

Structure, transcriptional signature, and regeneration of oscular rim in calcareous sponge *Leucosolenia corallorrhiza*

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ABSTRACT: The osculum is a structure through which filtered water leaves the aquiferous system of the sponge. The osculum is, however, not a mere opening, as it appears to participate in the water flow sensing and regulation. Additionally, the osculum is a central element of the axial patterning system of the sponge. Here we report data on the structure, gene expression profile, and regeneration of the osculum in the asconoid calcareous sponge *Leucosolenia corallorrhiza* (Haeckel, 1872). In this species, osculum openings are located on large, erect oscular tubes. The most distal part of the oscular tube, the oscular rim, differs structurally from the rest of the sponge body: here, the choanoderm is substituted by the endopinacoderm. The endopinacoderm forms a perpendicular two-layer fold called the oscular diaphragm. The diaphragm can regulate the effective size of the osculum opening, likely through the contractile activity of the supracellular actomyosin cable running along the inner edge of the diaphragm. The oscular rim also shows a unique transcriptional signature; when compared to other body regions, it shows the highest number of unique differentially expressed transcripts. Transcriptional profile of the oscular rim includes numerous components of the Wnt and TGF- β signaling pathways. Finally, the regeneration of the oscular tube represents a rapid epithelial morphogenesis occurring through morphallactic rearrangements of pre-existing tissues. The functional osculum is restored in 3 to 5 days after the amputation. During this process, the regenerative membrane transforms directly into the oscular diaphragm. The endopinacocyte lining of the oscular rim arises through transdifferentiation of the choanocytes near the wound. The preliminary experiments suggest that the course of the regeneration could depend on the amputation site.

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KEY WORDS: osculum, differential expression, Porifera, Wnt signaling pathway, TGF-beta signaling pathway.

Строение, профиль экспрессии и регенерация оскулярного кольца у известковой губки *Leucosolenia corallorrhiza*

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РЕЗЮМЕ: Оскулом — это структура, через которую профильтрованная вода выводится из водоносной системы губки. Однако оскулом — это не только выводное отверстие. Эта структура, вероятно, участвует в регуляции потока воды, выполняет сенсорную функцию и является частью системы осевой разметки тела у губок. В работе мы приводим данные о строении, профиле экспрессии генов и регенерации оскулома у асconoидной известковой губки *Leucosolenia corallorrhiza* (Haeckel, 1872). У этого вида оскулярные отверстия расположены на крупных оскулярных трубках, приподнимающихся над телом губки. Самая дистальная часть оскулярной трубки, оскулярное кольцо, отличается по структуре от остального тела губки: здесь хоанодерма замещается эндопинакодермой. Эндопинакодерма формирует перпендикулярную двухслойную складку, оскулярную диафрагму. Диафрагма может изменять эффективный размер оскулярного отверстия, вероятно, за счёт сократительной активности актомиозинового тяжа, идущего вдоль внутреннего края диафрагмы. Оскулярное кольцо имеет уникальный транскрипционный профиль. По сравнению с другими участками тела губки оскулярное кольцо имеет наибольшее количество уникальных дифференциально экспрессируемых транскриптов. Транскрипционный профиль оскулярного кольца включает многочисленные компоненты сигнальных путей Wnt и TGF-β. Наконец, регенерация оскулярной трубки происходит посредством быстрого эпителиального морфогенеза и морфаллактических перестроек существующих тканей. Восстановление функционального оскулома происходит через 3–5 дней после ампутации. В ходе этого процесса регенеративная мембрана напрямую трансформируется в оскулярную диафрагму. Эндопинакоциты оскулярного кольца восстанавливаются за

счёт трансдифференцировки хоаноцитов около раны. Предварительные эксперименты показали, что результат регенерации может зависеть от места ампутации.

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КЛЮЧЕВЫЕ СЛОВА: оскулум, дифференциальная экспрессия, Porifera, сигнальный путь Wnt, сигнальный путь TGF-бета.

Introduction

Sponges (phylum Porifera) are an ancient, early-branching lineage of Metazoa characterized by a unique anatomical and histological organization. In particular, sponges lack conventional metazoan organs and organ systems. The anatomy of these animals is built around the aquiferous system, a network of canals and flagellated chambers and tubes (Ereskovsky, Lavrov, 2021). Through the aquiferous system, sponges pump and filter large volumes of water to obtain food and oxygen and remove metabolic wastes. Sponges have numerous anatomical and cellular adaptations (Vogel, 1974; Weissenfels, 1992; Hammel, Nickel, 2014; Asadzadeh *et al.*, 2019) as well as behavioral patterns (Harrison, 1972; Elliott, Leys, 2007; Nickel *et al.*, 2011) to constantly sustain and regulate pumping. These animals even continuously rearrange their anatomy to adjust their aquiferous system to local hydrodynamic conditions (Bond, 1992; Gaino *et al.*, 1995; Bond, 1999; Ereskovsky, 2010; Lavrov, Kosevich, 2018).

The osculum is a large opening through which filtered water leaves the aquiferous system of the sponge. Studies on some demosponges show that the osculum can contract up to a complete closure, thus contributing to the regulation of the water flow (Prosser *et al.*, 1962; Reiswig, 1971; Kumala *et al.*, 2017). Some evidence implies that the osculum is not only an effector but also a sensory organ (Ludeman *et al.*, 2014): *e.g.*, the inner endopinacocyte lining of the osculum in freshwater *Ephydatia muelleri* (Lieberkühn, 1856) bears sensory cilia; these primary cilia are crucial for periodic coordinated contractions of the entire aquiferous system of the sponge. It is worth mentioning that all demosponges have the leuconoid aquiferous system, characterized by prolonged and branching canals and numerous discrete choanocyte chambers. Yet other organizations of the system (asconoid, solenoid,

syconoid, and sylleibid) with rather short canals and voluminous cavities lined with choanocytes can be found outside this class, particularly in calcareous sponges (Ereskovsky, Lavrov, 2021). It is thought that such open-type aquiferous systems could have functional differences compared to the leuconoid system (Asadzadeh *et al.*, 2020; Lavrov *et al.*, 2022). Possibly, the osculum utilizes distinct mechanisms and adaptations to regulate the water flow in open-type systems.

In addition to its flow-regulating function, the osculum is a part of the axial patterning system in sponges. The expression of certain components of the Wnt and TGF- β signaling pathways is associated with oscula in the demosponge *Halisarca dujardinii* Johnston, 1842 (Borisenko *et al.*, 2016) and the calcareous sponge *Sycon ciliatum* (Fabricius, 1780) (Leininger *et al.*, 2014). Furthermore, transplantation experiments with the freshwater demosponge *E. muelleri* demonstrated inductive capabilities of the osculum, which can guide canal reorganization in the host sponge upon transplantation. Again, it appears that these capabilities are at least partially controlled by the canonical Wnt signaling pathway (Windsor, Leys, 2010; Windsor Reid *et al.*, 2018).

Overall, the osculum appears to have essential developmental (controlling body patterning) and physiological (regulating water flow) functions, but available data are scarce and mostly restricted to demosponges. In the current study, we provide new data on the organization of the osculum in the calcareous sponge *Leucosolenia corallorrhiza* (Haeckel, 1872) with the open-type asconoid aquiferous system. We studied the histology and ultrastructure of the oscular rim with particular attention to the structure of the oscular diaphragm. The diaphragm seems to be able to change its area and regulate the size of the effective oscular opening. We also show that the osculum can exhibit distinct modes of regeneration: while the regeneration is always a rapid process occurring through epithelial

morphogenesis and rearrangements of tissues near the wound, the final result depends on the amputation site. Only the most distal amputations result in the restoration of the oscular rim, whereas the regeneration after amputations at more proximal sites ends with the transformation of the residual oscular tube into a blind outgrowth. The distinct modes of regeneration could be linked to the bulk RNA sequencing data, which reveals the differences in the transcriptional profiles of the body regions. We characterized the transcriptional signature of the oscular rim and revealed the oscular rim-specific expression of numerous transcripts related to the Wnt and TGF- β pathways.

Material and methods

Material collection

Specimens of *Leucosolenia corallorrhiza* (Haeckel, 1872) (Leucosolenida, Calcarea, Porifera) (Lavrov *et al.*, 2024) (Fig. 1A) were collected in Kandalaksha Bay of the White Sea in the environs of the Pertsov White Sea Biological Station (66°34'N, 33°08'E) from the upper subtidal zone at 0–2 m depth at low tide. Before the manipulations, the sponges were maintained in a 100 L laboratory aquarium with natural seawater (salinity 25–26‰) and biological filters at 8–12 °C for no longer than 1 week.

Histological and ultrastructural studies

For conventional histological and ultrastructural studies, oscular rims and cornus tubes (Fig. 1) were fixed with 2.5% glutaraldehyde (Electron Microscopy Science) in modified sodium cacodylate buffer (0.1 M sodium cacodylate, 85.55 mM NaCl, 5 mM CaCl₂, 5 mM MgCl₂, pH 7–7.5) for 2–12 h at 4 °C and post-fixed with 1% OsO₄ in the same buffer for 1 h at room temperature (RT) (Lavrov, Ereskovsky, 2022). Between fixation and post-fixation, specimens were rinsed three times with modified 0.1 M Na-Cacodylate buffer for 30 min each time at RT with constant orbital shaking at 70 rpm. In some specimens, 0.1% of ruthenium red (Sigma-Aldrich) was added to both the fixative and post-fixative to visualize the fine extracellular matrix. In this case, the time of fixation was 2 h at 4 °C and the time of post-fixation was 3 h at RT (Lavrov *et al.*, 2022). Further treatments were identical for both types of fixation.

Specimens for light microscopy and transmission electron microscopy (TEM) were subjected to spicule dissolution in 5% EDTA, pH 8.0–8.5 (Sigma-Aldrich) solution for 2 h at RT, dehydrated in an ethanol and acetone series at RT, and embedded in Epon/Araldite epoxy embedding media (Electron Microscopy Science) according to the manufacturer's protocol.

Semi-thin sections (1 μ m) and ultrathin sections (60–80 nm) were cut using an LKB V ultramicrotome (LKB). The semi-thin sections were stained with 1% toluidine blue — 0.2% methylene blue mixture for 1–1.5 min at 60 °C and studied with a Carl Zeiss Axioplan 2 microscope (Carl Zeiss) equipped with a digital camera AxioCam HRm (Carl Zeiss) and software AxioVision 3.1 (Carl Zeiss) or Nikon NiE and Ci microscopes (Nikon) equipped with a digital camera Digital Sight 10 (Nikon) and software NiS Elements Br 5.12 (Nikon). The ultrathin sections were double-stained with 4% aqueous uranyl acetate solution (40–60 min at 37 °C) and 0.4% lead citrate solution (25–30 min at RT in the dark) according to Reynolds (1963). The ultrathin sections were studied using JEM-1400 Flash (Jeol) and JEM-1011 (Jeol) transmission electron microscopes.

Specimens for scanning electron microscopy (SEM) were processed with spicules left intact. They were dehydrated in an ethanol and acetone series at RT and critical-point dried with a Hitachi Critical Point Dryer HCP-2 (Hitachi), gold-coated with an Eiko IB-3 (Eiko), mounted onto aluminum stubs, and studied using CamScan S-2 (Cambridge Instruments), Jeol JSM6380-LA (Jeol), and Jeol JCM-7000 (Jeol) scanning electron microscopes.

