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RESEARCH ARTICLE

An undescribed species within the *Gymnodinium* sensu stricto group found responsible for an exceptional bloom in the Southern Ocean

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Abstract

Naked dinoflagellates are a regular component of the Antarctic phytoplankton but remain poorly studied. In December 2016, the first massive bloom (9.5×10^6 cells·L^{-1}) of small (~15 µm) naked dinoflagellates was recorded in the western Antarctic Peninsula. To identify these organisms, we performed Illumina next-generation sequencing analysis on field samples to obtain genetic information (SSU rDNA 18SV9 and 16SV4-V5). In addition, we performed polymerase chain reaction (PCR) amplification and Sanger sequencing using dinoflagellate-specific primers (LSU rDNA D1-D3 and ITS/5.8S), as well as traditional light and scanning electron microscopy observations. Phylogenetic analyses revealed that these organisms belonged to the *Gymnodinium* sensu stricto group and may represent an undescribed species. These analyses also indicated that the observed organisms were closely related to the species *Gymnodinium dorsalisulcum*, *G. impudicum*, *Barrufeta bravensis*, and *B. resplendens*, as well as to the genera *Lepidodinium* and *Wangodinium*. This work has provided the LSU rDNA gene sequence from an Antarctic species belonging to the *Gymnodinium* sensu stricto group along with a description of the observed morphology of these Antarctic blooming dinoflagellates. We compared the 18S V9 amplicon sequence variant (ASV) that dominated the bloom with global databases and observed that it is widely distributed in the Antarctic Peninsula as well as in the global ocean. This study highlights the need for further efforts to identify and describe the diversity of naked dinoflagellates in Antarctic waters.

KEYWORDS
Antarctic Peninsula, *Barrufeta*, citizen science, FjordPhyto, Gymnodiniaceae, phylogeny

Abbreviations: ASV, amplicon sequence variants; BI, Bayesian inference; BS, bootstrap support; IAATO, International Association of Antarctica Tour Operators; LM, light microscopy; ML, maximum likelihood; PCR, polymerase chain reaction; PP, posterior probability; SEM, scanning electron microscopy; WAP, Western Antarctic Peninsula.

INTRODUCTION

In the Western Antarctic Peninsula (WAP), naked dinoflagellates are one of the main phytoplankton groups at the end of the growth season (Costa et al., 2020; Mascioni et al., 2023). However, before 2016, blooms had never been recorded in the area (Mascioni et al., 2019). In December 2016, a dense bloom of nano-sized (~15 μm) naked dinoflagellates was observed in the Errera Channel along the WAP, with cell densities reaching 9.5×10^6 cells · L^{-1} and a carbon biomass of 1597 μg C · L^{-1}. These dinoflagellates accounted for ≈90% of the total phytoplankton cell abundance and 97% of the total phytoplankton carbon biomass (Mascioni et al., 2019). Although the blooming organisms could not be identified by microscopy observations, this bloom offered a unique opportunity to explore the organisms' identities using molecular tools.

Naked dinoflagellates remain poorly studied in the Southern Ocean, with most species assigned to the genus *Gymnodinium* (Lange et al., 2018; Scott & Marchant, 2005). In the last 20 years, studies on Antarctic naked dinoflagellates, including molecular phylogenies, have been performed; however, none of them have included *Gymnodinium* taxa (De Salas et al., 2008; Gast et al., 2006, 2007). Since 2008, no new naked dinoflagellates have been described in the Southern Ocean, and no significant progress has been made in their taxonomic study. Given the recent taxonomic revision of the genus *Gymnodinium* (Daugbjerg et al., 2000; Romeikat et al., 2020), most naked dinoflagellates described from the Southern Ocean need re-assessment (Thessen et al., 2012).

The main objective of this work was to provide new insights into the characterization of the dinoflagellates that formed the unprecedented WAP bloom, as well as to provide information on their phylogenetic relationships and geographic distribution.

MATERIALS AND METHODS

Sampling

Since 2017, the FjordPhyto Citizen Science project (www.fjordphyto.org) has collected phytoplankton surface water samples for microscopy and molecular analysis in collaboration with International Association of Antarctica Tour Operators (IAATO) member vessels (protocols published in Cusick et al., 2020). Phytoplankton sampling is conducted at different locations along the WAP during the austral growth season between November and March.

Prior to the inauguration of the FjordPhyto program, sampling during the 2016–2017 season was limited to surface seawater for microscopy counts. The dinoflagellate bloom sample herein was collected in the Errera Channel near Danco Island (64°42′58.8″ S, 62°35’18.9″ W, Figure 1a) on December 18, 2016, and analyzed by microscopy and molecular methods.

