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Insights into ophiuroid coelomocytes: diversity and potential function in coelenterazine transfer in the bioluminescent brittle star *Amphiura filiformis*

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Abstract: The brittle star *Amphiura filiformis* is a keystone species within the burrowing fauna assemblage in the fine, muddy sediments along the European coasts. With high regeneration and bioluminescence capabilities, it species emerged as a model species for many studies. However, only a few studies have investigated the cellular components of its coelomic fluid, which play roles in immunity and metabolite transport. The present study provides a detailed morphological description of the different perivisceral coelomocytes found in the brittle star *A. filiformis*, including the cell proportions and concentrations. For this purpose, we present a simple method for collecting coelomic circulating cells from small echinoderms with a limited volume of perivisceral fluid, using a needle to inject artificial seawater in the perivisceral coelom followed by an immediate recovery of the fluid containing coelomocytes. Six cell types were identified based on optical and scanning electron microscopy. Morphological similarities with coelomocytes in other brittle star and echinoderm species are discussed. Moreover, the involvement of this coelomic fluid in the transfer of the luminous substrate, coelenterazine, from the stomach content to photogenic sites is investigated, adding a step towards the understanding of this brittle star bioluminescence.

Résumé : *Nouveaux aperçus des coelomocytes d'ophiures : de leur diversité cellulaire à leur rôle potentiel dans le transport de coelentérazine chez l'ophiure bioluminescente* Amphiura filiformis**.** L'ophiure *Amphiura filiformis* est une espèce clé des communautés benthiques, vivant dans les sédiments vaseux le long des côtes européennes. Grâce à ses capacités de régénération et de bioluminescense, cette espèce est devenue une espèce modèle pour un grand nombre d'études. Néanmoins, peu d'intérêt a été porté concernant les cellules circulantes présentes dans le fluide cœlomique, jouant un rôle dans l'immunité et le transport de métabolites. Cette étude fournit une description morphologique des différents types de cœlomocytes périviscéraux rencontrés chez

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l'ophiure *A. filiformis*, incluant les proportions et les concentrations cellulaires. À cette fin, nous présentons une méthode simple pour collecter les cellules circulantes du liquide cœlomique chez un échinoderme relativement petit et disposant d'un volume limité de fluide périviscérale, en utilisant une aiguille pour injecter de l'eau de mer artificielle dans le cœlome périviscéral, suivi d'une récupération immédiate du fluide contenant les cœlomocytes. Sur la base de critères morphologiques obtenus à l'aide de microscopes optique et électronique à balayage, six types cellulaires différents ont pu être distingués. Les similitudes morphologiques avec les coelomocytes d'autres espèces d'ophiures et d'échinodermes sont discutées. Finalement, l'implication de ce liquide cœlomique dans le transfert du substrat lumineux, la coelentérazine, depuis le contenu stomacal vers les sites photogéniques est investiguée, permettant une meilleure compréhension du processus de la bioluminescence chez cette espèce d'ophiure.

Keywords: Echinodermata ● Immune cells ● Immunity ● Electron Microscopy ● Coelenterazine ● Bioluminescence

Introduction

The brittle star *Amphiura filiformis* (O.F. Müller, 1776) is considered a keystone species in soft bottom areas within the European marine ecosystems (Duineveld & Van Noort, 1986; Rosenberg & Lundberg, 2004). The population dynamics and life cycle of this burrowing species have been extensively studied for decades (O'Connor et al., 1983; Sköld et al., 1994; Rosenberg & Lundberg, 2004). The exceptional population density of *A. filiformis* makes it an essential actor in the sediment bioturbation and oxygenation processes. Besides presenting a filter-feeding nutrition mode, the species actively captures and recycles the suspended matter. Additionally, it occupies a crucial position within the trophic chain as a prey for several species, such as *Limanda limanda* and *Nephrops norvegicus* (Sköld et al., 1994).

A. filiformis is renowned for its ability to emit blue light (λ max = 475 nm) at the level of the arm spines and tips (Delroisse et al., 2017a). This bioluminescent species relies on a coelenterazine-dependent luciferase expressed in specialized photocyte cells (Delroisse et al., 2017b). Besides, the bioluminescence of *A. filiformis* depends on the acquisition of the luciferin substrate, coelenterazine, through its diet (Mallefet et al., 2020; Coubris et al., 2024). Brittle stars kept in captivity and fed with a diet exempted from coelenterazine lose their luminous capacity; however, they regain their ability to emit blue flashes when provided with an exogenous supply of coelenterazine (Mallefet et al., 2020; Coubris et al., 2024). Light production was also demonstrated to be mainly elicited through calcium-related cholinergic control (Dewael & Mallefet, 2002; Vanderlinden et al., 2010; Mallefet et al., 2020).

The ophiuroid *A. filiformis* also serves as a model species for regeneration research (*e.g.*, Thorndyke et al., 2001; Bannister et al., 2005; Dupont & Thorndyke, 2006; Czarkwiani et al., 2016). Recently, the chromosome-scale genome of the species was published, enabling the in-depth investigation of molecular mechanisms underlying its exceptional regenerative capacities (Parey et al., 2024). The authors highlighted the early expression of numerous immune genes during the wound-healing process. In echinoderms, the highest expression of immune genes is observed in coelomocytes – cells that freely circulate in the body fluids and constitute the primary actors of immunity (Smith et al., 2018). While the diversity of coelomocytes has been extensively studied in sea urchins, sea cucumbers, and sea stars (Smith et al., 2018 & 2019; Jobson et al., 2022), research on the coelomocytes of brittle stars and feather stars remains limited (Smith et al., 2018), likely due to challenges in acquiring an adequate amount of cellular material as these non-fleshy organisms only present a small amount of coelomic fluid. The first attempts to describe brittle star coelomocytes were carried out in the context of the first research on echinoderm coelomocytes around the beginning of the 20th century (*e.g.*, Cuénot, 1888; Kindred, 1924; Boolootian & Giese, 1958). Other records of ophiuroids coelomocytes are often limited to highlighting their presence in various tissues (*e.g.*, Biressi et al., 2010; Giorgio et al., 2015). To our knowledge, only Boolootian & Giese (1962) and Jobson et al. (2022) provide pictures of circulating fresh cells (*i.e.,* not fixed cells). Some of these studies have demonstrated their presence in regenerative arms, suggesting that these cells play an essential role in the regeneration process of brittle stars (Biressi et al., 2010; Giorgio et al., 2015).

The aim of the present study is (*i*) to study the morphological diversity of circulating coelomocytes

found in the perivisceral coelomic fluid of *A. filiformis* and (*ii*) to investigate the role of this fluid in facilitating the transfer of the coelenterazine substrate, which is essential for light production.

Material and Methods

Organisms sampling

Adult brittle stars were collected with an Eckman grab at a depth of 30-40 m in the Gullmarsfjord near the Kristineberg Marine Research Station (University of Gothenburg, Fiskebäckskil, Sweden) in August 2023. The *A. filiformis* individuals were maintained in an aquarium with running seawater pumped directly from the adjacent fjord (12°C, 35 salinity, pH 8.2).

Coelomic fluid sampling

The animals were anesthetized by immersion in 3.5% $MgCl_2$ solution for 3 minutes (Dewael & Mallefet, 2002). The perivisceral coelomic fluid extraction was conducted using either the injection of 50-100 µL of two different types of artificial seawater: (*i*) calcium magnesium-free artificial seawater (CMFASW: 460 mM NaCl, 10.7 mM KCl, 7 mM Na_2SO_4, 2.4 mM $NaHCO_3$, 20 mM HEPES, pH = 7.4) to avoid coelomocytes clogging, allowing a proper morphological description (Smith et al., 2019), or (*ii*) artificial seawater (ASW: 400 mM NaCl, 9.6 mM KCl, 52.3 mM $MgCl_2$, 9.9 mM $CaCl_2$, 27.7 mM Na_2SO_4, 20 mM Tris; pH 8.2) for the luminometric assays (Coubris et al., 2024). After the injection, a slightly higher volume of fluid was sucked out immediately without removing the needle used for the injection. Injections and punctures were performed between two arms along the side of the central disk (Fig. 1). The needle (Hypodermic Microlance, 30G ½) was inserted obliquely at least 2 mm into the aboral surface to minimize the risk of collecting contaminating cells from other organs such as the stomach and bursae containing gonads, following the procedure of Jobson et al. (2022) (Fig. 1). It should be highlighted that the injection step is essential prior to the puncture, as several attempts failed to collect any perivisceral fluid without a preceding disk injection.