Immunocytochemical studies of actin distribution

For immunocytochemical studies, oscular rim samples were fixed with 2.5% glutaraldehyde in 1×PBS (ECO Service) and stored at 4 °C for several days. Fixed specimens were rinsed three times with 1×PBS for 30 min, incubated in ~0.5% fresh sodium borohydride solution in 1×PBS for 30 min, rinsed again three times with 1×PBS for 30 min, and finally incubated three times in Blocking solution (BS) (1% BSA (MP Biomedicals), 0.1% gelatin from cold water fish skin (Sigma-Aldrich), 0.5% Triton X-100 (Sigma-Aldrich), 0.05% Tween 20 (Sigma-Aldrich) in 1×PBS) for 30 min. The specimens were incubated in the mix of the primary antibodies: monoclonal anti- γ -actin antibodies produced in mouse (1:100) (clone 2A3, MCA5776, Bio-Rad) + polyclonal anti- β -actin antibodies produced in rabbit (1:100) (ab8227, Abcam) or monoclonal anti- β -actin antibodies produced in mouse (1:100) (clone 4C2, MCA5775, Bio-Rad) + monoclonal anti-Phospho-Myosin Light Chain 2 (pMLC2) antibodies produced in rabbit (1:200) (Cell Signaling). The samples were then rinsed three times with BS for several hours and incubated in the mix of the proper secondary antibodies: Donkey Anti-mouse IgG AlexaFluor 555 (1:100; final concentration 10 μ g/ml) (A-31570, Thermo Fisher Scientific), Goat Anti-mouse IgG FSD 647 (1:200; final concentration 5 μ g/ml) (RSA1165, BioActs), Donkey Anti-rabbit IgG AlexaFluor 488 (1:100; final concentration 10 μ g/ml) (A21206, Thermo Fisher Scientific), Goat Anti-rabbit

IgG AlexaFluor 555 (1:200; final concentration 5 µg/ml) (A-21248, Thermo Fisher Scientific). The incubations with primary and secondary antibodies were done in BS overnight at 4°C. After the incubation in the secondary antibodies, the specimens were rinsed several times with 1×PBS, and the nuclei were visualized by staining with 2 µg/ml 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich) for 1 h. The stained specimens were rinsed several times with 1×PBS, infiltrated with 30–60–90% glycerol-PBS/2.5% 1,4-diazabicyclo[2.2.2]octane (DABCO) (Sigma-Aldrich), and mounted in 90% glycerol-PBS/2.5% DABCO. Finally, the specimens were studied with a confocal laser scanning microscope (CLSM) Nikon A1 (Nikon) using lasers with wavelengths of 405 nm (DAPI), 488 nm (anti-β-actin pAbI + DAR IgG AlexaFluor 488 AbII), 561 nm (anti-γ-actin mAbI + DAM IgG AlexaFluor 555 AbII / anti-pMLC2 mAbI + GAR 555 AbII), and 640 nm (anti-β-actin mAbI + GAM IgG FSD 647 AbII).

RNA extraction, sequencing, and quality control

For RNA sequencing, four large sponges were manually cleaned of epibionts and debris using a stereomicroscope MSP-2 (Lomo), washed in 0.22-µm filtered seawater. Each sponge was split into cornus tubes (C), diverticula (D), and oscular rims (O) (cut slightly proximally to the choanoderm-endopinacoderm boundary, Fig. 1B line 3). The samples are denoted according to the body region (O, C, or D) and sponge specimen (1–4) (Table S1).

The samples were lysed in 1 ml of ExtractRNA (Evrogen, BC032). During the lysis, specimens were placed at RT for 15–20 min and homogenized with sterile pestles; then, they were cooled at 4 °C for 20 min, placed at –20 °C for at least 2 h, and finally stored at –80 °C. RNA extraction was performed using ExtractRNA (Evrogen, BC032) according to the manufacturer's protocol. The RNA samples in RNase-free water were frozen and stored at –80 °C until library preparation. The RNA concentration was low in three samples (C3, D1, and O1), and they were excluded from further analysis (Table S1).

For the rest of the samples, the polyadenylated fraction of total RNA was purified with NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs). Libraries were constructed with the NEBNext Ultra II RNA Library Prep Kit and sequenced on the Illumina NovaSeq6000 in paired-end mode with 150 bp read length, with an average output of 14 Gbp per library (Table S2).

Additionally, one large sponge was completely lysed in 1 ml of ExtractRNA. The subsequent preparation for sequencing was the same as described above. This reference sample was sequenced on the Illumina NovaSeq6000 in paired-end mode with 150 bp read length, with an output of 39.7 Gbp (Table S2).

Sequencing results were analyzed using FastQC v0.11.9 and multiQC v1.17. All major parameters (phred scores, sequence content per base, GC content distribution, etc.) were considered satisfactory, and no samples were discarded from the subsequent analysis. A total of three samples per body region (cornus tubes, diverticula, and oscular rim) were used in the subsequent analysis.

Transcriptome assembly and annotation

Raw paired-end reads were quality-filtered and adapter-trimmed using Trimmomatic (v0.39) with default parameters. Only properly paired reads with a minimum length of 36 bp were retained for downstream analyses. To remove ribosomal RNA contamination, the filtered libraries were additionally processed with SortMeRNA (v4.3.6; Kopylova *et al.*, 2012). Given the frequent presence of bacterial symbionts in sponges, a decontamination step was performed using Kraken2 (v2.1.3; Wood *et al.*, 2019), a k-mer-based taxonomic classification tool. Read classification was conducted against the plusPF database (including archaea, bacteria, viral, plasmid, human, protozoa, and fungal taxa (v. 1/12/2024)). The fraction of reads classified as contaminant sequences did not exceed 2.5–3% across all samples. Quality of the filtered reads was assessed using FastQC (v0.11.9) at each processing step. Summary statistics for raw and filtered libraries used for transcriptome assembly are provided in Table S2.

Adenovo transcriptome was assembled by Trinity (v2.15.1; Haas *et al.*, 2013) based on pooled paired-end reads from all sampled points (nine experimental and one reference samples) with default parameters and *in silico* read normalization. Highly similar contigs were clustered using cd-hit-est (v4.8.1; Li, Godzik, 2006), and transcripts sharing more than 95% sequence similarity were collapsed. Assembly completeness and quality were evaluated using BUSCO (v5.7.1; Manni *et al.*, 2021) with the metazoa_odb10 database and by assessing Ex90N50 statistics. The final non-redundant assembly at the “isoform level” contained 91.6% complete (47.1% single, 44.5% duplicated), 3.1% fragmented, and 5.3% missing BUSCO genes. When assessed at the Trinity “gene” level (retaining the longest isoform per gene), the assembly showed 88.6% complete BUSCOs (79.0% single-copy and 9.5% duplicated), with a reduced proportion of duplicated BUSCOs. Detailed assembly statistics and quality metrics are provided in Table S3.

Open reading frames (ORFs) were predicted for the assembled transcripts using TransDecoder (v5.7.1). Out of 322,094 assembled transcripts, 94,492 ORFs were predicted. Functional annotation was performed on the predicted amino acid sequences using InterProScan (v5.73-104.0; Jones *et al.*, 2014), DIAMOND (v2.1.14; Buchfink *et al.*, 2021) blastp search against the Swiss-Prot database (accessed

22.03.2025), and KofamScan (v1.3.0; Aramaki *et al.*, 2020). KEGG Orthology (KO) terms were assigned based on KofamScan search against the Kofam database (v2025-02-01; KEGG release 113.0). In addition, Gene Ontology (GO) terms were obtained using EggNOG-mapper (v2.1.12; Cantalapiedra *et al.*, 2021). Detailed parameters of the annotation pipeline were described previously (Melnikov *et al.*, 2025). Functional annotation was obtained for 25,939 unique Trinity “genes”, corresponding to 92.5% of predicted ORFs at the gene level and 10.5% of the total number of Trinity “genes” in the assembly.

Additionally, specific transcripts/proteins were annotated manually based on their similarity to transcripts/proteins known in *S. ciliatum*. For this purpose, we used blastp search of the predicted amino acid sequences against the *S. ciliatum* proteome (RefSeq assembly GCF_964019385.1, assembly version ocSycCili1.1) using online BLAST (blastp, Altschul *et al.*, 1997).

Differential expression analysis

Cleaned paired-end reads from nine body region samples were aligned to the assembled reference transcriptome using Bowtie2 (v2.5.4), and transcript abundance was estimated with RSEM (v1.3.3) with parameters implemented into Trinity pipeline scripts (Haas *et al.*, 2013).

Differential expression analysis was performed in R (v4.5.1) using DESeq2 package (v1.48.2; Love *et al.*, 2014) at the level of Trinity’s “genes” rather than “isoforms”. Unnormalized transcript-level count data were imported into the DESeq2 using the tximport package (v1.36.1; Soneson, 2016). A minimal pre-filtering step was applied following the DESeq2 recommended workflow, retaining only transcripts with at least 10 counts in a minimum of three samples. Exploratory data analyses revealed unwanted variation resulting in samples being grouped by the individual from which they were obtained (rather than by body region) (Fig. S1A, C). This fact was accounted for by including a batch variable in the DESeq2 design formula ($\sim$ individual+condition). For downstream analyses and data visualization, the batch effect of variance-stabilized transformed (vst) counts was further corrected using the limma package (v3.64.3) (Fig. S1B, D). Batch effect correction resulted in proper sample clustering corresponding to the studied body regions.

Differential expression testing was carried out using pairwise comparisons of the body regions (O vs C, O vs D, and D vs C). Additionally, we compared O vs the combined C+D condition by specifying numeric contrasts manually. Shrinkage of log2fold change (LFC) after Wald’s tests was performed using the *ashr* method (Stephens, 2016). Transcripts were considered differentially expressed (DET) if their absolute LFC was greater than 1 and adjusted P-value (FDR, padj) less than 0.01 (Fig. S2).

Functional enrichment analysis in GO and KEGG terms was performed using the clusterProfiler package (v4.16.0; Xu *et al.*, 2024). GO enrichment analyses were conducted on lists of DETs specific to each body region. These lists were obtained by combining DETs from all comparisons, including the respective body region (i.e., by combining O vs D and O vs C for osculum-specific DETs). KEGG enrichment analysis was performed using only unique region-specific DETs. GO enrichment analysis was restricted to the Biological Process (BP) category; KO terms were restricted to the “Environmental Information Processing”, “Genetic Information Processing”, and “Cellular Processes” KEGG categories. In both cases, the universe background included all non-zero-expressed transcripts (n = 26,607). In both analyses, categories were considered enriched if their adjusted p-value was < 0.1. Enrichment results were visualized using dotplots generated with the enrichplot package (v1.28.4).

Co-expression analysis was performed on the set of DETs identified across all pairwise comparisons (O vs C, O vs D, and D vs C). Variance-stabilized transformed (vst) and batch corrected transcript counts were centered and hierarchically clustered using hclust (Euclidean distance, Ward.D linkage method). Expression patterns were visualized as a heatmap, and transcript clusters were defined by cutting the hierarchical clustering tree into four clusters.

Experiments on oscular tube regeneration

For the regeneration studies, sponges 10–12 mm in size were used. Each sponge bears several (2–4) oscular tubes. One oscular tube per individual was amputated manually with Castroviejo scissors. A total of 71 sponges were used in regeneration experiments.

In the main experimental series, amputations of the oscular tubes were performed at their distal part, proximally to the choanoderm-endopinacoderm boundary (Fig. 1B line 3). After the operation, sponges were maintained in a 100 L laboratory aquarium with natural seawater and biological filters at 8–12 °C. For *in vivo* description of the regeneration, sponges were inspected daily using a stereomicroscope Nikon SMZ445 (Nikon) and photographed using a stereomicroscope Leica M165FC (Leica) equipped with a digital camera Leica DFC420 (Leica) and LAS Store and Recall v.4.1 software (Leica). For histological studies, regenerating oscular tubes (n=38) were fixed and processed as described in “Histological and ultrastructural studies”. During this series, samples were fixed at 6 hours post-operation (hpo), 12 hpo, 1 day post-operation (dpo), 1.5 dpo, 2 dpo, 3.5 dpo, 4 dpo, 6 dpo, 11 dpo, and 24 dpo. The regeneration was considered complete and successful if a specimen was able to restore the oscular rim with endopinacocyte lining and oscular diaphragm capable of change its area to regulate the size of the osculum opening.

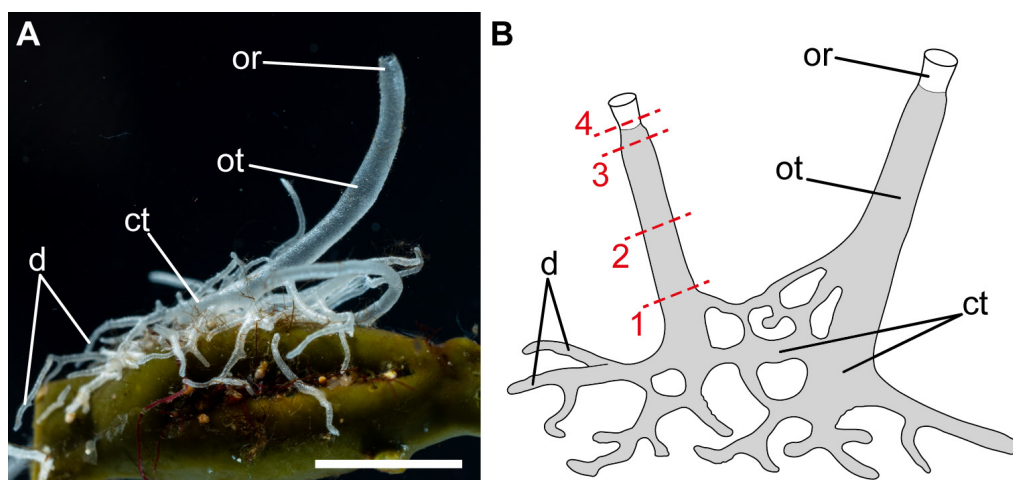


Fig. 1. Calcareous sponge *Leucosolenia corallorrhiza*. A — *in vivo* view of a sponge on the substrate. B — schematic representation of the sponge, 1–4 mark levels of oscular tube amputation.

