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Eastern Pacific scorpionfish *Scorpaena mystes* and *Scorpaena plumieri* (Perciformes: Scorpaenidae) from the coast of Brazil: two geminate species with very similar venoms

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Abstract

Some studies have shown phylogenetic proximity between the scorpionfish species *Scorpaena plumieri* and *Scorpaena mystes*, despite having inhabited different oceans for millions of years. The former occurs in the Western Atlantic Ocean, including the Caribbean Sea, and the latter inhabits the Eastern Pacific. The venom of *S. plumieri* from tropical Atlantic reefs has been widely studied, whereas that from *S. mystes* has not been characterized. To determine whether the venom of *S. mystes* reflects the previously shown phylogenetic proximity between these species, we performed a proteomics study and analysed its biochemical activities. We identified proteins with similarity to the characteristic cytolysin α- and β-subunits of *S. plumieri*, as well as putative pore-forming toxins from the venom of other scorpionfish species. As in many scorpaenoid taxa, several of the identified components are coming from the epidermal mucus, produced by mucus glands, leukocytes or other potential organisms living on their skin. This is the case of the mucus B- and C-type lectins identified in *S. mystes*, which are homologues to those from *S. plumieri*. The analysed venom also displays hyaluronidase and proteolytic activities, and can induce hemolysis, hemagglutination and nerve membrane permeabilization. In conclusion, regarding their venoms, *S. mystes* and *S. plumieri* appear to be closely related lineages inhabiting different marine ecosystems.

KEYWORDS
cytolysin, geminate species, lectin, Scorpaenidae, scorpionfish, venom

1 | INTRODUCTION

Coral reef ecosystems are among the most diverse and productive marine environments on the planet, playing crucial roles in coastal protection and nutrient cycling. Reef-associated fish represent an extraordinary component of their biodiversity, exhibiting taxonomic richness across multiple lineages. This remarkable diversity has resulted from a combination of evolutionary processes, ecological interactions (including anthropogenic influences) and historical biogeographic events that affect species composition and distribution (Dubuc et al., 2023).

The family Scorpaenidae comprises 388 valid species (Eschmeyer's Catalogue of Fishes, 2025), some of which are commonly known as scorpionfish, important members of reef communities (Poss, 1995, 2003). They are marine predatory fish characterized by large mouths and eyes, as well as bodies with cryptic features, including several skin ornaments (Paolino et al., 2023; Tripp-Valdez et al., 2023). Some species, such as *Scorpaena porcus* and *Scorpaena maderensis*, even respond to changes in habitat by adjusting body coloration through regulation of skin chromatophores. This would allow them to hide from predators and prey by wearing dynamic camouflage (John et al., 2024).

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Scorpionfish, like other scorpaenoid taxa, possess sharp spines on their dorsal, pelvic and anal fins (Wibowo & Motomura, 2021). The venom is produced in holocrine glands located in grooves inside the spines (Harris, 2023).

Only a few species have been studied regarding venom secretions and toxin composition (Borges et al., 2018; Schaeffer Jr et al., 1971; Xie et al., 2019). The stone scorpionfish, *Scorpaena mystes*, is a benthic fish found in the shallow waters (Jakes-Cota et al., 2017) of the Eastern Pacific coasts of Southern California and Baja California, extending throughout Central and South America to the shores of Chile (Poss, 1995). It is an opportunistic cryptic predator commonly present on sandy sea floors and seaweed-covered reefs (Tripp-Valdez et al., 2023) (Figure 1). Its average size is around 17 cm, and its coloration is described as a mottled pattern with dark and light brownish, grey and black areas (Poss, 1995) (Figure 2).

Cytogenetic and molecular data have suggested that *S. mystes* is a sister species of the spotted scorpionfish *Scorpaena plumieri*, which occurs in the western Atlantic, including the Caribbean Sea (Nirchio et al., 2016). Within *S. plumieri*, however, two different cytogenetic and molecular lineages have been identified: one in the Florida coast and the Caribbean, and the other in Venezuela and the Atlantic coast of South America, with the first group being more closely related to *S. mystes* than to its conspecific lineage (Nirchio et al., 2016). Even though the two *S. plumieri* cytogenetic lineages are currently considered only subspecies, these studies suggest that they could be sympatric cryptic species. The separation of *S. mystes* and the two *S. plumieri* populations has been attributed to the emergence of the Central American Isthmus and the Amazon-Orinoco Barrier (Araujo et al., 2022; Nirchio et al., 2016). In the case of the rise of land barriers such as the Isthmus, different conditions of salinity, temperature, productivity and seasonality between the two coasts of Central America, for instance, could have significantly impacted the evolution of the marine biota, including reef fish, inducing molecular divergence (Lessios, 2008; Marko, 2002). The first molecular evidence that suggested the rise of geminate species due to the Isthmus-related interruption of gene flow was provided by Vawter et al. (1980).

