

Full length article

Integrated transcriptomic and histological insights into immune and stress-related pathways in the Antarctic sea anemone

Urticinopsis antarctica[☆]

Claudia La Corte^{a,1} , Giuseppe Scapigliati^{b,1}, Marco Gerdol^c, Luca Bisanti^{a,*} ,
Mariano Dara^{a,**}, Matteo Cammarata^a, Maria Giovanna Parisi^a

^a Marine Immunobiology Laboratory, Department of Earth and Marine Sciences (DiTeM), University of Palermo, Palermo, Italy

^b Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF), University of Tuscia, Largo dell'Università snc, 01100, Viterbo, Italy

^c Department of Life Sciences, University of Trieste, Via Giorgieri 5, 34127, Trieste, Italy

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ABSTRACT

Antarctic sea anemones are dominant benthic predators, yet their molecular adaptive strategies to polar environments remain poorly understood. *Urticinopsis antarctica* is one of the most abundant Antarctic actinarians, but until now it has lacked any integrated molecular characterisation. This study provides the first comprehensive analysis combining *de novo* transcriptomics with histological and cytological observations to investigate immune and stress-response mechanisms in this azooxanthellate anthozoan. Gene Ontology and Pfam-based analyses revealed a broad and evolutionarily conserved innate immune repertoire, including Toll-like receptor, NF- κ B, JAK-STAT and NOD-like receptor pathways, together with numerous transcripts of pattern-recognition domains such as TIR, NACHT, LRR and C-type lectins. Integration with signal peptide prediction demonstrated a significant enrichment of immune- and stress-related genes within the secretome, indicating a strong bias toward extracellular defence strategies. Histological analyses revealed a typical a diploblastic organization with abundant cnidocytes, mucous cells and migratory amoeboid cells, consistent with an epithelial-centred immune system. The absence of algal symbionts was confirmed at both molecular and tissue levels. Together, these findings indicate that *U. antarctica* maintains a complex, flexible immune and stress-response architecture despite extreme thermal stability and resource limitation. This new molecular and cellular baseline establishes *U. antarctica* as a valuable reference for understanding resilience, cold adaptation, and the evolution of immunity in polar anthozoans, and provides a foundation for future functional and experimental work on the responses of Antarctic benthic species to environmental change.

1. Introduction

Cnidarians represent one of the earliest-diverging metazoan lineages endowed with a relatively sophisticated innate immune system. Although lacking adaptive immunity, they possess a rich repertoire of pattern-recognition receptors (PRRs) and conserved signalling pathways such as Toll-like receptors (TLRs), NF- κ B, NOD-like receptors and JAK/STAT cascades [1]. The interplay of these components enables recognition of microbial patterns, regulation of the microbiome, responses to

environmental stressors, maintenance of homeostasis at the epithelial interface and furthermore the modulation of interactions with symbiotic microorganisms [2,3].

Sea anemones (order Actiniaria) constitute valuable models for investigating evolutionary and functional aspects of innate immunity and environmental plasticity in non-bilaterian animals. Their relatively simple tissue architecture (epidermis, mesoglea, gastrodermis), presence of specialised cell types (e.g., cnidocytes, mucous cells, amoeboid cells) and sessile predator ecology make them amenable to studies of

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* Corresponding author.

** Corresponding author.

E-mail addresses: luca.bisanti@unipa.it (L. Bisanti), mariano.dara@unipa.it (M. Dara).

¹ These authors contributed equally.

cell-mediated defence and the epithelial immune system [4]. However, molecular investigations remain strongly biased towards temperate and tropical species (e.g., *Nematostella vectensis*, *Exaiptasia diaphana*), whereas cold-water and polar actinarian remain largely unstudied [5]. Among temperate anthozoans, the symbiotic anemone *Anemonia viridis* has clarified how algal symbionts influence host immunity and stress physiology. Transcriptomic studies under thermal stress, regeneration, and pollutant exposure revealed modulation of innate-immunity and extracellular-matrix pathways [6–11]. In contrast, most Antarctic anthozoans are azooxanthellate and entirely heterotrophic, offering a *symbiont-free* view of the cnidarian immune system. The absence of intracellular photosymbionts removes a major regulatory factor acting on immune gene expression and allows researchers to examine the intrinsic molecular defences of the host, reducing confounding influences of mutualistic regulation typical of tropical anthozoans. Moreover, recent syntheses show that a considerable fraction of anthozoans worldwide are naturally non-symbiotic [12], indicating that symbiosis is not an obligate strategy for success in this group.

The Antarctic marine environment, characterised by near-freezing and remarkably stable temperatures, pronounced seasonality in light and productivity and often minimal symbiont associations, provides a natural laboratory for studying adaptation of immune and stress-response systems. Over the past century, global climate change has led to pronounced alterations in the Southern Ocean's physical and chemical environment, including warming of +0.7 to +1.9 °C in seawater and an expected +3 °C increase in air temperature, accompanied by acidification and habitat shifts [13,14]. These rapid changes are expected to reshape benthic ecosystems and impose new selective pressures on Antarctic species. Recent transcriptomic and genomic studies in Antarctic invertebrates (e.g., scallop *Adamussium colbecki*, clam *Laternula elliptica*) highlight molecular signals of cold adaptation, such as enhanced expression of mRNA splicing/degradation genes, ubiquitin–proteasome pathways and cytoskeletal dynamics [15,16] and differentiated immune gene-expression responses to abrupt temperature shifts [17,18]. In this context, *Urticinopsis antarctica* (Actiniaria: Actiniidae) is among the most abundant and ecologically significant sea anemones on Antarctic continental shelves. Despite its key benthic role, it remains virtually uncharacterised at the molecular and cellular level.

U. antarctica is a sea anemone with a cylindric massive sandy-coloured body column up to 15 cm high and 11 cm in diameter [19]. It is among the largest and most conspicuous benthic invertebrates in the Antarctic community and was found from 6 to 223 m depth [20,21]. It is one of the most prevalent and abundant sea anemones on the Antarctic shore (in both Antarctic and Subantarctic waters). It has been observed in the Weddell Sea, South Shetland Islands, Prudz Bay, Cosmonauts Sea, and Haswell Archipelago (Fig. 1). *U. antarctica* has a massive, cylindrical body and pale tentacles arranged in several concentric rows around the oral disc, which is typically whitish to light beige. Small individuals (~1.2 cm in diameter) bear about 120 tentacles, whereas large specimens (~10 cm in diameter) can carry 600–800 (Fig. 1). When contracted, the tentacles appear short and conical, with faint longitudinal furrows and rounded tips [19,20,22]. Dietary analyses indicate that this generalist predator feeds mainly on echinoderms—sea stars and sea urchins accounting for nearly 77% of the stomach contents—but also on molluscs, crinoids, ophiuroids, and Antarctic fish (*Trematomus* sp.) [23]. Amphipods of the genus *Conicostoma* have occasionally been found undigested in the gastrovascular cavity, suggesting possible commensal associations. Medusae may represent up to 21% of the diet, and instances of cannibalism have also been reported [24,25].

In this study, we combine *de novo* transcriptomic sequencing and histological/cytological analyses of tentacle tissues of *U. antarctica* with the following aims: (1) to generate and assess the completeness of a *de novo* assembly; (2) to annotate genes and functional domains with emphasis on immune- and stress-related categories, including toxins, antimicrobial peptides and lectins; (3) to describe tentacular tissue organization and principal cell types in a functional-defensive context.

We hypothesise that *U. antarctica* possesses a broad and evolutionarily conserved innate immune toolkit, comparable to that of temperate or tropical Actiniaria, but likely reflecting polar-specific molecular adaptations (e.g., gene expansions, domain shifts or altered expression profiles). Rather than testing differential expression or adaptive responses under experimental conditions, this study aims to establish a high-quality molecular and histological baseline that can inform future investigations into stress responses and environmental resilience in Antarctic benthic fauna.

