



Ultrastructural description of surface receptors in the bioluminescent brittle star *Amphiura filiformis* (Müller, 1776)

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Abstract: Stäbchen are specific sensory receptors distributed along the integument of brittle stars (Ophiuroidea: Echinodermata). Found on the surface of spines and podia, these structures correspond to cuticular projections where one or more ciliated cellular processes terminate. Stäbchen are presumed to play a functional role in chemo- and/or mechanoreception. Structurally, the stäbchen of the bioluminescent brittle star *Amphiura filiformis* can be categorised into two types: (i) long-cilium cylindrical stäbchen, and (ii) short-cilium conical stäbchen. Electron microscopy analyses revealed a close and intricate association of the spine stäbchen with underlying luminous cells, known as photocytes. *In vivo* luminometry experiments have demonstrated that *A. filiformis* is sensitive to mechanical stimuli and to seawater containing injured conspecifics, responding by emitting blue luminescent flashes. These combined results suggest that spine stäbchen could be involved in initiating the light emission process.

Résumé : Description ultrastructurale des récepteurs de surface de l'ophiure lumineuse *Amphiura filiformis* (Müller, 1776). Les stäbchen sont des récepteurs spécifiques répartis le long du tégument des ophiures (Ophiuroidea : Echinodermata). Retrouvées à la surface des piquants et des podia, ces structures correspondent à des projections cuticulaires où aboutissent un ou plusieurs processus cellulaires ciliés. On suppose que les stäbchen jouent un rôle fonctionnel dans la chémoréception et/ou la mécanoréception. Structurellement, les

stäbchen de l'ophiure bioluminescente *Amphiura filiformis* peuvent être classés en deux catégories : (i) les *stäbchen* cylindriques à long cil, et (ii) les *stäbchen* coniques à court cil. Les analyses en microscopie électronique révèlent une association complexe et étroite entre les *stäbchen* et les cellules lumineuses sous-jacentes présentes au sein des piquants de l'ophiure. Des analyses luminométriques démontrent également qu'*A. filiformis* est sensible aux stimuli mécaniques et à l'eau de mer conditionnée avec des congénères blessés, réagissant en émettant des flashes luminescents. Ces observations combinées suggèrent l'implication des *stäbchen* des piquants dans le processus d'émission de lumière de l'ophiure.

Keywords: Echinoderm • Ultrastructure • Stäbchen • Sensory Receptors • Bioluminescence • Chemical communication

Introduction

Chemical signalling is a prevalent communication strategy used by many marine organisms (Zimmer & Butman, 2000; Hay, 2009). Prey species emit chemical alarm signals to warn conspecifics of potential predatory threats. In response, these signals activate defence strategies such as escape behaviour or adopting protective postures (e.g., Hagen et al., 2002; Smee & Weissburg, 2006; Majer et al., 2009). These chemical signals are particularly important in seascapes with dense vegetation or high turbidity (Pollock et al., 2003). The primary chemical alarm substances, known as damaged-released alarm signals, are released through physical injuries inflicted during predator handling, chewing, or ingestion of prey (Chivers & Smith, 1998). Prey conspecifics can assess and respond to the risk of predation through the detection of these alarm cues (Chivers & Smith, 1998). The perception of alarm substances and the associated escaping behaviour have been demonstrated for numerous sea urchins, sea stars, and some brittle stars (e.g., Sloan & Northway, 1982; Rosenberg & Selander, 2000; Hagen et al., 2002; Majer et al., 2009; Spyksma et al., 2020). Many sea urchins are repelled by the chemical cues present in conditioned sea water obtained from injured/preyed conspecifics or even heterospecifics (e.g., Hagen et al., 2002; Spyksma et al., 2020). Echinoderms possess a variety of specific chemical molecules that are potentially released in the environment and that could convey information between organisms. These molecules, known as semiochemicals, may play a role in anti-predatory and defense responses. These include, for example, saponins (e.g., Van Dyck et al., 2010; Kamyab et al., 2020) and quinones (e.g., Thomson, 1971; Brasseur et al., 2017; Feng et al., 2017; Zhukova, 2023). Brittle stars are characterized by the presence of 3 α -hydroxysterols, which are absent from the other four echinoderm classes (Riccio et al., 1985).

Ophiuroids have been shown to be sensitive to chemical cues (Sloan & Campbell, 1982; May & Mikalian, 2019). Majer et al. (2009) underlined the importance of the ophiuroid chemical cue concentration in the water for eliciting a defensive response. Many adult ophiuroids have either their entire body or their arms exposed to predation during feeding time. Despite their ability to autotomise and regenerate lost body parts rapidly, a clear trade-off exists between filter-feeding and predation risk, necessitating the development of efficient defensive responses and traits (Sköld & Rosenberg, 1996; Sköld, 1998). Ophiuroids exhibit a wide range of defensive traits, including morphological adaptations such as defensive arm spines, anti-predatory behaviours, and environmental strategies like burrowing, inhabiting cryptic habitats, or engaging in nocturnal activity (e.g., Hendler, 1984; Sköld, 1998; Henkel & Pawlik, 2005; Pereira Majer et al., 2009). Another possible defensive trait is bioluminescence (e.g., Mallefet, 2009; Jones & Mallefet, 2013). Bioluminescence, defined as the production of light by a living organism through a biochemical reaction (Harvey, 1952), offers various ecological advantages, including attracting prey, avoiding predation, and facilitating intraspecific communication (Haddock et al., 2010; Duchatelet & Dupont, 2024).

The burrowing European brittle star *Amphiura filiformis* (O.F. Müller, 1776) lives in muddy sediment along the coast of the North-eastern Atlantic and Mediterranean Sea feeding on plankton and particulate organic matter by extending two arms outside the sediments (Loo et al., 1996). *A. filiformis* produces blue light at 472 nm at the level of its arm spines under a cholinergic nervous control (Dewael & Mallefet, 2002; Delroisse et al., 2017b; Coubris et al., 2024). Light production can be easily induced by mechanical stimulation, such as pinching the brittle star arms. Cells responsible for light production, the photocytes, are located inside the spines and associated with

other cellular types such as glandular and pigmented cells (Delroisse et al., 2017a). Only a few ethological studies have investigated the ecological functions and advantages of light production in echinoderms (e.g., Grober, 1988; Deheyn et al., 2000; Jones & Mallefet, 2013), and the function of bioluminescence in *A. filiformis* remains speculative.

