy. Am. J. Bot. 101: 267–286.

Knox, G.A. (2000). *The Ecology of seashores*. CRC Press, Boca Raton.

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

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35: 1547–1549.

Lang, I. and Kaczmarska, I. (2011). A protocol for a single-cell PCR of diatoms from fixed samples: method validation using *Ditylum brightwellii* (West) Grunow. Diatom Res 26: 43–49.

Li, C.L., Witkowski, A., Ashworth, M.P., Przemyslaw, D., Sato, S., Zglobicka, I., Malgorzata, W., Khim, J.S., and Kwon, C.J. (2018). The morphology and molecular phylogenetics of some marine diatom taxa within the Fragilariaceae, including twenty undescribed species and their relationship to *Nanofrustulum, Opephora* and *Pseudostaurosira*. Phytotaxa 355: 001–104.

Macedo, C.X. (2007). Dinamica temporal de microalgas de zona de arrebentação na praia de Navegantes – SC, Master dissertation. Universidade Federal do Paraná. Colletion of Universidade Federal do Paraná (UFPR). Available at: <https://acervodigital.ufpr.br/handle/1884/17738> (Accessed May 2022).

MacGillivary, M.L. and Kaczmarska, I. (2011). Survey of the efficacy of a short fragment of the *rbc*L gene as a supplemental DNA barcode for diatoms. J. Eukaryot. Microbiol. 58: 529–536.

Mann, D.G. and Vanormelingen, P. (2013). An inordinate fondness? The number, distributions, and origins of diatom species. J. Eukaryot. Microbiol. 60: 414–420.

Mann, D.G., Thomas, S.J., and Evans, K.M. (2008). Revision of the diatom genus *Sellaphora*: a first account of the larger species in the British Isles. Fottea 9: 15–78.

Mann, D.G., Sato, S., Trobajo, R., Vanormelingen, P., and Souffreau, C. (2010). DNA barcoding for species identification and discovery in diatoms. Cryptogam. Algol. 31: 557–577.

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

Moniz, M.B.J. and Kaczmarska, I. (2009). Barcoding diatoms: is there a good marker? Mol. Ecol. Resour. 9: 65–74.

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

Montuori, E., Martinez, K.A., De Luca, D., Ianora, A., and Lauritano, C. (2023). Transcriptome sequencing of the diatom *Asterionellopsis thurstonii* and *in silico* identification of enzymes potentially involved in the synthesis of bioactive molecules. Mar. Drugs 21: 126.

Nakov, T., Beaulieu, J.M., and Alverson, A.J. (2018). Accelerated diversification is related to life history and locomotion in a hyperdiverse lineage of microbial eukaryotes (diatoms, Bacillariophyta). New Phytol. 219: 462–473.

Nanjappa, D., Kooistra, W.H.C.F., and Zingone, A. (2013). A reappraisal of the genus *Leptocylindrus* (Bacillariophyta), with the addition of three species and the erection of *Tenuicylindrus* gen. nov. J. Phycol. 49: 917–936.

Nei, M. and Kumar, S. (2000). *Molecular evolution and phylogenetics*. Oxford University Press, New York, p. 333.

Odebrecht, C., Bergesch, M., Rörig, L.R., and Abreu, P.C. (2010). Phytoplankton interannual variability at Cassino Beach, Southern Brazil (1992–2007), with emphasis on the surf zone diatom *Asterionellopsis glacialis*. Estuar. Coast 33: 570–583.

Odebrecht, C., Du Preez, D.R., Abreu, P.C., and Campbell, E.E. (2014). Surf zone diatoms: a review of the drivers, patterns and role in sandy beaches food chains. Estuar. Coast. Shelf Sci. 150: 24–35.

Pinseel, E., Hejduková, E., Vanormelingen, P., Kopalová, K., Vyverman, W., and Vijver, B.V. (2017). *Pinnularia catenaborealis sp. nov*. (Bacillariophyceae), a unique chain-forming diatom species from James Ross Island and Vega Island (Maritime Antarctica). Phycologia 56: 94–107.

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

Puillandre, N., Lambert, A., Brouillet, S., and Achaz, G. (2012). ABGD, automatic barcode gap discovery for primary species delimitation. Mol. Ecol. 21: 1864–1877.