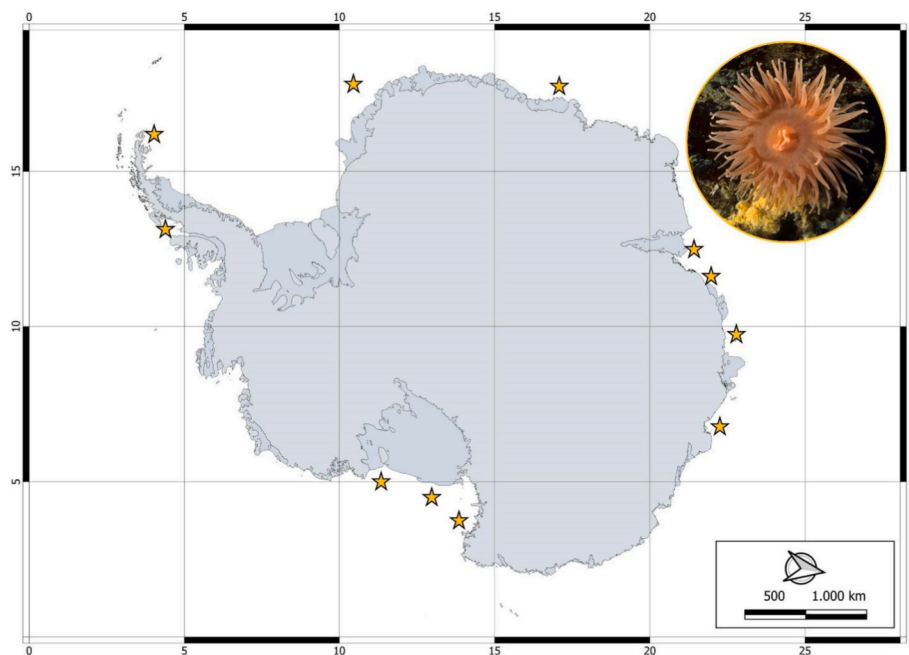


Fig. 1. Distribution of *Urticinopsis antarctica*. Yellow stars indicate localities from literature data. The map was created using QGIS 3.38.0 Grenoble. The reference system used is EPSG:3031. The grid coordinates are expressed in meters and represented in decimal format. Data from the SCAR Antarctic Digital Database, 2024. In the top right corner, an illustrative photo of *U. antarctica*.

2. Material and methods

2.1. Sample collection

Specimen of *U. antarctica* were collected during diving activities in the Gerlache Inlet, Ross Sea (74° 41' 42" S 164° 7' 23" E) in the 37° Campaign of Italian PNRA ("Programma Nazionale di Ricerche in Antartide", Project PNRA18_00077) in 2022 and maintained in aquaria in running sea water for observational purposes. Aquarium conditions were maintained at 0 ± 1 °C under continuous flow of natural seawater at native salinity. All tissue handling procedures were performed at the same maintenance temperature to minimize thermal stress. Tentacles (30 g) were dissected from a single animal that was left to recovery in aquaria and released in the sea after 20 days. Cells were obtained by tentacles by teasing the tentacles tissue over a stainless-steel mesh (100 µm) employing PBS (550 mOsm/kg), the homogenized tissue was then washed twice in PBS by centrifuging at 450×g, and obtained cells counted for viability in trypan blue and immediately employed for analyses. Cell dissociation and following procedures were performed at 0 ± 1 °C shortly after collection to minimize handling stress and avoid prolonged acclimation effects. We acknowledge that the osmolarity employed is lower than native Antarctic seawater conditions. Therefore, isolated cell morphology should be interpreted with caution, as osmotic conditions may influence cell shape, aggregation, and volume. The present cytological observations are thus intended as descriptive morphological documentation rather than functional characterization. A subsample of cell suspension was fixed in 70% ethanol and stored at 4 °C, air-dried, until cytological examination under light microscopy. Moreover, tentacles cells were collected for total RNA extraction and immediately frozen at -80 °C for transport and once in Italy (University of Tuscia, Viterbo) were processed. Finally, a fraction of tentacles was fixed in ice-cold Bouin's solution in order to preserve tissues for histological staining (see supplementary data, Fig. S1).

2.2. RNA extraction and quality assessment

A 2×10^7 fraction of tentacle cells of *U. antarctica* were dissolved by gentle pipetting in 1 ml of RNAzol RT (R4533, Sigma-Aldrich) for total RNA isolation and processed according to the manufacturer's technical procedures. Particularly, after thawing and homogenizing the sample, 400 µL of nuclease-free water were added and mixed for 15 s. The mixture was then incubated at room temperature for 15 min. The resulting mixture was centrifuged at 12,000×g for 15 min at 4 °C. The supernatant was transferred to a new RNase-free tube, and an equal volume of 100% isopropanol was added to precipitate total RNA under gentle mixing. After 10 min of incubation at room temperature, RNA was pelleted by centrifugation at 12,000×g for 10 min. The pellet was washed twice with 400 µL of 75% DEPC-treated ethanol, followed by centrifugation at 8000×g for 3 min at room temperature. After removal of residual ethanol, the RNA pellet was air-dried and resuspended in nuclease-free water. RNA purity and concentration were assessed using a BioTek Epoch 2 spectrophotometer, measuring absorbance at 260 nm and evaluating the $A_{(260/280)}$ ratio (protein contamination) and $A_{(260/230)}$ ratio (organic solvent contamination). RNA integrity was verified by agarose gel 1% (w/v) electrophoresis containing Gel red (SCT123 Sigma-Aldrich) using Hyperladder I (100 lanes; BIO-33053). Samples were prepared using DNA Loading Buffer Blue (Bioline; BIO-37045). A further samples control, capillary electrophoresis using the RNA Nano Bioanalysis chip (RNA 6000 Nano kit; Agilent 2100 Bioanalyzer, Agilent Technologies, Santa Clara, CA 95051, USA) was performed through an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA 95051, USA). RNA integrity number value > 6 was deemed suitable for library construction and thus, for *de novo* transcriptome assembly.

2.3. Library preparation and sequencing

Total RNA extracted from two tentacle cells samples of *U. antarctica* was used for poly(A)-enriched mRNA library construction. Library preparation and sequencing were performed by BMR Genomics (Padova, Italy) using the Illumina Stranded mRNA Prep Kit in 150 PE format (Illumina Inc., USA). Paired-end sequencing (2×100 bp) was conducted on a NovaSeq 6000 platform, targeting ~60 million reads per sample.

2.4. Transcriptome assembly

Quality control of raw reads was performed by the Trieste team using FastQC v0.11.9 and summarised with MultiQC v1.12. Adapter and quality trimming were conducted with fastp v0.23.2, applying parameters consistent with those recommended for Illumina paired-end datasets ($-f 15 -t 2 -T 2 -x -5 -3 -n 0 -l 75 -q 30 -w 32$). The overall quality of the raw reads was high, and no adapter contamination was detected. The *de novo* assembly followed the Oyster River Protocol [26], which integrates multiple assemblers to improve completeness and contiguity in non-model species. Two independent assemblies were produced using Trinity v2.11.0 and SPAdes v3.15.2 ($-rna -k 21,33,55,77$) and then concatenated. Redundant sequences were collapsed with CD-HIT-EST v4.8.1 at 98% similarity, and similar contigs were further clustered using the Orthofuser algorithm to merge isoforms and putative allelic variants [27–30]. Assembly completeness was assessed with BUSCO v5.3.2 employing the Metazoa ODB10 dataset. Quantification of transcript abundance was performed with Salmon v1.5.2, and within each Orthofuser (https://github.com/macmanes-lab/Oyster_River_Protocol/blob/master/orthofuser-README.md) cluster the transcript with the highest Transcripts Per Million (TPM) value was retained [31]. Putative coding sequences were annotated by BLASTx searches (e-value $\leq 1 \times 10^{-5}$) against the UniProt-SwissProt database, producing a non-redundant set of unique protein matches. A second Salmon quantification was then used to filter low-abundance transcripts, retaining those with TPM ≥ 1 or, if below this threshold, supported by a valid BLASTx match. Functional annotation was carried out with the AnnotaM pipeline, integrating Pfam, OrthoDB v10, and Gene Ontology (GO) term assignments. Quantitative assembly metrics were computed with the TrinityStats utility (see supplementary data, Fig. S2).