A. filiformis represents more than 60% of the diet of flatfishes such as *Limanda limanda* (Linnaeus, 1758) and *Hippoglossoides platessoides* (Fabricius, 1780) which makes these flatfishes the primary predators of this species (Pihl, 1994). These fish primarily consume the arms, and regenerating arms are commonly found in natural ophiuroid populations (Duineveld & van Noort, 1986; Sköld et al., 1994; Sköld & Rosenberg, 1996). *A. filiformis* can respond to environmental cues such as food availability and quantity, current direction and velocity (Sloan & Campbell, 1982; Loo et al., 1996). 'Stäbchen', specific ciliated cuticular projections, are believed to serve as sites for mechano- and/or chemoreception in ophiuroids (Buchanan, 1963; Whitfield & Emson, 1983; Harrison & Chia, 1994; Delroisse et al., 2017a). Rosenberg & Selander (2000) showed that *A. filiformis* responded to alarm signals released by conspecifics and, to a lesser extent, by individuals of the sympatric species *Amphiura chiajei* when faced with flat fish grazing. The feeding arms respond to potential alarm cues by bending towards the sediment surface, a behaviour interpreted as a defence mechanism against visual predation (Rosenberg & Selander, 2000).

In this article, we provide an ultrastructural description of the stäbchen distributed along the spines of *A. filiformis*. These sensory structures are shown to be co-localised with photogenic structures present in the spines. We also investigate the morphology and the relative abundance of the stäbchen present at the surface of the podia. Finally, *A. filiformis* responds to chemical cues from conspecifics by emitting light. We discuss the ecological implications of this alarm response on *A. filiformis* bioluminescence.

Materials and Methods

Organisms sampling

Adult *Amphiura filiformis* (Müller, 1776) 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) between fall 2021 and summer 2023. Individuals were maintained in an aquarium supplied with running seawater directly pumped from the

adjacent fjord (12°C, 35 salinity, pH 8.2). Individuals were either fixed for microscopy analyses or used fresh for physiological assays.

Scanning and transmission electron microscopy

Morphological visualisation of the stäbchen located along the podia, spines, and arm tip of *A. filiformis* was obtained through scanning and transmission electron microscopy (SEM and TEM; respectively).

For SEM, *A. filiformis* individuals ($n = 5$) were fixed in non-acetic Bouin's fluid for 65 hours at 4°C. The disc and one arm of each individual were dehydrated in sequential ethanol concentrations and dried by the critical-point method using CO₂ as the transition fluid (Lourtie et al., 2022, for technical details). Samples were mounted on aluminium stubs, coated with a mixture of gold/palladium (40/60) using a sputter coater, and observed with a JEOL JSM-7200F scanning electron microscope.

SEM micrographs of the spines were used to count stäbchen in three defined spine areas (basal, median and terminal; $n = 5$ each) to statistically evaluate their proportion according to spine zone. Spine areas were delimited by dividing the spine length equally.

For TEM observation of the mechano/chemoreception apparatus, the arms of three specimens of *A. filiformis* were fixed in a glutaraldehyde solution (3% glutaraldehyde, 0.1 M sodium cacodylate, 0.27 M sodium chloride; pH 7.8) for 3 hours, then rinsed in a cacodylate buffer (0.2 M sodium cacodylate, 0.31 M sodium chloride; pH 7.8) (Duchatelet et al., 2023, for technical details). Samples were then post-fixed in osmium tetroxide (1% osmium tetroxide, 0.1 M sodium cacodylate, 0.27 M sodium chloride; pH 7.8) for one hour and rinsed again in the cacodylate buffer. After the last wash, the pieces were decalcified for 30 hours in a 1:1 mixture of 2% ascorbic acid and 0.3 M NaCl and then progressively dehydrated in a graded ethanol concentration series. Arms were embedded in Spurr's resin (EM0300, Merck, Darmstadt, Germany) following the protocol of Renwart et al., 2014. Semithin sections of 400–500 nm were obtained using a Leica Ultracut UCT ultramicrotome (Leica microsystems, Wetzlar, Germany) and stained with an equal mixture (1:1) of 1% methylene blue and 1% azur II (Richardson et al., 1960). Semithin sections were observed using a Zeiss Axio Scope A1 microscope (Zeiss, Oberkochen, Germany) coupled with an Axiocam 305 colour camera (Zeiss). Ultrathin sections, 90 nm thick, were prepared using a Leica Ultracut UCT ultramicrotome (Leica microsystems, Wetzlar, Germany) and collected on copper grids. Sections were contrasted with a solution of uranyl acetate (0.18 M uranyl acetate

