Effector Repertoire and Host Transcriptomic Responses in the Tripartite Interaction Between the bloom-forming diatom *Coscinodiscus granii*, the oomycete *Lagenisma coscinodisci* and Co-occurring Pedinellale *Pteridomonas*.

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Abstract:

Parasitic interactions play a central role in shaping phytoplankton community dynamics. Understanding the host response is crucial to fully comprehend these interactions. In this article, we investigate the tripartite interaction between the bloom-forming centric diatom *Coscinodiscus granii*, its oomycete parasite *Lagenisma coscinodisci*, and a co-occurring heterotrophic flagellate (*Pteridomonas* sp.).

A major challenge in infection transcriptomics is that cells are often at different infection stages. To overcome this, we manually sorted *C. granii* cells at comparable infection stages to perform transcriptomic analyses. We assembled the first transcriptomes of both *C. granii* and *L. coscinodisci*. Our analysis reveals a sophisticated effector repertoire in *L. coscinodisci*, including canonical oomycete virulence factors such as Crinklers, RxLR effectors, cystatins, and transposon-associated proteins, as well as components of the RNAi machinery (Argonaute, Dicer, RdRP) and cyclophilins.

Notably, *C. granii* exhibits a transcriptional response involving proteases and exosome-related pathways, suggesting a deeply conserved, possibly ancient, mechanism of defense. In parallel, we analyzed the differential expression of *Pteridomonas*, which consistently co-occurred in culture, and identified a distinct transcriptional profile characterized by the upregulation of motility genes, highlighting a striking mobility strategy.

Thanks to the exceptionally large host size, together with available transcriptomic and metabolomic data, this tripartite system provides a unique marine model to study oomycete-diatoms interactions.

Introduction

Parasitism is acknowledged as one of the most prevalent ecological strategies among heterotrophic organisms [1]. Protist parasites account for over 50% of the richness and abundance within the heterotrophic pico-nanoplankton (0.8–5 µm) sequenced during the *Tara* Oceans expedition [2]. [2] Parasite organisms often act as parasitoids—killing their unicellular hosts—and can sometimes exert top-down control on microalgal populations, thereby influencing host's bloom dynamics and termination events [3,4]. In other cases, they are not directly lethal, but may lead to sterility or affect the host's physical condition and recruitment rates.

A number of eukaryotic parasites infecting microalgae have been identified in marine plankton. Such is the case of *Amoebophrya*, a wide-ranging and varied group of Syndiniales (also known as marine environmental honeycombs or MALVs [5]) infecting and controlling dinoflagellate populations. Exploring key elements in the interaction with the host throughout their life cycle has shed light on their infection strategies [6,7].

Diatoms, responsible for approximately 40% of the oceanic export of particulate organic carbon [8] are no exception. They are particularly susceptible to infection by a variety of parasitic eukaryotes, including dinoflagellates, rhizarian, aphelida, and stramenopiles such as hyphochytrids and labyrinthulomycetes [9–11]. Among diatoms, the class Coscinodiscophyceae includes large centric species such as *Coscinodiscus granii*, that commonly form dense, seasonal blooms in coastal environments [12]. These blooms are targeted by various parasitic organisms, including the oomycetes (stramenopiles), a group of heterokont parasites known for their roles in aquatic and terrestrial systems [13]. This is the case of *Pirsonia diadema*, a parasitoid nanoflagellate belonging to the Stramenophila group, which selectively infects C. granii by chemosensory stimulation, mechanical attachment and cell wall penetration. An intriguing feature of this complex interaction is the preference given to already infected cells, which increases the potential for subsequent infection [14], as does the influence of environmental factors such as turbulence in the infection.

One major marine oomycete, *Lagenisma coscinodisci*, has been identified as a specific parasite of *C. granii* species and proposed as a model organism [15]. Unlike most diatom-infecting oomycetes, *L. coscinodisci* develops true hyphae, an elongated filamentous structures that facilitate host penetration and nutrient absorption and has been molecularly characterized as an early-diverging member of crown oomycetes, indicating its evolutionary significance within the group [15,16]. *L. coscinodisci* contributes to *C. granii* bloom decline by massive host lysis and facilitate nutrient recycling within the ecosystem [12].

In the first molecular insight study of the oomycete *L. coscinodisci, a combination of co-cultivation experiments and comparative metabolomics approach revealed how the parasite* manipulates the metabolome of its diatom host *C. granii* to enhance its infection success [17]. During infection, the oomycete produces specific alkaloids, such as β-carboline and 2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid, which accumulate in the reproductive form of the parasite. These compounds inhibit algal cell division, promote plasmolysis, and increase the infection rate, demonstrating a sophisticated hijacking of host metabolic pathways to facilitate the oomycete's proliferation [17].

In parasitism studies, a substantial limitation to classical approaches of gene expression analysis is the presence of asynchronous infection stages in batch cultures [18]. In our study, we overcome this obstacle by performing sorted-cell transcriptomics on individually infected *C. granii* cells. After sampling the natural bloom samples from the bloom at Helgoland, Germany, in November 2019, the

diatom host *C. granii*, the oomycete *L. coscinodisci* strain LagC19 and associated native protists were isolated characterized and maintained continuously in laboratory cultures. Diatom infected cells in the reproduction stage, i.e., the sporangium (Figure 1), presenting a grown filamentous within its cytoplasm were one-by-one harvested with a Pasteur pipette, making up the infected samples further processed for transcriptome analysis. To apprehend community chemical sensing, we also exposed *C. granii* healthy cells to infected *C granii* cells in the lytic phase using dual cultivation chambers. We also observed and characterized a Pedinellales (Dictyochophyte), a flagellate protist consistently present in *C. granii* cultures. Pedinellales display distinct morphological features that may have an impact on their ecological niches and the way they interact with other microorganisms. In addition, their mixotrophic abilities, permitting them to trap food particles, may point to possible interactions with other protists or diatoms in shared environments [19]. To explore the molecular response of the diatom host to its associated protists, we conducted a differential expression analysis of the Pedinellales, aiming to characterize potential opportunist, escape, or defense responses and their role within this tripartite microbial interaction.