Microscopy observations

The dinoflagellate bloom sample was analyzed under an inverted microscope using the cell counting procedure described in Mascioni et al. (2019). Furthermore, the organisms were observed in detail under a light microscope (Leica DM 2500) and scanning electron microscope. For the scanning electron microscopy (SEM) observations, sample aliquots were filtered onto 0.2-μm polyamide filters and dehydrated through an ethanol dilution series with final critical point dehydration. Specimens were sputter-coated in gold–palladium and then examined with two SEM microscopes, a Jeol JSM-6360 LV and a Carl Zeiss NTS SUPRA 40.

DNA extraction and amplification

To identify the organisms, we performed Illumina next-generation sequencing (SSU rDNA 18S V9—nuclear—and 16S V4–V5—plastidic) and Sanger sequencing (D1–D3 LSU rDNA and ITS rDNA region) on the same bloom sample to obtain genetic information. All procedures took place at the J. Craig Venter Institute (JCVI), San Diego, California, United States. Genomic deoxyribonucleic acid (gDNA) was extracted from the Lugol-preserved sample following Auinger et al. (2008) and quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific).

DNA was amplified via polymerase chain reaction (PCR) using the TruFi DNA Polymerase PCR kit (Azura, United States) following the manufacturer's recommendations. The PCR products were purified using the Zymo Research OneStep PCR Inhibitor Removal Kit and quantified using the Invitrogen Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, United States). For the hypervariable region 9 of the nuclear 18S rDNA gene (18S V9), the 1389 F/1510 R primer set was used (Amaral-Zettler et al., 2009). For the hypervariable V4–V5 region of the plastid 16S rDNA gene, the 515 F/926 R primer set was used (Parada et al., 2016). For the domain D1–D3 of the LSU rDNA and both internal transcribed spacer (ITS1 and ITS2) rDNA regions including the 5.8S rDNA gene, the pairs of primers D1R/LSUB (Lee et al., 2013) and ITSF01/PERK-ITS-AS (Sunesen et al., 2020) were used. The 18S V9 and 16S V4-V5 rDNA regions were sequenced at the Institute for Genomics Medicine (IGM) at the University of California, San Diego, using Illumina MiSeq lanes (PE150, 2 × 150 bp for 18S,