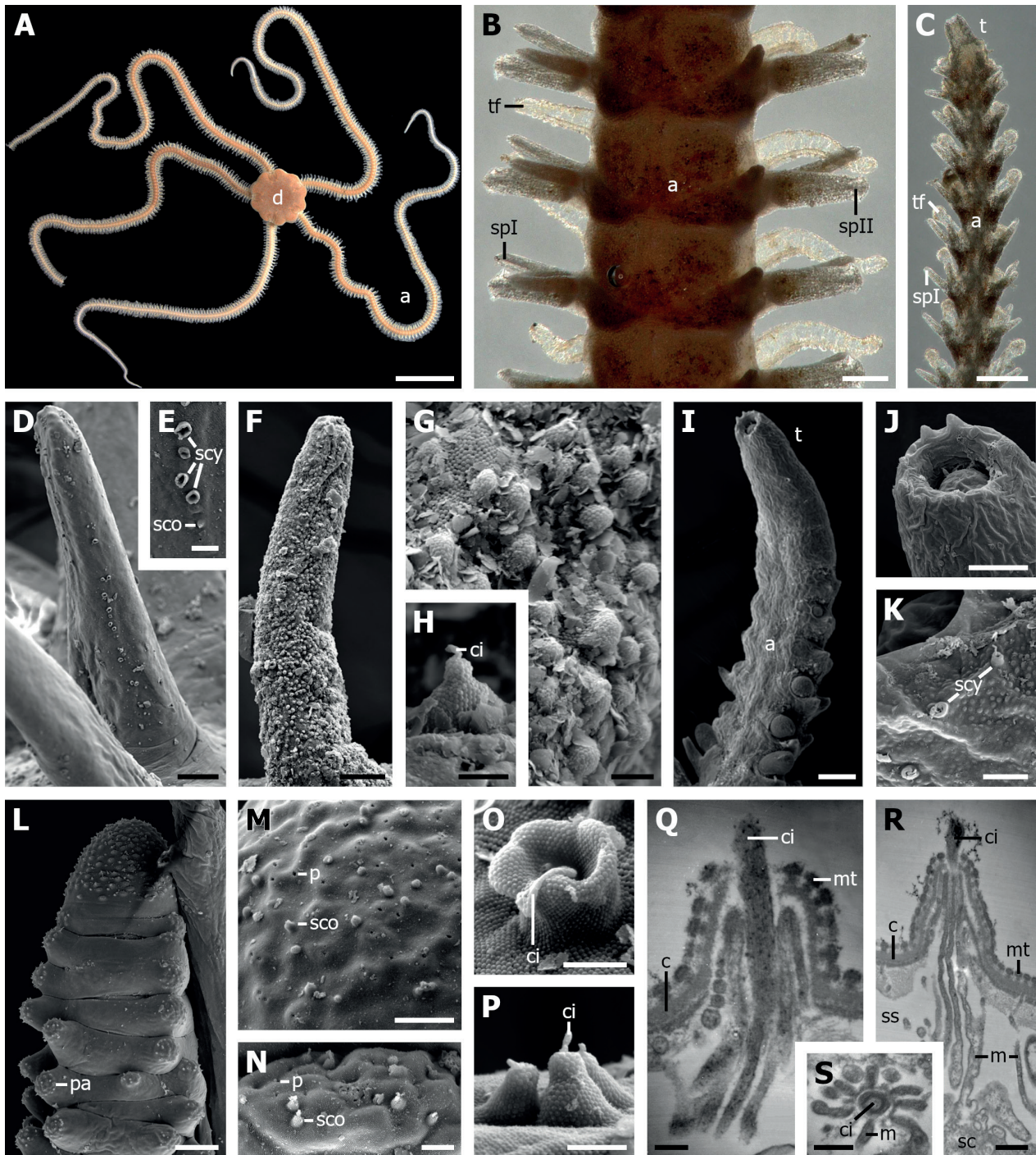


Figure 1. Localisation and morphology of *Amphiura filiformis* stäbchen. **A.** Aboral view of the brittle star. **B.** Aboral view of arm proximal segments. **C.** Aboral view of an arm tip. **D.** Aboral view of a spine from proximal arm segment showing a row of stäbchen. **E.** Close-up of the stäbchen row in **D.** **F.** Spine of a distal arm segment. **G.** The surface of the spine in **F.** is covered with cuticular projections. **H.** Close-up of a projection in **G.** **I.** Side view of the arm tip. **J.** Close-up of the arm tip in **I.** **K.** Stäbchen of the arm tip forming a crown around the arm tip pit. **L.** Oral view of an arm tube foot with papillae forming two rows. **M.** Close-up of the podial tip surface presenting stäbchen and pores. **N.** Close-up of the podial papillae. **O.** Cylindrical stäbchen characterised by a long cilium. **P.** Conical stäbchen characterised by a short cilium. **Q & R.** Longitudinal section of a stäbchen. **S.** Cross section of a stäbchen. **D-P** are SEM micrographs; **Q-S** are TEM micrographs. a: arm, c: cuticle, ci: cilium, d: disc, m: microvilli, mt: microvilli's tip, p: pores, pa: papilla, sc: sensory cell, sco: conical stäbchen, scy: cylindrical stäbchen, ss: subcuticular space, spl: type I spine, spll: type II spine, t: arm tip, tf: tube feet. Scale bars: **A** = 1 cm; **B, C** = 200 µm; **D** = 25 µm; **E, K & M** = 5 µm; **F** = 10 µm; **G, H, O & P** = 1 µm; **I** = 50 µm; **J & L** = 20 µm; **N** = 2 µm; **Q & S** = 0.2 µm; **R** = 0.5 µm.

solution:ethanol (2:1)) in dark chambers for 45 min, followed by a lead citrate bath (0.08 M lead nitrate, 0.12 M sodium citrate, 0.16 M sodium hydroxide) for 4.5 min, and left to dry. Sections were observed using a Zeiss Leo 906E transmission electron microscope (Zeiss, Oberkochen, Germany) coupled with a Jenoptik camera (Jenoptik ProgRes CFcool, Jena, Germany).

Luminescent response to alarm cues

The conditioned water was prepared using four *A. filiformis* animals placed in a petri dish with 2 mL of filtered seawater (FSW). The central discs were removed and the remaining arms (20 in total) were cut into small pieces with a scalpel blade, and then crushed with forceps. The conditioned seawater was then filtered through a 30 µm sieve to remove the remaining large cellular debris. After filtration, 1 mL of the conditioned water (CW) was collected for the luminometric experiment.

Light emission measurements were carried out with the same protocol as Coubris et al. (2024) in a dark room with a FB12 tube luminometer (Tirtertek-Berthold, Pforzheim, Germany) calibrated using a standard 470 nm light source (Beta light, Saunders Technology, Hayes, UK). Light responses were recorded using FB12-Sirius PC Software (Tirtertek-Berthold). All data were standardised per unit of specimen mass (g).