These insights contribute to a more integrated understanding of parasite-driven regulation in phytoplankton communities and their implications for marine ecosystem functioning, and they pave the way to future research on molecular interactions within these communities.

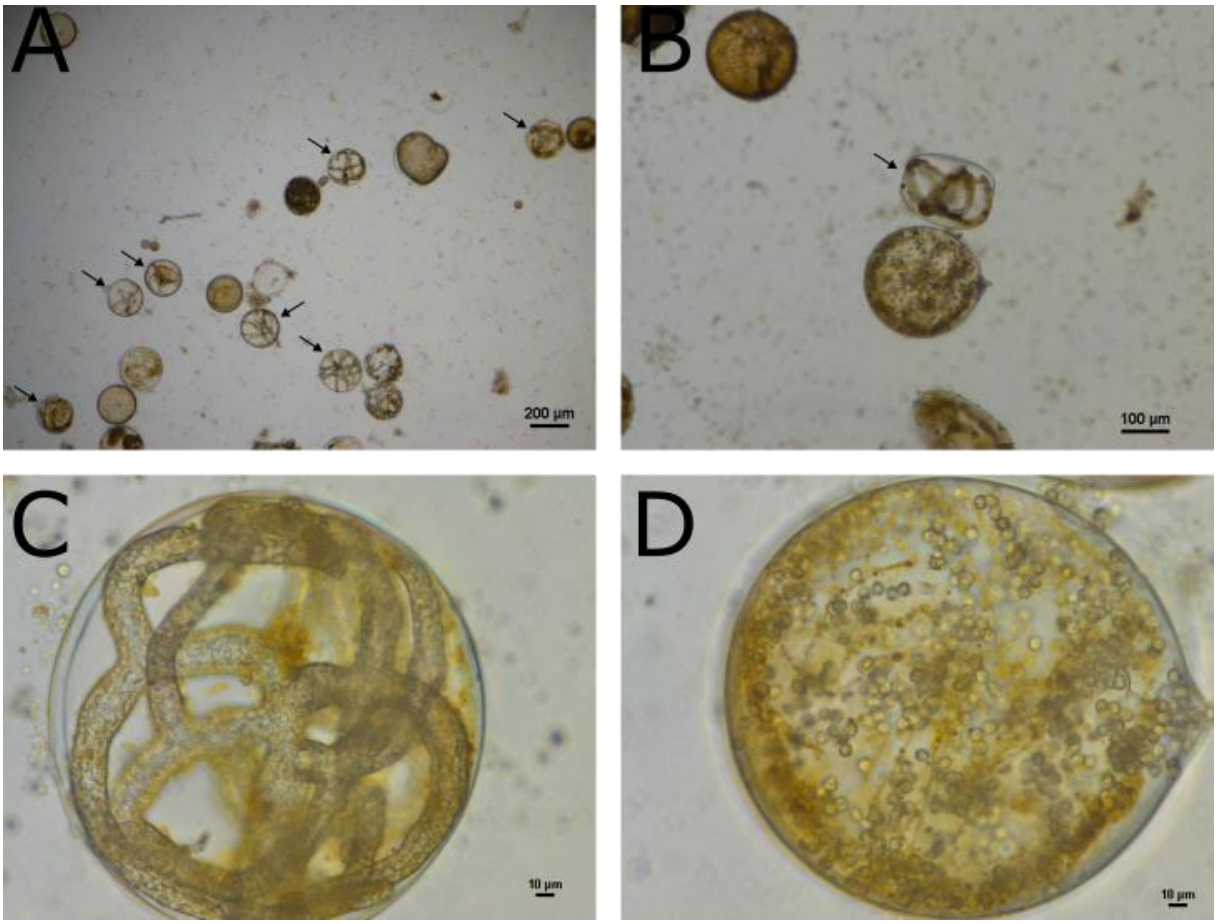

Figure 1. *C. granii* culture observed under bright field microscope. Infected algal cells in the sporangium stage were selected and manually isolated when a fully developed hyphae (C) was visible within (cells with black arrow). Mature stage cells were not sampled (D).

Material and methods:

Strains and cultivation

Marine diatom *C. granii* and the pathogenic oomycete *L. coscinodisci*, specifically the strain LagC19 were collected from Helgoland, Germany, a location in the North Sea, during the phytoplankton bloom in November 2019. Guillard's f/2 enrichment medium (G9903, Sigma-Aldrich, Munich, Germany) was prepared by adding 1% of the enrichment solution to 0.22 µm filtered and autoclaved natural seawater (Helgoland, AWI, Bremenhaven, Germany). The cultures were maintained under LED lights at 100 µE m-2 s-1 of irradiance (16-12 °C, photoperiod 14h: 10h, day-night). Microscopy was performed using an inverted microscope (AE30, Motic) for regular checks and sub-cultivation, and a Zeiss Imager 2 (Carl Zeiss) to record microscopic images

Infection experiments

To initiate infection in the diatom host, healthy *C. granii* cells were co-cultivated with infected cells of *L. coscinodisci* by transferring 40 µL of cells in late infection stage (sporangia) into 40 mL of healthy algal cell culture. This involved transferring single diatom cells into a culture with the pathogen, allowing the oomycete to attach and penetrate the algal cells. The infection was maintained by regularly inoculating healthy cultures with aliquots of infected cells every 7 days. The infection progressing in diatom cells was monitored using light microscopy. For the exposition experiment, non-infected *C. granii* were cultivated in parallel to an infected culture using a dual-chamber cultivation system, following established protocol in Vallet et al., 2019 [17].

Sampling, RNA extraction, cDNA libraries construction and sequencing.

