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RECEIVED 03 October 2023

ACCEPTED 04 December 2023

PUBLISHED 07 February 2024

CITATION

Almandoz GO, Fabro E, Sprong P,
Mascioni M, Antoni J, Ferrario M,
Metfies K and Barrera FM (2024)
Metabarcoding and microscopy
characterization of phytoplankton
from frontal areas of the Argentine Sea.
Front. Mar. Sci. 10:1306336.
doi: 10.3389/fmars.2023.1306336

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Metabarcoding and microscopy characterization of phytoplankton from frontal areas of the Argentine Sea

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The Argentine Sea is worldwide recognized as a highly productive area, characterized by extensive phytoplankton blooms during spring and summer. Despite the well-known importance of frontal areas for biomass accumulation, phytoplankton diversity remains poorly studied. In an unprecedented approach for the Argentine Sea, we combined microscopy and 18Sv4 metabarcoding analyses for a refined assessment of summer phytoplankton composition in three understudied frontal areas of the Argentine Sea ($\approx 43^{\circ}$ – 55° S), with contrasting oceanographic conditions. Metabarcoding and microscopy analyses agreed on the detection of the dominant phytoplanktonic groups in the different frontal areas studied; chlorophytes in Valdés Peninsula, dinoflagellates in waters off Blanco Cape, and diatoms in de los Estados Island. The analysis of the phytoplankton community was significantly enriched by combining both techniques, microscopy provided cell abundances and biomass data and metabarcoding provided greater detail on species composition, revealing an important specific richness of dinoflagellates, diatoms and other delicate groups, such as chlorophytes. However, we also considered differences between the methods for certain taxa at a lower taxonomic level (species/genus) of the dominant taxa, such as the underestimation of the diatoms *Asterionellopsis glacialis* and *Pseudo-nitzschia* spp. and the overestimation of *Chaetoceros contortus* by metabarcoding in comparison to microscopic counts. The detection of several taxa belonging to small and delicate groups, previously overlooked due to the lack of distinct morphological features, establishes a baseline for future studies on phytoplankton diversity in the Argentine Sea.

KEYWORDS

metabarcoding, dinoflagellates, unidentified *Gyrodinium*, chlorophyte blooms, *Pycnococcus provasolii*

1 Introduction

The Argentine Sea, located in the southwestern Atlantic Ocean ($\approx 37\text{--}55^\circ\text{S}$), is recognized as an area of high phytoplankton biomass accumulation based on multi-year satellite observations (Rivas et al., 2006). Large phytoplankton blooms occur during austral spring and summer, mainly associated with the occurrence of oceanographic fronts (Acha et al., 2004; Piola, 2008). High chlorophyll *a* (Chl-*a*) concentrations have been mostly associated with diatoms and dinoflagellates (Carreto et al., 2016; Gonçalves-Araujo et al., 2016; Guinder et al., 2018; Nunes et al., 2019), and also with coccolithophorids (García et al., 2011). Blooms of small (2–5 μm) flagellated or coccoid cells has also been observed, during late summer (Almandoz et al., 2007; Silva et al., 2009; Antacli et al., 2018), although their taxonomic identity has not been clearly resolved.

Several microscopy-based studies revealed a high diatom and dinoflagellate diversity in different regions of the Argentine sea (Balech, 1988; Cefarelli et al., 2010; Antacli et al., 2018). Moreover, detailed taxonomic studies, based on molecular and/or ultrastructural analysis of isolated strains or field organisms, have recently led to the description of several new diatom and dinoflagellate species (e.g., Sar et al., 2014; Boutrup et al., 2017; Ferrario et al., 2018; Tillmann et al., 2018; Sunesen et al., 2020). By contrast, little is known about species diversity from other phytoplankton groups that include delicate and small organisms that are hard to identify to species level by microscopy (Silva et al., 2009).

Despite the well-known importance of frontal areas for biomass accumulation in the Argentine Sea, only a few studies have dealt with phytoplankton composition and dynamics in these areas. Most of them have been focused on the shelf-break front (e.g., Carreto et al., 1995; Balch et al., 2014; Carreto et al., 2016), but little is known about tidal fronts, which also play a significant role in biological production and enhanced activity at higher trophic levels (Acha et al., 2004). Among them, the Valdés front has been previously studied because of the occurrence of intense harmful phytoplankton blooms (Carreto et al., 1986). Located in shelf waters around Valdés Peninsula, the Valdés front separates well-mixed and nutrient rich coastal waters from highly stratified and poor nutrient waters offshore. Phytoplankton composition differs at both sides of the front. Chain forming diatoms and dinoflagellates usually dominate at the homogeneous and stratified sides of the front, respectively (Carreto et al., 2007). By contrast, phytoplankton dynamics from other southern frontal areas such as Blanco Cape and de los Estados Island remain almost unexplored (Guerrero et al., 1999; Papparazzo et al., 2010).

Metabarcoding stands as a powerful tool for assessing plankton diversity, particularly in taxonomic groups lacking conspicuous morphological characteristics or with numerous cryptic species (De Vargas et al., 2015). Furthermore, it proves valuable in detecting species that occur at low abundances or are simply beyond the reach of traditional microscopic analyses (Grzebyk et al., 2017). A good correspondence between metabarcoding and microscopy-based abundance and biomass suggests its potential for semiquantitative analysis of plankton, although marked

discrepancies have also been noticed (Abad et al., 2016; Gran-Stadniczeńko et al., 2019; Santi et al., 2021; Andersson et al., 2023). Although metabarcoding has been intensively implemented worldwide in the last two decades for phytoplankton analysis (Burki et al., 2021), in the Argentine Sea there is a lack of this type of studies. As the current knowledge we have of the phytoplankton of the Argentine Sea comes mainly from microscopic studies, it is timely to complement both approaches to get a better and more detailed understanding of the phytoplankton community composition in the area.

Here we analyzed for the first-time eukaryotic phytoplankton diversity and community composition in three understudied frontal areas of the Argentine Sea by a combination of 18S metabarcoding and microscopy, considering the strengths and weaknesses of metabarcoding for the study of species richness, dominant taxa, and species assemblages.

2 Methods

2.1 Field sampling and data acquisition

Sampling was carried out in early austral summer on the R/V Bernardo Houssay from January 6th to 12th 2016, along three study areas (Figure 1A) covering a latitudinal gradient: a) Stations 1-2 located in front of Valdés Peninsula ($\approx 43^\circ\text{S}$), b) Stations 7, 8, 9 located in front of the southern tip of San Jorge Gulf, i.e. Blanco Cape ($\approx 47^\circ\text{S}$), and c) Stations 15-16 located in front of de los Estados Island ($\approx 55^\circ\text{S}$). Samples for both metabarcoding and microscopy analyses were simultaneously taken at each sampling station (see below).

Physical data on conductivity (salinity)/temperature/depth were determined *in situ* with a profiling conductivity-temperature-density (CTD) device (Sea Bird SBE 911 plus, Bellevue, WA, USA) for each sampling station. Moreover, the World Ocean Atlas (WOA-18) from the NOAA/National Center for Environmental Information website (<https://www.nodc.noaa.gov/OC5/woa18/>) was used to download January 2016 gridded mean fields of Sea Surface Temperature (Locarnini et al., 2019) and Sea Surface Salinity (Boyer et al., 2018; Zweng et al., 2018). Satellite chlorophyll *a* (Chl-*a*) concentration was taken from https://neo.gsfc.nasa.gov/view.php?datasetId=MY1DMW_CHLORA&year=2016. All data were plotted with ODV software version 5.6.3 (odv.awi.de).