The venom of *Scorpaena plumieri* from the Brazilian coast has been widely studied due to its medical importance (Campos et al., 2016; Carrijo et al., 2005; Lopes-Ferreira et al., 2025). In contrast, the venom of the *S. plumieri* population from the Caribbean and *S. mystes* has not been analysed. *Scorpaena brasiliensis*, commonly known as barbfish, is another scorpionfish associated with human envenomation accidents (Bernardes Filho et al., 2019; Haddad et al., 2003), whose venom has not been characterized.

The most prominent toxins in scorpaenoid fish venoms are the pore-forming cytolysins (Kiriake et al., 2013). *S. plumieri* scorpionfish venom contains these toxins (Andrich et al., 2015; Borges et al., 2018; Costa et al., 2018; Gomes et al., 2013, 2016). Cytolysins have been associated with several aspects of scorpionfish envenomation, including pain, inflammation, oedema and cardiovascular alterations (Andrich et al., 2010; Campos et al., 2021; Malacarne et al., 2018). The genes of this family of cytolysins have also been identified in other scorpionfish species, such as *Sebastapistes strongia*, *Scorpaenopsis oxycephala* and *Sebasticus marmoratus* (Chuang & Shiao, 2014), but no functional studies have been performed in their venoms. Interestingly, transcriptomics analysis of three *Scorpaenopsis* species revealed the presence of cytolysin transcripts in Indo-Pacific *S. possi*, corresponding to neoverrucotoxin from the stonefish *Synanceia verrucosa*, and PI toxin-b, from the lionfish *Pterois volitans* (Xie et al., 2019).

The other representative toxins present in *S. plumieri* spine extracts are B- and C-type lectins, which could display pro-inflammatory and defence activities against pathogens (Andrich et al., 2015; de Santana Evangelista et al., 2009; Fiorotti et al., 2022). Borges et al. (2018) have suggested that the skin mucus glands are the ones producing these proteins since only cytolysins and hyaluronidase have been identified exclusively in venom gland secretions. Moreover, several lectins have been identified in venomous and non-venomous fish skin mucus (Brinchmann, 2016).

We aimed to determine the potential similarities in venom composition of the tropical Eastern Pacific *S. mystes* from the coasts of Costa Rica, compared to the available data of the very well-characterized *S. plumieri* venom from the shallow waters of the tropical Atlantic shores of Brazil. The presence of potential similar venoms could be

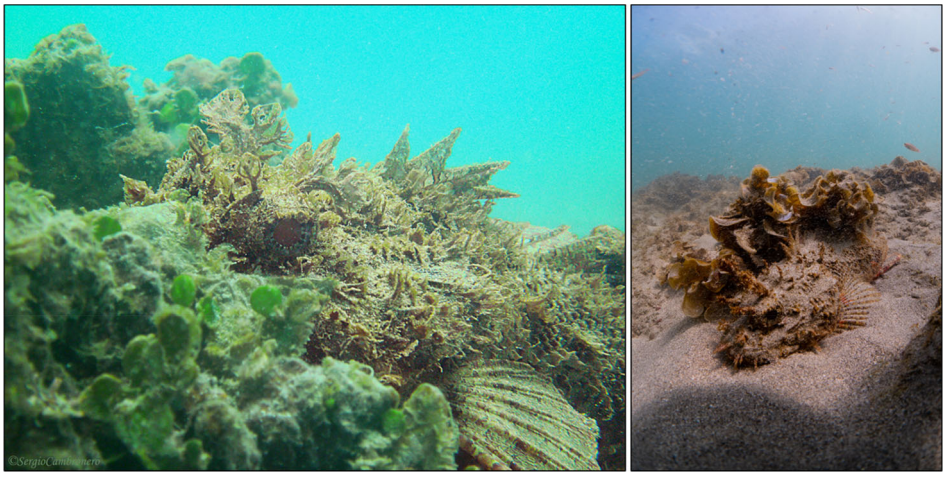


FIGURE 1 Scorpionfish *Scorpaena mystes* from the Pacific coasts of Costa Rica. Scorpionfish observed at seaweed-covered sandy reefs from Samara beach, Guanacaste Province, Costa Rica. The pictures are courtesy of Sergio Cambronero-Solano. Their cryptic characteristics and sedentary behaviour make them very difficult to photograph in their natural habitats.