Abbreviations: 1 — base of the oscular tube, 2 — middle part of the oscular tube, 3 — distal part of the oscular tube (proximally to the choanoderm–endopinacoderm boundary), 4 — distal part of the oscular tube (distally to the choanoderm–endopinacoderm boundary), ct — cormus tubes, d — diverticula, or — oscular rim, ot — oscular tube. Scale bar: A — 1 cm.

In the supplementary experimental series, oscular tubes were amputated at various levels along the proximal-distal axis (Fig. 1B): 1) at the base of the oscular tube, line 1 ($n=2$); 2) in the middle of the oscular tube, line 2 ($n=2$); 3) at the distal part of the oscular tube proximally to the choanoderm–endopinacoderm boundary, line 3 ($n=2$); 4) at the distal part of the oscular tube distally to the choanoderm–endopinacoderm boundary, line 4 ($n=2$). Sponges were maintained, inspected, and photographed after the operation as described above.

Results

Structure of the oscular rim

The body of *Leucosolenia corallorrhiza* is represented by cormus, a tangled and anastomosing network of thin-walled tubes (Fig. 1A). The most conspicuous parts of the *L. corallorrhiza* body, however, are the oscular tubes rising from the cormus. They appear as rather thick tubes, and a sponge bears from one to dozens of oscular tubes depending on the cormus size. Additionally, the periphery of the cormus bears numerous blind tubes, diverticula (Fig. 1A).

The structure of the oscular tube varies along its proximal-distal axis. For most of its length, the oscular tube shows no principal differences in organization from regular cormus tubes. The outer surface of the body wall is represented by a layer of T-shaped exopinacocytes, the inner

surface — by a layer of prismatic flagellated choanocytes. The rather thin mesohyl is located between the exopinacoderm and choanoderm layers. The mesohyl is rich in fibrillary extracellular matrix and contains some wandering cells. The body wall is pierced by numerous tube-shaped porocytes responsible for delivering the water inside a sponge (Lavrov *et al.*, 2022, 2024).

The structure of the oscular tube changes at its distal end, where water is expelled from the sponge body. This region represents the oscular rim. *In vivo*, the oscular rim appears as a more transparent zone of the osculum. The outer surface of the oscular rim, similarly to other parts of the oscular tube, is covered by T-shaped exopinacocytes (Fig. 2A–D). They have a submerged cell body containing the majority of organelles and thin cytoplasmic plates covered by conspicuous glycocalyx (Fig. 2D). The mesohyl of the oscular rim also has a regular structure (Fig. 2A–C). The differences in the structure of the oscular rim concern mainly the inner layer of cells: here, choanocytes are substituted by endopinacocytes (Fig. 2A–C). Endopinacocytes are flat, non-flagellated cells that are fusiform in section. The nucleus and the majority of other organelles are located in the central, slightly bulged part of the cell. The surface of endopinacocytes facing the external environment is covered by a thin glycocalyx

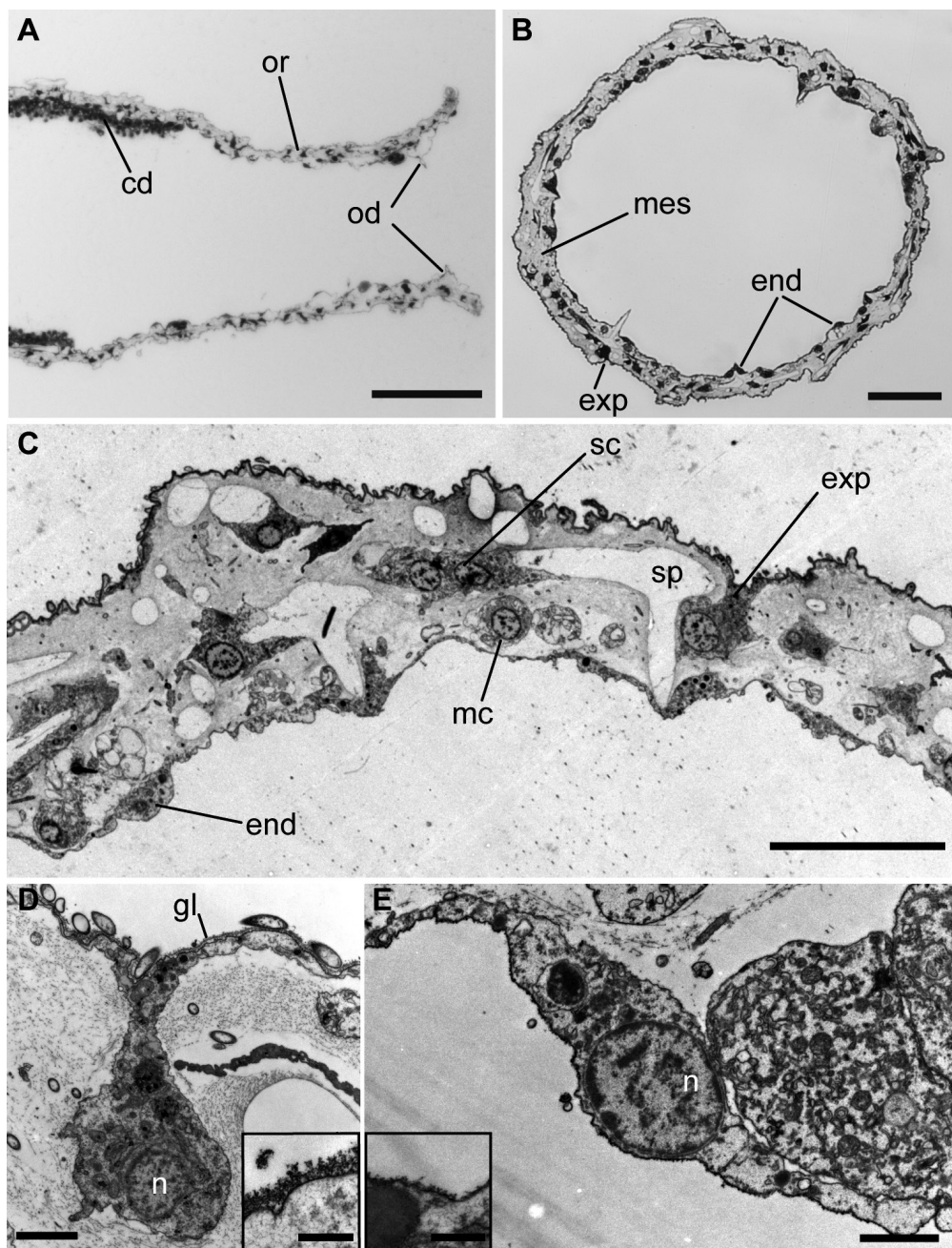


Fig. 2. Histology and ultrastructure of the oscular rim in *Leucosolenia corallorrhiza*. A — longitudinal section of the oscular rim, light microscopy. B — transverse section of the oscular rim, light microscopy. C — structure of oscular body wall in transverse section, transmission electron microscopy. D — exopinacocyte, inset — surface glycocalyx of the exopinacocyte, transmission electron microscopy. E — endopinacocyte, inset — surface glycocalyx of the endopinacocyte.

Abbreviations: cd — choanoderm, end — endopinacocyte, exp — exopinacocyte, gl — surface glycocalyx, mc — mesohyl cell, mes — mesohyl, od — oscular diaphragm, or — oscular rim, n — nucleus, sc — sclerocyte, sp — space from an etched spicule. Scale bars: A — 100 μ m, B — 50 μ m, C — 20 μ m, D, E — 2 μ m, D, E insets — 0.5 μ m.

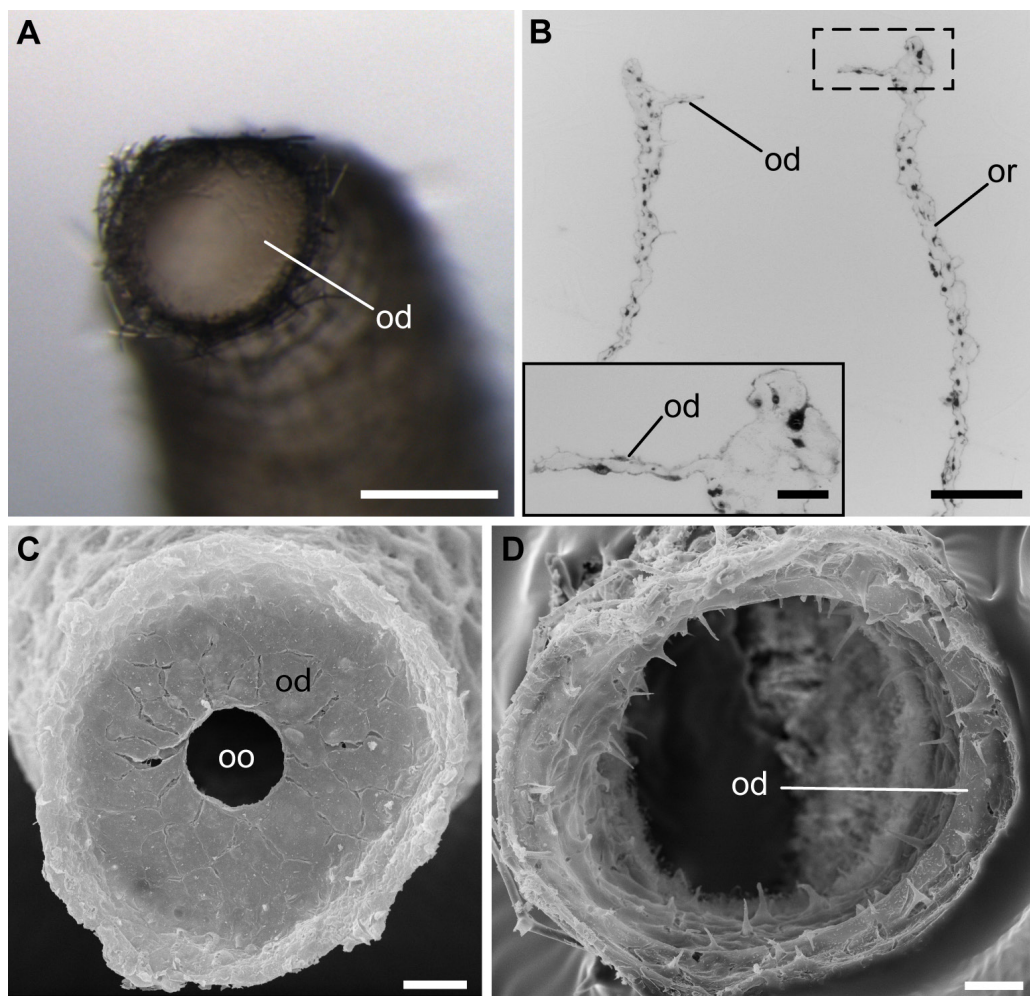


Fig. 3. Ocular diaphragm in *Leucosolenia corallorrhiza*. A — *in vivo* view of the oscular diaphragm, light microscopy. B — transverse section of the oscular rim, light microscopy; inset — zoomed view of the zone in the black dashed rectangle. C, D — oscular diaphragms of various sizes, scanning electron microscopy. Abbreviations: od — oscular diaphragm, oo — oscular opening, or — oscular rim. Scale bars: A — 200 μ m, B — 100 μ m, B inset — 20 μ m, C, D — 30 μ m.

(Fig. 2E). In addition, no porocytes are present in the oscular rim.