To evaluate the effect of CW application on light production, one *A. filiformis* individual, preliminarily weighed, was placed in a luminometric tube containing 1 mL of FSW and placed in a FB12 tube luminometer. CW was injected into the chamber, and light emission was recorded for one hour. This experiment was repeated 15 times using different individuals. The total light emission (L_{tot}) was recorded and converted into 10^9 quanta per gram of *A. filiformis* tissue (10^9 q g^{-1}). Several controls were performed: (i) a negative control replacing CW with FSW application ($n = 15$); (ii) a positive control using a solution of acetylcholine (ACh) at $10^{-6} \text{ mol l}^{-1}$ instead of CW ($n = 12$); (iii) CW was injected without the presence of *A. filiformis* specimen ($n = 6$) to ensure that it was not bioluminescent.

Statistical analyses

All the statistical analyses were performed with R Studio (version 2023.06.1, 2022, Posit Software, USA). The normality and equality of variance were assessed using the Shapiro-Wilk test and Levene's test, respectively. When these parametric assumptions were met, an ANOVA coupled with Tukey's test was used. When logarithmic transformation did not achieve normality or homoscedasticity, non-parametric Kruskal-Wallis ANOVA and Wilcoxon multiple comparisons

tests were used to assess the significant differences between treatments. Differences were considered significant when p-values were below 0.05. Values were graphically illustrated with mean and standard error of the mean (SE).

Results

*Localisation and morphological characterisation of stäbchen in the brittle star *Amphiura filiformis**

In *A. filiformis*, each arm segment bears lateral spines and a pair of ventrolateral podia. The spines borne by the proximal arm segments are more numerous and longer (up to 6 pairs of spines approximately 400 µm in length), while these values progressively decrease towards the distal part of the arm (2 pairs of spines approximately 60 µm long) (Fig. 1A-C). Two distinctive spine morphotypes are observed: the tapered spines assigned as type I spines, and the rectangular spines, classified as type II spines (Fig. 1B). Only type I spines are present on the most distal part of the arm (Fig. 1C). One pair of spines and podia are also observed at the base of each of the five jaws encircling the mouth (Fig. S1A & B).

Rows of stäbchen along the length of all arm spines were observed using SEM (Fig. 1D & E). However, the organisation of the stäbchen differs for the most distal arm spines, with the projections uniformly covering the entire surface of the spine and not forming rows (Fig. 1F-H). Conversely, the buccal spines of the brittle star have stäbchen confined almost exclusively at the spine tip (Fig. S1C & D). At the most distal part of the arm, the arm tip, a pit is observed (Fig. 1C, I & J). Stäbchen border this pit, forming a crown-like structure (Fig. 1I-K).

Stäbchen are also visible on the tip and papillae of podia, alongside slightly raised pores (Fig. 1L-N). At the level of the buccal podia in addition to the tip and papillae, the surface between the papillae, facing the mouth is also covered with stäbchen (Fig. S1E-H).

A. filiformis stäbchen can exhibit a slightly different morphology but they are all characterised by a cuticular projection, forming a small hillock, with a single cilium protruding from the apex (Fig. 1O & P). Yet, they can be sub-clustered depending on their shape (cylindrical or conical), their size, and the size of their projecting cilium. The height of cylindrical projections ranges from 2 to 3 µm, while conical projections are shorter, varying from 400 nm to 2 µm (Fig. 1O & P). The cylindrical stäbchen are usually associated with a long projecting cilium varying in size from 2 to 7 µm (Fig. 1O). The conical stäbchen have a short projecting cilium that