Puillandre, N., Brouillet, S., and Achaz, G. (2021). ASAP: assemble species by automatic partitioning. Mol. Ecol. Resour. 21: 609–620.

Quijano-Scheggia, S.I., Garcés, E., Lundholm, N., Moestrup, Ø., Andree, K., and Camp, J. (2009). Morphology, physiology, molecular phylogeny and sexual compatibility of the cryptic *Pseudo-nitzschia delicatissima* complex (Bacillariophyta), including the description of *P. arenysensis* sp. nov. Phycologia 48: 492–509.

Rambaut, A., Drummond, A.J., Xie, D., Baele, G., and Suchard, M.A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst. Biol. 67: 901–904.

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

Rörig, L.R., Honorato, M.C., Itakazu, A.G., Macedo, C.X., Deschamps, F., Lins, J.V.H., Maraschin, M., Ramlov, F., Gressler, P.D., and Filho, J.P. (2017). Ecophysiological and biochemical variation of the surf zone diatom *Asterionellospsis glacialis* sensu lato from Santa Catarina, Southern Brazil. Braz. J. Oceanogr. 65: 695–708.

Round, F.E., Crawford, R.M., and Mann, D.G. (1990). *The diatoms. Biology and morphology of the genera*. Cambridge University Press, Cambridge.

Sarno, D., Kooistra, W.H.C.F., Medlin, L.K., Percopo, I., and Zingone, A. (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.

Schluter, D. and Pennell, M.W. (2017). Speciation gradients and the distribution of biodiversity. Nature 546: 48–55.

Seddon, A.W.R., Froyd, C.A., and Wikowski, A. (2011). Diatoms (Bacillariophyta) of isolated islands: new taxa in the genus *Navicula* sensu stricto from the Galápagos Islands. J. Phycol. 47: 861–879.

Simonsen, R. (1974). *The diatom plankton of the Indian Ocean Expedition of RV "Meteor" 1964–1965 (Meteor Forschungsergebnisse)*. Gebrüder Borntraeger, Berlin.

Sims, P.A., Williams, D.M., and Ashworth, A. (2018). Examination of type specimens for the genera *Odontella* and *Zygoceros* (Bacillariophyceae) with evidence for the new family *Odontellaceae* and a description of three new genera. Phytotaxa 382: 01–56.

Takano, Y. and Horiguchi, T. (2006). Acquiring scanning electron microscopical, light microscopical and multiple gene sequence data from a single dinoflagellate cell. J. Phycol. 42: 251–256.

Tedesco, E.C., Ribeiro, S.M.M.S., Pompeu, M., Gaeta, S.A., and Cavalcante, K.P. (2017). Low-latitude accumulation of the surf-zone diatoms *Anaulus australis* Drebes & Schulz and *Asterionellopsis glacialis* (Castracane) Round species complex in the eastern coast of Brazil. Braz. J. Oceanogr. 65: 324–331.

Theriot, E.C., Ashworth, M., Ruck, E., Nakov, T., and Jansen, R.K. (2010). A preliminary multigene phylogeny of the diatoms (Bacillariophyta): challenges for future research. Plant Ecol. Evol. 143: 278–296.

Theriot, E.C., Ashworth, M., Nakov, T., Ruck, E., and Jansen, R.K. (2015). Dissecting signal and noise in diatom chloroplast protein encoding genes with phylogenetic information profiling. Mol. Phylogenet. Evol. 89: 28–36.

Trobajo, R., Clavero, E., Chepurnov, V.A., Sabbe, K., Mann, D.G., Ishihara, S., and Cox, E.J. (2009). Morphological, genetic and mating diversity within the widespread bioindicator *Nitzschia palea* (Bacillariophyceae). Phycologia 48: 443–459.

Trobajo, R., Mann, D.G., Clavero, E., Evans, K.M., Vanormelingen, P., and McGregor, R.C. (2010). The use of partial *cox*1, *rbcL* and LSU rDNA sequences for phylogenetics and species identification within the *Nitzschia palea* species complex (Bacillariophyceae). Eur. J. Phycol. 45: 413–425.