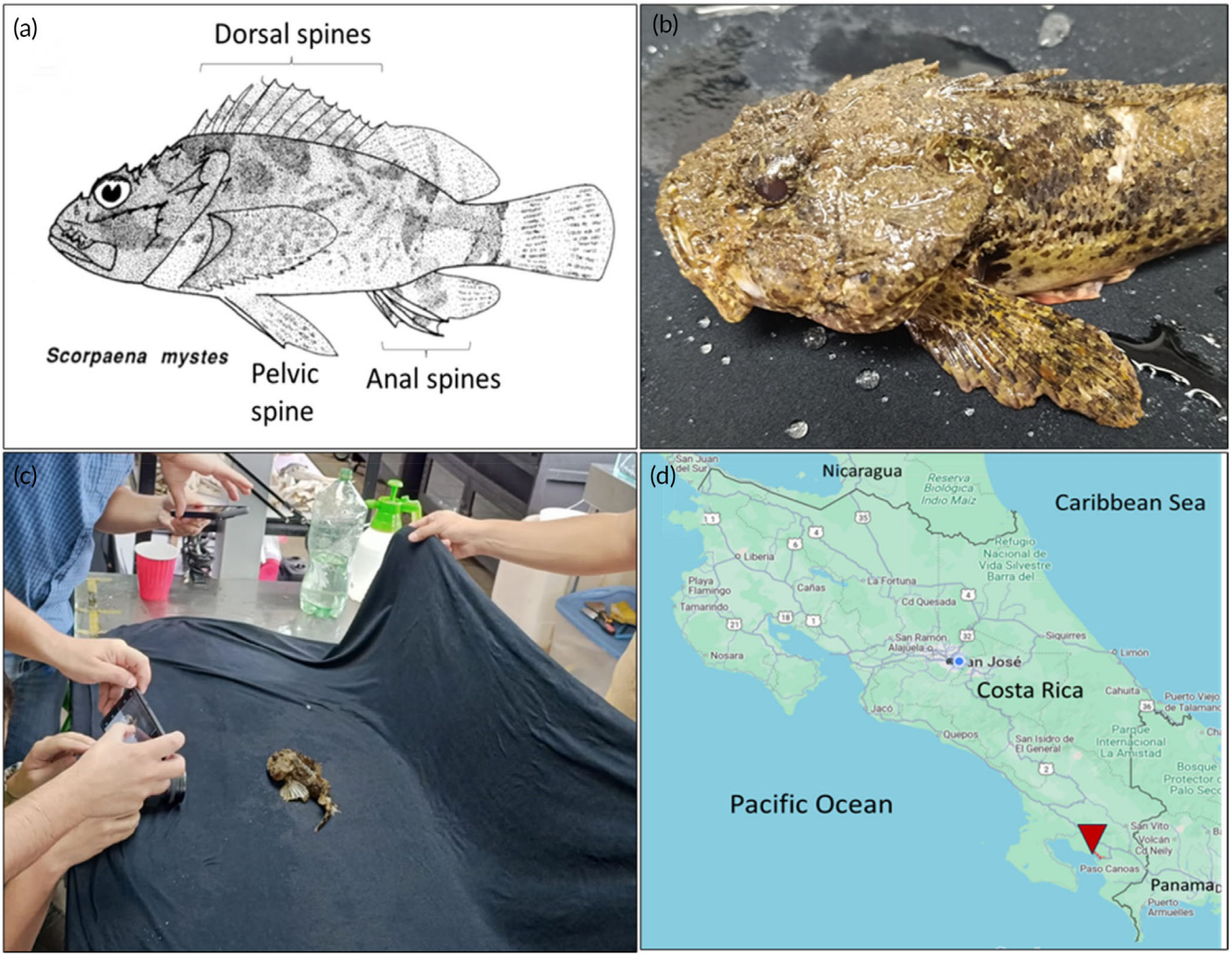


FIGURE 2 Collected *Scorpaena mystes* scorpionfish from the Pacific coasts of Costa Rica. (a) Scorpionfish drawing modified from Bussing and López (1999). (b) Scorpionfish specimen collected in Golfito Bay (Puntarenas Province, at the Pacific South of Costa Rica), photographed at the Laboratorio para la Investigación de Animales Peligrosos facilities of the Instituto Clodomiro Picado, Universidad de Costa Rica. (c) Image of a collected specimen shown as a reference for the appreciation of the fish size. (d) Map of Costa Rica with the collecting location indicated. This image was modified from Google Maps.

strengthened by their phylogenetic relationships and the possibility that both species derived from a common ancestral fish that separated into two different populations at least 3–15 million years ago.