This design enabled the comparison of transcriptomic profiles across three distinct groups: healthy control algal cells, non-infected algal cells exposed to infection-derived exudates, and infected alga cells. Due to the asynchronous nature of the infection process, we manually isolated 3 x 100 infected cells at the sporangium stage using an aspiration micro-syringe under the microscope to ensure homogeneity and synchronous samples in the same infection stage, making up the infected algal samples. Infected algal cells in the sporangium stage were selected and isolated when a fully developed hyphae was visible within (Figure 1). The strains used in this study are publicly available and can be obtained free of charge for research purposes upon request to M. Vallet.

Flash-frozen pellets were extracted using RNeasy Plus Universal Mini Kits (Qiagen, Ref 73404) following the manufacturer's instructions. All extracted RNA samples were treated with 6U of TURBO DNase (2 U/µL) (Thermo Fisher Scientific, Ref. AM2238), then purified with RNA Clean and Concentrator-5 kit (Zymo Research, Ref. ZR1016), keeping only large RNA fractions (>200 nt) for RNAseq library preparation. A total of 30 ng of treated RNA was used to produce Illumina libraries (Illumina Stranded mRNA Prep, Ligation). Briefly, poly(A)+ RNAs were selected with oligo(dT) beads, chemically fragmented by divalent cations under high temperature, converted into single-stranded cDNA using random hexamer priming, followed by second-strand synthesis and 3'-adenylation. A pre-index anchor was ligated, and a PCR amplification step with 18 cycles was conducted to add 10 bp unique dual index adapter sequences (IDT for Illumina RNA UD Indexes, Ligation). All libraries were quantified using Qubit dsDNA HS Assay measurement. A size profile analysis was performed in an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). The library preparation failed for one sample of RCC697 (200 µM NO_3). Libraries were sequenced in 2 × 150 bp on an Illumina NovaSeq 6000 sequencer (Illumina, San Diego, CA, USA) in order to obtain 50 million paired-end reads. After Illumina sequencing, an in-house quality control process was applied to the reads that passed the Illumina quality filters [20]. Briefly, Illumina sequencing adaptors and primer sequences

were removed, then low-quality nucleotides ($Q < 20$) were discarded from both ends of the reads. Sequences between the second unknown nucleotide (N) and the end of the read were also trimmed. Reads shorter than 30 nucleotides were discarded after trimming with an adaptation of the fastx_clean tool (https://www.genoscope.cns.fr/fastxtend/). In the last step, reads that were mapped to the Enterobacteria phage PhiX174 genome (GenBank: NC_001422.1) were discarded using bowtie2 v2.2.9 (-L 31-mp 4-rdg 6,6-local-no-unal) [21]. Remaining rRNA reads were removed using SortMeRNA v2.1 and SILVA databases version 119 [22,23].

Transcriptome assembly

Healthy, exposed and infected readsets were co-assembled using Trinity v2.11.0 [24], then isoforms were clustered using SuperTranscripts [25]. Transcripts larger than 250 bp were retained. Taxonomic affiliation of assembled transcripts was performed with DIAMOND BLASTx (v2.1.8) against MaRRFeret v1.1 [26] and NR v2025-02-12 [27] databases. For each transcript, a lowest common ancestor (LCA) was calculated with all matches with an e-value <10-5 and above 95% of the best match (diamond option -outfmt 102 and -top 5). Functional annotation was conducted on six-frame translated sequences using multiple databases and tools: DIAMOND blastP against the KEGG protein database v2024-07-26 InterProScan v5.61-93.0 including Gene Ontology (GO) term search, eggNOG mapper v2.1.12 and its database v 5.0.2, and the HMM search tool KofamKoala v1.3.0 to find KEGG Orthologues. We used [28] KEGG BRITE hierarchical classification systems [28] to attribute a global functional classification to each transcript. Functional and taxonomic information for each transcript are available in SupTableX.

Sequenced reads were mapped back to the reference transcriptome using BWA-MEM2 v2.2.1, then sorted with SAMtools v1.18. Primary read alignments with more than 95% of identity over 80% of their length and a minimum of 100bp were selected. Read counts for each transcript were calculated with samtools idxstat. In R v4.4.1, we selected transcripts assigned to the three dominant taxonomic groups: Bacillariophyta (mostly assigned to *C. granii* l *Bacillariophyta*), Oomycetes (mostly assigned to *L. coscinodisci)*, and [17]. The Sankey plot was done with R package network3D.

Differential expression analyses

Differential expression analyses were performed using the DESeq2 package [29].

All subsequent analyses were conducted in R. Gene expression data were first filtered to retain only transcripts with sufficient abundance, defined as having at least 100 reads in one or more samples. Taxonomic assignments were based on Diamond LCA annotations, and genes were classified into major groups of interest, including *C. granii* (Bacillariophyta), *L. coscinodisci*-related oomycetes (Oomycota, Saprolegniaceae), and Pedinellales (Dictyochophyceae). For interspecies comparisons such as Pearson correlation analyses, genes were further filtered to include only those with a minimum expression level of 500 reads in at least one sample. Photosynthesis-related genes within the Pedinellales group were identified based on our annotation with specific Gene Ontology (GO) terms associated with photosynthetic processes.

Structure prediction

The protein structure of the *C. granii* SSL protein was predicted using AlphaFold 3 (Jumper et al., 2021). The input amino acid sequence TRINITY_DN10640_c0 was used to generate a 3D structural model in PDB format.

Pedinellale identification from RNAseq reads.

To identify the Pedinellale protist present in C. granii culture, we aligned RNAseq reads of the HEALTHY condition (replicate 1) on the full length 18S rRNA sequence of Pteridomonas danica (GenBank: PQ770944.1) and C. granii (AY485495.1) with BWA-mem2 (version 2.2.1). Reads aligned on Pteridomonas danica 18S rRNA with more than 90% of identity over 80% of their length were retained then a consensus sequence was build with bcftools consensus version 1.21 [30]. Complete 18S rRNAs sequences of Dictyochophytes available in the PR2 database [31] were aligned with mafft v7.49 using default options [32]. A maximum likelyhood phylogeny with 100 bootstrap was performed on MEGA version 11 [33].