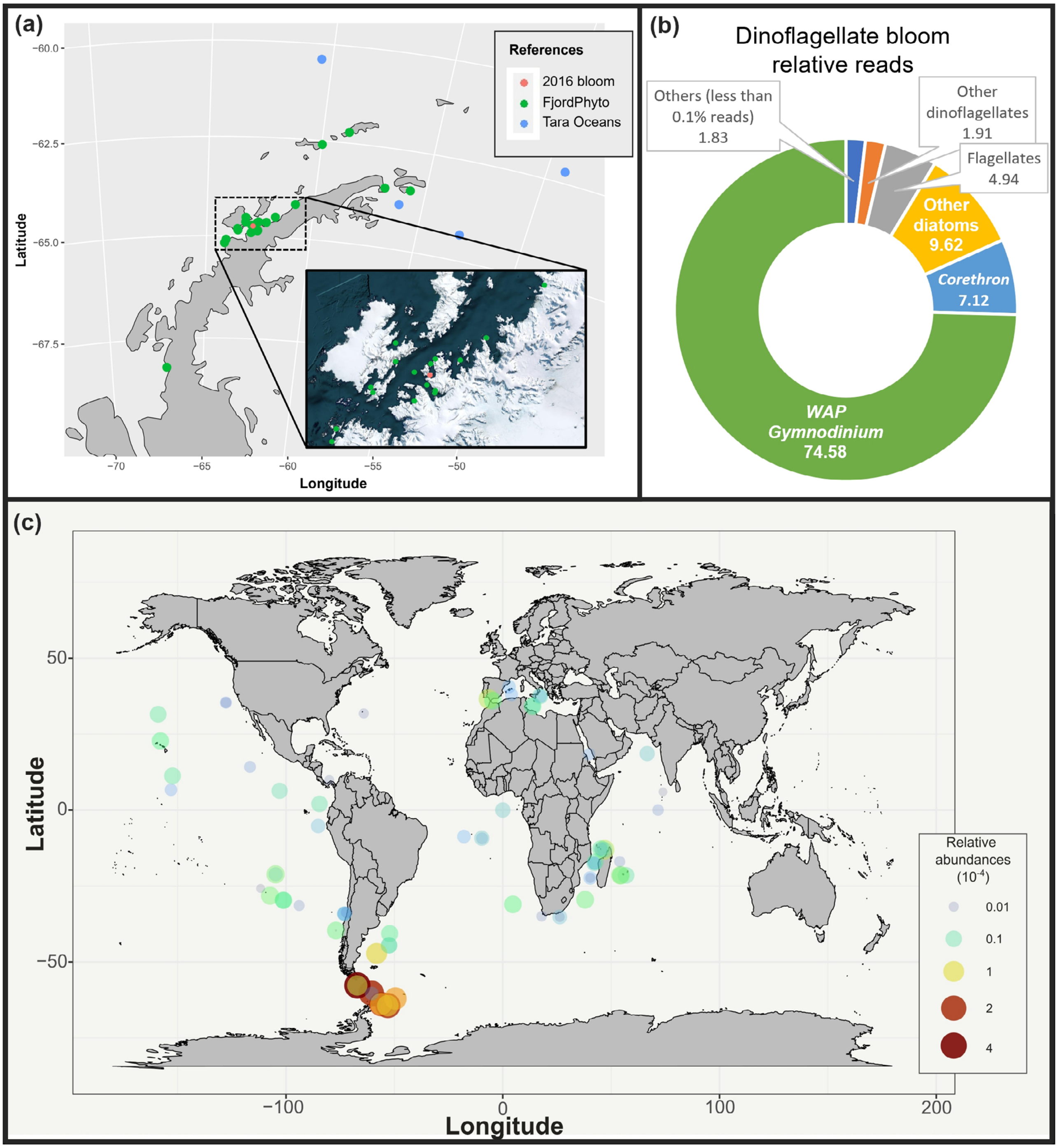

FIGURE 1 (a) Location of the 2016 dinoflagellate bloom near Danco Island, and additional sampling locations where the WAP *Gymnodinium* 18S V9 rDNA region sequence was detected by FjordPhyto (2017–2022 seasons) and Tara Oceans (ASV26740). (b) Metabarcoding nuclear SSU 18S V9 rDNA region analysis of the bloom sample, showing a clear dominance of the WAP *Gymnodinium* ASV. (c) Global map showing the relative reads (of the total sample) of the WAP *Gymnodinium* inferred from the Tara Oceans 18S V9 metabarcoding dataset.

2×300 bp for 16S) with a 15% PhiX spike-in. The amplified LSU rDNA and ITS rDNA region products were sequenced at Eurofins Genomics using Sanger sequencing (Louisville, United States).

The DNA extraction and amplification for 2017–2022 FjordPhyto samples followed the protocols aforementioned for 18S V9 rDNA region.

Bioinformatics analysis

Raw paired-end Illumina sequence data were analyzed in the QIIME2 (v2022.11) environment with DADA2, in which data were demultiplexed, trimmed, denoised, and annotated using the protocol from Allen Lab GitHub to produce amplicon sequence variants

(ASVs; 16S: https://github.com/allenlab/QIIME2_16S_ASV_protocol and 18S: https://github.com/allenlab/QIIME2_18Sv9_ASV_protocol). Taxonomic annotation of the 16S ASVs was performed against the SILVA database (https://www.arb-silva.de/) and of the 18S ASVs was performed against the Protist Ribosomal Reference database (PR2, v4.14.0; Guillou et al., 2012), generating a taxa table for each unique ASV. Amplicons with greater than a 97.0% confidence interval were used for downstream analysis. For a detailed list of all the ASVs in the bloom sample, please refer to Table S1.

Phylogenetic inference

Newly obtained LSU and SSU rDNA region sequences were deposited in GenBank with accession numbers PQ578732, PQ589805, PQ578753, PQ578754. Partial LSU rDNA region sequences (D1-D3; 1336 bp), concatenated sequences of LSU and ITS/5.8S rDNA region sequences (1895 bp), and partial SSU rDNA region plastid sequences (641 bp) were aligned with homologous sequences available in GenBank (Table S2) using Aliview (Larsson, 2014).

Maximum likelihood (ML) phylogenetic analyses of each of the three alignments were conducted with IQtree2 v2.2.2.6 (Nguyen et al., 2015) with 100 standard bootstrap replicates using the best fit model according to the Bayesian information criterion. Bayesian inference (BI) was conducted with MrBayes v3.2.7 (Ronquist & Huelsenbeck, 2003) using the GTR + I + G model. Four Markov chain Monte Carlo chains ran for as many generations as needed for chain convergence, sampling every 100 generations with a burn-in of 25%. A majority rule consensus tree was created to examine the Bayesian posterior probabilities (PPs) and bootstrap support (BS) of each clade.

Distribution assessment

To assess the distribution of the bloom-forming dinoflagellates in the WAP, genomic samples from the FjordPhyto metabarcoding dataset, covering four consecutive seasons (2017–2022, excluding the COVID-19 pandemic season 2020–2021 when no sampling took place), were also considered. The FjordPhyto metabarcoding data used can be found in GenBank under accession number PRJNA1314243.