2 | MATERIALS AND METHODS

2.1 | Fish collecting and preparation of spine extracts

Two adult scorpionfish specimens were collected at the Pacific coast of Costa Rica, at Golfito Bay (Province of Puntarenas) (Figure 2). Fish were anaesthetised and euthanized by introducing them into clove oil-containing water. After that, specimens were frozen and spines (dorsal, anal and pelvic) were cut and placed in cold distilled water for processing. Then, maceration was performed in an ice-cold ceramic mortar, preserving toxin activity. To eliminate debris, samples were centrifuged and frozen at −80°C.

2.2 | Electrophoresis and zymography

Venom samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12%) under reducing conditions (5% 2-mercaptoethanol) in a Bio-Rad Mini-Protean system at 150–180 V, and proteins were stained with Coomassie blue R-250.

To study the proteolytic properties of the spine extract, gelatin zymography was carried out. Briefly, we analysed venom samples by SDS-PAGE under non-reducing conditions on 12% gels containing 0.25 mg ml^{-1} of gelatin. Gels were washed with 1% Triton X-100 at room temperature to remove the SDS detergent, and then an overnight incubation in 50 mM Tris–HCl buffer at 37°C was performed. Gels were stained with Coomassie Blue R-250.

Hyaluronidase activity present in the venom was determined by the method reported by Cevallos et al. (1992) based on SDS-PAGE in a 12% gel containing 0.5 mg ml^{-1} hyaluronic acid from rooster comb. The incubation buffer (0.1 M NaCl, 0.1 M sodium phosphate) was

adjusted to pH 6.6, the optimal pH for fish hyaluronidases. Gels were stained with Alcian Blue.

2.3 | Direct haemolytic activity and haemagglutination

Rabbit blood was collected by ear vein cannulation, and erythrocytes were obtained by centrifugation of the citrated blood, previously washed with phosphate-buffered saline (PBS), and used for the hemolytic assay. Venom, previously dissolved in PBS, was incubated with a 10% erythrocyte suspension and absorbances were read at 545 nm to determine haemolysis.

The haemagglutination activity was performed according to the method of Fiorotti et al. (2022). Briefly, we incubated 2% rabbit erythrocytes in PBS with different venom concentrations and observed the net formation.

2.4 | Snail neuron experiments of membrane permeabilization

Juvenile specimens of *Helix aspersa* snails were provided by a local breeder. The animals were kept in ventilated plastic boxes at 20°C and under a 12-h light–dark cycle. They were fed with vegetables and chicken supplemented with calcium and water ad libitum. The experiments were conducted under the approval of the Institutional Animal Care and Use Committee of the University of Costa Rica.

Cell cultures were performed as previously described by Ghirardi et al. (1996) and modified by Brenes et al. (2015). Briefly, the snails were anaesthetised with a 0.1 M $MgCl_2$ isotonic solution injected into the foot and euthanized by evisceration through shell removal. Cerebral and buccal ganglia were surgically isolated and incubated at 34°C for 4 h in modified L15 solution containing 0.4 U ml^{-1} protease type XIV. After enzymatic digestion, ganglia were washed twice in modified L15 solution and neurons (C1, B1, B2, B3 and B4) were individually isolated based on their anatomical localization and morphology. After isolation, they were immediately plated on Petri dishes pretreated with poly-L-lysine and *Aplysia* hemolymph.

S. mystes venom and propidium iodide were directly applied to the culture dish containing the neurons and incubated at 37°C for 1–2 h, followed by a period at room temperature for 6 and 24 h. Fluorescence was monitored at 600 nm, and cell images were captured through an inverted microscope (Olympus CKX53) equipped with optical epifluorescence.

2.5 | Size-exclusion HPLC

Anal spine extract (2 mg) was separated by size-exclusion high-performance chromatography (SEC-HPLC) in a S2000 BioSepSEC column (10 × 300 mm), equilibrated with 0.06 M NaCl, 0.02 M sodium phosphate buffer (pH 7.2), at 1 ml min^{-1}, in an Agilent 1220 chromatograph, monitoring at 215 nm. Fractions were dried in a SpeedVac and separated by SDS-PAGE under reducing conditions. Some of the gel bands were analysed by mass spectrometry (MS), as indicated below, to identify the proteins present in the fractions.