Results and discussion

C. granii/*L. coscinodisci* is an emerging pathosystem, which includes non-model organisms for which no reference genome is yet available, and which represent one of the first models for studying phytoplankton-eukaryotic parasitoid interactions in marine ecosystems. This makes the system highly relevant for research on host-pathogen co-evolution and the ecological impact of oomycete parasitesi in oceanic primary producers. L. coscinodisci is among the first described parasitoids; L. coscinodisci is a parasite of centric diatoms of the genus *C. granii*, first described by Drebes in 1968 [16], and is a representative of the little-studied marine holocarpic pathogenic Oomycota [34]. Only a transcriptome exists for the diatom *C. wailesii* in the MMETSP database [35]. Thus we conducted a *de novo* transcriptome assembly and obtained 470,730 transcripts from the co-assembly of the 9 RNA readsets (healthy, exposed, and infected conditions in triplicates), subsequently clustered into 342,581 non-redondant super-transcripts larger than 250bp. We identified 15,712 transcripts affiliated with diatoms, including 7,549 with *C. wailesii* as best match and the others matching distant diatoms transcriptomes and genomes and therefore affiliated more broadly to Coscinodiscophyceae (2,188 transcripts) or Bacillariophyta (5,838 [36] transcripts) (Figure 2). Similarly, 12,130 transcripts correspond to *L. coscinodisci* (Oomycota phylum) with 3,807 transcripts attributed to Oomycota and 4,542 to Saprolegniaceae. Finally, 15,300 transcripts were attributed to the Pedinellales (Dictyochophyceae), with 11,800 of them affiliated to *Pteridomonas danica*. An additional 5,045 eukaryotic transcripts could not be precisely assigned, likely due to incomplete reference transcriptomes or highly conserved proteins, identical between distant taxa.

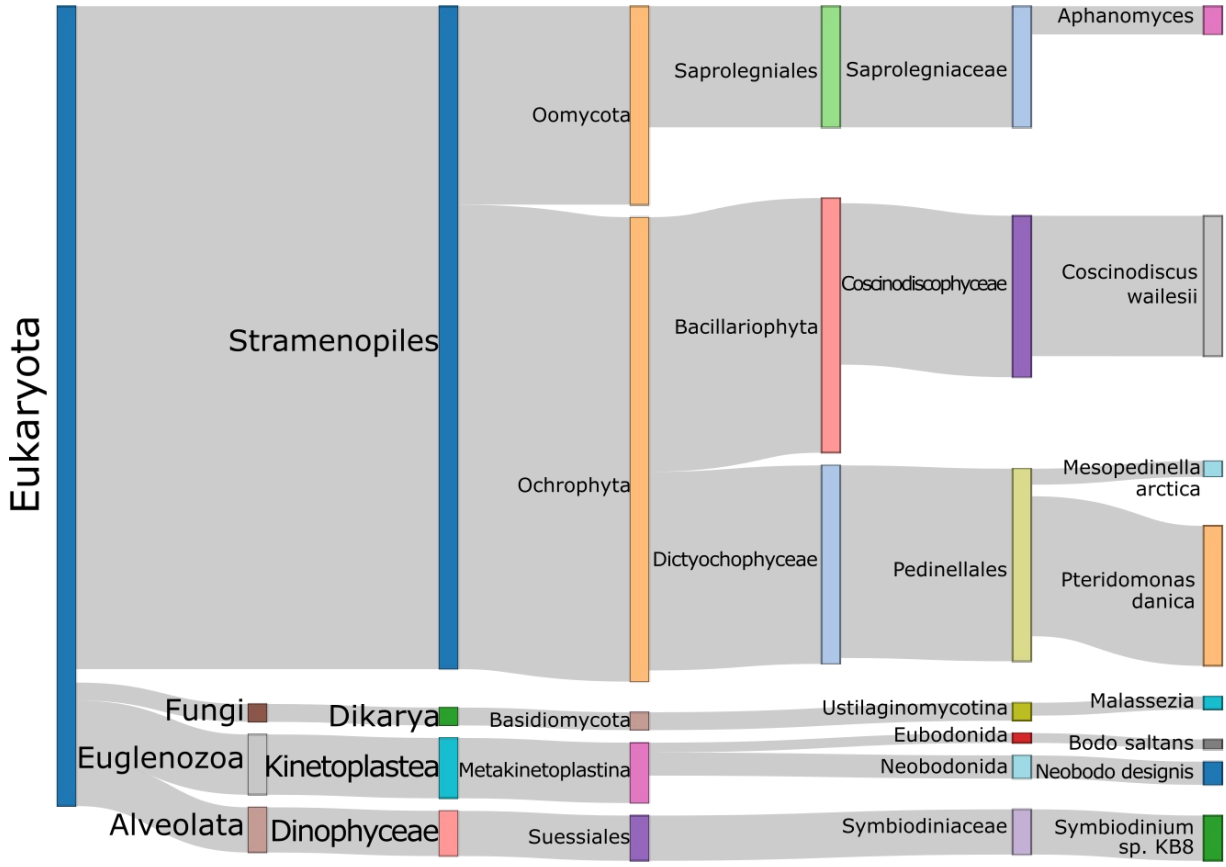

Figure 2 : Taxonomic affiliation of eukaryote transcripts assembled from *C. granii* cultures (Healthy, Exposed, and Infected). Taxa with less than 500 genes were removed.

To precise the taxonomy of the co-occuring Pedinellales, we built an 18S rRNA consensus sequence from RNAseq reads and performed a phylogeny of all Dictyochophyceae 18S rRNA. The Pedinellale sequenced in this experiment is closely related to *Pteridomonas danica* but with 3% of divergence on the 18S sequences. Therefore, we named it *Pteridomonas* sp.