To assess their global distribution, we performed a search on Ocean Barcode Atlas (Vernette et al., 2021) Tara Oceans V9 region SSU rDNA database (vsearch, threshold 100%), which provided only one hit. The Tara Oceans samples in the WAP correspond to January 2011 (Ibarbalz et al., 2019). The matched data were downloaded from the Ocean Barcode Atlas database, and maps were created using R and Rstudio (R Core Team, 2021).

RESULTS

Dinoflagellate morphology

Mascioni et al. (2019) previously described the blooming organisms as naked cells, rounded to slightly hexagonal outline, and slightly flattened dorsoventrally. Cells were 12.6 ± 1.3 μm wide and 14.3 ± 1.4 μm long (mean ± standard deviation of 20 individuals) with a conical to rounded epicone and hemispherical rounded hypocone, slightly larger than the epicone. The cingulum displacement was approximately one cingulum width. Cell morphology of the blooming dinoflagellates by light microscopy and SEM is shown in Figure 2a–d. Further SEM observations suggested the occurrence of a horseshoe-shaped apical groove running counterclockwise, typical of the *Gymnodinium* sensu stricto group (Figure 2e). However, the state of the Lugol's preserved cells prevented a more thorough observation of the amphiesma vesicles or any further details.

Metabarcoding (18S V9 rDNA region) of the dinoflagellate bloom

Metabarcoding analysis showed a clear dominance of one ASV that made up almost 75% of the total phytoplankton reads in the bloom sample, corresponding with the microscopy counts (Figure 1b). This ASV did not match any previously described species and could only be identified to the family Gymnodiniaceae. Thus, we assume that this dominant ASV corresponded to the dominant (~90% of the cells) *Gymnodinium*-like cells observed by microscopy and will be referred to from now on as WAP *Gymnodinium*. The remaining dinoflagellate taxa represented only 1.91% of the phytoplankton reads.

We compared the 18S V9 rDNA region sequence with different taxa from the *Gymnodinium* sensu stricto group (Figure S1). The WAP *Gymnodinium* sequence differed from *Lepidodinium chlorophorum*, *L. viride, G. litoralis, G. aureolum*, and *Wangodinium sinense* by only one nucleotide. This sequence also differed from *Nematodinium* sp. and *Pheopolykrikos beauchampii* by three nucleotides and from *G. dorsalisulcum, Warnowia* sp., and the more distantly related species *Karlodinium veneficum* by four nucleotides.