Light microscopy and cell count

The freshly collected cells from three individuals were examined and photographed under a microscope (Axio Scope A1, Zeiss) to distinguish various cell morphotypes. To determine the concentration and proportion of these cell morphotypes, 10 µL of the collected fluid was loaded onto a hemocytometer, and the cells were counted within the volume corresponding to 0.1 mm^3. This concentration was then converted into cells per milliliter of fluid. The proportion of the different cell types was then calculated as the ratio between the number of cells of the specific morphotype and the total number of cells. When obvious non-coelomocyte cells were also detected (*i.e.,* spermatozoa), they were not considered in the ratios.

Scanning electron microscopy

Scanning electron microscopy was used to obtain a detailed morphological visualization of the coelomic fluid cells in *A. filiformis.* Cells were prepared following the protocol of Caulier et al. (2024). Briefly, 30-50 µL of fluid from three individuals was pipetted onto a precut slide and incubated for 30 minutes to enable cells adhesion to the slide before their fixation in a fixative solution for electron microscopy (3% glutaraldehyde in cacodylate buffer). After successive rinses in cold phosphate-buffered saline and distilled water, the slides were dehydrated through a series of baths in ethanol solutions of increasing concentrations, chemically dried in sequential hexamethyldisilazane (HMDS) concentrations, and coated with a mixture of gold/palladium (40/60) in a sputter coater. Eventually, slides were visualized and photographed via a JEOL JSM-7200F scanning electron microscope.

Coelenterazine detection

For coelenterazine detection, coelomic fluid extracted on either male or female with ASW was purified with a 20 µm cell strainer (pluriStrainer) to eliminate the cellular debris and limit the contamination from the brittle star tissues. The extracted fluid was then mixed with an equal volume of cold methanol (-20°C; CH_3OH 99.8%; Merck, Darmstadt, Germany) (1:1 dilution) in an Eppendorf tube. The mixture was then crushed using a micro-pestle (Micropestles, 211-2100, VWR International, Leuven, Belgium). Subsequently, 5 µL of the methanolic extract was transferred into a tube containing 195 µL of Tris buffer (20 mM Tris, 0.5 M NaCl; pH 7.4) and placed in the FB12 tube luminometer (Tirtertek-Berthold, Pforzheim, Germany). Afterward, 200 µL of *Renilla* luciferase solution containing 4 µL of *Renilla* luciferase (Prolume Ltd., working dilution of 0.2 g L^{-1} in a Tris-HCl buffer 10 mM, NaCl 0.5 M, BSA 1%; pH 7.4), diluted in 196 µL of Tris buffer, was injected into the luminometer tube. The luminometer was kept in the dark room and calibrated using a standard 470 nm light source (Beta light, Saunders Technology, Hayes, UK). Light responses (*i.e.,* Ltot) were recorded using FB12-Sirius PC Software (Tirtertek-Berthold) to calculate the coelenterazine content per µL of coelomic

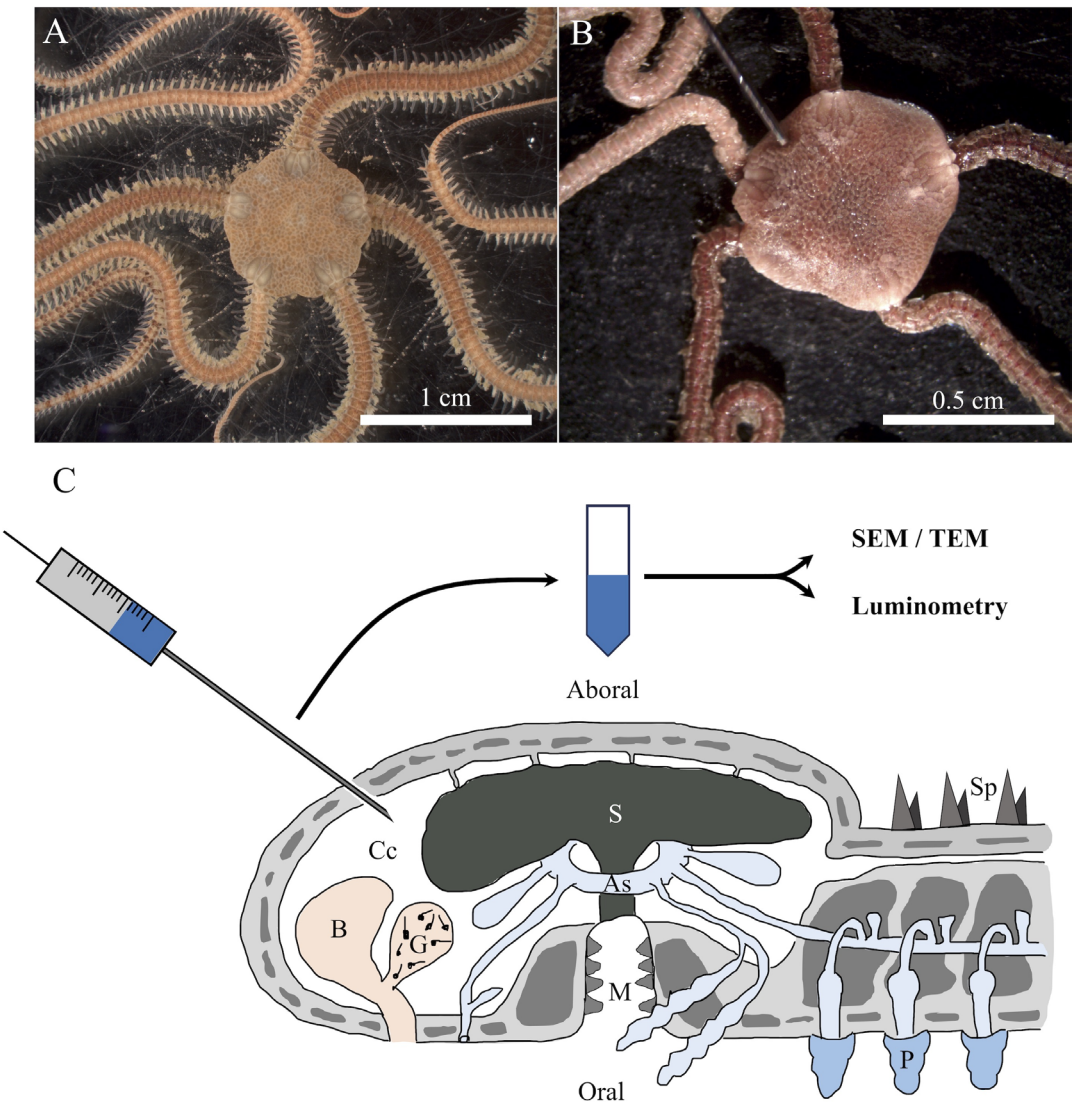

Figure 1. *Amphiura filiformis.* Sampling of perivisceral fluid. **A.** Aboral view of the brittle star. **B.** Aboral view of the brittle star showing coelomic punction with the needle insertion (green arrowhead). **C.** Scheme of the brittle star disk anatomy showing the needle insertion. As: ambulacral (*i.e,* hydrovascular) system, B: bursae, Cc: perivisceral coelomic cavity, M: mouth, P: podia, S: stomach, Sp: spine.

fluid sampled (ng μL^{-1}). This calculation assumed that 1 ng of pure coelenterazine coupled with *Renilla* luciferase emits 2.52 x 1011 photons (Shimomura, 2012). To ensure the accuracy and reliability of the measurement, a control experiment was performed by replacing coelomic fluid with ASW.