This multi-assembler strategy, implemented within the Oyster River Protocol, was selected to maximise transcript recovery and minimize potential biases associated with single-assembler pipelines. The approach is particularly suitable for *de novo* transcriptomes of non-model marine invertebrates, where RNA integrity can be variable due to field sampling under polar conditions.

2.5. Functional annotation and contamination filtering

Functional annotation of the final transcriptome was performed using the AnnotaM pipeline (<https://gitlab.com/54mu/annotaM/-/tree/master>), which integrates multiple reference databases and assigns functional terms to each predicted coding sequence. Homology searches were carried out with DIAMOND BLASTx (e-value $\leq 1 \times 10^{-5}$) against the UniProt-SwissProt protein database, while conserved domains were identified using Pfam, and orthologous groups were assigned through OrthoDB v10 (Metazoa). The Gene Ontology (GO) framework was employed to classify annotated transcripts by biological process, molecular function, and cellular component categories.

Potential cross-species contamination was evaluated using the taxonomic distribution of top BLASTx hits. Transcripts showing best matches to Antarctic teleosts (*Trematomus bernacchii*, *Chionodraco hamatus*, *Pleuragramma antarctica*), echinoderms (*Sterechinus neumayeri*, *Ophioparte* sp.), molluscs (*Adamussium colbecki*, *Limacina* sp.), and the diatom *Porosira glacialis* were considered environmental contaminants. Additional hits associated with *Homo sapiens*, copepods, or sponges were

interpreted as likely sampling or laboratory contamination.

Because these non-cnidarian sequences represented a negligible fraction of the total contigs and did not affect overall annotation results, they were excluded from GO enrichment analyses but retained in the master dataset for transparency. No molecular evidence of algal symbionts was detected, as no reads or contigs matching Symbiodiniaceae marker genes (rRNA, COI) were identified.

All annotation and contamination-screening steps were conducted by the Trieste team as part of the integrated assembly pipeline. The resulting high-confidence *U. antarctica* transcriptome was then used for downstream analyses described in the Results section.

2.6. Secretome prediction (SignalP 6.0) and transcript-level integration

Prediction of signal peptides was performed using SignalP v6.0 on a Linux Ubuntu environment. The input consisted of all predicted protein-coding Open Reading Frames (ORFs) derived from the curated transcriptome. Since SignalP outputs multiple ORF-level predictions per transcript (e.g., p1, p2 isoforms), FASTA headers were normalised by: extracting the first token of each header, and removing isoform suffixes to collapse predictions to transcript-level identifiers. For transcripts with multiple ORFs, a conservative rule was applied: a transcript was considered secreted if at least one associated ORF was predicted as SP (Sec/SPI). The SignalP output was processed and merged with the functional annotation table in R using custom scripts (dplyr, tidyr). This produced a unified secretome classification for downstream analyses.

2.7. GO-based identification of immune- and stress-related transcripts

Gene Ontology terms assigned by AnnotaM were parsed into long format (one GO per row).

A curated set of GO categories associated with: innate immunity (e.g., GO:0045087, GO:0006954, GO:0002376), pathogen/stress response (e.g., GO:0009617, GO:0006979), epithelial integrity and defence (e.g., GO:0007155, GO:0030198), was compiled based on established metazoan immune frameworks. Transcripts annotated with ≥ 1 GO term from this list were classified as immune/stress-associated. Descriptive counts were generated for each GO term, quantifying (i) the total number of associated transcripts and (ii) how many belonged to the immune/stress gene set.

2.8. Pfam domain-based identification of immune and stress-related protein families

Pfam domains were extracted from AnnotaM output and standardised by retrieving only the core accession (PFxxxxx). Seven domain families relevant to cnidarian innate immunity and cellular stress responses were pre-selected: TIR (PF01582), NACHT (PF05729), C-type lectin-like domain (CTL) (PF00059), LRR families (PF00560, PF07723, PF07725), Heat-shock proteins, in particular, HSP20 (PF00011), HSP70 (PF00012), HSP90 (PF00183). All transcripts containing ≥ 1 of these domains were grouped by family. Both total counts and the subset overlapping with the immune/stress GO-defined gene set were computed. A summary table was generated, together with a barplot of per-family transcript abundance using ggplot2.

2.9. Integration of secretome, GO terms, and Pfam domains

A final integrated table was assembled by merging annotation data, SignalP predictions, Pfam domains, and immune/stress GO classifications. For each transcript, the following fields were included: secreted (TRUE/FALSE, based on SignalP6), immune/stress-associated (TRUE/FALSE, based on GO), number and type of Pfam, immune/stress domains, UniProt annotation fields. Transcripts were retained if they satisfied at least one of the following: 1. contained an immune/stress-related GO term, 2. contained an immune/stress-related Pfam domain,

3. were predicted to be secreted and the final dataset consisted of 13,843 transcripts.

2.10. Enrichment analysis of immune/stress genes in the secreted fraction

To evaluate whether immune/stress-associated genes are over-represented among secreted proteins, a 2×2 contingency table was constructed using: secreted vs non-secreted transcripts (from SignalP6), presence/absence of immune/stress GO terms. Only transcripts with GO annotation were included to avoid bias from unannotated genes.

2.11. Code availability

All steps described above were executed using custom R scripts (R v4.3.1) employing the packages dplyr, tidyr, stringr, readxl, ggplot2. Scripts include parsing of SignalP6 output, Pfam/GO restructuring, summarisation, and statistical testing.

2.12. Histology & histochemistry

Tentacles were fixed in Bouin's solution until complete sinking. Subsequently, samples were dehydrated in an increasing scale of ethanol (30%, 50%, 70%, 90%, 96%, 100%) and after two steps in toluene (10 min each) were paraffin-embedded. Longitudinal histological sections (7 μ m thick) were cut with a rotary automatic microtome (Microm HM 350S, GMBH, Walldorf, Germany) and processed for the Trichrome stains. Gomori's Trichrome sections were stained with Carazzi's emalume (200 mL—10 g KAl(SO₄), 0.2 g hematoxylin, 0.04 g KIO₃, 40 mL glycerol) for 10 min at room temperature, washed in distilled water for 15 min, and immersed in Gomori's trichrome solution (100 mL—0.6 g chromotrope 2R, 0.3 g fast green FCF, 0.6 g of phosphotungstic acid, 1 mL acetic acid, pH 3.4) and finally washed in acidified water (0.2% acetic acid). For cytological analysis, freshly dissociated tentacle cells were fixed in 4% paraformaldehyde prepared in osmoconforming seawater, spread on glass slides, and stained with May–Grünwald–Giemsa (MGG) for subsequent morphological examination under light microscopy.