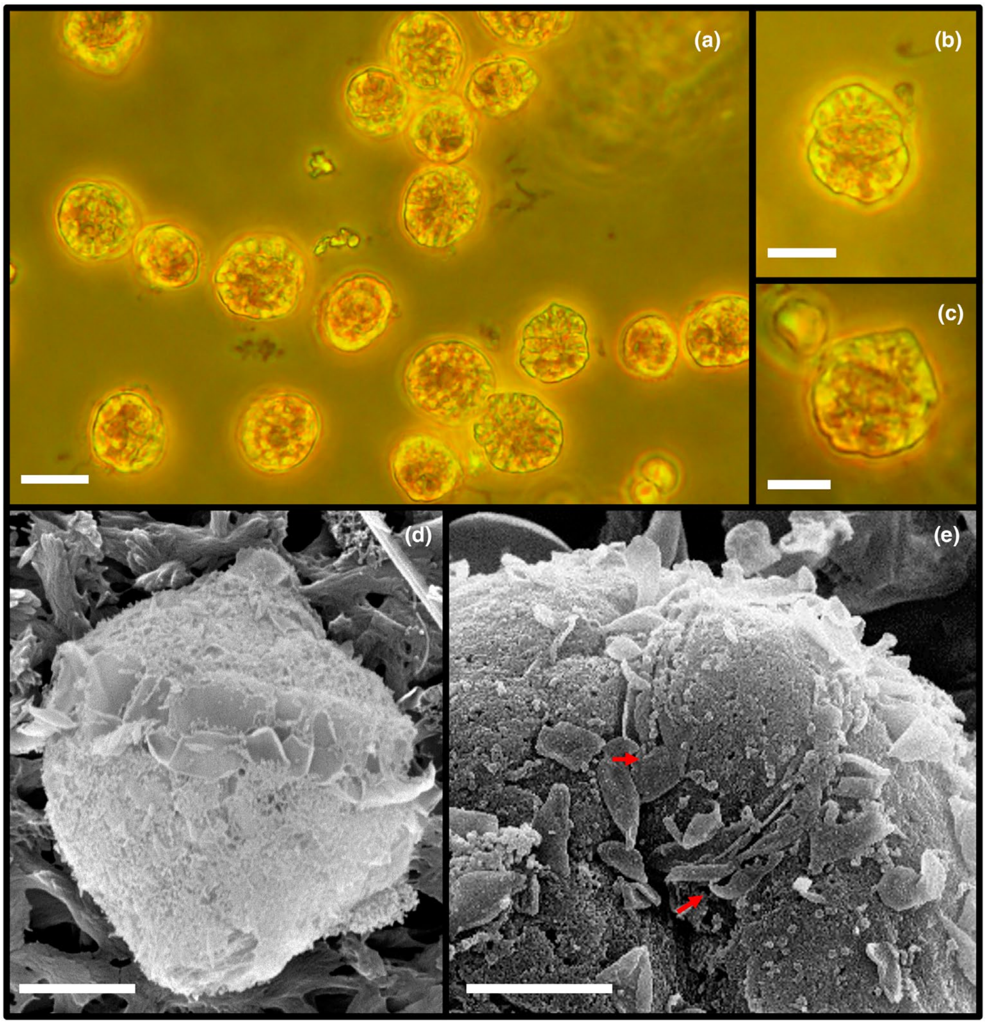

FIGURE 2 Light microscope and scanning electron microscope photos of the WAP *Gymnodinium*. Scale bars: 10 μm (a); 5 μm (b and c); 2 μm (d); 1 μm (e). (a) Light microscope WAP *Gymnodinium* bloom. (b, c) Light microscope WAP *Gymnodinium* cells. (d) Scanning electron microscopy (SEM) dorsal view of the WAP *Gymnodinium* cell. (e) SEM detail of the apical structure complex. The lower-right red arrow indicates the start of the apical groove, and the upper-left red arrow indicates the ending point.

Phylogenetic analysis

The LSU rDNA region sequences (D1-D3) obtained from the WAP *Gymnodinium* were markedly dissimilar from available sequences from species within the *Gymnodinium* sensu stricto group (Table S2). The LSU rDNA region sequence similarity with *Lepidodinium viride* was 89.98%, with *L. chlorophorum* sequences was 89.60%, with *Wangodinium sinense* sequences was 89.36%, with *G. impudicum* sequences was 87.73%, and with *G. dorsalisulcum* sequences was 89.55%.

Bayesian inference and ML analyses determined similar best trees based on LSU rDNA region sequences (Figure 3) and based on concatenated data of partial LSU rDNA and ITS/5.8S rDNA regions (Figure S2), differing only at a few not well-supported internal nodes. The *Gymnodinium* sensu stricto group comprised *Gymnodinium* as well as other genera. The WAP *Gymnodinium* sequence was observed in a polytomy with two well-supported clades—*Lepidodinium*/*Gymnodinium* sp. 1/*Wangodinium* (92% BS; 0.99 PP based on the LSU rDNA region and 87% BS; 1.0 PP based on the concatenated dataset) and *G. impudicum*/*G. dorsalisulcum*/*Barrufeta* (95% BS; 1.0 PP based on the LSU rDNA region and 91% BS; 1.0 PP based on the concatenated dataset)—with moderate statistical support (71% BS; 0.82 PP based on the LSU rDNA region and 60% BS; 0.54 PP based on the concatenated dataset). In addition, this clade was sister to *G. litoralis* with 69% BS and 0.99 PP (based on the LSU rDNA region) and 75% BS and 0.97 PP (based on the concatenated dataset).

Based on the SSU rDNA region plastid sequences, we inferred that the plastids of the WAP *Gymnodinium* were within the highly supported clade of peridinin-containing dinoflagellates (Figure S3). This clade is part of a larger clade that includes red algae (Rhodophytes) and several