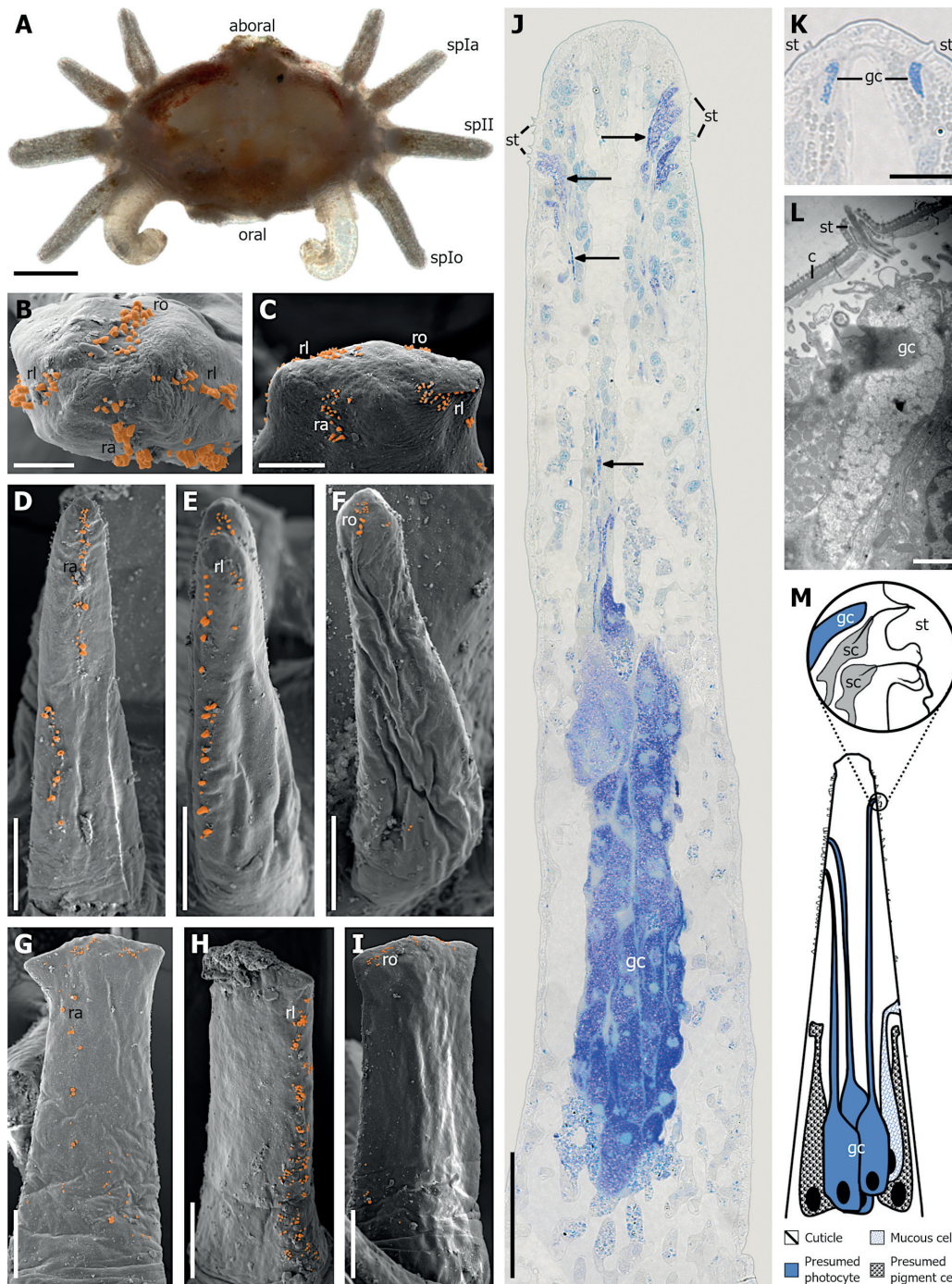


Figure 2. Stäbchen spatial arrangement on the proximal arm spines and morphological link with granular cells. **A.** Cross section of a proximal arm segment showing the position of the two spine morphotypes. **B.** Apical view of a type I spine showing four rows of stäbchen. **C.** Apical view of a type II spine showing four rows of stäbchen. Stäbchen rows on the aboral (**D**), lateral (**E**), and oral (**F**) sides of a type I spine. Stäbchen rows on the aboral (**G**), lateral (**H**), and oral (**I**) sides of a type II spine. **J.** Semithin longitudinal section of the spine inner tissues under 1:1 mixture of methylene blue and azure II staining showing granular cell clusters (arrows indicate granular cell processes approaching the cuticle where the stäbchen are located). **K.** High magnification of two granular cell process tips, each oriented toward one stäbchen, in the apical part of a spine (1:1 mixture of methylene blue and azure II staining). **L.** TEM micrograph of granular cell process oriented toward a stäbchen in the apical part of a spine. **M.** Schematic longitudinal section of a spine showing the proximity between photocyte processes and stäbchen-associated sensory cells. B-I are SEM micrographs with stäbchen highlighted in orange. c: cuticle, gc: granular cells, ra: aboral row of stäbchen, rl: lateral row of stäbchen, ro: oral row of stäbchen, sc: sensory cell, spIIa: aboral type I spine, spIo: oral type I spine, spII: type II spine, st: stäbchen. Scale bars: A = 250 μ m; B & K = 10 μ m; C = 20 μ m; D, E, F, G, H, I & J = 50 μ m; L = 1 μ m.