Results

Coelomocyte type morphology, concentration, and proportion

An average of 1.17 ± 0.89 10^6 cells mL^{-1} was collected in the coelomic cavity of three *A. filiformis* specimens (excluding spermatozoa). Given that the volume collected after injection of 100 µL of CMFSW ranged between 150 and 200 µL, the maximum number of cells that could be obtained was 2.4 10^5.

Thanks to the microscopic observations, seven distinct cellular types were characterized in the collected coelomic fluid: small corpuscles, undifferentiated cells, spherule cells, red corpuscles, amoebocytes, pigmented amoebocytes, and spermatozoa (Fig. 2A-G). These free coelomocyte types exhibited varying proportions, morphologies, and size ranges, described below in descending order of abundance.

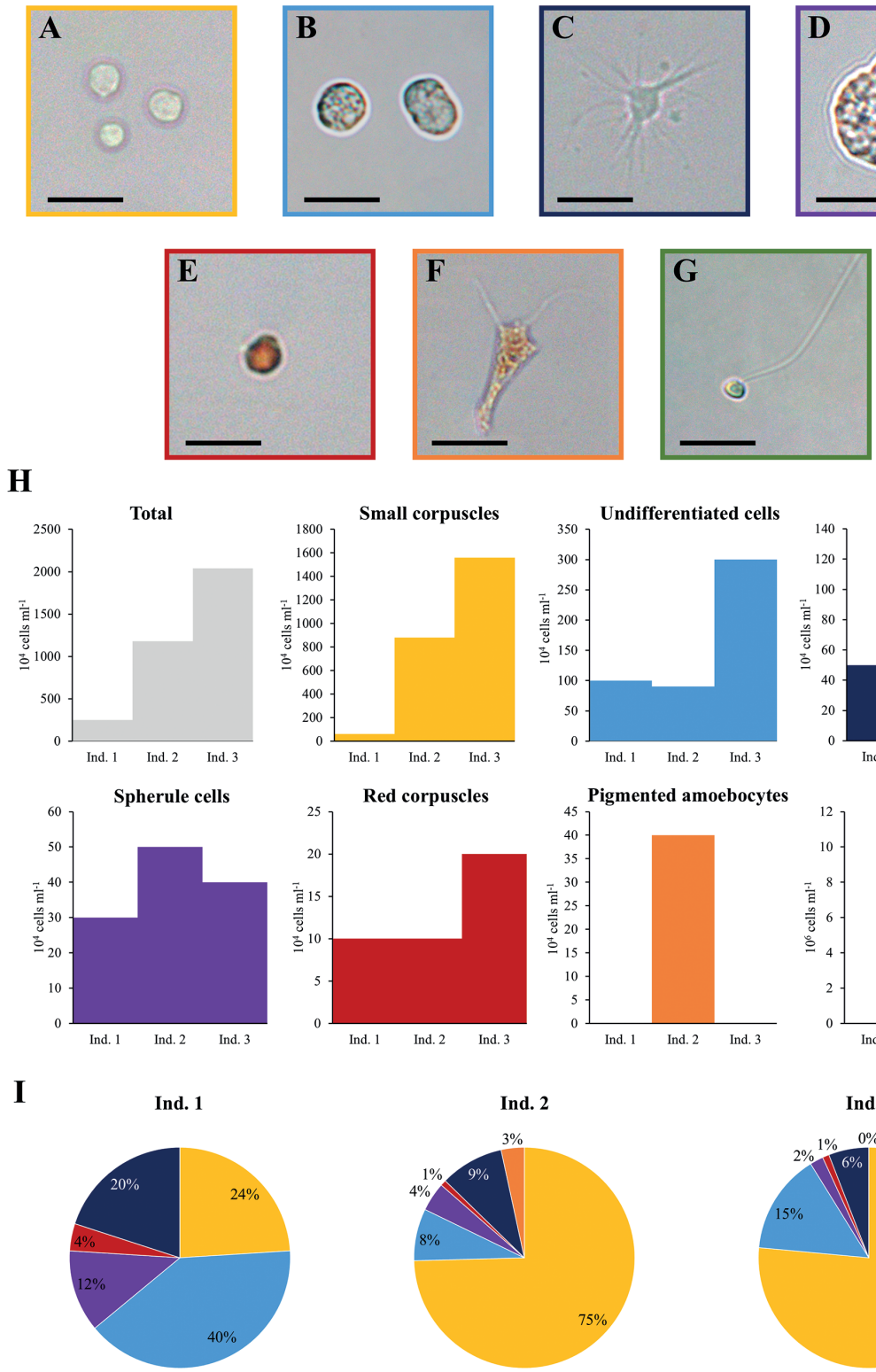

Figure 2. *Amphiura filiformis*. Diversity, concentration, and proportion of cellular elements found in the perivisceral fluid. **A-G.** Different cellular elements (*i.e.,* coelomocytes and spermatozoa). **A.** Small corpuscles. **B.** Undifferentiated cells. **C.** Amoebocyte. **D.** Spherule cell. **E.** Red corpuscle. **F.** Pigmented amoebocyte. **G.** Spermatozoon (scale bars represent 6.5 µm in A, 8 µm in B, 12.5 µm in C, 6.5 µm in D, 10 µm in E, 11 µm in F, 15 µm in G). **H.** Cell concentration in three individuals of *A. filiformis*. **I.** Cell proportion of the different coelomocyte types in the same three individuals (colors correspond to the concentration graph in H; percentages are included in the graph; spermatozoa were not considered in calculating coelomocyte proportions).

Table 1. *Amphiura filiformis.* Comparison of coelomocytes with coelomocyte types of other ophiuroid species and other echinoderm classes. The first two columns show the cell types described in this study with their schematic representations and names. The table shows for each coelomocyte type: its size (* means including pseudopods), and its range in proportion (prop. – proportion). The second column includes different synonyms that were found in the literature related to ophiuroid coelomocytes and the species for which similar cell types were reported with their specific reference (N.R. - no reference; see Table S1 for further detail on ophiuroid coelomocyte literature). The third column lists analogies of morphology with coelomocyte types in other echinoderm classes (legend corresponds to the class in question: CR - crinoid; ST - sea star; SU - sea urchin; SC - sea cucumber) with an example of a representative species in which the cell type was described with the associated references.