Surface (5 m) seawater samples of 4-5 L were collected at each station with Niskin bottles. Aliquots of 250 mL were fixed with acidic Lugol's iodine solution (4%) for microscopic analysis of phytoplankton composition. The rest was sieved through a 200 μm Nitex mesh to remove large zooplankton. Aliquots of 2 L were then sequentially filtered through 5 μm and 0.4 μm pore polycarbonate filters for molecular analysis. These filters were bent and wrapped into foil, flash frozen in liquid nitrogen and kept at -20°C for 4-10 days. The upper surface of the filters was then completely scraped using a 2 ml lysis buffer and a sterile scraper. The collected fluid was stored in Eppendorf tubes and frozen at -20°C until DNA extraction.

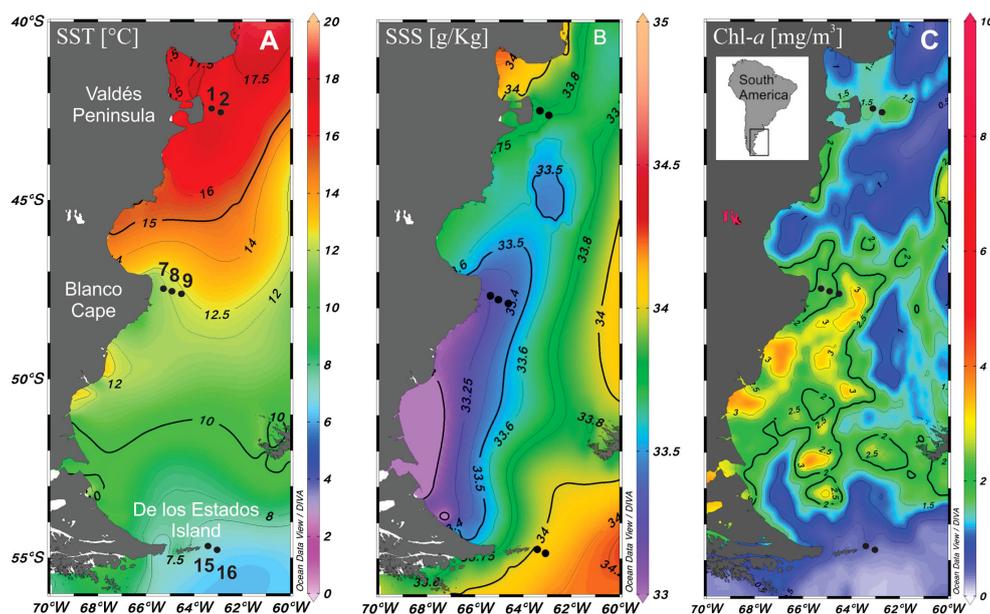


FIGURE 1

Map of the study area showing the location of sampling stations in the Argentine Sea (black dots). (A) Sea surface temperature (SST), (B) salinity (SSS) and (C) Chl-*a* distribution.

2.2 Molecular analysis

Genomic DNA was isolated from the filters using the Nucleospin Plant II kit (Machery-Nagel, Düren, Germany) according to the manufacturer's protocol. DNA concentrations were determined with a Quantus Fluorometer according to the manufacturer's protocol for measuring double stranded DNA (QuantiFluor dsDNA System, Promega, USA).

The variable V4 region of the 18S rDNA was amplified using the following primer-set: 528iF (GCG GTA ATT CCA GCT CCA A) and 964iR (AC TTT CGT TCT TGA TYR R) (Metfies et al., 2020). The PCR reaction mixture consisted of 12.5 μ L master mix (KAPA HiFi HotStart ReadyMix, KAPABiosystems, Roche), 5 μ L of each primer (1 μ M) and 2.5 μ L template (~5 ng/ μ L). Amplification was carried out on a MasterCycler (Eppendorf, Hamburg, Germany) with the following program: 95°C for 3 min, 25 cycles consisting of: 95°C for 30 s, 55°C for 30 s and 72°C for 30 s. Followed by a final five minutes at 72°C. The products were kept at 4°C once the program had ended. Samples were stored at -20°C until further analysis. The PCR products were purified from an agarose gel 1% [w/v] with the AMPure XP PCR purification kit (Beckman Coulter, Ing., USA) according to the manufacturer's protocol. After purification of the 18S rDNA fragment the DNA concentrations of the samples were determined using the Quantus Fluorometer (Promega, USA). Indices and sequencing adapters of the Nextera XT Index Kit (Illumina, USA) were attached via the Index PCR. All PCRs had a final volume of 50 μ L and contained 25 μ L of KAPA HIFI Mix (Kapa Biosystems, Roche, Germany), 5 μ L of each Nextera XT Index Primer [1 μ mol/L], 5 μ L DNA-template [~5ng] and 10 μ L PCR grade water. PCR amplification was performed in a thermal cycler (Eppendorf, Germany) with an initial denaturation (95°C, 3 min)

followed by 8 cycles of denaturation (95°C, 30 sec), annealing (55°C, 30 sec), and extension (72°C, 30 sec) with a single final extension (72°C, 5 min). Prior to quantification of the PCR products with the Quantus Fluorometer (Promega, USA) for sequencing with the MiSeq-Sequencer (Illumina, USA), the final library was cleaned up using the AMPure XP PCR purification kit (Beckman Coulter, Ing., USA). Sequencing of the DNA-fragments was carried out with the MiSeq Reagent Kit V3 (2 x 300 bp) according to the manufacturer's protocol (Illumina, USA).

Raw reads were quality trimmed with Trimmomatic (Bolger et al., 2014). Thus, reads were scanned with a 4-base wide sliding window and cut when the average quality dropped below 15. For merging paired-end reads, the script join-paired-ends within the open-source bioinformatics pipeline QIIME v.1.8.0 was used with a minimum read overlap of 20 bases. Further analyses were performed following a pipeline developed in-house using QIIME v.1.8.0 (Caporaso et al., 2010). In short, reads were quality-filtered according to recommended settings (Bokulich et al., 2013). Only sequences that fully matched the primer sequences at the beginning and end of the sequence, respectively, and which were between 200 and 500 bp in length were further processed. For chimera detection and clustering of sequences into OTUs we used the QIIME workflow 'usearch.qf', which incorporates UCHIME (Edgar et al., 2011). Pre-clustered sequences were checked for chimeras (*de novo* and with Silva 119 SSU). The remaining sequence set was clustered (*de novo*) into OTUs with a similarity threshold of 98%. Sequences from all samples were analyzed together to define operational taxonomic units (OTUs) for 18S rRNA genes using SWARM (Mahé et al., 2015), with a cut-off value of 0.005% of the total amount of reads. OTUs were assigned to taxa based on their similarity with SILVA database. In addition, BLAST searches

were performed to further explore identities of the most abundant OTUs.

The database initially contained a set of 3,460 OTUs. After applying a cut-off value of 0,005% and selecting those assigned to Chlorophyta, Haptophyta, Cryptophyta, Stramenopiles (excluding Phaeophyceae, Labyrinthulomycetes, and Peronosporomycetes), Dinophyta, Raphidophyceae, Picozoa, and Telonemians, the number of OTUs was reduced to 382.