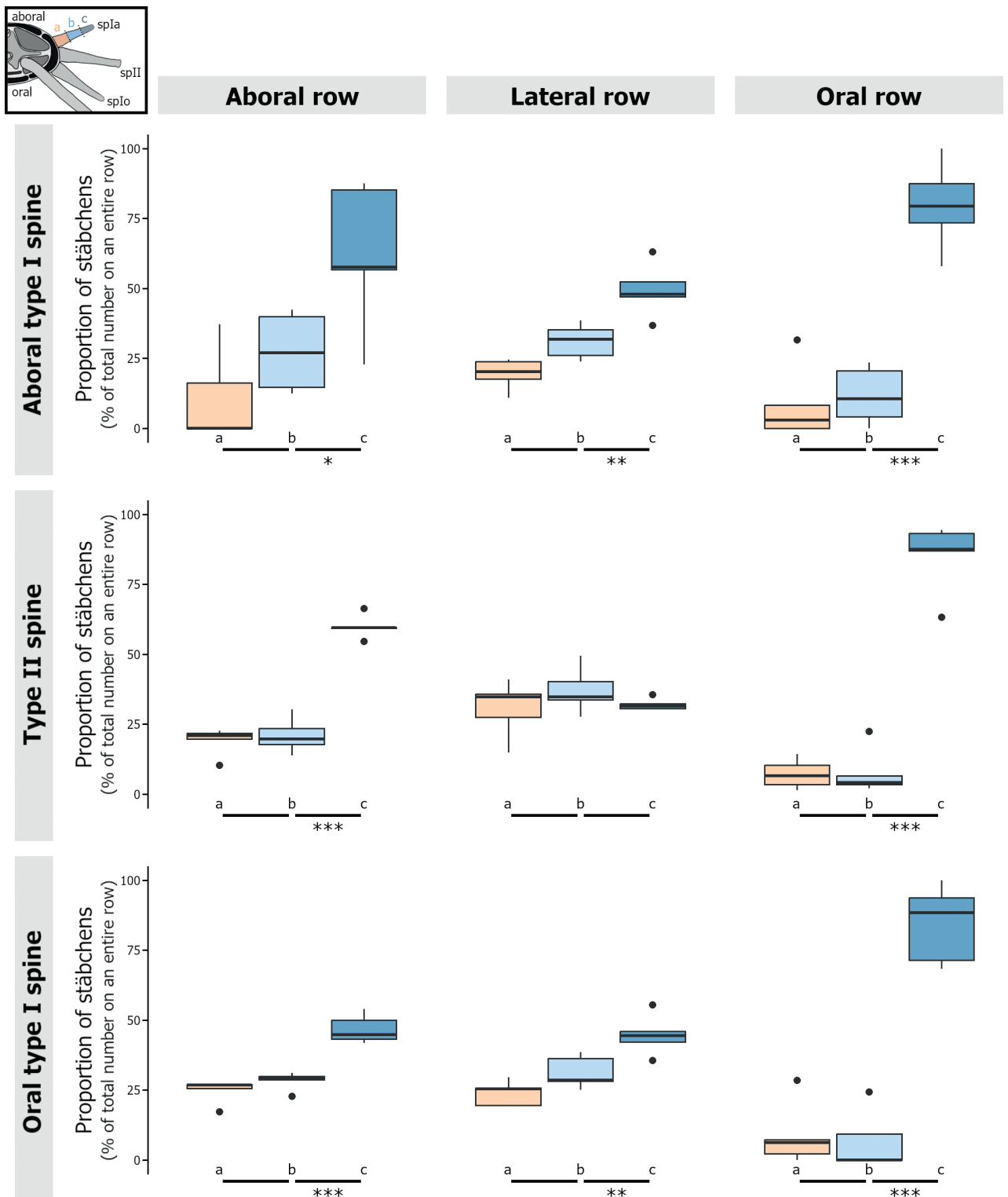


Figure 3. Proportion of stäbchen by spine zone as a function of stäbchen row for each proximal arm spine. The proportion of stäbchen is expressed as the percentage of their total number on a row. The spine zone is defined by dividing the total spine length into three equal parts; basal area (a), middle area (b), and terminal area (c). Stäbchen were counted on each of the three row types observed on the spines; aboral row, lateral row, and oral row of stäbchen. The investigated proximal arm spines are the aboral type I spine (spla), the type II spine (splI), and the oral type I spine (splO). Parameters are shown in the scheme of an arm cross-section in the black box. Asterisks indicate a statistical difference between values for the two spine zones (ANOVA and Kruskal-Wallis test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Number of replicates for each boxplot is 5.

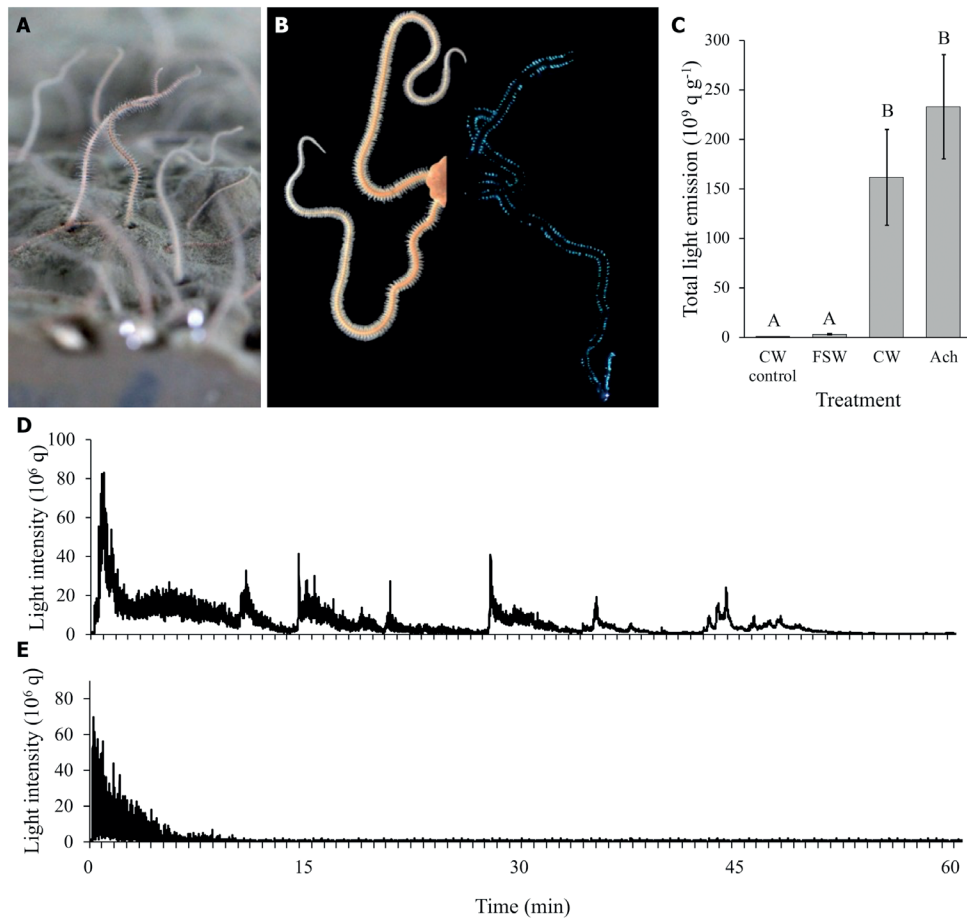


Figure 4. *Amphiura filiformis* luminescence and physiological response to conspecific injuries. **A.** *In vivo* pictures of *A. filiformis* burrowed in the sediment with arms protruding from the mud. **B.** Composite image of a daylight (left) and blue bioluminescence (right) pictures of *A. filiformis*. **C.** Mean total amount of light produced by *A. filiformis* under different treatments: Ach, acetylcholine; CW, conditioned sea water with conspecific chopped arm portion; FSW, filtered sea water; CW control, CW applied without samples. Typical luminometric curves obtained after **(D)** CW and **(E)** Ach application on *A. filiformis* individuals showing the peaks of light emission.

measured 400–500 nm or did not show any visible cilium (Fig. 1P). Stäbchen from both sub-clusters are observed on all proximal arm spines, buccal spines and at the arm tip (Figs 1E, S1D & 1K, respectively). Only conical stäbchen were observed on most-distal arm spines and on all tube feet (Figs 1G, H, M & N, S1F & H). No stäbchen were observed on the surface of the arm plates or of the disc.

TEM micrographs revealed that the single cilium (approximately 160 nm in diameter), originating from the underlying sensory cell, traverses the centre of the cuticular projection and protrudes from the apex into the external environment (Fig. 1Q & R). The cilium is surrounded by microvilli, each approximately 75 nm in diameter, derived from the same sensory cells (Fig. 1R & S). The outer layer of the cuticular projections, like the entire cuticle surface, is encrusted with ovoid structures of around 100 nm diameter, as observed

on both SEM surface images (Fig. 1O & P) and TEM micrographs (Fig. 1Q & R). These correspond to the modified ends of epidermal cell microvilli. The microvilli extend through the subcuticular space and penetrate the spine cuticle to protrude from the surface (Fig. 1Q & R).