This study				Ophiuroid literature		Other echinoderm class literature	
Cell types		Size (µm)	Prop. (%)	Synonyms in ophiuroids	Species and associated references	Analogies with other echinoderm classes	Species and associated references
	Small corpuscles	1.5 - 5	25 - 75	Small spherical corpuscles	*Gorgonocephalus* sp. (Boolootian and Giese, 1958)	Minute corpuscles (SC)	*Apostichopus japonicus* (Xing et al., 2008)
	Undifferentiated cells	4 - 7	10 - 40	Undifferentiated coelomocytes, Coelomocytes, Dedifferentiated cells	*Ophioderma longicaudum* (Biressi et al., 2010), *Amphiura filiformis* (Biressi et al., 2010), *Ophioplocus januarii* (Di Giorgio et al., 2015), *Gorgonocephalus arcticus* (Jobson et al., 2022), *Ophiopholis aculeata* (Jobson et al., 2022)	Progenitor cells (ST, SC), Small round cells (SC), Lymphoid cells (SC), Undifferentiated cells (CR)	*Apostichopus japonicus* (Xing et al., 2008; Taguchi et al., 2016), *Asterias rubens* (Sharlaimova et al., 2021), *Holothuria scabra* (Wambreuse et al., 2023), *Antedon mediterranea* (Di Benedetto et al., 2014)
	Amoebocytes	20 - 30*	5 - 20	Leucocytes, Amoebocytes, Phagocytes	*Ophiactis* sp. (Cuénot, 1891), *Ophiopholis aculeata* (Kindred, 1924), *Ophioderma longicaudum* (Biressi et al., 2010), *Amphiura filiformis* (Biressi et al., 2010), *Amphipholis kochii* larvae (Gliznutsa and Dautov, 2011), *Ophioplocus januarii* (Di Giorgio et al., 2015), *Gorgonocephalus arcticus* (Jobson et al., 2022), *Ophiopholis aculeata* (Jobson et al., 2022)	Phagocytes (SC, ST, SU, CR) Amoebocytes (CR)	*Apostichopus japonicus* (Xing et al., 2008), *Asterias rubens* (Pinsino et al., 2007), *Strongylocentrotus purpuratus* (Majeske et al., 2013), *Antedon mediterranea* (Di Benedetto et al., 2014)
	Spherule cells	10 - 12	2 - 12	Granular ameboid corpuscles, Spherul cells, Amcoebocyte with colorless spherules, Granular cells, Granulocytes,	*Ophiopholis aculeata* (Cuénot 1888), *Ophiactis* sp. (Cuénot, 1891), *Ophiopholis aculeata* (Kindred, 1924), *Ophionereis reticulata* (Andrew, 1962), *Ophioplocus januarii* (Di Giorgio et al., 2015), *Gorgonocephalus arcticus* (Jobson et al., 2022)	Spherulocytes (ST, SC), Colorless spherule cells (SU), Granulocytes (CR)	*Holothuria* spp. (Queiroz et al., 2022a; Wambreuse et al., 2023), *Asterias rubens* (Pinsino et al., 2007), *Paracentrotus* spp. (Queiroz et al., 2022b), *Antedon mediterranea* (Di Benedetto et al., 2014)
	Red corpuscles	4 - 6	< 5	Red blood cells, Red corpuscles	*Ophiactis virens* (Foettinger, 1880), *Ophiactis* sp. (Cuénot, 1891)	Hemocytes (SC, ST)	*Paracaudina chiliensis* (Baker and Terwilliger, 1993), *Cucumaria frondosa* (Caulier et al., 2020), *Asterias rubens* (Pinsino et al., 2007)
	Pigmented amoebocytes	10 - 25*	< 5	N.R.	N.R.	Red spherule cells (SU); Probably another pigment	*Paracentrotus lividus* (Coates et al., 2018), *Strongylocentrotus droebachiensis* (Hira et al., 2020)

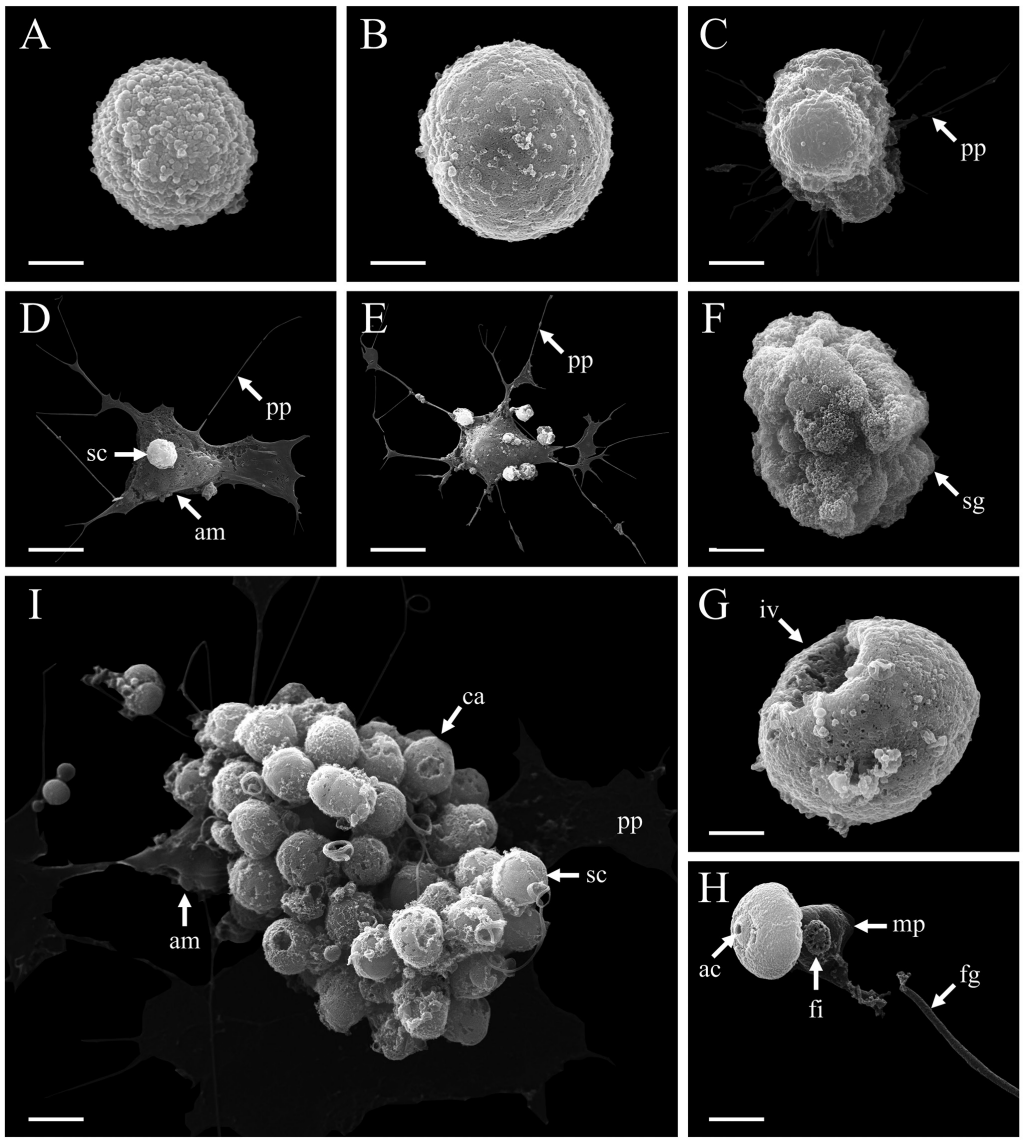

Figure 3. *Amphiura filiformis*. SEM pictures of cellular elements (*i.e.,* coelomocytes and spermatozoa) found in the perivisceral fluid. **A.** Small corpuscle. **B.** Undifferentiated cell; note that the cell size is larger than the small corpuscles. **C.** Large semi-granular cell showing some pseudopodia; this cell might be considered as an intermediate cell between undifferentiated cells and other spherule cells or amoebocytes. **D-E.** Amoebocytes displaying numerous pseudopodia that are more or less elongated; some small corpuscles around are visible and directly in contact with amoebocytes. **F.** Spherule cell; bumps are visible all around the cell, reflecting a cytoplasm filled with large granules. **G.** Undifferentiated cell exhibiting a putative phagocytic activity suggested by the invagination at the apex of the cell. **H.** Isolated spermatozoon: the broken flagellum allows the visualization of the flagellum basal insertion at the midpiece level; the acrosome is visible at the apex of the cell. **I.** Coelomocyte aggregate mostly composed of small corpuscles (4 µm) and amoebocytes showing large pseudopodia adhering to the slide. Legend: ac - acrosome; am - amoebocyte; ca - cell aggregate; fg - flagellum; fi - flagellum insertion (basal body); iv - invagination; mp - midpiece; pp - pseudopodia; sg - secretion granule; sc - small cell. Scale bars represent 0.5 µm in A, 1.2 µm in B, 2.9 µm in C, 7.1 µm in D, 8.7 µm in E, 3 µm in F, 1 µm in G, 1.1 µm in H and 3.8 µm in I.