The *C. granii* and *Pteridomonas* transcriptomes exhibited high completeness (80.6% complete BUSCOs) and low fragmentation (9% and 6.3% fragmented genes, respectively). In contrast, the *L. coscinodisci* transcriptome displayed slightly lower completeness (66.2%) and higher fragmentation (12.7%).

Global transcriptional patterns were compared across sample types using Pearson correlation of gene expression levels within each lineage. For both *C. granii* and *Pteridomonas*, healthy and exudate-exposed samples showed highly similar profiles (persons mean 0.97 and 0.93, respectively), indicating limited transcriptomic response in the absence of direct infection. Infected samples, however, displayed markedly distinct expression patterns (persons mean 0.9 and 0.76, respectively), consistent with expected major transcriptional reprogramming in the *L. coscinodisci* infected samples.

To determine the trophic mode of the co-occurrent *Pteridomonas*, we searched for photosynthetic genes in its transcriptome. GO term analysis revealed no genes associated with photosynthesis in this lineage, in stark contrast to C. granii, which shows a full set of photosynthesis-related GO terms including "photosynthesis" (GO:0015979), "photosynthetic electron transport" (GO:0019684), and "chlorophyll binding" (GO:0016168). Thus, the Pteridomonas present in our culture is likely a

heterotrophic or mixotrophic lineage that do not express photosynthetic genes in our conditions, consistent with known diversity of trophic strategies within the group.

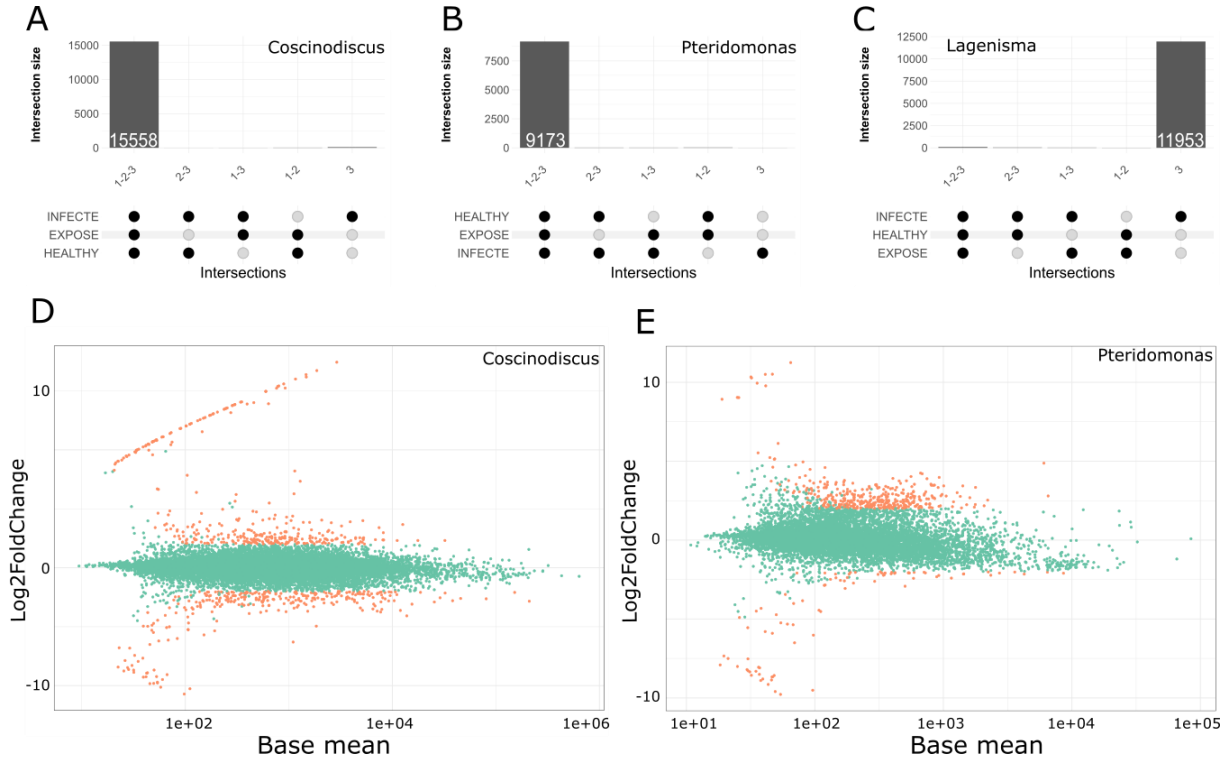

Figure 3: **(A–C)** Taxonomic affiliation of reads across sample: UpSet plots showing the number of differentially expressed genes (DEGs) unique to or shared between conditions for C. granii (A), Pteridomonas (B) and L. coscinodisci (C). Bar heights represent the size of each intersection, and dots below indicate the conditions involved in each intersection. **(D–E)**: MA plots displaying log2 fold changes versus gene abundance (log counts). Orange points represent significantly up- or down-regulated genes, while green points indicate non-significant genes for C. granii (D) and Pteridomonas (E)

Following *L. coscinodisci* infection, we identified 340 up-regulated genes and 304 down-regulated genes in *C. granii*, and 392 up and 87 down in *Pteridomonas*. The analysis of Brite C functional categories reveals extensive transcriptomic landscape remodelling characterized by upregulation in DNA/RNA processing pathways, including Chromosome and associated proteins (br:ko03036),DNA replication proteins (br:ko03030), and Ribosome biogenesis (br:ko03009). Additionally, functions related to membrane trafficking (br:ko04131) and transporters (br:ko02000) are also significantly affected, exhibiting both upregulation and downregulation depending on the cellular context. The differential gene expression analysis revealed no significant gene expression difference between healthy and exposed sample. Both *C. granii* and the *Pteridomonas* appear unable to sense the cellular lysate of *C. granii* infected by *L. coscinodisci*. The *Pteridomonas* also responds with changes at chromosome and associated protein level but shows a high mobility response with striking upregulation of cilium and associated proteins, cytoskeleton proteins, membrane trafficking, and motor proteins.