3. Results

3.1. Sequencing output and transcriptome assembly

A total of ~230.3 million paired-end reads were generated from the two tentacle RNA samples of *U. antarctica* (113.5 million and 116.8 million reads, respectively; Table 1). After trimming and quality filtering, more than 92% of bases showed Phred quality scores above Q30, indicating high-quality sequencing output. The overall metrics of read duplication, GC content, and adapter removal are reported in Table 2. The raw reads have been deposited in the NCBI Sequence Read Archive under accession number PRJNA1285170.

The *de novo* transcriptome assembly, obtained through the Oyster River Protocol, which integrates the Trinity and SPAdes assemblers, produced a comprehensive dataset with excellent completeness and contiguity (Table 1). The high percentage of complete BUSCO genes (98.3%) demonstrates the reliability of the assembly, while the observed duplication rate likely reflects the presence of multiple isoforms or

Table 1
Sequencing, *de novo* assembly statistics.

Number of paired-end reads sample 1	113472968
Number of paired-end reads sample 2	116786307
Total sequencing output	33.2 Gb
Number of assembled contigs	124184
Median contig length	1139
Average contig length	1775.11
N50 (nt)	3098

Table 2
Control quality and trimming.

	Sample 1	Sample 2
Duplication %	28.3%	32.5%
% > Q30	92.3%	92.3%
Mb Q30 bases	24312	25385.9
M Reads After Filtering	215.7	221.9
GC content	42.7%	43.5%
% PF	95%	95%
Adapter %	37.2%	28.2%

uncollapsed allelic variants—features commonly observed in transcriptomes of non-model invertebrates. Only a small fraction of fragmented or missing BUSCOs was detected, confirming the robustness of the pipeline.

These results indicate that the combined multi-assembler approach successfully maximised transcript recovery and accuracy, yielding a high-quality reference resource for subsequent annotation and functional analyses.

3.2. Functional annotation

Functional annotation of the *U. antarctica* transcriptome was performed using the AnnotaM pipeline, which integrates multiple reference databases to assign gene functions and conserved domains. In total, 71,920 transcripts received at least one annotation across the UniProt-SwissProt, Pfam, and OrthoDB v10 (Metazoa) databases. Among these, the majority had hits in both UniProt-SwissProt and Pfam, indicating a high proportion of protein-coding transcripts with well-characterised homologues (Fig. 2).

The overlap among databases, illustrated by the Venn diagram (Fig. 2), reflects the broad functional coverage and internal consistency of the annotation process. The high annotation rate and the prevalence of transcripts associated with conserved protein domains confirm the overall quality and completeness of the *U. antarctica* transcriptome. This level of annotation is comparable to, or higher than, that reported for other anthozoan transcriptomes, such as *Nematostella vectensis* and *Exaiptasia diaphana*, underscoring the reliability of the dataset for

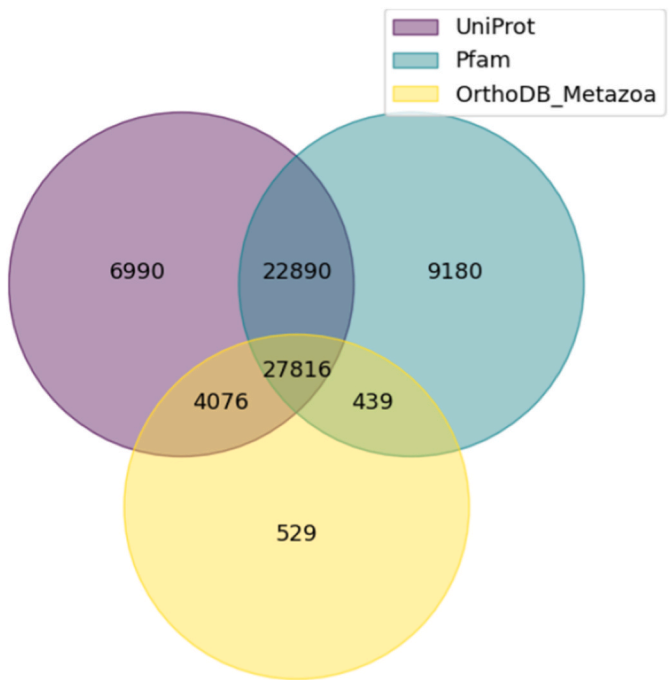


Fig. 2. Venn diagram shows the intersection between the annotation sets.

downstream comparative analyses.

3.3. Immune-related gene ontology categories

To investigate the molecular basis of the immune system in *U. antarctica*, all annotated transcripts associated with Gene Ontology (GO) terms related to defence and immunity were examined (Table 3). The largest categories included genes involved in the innate immune response (GO:0045087) and inflammatory processes (GO:0006954), representing the core of the cnidarian defence repertoire. Several transcripts were linked to NF- κ B signalling, including positive and negative regulation of NF- κ B activity (GO:0051092, GO:0032088, GO:0038061), a central pathway controlling stress and immune responses. A substantial number of contigs were also associated with cytokine-mediated

Table 3
List of the major Gene Ontology biological processes relevant for immunity and number of corresponding annotated contigs in the *U. antarctica* transcriptome.

GO category	Description	Annotated contigs
GO:0045087	Innate immune response	1809
GO:0006954	Inflammatory response	659
GO:0006955	Immune response	264
GO:0051607	Defense response to virus	664
GO:0051092	Positive regulation of NF-kappaB transcription factor activity	297
GO:0019221	Cytokine-mediated signaling pathway	44
GO:0006950	Response to stress	8
GO:0007179	Transforming growth factor beta receptor signaling pathway	137
GO:0002376	Immune system process	2080
GO:0032088	Negative regulation of NF-kappaB transcription factor activity	142
GO:0002224	Toll-like receptor signaling pathway	90
GO:0050727	Regulation of inflammatory response	172
GO:0042742	Defense response to bacterium	654
GO:0034138	Toll-like receptor 3 signaling pathway	7
GO:0034142	Toll-like receptor 4 signaling pathway	20
GO:0050728	Negative regulation of inflammatory response	153
GO:0035666	TRIF-dependent toll-like receptor signaling pathway	7
GO:0034097	Response to cytokine	55
GO:0032722	Positive regulation of chemokine production	28
GO:0070102	Interleukin-6-mediated signaling pathway	10
GO:0002755	MyD88-dependent toll-like receptor signaling pathway	16
GO:0032755	Positive regulation of interleukin-6 production	93
GO:0032743	Positive regulation of interleukin-2 production	39
GO:0045088	Regulation of innate immune response	27
GO:0002474	Antigen processing and presentation of peptide antigen via MHC class I	4
GO:0007259	JAK-STAT cascade	28
GO:0038061	NIK/NF-kappaB signaling	69

signalling and interleukin production (GO:0019221, GO:0070102, GO:0032755, GO:0032743), suggesting the presence of conserved elements of the JAK–STAT and interleukin pathways. Furthermore, a suite of transcripts corresponded to Toll-like receptor (TLR) signalling (GO:0002224, GO:0002755, GO:0034138, GO:0034142, GO:0035666), including both MyD88- and TRIF-dependent cascades. These findings indicate that *U. antarctica* possesses the canonical pattern-recognition machinery typical of cnidarian innate immunity.

3.4. Environmental signals and contamination check

No genetic evidence of algal symbionts was detected in the *U. antarctica* transcriptome: no reads or contigs corresponding to Symbiodiniaceae molecular markers (rRNA or COI) were identified, confirming the absence of endosymbiotic algae within the analysed specimens. A small number of transcripts, however, showed best BLAST hits to taxa common in Antarctic benthic environments, including teleost fishes (e.g., *Trematomus bernacchii*, *Chionodraco hamatus*, *Pleuragramma antarctica*), echinoderms (e.g., *Sterechinus neumayeri*, *Ophioparte sp.*), and molluscs (e.g., *Adamussium colbecki*, *Limacina sp.*). Occasional matches to human sequences, copepods, sponges and the diatom *Porosira glacialis* were also detected. These sequences are interpreted as environmental or technical contaminants, rather than genuine components of the *U. antarctica* transcriptome. Because their abundance was negligible and they did not influence functional annotation or GO analyses, they were excluded from downstream enrichment analyses but retained in the full dataset for completeness and transparency. To complement the molecular data, histological and cytological analyses were performed to characterize tissue organisation and cellular components potentially involved in defensive functions.