We rarefied all samples to 43,456 reads, the smallest phytoplankton reading determined by the rarefaction curves (Cameron et al., 2021). Rarefaction curves were created and calculated using the R package *vegan* (Oksanen, 2015).

2.3 Microscopic analysis

The abundance of nano- (5–20 μm) and microplankton (20–200 μm) was estimated following the Utermöhl (1958) method, with a phase-contrast Leica DMIL LED equipped with a Mshot MS60 camera. Subsamples (50 mL) from the water obtained by Niskin bottles were left to settle for 24 h in a composite sedimentation chamber before cell counting. At least 300 cells of the dominant taxa were counted in random fields or in half the chamber to estimate general phytoplankton composition, whereas the whole chamber bottom was scanned to count less abundant species. All taxa, including photosynthetic and heterotrophic groups, were identified to the highest possible taxonomic level (i.e., species). However, since flagellates generally lose their flagella by the addition of fixatives, unidentified phytoflagellates and round-shaped organisms with or without flagella, were included in a single group as small unidentified flagellates (< 10 μm), referred herein as small flagellates. Further morphological examination was conducted by phase-contrast, differential interference contrast and UV epifluorescence microscopy with a Leica DM2500 microscope equipped with a DFC420C camera. For the estimation of phytoplankton biomass, we utilized cell carbon content ($\mu\text{g C L}^{-1}$) data from the most abundant taxa, obtained from previous studies conducted in the area by the authors (e.g., Almandoz et al., 2011).

2.4 Data analysis

Cluster analyses based on Euclidean distances and Unweighted Pair Group Average (UPGMA) algorithm were carried out with the software PRIMER v6. Relative biomass values of taxa representing at least 0.5 of total biomass (N=23) and normalized OTU values (N=382) were used. The Similarity Profile routine (SIMPROF) test was further applied for detecting significant clusters of samples.

3 Results

3.1 Hydrography

The three studied zones differ in their sea surface temperature and salinity, with warmer ($\approx 16^\circ\text{C}$) and saline (33.8) waters at Valdés

Peninsula, colder ($\approx 8^\circ\text{C}$) and saline (34) waters at de los Estados Island, and waters with intermediate temperature ($\approx 12^\circ\text{C}$) and lower salinity (33.4) at Blanco Cape (Figures 1B, C). Satellite Chl-*a* estimations showed higher values ($\approx 2 \text{ mg m}^{-3}$) in the two northern frontal areas, and lower values to the south (Figure 1C). Vertical profiles showed a typical tidal front structure at Valdés Peninsula and Blanco Cape zones, with well-mixed waters near shore and enhanced stratification offshore (Figure 2). By contrast, a topographically controlled front is observed at de los Estados Island, when the low salinity water coming from the Beagle Channel converges with the cool and salty water from the upper stratum of the Malvinas Current (Figure 2), generating a surface front at the edge of the continental slope (Guerrero et al., 1999).

3.2 Overall phytoplankton composition

Microscopy cell counts revealed that phytoplankton was mainly composed of small flagellates in terms of cell abundance, reaching up to $11.6 \times 10^6 \text{ cells L}^{-1}$ at Valdés Peninsula (St. 2), that represented 90.6% of total cells (Figure 3A). Small flagellates were only outnumbered by diatoms at de los Estados Island, where diatoms reached $7.0 \times 10^5 \text{ cells L}^{-1}$ (St. 16), accounting for 56.4% of total cell abundance (Figure 3A). Other taxonomic groups, i.e., dinoflagellates, prasinophytes, haptophytes, raphidophytes, and silicoflagellates were detected in low values (< 7%), except for cryptophytes which accounted for up to 15.2% of total cells at Blanco Cape (St. 9).

Carbon biomass estimations, based on cell volumes, showed conspicuous differences with respect to cell abundance distribution (Figure 3B). Because of their bigger cell size, dinoflagellates dominated at Valdés Peninsula (St. 9), reaching up to $48.7 \mu\text{g C L}^{-1}$ (50.3% of total biomass), whereas diatoms mainly dominated at de los Estados Island, reaching up to $28.3 \mu\text{g C L}^{-1}$ (64.7% of total biomass) at St. 16 (Figure 3B). Small flagellates reached a maximum of $62.8 \mu\text{g C L}^{-1}$ (43.6% of total biomass) at St. 2. The other taxonomic groups represented less than 10% in all samples.

Metabarcoding analysis showed that total reads were dominated by chlorophytes, dinoflagellates, and diatoms (Figure 3C), in accordance with what was observed for microscopic biomass estimations. Other groups, such as cryptophytes, silicoflagellates, raphidophytes, and haptophytes were detected in low relative read numbers. Moreover, Telonemians, Picozoa, and other non-diatom Stramenopiles, which could not be identified by microscopy, were also detected in low read numbers. Chlorophytes dominated at Valdés Peninsula and at St. 7–8 from Blanco Cape, where they represented 52%–57% of total reads, similar to the dominance of small flagellates observed by microscopy. This observation suggests that most cells identified during cell counting as small flagellates in these samples may correspond to chlorophytes. Chlorophytes were mostly detected in the 0.4 μm filters than in 5 μm filters (Supplementary Figure 1A), reaching up to 97.3% of total reads (St. 8). By contrast, dinoflagellates and diatoms were more important in the 5 μm filters (Supplementary Figure 1B), reaching up to 70.3% (St. 9) and 93.5% (St. 16), respectively.

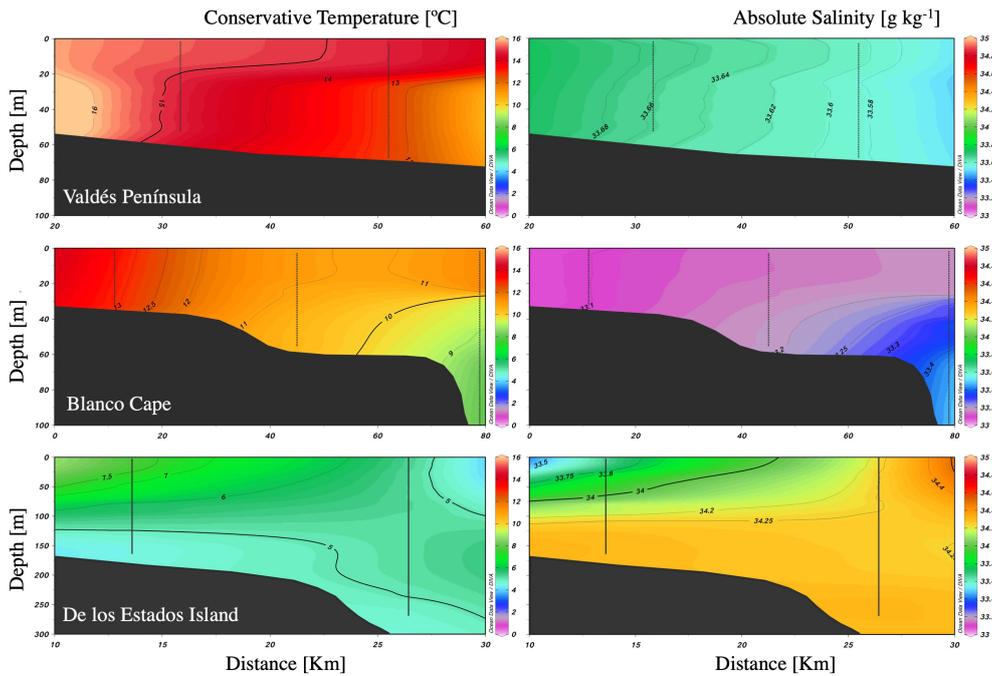


FIGURE 2 Vertical profiles of temperature and salinity along three frontal areas in the Argentine Sea.