The opening of the osculum is partially closed by a thin, semi-transparent oscular diaphragm (Fig. 3A). It is located at the distal part of the inner surface of the oscular rim (Fig. 3B). The oscular diaphragm is a two-layered structure built by endopinacocytes and could be described as a fold of the oscular rim endopinacoderm. Two layers of endopinacocytes are separated by a thin acellular mesohyl inlay. *In vivo* studies of different individuals (Fig. 3C–D) and repetitive

observations of a single osculum reveal that the oscular diaphragm can change its area, thus regulating the size of the osculum opening. Immunolabelling shows that the cells at the inner edge of the oscular diaphragm form a prominent supracellular actin cable (Figs. 4, 5). The cable is apparently immunoreactive with anti- β -actin antibodies but not with anti- γ -actin antibodies; anti- γ -actin immunoreactivity is characteristic of some mesohyl cells (Figs. 4, 5). The cable also shows anti-phosphorylated myosin light chain 2 immunoreactivity (Fig. 6).

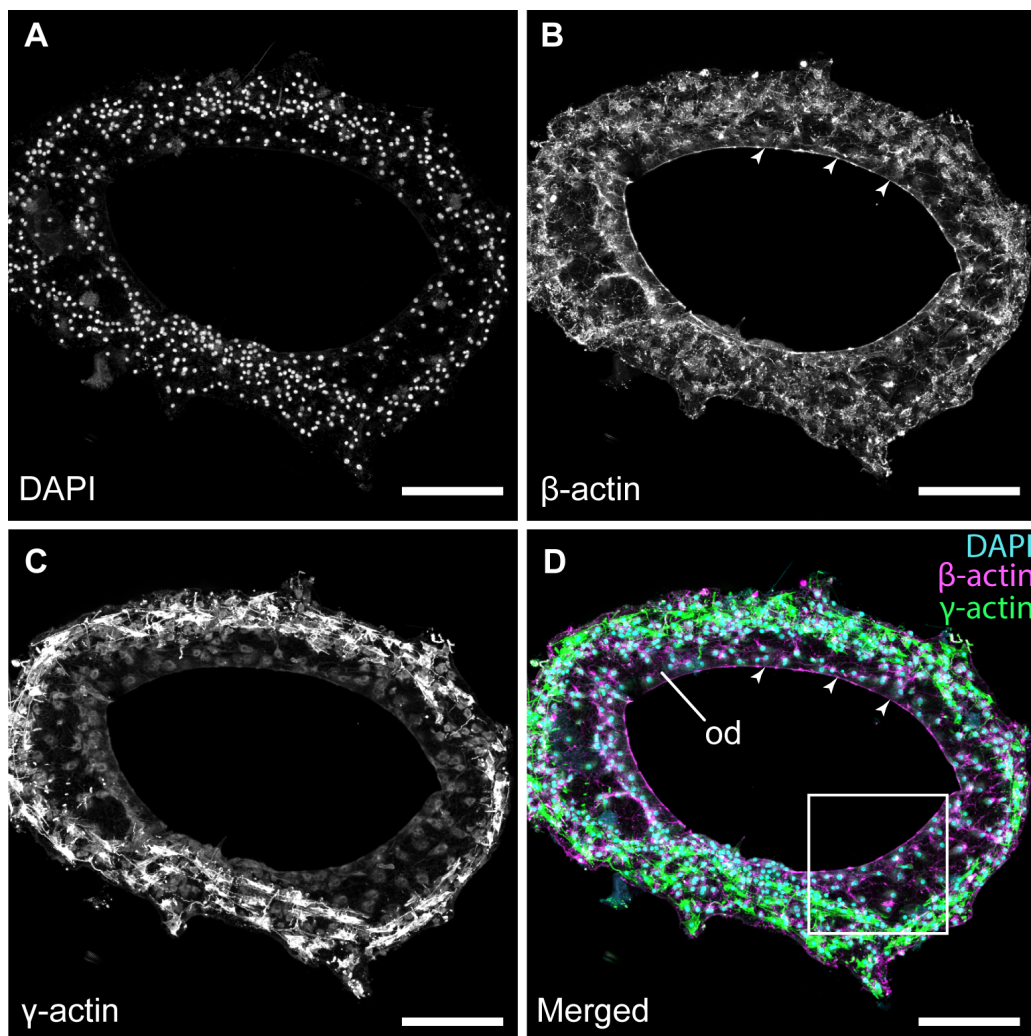


Fig. 4. Actin distribution in the oscular diaphragm of *Leucosolenia corallorrhiza*, confocal laser scanning microscopy (maximum intensity projection). A — cell nuclei (DAPI), B — anti- β -actin labelling, C — anti- γ -actin labelling, D — merged image. White arrowheads — actin cable revealed by anti- β -actin immunoreactivity, white square — area shown in Fig. 5.

Abbreviations: od — oscular diaphragm. Scale bars: 100 μ m.

Gene expression profile of the oscular rim

We used bulk mRNA sequencing to assemble a *de novo* transcriptome of *L. corallorrhiza* and describe transcript expression in different regions of the sponge body: the oscular rim, diverticulum, and regular cormus tubes (Fig. 1). After the batch correction, the principal component analysis and hierarchical clustering of gene expression levels showed that samples grouped according to the body region (Fig. S1), implying that our

RNA libraries reflect the differences in the global transcriptional profiles of these regions.

Subsequent pairwise comparisons of the regions revealed 1106 differentially expressed transcripts (DETs) (Wald's test, p -value_{adj} < 0.01, $|\text{Log}_2\text{FoldChange}| > 1$) (Fig. S2). Notably, more than half of the DETs (582) were upregulated specifically in the oscular rim (Fig. S2A). The cormus tubes and diverticula shared a considerable number of upregulated DETs (160) and had

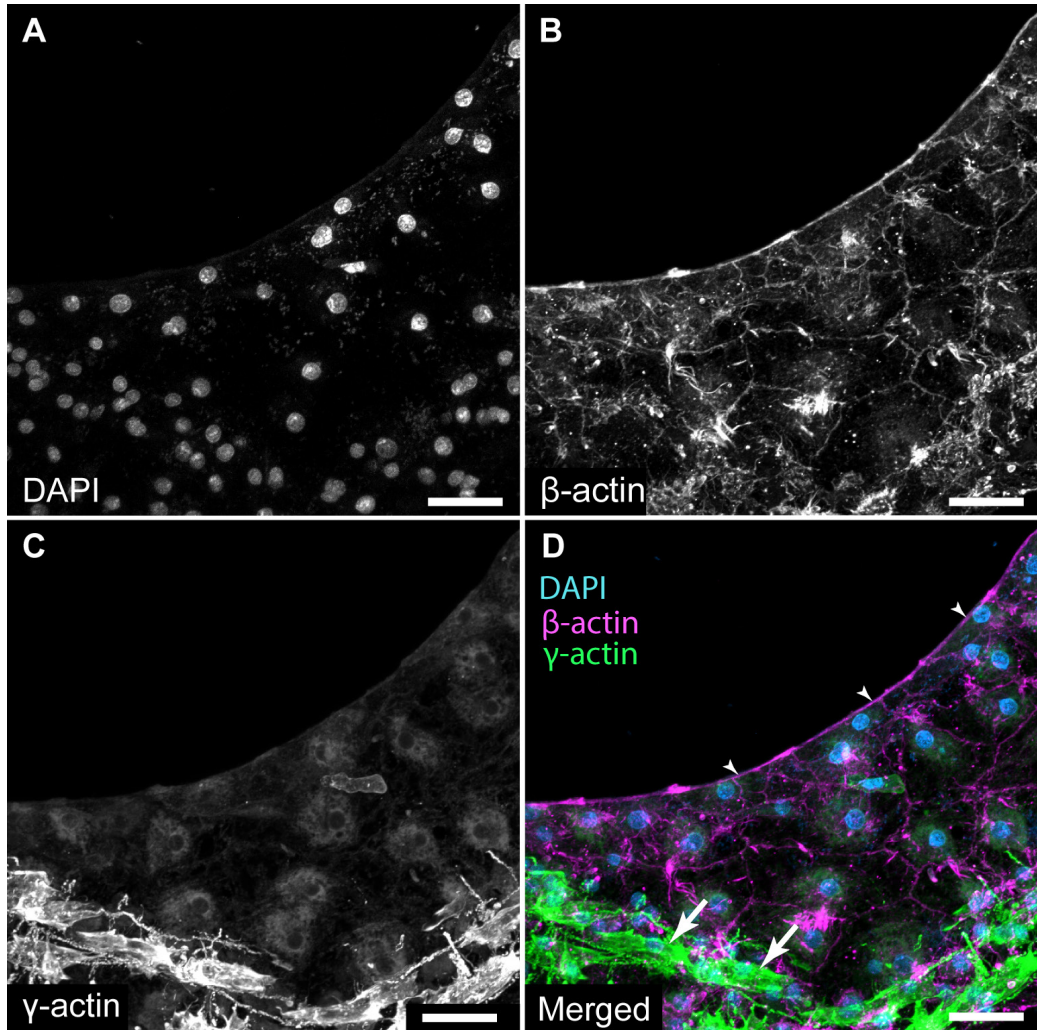


Fig. 5. Actin distribution in the oscular diaphragm of *Leucosolenia corallorrhiza*, zoomed view, confocal laser scanning microscopy (maximum intensity projection). A — cell nuclei (DAPI), B — anti- β -actin labelling, C — anti- γ -actin labelling, D — merged image. White arrowheads — actin cable revealed by anti- β -actin immunoreactivity; white arrows — mesohyl cells revealed by anti- γ -actin immunoreactivity. Scale bars: 20 μ m.

much fewer unique upregulated DETs (266 and 50, respectively) compared to the oscular rim (Fig. S2A). The distribution of the downregulated DETs across the body regions was generally the same: the majority of the underexpressed DETs were specific to the oscular rim (425), while the cormus tubes and diverticula shared most of the downregulated DETs (280) (Fig. S2B).

The coexpression analysis supports the results of the DE analysis: judging by the general transcriptional landscape, the oscular rim appears

to be distinct from the cormus tubes and diverticula. With minor exceptions, clusters that show upregulation in the oscular rim (clusters 3 and 4; 185 and 439 transcripts, respectively) consist of transcripts underexpressed in both cormus tubes and diverticula (Fig. 7). *Vice versa*, clusters 1 and 2 (327 and 155 transcripts, respectively) show downregulation in the oscular rim while being generally upregulated in cormus tubes (Fig. 7). Notably, the expression profiles of these clusters in diverticula are somewhat noisy and can be

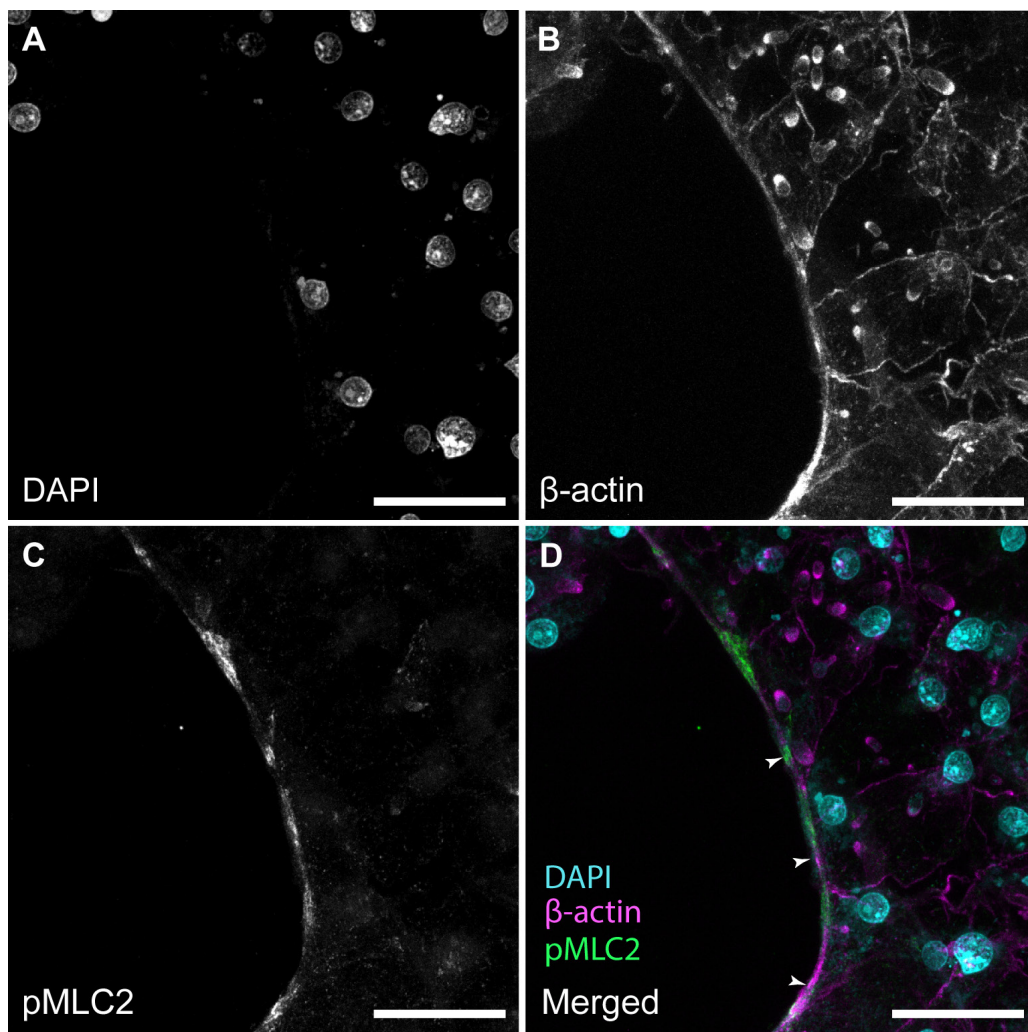


Fig. 6. Actin and phosphorylated myosin light chain distribution in the oscular diaphragm of *Leucosolenia corallorrhiza*, confocal laser scanning microscopy (maximum intensity projection). A — cell nuclei (DAPI), B — anti- β -actin labelling, C — anti-phosphorylated myosin light chain 2 labelling, D — merged image. White arrowheads — actomyosin cable. Scale bars: 20 μ m.

interpreted as intermediate between the oscular rim and cormus tubes.

Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of non-unique DETs shows that both up- and downregulated transcripts across all regions were mainly enriched in terms related to various signaling pathways. In particular, the upregulated DETs of the oscular rim were enriched in transcripts associated with the Wnt, TGF- β , mTOR, and calcium signaling pathways (Fig.

S3). We were also surprised to see the term “Signaling pathways regulating pluripotency of stem cells” among DETs upregulated in the osculum. Judging by the manual inspection of the results of the enrichment test, the presence of this term can be explained by the expression of components of Wnt- and TGF- β pathways, as they are often associated with the maintenance of stem cells. No *sensu stricto* stem cell markers contributed to the enrichment. Thus, currently there is no evidence to suggest the

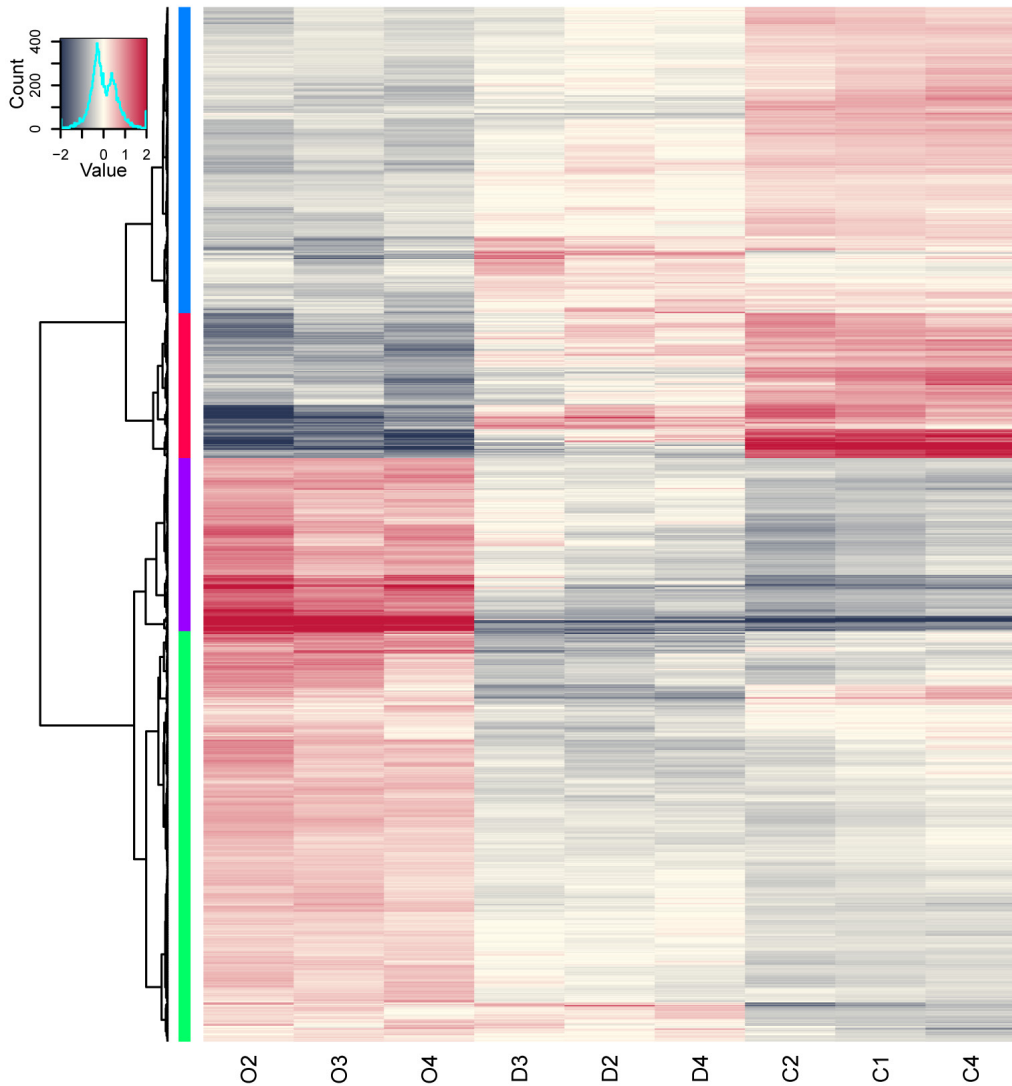


Fig. 7. Heatmap of scaled normalized counts of 1106 differentially expressed transcripts (DETs) (Wald's test, $p\text{-value}_{\text{adj}} < 0.01$, $|\text{Log}_2\text{FoldChange}| > 1$). Clustering was performed on counts after variance stabilizing transformation (VST) implemented in DESeq2 using Pearson correlation and Ward.D2 link. Blue — cluster 1 (327 transcripts), red — cluster 2 (155 transcripts), purple — cluster 3 (185 transcripts), green — cluster 4 (439 transcripts).

Abbreviations: C — cornus tubes, D — diverticula, O — oscular rim.

presence of a specific stem population in the osculum (Fig. S3).

We further specifically analyzed the expression profiles of Wnt and TGF- β pathway components, as these signaling pathways are known to be involved in axial patterning across Metazoa (Weiss, Attisano, 2013; Hayat *et al.*, 2022).

Regarding the Wnt signaling pathway, we have recovered 20 *Wnt ligands*, four *Frizzled receptors* (*Fzd*), and five Wnt-ligand inhibitors of the *secreted frizzled related protein* family (*sFRP*) (Table S4). *Wnt ligands* and *Frizzled receptors* were named after the genes known in *S. ciliatum* (Leininger *et al.*, 2014) using reciprocal

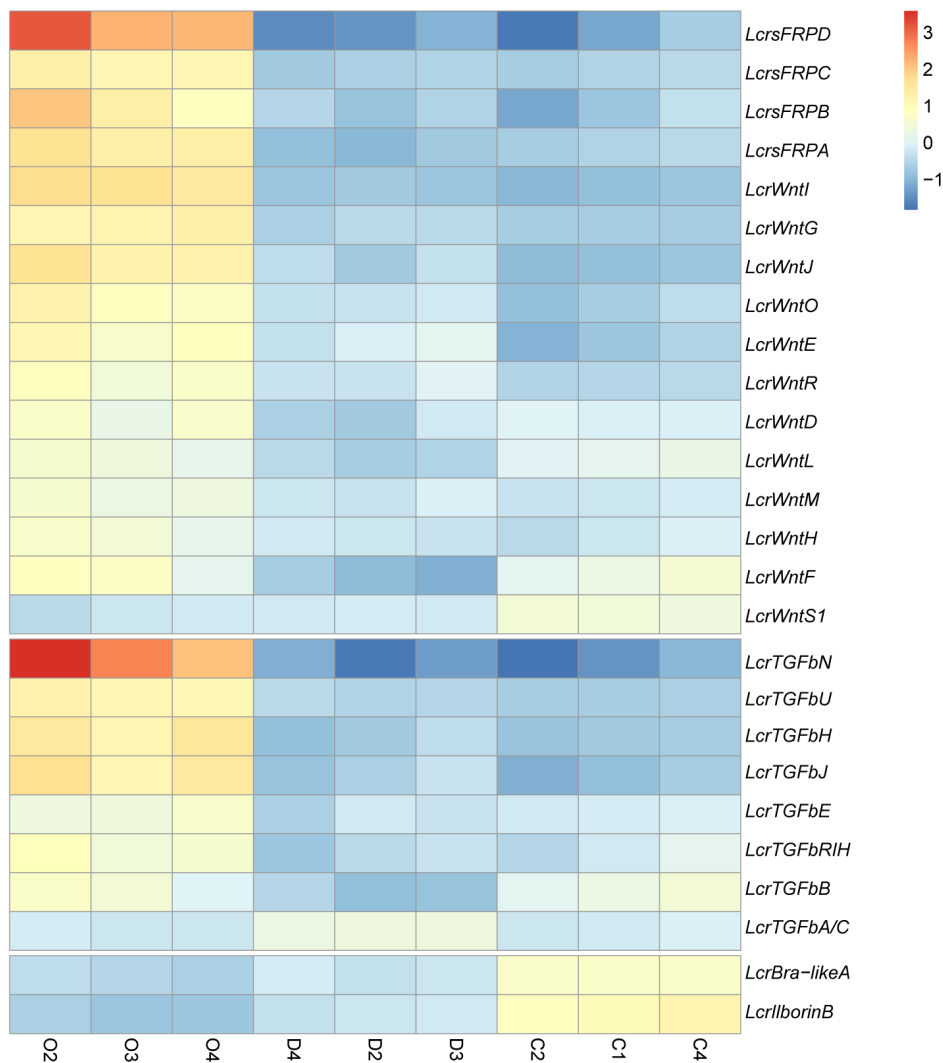


Fig. 8. Expression profiles of manually selected differentially expressed transcripts (DETs) (Wald’s test, $p\text{-value}_{\text{adj}} < 0.01$, $|\text{Log}_2\text{FoldChange}| > 1$) across *Leucosolenia corallorrhiza* body regions. Heatmap colors reflect the scaled normalized counts of selected transcripts. Abbreviations: C — cormus tubes, D — diverticula, O — oscular rim.

blast for inferring orthology relationships, while *sFRPs* were designated with consecutive letters. Additionally, we identified the major downstream components of the pathway: two β -catenin, two *Dishevelled*, two *TCF/LEF*, and one *Groucho*. Among TGF- β signaling pathway components, we have recovered 15 TGF- β ligands, 13 TGF- β receptors (*TGF β R*), and seven *Smads* (Table S4). The components of the pathway were named

after the genes known in *S. ciliatum* (Leininger *et al.*, 2014) using reciprocal blast for inferring orthology relationships.