3.5. Immune and stress-associated transcripts

A total of 60,978 transcripts annotated with at least one Gene Ontology (GO) term were considered for downstream functional analyses. Among these, 8285 transcripts ($\approx 13.6\%$) were associated with immune- and stress-related biological processes, including innate immune response, response to stimulus, apoptosis, and cell adhesion. The GO terms represented by the highest number of immune/stress transcripts were: GO:0005737 – cytoplasm (3889 genes), GO:0016020 – membrane (3851 genes), GO:0005886 – plasma membrane (2314 genes), GO:0007155 – cell adhesion (2010 genes; notably all genes within this category were in the immune/stress set), GO:0002376 – immune system process (1665 genes), GO:0045087 – innate immune response (1460 genes), GO:0006915 – apoptotic process (1359 genes). GO term distribution highlighted a broad representation of cellular component and molecular function categories typically involved in immune signalling and stress responses, such as membrane-associated proteins, signal transduction components, and intracellular response mediators.

3.6. Pfam domains associated with innate immune receptors and stress responses

Pfam domain analysis revealed a diverse repertoire of immune- and stress-associated protein families in the *U. antarctica* transcriptome (Fig. 3).

The highest transcript counts were observed for: NACHT domains (722 transcripts), typical of NOD-like receptors, LRR domains (493 transcripts), typical of Toll-like and NOD-like signalling, CTLD lectins (156 transcripts), commonly involved in pathogen recognition. Heat shock protein families were also detected, including HSP70 (62 transcripts), HSP90 (20 transcripts), and a smaller number of HSP20 transcripts (11), reflecting conserved cellular stress response mechanisms. A substantial fraction of these Pfam-associated transcripts overlapped with the immune/stress GO gene set, particularly for TIR and NACHT

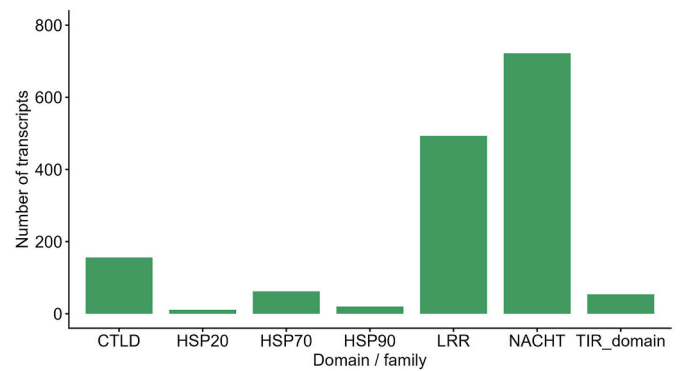


Fig. 3. Distribution of immune- and stress-related Pfam domains in the *Urticinopsis antarctica* transcriptome. Bar plot showing the number of transcripts containing selected Pfam domains associated with immune recognition and cellular stress responses.

domains (Table 4).

3.7. Secretome and enrichment of immune/stress genes in secreted proteins

SignalP-based prediction identified 4881 secreted transcripts, while 56,097 were classified as non-secreted. Among immune/stress-associated genes, 1240 were secreted (25.4%), compared to 7045 non-secreted transcripts (12.6%). Overall, approximately 15% of all immune/stress-related genes encoded predicted secreted proteins. This proportion was significantly higher than expected based on the background distribution of secreted versus non-secreted transcripts (Fisher's exact test, $p < 2.2 \times 10^{-16}$; odds ratio = 2.37), indicating a strong enrichment of immune and stress functions within the secretome. This indicates that *U. antarctica* disproportionately deploys secreted immune effectors, consistent with the importance of extracellular defences (lectins, antimicrobials, proteases, signalling peptides) in anthozoan innate immunity (Fig. 4).

3.8. Histological analysis

In order to shed light on the morphological structure of *U. antarctica*, histological analysis was conducted on tentacles paraffin-embedded and stained with Gomori's Trichrome. Longitudinal tentacle sections revealed the typical a diploblastic organisation of actinarian tissues, consisting of an outer epidermis, a mesoglea, and an inner gastrodermis (Fig. 5A). The epidermis appeared as a continuous epithelium containing abundant cnidocytes and mucous cells, interspersed with smaller amoeboid and granular cells. The mesoglea was relatively thick and eosinophilic, showing scattered fibrous elements and elongated mesogleal cells. The gastrodermis displayed a well-developed epithelial layer rich in digestive and vacuolated cells, with evident intracellular granules.

Table 4

Distribution of selected Pfam domain families across the *Urticinopsis antarctica* transcriptome and within the immune/stress-related gene subset. The table reports the total number of transcripts containing each Pfam domain and the number retained after functional filtering.

Pfam family	Total transcripts	Immune/stress subset
TIR domain	54	39
NACHT	722	270
LRR	493	106
CTLD (C-type lectin)	156	71
HSP20	11	0
HSP70	62	7
HSP90	20	0

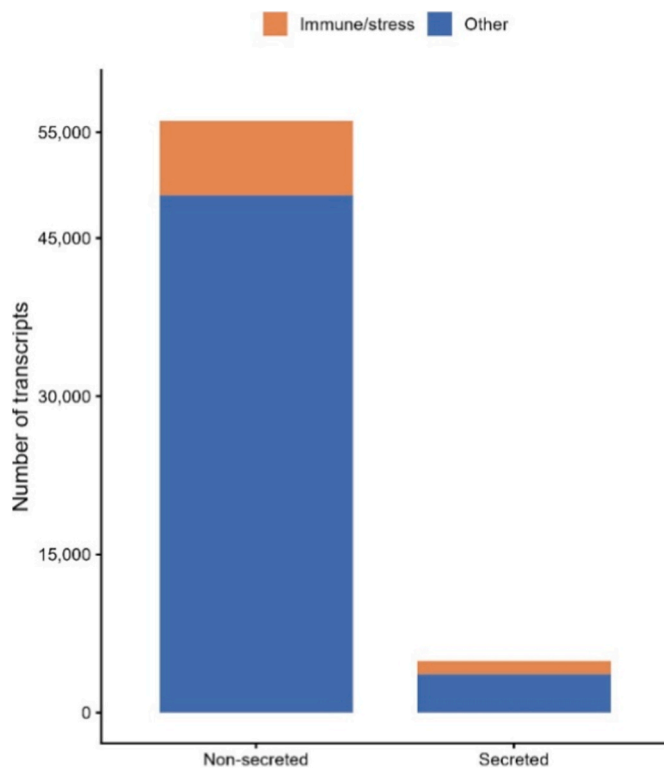


Fig. 4. Stacked bar plot showing the number of transcripts classified as immune/stress-associated (orange) and non-immune (“Other”, blue) within the predicted secreted and non-secreted protein sets. Secretion was inferred using SignalP v6.0, while immune and stress functions were assigned based on a curated set of Gene Ontology (GO) terms.

Gomori's trichrome staining highlighted the structural differentiation of the tissue layers, with the mesoglea staining intensely blue-green and muscle fibres distinctly visible along the longitudinal plane. The organisation of circular and longitudinal muscle bundles was clearly discernible at the base of the tentacles, suggesting effective contractile activity.