The **small corpuscles** measured between 1.5 and 5 µm and displayed a spherical shape with no discernible nucleus and cytoplasmic granules (Figs 2A & 3A). Their proportions in the coelomic fluid varied considerably among the brittle star individuals. Still, these cells represented at least 75% of the identified coelomocytes in two individuals (Fig. 2H-I). The **undifferentiated cells** were slightly larger (4-7 µm) and displayed small cytoplasmic granules (around 1 µm), sometimes with a conspicuous central nucleus. These coelomocytes represented the second largest proportion (between 8 and 40%; Fig. 2I), with a

mean cell abundance of 1.6 10^5 cells mL^{-1} (Figs 2B & 3B). Scanning electron microscopy (SEM) revealed that cells of this size could have an invaginated membrane (Fig. 3G). Moreover, some cells seemed to be intermediate between the undifferentiated cells, spherule cells, and amoebocytes, as cells of this size (6-7 µm) were observed with some pseudopodia and some cytoplasmic protrusions (Fig. 3C). In light microscopy and SEM, the **amoebocytes** showed an irregular shape with elongated pseudopodia (Figs 2C & 3D-E). They were the biggest cell type with a diameter reaching around 30 µm by considering their pseudopodia. These cells represented between 6 and 20% of all coelomocytes (Fig. 2I). In some cases, they could interact with other cell types to form large cell aggregates attached to the slide with large pseudopodia. (Fig. 3I). The **spherule cells** were large cells, measuring around 10 to 20 µm, with numerous secretion granules visible inside the cytoplasm under light microscopy (Fig. 2D). SEM also revealed cytoplasmic protrusions corresponding to large secretion granules (2 µm) in the cytoplasmic region (Fig. 3F). These cells represented between 2 and 12% of coelomocytes (Fig. 2I). They were easily found under light microscopy but were difficult to find in SEM preparations. The **red corpuscles** were distinguishable in light microscopy by their red pigmentation and small size (between 4 and 6 µm) (Fig. 2E). No nucleus was distinguishable in these cells. Red corpuscles displayed one of the lowest concentrations compared to the other coelomocyte types, with a proportion between 1 and 4% (Fig. 2I), but were observed in all individuals examined (Fig. 2H). While most amoebocytes were colorless, **pigmented amoebocytes** were observed in only one of the three individuals analyzed (Fig. 2F), representing 3% of all coelomocytes (Fig. 2H-I). These amoebocytes were generally slightly smaller, measuring between 20 and 25 µm by including their pseudopodia. They were orange and showed a few small granules in their cytoplasm. In addition to coelomocyte cell types, spermatozoa are the last cell type observed in *A. filiformis* perivisceral fluid. The latter, found in considerable numbers in two of the three sampled specimens (Fig. 2H), is characterized by a long flagellum (20 µm) and a small cellular body (4 µm) showing a conspicuous midpiece and acrosome when observed by SEM (Figs 2G & 3H).

Coelomic fluid as a transfer medium for the luminous substrate

The concentration of coelenterazine, the luminous substrate involved in *A. filiformis* luminescence, was evaluated in the coelomic fluid. Present preliminary results underline the presence of coelenterazine in the coelomic fluid, even though the concentration is low. The mean coelenterazine concentration was $12.4 \pm 6.05\ 10^{-7}$ ng μL^{-1} of coelomic fluid. Comparatively, the control performed with ASW showed a mean coelenterazine concentration of $4.8\ 10^{-9}$ ng μL^{-1}. A typical enzymatic reaction curve, confirming the presence of coelenterazine using synthetic *Renilla* luciferase, was obtained for the coelomic fluid extract (Fig. S1). https://cbm.sb-roscoff.fr

Discussion

For over two decades, *A. filiformis* has been extensively studied across various fields, providing fundamental insights into its genome, regeneration process, ability to emit and perceive light, and the physiological stress it faces due to current global changes. However, the cellular composition of its coelomic fluid remains uncharacterized. Using the successive injection and immediate puncture method, more than 10^5 circulating coelomocytes were successfully collected. Although this number remains low for certain applications, such as some types of omics analyses, pooling several individuals could provide a solution while keeping the number of individuals pooled reasonable. The experiments enabled us to assess the concentration and proportion of circulating coelomocyte types in an ophiuroid and to perform scanning electron microscopy on these cells. Although these results are informative in terms of quantification of coelomocyte concentration, it is important to note that this concentration may be variable depending on the stress state of the individuals (e.g., Caulier et al., 2020; Wambreuse et al., 2023), which was not investigated in this study, and also between individuals, as demonstrated by the high inter-individual variability in our results.

Based on morphological criteria, the present study attempts to establish analogies between ophiuroid species and coelomocyte types from the four other echinoderm classes (Table 1). Nevertheless, categorizing coelomocytes at either species or class level remains challenging. Firstly, the morphology of a particular coelomocyte is not fixed over time, and this temporal variability has not been considered in the present study. Secondly, the coelomocytes were not studied equally across the five echinoderm classes; while coelomocytes from sea cucumbers, sea stars, and sea urchins have been extensively studied using a variety of techniques, ophiuroid and crinoid coelomocytes remain understudied in terms of species diversity, with only a limited number of techniques applied (see Table S1 https://cbm.sb- roscoff.fr for a list

of ophiuroid coelomocyte mentions in the literature and associated techniques). Finally, several nomenclatures exist in the literature; hence, establishing a consensus on each coelomocyte type within ophiuroids and among echinoderm classes is difficult.

The first cell type described in *A. filiformis,* the **small corpuscles***,* appears similar to those reported by Boolootian & Giese (1958) in the ophiuroid species *Gorgonocephalus* sp., although it might also correspond to a smaller group of undifferentiated cells reported in diverse species of ophiuroid. Regarding morphological analogies with other classes, the inability to distinguish their nucleus is consistent with the minute corpuscles reported in some sea cucumber species (Hetzel, 1963; Xing et al., 2008). There is an ongoing debate regarding the coelomocyte status of the small corpuscles. Their small size and increasing number over time post-fluid collection have led some authors to consider them as cellular debris (*e.g.*, Wahltinez et al., 2023; Queiroz & Custódio, 2024). These hypotheses could be considered for *A. filiformis*, with possible damage resulting from our puncture technique, which may lead to increased cellular debris in the sample and/or apoptotic processes of some other coelomocytes. However, other authors report coelomocytes of this size and consider them simply as small undifferentiated coelomocytes (*e.g.,* undifferentiated coelomocytes with a size < 3 µm in Di Benedetto et al., 2014 and Giorgio et al., 2015). While transmission electron microscopy analyses are required to answer this question, morphological similarities shown for undifferentiated cells in table 1 can also be applied to small corpuscles. These **undifferentiated cells** were reported in diverse ophiuroid species (Giorgio et al., 2015; Jobson et al., 2022), including *A. filiformis* (Biressi et al., 2010). While in our study, these cells were generally small (4-7 µm), their size is highly variable in the literature, ranging from 3 to 15 µm (Biressi et al., 2010; Giorgio et al., 2015, respectively). Undifferentiated cells encountered in our samples are identical to the widespread so-called progenitor, lymphoid, and undifferentiated cells in sea cucumbers, sea stars, and crinoids, *e.g., Cucumaria frondosa* (Gunnerus,1767), *Asterias rubens* Linnaeus, 1758, *Antedon mediterranea* (Lamark, 1816) (Caulier et al., 2020; Sharlaimova et al. 2021; Di Benedetto et al., 2024, respectively). Although this has not been definitively proven, the undifferentiated cells described in echinoderms have been suggested as precursor cells responsible for generating other coelomocyte types (Eliseikina & Magarlamov, 2002). This hypothesis is supported by observing intermediate cells resembling this type and more differentiated cells. However, some undifferentiated cells showed membrane invagination. Although care must be taken when interpreting these morphological features, this type of invagination is reminiscent of the formation of a phagosome during a phagocytosis process (Flannagan et al., 2012). The potential phagocytic activity displayed by these cells, which indicates a role in the cellular immune response, would, therefore, appear to contradict the hypothesis that these cells are progenitor cells. The **spherule cells** and **amoebocytes** were observed in most ophiuroid species investigated with many different terminologies (see Tables 1 & S1). In *A. filiformis*, while Biressi et al., 2010 reported phagocytes on histological and TEM sections corresponding to amoebocytes in this study, they did not report any granular cells. These two cell morphotypes appeared to be conserved in coelomocytes of all echinoderm classes, generally, named spherule cells (colorless in sea urchins) or spherulocytes and phagocytes, respectively. (Boolootian & Giese, 1958; Gross et al., 1999; Di Benedetto et al., 2014; Smith et al., 2018; Caulier et al., 2024). While phagocytes ensure phagocytosis, the production of humoral factors, and lead the aggregation and encapsulation processes by recruiting other cell types (Arizza et al., 2007; Caulier et al., 2020), spherulocytes participate in the humoral factors production by degranulating within cell aggregates and would release extracellular matrix elements to consolidate cell aggregates or participate in wound healing (Pagliara et al., 2003; San Miguel-Ruiz & Garcia-Arraràs, 2007). Concerning the **red corpuscles***,* anucleate red cells were first described by Foettinger (1880) and Cuénot (1891) in ophiuroids belonging to the genus *Ophiactis* and were thought to facilitate the transport of oxygen thanks to their high intracellular haemoglobin concentration. This haemoglobin was thought to confer this particular red pigmentation to the cells. Later, haemoglobin-containing cells were also reported within the hydrovascular fluid of *Hemipholis elongata* (Say, 1825) by Christensen et al. (2003) in which they characterized the oxygen ligand properties of their haemoglobin. However, the method used to collect these cells from the radial canal by mixing pieces of arms in a collection medium seems questionable in terms of accuracy, and no picture or description of the cells is provided in this study. In our study, small red-pigmented cells, potentially enucleated, could be observed but in low abundance, which is surprising in a view of their putative function of oxygen transport. However, this low abundance could be explained by the fact that these cells could be restricted to the hydrovascular system. In other classes, some red cells called hemocytes, which would contain haemoglobin, were reported in sea cucumbers, for which they can be