Twelve of the identified *Wnt* ligands showed differential expression (Figs 8, 9, S4; Table S4). Ten of these *Wnt* ligands (*LcrWntD*, *LcrWntE*, *LcrWntG-J*, *LcrWntL*, *LcrWntM*, *LcrWntO*, and *LcrWntR*) were upregulated in the oscular rim, while *WntS1* was upregulated in the cormus tubes,

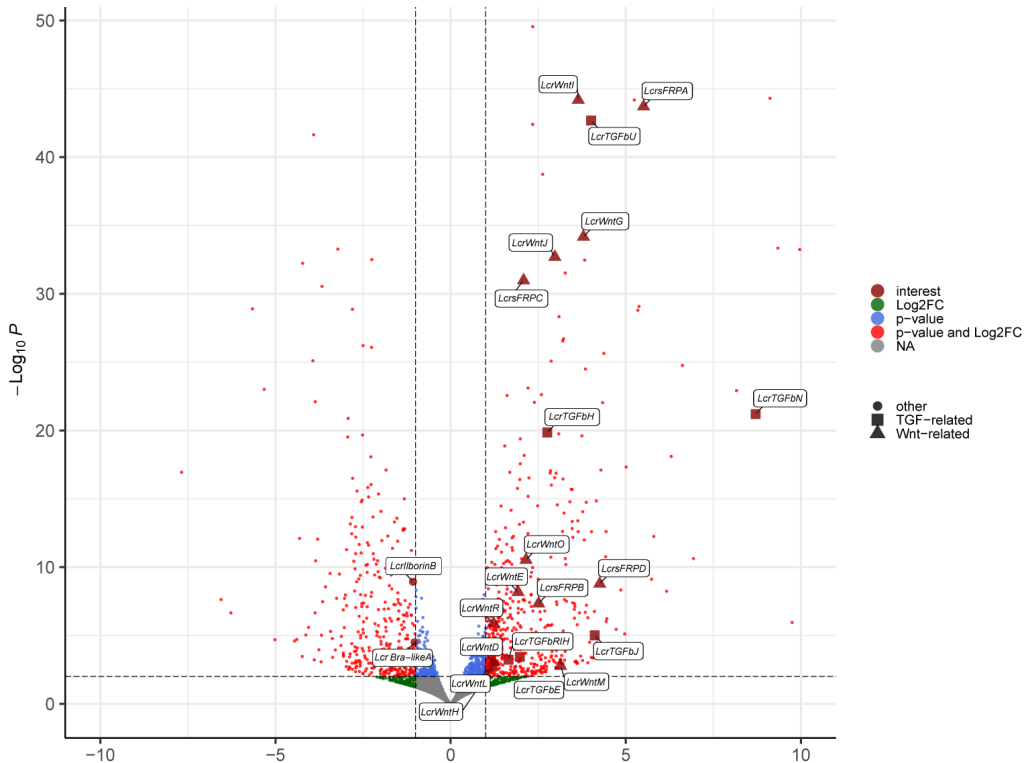


Fig. 9. Volcano plot of differentially expressed transcripts (DETs) (Wald's test, $p\text{-value}_{\text{adj}} < 0.01$, $|\text{Log}_2\text{Fold-Change}| > 1$) of the oscular rim samples versus the pooled cornus tubes and diverticula samples. Labels mark manually selected DETs.

and *LcrWntF* was downregulated in the diverticula (Figs 8, 9, S4; Table S4). *sFRPs* also showed differential expression as four of them (*LcrsFRPA-D*) were upregulated in the oscular rim (Figs 8, 9, S4; Table S4). *Fzds* and the intracellular components of the pathway were not differentially expressed across the studied body regions (Table S4). Finally, one of the Brachyury paralogs (*LcrBraA*), which is a widely known target of the Wnt signaling pathway, was downregulated in the oscular rim. The other paralog (*LcrBraB*) shows no differential expression (Figs 8, 9, S4; Table S4).

Among identified TGF- β signaling pathway components, five TGF- β ligands (*LcrTGFbE*, *LcrTGFbH*, *LcrTGFbJ*, *LcrTGFbN1*, and *LcrTGFbU*) and one Type I receptor *LcrTGFbRIH* showed differential expression and were upregulated in the oscular rim (Figs 8, 9, S4; Table S4). *Smads* were not differentially expressed across the studied body regions (Table S4).

Additionally, we have identified two paralogs of *Ilborin* (designated with consecutive letters).

The expression of *Ilborin* marks the flagellated larval cells and choanocytes in the demosponge *H. dujardinii* (Borisenko *et al.*, 2022). In concordance with this fact, the expression of one paralog (*LcrIlborinB*) was downregulated in the oscular rim, which lacks choanocytes. The other paralog (*LcrIlborinA*) shows no differential expression (Figs 8, 9, S4; Table S4).

Regeneration of the oscular tube

The regeneration of the oscular tube was studied in detail (Figs 10, 11, 12) after excision proximally to the choanoderm–endopinacoderm boundary (Fig. 1B line 3). The functional osculum was restored in 3 to 5 days. The process began with the isolation of the sponge's internal milieu (mesohyl) from the external environment. The isolation occurred through the joining of the exopinacoderm and choanoderm, which was already finished at 6 hours post-operation (hpo) (Fig. 12A). It seems that exopinacocytes played a leading role during the isolation: exopinacocytes near the wound changed

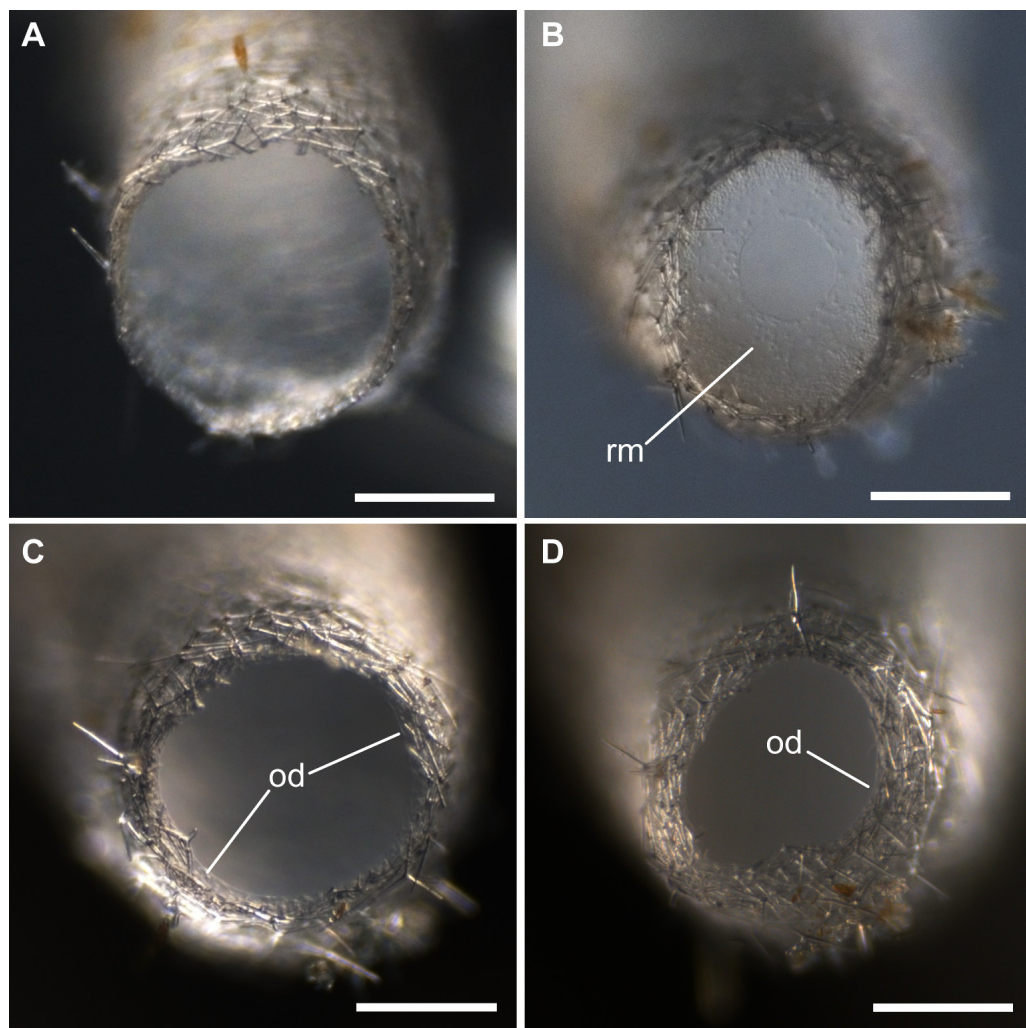


Fig. 10. Oscular rim regeneration after the amputation at the distal part of the oscular tube proximally to the choanoderm-endopinacoderm boundary, *en face* view. A — 0 hours post-operation, B — 1 day post-operation (dpo), C — 2 dpo, D — 3 dpo.

Abbreviations: od — oscular diaphragm, rm — regenerative membrane. Scale bars: 200 μm .

their T-shape to a flat one, extending their area. The exopinacoderm bent over the wound and contacted the choanoderm. In contrast, choanocytes seemed to be passive at this stage: the vast majority of these cells retained their intact prismatic shape even in very close proximity to the wound; few choanocytes contacting exopinacocytes showed first signs of future transdifferentiation by acquiring a pillow-like shape (Fig. 12A).

At 12–24 hpo, regenerating tubes slightly contracted near the amputation sites, reducing their diameter. At the same time, the regenera-

tive membrane appeared (Fig. 10B) and grew perpendicular to the intact walls of the tube in a centripetal direction. It was built by two layers of flat cells separated by the mesohyl, which was very narrow and almost acellular at this stage (Fig. 12B). In 18 sponges (72%), the regenerative membranes reached their maximum area at 24 hpo but remained incomplete, having an orifice in the central part; in 7 sponges (28%), complete regenerative membranes formed.

Simultaneously, the active transdifferentiation of the choanocytes into endopinacocytes

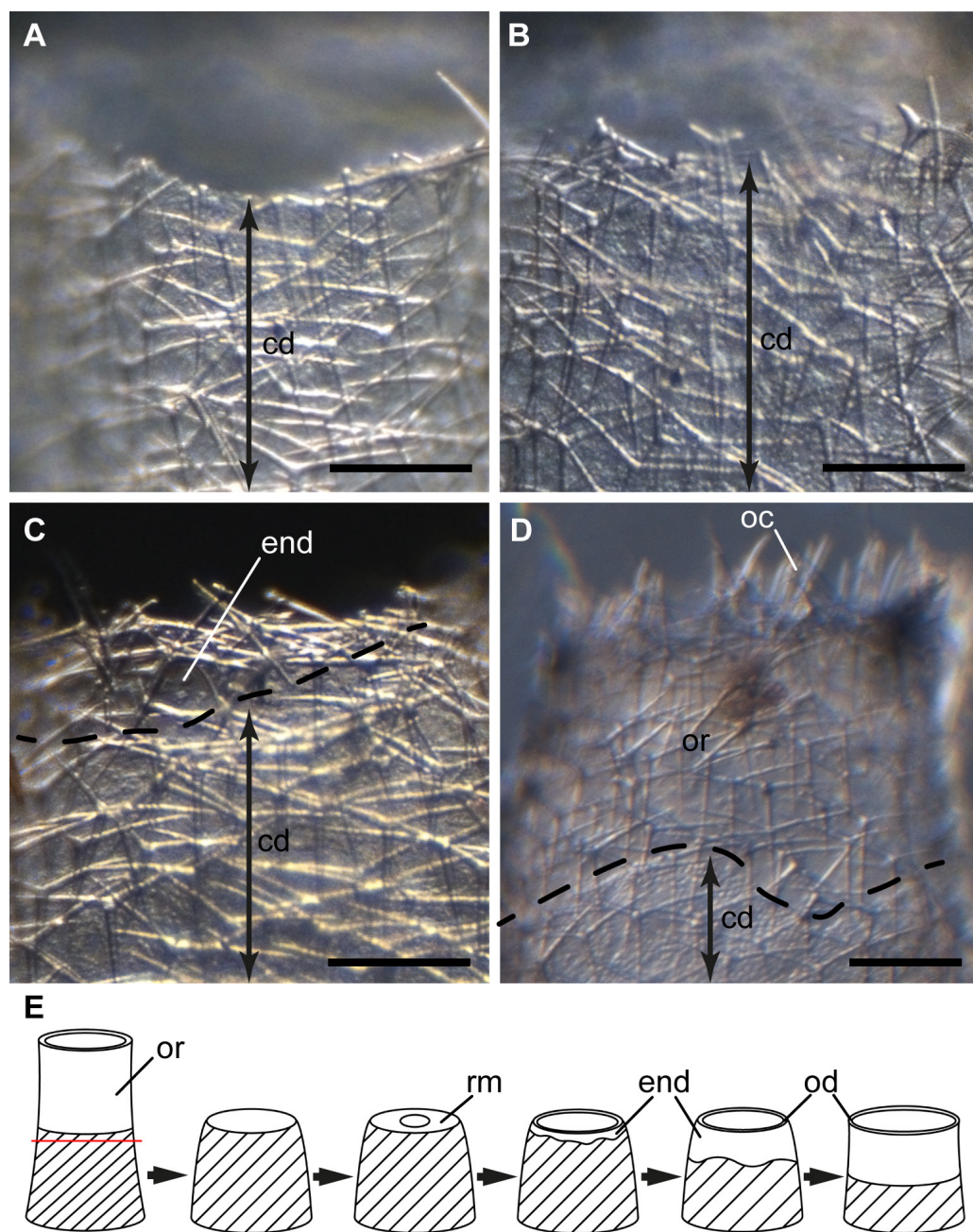


Fig. 11. Ocular rim regeneration after the amputation at the distal part of the oscular tube proximally to the choanoderm-endopinacoderm boundary, side view. A — 0 hours post-operation, B — 1 day post-operation (dpo), C — 2.5 dpo, D — 6 dpo, E — scheme of the main events during the oscular rim regeneration after the amputation (red line). Black dashed line marks the border between choanoderm and endopinacoderm. Abbreviations: cd — choanoderm, end — endopinacoderm, oc — oscular crown, od — oscular diaphragm, or — oscular rim, rm — regenerative membrane. Scale bars: A–D — 100 μ m.