The thickness of the three body layers was measured and the mesoglea ($11.276 \pm 5.47 \mu\text{m}$) represent the thinner layer in all sections studied (Fig. 5B). Finally, epidermis ($57.713 \pm 15.45 \mu\text{m}$) and gastrodermis ($60.207 \pm 21.48 \mu\text{m}$) did not show a significant difference in their size. Welch's ANOVA test was performed ($W = 60.25$; $DFn = 2.000$; $DFd = 12.99$; $P \text{ value} = < 0.0001$). Mesoglea showed a $P \text{ value} < 0.0001$ compared to epidermis and 0.0003 compared to gastrodermis layer.

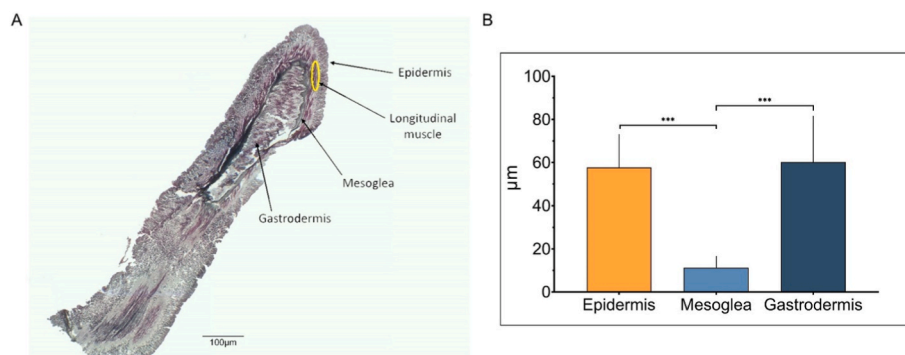


Fig. 5. (A) Microphotograph of the epithelial layers of *Urticinopsis antarctica* tentacle. The outermost layer is the epidermis and the innermost layer is the gastrodermis, both rich in cells. The mesoglea is the central amorphous gelatinous thin layer in blue-green. Scale bar: 100 μm . (B) Plot reports the thickness of the three body layers (epidermis, mesoglea and gastrodermis). Data are presented as mean $\pm$ standard deviations. Differences between means were considered significant for $p < 0.05$. Significant differences were detected by Two stage step up method of Benjamini, Krieger and Yekutieli post hoc test—* $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$.

Epidermis is formed by a single layer of densely packed columnar cells and among them spirocysts (sp) have been identified (Fig. 6A). The basal part mounted on the mesoglea and the two layers are bounded by a longitudinal muscle. The mesoglea appeared as an amorphous fibrous matrix containing a large number of rounded and linear cells that migrate within it (Fig. 6B).

On the opposite side, the gastrodermis layer with only some cells clearly distinguishable and in differentiation, like some nematoblasts (Fig. 7A), some oblong (Fig. 7B) and fibroblasts-like cells (Fig. 7C). Observing each layer, there is no sign of endosymbionts.

3.9. Cytological observations

Cytological preparations from isolated tentacle cells stained with May–Grünwald–Giemsa (MGG) revealed a heterogeneous population. Besides typical cnidocytes with well-defined capsules, several mucous cells and cells displaying irregular or amoeboid-like morphology were identified. The latter exhibited variable morphology—from round to spindle-shaped—with lightly basophilic cytoplasm and occasional cytoplasmic vacuoles.

MGG cells stained were observed under optical microscope and measured. Spirocysts length is $43.60 \pm 20.43 \mu\text{m}$. Rounded to elongated cells measured $27.02 \pm 0.35 \mu\text{m}$ in length and $12.14 \pm 4 \mu\text{m}$ in width with the nucleus occupying the 29.6% of the total cell area. Cells containing visible cytoplasmic granules showed an average area of $20.41 \pm 0.19 \mu\text{m}^2$ (Fig. 8A–C).

Live cells, extracted from the sea anemone tentacles were directly observed under the light microscope. Most of the cells were rounded and isolated, the presence of granules in some of them may indicate that there are more cell populations. The occurrence of some clotted cells allowed to highlight yellowish granules with intrinsic autofluorescence, with signal detected. After 24 h *in vitro* under the same dissociation conditions, occasional aggregates were observed. Given the dissociation procedure and osmotic conditions employed, these observations are presented in supplementary data (Fig. S3) as qualitative morphological documentation only and should be interpreted cautiously.

4. Discussion

This study represents the first integrated molecular, histological, and cytological characterisation of the Antarctic sea anemone *U. antarctica*, providing a baseline molecular and histological characterization of a non-symbiotic anthozoan inhabiting extreme environments [14,32]. The combination of transcriptomic and histological analyses establishes a comprehensive framework for interpreting how basal metazoans cope with the selective pressures of the Southern Ocean [15,16]. These findings expand current knowledge of this species' biology and offer a

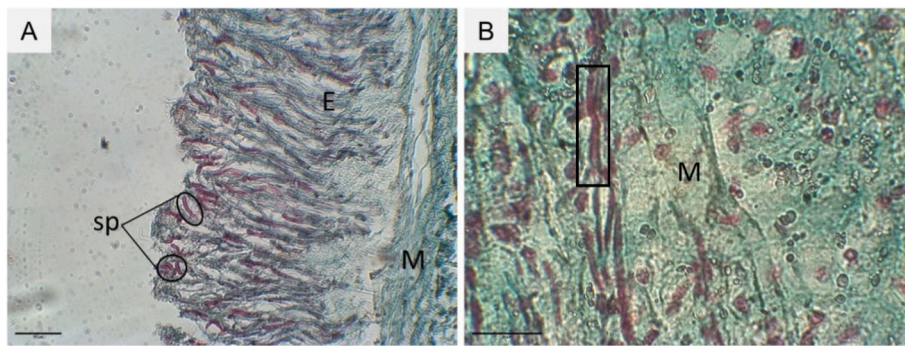


Fig. 6. *U. antarctica* longitudinal sections of (A) epidermis and (B) mesoglea layers. E = epidermis, M = mesoglea, sp = spirocysts, black boxes = linear cells. Scale bar: 50 µm (a) and 25 µm (b).

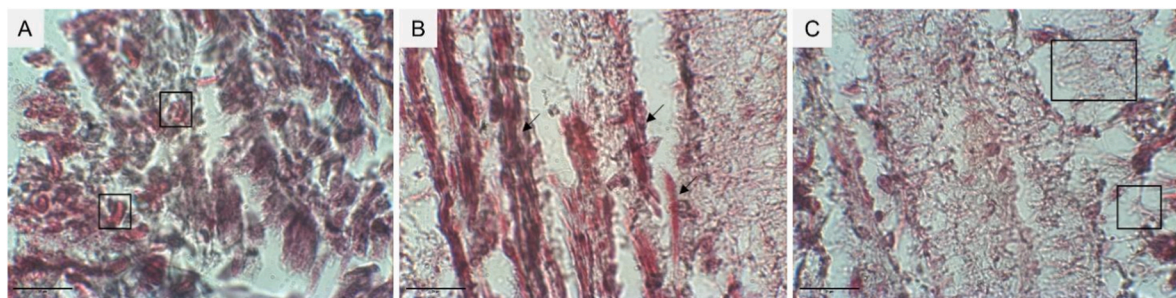


Fig. 7. *U. antarctica* longitudinal sections of (A–C) gastrodermis layer. Black boxes in (A) highlight spirocyst cells in differentiation. In (B) arrows indicate linear cells, like fibers. In (C) boxes evidenced a network of fibroblasts-like cells. Scale bar: 25 µm.

foundation for comparative research on anthozoan immunity, stress responses, and environmental adaptation [2,7,33].