the predominant cell types (*e.g.*, *Cucumaria miniata* (Brandt, 1835), Fontaine & Lambert, 1973) and in sea stars (*e.g.*, *A. rubens*, Pinsino et al., 2007). However, the distribution of these cells within these classes remains poorly understood (Caulier et al., 2024). The sea cucumber hemocytes would be mainly involved in oxygen transport (Andrew, 1962; Caulier et al., 2024), which would be essential for some species living in oxygen-poor environments (Baker & Terwilliger, 1993). This hypothesis is consistent given the ecological characteristics of *A. filiformis*, living burrowed in the mud sediment. However, further investigation is needed to confirm whether red corpuscles in *A. filiformis* contain haemoglobin and elucidate their specific function.

The last coelomocyte type identified in *A. filiformis* is **pigmented amoebocytes**. Although pigmented cells with this morphology have never been reported in ophiuroids, their orange color might be due to haemoglobin as for red corpuscles. However, some coelomocytes in other classes are known to contain pigment, such as red spherule cells of sea urchins that contain echinochrome A, a specific naphthoquinone known for its antibacterial activity (Coates et al., 2018). As this pigment has never been detected in ophiuroids, it could rather be another type of molecule, such as carotenoids, which have already been detected in tissue of *Ophiocomina nigra* (Abildgaard in O.F. Müller, 1789) (Fontaine, 1962), for example. Also, the fact that this cell type has only been found in one individual suggests potential contamination from another compartment or from tissues due to our method using a needle (Caulier et al., 2024). Two other cell types have already been described in ophiuroids but were not identified in this study. These are reniform cells in *Ophioderma panamensis* (Boolootian, 1962) and fusiform cells in *Ophiopholis aculeata* (Linnaeus,1767) and *Gorgonocephalus* sp. (Boolootian & Giese, 1958; Jobson et al., 2022). This last cell type has a morphology similar to fusiform cells described in different sea cucumber species (*e.g., Cucumaria frondosa*, *Holothuria scabra* Jaeger, 1833, Caulier et al., 2020; Wambreuse et al., 2023). However, their function remains unknown. Otherwise, although spermatozoa were not considered a coelomocyte type in this study, a large number of these cells could be found in the coelomic fluid of *A. filiformis*. These exhibit a morphology consistent with other ophiuroid spermatozoa, with a specific shape of the acrosomal ending and a large midpiece (Chia et al., 1975; Fontaine & Lambert, 1976). While their presence is probably due to the puncture method used to collect coelomocytes that can damage surrounding organs, including gonads, it is important to note their presence since a debate is ongoing considering the status of vibratile cells in echinoderm classes (Caulier et al., 2024). In ophiuroids, notably, vibratile corpuscles were described by Kindred 1924, and their description is compatible with the morphology of spermatozoan cells that we observed by light microscopy. However, under SEM, the specific characteristic of spermatozoa are easily identifiable. The same observation was made by Caulier et al. (2024) in the species *G. arcticus* and *O. aculeata*, although SEM was not performed on these species, preventing a detailed visualization of their morphology. The present study, therefore, provides additional evidence casting doubt on the existence of vibratile cells or corpuscles in ophiuroids. Despite that this study aimed to comprehensively describe coelomocyte types, it is important to specify that it focused solely on perivisceral coelomocytes. However, coelomocytes can also be found in various tissues and compartments, particularly in the hydrovascular and hemal systems, notably in ophiuroids (Ferguson, 1995). Although no comparative information is available about the spatial distribution (*i.e.,* across tissues and compartments) of coelomocytes in ophiuroids, it is known that certain cell types are specific to certain compartments in sea cucumbers (*e.g.,* hemocytes specific to the hydrovascular system in *C. frondosa*, Caulier et al., 2020). In this regard, future research aimed at providing a comprehensive description of coelomocyte cell types in ophiuroids will need to consider this.

Finally, *A. filiformis* bioluminescence has been demonstrated to depend on a dietary acquisition of the coelenterazine substrate (Mallefet et al., 2020; Coubris et al., 2024). Recently, Coubris et al. (2024) demonstrated that the green autofluorescent signal at the photogenic sites (*i.e.,* photocytes on the arm tips and spines) depends on the exogenous coelenterazine supply. When coelenterazine was not provided during captivity, this signal disappeared. This green autofluorescence at the arm spines and tips level was attributed to the autofluorescence of coelenterazine acquired through the diet (Coubris et al., 2024). Therefore, coelenterazine must be transferred to the light emission site after ingestion to allow it to react with the luciferase. Coelomic fluid, known as a metabolite transfer fluid in echinoderms (Ferguson, 1982), would be the primary candidate involved in coelenterazine transport. The results, illustrating the presence of coelenterazine in the circulating body fluid, support the potential for coelenterazine transport through the coelomic fluid from the stomach content to the photogenic sites within the arm spines and tips. However, further studies are warranted to explore the involvement and uptake of the luminous substrate by

specific cells or whether it circulates freely within the coelomic fluid.

Conclusion

The present study provides a fine characterization of circulating coelomocyte types in *A. filiformis* and will serve as a basis for investigating the diversity and function of coelomocytes in ophiuroids. The injection and immediate puncture method developed to collect coelomic circulating cells from a brittle star was essential to describe the coelomocytes in *A. filiformis* morphologically and could be extrapolated to other species. Additionally, luminometric assays indicate the coelomic fluid's potential involvement in the coelenterazine transfer from the stomach content to the photocytes.

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References

Andrew W. 1962. Cells of the blood and coelomic fluids of tunicates and echinoderms. *American Zoologist*, 2: 285-297.