*Distribution of stäbchen on the spines of the brittle star *Amphiura filiformis* and their potential interaction with photogenic cells*

SEM micrographs revealed a stäbchen pattern on all proximal arm spines of *A. filiformis*. The two types of stäbchen are arranged in four longitudinal rows along the spine surface, independently of the spine morphotype (Fig. 2A–C). Rows were localised as follows: one aboral, one oral, and two on each lateral side of the spine (Fig. 2D–I). The oral row is always shorter than the three others (Fig. 2F & I).

The stäbchen abundance was significantly higher in the terminal third of all proximal arm spines (aboral and oral type I spine and type II spine) for each row (ANOVA, Tukey's comparison test; Fig. 3, area c), except for the lateral row of type II spines (Kruskal-Wallis, Wilcoxon comparisons test; p-value middle area b versus terminal area c = 0.93; Fig. 3). This higher proportion was most marked for the oral row of stäbchen, with an average of 80 to 85% of stäbchen located in the terminal third of the three spine types (Fig. 3; Oral row, area c). It is also well marked for the aboral row of stäbchen on aboral type I spines and type II spines, with an average of 60 and 62% of stäbchen located in the terminal third, respectively (Fig. 3; Aboral row, area c). Finally, the aboral row on oral type I spines and the lateral rows of both type I spines present an average of 45 to 49% of stäbchen in the terminal third (Fig. 3; Aboral row and Lateral row, area c). Although the proportion of stäbchen located in the middle area of the spine tends to be slightly higher than that at the base of the spine, the difference is not significant whatever the type of spine and the row of stäbchen investigated (ANOVA and Kruskal-Wallis; Fig. 3, areas a and b).

Semi-thin sections show mid-blue cells with cell bodies at the base of the spine and projections directed towards the cuticle at the spine tip, more precisely towards an area rich in stäbchen (Fig. 2J & K). On TEM micrographs, processes of two types of non-pigmentary cells filled with granules of variable electron-density and oriented towards the cuticle can be seen. The end of these processes is usually seen in the distal or medial area of the arm spine. Some of these extremities are anatomically close to the cuticular stäbchen (Fig. 2L; schematised in Fig. 2M).

*Mechanical and chemical stimuli elicit luminescent responses in the brittle star *Amphiura filiformis**

The burrowing brittle star *A. filiformis* is known to produce light at the level of the arm spines (Fig. 4A & B). Application of CW on *A. filiformis* led to production of light (Fig. 4C) comparable to the light produced through acetylcholine (Ach) stimulation (Kruskal-Wallis, Wilcoxon comparisons test; p-value CW versus Ach = 0.065; Fig. 4C). Application of FSW did not trigger any visible light production (Kruskal-Wallis, Wilcoxon comparisons test; p-value FSW versus Ach = $2.7 \cdot 10^{-7}$; p-value FSW versus CW = $7.7 \cdot 10^{-8}$; Fig. 4C). CW itself did not produce any visible light (Kruskal-Wallis, Wilcoxon comparisons test; p-value CW control versus Ach = 0.0021; p-value CW control versus CW = 0.0021; p-value CW control versus FSW = 0.9654). Multiple peaks of light, with decreasing intensity,

were observed over an hour following CW application (Fig. 4D), while Ach application only resulted in a single series of flashes emitted in short intervals within the first 10 min (Fig. 4E).

Discussion

The bioluminescent brittle star *A. filiformis* features five arms, each divided into numerous segments adorned with spines and podia. While previous research has examined the morphology and ultrastructure of the spines, including the description of various spine morphotypes (Delroisse et al., 2017a), there remained a gap in understanding the morphology and ultrastructure of stäbchen, as well as the co-localisation of these sensory receptors with underlying internal structures and cells. Our morphological and ultrastructural description of the stäbchen from the spines, arm tips and podia is consistent with already described stäbchen in other species of ophiuroids. However, variations in the size of cuticular projections have been observed (e.g., Whitfield & Emson, 1983; Harrison & Chia, 1994; Ball & Jangoux, 1996; Morgan & Jangoux, 2004). Furthermore, the distinction between the long-cilium cylindrical and the short-cilium conical stäbchen is consistent with the stäbchen description made for the arm terminal segment of *Ophiothrix fragilis* (Abildgaard in O.F. Müller, 1789) (Morgan & Jangoux, 2004). The size of the projecting cilia could be indicative of the type of stimulus detected. It has been suggested that the long cilium receptors of the sea cucumber *Holothuria forskali* Delle Chiaje, 1824 (VandenSpiegel et al., 1995) and the brittle star *O. fragilis* (Morgan & Jangoux, 2004) are better candidates for distant chemoreception, as the long cilium increases the sensory surface area. It has also been suggested that short-cilia receptors are better suited to mechanoreception (VandenSpiegel et al., 1995) or contact chemoreception (Morgan & Jangoux, 2004).

Our micrograph analyses showed a distinct spatial arrangement of the stäbchen in four longitudinal rows positioned along the surface of the spines. In a previous ultrastructural description of the inner tissues of the arm spines in *A. filiformis*, Delroisse et al. (2017a) observed two rows of stäbchen along the spine lateral sides. Our results highlight a differential arrangement of stäbchen depending on the position of the spine along the arm as well as the spine morphotype. Stäbchen distribution in the integument of *A. filiformis* podia is congruent with the description of Keogh & Keegan (2006) on two *Amphiura* species. Our results highlight different stäbchen numbers

depending on the podia location (*i.e.*, along the arms versus close to the mouth). Finally, the stäbchen crown observed on the *A. filiformis* arm tip coincides with the crown distribution reported for the arm tip of *O. fragilis* (Morgan & Jangoux, 2004). Nevertheless, only one row was present around the terminal arm tip of *A. filiformis*. The presence and differing arrangement of the two distinct types of stäbchen raise questions about their potentially different functions, their respective intra-organ targets, and the signals they trigger.