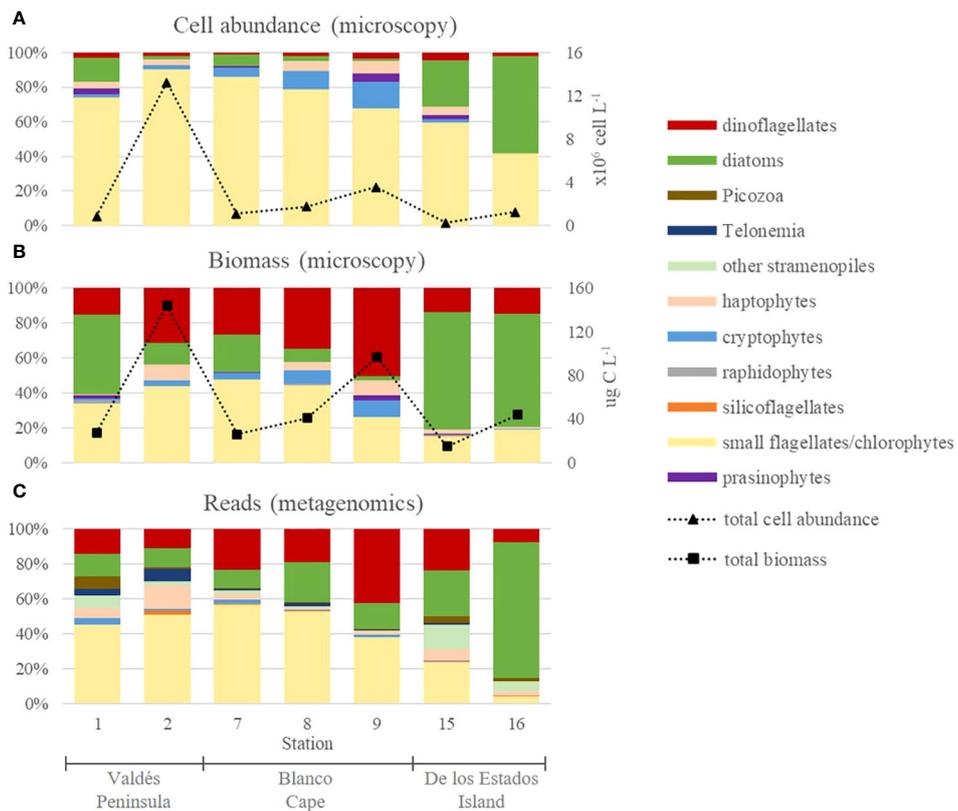


FIGURE 3 Overall phytoplankton composition in frontal areas of the Argentine Sea expressed in relative cell abundances (A) and relative carbon biomass (B) of main phytoplankton groups obtained by microscopy, and relative reads of main phytoplankton groups obtained by metabarcoding (C). Note that small flagellates that could not be identified by microscopy were mostly assigned to chlorophytes based on metabarcoding analysis.

3.3 Species richness

Microscopy analysis revealed the presence of 91 taxa, most of them diatoms (40) and dinoflagellates (40), (Figure 4). The richest taxa include dinoflagellates from the genus *Protoperidinium* (8), *Ceratium* (4), and *Prorocentrum* (3), and the diatoms *Pseudo-nitzschia* (5), *Guinardia* (3), and *Thalassiosira* (3) (Supplementary Table 1). By contrast, the other groups were mostly assigned to class level.

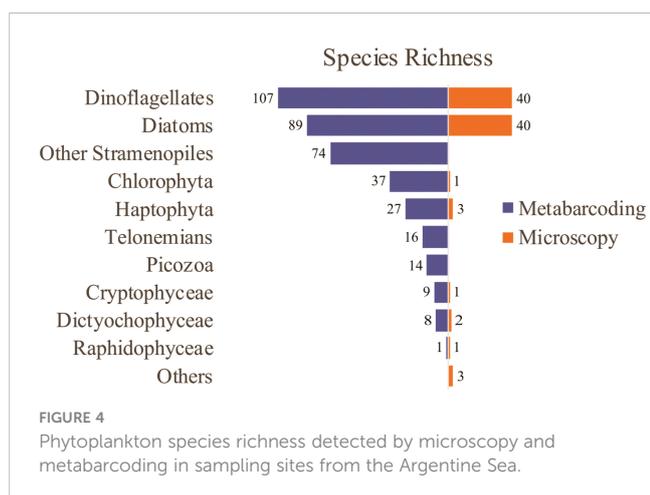
Metabarcoding analysis revealed a notably higher number of taxa, with a total of 382 environmental OTUs, most of them assigned to dinoflagellates (107), diatoms (89), and other Stramenopiles (74), followed by Chlorophyta (37), Haptophyta (27), Telonemians (16), Picozoa (14), Cryptophyceae (9), Dictyochophyceae (8), and Raphidophyceae (1) (Figure 4). The richest taxa include the diatoms *Chaetoceros* (21), *Thalassiosira* (16), *Pseudo-nitzschia* (8), and *Guinardia* (7), the dinoflagellates *Gyrodinium* (10), the *Gymnodinium* clade (8), and *Symbiodinium* (7), other Stramenopiles such as uncultivated MARine STramenopiles (MAST) lineages (10) and *Triparma* (5), the Chlorophyta genus *Micromonas* (6), and the Haptophyta genera *Chrysochromulina* (6) and *Phaeocystis* (4) (Supplementary Table 2).

3.4 Species assemblages and dominant taxa

Cluster analysis based on relative biomass estimations (microscopy) showed three main groups (Figure 5A). Stations from Blanco Cape (St. 7-9) grouped together with the offshore station from Valdés front (St. 2) and were characterized by the presence of an assemblage dominated by small flagellates, which accounted for up to 44.2% of total biomass at St. 2 (Supplementary Table 3). In terms of relative biomass, small flagellates were followed, at St. 2 by the dinoflagellate aff. *Peridiniella/Scropsiella* (21.5%) and unidentified small centric diatoms (10.7%), and at St. 7 by the diatom *Guinardia delicatula* (10.1%), and the dinoflagellates *Protoperidinium granii* (9.2%) and *P. conicum* (8.5%). A high contribution of *Gyrodinium* sp. ($\approx 30 \mu\text{m}$) was observed at

stations 8-9, with increased cell abundance (9.5×10^4 cells L^{-1}) and biomass contribution (46.3%) towards offshore waters (St. 9). Cryptophytes were also observed in high densities at stations 8-9, with cell densities up to 5.4×10^5 cells L^{-1} and biomass contribution of 9.6% at St. 9 (Figures 3A, B). A second group was formed with samples from de los Estados Island (St. 15-16), here diatoms outnumbered small flagellates in terms of relative biomass. *Stephanophysix nipponica* (26.4%) and *Cerataulina pelagica* (22.7%) were the most important diatoms at St. 15, whereas *Pseudo-nitzschia* spp. dominated at St. 16 (60.9%), reaching 4.6×10^5 cells L^{-1} . Other diatom species, such as *Fragilariopsis kerguelensis*, *Pseudo-nitzschia turgiduloides*, and single cells resembling *Attheya septentrionalis* (Figure 6), were also observed by microscopy at St. 16. Finally, St. 1 did not group with any other stations, presented an assemblage dominated by the diatom *Asterionellopsis glacialis* (47.5%), followed by small flagellates (37.5%) and the dinoflagellate *Prorocentrum micans* (10.6%). The most abundant taxa found in the three frontal areas by microscopy are illustrated in Figure 6.