During parasite infection, there is an intricate response of organisms to each other. To better understand the involvement of the *L. coscinodisci* weapon repertoire during the infection, we looked for sequences with annotation as plant-infecting oomycetes effectors in the *L. coscinodisci* transcriptome notably from the Crinkler, cystatin, and RxLR families, as well as transposable element machinery, silencing machinery and cyclophilins (Table I).

	Proposed function	Number of sequences
Effectors	Crinkler	5
	Cystatin	7
	RxLR	13
Silencing	Argonaute	3
	Dicer	4
	RdRP	1
PPIases	Cyclophilins	18

Table I: Reads attributed to oomycete and their putative function as virulence effector after gene annotation (PPIases: peptidyl-prolyl cis-trans isomerases, RdRP RNA-dependent RNA polymerase)

Crinkler in *L. coscinodisci*

Secreted by oomycete pathogens, such as *Phytophthora* species, they play a crucial role in promoting infection by manipulating host plant cellular processes. Cinkler effectors like CRN12_997 from *P. capsici* target transcription factors such as SlTCP14-2 in tomatoes, inhibiting their DNA-binding ability and thus diminish the plant's immune response [37]. targeting specific sub-nuclear compartments, as seen in *P. capsici* infection [38]. PsCRN108 from *Phytophthora sojae* can reprogram plant gene expression by targeting promoters of heat shock proteins, thereby suppressing stress responses and enhancing pathogen virulence [39].

RxLR in *L. coscinodisci*

Pi22798 (RxLR) from *Phytophthora infestans*, promotes the homodimerization of the host transcription factor StKNOX3, enhancing plant susceptibility by modulating transcriptional regulation [40]. HaRxL10 from *Hyaloperonospora arabidopsidis* targets the transcriptional repressor JAZ3 to activate jasmonic acid signalling and suppress salicylic acid-mediated defences [41]. In *Hyaloperonospora arabidopsidis*, the oomycete RxLR effector HaRxL10 directly targets the central *Arabidopsis* circadian clock component CHE (CCA1 HIKING EXPEDITION), stabilizing it by inhibiting its degradation via the E3 ligase ZEITLUPE, which paradoxically suppresses CHE function, reprograms circadian gene expression, and enables the pathogen to repress plant immunity while manipulating growth and flowering processes [42].

Cyclophilin in *L. coscinodisci*

Certain cyclophilins (CYPs) are implicated as virulence factors in oomycete infections as peptidyl-prolyl cis-trans isomerase (PPIase) [43]. This study identified 16 CYP orthogroups across 21 oomycete species, with each species encoding 15 to 35 CYP genes. Notably, three orthogroups contained proteins with signal peptides, indicating a potential role in secretion. Expression analysis in *Plasmopara infestans* and *Pl. halstedii* revealed distinct expression profiles during different asexual life stages, highlighting their importance in pathogenicity. We identify 18 cyclophilins transcripts in *L. coscinodisci* transcriptome.

***L. coscinodisci* siRNA / Piwi**

SiRNA are described in plants oomycete as tuning oomycetes effectors expression and directly implicated for virulence targetting host mRNA. We search for homologous genes cues for the presence of an active mRNA silencing pathway. Among L. coscinodisci expressed genes, we could identify several key components of the RNA silencing machinery, including RNA-dependent RNA polymerases (RdRP, RDR2, RDR6). Additionally, Argonaute1 (AGO1) a central component of the RNA-induced silencing complex (RISC), as well as argonaute linker 1 and 2 were detected, suggesting active silencing in *L. coscinodisci*. In addition, 2 genes have DUF1785 domain which is require in *A. thaliana* sRNA Duplex Unwinding [44].

Oomycete pathogens, such as *P. sojae*, secrete hundreds of effector proteins, including avirulence (Avr) proteins, that manipulate host immunity to promote infection. In a study by [45], it was discovered that endogenous small RNAs (sRNAs), derived from natural antisense transcripts, regulate the expression of the Avr1b gene in *P. sojae*. The study demonstrated that sense and antisense expression of Avr1b is influenced by 10-base deletions in promoter regions, affecting the generation of these sRNAs. Further genome-wide analysis revealed that up to 31% of RXLR effector genes in *P. sojae* are associated with sRNAs. These sRNAs likely contribute to effector gene silencing and expression plasticity, suggesting a broader role in the pathogen's ability to adapt and evade host immunity. Moreover, insertion/deletion variants of 9–10 base pairs were significantly enriched in the regulatory regions of RXLR genes, highlighting a mechanism by which bidirectional transcription and sRNA-mediated regulation shape effector expression patterns and, potentially, virulence.

In *A.thaliana*, the exchange of small RNAs (sRNAs) between the pathogen and the host, known as cross-kingdom RNA interference (ck-RNAi), leads to the silencing of hostspecific function [46]. For instance, the oomycete *Hyaloperonospora arabidopsidis* utilizes its sRNAs to bind to the plant's Argonaute (AGO) proteins, part of the RNA-induced silencing complex, to suppress host immunity and promote virulence [46].