Although further studies are needed to fully understand the exact function of these structures, their ultrastructural morphology and preliminary physiological analyses provide valuable insights into their role. Different cell types are present in the inner part of the spine, including the cells forming the spine nerve cord, mucocytes and two different types of granular cells that are mainly present at the base of the spine (Delroisse et al., 2017a). The granular cells were clustered into two distinctive types (*i.e.*, types I and II). Through the immunolocalisation of the luciferase (*i.e.*, the specific enzyme involved in the light emission process) within type II granular cells, these particular spine cells have been assigned as the photocytes of *A. filiformis* (Delroisse et al., 2017a). Besides, the spine photogenic region has been depicted as being surrounded by a pigmentary sheath (Delroisse et al., 2017a; Coubris et al., 2024). Delroisse et al. (2017a) hypothesised that this sheath, along with the spine skeleton, guides the light produced from the photocyte to the tip of the spine.

The internal cellular components (*i.e.*, cell body or cell projection of the sensory cell) of the spine stäbchen were located close to the granular cells resembling the type II granular cells (*i.e.*, photocytes) described by Delroisse et al. (2017a). The proximity between the granular cells (putative photocytes) and the presumed mechano/chemoreceptors is consistent with the histological description made for the same species by Buchanan (1963). In addition to the histological and ultrastructural observations, the significantly higher stäbchen number in the tip of the spine, highlighted by stäbchen distribution analyses, correlates with the localisation of the tips of photocyte processes in the same area of the spine (Delroisse et al., 2017a). The close physical association between the stäbchen-associated sensory cell and the photocyte led to the hypothesis of a functional role of these receptors in the physiological control of light emission. Stäbchen are thought to be involved in mechanical perception (*e.g.*, mechanical disturbance due to water flow, predation, contact with other animals) or chemoreception (*i.e.*, detection of specific molecules in the water surrounding the animal). Light production in *A. filiformis* can be

elicited by both mechanical and chemical signalling. A simple pinching of an arm induces a flash response (Duchatelet, personal communication). Moreover, our results demonstrate the sensitivity of *A. filiformis* to injured conspecifics, as it produces flashes of light in response to water conditioned with crushed individuals. The pattern of light emission in response to an alarm substance, with multiple repeated flashes, is different from the one obtained via the application of the classical neurotransmitter acetylcholine. Acetylcholine was previously demonstrated to be the main neurotransmitter triggering light emission in the brittle star *A. filiformis* (Dewael & Mallefet, 2002; Coubris et al., 2024). The arm tip of *A. filiformis* also contains luciferase immunoreactive cells arranged in a cluster of about 20 putative photocytes (Delroisse et al., 2017a). The presence of a crown of stäbchen at the level of the arm tip, where the large photocytes cluster is located, correlates with a role in light emission control.

As demonstrated by Rosenberg & Selander (2000), the perception of chemical cues from injured conspecifics by *A. filiformis* triggers a behavioural response in which the ophiuroids lower their arms along the substrate. Our results also highlight the production of flashes over an extended period in response to the same cues. This pattern of light emission contrasts with that induced by acetylcholine. While a single flash is often considered as a way to startle predators, an extended succession of flashes may attract attention to the emitter. Although this may seem counter-intuitive as a response to an alarm substance from an individual point of view, it can make sense in high-density populations. It can be interpreted in the context of the burglar alarm hypothesis, defined as the production of light by an organism during a predation event to attract the attention of a higher predator and thus reduce the predation pressure on a community or population (Haddock et al., 2010). The burglar alarm stratagem has been experimentally demonstrated for different luminous species, notably dinoflagellates (*e.g.*, Abrahams & Townsend, 1993; Huang et al., 2024). As *A. filiformis* lives in dense populations, with hundreds of individuals per square metre (Sköld et al., 1994; Rosenberg et al., 1997), warning conspecifics of predation events by releasing chemical cues and triggering a luminous response from conspecifics may help reduce the predation risk by (i) disturbing the predators or (ii) attracting the attention of higher predators. Further ethological studies are needed to test these hypotheses.

While we provide morphological evidence suggesting the involvement of stäbchen in the regulation of bioluminescence, other functions cannot

be excluded. In *O. fragilis*, the crown of stäbchen at the tip of the arm senses the presence of conspecifics through chemosensitivity (Morgan & Jangoux, 2004). This is used to favour the aggregation of conspecifics. Stäbchen located within the podial integument, a non-photogenic structure, may function in the detection of dietary sources and regulation of mucus production, as suggested for the stäbchen of the podial papillae of *Ophiocomina nigra* (Abildgaard in O.F. Müller, 1789) (Ball & Jangoux, 1990). Podial papillae, covered by sensory-secretory complexes, function as adhesive surfaces for food collection in suspension-feeding brittle stars (e.g., Warner & Woodley, 1975; Keogh & Keegan, 2006). Captured particles may trigger a signal that stimulates mucus release, which then packages the particles for transfer to the mouth (Ball & Jangoux, 1990; Keogh & Keegan, 2006), and captured particles might send a signal to initiate release of mucus that package those particles for transfer to the mouth (Ball & Jangoux, 1990; Keogh & Keegan, 2006). The potential involvement of these stäbchen in food perception would also explain their greater number on the surface of the buccal podia.

In conclusion, based on the pattern of distribution and morphology of stäbchen, we can hypothesise their functions: (i) the spine and arm tip associated stäbchen could be used to assess environmental cues through the perception of mechanical disturbances and chemical substances (possibly thanks to the short- and long-cilium types, respectively) and, in response, trigger light emission; (ii) long-cilium stäbchen of the arm tip might also enable recognition of conspecifics; and (iii) tube foot-associated short cilium stäbchen (and to a lesser extent, the spines and the arm tip ones; Woodley, 1975) could be involved in the perception of food sources, through chemical contact and/or mechanoreception and respond by producing mucus.

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