2.6 | Mass spectrometry

A shotgun MS approach was performed to analyse the dorsal, anal and pelvic spines' venom composition. The SDS-PAGE concentrated band containing the whole venom was reduced in solution with 10 mM dithiothreitol for 30 min at 56°C, alkylated with 50 mM iodoacetamide for 20 min and digested overnight with trypsin at 37°C. After adding formic acid, the resulting tryptic peptide mixture was centrifuged and subjected to reverse phase chromatography on a nano-Easy 1200R chromatograph (Thermo) in line with a Q-Exactive PlusR mass spectrometer (Thermo). Then, 10 µL of peptide mixture was injected on a C18 trap column (75 µm × 2 cm, 3-µm particle; PepMap, Thermo), washed with 0.1% formic acid (solution A) and separated at 200 nL min^{-1} on a C18 EasysprayR column (75 µm × 15 cm, 3-µm particle; Thermo). A gradient toward solution B (80% acetonitrile, 0.1% formic acid) was developed in a total of 120 min (1%–5% B in 1 min, 5%–26% B in 84 min, 26%–80% B in 30 min, 80%–99% B in 1 min and 99% B for 4 min). MS spectra were acquired in positive mode at 2.0 kV, with a capillary temperature of 200°C, using one µscan in the range 400–1600 *m/z*, maximum injection time of 50 ms, automatic gain control (AGC) target of 1 × 106 and resolution of 70,000. The top 10 ions with two to five positive charges were fragmented with an AGC target of 3 × 106, minimum AGC 2 × 103, maximum injection time 110 ms, dynamic exclusion time 5 s and resolution 17,500. MS/MS spectra were processed against protein sequences in the UniProt/SwissProt database using Peaks XR (Bioinformatics Solutions) and matches were assigned to known protein families based on sequence similarity. Cysteine carbamidomethylation was set as a fixed modification, while deamidation of asparagine or glutamine and methionine oxidation were set as variable modifications, allowing up to three missed cleavages by trypsin. Parameters for match acceptance were set to false discovery rate <0.1%, detection of at least one unique peptide and −10 lgP protein score ≥50.

The same procedure was followed to analyse the proteins present in SEC-HPLC fractions obtained from the anal spine extract.

2.7 | Ethical statement for any submissions to the *Journal of Fish Biology*

The care and use of experimental animals complied with the National System of Conservation Areas (SINAC) guidelines from the Ministry of Environment and Energy (R-SINAC-SE-DT-PI-031-2024), as well as animal welfare laws, guidelines and policies. The study was also approved by the Biodiversity Committee of the University of Costa Rica (CBio-46-2022 and CBio-29-2025).

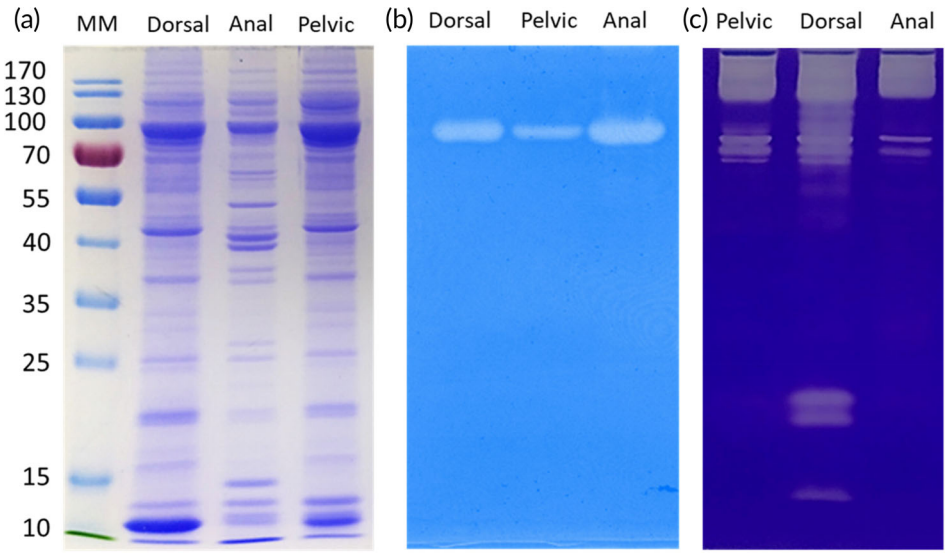


FIGURE 3 SDS-PAGE and zymography of *Scorpaena mystes* venom extracts. Extracts from three different scorpionfish spines (dorsal, anal and pelvic) were analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions (a). Zymography on hyaluronic acid (b) and gelatin (c) was performed with the scorpionfish spines' extract to determine hyaluronidase and proteolytic activities.