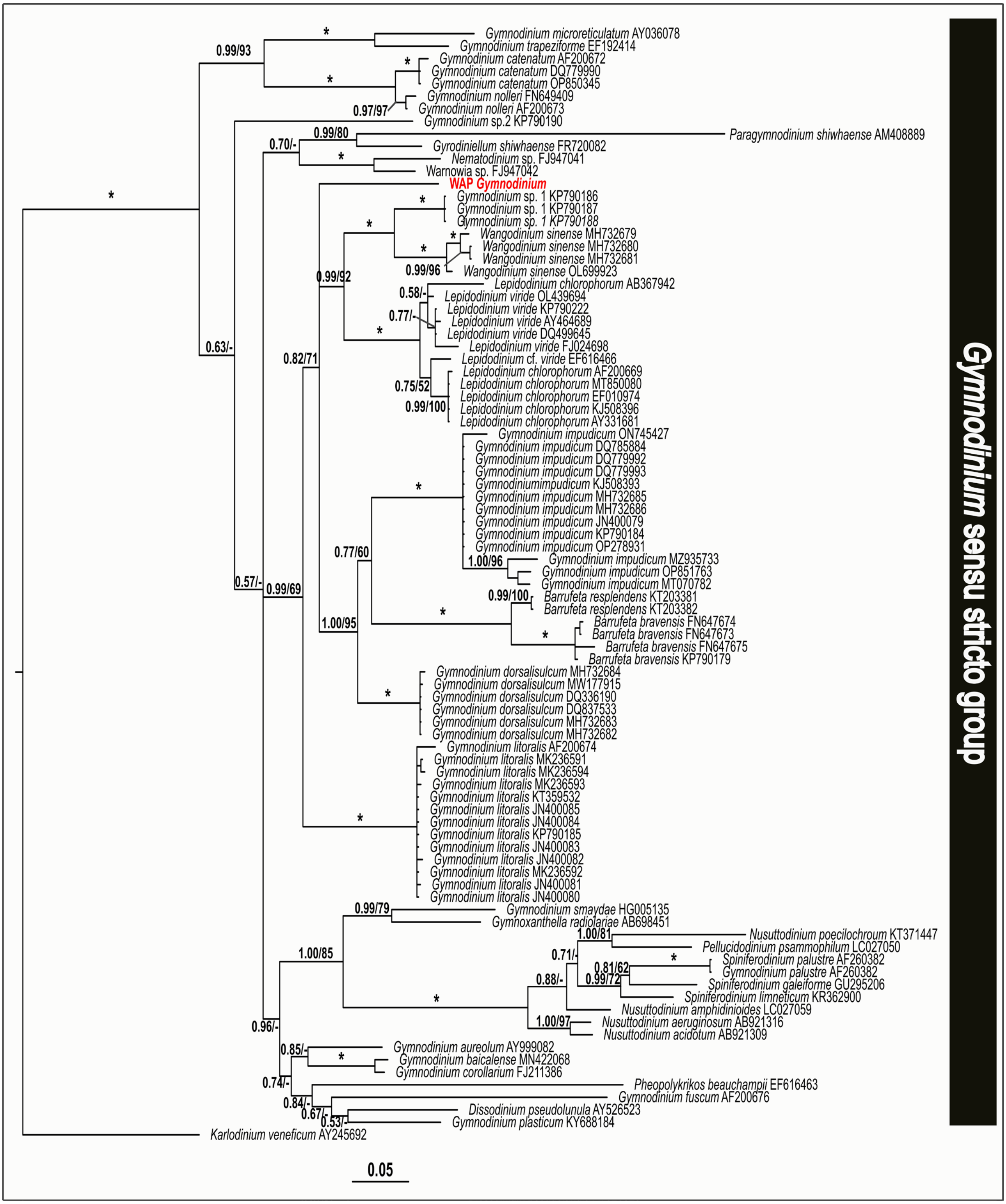

FIGURE 3 A phylogenetic tree of *Gymnodinium* sensu stricto group including the WAP *Gymnodinium* sequence inferred from partial LSU rDNA region sequences using Bayesian inference, with *Karlodinium veneficum* as the outgroup. Branch lengths are drawn to scale, with the scale bar indicating the number of the substitutions per site. Numbers on branches are statistical support values (PP/ML BS). Bootstrap support values >50% and PPs above 0.5 are shown. *Indicates maximal support (ML BS: 100% and PP: 1.00).

organisms with plastids derived from them (i.e., prymnesiophytes, diatoms, cryptophytes, dinoflagellates, and apicomplexans). However, *Lepidodinium*, a dinoflagellate genus closely related to the WAP *Gymnodinium*, was in an entirely different clade closely related to chlorophytes due to its chloroplast of prasinophyte origin.

Distribution

Antarctic Peninsula distribution

In the FjordPhyto metabarcoding dataset, the WAP *Gymnodinium* ASV was detected in 77% of samples ($n=233$) collected during 2017–2022. This ASV was detected throughout the WAP (Figure 1a) in 70% of the sampling sites and throughout all months in the four sampling seasons, showing an increase in its relative frequency from November to March (Figure S4). The highest relative abundance peak of the WAP *Gymnodinium* ASV was detected in March 2018 (9.5%), but relative abundances rarely exceeded 5% of total reads (Figure S5).

Global distribution

In the Tara Oceans 18S V9 ASV metabarcoding dataset, ASV26740 was identical to the WAP *Gymnodinium* ASV sequence. This ASV presented a wide distribution in both hemispheres, with the maximum relative reads in the Southern Ocean (Figure 1c). ASV26740 was more frequently observed in surface waters and in the 0.8–5 μm and 5–20 μm size-fraction samples (Figure S6).