Arizza V., Giaramita F.T., Parrinello D., Cammarata M. & Parrinello N. 2007. Cell cooperation in coelomocyte cytotoxic activity of *Paracentrotus lividus* coelomocytes. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 147: 389-394. Doi: 10.1016/j.cbpa.2007.01.022

Baker S.M. & Terwilliger N.B. 1993. Hemoglobin structure and function in the rat-tailed sea cucumber, *Paracaudina chilensis*. *The Biological Bulletin*, 185: 115-122. Doi: 10.2307/1542135

Bannister R., McGonnell I.M., Graham A., Thorndyke M.C. & Beesley P.W. 2005. Afuni, a novel transforming growth factor-β gene is involved in arm regeneration by the brittle star *Amphiura filiformis*. *Development Genes and Evolution*, 215: 393-401. Doi: 10.1007/s00427-005-0487-8

Biressi A.C., Zou T., Dupont S., Dahlberg C., Di Benedetto C., Bonasoro F., Thorndyke M. & Candia Carnevali M.D. 2010. Wound healing and arm regeneration in *Ophioderma longicaudum* and *Amphiura filiformis* (Ophiuroidea, Echinodermata): comparative morphogenesis and histogenesis. *Zoomorphology*, 129: 1-19. Doi: 10.1007/s00435-009-0095-7

Boolootian R.A. & Giese A.C. 1958. Coelomic corpuscles of echinoderms. *The Biological Bulletin*, 115: 53-63. Doi: 10.2307/1539092

Boolootian R.A. & Giese, 1962. The perivisceral elements of echinoderm body fluids. *American Zoologist*, 2: 275-284.

Caulier G., Hamel J.F. & Mercier A. 2020. From coelomocytes to colored aggregates: cellular components and processes involved in the immune response of the holothuroid *Cucumaria frondosa*. *The Biological Bulletin*, 239: 95-114. Doi: 10.1086/710355

Caulier G., Jobson S., Wambreuse N., Borrello L., Delroisse J., Eeckhaut I., Mercier A. & Hamel J.-F. 2024. Vibratile cells and hemocytes in sea cucumbers-Clarifications and new paradigms. In: *The world of sea cucumbers*, pp. 403-412. Academic Press. Doi: 10.1016/B978-0-323-95377-1.00024-2

Chia F.S., Atwood D. & Crawford B. 1975. Comparative morphology of echinoderm sperm and possible phylogenetic implications. *American Zoologist*, 15: 553-565. Doi: 10.1093/icb/15.3.553

Christensen A.B., Colacino J.M. & Bonaventura C. 2003. Functional and biochemical properties of the hemoglobins of the burrowing brittle star *Hemipholis elongata* Say (Echinodermata, Ophiuroidea). *The Biological Bulletin*, 205: 54-65. Doi: 10.2307/1543445

Coates C.J., McCulloch C., Betts J. & Whalley T. 2018. Echinochrome A release by red spherule cells is an iron-withholding strategy of sea urchin innate immunity. *Journal of Innate Immunity*, 10: 119-130. Doi: 10.1159/000484722

Coubris C., Duchatelet L., Delroisse J., Bayaert W.S., Parise L., Eloy M.C., Pels C. & Mallefet J. 2024. Maintain the light, long-term seasonal monitoring of luminous capabilities in the brittle star *Amphiura filiformis*. *Scientific Reports*, 14 : 13238. Doi: 10.1038/s41598-024-64010-x

Cuénot L. 1888. Études anatomiques et morphologiques sur les ophiures. *Archives de Zoologie Expérimentale et Générale*, 2: 33-82.

Cuénot L. 1891. Études sur le sang et les glandes animale dans la série animale. Série 2: Invertébrés. *Archives de Zoologie Expérimentale et Générale*, 9: 13-90.

Czarkwiani A., Ferrario C., Dylus D.V., Sugni M. & Oliveri P. 2016. Skeletal regeneration in the brittle star *Amphiura filiformis*. *Frontiers in Zoology*, 13: 1-17. Doi: 10.1186/s12983-016-0149-x

Delroisse J., Ullrich-Lüter E., Blaue S., Ortega-Martinez O., Eeckhaut I., Flammang P. & Mallefet J. 2017a. A puzzling homology: a brittle star using a putative cnidarian-type luciferase for bioluminescence. *Open biology*, 7: 160300. Doi: 10.1098/rsob.160300

Delroisse J., Ullrich-Lüter E., Blaue S., Eeckhaut I., Flammang P. & Mallefet J. 2017b. Fine structure of the luminous spines and luciferase detection in the brittle star *Amphiura filiformis*. *Zoologischer Anzeiger*, 269: 1-12. Doi: 10.1016/j.jcz.2017.05.001

Dewael Y. & Mallefet J. 2002. Luminescence in ophiuroids (Echinodermata) does not share a common nervous control in all species. *Journal of Experimental Biology*, 205: 799-806. Doi: 10.1242/jeb.205.6.799

Di Benedetto C., Parma L., Barbaglio A., Sugni M., Bonasoro F. & Candia Carnevali M. D. 2014. Echinoderm regeneration: an *in vitro* approach using the crinoid *Antedon mediterranea*. *Cell and Tissue Research*, 358: 189-201. Doi: 10.1007/s00441-014-1915-8

Duineveld G.C. A. & Van Noort G.J. 1986. Observations on the population dynamics of *Amphiura filiformis* (Ophiuroidea: Echinodermata) in the southern North Sea and its exploitation by the dab, *Limanda limanda*. *Netherlands Journal of Sea Research*, 20: 85-94. Doi: 10.1016/0077-7579(86)90064-5

Dupont S. & Thorndyke M.C. 2006. Growth or differentiation? Adaptive regeneration in the brittle star *Amphiura filiformis*. *Journal of Experimental Biology*, 209: 3873-3881. Doi: 10.1242/jeb.02445

Eliseikina M.A. & Magarlamov T.Y. 2002. Coelomocyte morphology in the holothurians *Apostichopus japonicus* (Aspidochirota: Stichopodidae) and *Cucumaria japonica* (Dendrochirota: Cucumariidae). *Russian Journal of Marine Biology*, 28: 197-202. Doi: 10.1023/A:1016801521216

Ferguson J.C. 1982. Nutrient translocation. In *Echinoderm nutrition,* pp. 373-393. CRC Press: Boca Raton.

Ferguson J.C. 1995. The structure and mode of function of the water vascular system of a brittle star, *Ophioderma appressum*. *The Biological Bulletin*, 188: 98-110. Doi: 10.2307/1542072

Flannagan R.S., Jaumouillé V. & Grinstein S. 2012. The cell biology of phagocytosis. *Annual Review of Pathology: Mechanisms of Disease*, 7: 61-98. Doi: 10.1146/annurev-pathol-011811-132445

Fontaine A.R. 1962. The colours of *Ophiocomina nigra* (Abildgaard): III. Carotenoid pigments. *Journal of the Marine Biological Association of the United Kingdom*, 42: 33-47. Doi: 10.1017/S0025315400004446

Fontaine A.R. & Lambert P. 1973. The fine structure of the haemocyte of the holothurian, *Cucumaria miniata* (Brandt). *Canadian Journal of Zoology*, 51: 323-332. Doi: 10.1139/z73-046

Fontaine A.R. & Lambert P. 1976. The fine structure of the sperm of a holothurian and an ophiuroid. *Journal of Morphology*, 148 : 209-225. Doi: 10.1002/jmor.1051480207

Foettinger A., 1880. Sur l'existence de l'hemoglobine chez les echinodermes. *Archives de Biologie de Paris*, 1: 405-415.