3 | RESULTS

3.1 | Biochemical characterization of *S. mystes* venom

The extract obtained from the maceration of *S. mystes* dorsal, anal and pelvic spines, which contain venom and epidermal mucus, was separated by SDS-PAGE under reducing conditions. Figure 3a shows that the three types of spines contain several proteins, including significant bands with molecular weights corresponding to cytolysins (~65–80 kDa) (Campos et al., 2021). There are also low-mass peptides between 10 and 15 kDa, some of which may correspond to venom components such as lectins (Campos et al., 2016; de Santana Evangelista et al., 2009). We observed that all *S. mystes*-tested spines display hyaluronidase activity through a single band on hyaluronic acid zymography (Figure 3b).

Since the scorpionfish sister species *S. plumieri* contains several proteases (Borges et al., 2018; Carrijo et al., 2005; Tenorio et al., 2016), we determined the proteolytic activity of *S. mystes* spine extracts on gelatin. The three types of spines display proteolytic activity. The pattern primarily indicates the presence of high-molecular-weight proteases, with some low-mass components observed only in the dorsal scorpionfish spines (Figure 3c). These differences could be explained by variations in epidermal mucus composition at different parts of the fish anatomy, and the presence of skin parasites and symbiotic organisms could influence that (Ballantine et al., 2001; Fernández-Del Valle et al., 2017).

3.2 | Haemolysis, haemagglutination and neuron permeabilization of *S. mystes* venom

Direct haemolytic activity of the spine extract (from the three types of spines) was observed on rabbit erythrocytes, as reported in the literature for most fish venoms (Ziegman & Alewood, 2015). These extracts could also agglutinate rabbit erythrocytes (not shown). The dorsal spine extract was also tested on snail neurons to determine the effect of the venom on cell membrane integrity. At 6 h, the venom induced a weak effect permeabilizing the membranes to propidium iodide (by labelling the nuclei), but its entrance was more evident after 24 h of treatment (Figure 4).

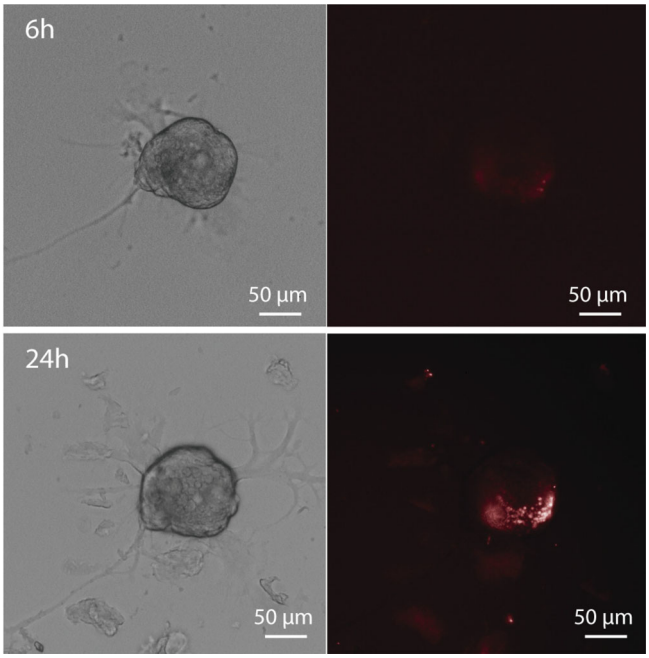


FIGURE 4 Membrane permeabilization to propidium iodide of primary snail neuron incubated with *Scorpaena mystes* dorsal spine extract. Two different time points of a representative experiment performed in nine independent neurons are presented. Fluorescence was monitored at 600 nm and cell images were captured through an inverted microscope equipped with optical epifluorescence.

3.3 | Proteomics analysis of *S. mystes* venom

The shotgun-MS analysis of *S. mystes* spine extracts revealed the presence of cytolysins with similarity to *S. plumieri* α- and β-subunits

TABLE 1 Proteomic analysis of *Scorpaena mystes* venomous spine extract.