Cluster analysis based on metabarcoding also showed three main groups that had some differences with the groups previously found by microscopy (Figure 5B). An assemblage mainly represented by the prasinophyte species *Pycnococcus provasolii* was observed at St. 1-2 at Valdés front (Supplementary Table 4). *Pycnococcus provasolii* accounted for 54.7% and 85.4% of total Chlorophyta reads at St. 1 and 2, respectively. Other species with high contribution at Valdés front included *Phaeocystis globosa* (Prymnesiophyceae), *Chloropicon roscoffensis* (Prasinophytes, Clade VIII), *Picomonas judraskeda* (Picozoa), the dinoflagellate *Prorocentrum micans*, an unclassified Dinophyceae, and the diatom *Thalassiosira profunda*. In contrast with the large contribution to total biomass of the diatom *Asterionellopsis glacialis* at St. 1 (47.5%) detected by microscopy, this species accounted for less than 0.2% of total reads. A second group was formed by samples from Blanco Cape (St. 7-9), and the inner station from de los Estados Island (St. 15). Here the assemblage was mainly represented by OTUs assigned to the Clade VIII (subclade B) of prasinophytes, *Picochlorum* sp. (Trebouxiophyceae), the diatom *Minidiscus trioculatus*, and the dinoflagellate *Gyrodinium fusiforme*. Finally, the offshore station from the los Estados Island (St. 16) did not group with any other sample, here the assemblage was dominated by the diatom species *Chaetoceros contortus*, accounting for 63.8% of total reads. This observation markedly differs with what we found by microscopy at St. 16, where total biomass was dominated by *Pseudo-nitzschia* spp. (60.9%), and *Chaetoceros* spp. contributed less than 5%.



4 Discussion

4.1 Main phytoplankton groups

Microscopy and 18S metabarcoding both identified small flagellates, dinoflagellates and diatoms to be the dominant taxonomic groups within the three different frontal areas sampled along the Argentine Sea. Here, 18S metabarcoding provided a more

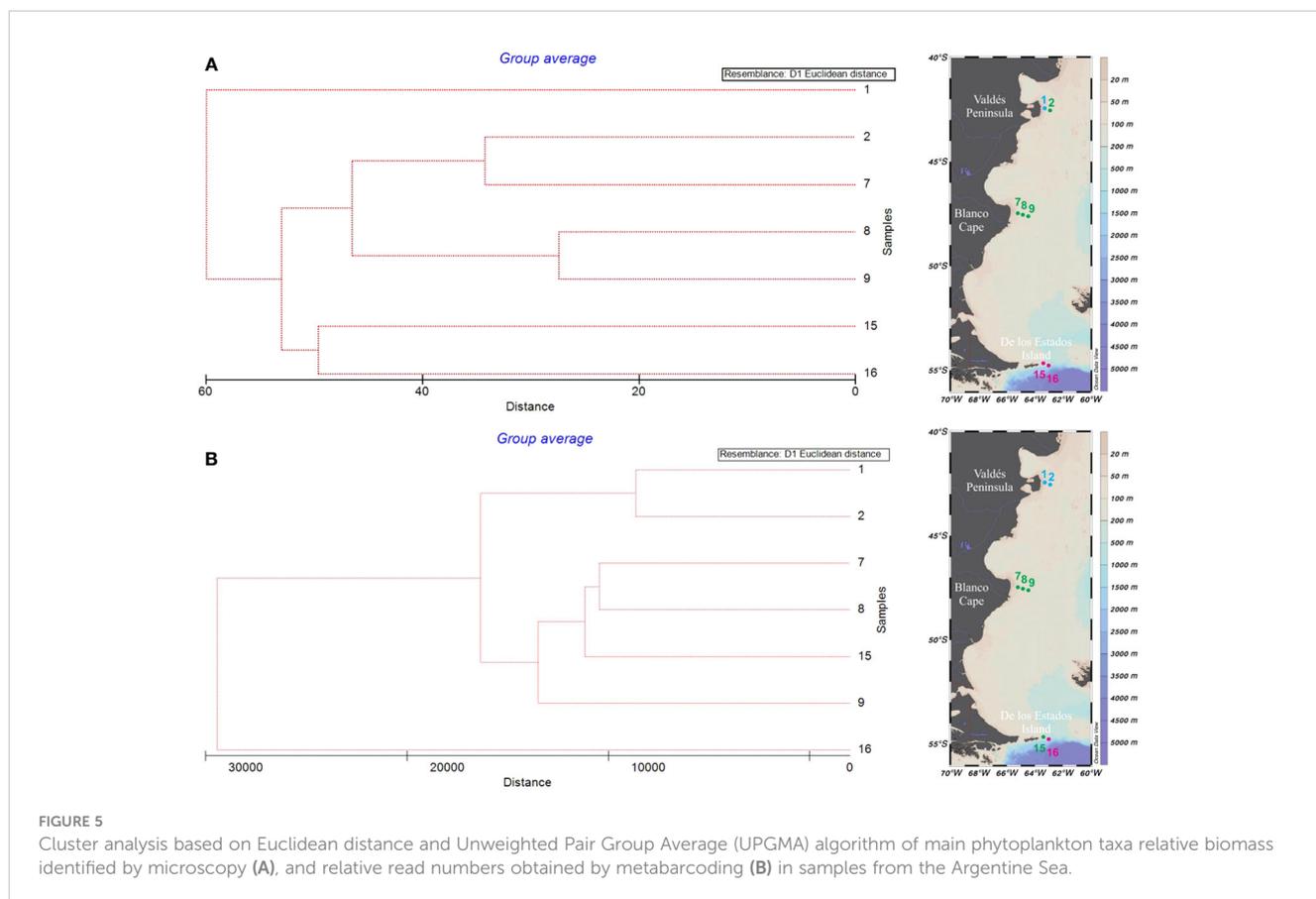


FIGURE 5

Cluster analysis based on Euclidean distance and Unweighted Pair Group Average (UPGMA) algorithm of main phytoplankton taxa relative biomass identified by microscopy (A), and relative read numbers obtained by metabarcoding (B) in samples from the Argentine Sea.

detailed insight into the taxonomic composition of the small flagellates, suggesting small chlorophytes to be main contributors to this taxonomically unidentified group. In combination, the two methods indicate a prevalence in the predominance of small chlorophytes at Valdés Front, an increase in dinoflagellates in offshore waters near Blanco Cape, and a dominance of diatoms at de los Estados Island. More particular, we observed agreement on the main taxonomic groups when comparing metabarcoding reads and carbon biomass measurements in samples with high biomass (Figure 3, St. 2, 9, and 16). As expected, there was less agreement between metabarcoding reads and cell abundance. Notably, cell abundance did not reflect the increased presence of dinoflagellates in the waters near Blanco Cape. In this context, our findings align with recent previous research that suggest a stronger correlation between metabarcoding reads and carbon biomass compared to cell abundance in microbial communities (Andersson et al., 2023). This correlation may be attributed to a positive relationship between the genome size of eukaryotes (and the number of rDNA copies) and their cell size, as highlighted by Lavrinienko et al. (2021).