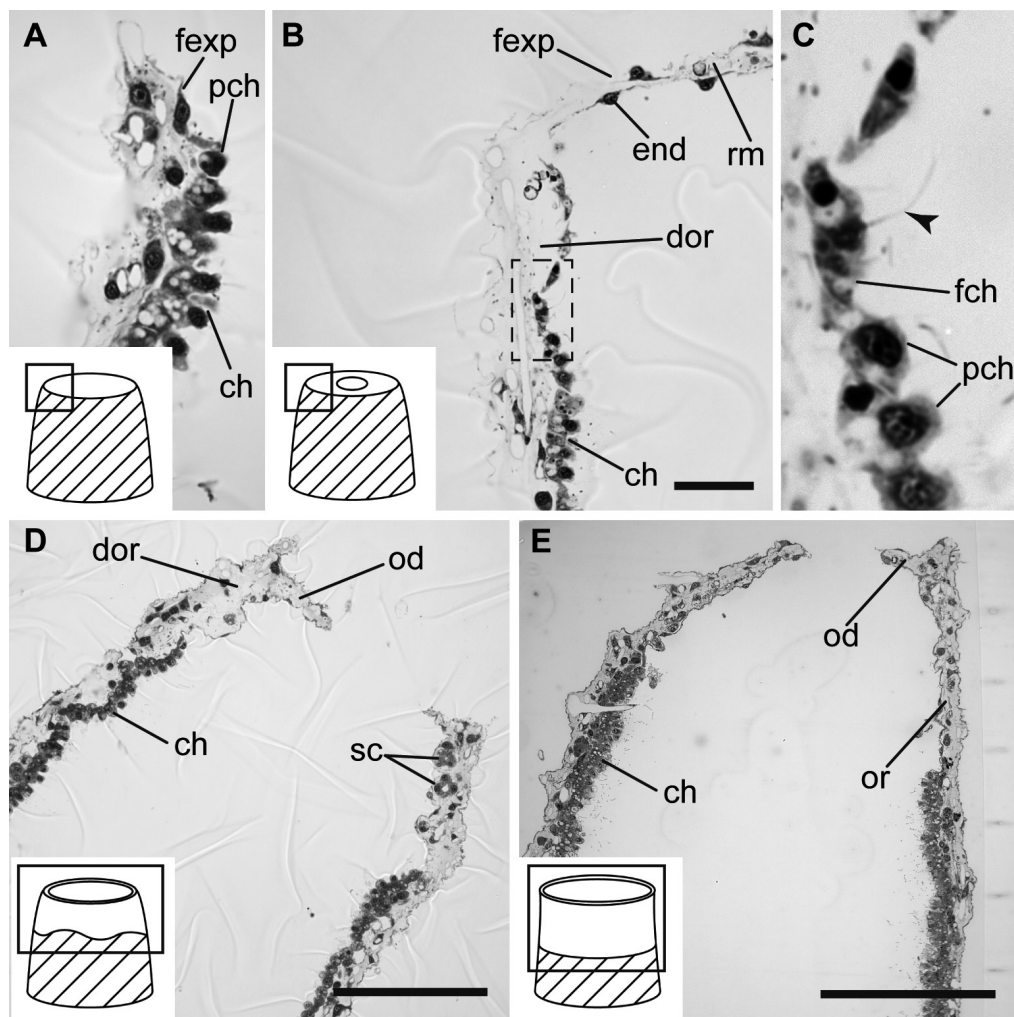


Fig. 12. Histology of the main stages of the oscular rim regeneration, longitudinal sections, light microscopy. A — 6 hours post-operation, B — 1 day post-operation (dpo), C — transdifferentiating choanocytes in the developing oscular rim (zoomed view of the zone in the black dashed square in B), black arrowheads mark flagella, D — 2 dpo, E — 4 dpo. Schemes denote the regeneration stage and approximate area of the histological studies.

Abbreviations: ch — choanocytes, dor — developing oscular rim, end — endopinacocytes, fch — flattened transdifferentiating choanocytes, fexp — flat exopinacocytes, od — oscular diaphragm, or — oscular rim, pch — pillow-shaped transdifferentiating choanocytes, rm — regenerative membrane, sc — sclerocytes. Scale bars: A–B — 20 μ m, B inset — 10 μ m, C, D — 100 μ m.

occurred near the regenerative membrane. More cells were involved in this process compared to the 6 hpo stage. The full range of the intermediate stages of the choanocyte transdifferentiation could be found in the developing oscular rim (Fig. 12C): the typical prismatic choanocytes were followed by the choanocytes of pillow-like shape; they, in turn, were followed by flat-

tened cells, occasionally retaining their flagella and microvilli collars. Finally, the typical flat endopinacocytes comprised the inner layer of the regenerative membrane. The transdifferentiation zone of the developing oscular rim was clearly distinguishable in histological sections but not during *in vivo* observations (Figs 11B, 12B).

In 22 sponges (88%), the regenerative membrane shrank and reduced its area at 1.5–2 days post-operation (dpo) (Fig. 10C). In the case of a complete membrane, the shrinkage ended with the appearance of an orifice. The shrinkage of the membrane was very pronounced in the majority of cases, and the regenerative membrane appeared as a very narrow ring after it (Fig. 10C). Essentially, the regenerative membrane directly transformed into the oscular diaphragm at this stage; later on, the membrane only occasionally changed its area and did not directly contribute to the further regenerative process (Fig. 10C–D). The endopinacocyte zone of the developing oscular rim became visible during *in vivo* observation as it extended proximally (Fig. 11C). It seems that this extension occurred mainly due to the further flattening of transdifferentiating choanocytes (involved in this process at 6 and 24 hpo), rather than due to transdifferentiation of additional cells: the endopinacocytes of the developing oscular rim obtained typical flat shape comparing to the flattened shape at the previous stage (Fig. 12B–C). The mesohyl of the developing oscular rim contained more cells, including groups of sclerocytes synthesizing new spicules (Fig. 12C).

Further gradual extension of the endopinacocyte zone in the proximal direction was the main process occurring after 2 dpo (Fig. 11D). This phase of the extension likely proceeded due to the involvement of more choanocytes in the transdifferentiation process (Fig. 12D). The regenerating oscular rim reached its final size at 3–5 dpo when extension of the endopinacocyte zone stopped (Figs 10D, 12E). This timepoint was considered the essential end of oscular regeneration.

In 3 sponges (12%), the regenerative membrane transformed into the oscular rim walls rather than into the oscular diaphragm. In this case, the membrane slightly changed its orientation, lifting the central part with the orifice above the excision plane. Numerous new spicules (tri- and tetractins) appeared in the transforming membrane. Under such a developmental trajectory, no additional transdifferentiation of choanocytes into endopinacocytes occurred to form the oscular rim.

Additionally, in 8 sponges, we observed the synthesis of diactins of the oscular crown at 4–6 dpo (Fig. 10D).

In the supplementary experimental series, the possible effect of the amputation site (Fig. 1B)

on the course of the oscular tube regeneration was preliminarily evaluated. The amputation at each site initiates a regenerative process, however the outcome of the process depends on the amputation site. When the oscular tube was amputated at its basal or middle part (Fig. 1B lines 1 and 2, respectively), the regeneration occurred through the formation of a fully sealed regenerative membrane growing perpendicular to the intact body walls and covering the tube lumen. During the next 3–4 days, the regenerative membrane transformed into an intact body wall, while oscular tubes lost functionality and became blind outgrowths of the cornus. In contrast, amputation of the oscular tube in its distal part (irrespective of whether the cut was made proximally (Fig. 1B line 3) or distally to the choanoderm–endopinacoderm boundary (Fig. 1B line 4)) initiated a regenerative process ending with the restoration of a functional oscular tube with oscular rim and diaphragm.

Discussion

Osculum in the regulation of the water flow

The constant pumping of water is the most prominent physiological activity of sponges, and their survival crucially depends on the efficacy of this process. The aquiferous system of sponges has numerous adaptations for the generation and regulation of the constant water flow. These adaptations include the general organization of the system that can induce an effective passive flow (Vogel, 1974, 1977, 1978), as well as structural characteristics of various elements of the system contributing to flow generation and regulation. The ability of the flagellated choanocytes to effectively generate a pressure drop driving water through the aquiferous system seems to rely on the fine features of the collar-flagellum apparatus (Weissenfels, 1992; Leys *et al.*, 2007, 2018; Mah *et al.*, 2014; Lavrov *et al.*, 2022; Larsen, Riisgård, 2025). In turn, the water-conducting elements of the aquiferous system (ostia, canals, and oscula) are involved in the regulation of the water flow by changing their diameter (Harrison, 1972; Weissenfels, 1980; Willenz, Van de Vyver, 1982; Eerkes-Medrano, Leys, 2006; Nickel *et al.*, 2011).

The osculum is essentially an exit for water from the aquiferous system; still, it also seems to contribute to the water flow regulation. First

of all, the osculum can change its effective diameter (Elliott, Leys, 2007; Kumala *et al.*, 2017). According to the scarce available data, some sponges can directly contract the walls of the oscular tube, while others utilize the oscular diaphragm to constrict the opening. Both mechanisms seem to be able to completely cease the water flow (Prosser *et al.*, 1962; Reiswig, 1971; Kumala *et al.*, 2017; Goldstein *et al.*, 2024).

To date, the presence of the oscular diaphragm has been reported only for a few demosponges (Bagby, 1966; Reiswig, 1971, 1975; Thiney, 1972; Thompson *et al.*, 1987; van Soest, Sten-toft, 1988; Maldonado, Young, 1998; Manconi *et al.*, 2006) and calcareous sponges (Minchin, 1892; Dendy, 1893; Jones, 1954, 1957, 1971; Ledger, 1974; Sarà *et al.*, 1974; Leininger *et al.*, 2014; Goldstein *et al.*, 2024), so the actual occurrence of this structure may be considerably underestimated. However, it is reasonable to assume that the mechanism for the regulation of the oscular opening diameter depends on the structural characteristics of the osculum. In the case of demosponges, it seems that species that have thin-walled oscular tubes with few or no skeletal elements (*i.e.*, oscular chimneys) can effectively contract the walls of the tube to reduce its opening (Elliott, Leys, 2007; Kumala *et al.*, 2017). In contrast, the crater-like oscula present in other species cannot be directly contracted as they lack chimneys and are surrounded by a mass of tissues with a pronounced skeleton. These demosponges are reported to have oscular diaphragms instead (Bagby, 1966; Reiswig, 1971; Maldonado, Young, 1998). In line with this, the need for the oscular diaphragm in calcareous sponges can be explained by the presence of a dense mesh of tri- and tetractine spicules, which may preclude the complete contraction of the oscular tube walls.

Despite the superficial resemblance, the oscular diaphragms in demosponges and calcareous sponges seem to differ anatomically. In all studied calcareous sponges, the oscular diaphragm represents a delicate two-layered structure built by flat endopinacocytes (Minchin, 1892; Dendy, 1893; Jones, 1954, 1957, 1971; Ledger, 1974; Sarà *et al.*, 1974; Leininger *et al.*, 2014; Goldstein *et al.*, 2024). Here, we reported the presence of a supracellular actomyosin cable running in the endopinacocytes along the inner edge of the diaphragm. The cable is composed of the actin

immunoreactive with anti- β -actin antibodies. This actin also occurs in the cell cortex and protrusions (Skorentseva *et al.*, 2023). The actin immunoreactive with anti- γ -actin antibodies does not form any apparent structures in most cells of *L. corallorrhiza* except for the particular mesohyl cells with yet unknown function, in which it is the main actin isoform. These data suggest that sponges have several actin paralogs with different functions similar to other metazoans (Wagner *et al.*, 2002; Dugina *et al.*, 2009).