Giorgio G.D., Rubilar T. & Brogger M.I. 2015. Histological analysis after arm tip amputation in the brittle star *Ophioplocus januarii* (Echinodermata: Ophiuroidea). *Revista de Biología Tropical*, 63: 297-308. Doi: 10.15517/rbt.v63i2.23164

Gliznutsa L.A. & Dautov S.S. 2011. Cell differentiation during the larval development of the ophiuroid *Amphipholis kochii* Lütken, 1872 (Echinodermata: Ophiuroidea). *Russian Journal of Marine Biology*, 37: 384-400. Doi: 10.1134/S1063074011050051

Gross P.S., Al-Sharif W.Z., Clow L.A. & Smith L.C. 1999. Echinoderm immunity and the evolution of the complement system. *Developmental & Comparative Immunology*, 23: 429-442. Doi: 10.1016/S0145-305X(99)00022-1

Hetzel H.R. 1963. Studies on holothurian coelomocytes. I. A survey of coelomocyte types. *The Biological Bulletin*, 125: 289-301. Doi: 10.2307/1539404

Hira J., Wolfson D., Andersen A.J.C., Haug T. & Stensvåg K. 2020. Autofluorescence mediated red spherulocyte sorting provides insights into the source of spinochromes in sea urchins. *Scientific reports*, 10: 1149. Doi: 10.1038/s41598-019-57387-7

Jobson S., Hamel J.-F. & Mercier A. 2022. Rainbow bodies: Revisiting the diversity of coelomocyte aggregates and their synthesis in echinoderms. *Fish & Shellfish Immunology*, 122, 352-365. Doi: 10.1016/j.fsi.2022.02.009

Kindred J.E. 1924. The cellular elements in the perivisceral fluid of echinoderms. *The Biological Bulletin*, 46: 228-251. Doi: 10.2307/1536725

Majeske A.J., Bayne C.J. & Smith L.C. 2013. Aggregation of sea urchin phagocytes is augmented in vitro by lipopolysaccharide. *PLoS One*, 8 : e61419. Doi: 10.1371/journal.pone.0061419

Mallefet J., Duchatelet L. & Coubris C. 2020. Bioluminescence induction in the ophiuroid *Amphiura filiformis* (Echinodermata). *Journal of Experimental Biology*, 223: jeb218719. Doi: 10.1242/jeb.218719

O'Connor B., Bowmer T. & Grehan A. 1983. Long-term assessment of the population dynamics of *Amphiura filiformis* (Echinodermata: Ophiuroidea) in Galway Bay (west coast of Ireland). *Marine Biology*, 75: 279-286. Doi: 10.1007/BF00406013

Pagliara P., Candia Carnevali M.D., Burighel P. & Ballarin L. 2003. The spherule cells of *Holothuria polii* Delle Chiaie, 1823 (Aspidochirota, Holothuroidea) during brown body formation: an ultrastructural study. *Journal of Submicroscopic Cytology and Pathology*, 35: 295-302.

Parey E., Ortega-Martinez O., Delroisse J., Piovani L., Czarkwiani A., Dylus D., Arya S., Dupont S., Thorndyke M., Larsson T., Johannesson K., Buckley K. M., Martinez P., Oliveri P. & Marlétaz F. 2024. The brittle star genome illuminates the genetic basis of animal appendage regeneration. *Nature Ecology & Evolution*, 8: 1505-1521. Doi: 10.1038/s41559-024-02456-y

Pinsino A., Thorndyke M.C. & Matranga V. 2007. Coelomocytes and post-traumatic response in the common sea star *Asterias rubens*. *Cell stress & chaperones*, 12: 331. Doi: 10.1379/CSC-288.1

Queiroz V., Mauro M., Arizza V., Custódio M.R. & Vazzana M. 2022a. The use of an integrative approach to identify coelomocytes in three species of the genus *Holothuria* (Echinodermata). *Invertebrate Biology*, 141: e12357. Doi: 10.1111/ivb.12357

Queiroz V., Arizza V., Vazzana M. & Custódio M.R. 2022b. Comparative evaluation of coelomocytes in *Paracentrotus* sea urchins: Description of new cell types and insights

on spherulocyte maturation and sea urchin physiology. *Zoologischer Anzeiger*, 300: 27-40. Doi: 10.1016/j.jcz.2022.06.008

Queiroz V. & Custódio M.R. 2024. Diversity of coelomocytes in the class Holothuroidea. In *The world of sea cucumbers*. Academic Press, pp. 377-401. Doi: 10.1016/B978-0-323-95377-1.00011-4

Rosenberg R. & Lundberg L. 2004. Photoperiodic activity pattern in the brittle star *Amphiura filiformis. Marine Biology*, 145: 651-656. Doi: 10.1007/s00227-004-1365-z

San Miguel-Ruiz J.E. & García-Arrarás J.E. 2007. Common cellular events occur during wound healing and organ regeneration in the sea cucumber *Holothuria glaberrima*. *BMC Developmental Biology*, 7: 1-19. Doi: 10.1186/1471-213X-7-115

Sharlaimova N., Shabelnikov S., Bobkov D., Martynova M., Bystrova O. & Petukhova O. 2021. Coelomocyte replenishment in adult *Asterias rubens*: the possible ways. *Cell and Tissue Research*, 383: 1043-1060. Doi: 10.1007/s00441-020-03337-z

Shimomura O. 2012. *Bioluminescence: chemical principles and methods*, 367 pp. World Scientific.

Sköld M., Loo L.O. & Rosenberg R. 1994. Production, dynamics and demography of an *Amphiura filiformis* population. *Marine Ecology Progress Series*, 103: 81-90.

Smith L. C., Arizza V., Barela Hudgell M.A., Barone G., Bodnar A.G., Buckley K.M., Cunsolo V., Dheilly N.M., Franchi N., Fugmann S.D., Furukawa R., Garcia-Arraras J., Henson J.H., Hibino T., Irons Z.H., Li C., Man Lun C., Majeske A.J., Oren M., Pagliara P., Pinsino A., Raftos D.A., Rast J.P., Samasa B., Schillaci D., Schrankel C.S., Stabili L., Stensväg K. & Sutton E. 2018. Echinodermata: The Complex Immune System, pp. xx-xx *in* Echinoderms. In: Cooper E. (Ed.) Advances in Comparative Immunology. Springer, Cham. Doi: 10.1007/978-3-319-76768-0_32

Smith L.C., Hawley T.S., Henson J.H., Majeske A.J., Oren M. & Rosental B. 2019. Methods for collection, handling, and analysis of sea urchin coelomocytes. *Methods in cell biology*, 150: 357-389. Doi: 10.1016/bs.mcb.2018.11.009

Taguchi M., Tsutsui S. & Nakamura O. 2016. Differential count and time-course analysis of the cellular composition of coelomocyte aggregate of the Japanese sea cucumber *Apostichopus japonicus*. *Fish & Shellfish Immunology*, 58: 203-209. Doi: 10.1016/j.fsi.2016.06.060

Thorndyke M.C., Patruno M.V., Dewael Y., Dupont S. & Mallefet J. 2001. Regeneration in the ophiuroid *Amphiura filiformis*: cell biology, physiology and luminescence. In *Echinoderm Research 2001*, pp. 193-199. Balkema Publishers: Amsterdam.

Vanderlinden C., Mallefet J. & Gailly P. 2010. How do brittle stars control their light emission? *Echinoderms: Durham*, pp. 419-422.

Wahltinez S.J., Byrne M. & Stacy N.I. 2023. Coelomic fluid of asteroid echinoderms: Current knowledge and future perspectives on its utility for disease and mortality investigations. *Veterinary Pathology*, 60: 547-559. Doi: 10.1177/03009858231176563

Wambreuse N., Caulier G., Eeckhaut I., Borrello L., Bureau F., Fievez L. & Delroisse J. 2023. Morpho-functional Characterisation of Cœlomocytes in the Aquacultivated Sea Cucumber *Holothuria Scabra*: From Cell Diversity to Transcriptomic Immune Response. *Available at SSRN 4658658*. (preprint repository). Doi: 10.2139/ssrn.4658658

Xing K., Yang H.S. & Chen M.Y. 2008. Morphological and ultrastructural characterization of the coelomocytes in *Apostichopus japonicus*. *Aquatic Biology*, 2: 85-92. Doi: 10.3354/ab00038