Pteridomonas **but not C. *granii* has a typical pathogen-exposition response**

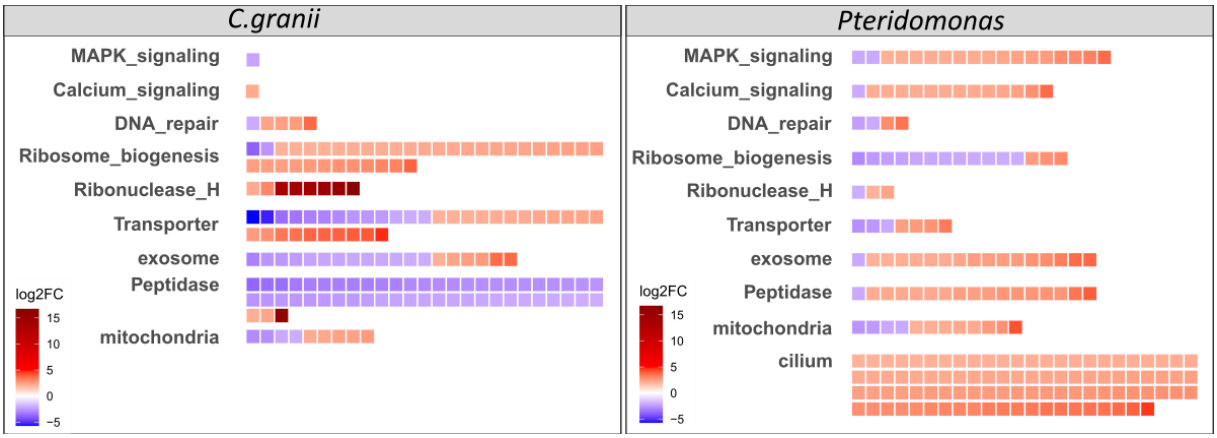

Figure 4: Overexpressed functions in *C. granii* and *Pteridomonas* in *C. granii*-infected sample. Each colored box represents a differentially regulated gene, classified according to its functional annotation using InterPro (IPR) domains (*Ribonuclease H*, *Peptidase*) or KEGG Brite category (Brite C) (*Transporter [BR:ko02000]*, *DNA repair [BR:ko03400]*, *Ribosome biogenesis [BR:ko03009]*, *Peptidase [BR:ko01002]*, *MAPK signaling [BR:ko04010]*, *Calcium signaling [BR:ko04020]*, *Cilium [BR:ko04961]*, *Exosome [BR:ko04147]*, *Mitochondria [BR:ko00190]*)

Eukaryotic cells have evolved sophisticated signalling mechanisms to respond to various biotic stresses, including infections by oomycete pathogens. In plants, a key signalling pathway involved in

this response is the Mitogen-Activated Protein Kinase (MAPK) cascade [47]. The MAPK signalling pathway is activated in *Pteridomonas* (16 genes, FC up to 3.7) but not in C. granii. These findings could suggest that Pteridomonas detect the parasite and mount a MAPK-mediated defense response.

Treatment of plant cells with a *Phytophthora sojae* protein elicitor induces a biphasic cytosolic Ca^{2+} increase, which coincides with a burst of reactive oxygen species (ROS) and phytoalexin accumulation [48]. Calcium-dependent protein kinases (CDPKs) then transduce the signal to defense effectors. In potatoes, StCDPK7 is upregulated upon *P. infestans* infection and phosphorylates phenylalanine ammonia -lyase in a Ca^{2+}-dependent manner [48]. Similarly, in pepper, the Ca^{2+} sensor CBL1 and its interacting partner CIPK1 are essential for resistance to *P. capsici. A* loss of CaCIPK1 increases susceptibility, while its overexpression enhances defence responses, including ROS production and cell death [48]. In our study, a strong calcium signalling response is observed in *Pteridomonas* exposed to *L. coscinodisci*, with 13 calcium-related genes upregulated (fold change (FC) up to +3.7). In contrast, *C. granii* shows a minimal response, with only one gene up-regulated (FC +2.17).

The role of extracellular vesicles (EVs) in plant immunity has gained growing interest in recent years. [49–51]. For decades, plant EVs have been observed to accumulate in infected cells, particularly beneath sites of pathogen attack and at papillae formation zones, where they are believed to contribute to the establishment of defensive barriers [49]. EVs have also been detected surrounding fungal haustoria and within the extrahaustorial matrix. These vesicles, which may carry antimicrobial compounds or RNAs, are hypothesized to deliver their contents into fungal haustoria either through membrane fusion or endocytic uptake. Interestingly, our transcriptomic data reveal that 16 genes associated with exosome-related functions (as annotated by BRITE category C) are upregulated in the *Pteridomonas*, and six related genes are upregulated in *C. granii*. This raises the question of whether Extracellular Vesicles (EVs) also exist in algae and what roles they might play. It is plausible that algal EVs carry defense-related molecules such as enzymes or small RNAs. Interestingly, Deng et al. (2024) showed that the diatom *C. granii radiatus* produces EVs during recovery from nutrient stress, which help eliminate harmful metabolites and support cell rejuvenation. Notably, this vesicle production is triggered by bacterial signals, suggesting a broader role for EVs in stress responses and microbial interactions [52].

In *Pteridomonas, a* substantial transcriptional reprogramming of motility-related genes is observed, with strong induction (up to +4.6-fold change) of 47 cytoskeleton-associated genes and 93 cilium-related genes, including members of the dynein and kinesin families. This shift in gene expression likely reflects one or both of two non-mutually exclusive strategies: an evasive behavioural response triggered by chemical cues released following parasite-induced cell lysis and/or a broader adaptation of motility and signaling pathways in response to environmental changes. Notably, the absence of significant upregulation in nutrient transporter genes suggests that this response is not driven by opportunistic feeding behaviour but aligns more closely with an active escape mechanism in reaction to infection-associated exudates. In parallel, several mitochondrial genes also exhibit strong upregulation, with log2 fold changes ranging from +2.2 to +4.9, indicating a possible increase in energy demand. This mitochondrial activation may support enhanced motility and cellular remodelling.

Genome stress, reparation, and ribonucleases

C. granii does not exhibit this early infection-related signalling response despite showing phenotype of infected cells. Instead, *C. granii* upregulates a variety of ribonucleases, notably members of the Ribonuclease H (RNase H) superfamily, with eight proteins showing fold changes up to +16.7. These

enzymes specifically target RNA/DNA hybrid structures, indicating genomic stress. The response also includes upregulating four DNA repair genes, with fold changes ranging from +2.3 to +3.7.