Villac, M.C. and Noronha, V.A.P.C. (2008). The surf-zone phytoplankton of the State of São Paulo, Brazil. I. Trends in space-time distribution with emphasis on *Asterionellopsis glacialis* and *Anaulus australis* (Bacillariophyta). Nova Hedwigia 133: 115–129.

Villar, E., Farrant, G.K., Follows, M., Garczarek, L., Speich, S., Audic, S., Bittner, L., Blanke, B., Brum, J.R., Brunet, C., et al. (2015). Ocean plankton. Environmental characteristics of Agulhas rings affect interocean plankton transport. Science 348: 1261447.

Williams, D.M. (1985). *Morphology, taxonomy and inter-relationships of the ribbed araphid diatoms from the genera Diatoma and Meridion (Diatomaceae: Bacillariophyta)*. Bibliotheca Diatomologica 8. J. Cramer.

Witkowski, A., Ashworth, M., Li, C., Sagnad, I., Yatte, D., Górecka, E., Franco, A.O.R., Kusber, W.H., Klein, G., Lange-Bertalota, H., et al. (2020). Exploring diversity, taxonomy and phylogeny of diatoms (Bacillariophyta) from marine habitats. Novel taxa with internal costae. Protist 171: 125713.

Zhang, J., Kapli, P., Pavlidis, P., and Stamatakis, A. (2013). A general species delimitation method with applications to phylogenetic placements. Bioinformatics 29: 2869–2876.

Zingone, A., Percopo, I., Sims, P., and Sarno, D. (2005). Diversity in the genus *Skeletonema* (Bacillariophyceae). I. A reexamination of S. costatum with the description of *S. grevillei* sp. nov. J. Phycol. 41: 140–150.

Supplementary Material: This article contains supplementary material (https://doi.org/10.1515/bot-2022-0067).

Bionotes

Andréa de Oliveira da Rocha Franco
Institute of Oceanography, Federal University of Rio Grande – FURG, Av. Italia, km 8, CEP 96203-900, Rio Grande, Brazil
andrea.ecomarine@gmail.com
https://orcid.org/0000-0002-6656-8358

Andréa de Oliveira da Rocha Franco is a PhD candidate in Biological Oceanography at Universidade Federal do Rio Grande in Brazil. Her research expertise is ecology and taxonomy (morphological and molecular data) of marine phytoplankton, currently focusing on plankton communities from surf zones associated to events of diatom accumulations.

Matt Peter Ashworth
Department of Molecular Biosciences, UTEX Culture Collection of Algae, University of Texas at Austin, Austin, TX 78712, USA

Matt Peter Ashworth earned his PhD in Plant Biology from the University of Texas at Austin and is currently employed as a research associate at the UTEX Culture Collection of Algae at the University of Texas in the United States. His research is focused on the evolution, systematics and mechanisms of diversification in diatoms, using morphological, molecular and natural history data.

Debbie Du Preez
Department of Botany, Nelson Mandela University, Port Elizabeth 6031, South Africa

Debbie Du Preez is a PhD candidate in Botany at Nelson Mandela University, School of Enviromental Sciences, Deparment of Botany, Gqeberha, South Africa. Her research expertise is in the field of marine botany focusing on surf-zone diatoms along the South African coastline.

Eileen Campbell
Department of Botany, Nelson Mandela University, Port Elizabeth 6031, South Africa

Eileen Campbell is an Associate Professor at Nelson Mandela University, School of Enviromental Sciences, Deparment of Botany, Gqeberha, South Africa. She is one of the leaders in the biology of surf-diatoms in general and *Anaulus australis* in particular. She has worked on most temperate surf-diatom beaches on four continents.

Clarisse Odebrecht
Institute of Oceanography, Federal University of Rio Grande – FURG, Av. Italia, km 8, CEP 96203-900, Rio Grande, Brazil
claodebrecht@gmail.com
https://orcid.org/0000-0001-7159-4713

Clarisse Odebrecht has a PhD in Natural Sciences/Biological Oceanography and is Volunteer Professor at the Post Graduation Programme in Biological Oceanography. Her research expertise is within the field of marine ecology and biological oceanography focusing on phytoplankton taxonomy and ecology in estuaries and coastal environments.