4.2 Spatial variability of main species assemblages

Cluster analyses performed on both relative reads (metabarcoding) and relative biomass (microscopy), grouped the samples into three main groups well related to the geographic

location of the sampling stations. Stations 7-9 (Blanco Cape) clustered closely, while the most norther station 1 (Valdés Peninsula) and the most southern station 16 (De los Estados Island) clustered both separately outside the Blanco Cape cluster. Differences in the clustering were observed between the methods for stations 2 and 15. Station 2 clustered with station 1 based on the metabarcoding data, but with stations 7-9 based on the microscopic data, while Station 15 clustered with station 16 based on the microscopic data and with stations 7-9 based on the molecular data. We consider that these differences were largely due to the higher taxonomic resolution provided by metabarcoding, particularly of small flagellates identified as a single group by microscopy, and to differences in the detection of dominant diatoms by both methods (St. 1 and 16). Microscopy separated St. 1, north of Valdés Peninsula, from the rest of the stations mainly because of the high relative biomass of the diatom *Asterionellopsis glacialis*, which was not reflected in terms of relative reads. This is a major bloom-forming species in coastal areas worldwide, for which high cryptic diversity has recently been observed (Kaczmarek et al., 2014). The low values of relative reads corresponding to *Asterionellopsis* could be associated, among other factors, to its particularity of producing extracellular mucous-like compounds, which has been observed to interfere with DNA amplification (Odebrecht et al., 2013; Uyua et al., 2014). However, we have no way of testing whether these compounds were indeed present and if so whether they affected other species in the sample as well as *Asterionellopsis* causing a reduction in total reads. In any case, this is

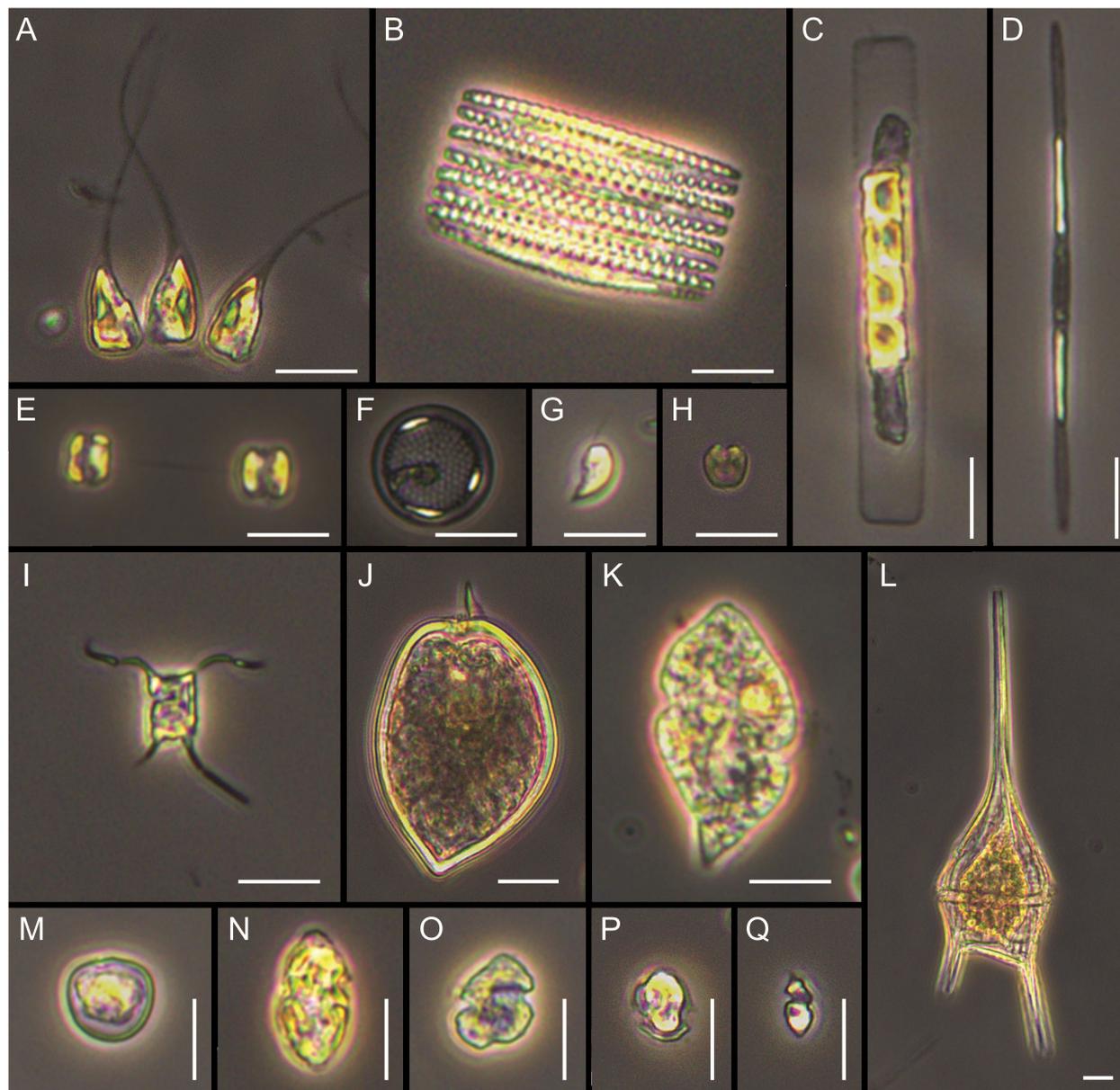


FIGURE 6
Main taxa observed under light microscopy. (A) *Asterionellopsis glacialis*, (B) *Fragilariopsis kerguelensis*, (C) *Guinardia delicatula*, (D) *Pseudo-nitzschia* aff. *turgiduloides*, (E, F) unidentified small centric diatoms, (G) unidentified cryptophyte, (H) unidentified prasinophyte, (I) *Attheya* aff. *septentrionalis*, (J) *Prorocentrum micans*, (K) *Gyrodinium* sp., (L) *Tripos lineatus*, (M) *Prorocentrum cordatum*, (N, O) gymnodinoid dinoflagellates, (P) small thecate dinoflagellate, (Q) small naked dinoflagellate. Scale bars = 10 μm .