This observation raises the intriguing possibility that *C. granii* may contribute to host defense by degrading foreign RNA hybridized to regulatory regions of host DNA. R-loops are structures where RNA anneals to one strand of DNA leaving the other strand single-stranded, they are known to induce genomic stress and require timely resolution to maintain genome [53]. Additionally, ribosome biogenesis in *C. granii* appears significantly upregulated during oomycete infection, with 35 genes upregulated (maximum fold change +3.85). This observation mirrors findings in plants, where ribosome biogenesis is a crucial cellular process frequently targeted by oomycete effectors. For example, the *Phytophthora infestans* effector Pi23226 localizes to the plant nucleolus and binds the 3' end of 25S rRNA precursors, disrupting pre-rRNA processing, inducing nucleolar stress, and impairing global translation [54].

The ribosomal upregulation observed in *C. granii* is likely a compensatory mechanism in response to parasite-mediated inhibition of ribosome function, possibly involving parasite-derived cyclophilins that could interfere with ribosomal activity and protein synthesis.

Peptidase from *C. granii*

We could identify 460 peptidase gene in the *C. granii* transcriptome, that is consistent with the 461 peptidase genes found in *P. tricornutum* [55]. 32 peptidases are downregulated and 3 of them upregulated. A Papain-like cysteine peptidase is one of the most upregulated proteins during infection (log2fold change +16). Papain-like cysteine peptidases are crucial in plant-pathogen interactions, notably during oomycete infections [56]. These enzymes are part of a broader family of cysteine proteases involved in plant defense mechanisms by degrading pathogen proteins and activating immune responses.

However, pathogens such as *Phytophthora* species have evolved mechanisms to counteract these defenses by producing cystatin-like protease inhibitors. For instance, *Phytophthora palmivora* secretes PpalEPIC8, a cystatin-like inhibitor that targets papain in papaya, thereby enhancing the pathogen's virulence by inhibiting the plant's defense protease activity [57]. Similarly, *P. infestans*, responsible for late blight in tomatoes and potatoes, produces EPIC2B, which inhibits a novel papain-like protease, PIP1, involved in plant defense [58]. In *Arabidopsis*, the XYLEM CYSTEINE PEPTIDASE 1 (XCP1) and its inhibitor CYSTATIN 6 (CYS6) regulate pattern-triggered immunity by modulating the stability of the NADPH oxidase RBOHD, which is crucial for reactive oxygen species (ROS) production during immune responses [59]. The pathogen *Plasmodiophora brassicae* also employs a cystatin-like effector, SSPbP53, to inhibit cruciferous papain-like cysteine proteases (PLCPs), reducing plant immunity and promoting disease susceptibility [60]. In the *L. coscinodisci* transcriptome, we could identify 7 cystatin like proteins that could represent potential candidates to counter *C. granii* Papain-like cysteine peptidases.

In contrast, we observed a substantial downregulation of most peptidases in the *C. granii* transcriptome. Proteases are known to play key roles in numerous cellular processes, including protein turnover and stress response pathways. Notably, eight of these proteases are also classified as molecular chaperones, suggesting that the observed response is more likely indicative of a broad physiological shift linked to cellular stress rather than a specific reaction to pathogen attack [61]. Further, a study on the parasite *Amoebophrya* revealed that dinoflagellates can produce lytic compounds as a defensive strategy [62]. However, the precise structure and class of these compounds remained unidentified. This raises the possibility that proteases could be among such an elusive defensive agents.

β-carbolines biosynthesis putative enzyme

In plants, β-carboline alkaloids are synthesised by the Strictosidine synthase enzyme, which catalyses the condensation of secologanin and tryptophan to form strictosidine, a β-carboline intermediate in the monoterpenoid indole alkaloid [63,64]. It presents a stable architecture composed of six radially arranged β-sheets. Conserved across kingdoms, this fold supports a broad spectrum of enzymatic activities, including catalysis, molecular recognition, and signal transduction [65]. Despite this structural insight, functional inference remains challenging particularly when comparing microalgae to land plants due to the broad conservation of β-propeller structures across diverse taxa and their involvement in many unrelated biological processes.

By generating *in-silico* protein structure of unannotated or DUFs proteins in our dataset we identified as a six-bladed β-propeller fold one of the most abundant (5th by basemean) and strongly upregulated protein ($\log_2$ fold change = +8.2) annotated as DUF839 (TRINITY_DN10640_c0_g2). Thus, it is conceivable that this DUF839-domain protein may participate in a related biosynthetic pathway, potentially linked to β-carboline derivatives previously detected by mass spectrometry in algal cells infected with *L. coscinodisci* [17]. However, this hypothesis remains speculative and requires further biochemical and functional validation. Interestingly, strictosidine synthase-like (SSL) enzymes in *A. thaliana* have also been implicated in biotic stress responses [66].

Conclusion

The *C. granii/L. coscinodisci* pathosystem presents a highly promising model for studying marin oomycete-diatoms interactions, particularly due to its notable parallels with well-characterized plant–pathogen interactions. *L. coscinodisci* possesses a rich genetic toolkit that enables a sophisticated infection strategy resembling those of plant-infecting oomycetes, including hyphal development and the deployment of conserved molecular effectors such as Crinklers, cystatins, RxLR proteins, and transposable elements. Additionally, it encodes components of the RNA interference machinery: Argonaute, Dicer, and RdRP as well as cyclophilins.

The activation of an exosome-mediated response to infection or pathogen-associated elicitors suggests an ancient defense mechanism, potentially predating and informing those found in terrestrial plants. This intriguing possibility warrants further investigation.

Moreover, this pathosystem opens new avenues for characterizing enigmatic protein families, such as the SSL-like proteins and DUF839, not only in algae but also within broader lineages.

In conclusion, this model stands out for its relative simplicity compared to land plant systems while still encompassing key aspects of host-parasite interactions, particularly within the context of exceptionally large diatom cells, which can span hundreds of microns. Establishing genomic resources for the host (*C. granii*) and the parasite (*L. coscinodisci*) will be crucial to realizing the system's full potential. Ultimately, this marine microalgal model offers a unique and powerful platform to investigate fundamental questions in evolutionary parasitology, effector biology, and host manipulation.

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Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

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