Matching protein, species	Protein family	Accession	MW
1. Cytolytic toxin-α, *Scorpaena plumieri*	Toxin, cytolysin	A0A2P1BRQ0	79.8
2. Cytolytic toxin-β, *Scorpaena plumieri*	Toxin, cytolysin	A0A2P1BRP3	80.1
3. Tx α-subunit, *Scorpaenopsis oxycephala*	Toxin, cytolysin	A0A068BD77	79.7
4. Tx β-subunit, *Scorpaenopsis oxycephala*	Toxin, cytolysin	A0A068BD90	79.7
5. Tx α-subunit, *Sebastapistes strongia*	Toxin, cytolysin	A0A068BIV4	79.4
6. Tx β-subunit, *Sebastapistes strongia*	Toxin, cytolysin	A0A068BIW2	79.6
7. Tx α-subunit, *Sebastiscus marmoratus*	Toxin, cytolysin	A0A068BET6	79.2
8. Tx α-subunit, *Dendrochirus zebra*	Toxin, cytolysin	A0A068BGX9	66.1
9. Tx β-subunit, *Dendrochirus zebra*	Toxin, cytolysin	A0A068BD83	66.9
10. Bryoporin, *Gasterosteus aculeatus*	Toxin, porin	G3P0X9	19.8
11. SCP-domain containing protein, *Perca fluviatilis*	GAPR1-like	A0A6A5EHY1	16.8
12. B-type lectin plumieribetin, *Scorpaena plumieri*	Lectin	P0DQV9	12.8
13. C-type isolectin Sp-CL4, *Scorpaena plumieri*	Lectin	P0DQV8	16.5
14. Galectin, *Blennius fasciatus*	Lectin	A0A673AQX0	15.0
15. Galectin, *Triplophysa rosa*	Lectin	A0A672GB85	25.4
16. Bulb-type lectin domain-containing protein, *Gasterosteus aculeatus*	Lectin	A0AAQ4QJJ6	13.6
17. Bulb-type lectin domain-containing protein, *Amphiprion percula*	Lectin	A0A3P8SWS8	13.4
18. Lysozyme C-like, *Labrus bergylta*	Glycosidase	A0A3QGBQ4	16.0
19. Glyceraldehyde-3-phosphate dehydrogenase, *Stegastes partitus*	Oxidoreductase	A0A3B5BBR4	36.0
20. Glutathione peroxidase, *Acanthochromis polyacanthus*	Oxidoreductase	A0A3Q1FA52	19.9
21. Superoxide dismutase, *Lepisosteus oculatus*	Oxidoreductase	W5MS000	16.0
22. Peroxiredoxins 1–6, *Gasterosteus aculeatus*	Oxidoreductase	G3PNW3	24.5
23. Serpin peptidase inhibitor, *Dicentrarchus labrax*	Protease inhibitor	A0A8C4F6W7	57.5
24. Serine peptidase inhibitor Kunitz, *Lates calcarifer*	Protease inhibitor	A0A4W6ED60	40.7
25. Cystatin B, *Anoplopoma fimbria*	Protease inhibitor	C3KHH1	11.2
26. Alpha-2 macroglobulin like-, *Gymnodraco anticeps*	Protease inhibitor	A0A6P8VXK9	161,1
27. Peptidase M14 carboxypeptidase A	Proteinase	A0A6A5E111	49.5
28. Thimet oligpeptidase, *Labrus bergylta*	Proteinase	A0A3Q3GNY2	71.7
29. Prolyl endopeptidase, *Anabas testudineus*	Proteinase	A0A7N6BGM0	77.3
30. Cathepsin B, *Oplegnathus fasciatus*	Proteinase	F4YIN3	36.3
31. Aspartyl aminopeptidase, *Cyclopterus lumpus*	Proteinase	A0A8C2XR70	48.9
32. Peptidase S1, *Synaphobranchus kaupii*	Proteinase	A0A9Q1IAR5	81.7
33. Peptidase M20, *Hemibagrus wyckioides*	Proteinase	A0A9D3N9G4	53.0
34. Peptidase D, *Mastacembelus armatus*	Proteinase	A0A3Q3LDD6	55.1
35. Dipeptidyl peptidase 1, *Fundulus heteroclitus*	Proteinase	Q6JZZ3	49.9
36. Fatty acid binding protein, *Kryptolebias marmoratus*	Lipocalin	A0A3Q3A20	15.1
37. Phosphatidylethanolamine-binding protein, *Lates calcarifer*	Regulator	A0A4W6EA57	19.7
38. Hemopexin, *Seriola dumerili*	Haeme transport/toxin	A0A3B4U5F8	46.8
39. Serotransferrin, *Perca fluriatilis*	Iron transport	A0A6A5F5F9	73.6

Note: The list includes the putative toxins found in dorsal, anal and pelvic spines. Accession numbers correspond to the best matches in the UniProt/SwissProt database.
Abbreviation: SCP, cysteine-rich secretory.

as well as putative venom pore-forming toxins from other scorpionfish species, such as *Scorpaenopsis oxycephala*, *Sebastapistes strongia*, *Sebasticus marmoratus* and lionfish *Dendrochirus zebra*, all members of the Scorpaenidae family (Table 1).