DISCUSSION

Morphology

The presence of a horseshoe-shaped apical groove in the blooming organisms suggests their inclusion in the *Gymnodinium* sensu stricto group, yet it has already been mentioned that this characteristic is not sufficient to group these organisms together (Daugbjerg et al., 2000; Hansen et al., 2000). Unfortunately, the limited volume of the available field sample (120 mL) did not allow us to perform ultrastructural analyses and prevented us from assessing further characteristics in the WAP *Gymnodinium*. It should be noted that new genera and species within the *Gymnodinium* sensu stricto group have recently been established from cultures, for example, *Wangodinium* (Luo et al., 2018) and *G. plasticum* (Wang et al., 2017), among others. Therefore, efforts should be made to establish cell cultures of the WAP *Gymnodinium*, as well as other Antarctic naked dinoflagellates, to better assess the ultrastructure of dinoflagellates in Southern Ocean waters.

Phylogeny

The phylogenetic results of this study suggest that the first bloom of small naked dinoflagellates recorded in the WAP was monospecific and caused by an undescribed species—here referred to as WAP *Gymnodinium*—which belongs within the monophyletic group *Gymnodinium* sensu stricto. The *Gymnodinium* sensu stricto group, although it is a highly supported monophyletic group based on LSU rDNA region sequences (Gu et al., 2013, 2015; Luo et al., 2018; this study), is a very diverse group including several genera (e.g., *Lepidodinium, Warnowia, Nematodinium*, and *Polikrykos*).

The phylogenetic distance between the WAP *Gymnodinium* and the sister clades suggests it forms a separate species probably belonging to an undescribed genus. In addition, the trichotomy in which the WAP *Gymnodinium* was observed shows that the relationships within this clade are not yet resolved. The monophyletic groups formed by *Lepidodinium* and *Wangodinium*, and by *Barrufeta* with *G. dorsalisulcum* and *G. impudicum*, have been previously described (Gu et al., 2015; Luo et al., 2018; Reñé et al., 2011, 2015; Sampedro et al., 2011). Close species in the same clade as the WAP *Gymnodinium* include taxa that have been recently established (e.g., *Wangodinium* in Luo et al., 2018) as well as taxa transferred from *Gymnodinium* (e.g., *Barrufeta*, Sampedro et al., 2011, Gu et al., 2015). However, there were still *Gymnodinium* species within the same clade (i.e., *G. impudicum*, *G. dorsalisulcum*), despite being widely separated from the type species of the genus, *G. fuscum* (Romeikat et al., 2020). As already suggested by Sampedro et al. (2011), the position of *G. dorsalisulcum* and *G. impudicum* within the *Gymnodinium* genus should be revisited. This work has demonstrated that there are still intermediate genera to be discovered in the *Gymnodinium* sensu stricto group, so further exploration could help to resolve the phylogeny (Romeikat et al., 2020).

The fact that this WAP *Gymnodinium* was an undescribed species is not surprising, since our knowledge about naked dinoflagellates in polar areas is very limited, and there are no LSU rDNA D1–D3 region sequences from Antarctic *Gymnodinium* sensu stricto species in online libraries or repositories. The sequences produced in this research are the first Antarctic ones to be incorporated in the phylogeny of the *Gymnodinium* sensu stricto group. To fully understand *Gymnodinium* sensu stricto evolutionary history, it is crucial to incorporate sequences from diverse environments (Wang et al., 2017), including sequences from all over the world.

Phylogenies with partial SSU rDNA region plastid sequences showed that the plastid sequence of the WAP *Gymnodinium* is related to dinoflagellates with peridinin (Figure S3). Unfortunately, plastid sequences from dinoflagellates available in libraries are very scarce; therefore, more studies on this topic are necessary, to better understand the evolutionary history of these plastids (Klinger et al., 2018). For further comparisons, the morphological differences between the WAP *Gymnodinium* and closely related taxa are in Table S3.

Distribution

Our results showed that the ASV dominating the bloom has a wide global distribution, suggesting it is not restricted to Antarctic waters. However, the higher relative reads of this dinoflagellate detected in Antarctic waters, including this first bloom recorded to date, indicate a strong affinity for the Southern Ocean. Other authors have noted that naked dinoflagellates could be more diverse in the Antarctic than previously believed (Le Bescot et al., 2016). Although the high relative reads observed by metabarcoding do not necessarily indicate an actual high biomass of these organisms, we can confirm their conspicuous presence in the WAP during the summer months, with an increasing presence toward the end of summer. This agrees with previous observations of the increasing abundance of naked dinoflagellates in the WAP at the end of the season (Costa et al., 2020; Mascioni et al., 2023), which may be related to climate change effects in this region (Lange et al., 2018). This first assessment of the distribution of these organisms is key for future studies, as the distribution of planktonic organisms remains largely unexplored (Ibarbalz et al., 2019).