something we cannot determine due to the compositional nature of metabarcoding, which prevents us from comparing the numbers of total reads obtained in different samples (Gloor et al., 2017). Moreover, our metabarcoding data suggest that small flagellated prasinophyte species could represent a widespread and important component of phytoplankton assemblages in the Argentine Sea during late spring or summer, further contributing to the differences in the clustering observed between the methods. An important bloom of the chlorophyte species *Pycnococcus provasolii* was observed on the stratified side of Valdes Peninsula, in accordance with microscopic observations. This is a little known, small ($\approx 1.5\text{--}4\ \mu\text{m}$) and coccoid species described based on cell

cultures from the northwestern Atlantic and the Gulf of Mexico (Guillard et al., 1991), which has been tentatively identified by transmission electron microscopy in the Strait of Magellan ($\approx 52^\circ\text{--}54^\circ\text{S}$) dominating phytoplankton during summer (Zingone et al., 2011). Likewise, pigments mostly associated with chlorophytes in coincidence with a massive bloom of up to $59.1 \times 10^6\ \text{cells L}^{-1}$ of a picoplanktonic ($<3\ \mu\text{m}$) coccal cells of an unidentified chlorophyte, were detected in the shelf-break frontal area of the Argentine Sea during late spring (Carreto et al., 2008). Moreover, a small coccal cell ($3\ \mu\text{m}$) possibly belonging to the group of prasinophytes was an important component of phytoplankton assemblages in stratified waters north of 52°S during a summer oceanographic cruise in the

southern Argentine Sea (Antacli et al., 2018). Thus, we consider the *Pycnococcus provasolii* bloom detected during January in our study could represent a later stage of phytoplankton succession at Valdés Front area.

Based on both methods, the frontal area of Blanco Cape presented the typical phytoplankton composition observed in local tidal fronts (Carreto et al., 2007; Papparazzo et al., 2010), with a predominance of diatoms and dinoflagellates in coastal (mixed) and offshore (stratified) waters, respectively. Vertical mixing in the coastal side of the front brings bottom nutrients up to the upper layers, which promotes the dominance of diatoms. By contrast, dinoflagellates dominate in nutrient-depleted and stratified waters offshore (Ramond et al., 2021). Dinoflagellates reads were particularly abundant in the more external station, where the species *Gyrodinium fusiforme* represented 88.2% of total dinoflagellates reads and a peak cell abundance of 9.5×10^4 cells L^{-1} of *Gyrodinium* sp. was observed by microscopy. However, the dominant *Gyrodinium* cells observed with microscopy did not match completely with the description of *G. fusiforme* (Takano & Horiguchi, 2004) as they were smaller ($\approx 30 \mu m$ long) and had a more rounded epicone. Likewise, smaller *Gyrodinium* cells recently observed in shelf-break waters of the Argentine Sea, could not be assigned to species level by detailed microscopy, due to their differences with other described *Gyrodinium* species, and probably represents a new species (Fabro & Almandoz, 2021). Previous phytoplankton studies at Valdés Front area revealed the occurrence of a conspicuous dinoflagellate bloom dominated by the toxic species *A. catenella* causing water discoloration from the beginning of October until January, with cell densities up to 1.8×10^6 cells L^{-1} during November 1981 in the stratified side of the front (Carreto et al., 1986; Carreto et al., 2007).

Stations at de los Estados Island were also grouped differently, microscopy results grouped together stations 15 and 16, based on the high contribution of diatom species to total biomass, mainly *Stephanophysix nipponica* and *Cerataulina pelagica* at St. 15 and *Pseudo-nitzschia* spp. at St. 16. By contrast, metabarcoding results showed that St. 15 presented a composition similar to that of Blanco Cape, with high relative reads of an uncultured unidentified eucaryote, *Pycnococcus provasolii*, *Minidiscus trioculatus*, and *Gyrodinium fusiforme* whereas offshore waters (St. 16) were dominated by the diatom species *Chaetoceros contortus*. The differences in the clustering between the methods are probably related to missing sequence information for the 18S gene of *Stephanophysix nipponica* in the reference data-base (Santoferrara, 2019). Beyond this, we could not find a clear explanation for the discrepancy observed in the identification of dominant species at St. 16 by microscopy (*Pseudo-nitzschia* spp.) and metabarcoding (*C. contortus*). Discrepancies in the identification of dominant taxa between microscopy and metabarcoding have been previously documented in the scientific literature (Abad et al., 2016; Mäki et al., 2017; Hanžek et al., 2023). These disparities can often be attributed to multiple factors, including potential error stemming from various stages of the analysis process. These factors encompass sample preservation, the volume of the sample analyzed, the DNA extraction process, primers selection, PCR procedures, incomplete reference databases, data analysis inaccuracies, and others relevant variables (Santoferrara, 2019; Burki

et al., 2021; Santi et al., 2021). Particularly for the genus *Pseudo-nitzschia*, Ruggiero et al. (2015) found an under-estimation of its relative proportion of metabarcoding reads compared with microscopy observations in the Gulf of Naples. By contrast, Turk Dermastia et al. (2023) found a large proportion of *Pseudo-nitzschia* by 18S-v9 and *rbcL* metabarcoding that was not observed by microscopy in the Gulf of Trieste, mainly attributed to misidentification of small and delicate species by microscopy. Therefore, our results show that there could be notable differences in the identification of dominant organisms at species level with microscopy and metabarcoding, which could lead to different ecological interpretations. This highlights the need for more comparative studies to attempt to bridge this gap, but on the other hand our results demonstrate that both methodologies are currently complementary (Pierella Karlusich et al., 2020).

Samples from de los Estados Island are influenced by a topographically controlled front with strong horizontal salinity and temperature gradients as a result of the mix of the low salinity water coming from the Beagle Channel with the cool and salty water from the upper stratum of the Malvinas Current (Guerrero et al., 1999). Consequently, a stratified water column was found in shallow coastal waters and a mixed water column was observed offshore. In a previous phytoplankton study covering a transect from the Beagle Channel to the Burdwood Bank, high cell densities of *Chaetoceros* spp. and *Pseudo-nitzschia* spp. were also recorded to the east of de los Estados Island during December, associated with high nutrients and low light conditions (Guinder et al., 2020). The high contribution of diatoms and the different species composition found between inshore and offshore waters in de los Estados Island frontal area, could be then associated with the presence of diatom-rich Antarctic waters moving northward through the Malvinas current (Guerrero et al., 1999).

4.3 Phytoplankton richness in the Argentine Sea

One of the most conspicuous feature of phytoplankton composition in the study area with the high dinoflagellate richness, compared with other groups. This observation agrees with the Tara Ocean global survey, which has shown that overall dinoflagellate 18S richness accounted for $\sim 49\%$ of total protistan richness (Le Bescot et al., 2016). Dinoflagellate diversity assessments in the Argentine Sea have been previously confined to microscopic observations showing also a high morphological diversity (e.g., Balech, 1988; Balech, 2002). In a more recent study of summer phytoplankton diversity in the southern Argentine Sea (47° – $55^\circ S$), dinoflagellates represented 36% of total species/morpho-species detected by microscopy (Antacli et al., 2018). According to these authors, only 46 of 148 dinoflagellate taxa could be identified to genus or species level, most of them corresponding to the genus *Protoperidinium*. Our summer metabarcoding analysis showed a total of 107 OTUs assigned to dinoflagellates, from which a large number was assigned to *Gyrodinium*, the *Gymnodinium* clade, and *Symbiodinium*, but also a high percentage was assigned to unclassified Dinophyceae and Peridiniphyceae. By contrast, only one OTU was assigned to the genus *Protoperidinium*, which