Similar to the actomyosin cable of the regenerative membrane in calcareous sponges (Skorentseva *et al.*, 2023), the diaphragm cable is likely contractile, as it shows anti-phosphorylated myosin light chain 2 immunoreactivity. The force generated by the cable could potentially pull the edges of the diaphragm together, thus increasing the area of the diaphragm and closing the oscular opening.

In contrast, the oscular diaphragm in demosponges appears to be a sturdy structure (Bagby, 1966; Maldonado, Young, 1998). The histological and ultrastructural studies of oscula in *Tedania ignis* (Duchassaing et Michelotti, 1864) and *Hippospongia communis* (Lamarck, 1814) showed that its diaphragm has developed mesohyl with numerous cells. The myocytes (potentially contractile cells) represent a considerable part of the mesohyl cells in the diaphragm and constitute concentric layers around its inner edge (Bagby, 1966; Thiney, 1972). Such an organization implies that the diaphragm in demosponges increases its area also due to actomyosin contractility, but is conveyed by the layers of the mesohyl myocytes.

The *in situ* observations on filtration in *Tectitethya crypta* (de Laubenfels, 1949) showed that the closure of the osculum orifice by the diaphragm occurs only after a decrease in exhalant water velocity (Reiswig, 1971). This fact possibly implies that the upstream components of the aquiferous system have the main control over the water flow, while changes in the oscular effective diameter control the final acceleration of the exhalant water. The appropriate speed of the exhalant jet guarantees that the filtered water is expelled far enough not to be refiltered by the sponge.

Proper regulation of the effective diameter should rely on sensing ability. The sensing structures were shown for the freshwater demo-

sponge *E. muelleri*: the endopinacocytes lining the oscular tube bear pairs of immotile sensory primary cilia (Ludeman *et al.*, 2014). We found no obvious sensory structures in the oscular rim of *L. corallorrhiza*, yet it is still possible that the endopinacocyte could directly sense the water flow shear stress by mechanosensory channels in its cytoplasmic membrane similar to endothelial cells of mammals (Cheng *et al.*, 2025).

Transcriptional signature of the oscular rim region

The conducted analysis showed that different regions of the *L. corallorrhiza* body are characterized by certain transcriptional landscapes. The transcriptional landscapes of cornus tubes and diverticula share numerous transcripts. Such transcriptional similarity is in concordance with the general structural and functional similarity of these regions. In contrast, the oscular rim has a considerably distinct transcriptional profile that is rich in signaling pathway components, *e.g.*, Wnt and TGF- β pathways. This fact is corroborated by both the enrichment analysis and the manual inspection of the expression of individual transcripts related to the respective pathways.

Wnt and TGF- β signaling pathways are important components of the intercellular communication system in metazoans. These pathways regulate numerous aspects of cellular behavior, *e.g.*, proliferation, migration, differentiation, and apoptosis. Such a wide control over cellular behavior allows Wnt and TGF- β pathways to play an essential role in the histogenesis and body patterning during embryogenesis, adult life, and various restorative processes in all animals — from sponges to vertebrates (Johnson, Newfeld, 2002; Petersen, Reddien, 2009; Weiss, Attisano, 2013; Hayat *et al.*, 2022).

According to our analysis, *L. corallorrhiza* has a complete set of the core components of both pathways and a rich repertoire of Wnt (20 paralogs) and TGF- β (15 paralogs) ligands, similarly to *S. ciliatum*, having 21 and 22 paralogs, respectively (Leininger *et al.*, 2014). At least for the Wnt pathway, the expansion of the ligand number seems to be a specific feature of calcareous sponge lineage, as both demosponges and homoscleromorphs have fewer Wnt ligands (up to eight) (Adamska *et al.*, 2010; Leininger *et al.*, 2014; Borisenko *et al.*, 2016), while hexactinellids completely lack these

components (Schenkelaars *et al.*, 2017; Windsor Reid *et al.*, 2018; Santini *et al.*, 2023). The number of TGF- β ligands in calcareous sponges is also considerably higher in comparison with demosponges and hexactinellids (Schenkelaars *et al.*, 2017; Pozzolini *et al.*, 2019), but data for homoscleromorphs are still lacking. Surprisingly, we identified 13 TGF- β receptors in the *L. corallorrhiza* transcriptome, which also looks unusual as demosponges and hexactinellids seem to have fewer paralogs (Schenkelaars *et al.*, 2017). Moreover, the relatively low number of TGF- β receptors is characteristic of Metazoa with the exception of the vertebrates due to lineage-specific genome duplications (Huminiński *et al.*, 2009; Pang *et al.*, 2011).

Our differential expression analysis has shown the upregulation of numerous Wnt and TGF- β ligands in the oscular rim of *L. corallorrhiza*, but not the respective receptor and intracellular messengers. This is highly concordant with the results of *in situ* hybridization in *S. ciliatum*, which showed the osculum-specific expression pattern for the ligands and more uniform expression of other components of Wnt and TGF- β signaling pathways (Leininger *et al.*, 2014). Notably, *L. corallorrhiza* shows overexpression of the very same Wnt and TGF- β orthologs that were expressed in the osculum of *S. ciliatum* (*i.e.*, *WntI*, *WntJ*, *WntR*, or *TGFbN*, *TGFbU*, and *TGFbH*). The osculum-specific expression of some Wnt ligands is also characteristic of the demosponge *H. dujardinii* (Borisenko *et al.*, 2016).

As Wnt and TGF- β are well known for regulating body patterning in a broad range of metazoans (Johnson, Newfeld, 2002; Petersen, Reddien, 2009), we would like to hypothesize that the expression of the ligands of these pathways in the oscular rim of *L. corallorrhiza* could also be related to the axial patterning. Surely, further studies are needed to verify the involvement of the identified transcripts in the establishment of the oscular axis in this species. The establishment of the non-redundant list of the patterning transcripts is also a prospective issue, as the differential expression analysis is limited to describing the association between transcript expression and body parts — rather than directly implying the functional role of transcripts.

The unique transcriptomic signature of the osculum, particularly the expression of various

signaling pathway components, may be important in specifying osculum identity and contribute to development and maintaining the apico-basal axis in the adults of other sponge species. Indeed, treating the developing (hatching from the gemmule) freshwater demosponge *Ephydatia muelleri* with pharmacological activators of the canonical Wnt pathway (LiCl and Alsterpaullone), as well as the RNAi downregulation of Gsk-3, caused the formation of the ectopic oscula (Windsor, Leys, 2010; Windsor Reid *et al.*, 2018). Such an increase in the osculum number could be interpreted as the apico-basal axis multiplication (Windsor, Leys, 2010). On the contrary, the inhibitors of this pathway (niclosamide and quercetin) blocked the formation of the oscula during hatching of another freshwater demosponge, *Spongilla lacustris*. This treatment also prevents the osculum regeneration in the adult individuals (Windsor Reid *et al.*, 2018). Finally, the osculum of freshwater sponges has the inductive properties, as its transplantation leads to the reorganization of the exhalant canal system in the host sponge (Windsor, Leys, 2010).

Intriguingly, the transplantation of the osculum in *S. ciliatum* did not lead to any reorganization of the body, possibly because *Sycon* are monosculum sponges with effective mechanisms for preserving a sole body axis (Leininger *et al.*, 2014). In this sense, *L. corallorrhiza* represents a promising model species for future studies of the apico-basal axis specification, the osculum inductive properties, and the involved molecular regulation in calcareous sponges.

The regeneration of the oscular tube

In general, the regeneration of the oscular tube in *L. corallorrhiza* is similar to the other regenerative processes described for this (Ereskovsky *et al.*, 2017; Lavrov *et al.*, 2018; Skorentseva *et al.*, 2023, 2025) and other calcareous sponges (Jones, 1957; Korotkova, 1961a, b, 1962; Caglar *et al.*, 2021). It is a rapid process ending in 3 to 5 days after the amputation and occurring through the epithelial morphogenesis, cell shape changes, and transdifferentiations (Lavrov *et al.*, 2018; Skorentseva *et al.*, 2023, 2025).

The main morphogenetic mechanisms and the early stages are the same as during body wall regeneration (Lavrov *et al.*, 2018; Skorentseva *et al.*, 2023, 2025). The regeneration begins with the isolation of the mesohyl by flattening

exopinacocytes, which come into contact with the choanocytes. This event is followed by the formation of the regenerative membrane through ongoing flattening of the exopinacocytes and transdifferentiation of the prismatic flagellated choanocytes into the flat aflagellated endopinacocytes. However, during the oscular rim restoration, the regenerative membrane remains incomplete in most of the regenerating sponges and does not transform into the body wall. Instead, it decreases its area and becomes the oscular diaphragm. Under this scenario, the actomyosin cable (Skorentseva *et al.*, 2023), which drives the growth of the regenerative membrane, directly transforms into the cable of the diaphragm.

At the cellular level, the oscular rim regeneration differs from the regular body wall regeneration in the fate of the endopinacocytes. During body wall regeneration, only choanocytes required to build a fully sealed regenerative membrane undergo transdifferentiation into the endopinacocytes (Lavrov *et al.*, 2018). In contrast, the oscular rim regeneration includes the additional transdifferentiation of the choanocytes: this process spans the intact walls beneath the regenerative membrane and leads to the formation of the oscular rim walls with the inner endopinacocyte lining. Moreover, endopinacocytes represent a transient state during body wall regeneration, as they redifferentiate back into choanocytes in the course of the transformation of the regenerative membrane into intact body wall (Lavrov *et al.*, 2018). During oscular rim regeneration, the endopinacocytes represent the final stage of cell differentiation.

The preliminary experiments suggest that the course of the regeneration could depend on the amputation site. These distinct regenerative trajectories could be underpinned by differences in transcriptomic profiles of the proximal/middle and distal regions of the oscular tube. The proximal and middle parts likely share gene expression patterns with regular cornus tubes. In contrast, regeneration after the distal amputation occurs in the vicinity of the oscular rim, within a different signaling context, characterized by increased activity of the Wnt and TGF β pathways. This signaling context may guide the alternative regeneration course where the regenerative membrane does not fully seal but instead transforms into the oscular diaphragm rather than forming a regular body wall.

Conclusion

The oscular rim of *L. corallorrhiza* is distinguished from other parts of the sponge body in its organization: it has the endopinacocyte internal lining and is equipped with the oscular diaphragm. It seems that the oscular diaphragm can change its area, thereby controlling the size of the oscular opening. These changes are likely driven by the contractions of the actomyosin cable running along its inner edge.

The oscular rim is also considerably different in its transcriptomic profile from the regular cormus tubes and diverticula. In particular, the differences concern the increased expression of the Wnt and TGF- β ligands in the oscular rim.

The regeneration of the oscular rim in *L. corallorrhiza* represents a typical regenerative process for calcareous sponges, characterized by rapid restoration based on epithelial morphogenesis and morphallactic rearrangements of the intact tissues near the wound. However, the oscular rim regeneration is distinct from the regular body wall regeneration in terms of cell fate decisions and the transformations of the regenerative membrane. The morphallactic nature of the process and the necessity to restore control over the effective size of the oscular opening determine the fast rate of the regeneration.

Supplementary information

Supplementary Data. Supplementary tables S1–S4 and figures S1–S4.

Table S1. List of the samples used for transcriptomic studies.

Table S2. Total number of read pairs in each library used in the *de novo* assembly.

Table S3. Summary statistics of the *Leucosolenia corallorrhiza* transcriptome *de novo* assembly.

Table S4. List of the components of Wnt and Tgf β signaling pathways recovered from *Leucosolenia corallorrhiza* transcriptome and log-fold changes in their expression across different regions of the sponge body.

Fig. S1. Sample clustering across different body regions of *Leucosolenia corallorrhiza*.

Fig. S2. Venn's diagrams of 1106 differentially expressed transcripts (DETs).

Fig. S3. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichments of up- and downregulated non-unique differentially expressed transcripts (DETs).

Fig. S4. Volcano plot of differentially expressed transcripts (DETs).

Conflict of interests

The authors declare that they have no conflict of interest.

Data availability statement

Transcriptome, its annotation, and table with transcript counts are uploaded to the Mendeley Data repository (<https://data.mendeley.com/datasets/g44jm9yts4/1>). Raw reads are available at the SRA database under accession nos. SRX32678815–SRX32678806 (BioProject accession no.: PRJNA1443665).

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