The specific identity of the naked dinoflagellates in the WAP cannot be assessed by traditional microscopy in most cases (Mascioni et al., 2019). The use of metabarcoding, and particularly 18S V9 rNDA region ASVs, was useful for identifying the potential distribution of WAP *Gymnodinium*. Despite the 18S rDNA gene having relatively low variability for dinoflagellates, it remains advantageous for a widespread, first-level evaluation of protist biodiversity due to its ease of comparison and its large representation in reference databases (Burki et al., 2021; de Vargas et al., 2015). In this sense, this work highlights the contribution of phytoplankton monitoring studies using metabarcoding, such as the FjordPhyto citizen science program (Cusick et al., 2020). However, the 18S V9 rDNA region is inconsistent with the phylogenetic relationships we observed with the partial LSU rDNA region. There is no agreed universal primer set specifically for all protists, yet the 18S rRNA gene has been chosen as a general marker (Vaulot et al., 2022), and combining sequences can help better understand their evolutionary history (Ott et al., 2022). Including microscopy observations into metabarcoding results is of great importance for databases, to improve the accuracy of molecularly identified organisms (Pierella Karlusich et al., 2020).

AUTHOR CONTRIBUTIONS

Martina Mascioni: Conceptualization (lead); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); writing – original draft (lead). **Allison Cusick:** Data curation (supporting); formal analysis (supporting); investigation (supporting); writing – review and editing (equal). **María Virginia Sanchez-Puerta:** Formal analysis (supporting); investigation (supporting); supervision (supporting); writing – review and editing (equal). **Christian Johnson:** Methodology (supporting); writing – review and editing (equal). **Hong Zheng:** Methodology (supporting); writing – review and editing (equal). **Rick A. Reynolds:** Project administration (equal); writing – review and editing (equal). **Andrew E. Allen:** Resources (supporting); writing – review and editing (equal). **Gastón O. Almandoz:** Conceptualization (supporting); funding acquisition (equal); investigation (supporting); project administration (equal); supervision (supporting); writing – review and editing (equal).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Part alignment of the SSU 18S V9 rDNA region sequences from several taxa included in the *Gymnodinium* sensu stricto group and a sister clade from the Gymnodiniales (*Karlodinium veneficum*). The positions that differ are highlighted with colored background

Figure S2. A phylogenetic tree of *Gymnodinium* sensu stricto group including the WAP *Gymnodinium* sequence inferred from concatenated data of partial LSU rDNA and both internal transcribed spacer (ITS1 and ITS2) rDNA regions including the 5.8S rDNA gene sequences using BI with *Karlodinium veneficum* as the outgroup. Branch lengths are drawn to scale, with the scale bar indicating the number of the substitutions per site. Numbers on branches are statistical support values to clusters on the right of them (PP/ML BS). BS values >50% and PP above 0.7 are shown. *Indicates maximal support (ML BS: 100/PP: 1.00).

Figure S3. Phylogenetic relationships between plastids of dinoflagellates and chlorophytes, rhodophytes, diatoms, prymnesiophytes, cryptophytes, and apicomplexans (apicoplast), including the WAP *Gymnodinium* sequence (in red), inferred from partial SSU rDNA plastid sequences by Bayesian inference. Note that dinoflagellate species are in bold. The red arrow shows the position of the plastidial ancestor of the red algal group. Branch lengths are drawn to scale, with the scale bar indicating the number of the substitutions per site. Numbers on branches are statistical support values (PP/ML BS). BS values >50% and PP above 0.7 are shown. *Indicates maximal support (ML BS: 100/PP: 1.00).

Figure S4. Box plot of the relative frequency of the WAP *Gymnodinium* ASV in samples collected from November to March in four summer growth seasons (2017-2022). The relative frequency was calculated as number of samples with WAP *Gymnodinium*/total number of samples per month per year. Note that the boxplot represents the quartiles (Q1, Q2—median, Q3), the height of the box is the difference between Q3 and Q1 and the black line inside the box divides the distribution into two equal parts (median).

Figure S5. Relative reads by month and season of the ASV corresponding to WAP *Gymnodinium* in the FjordPhyto dataset (2017–2022). Panel **a** shows the total distribution of values and Panel **b** shows a trim of the values on the Y-axis between 0.0 and 2.5.

Figure S6. Frequency of ASV26740 in the Tara Oceans dataset according to (a) different depths DCM (Deep Chlorophyll Maximum), MES (mesopelagic, >200 m), SRF (Surface) ZZZ (no information), and (b) different size fractions. See Ibarbalz et al. (2019) for more details on Tara Oceans sampling.

Table S1. Sequences downloaded from GenBank used in phylogenethic analyses

Table S2. Sequences downloaded from GenBank used in phylogenethic analyses

Table S3. ASVs Feature ID from the bloom sample, its taxonomic annotation according to Protist Ribosomal Reference database (PR2, v4.14.0) along with the confidence interval.

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