The high-quality *de novo* transcriptome obtained for *U. antarctica* displayed completeness comparable to that of well-established anthozoan models such as *Nematostella vectensis* and *Exaiptasia diaphana* [1,5]. The elevated proportion of duplicated BUSCOs likely reflects naturally occurring isoforms and allelic variants rather than assembly artefacts, consistent with patterns observed in other cnidarian transcriptomic resources [1]. These results demonstrate that *U. antarctica* retains a rich gene repertoire despite inhabiting an ecosystem often described as evolutionarily conservative [14,32]. Gene Ontology analyses revealed an extensive representation of innate immune processes, including NF-κB, Toll-like receptor, and JAK-STAT signalling pathways. Such pathways form the highly conserved core of anthozoan immunity and are involved in wound healing and stress modulation in species like *A. viridis* and *N. vectensis* [2,6,8,11]. The identification of cytokine-like and interleukin-associated transcripts suggests that *U. antarctica* may use complex signalling networks despite lacking adaptive immunity, in line with what is known for other anthozoans [34]. Altogether, these features support the hypothesis that anthozoan innate immunity predates bilaterian immunity in its functional sophistication [35,36]. Although the high number of transcripts containing NACHT, LRR and TIR domains indicates a complex repertoire, transcriptome-based abundance does not necessarily reflect gene copy number. Redundancy due to alternative splicing, allelic variants, or assembly fragmentation cannot be entirely excluded. Dedicated phylogenetic reconstruction and comparative genomic analyses will be required to determine whether these patterns reflect lineage-specific diversification.

Delgado et al.ii (2022), using transcriptomic data identified differences and similarities in venom profiles of six sea anemone species of three clades of clownfish-hosting and have found 1121 transcript that match with haemolytic and haemorrhagic toxins across all species. In *U. antarctica* we have found 314 transcripts inherent to toxins (GO: 0090729), of which 111 strictly linked to Cnidarians, the rest are mainly

related to reptiles and arthropods toxins. In *Nematostella vectensis*, it has been observed that the toxin Nv1 (the most abundant) under thermal stress conditions was downregulated in the populations studied, suggesting a differential modulation of the production of venom linked to the geographical position. These findings suggest that toxin expression may be dynamically adjusted to balance defence, predation, and energetic constraints, particularly important in polar environments with strong resource limitations [14,32,37].

This enrichment supports the hypothesis that extracellular effector mechanisms represent a central component of the immune strategy of *U. antarctica*. In cnidarians, immunity is largely integrated with epithelial function, and secreted molecules such as lectins, antimicrobial peptides, and cytokine-like factors play a key role in host–environment interactions [2,34]. In the cold, oxygen-rich Antarctic environment, reliance on secreted immune effectors may provide a rapid and energetically efficient means of defence, reducing the need for prolonged cellular inflammatory responses.

The absence of zooxanthellae in *U. antarctica*, confirmed through histological observations, aligns with the general azooxanthellate condition of polar anthozoans and cold-water corals [14,32]. In extreme environments, maintaining a symbiont can impose significant metabolic costs and increase oxidative stress through photosynthesis-derived reactive oxygen species [10,38]. In resource-limited settings, symbiosis can lead to competition for nutrients, especially if the host must allocate substantial energy to sustain the symbiont. In a nutrient-scarce environment like Antarctica, this additional demand could become a significant burden [39,40]. Furthermore, symbiont photosynthesis produces oxygen, potentially requiring the host to develop protective mechanisms against reactive oxygen species (ROS) generated in the process. Consequently, cnidarians hosting photosynthetic symbionts must invest in oxidative stress mitigation [41]. Studies on holobiont thermal acclimation indicate that symbiont responses to heat can influence the overall acclimation capacity of the host-symbiont system [42]. As shown for Mediterranean cnidarians under warming, symbiosis

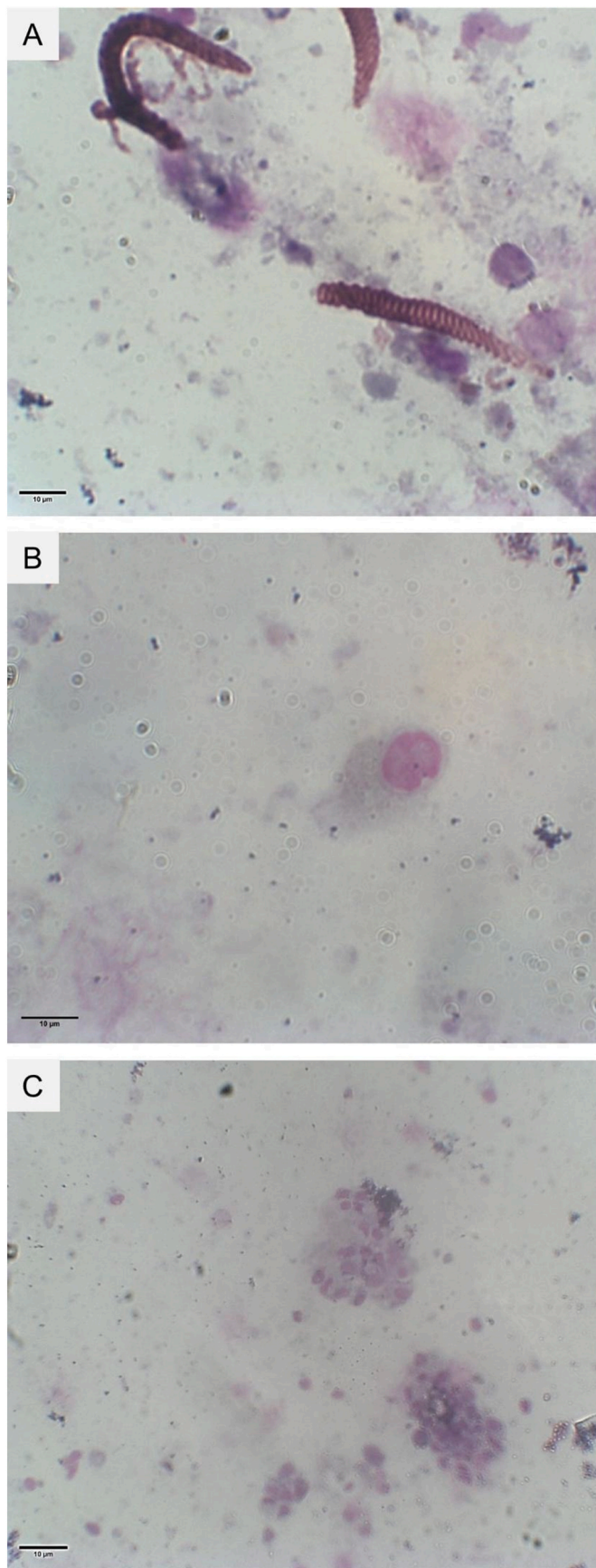


Fig. 8. Representative *U. antarctica* cells stained in May–Grünwald–Giemsa. (A) spirocysts; (B) amoebocyte; (C) granulocytes. Images are intended for qualitative morphological illustration only. Scale bar: 10 µm.

can also constrain physiological plasticity and alter thermal thresholds [43,44]. Therefore, the absence of symbiosis in *U. antarctica* may reflect an adaptive strategy favouring metabolic economy and stress resilience.

The coexistence of broad immune capacities and specific regulatory adjustments suggests that *U. antarctica* balances defence readiness with metabolic efficiency, a strategy common among polar invertebrates such as *Adamussium colbecki* and *Laternula elliptica* [15,16]. In these taxa, transcriptional modulation of protein folding, cytoskeletal dynamics, and ubiquitin–proteasome pathways supports survival in cold, oxygen-rich waters [15]. Similar patterns have been described in other polar invertebrates, suggesting potential common physiological constraints in cold environments [14,32].