contrasts with microscopic observations where at least eight different species were detected in very low cell abundances. Molecular identification of *Protoperidinium* species is limited by the low number of available sequences in databases, because of their heterotrophic conditions that difficult their culture and growth (Phan-Tan et al., 2016). Up to ten dinoflagellate genera detected by metabarcoding in the present study (see below) have not been mentioned before in the Argentine Sea by previous microscopic observations. They mostly include small size (< 15 μm), with a very thin theca or naked organisms, which require detailed ultrastructural observations for their identification. Moreover, some of them also show complex life cycles involving parasite stages or long cyst stages, reducing the planktonic motile stage, so they could be overlooked in routine monitoring or abundance estimations. This is the case of the genera *Symbiodinium*, *Gymnoxanthea*, *Chytriodinium*, *Paragymnodinium*, *Pfiesteria*, *Cryptocodinium*, *Biecheleria*, and *Pelagodinium*, which might be considered in this study as “small naked dinoflagellates” or included as *Gymnodinium* sp. by microscopic analysis (Bhaud et al., 1991; Burkholder et al., 2001; Gómez et al., 2009; Moestrup et al., 2009; Kang et al., 2010; Siano et al., 2010; Yuasa et al., 2016; Decelle et al., 2018). Conversely, the genera *Gotoius* and *Sinophysis* consist of large thecate dinoflagellates, with cell sizes exceeding 30 μm and distinct morphological characteristics such as cell shape and theca plate pattern. These characteristics make their identification considerably more straightforward and reliable when employing optical methods (Dodge and Toriumi, 1993; Hoppenrath, 2000). However, it is noteworthy that these genera have not been previously documented in the Argentine Sea, which suggests that they may exist in exceedingly low abundances, consistent with their limited relative read numbers. In this sense, it is important to consider that the significantly larger sample volume used for metabarcoding (4–5 L) in comparison to microscopy (50 mL) could have played a pivotal role in enabling the detection of rare species. These species, although present might have eluded observation under the light microscope techniques.

Diatoms represented the second group in terms of phytoplankton richness, with most OTUs assigned to the genera *Chaetoceros*, *Thalassiosira*, and *Pseudo-nitzschia*. These are among the most diverse marine diatom genus worldwide and have also shown a high species diversity by detailed microscopy studies in the Argentine Sea (Ferrario and Sar, 1988; Sar et al., 2002; Sunesen et al., 2008; Almandoz et al., 2017). By contrast, the finding of the genus *Plagiotriata* in the metabarcoding analysis represents, as far as we know, a new record for the Argentine Sea. Non-diatom stramenopiles richness was mostly composed of MAST, a group of world-wide distributed marine heterotrophic picoeukaryotes that are mostly phagotrophic free-living flagellates (Massana et al., 2004). Likewise, five OTUs were assigned to the picophytoplanktonic genus *Triparma* (ex *Bolidomonas*), which to the present day only includes two described species (Guillou et al., 1999), suggesting this group might have a greater diversity.

Other poorly studied taxonomic groups of nano- and picoplankton showed a great diversity previously ignored in the Argentine Sea. A high OTU richness was found for the heterotrophic pico- and nano-eukaryotes genera *Picomonas* (5) and *Telonema* (16), members of two

new recently described eukaryotic phyla (i.e., Picozoa and Telonemia) with uncertain evolutionary position and unknown diversity (Shalchian-Tabrizi et al., 2007; Seenivasan et al., 2013). Chlorophytes richness was mainly represented by the genera *Micromonas* and *Prasinoderma*, which includes tiny (< 3 μm) flagellates (*Micromonas*) and nanoplanktonic (\approx 3–5 μm) coccoid organisms (*Prasinoderma*), which are hardly detected during microscopic observations (Hasegawa et al., 1996; Guiry, 2022). Moreover, two OTUs were assigned to the genus *Pyramimonas*, which include bigger (\approx 5–10 μm) nanoflagellates that can be usually identified by microscopy by the presence of four flagella (Thronsdson, 1996). The detection of OTUs assigned to four different cryptophyte genera (i.e., *Leucocryptos*, *Teleaulax*, *Hanusia*, and *Katablepharis*), in the Argentine Sea is noteworthy, as to our knowledge, these genera had not been previously recorded in this region. This finding suggests a greater diversity of Cryptophytes than previously documented. Despite their relatively large cell size (\approx 10 μm) and their significant contribution to phytoplankton biomass in cold-water regions (Brown et al., 2021), cryptophytes have received limited attention in the Argentine Sea. With the exception of Silva et al. (2009), who mentioned the occurrence of the genera *Hemiselmis* and *Plagioselmis* in the northern Argentine Sea, other microscopic studies have typically referred to cryptophytes in this region as “unidentified cryptophytes” (e.g., de Souza et al., 2012; Antacli et al., 2018; Guinder et al., 2018). However, it is important to note that, thus far, no comprehensive examination of cryptophytes using electron microscopy has been conducted in the Argentine Sea.

5 Conclusions

This study provided refined information on biodiversity and environment related variability of phytoplankton communities during austral summer for three different frontal areas in an unprecedented approach for the Argentine Sea. Based on integrated information from microscopic counts, biomass estimation, and 18S sequences phytoplankton community variability could be mainly attributed to the latitudinal gradient along the three frontal areas. To the north, there was a predominance in terms of biomass and taxonomic composition of chlorophytes (small flagellates), dinoflagellates increased at mid latitude, while to the south, diatoms dominated. The use of metabarcoding significantly increased the detection of phytoplankton species richness and our understanding of biodiversity for the Argentine Sea. It allowed the identification of a broad range of dinoflagellates, diatoms, as well as smaller flagellate or coccoid organisms that are beyond the scope of light microscopy, and which have not been described for the Argentine Sea. Some of these smaller organisms, such as the chlorophyte *Pycnococcus provasolii*, play a crucial role in the southwestern Atlantic ecosystem, particularly in the formation of significant summer blooms. Our study shows the value of combining the strengths of metabarcoding and microscopy to improve our understanding of marine ecosystems, although the weaknesses of the methods should always be considered.

Data availability statement

Raw sequences generated in this study have been deposited at the European Nucleotide Archive (ENA) using the data brokerage service of the German Federation for Biological Data (Diepenbroek et al., 2014) with the accession number PRJEB72201.

Author contributions

GA: Conceptualization, Formal analysis, Funding acquisition, Investigation, Writing – original draft, Writing – review & editing. EF: Formal analysis, Writing – original draft, Writing – review & editing. PS: Formal analysis, Writing – review & editing. MM: Formal analysis, Writing – review & editing. JA: Formal analysis, Writing – review & editing. MF: Writing – review & editing. KM: Formal analysis, Writing – review & editing. FB: Formal analysis, Funding acquisition, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the National Council for Scientific and Technical Research [CONICET PIP 1195], the National Agency for Scientific and Technological Promotion [ANPCyT PICT 2719], and FONDECYT 11230767.

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Acknowledgments

We thank the support of the crew of the R/V Bernardo Houssay (Prefectura Naval Argentina), Captain Fernando Dos Santos. Furthermore, we thank Kerstin Korte and Swantje Ziemann for excellent technical assistance, and Stefan Neuhaus and Stephan Frickenhaus (Scientific Computing, AWI) are acknowledged for excellent technical support of the sequence analyses. Finally, we want to thank both reviewers for their constructive comments on the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1306336/full#supplementary-material>

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