The transcriptome of *U. antarctica* also revealed numerous sequences associated with antifreeze proteins, lectins, and antimicrobial peptides—molecules known to mediate cold adaptation and immune defence in a variety of polar organisms [45–47]. For example, fucose-binding lectins such as TbFTL in *Trematomus bernacchii* exhibit carbohydrate-binding and bacterial agglutination activity at low temperatures [45,48]. In some fish species, for instance, the presence of antifreeze glycoproteins lowers the freezing point of bodily fluids by binding to ice crystals and preventing their growth [47]. The presence of analogous lectin and AMP transcripts in *U. antarctica* suggests that similar mechanisms may contribute to immune surveillance and pathogen defence in this species [41,49]. Antimicrobial peptides, which are small molecules produced by various organisms, particularly cnidarians, in fact play a fundamental role in immune defense against bacteria, fungi, and viruses. As such, they are crucial for survival and protection against infections and pathogens [34,49,50]. Given their importance, transcripts corresponding to these key molecules were searched for in the *U. antarctica* transcriptome. A total of 12 transcripts related to antimicrobial peptides, 254 linked to lectins, and 252 associated with glycoproteins were identified. These findings pave the way for new comparative and evolutionary studies, as well as further investigations into the functions of some of these molecules. Beyond the overall diversity of immune-related pathways, the integration of signal peptide prediction with functional annotation highlights a marked bias of the immune repertoire of *U. antarctica* towards extracellular deployment. A substantial fraction of immune- and stress-associated transcripts encode predicted secreted proteins, a pattern that is significantly enriched compared to non-immune genes. This organisation supports the view that, in anthozoans, immune defence is largely mediated at the epithelial interface through the release of soluble effectors, rather than relying exclusively on intracellular signalling cascades [2,36]. Such an epithelial-centred immune strategy is consistent with the biology of sessile cnidarians, which are continuously exposed to environmental microbes and mechanical damage and therefore benefit from rapid, localised defensive responses [6,8].

In polar environments, this strategy may be particularly advantageous. The production of secreted immune molecules allows an efficient allocation of resources by concentrating defence at the host–environment boundary, potentially reducing the metabolic costs associated with sustained intracellular immune activation [32,51]. Similar extracellular immune biases have been described in other marine invertebrates inhabiting cold or variable environments, where immune readiness must be balanced with energetic constraints [15,46]. In this context, the enrichment of secreted immune-related transcripts in *U. antarctica* supports the hypothesis that polar anthozoans rely on a functionally sophisticated yet energetically economical immune system, integrating conserved signalling pathways with a strong investment in secreted effectors. This provides a framework for future functional investigations.

Histological observations revealed a typical anthozoan a diploblastic organization consisting of epidermis and gastrodermis separated by a mesogleal layer, with abundant cnidocytes, mucous cells, and amoeboid cells across the epidermis and mesoglea [8]. The distribution of these morphotypes corresponds to those implicated in defence and wound repair in other anthozoans, including *A. viridis* [9,52]. The abundance of

amoeboid cells may suggest active immune surveillance, consistent with transcriptomic evidence of inflammatory and cytokine-mediated signalling [7]. The thinner connective layer and elevated number of migratory cells compared to temperate species may reflect structural adaptations to polar environmental constraints. At the tissue level it has not been easy to characterize all the different cell populations, but there are clearly spirocysts in the epidermis layer, nematoblasts in the gastrodermis and fibroblast-like cells in the areas of conjunction between the two epithelial layers and the mesoglea. Elongated and rounded cells are visible. Further ultrastructural analyses would help clarify the functions of these cell types. In addition, from the extracted cells it was possible to detect and measure spirocysts, granulocytes and amoebocytes as was done in a study conducted on *A. viridis* regeneration with results in line with the observed [9]. It should be noted that cells isolated *in vitro* have been maintained at an available osmolarity value known for Antarctic fish species and further investigation is needed to better elucidate the optimal osmotic values for *U. antarctica*. The differences between *A. viridis* and *U. antarctica* related to the biometric measurements, could be attributed to the intrinsic characteristics of the two specimens, but also to the different experimental conditions to which they have been subject. The obtained outputs can be of considerable support for future investigations that consider tissue, cellular and molecular markers. Pigmentation may also contribute to environmental resilience: the orange coloration of *U. antarctica* could derive from carotenoids, which function as antioxidants and UV-protective molecules in several anthozoans [53]. Carotenoids are also implicated in mitigating damage from bacterial lesions, as documented in *Gorgonia ventalina* [54]. If supported by pigment and gene-expression analyses, carotenoid accumulation could represent an additional layer of oxidative stress protection in polar environments [53].

Comparisons with cold-water corals provide further context: species such as *Desmophyllum dianthus* exhibit phenotypic plasticity and distinct stress-response modules under warming, acidification, and pollutants [55,56]. These studies consistently identify temperature as the dominant stressor, underscoring the vulnerability of cold-adapted taxa to warming [55]. The parallels between *U. antarctica* and cold-water scleractinians reinforce the idea of conserved innate immune modules and flexible stress responses across polar anthozoans. From the transcriptome data of *U. antarctica*, it is evident that many transcripts are highly conserved and among them, 7571 concern immune system responses and 173 transcripts correspond to Heat shock proteins (HSPs), molecules not strictly linked to heat and cold stress, involved also in regeneration or detoxification from metals, which are also expressed in a differential way when the survival of the individual is at stake, cutting the investment of energy in physiological processes at high cost [37,41]. Although the present study provides a comprehensive annotation of immune- and stress-related pathways, functional validation under controlled environmental challenges will be necessary to assess transcriptional dynamics and adaptive relevance. Targeted qRT-PCR analyses and experimental stress exposures will therefore represent essential next steps to further elucidate regulatory mechanisms in *U. antarctica*.

5. Conclusions

This study provides the first integrated molecular and histological characterisation of *U. antarctica*, revealing a conserved and functionally rich innate immune toolkit in an azooxanthellate Antarctic anthozoan. The combination of a high-quality transcriptome and detailed cytological observations highlight how immune, stress-response, and structural pathways are maintained and modulated in a species living under extreme thermal stability and resource limitation. These results position *U. antarctica* as a valuable reference for understanding the evolution, plasticity of innate immunity, cold adaptation, and symbiont-free strategies among anthozoans. Although this represents a pioneering zoological exploration, the findings establish a solid foundation for

functional and comparative studies aimed at clarifying how polar actinarians respond to environmental stressors, particularly warming. Future work integrating single-cell approaches, experimental stress exposures, and refined ultrastructural analyses will be essential to functionally validate the molecular repertoire described here and finally to determine the resilience and vulnerability of Antarctic benthic communities in a rapidly changing ocean.

CRedit authorship contribution statement

Claudia La Corte: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Validation, Writing-original draft preparation, Writing-review and editing; Giuseppe Scapigliati: Conceptualization, Supervision, Methodology, Writing-review and editing; Marco Gerdol: Investigation, Formal analysis, Validation; Luca Bisanti: Formal analysis, Writing-review and editing; Mariano Dara: Writing-review and editing; Matteo Cammarata: Supervision, Validation, Writing-review and editing; Maria Giovanna Parisi: Conceptualization, Supervision, Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fsi.2026.111295>.

Data availability

Raw sequencing reads have been deposited in the NCBI Sequence Read Archive under BioProject PRJNA1285170 (<http://www.ncbi.nlm.nih.gov/bioproject/1285170>). The curated *de novo* transcriptome assembly and associated annotation tables are publicly available in Zenodo (<https://doi.org/10.5281/zenodo.18849852>).

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