Figure 5 shows the cytolysin homologue sequences recovered from *S. mystes* venomous spines with the identified peptides. Highlighted in blue are the unique peptides and in grey are those identified in more than one UniProt/SwissProt database-deposited

Cytolytic toxin-alpha, *Scorpaena plumieri*

MSSDIIMAGLGRPFTLGFLYDARREKLIPGFSLFGDETLQKYATSTPQIISSDFQIVASDSVESKSNVMDIEASLGVSFLGGLVEVGGSAKYLNNTKKYQNQSR
VTLQYKVTTTFKQFKAPPGKVNVQQTAISDKNVATHVVTAILFGANAFFVFDSDKVEDSNLQDIQGKMEAVIKKIPSVSIEGSGSVQLTDEEKSLASNLSCKF
HGDFLLESLPTTFEEAVMTYQKLPELVGEEASDGVPMKVWLVPLTRFYSKADLLVRDISQGLVRKVHSILEDLHKLKRRANDSLEDDTVKLFPLLEKKLKN
FQKNYSDYMTTFRRTISQKLQSIRKGDEDETAMLQLFEDRLRSPFNIDSLNMWMEITEREINVLRSCIDIIEETKHKAVLSQSQMVKDLLDSEVMHAVCYVFT
YVTDKDHYLDALRDYLKSPNSRPARVRPVVTWVASNTVLETMREKAHLFRSLAKDMENRCVHFLVASIVNLKVEGAAIHYYRESVLIEPNFKHPIISAVEKI
VDRRDLLWYDCELTLDPNTSHPSLYLSEGNKKAVTGTLRAFDNNPERFGLWQQVLCNKGLSRRHYWEVEWNGYVIVGVTYSSIGRKNIDIQSFIGFSETSW
TLMFIPKNGVAVKGARRSVSYYFISDLAFPPLGLYHDCHASTLSFYKVSDNVLNHFHTIEIKPKLSEPVYAIIRIGDEDRPYHGTVRLL

Cytolytic toxin-beta, *Scorpaena plumieri*

MPSDILVVAALGRPFTLGALYDARKDKLYPGFTLWEHEVLEESTVESDQPSSTFEITASDSIDDKSSLMDIEASLKASFLGGLIEVGGSAKYLNDTKKFKNQS
RVTLQYKATTSFKQLMTNLETKHVEYSEYFQNIEATHVVIGILYGANAFFVFDSDKVDSSNVQDIQGSMEAVIKKIPSVEISGQGSVQLTSEESDITNSFSCKF
HGDFHLPSNPTTFEDAVKTYQQLPQMMGKETAVPMTVWLVPMTNFYSEAPQLMADSSTPILRKVRNTLEAMRQLDMRCNDSLERRHSEAGFHCLKKKLK
TFQKHYERLHVNPFRKNHFPETFSPSGKGTKMKLQCLPTFRNKLRSPSNINSLNMWMDCAEREINVLRSCIDIIEEAKHKVVLSKSQMARELDDSEVKHAVC
YVFTYVTDYDPFLNALSDFSKSIKPKKYSPSKKDYWYTSDDVPEMMREKAHHFYNLAKDMENRCVRFLVASIVNPKEEGAAIHYYREGIQIINDFSNPRIPP
VETIQDQESYSGMTVSSPWKETAHPALHLSEGNKKAMSGKPQPSDNNPKRFDHYQQVLCNKGLSKRHYWEVEWCGYVRAGITYKGIQRKTFASECSLGHT
DMSWVFDYYPKSGYHHIYNNKKVRVKVASPGFDRLGVYLDWPAGTLSFYMVTSTWVTHLHTFSIRFNEAVYPAFLIGHGQKNANGQIKLKGE

Tx alpha-subunit, *Scorpaenopsis oxycephala*

MSSDIMIMAGLGRPFTLGMLYDARREKLIPGFSLFGDETLQQYATSTPQRSSEFQIVASDSVESKSNLMDIEASLGVSFLGGLVEVGGSAKYLNNTKKYQNQS
RVTLKYKVTTTYQQFKAPPGKVNVQQTAITDKDVATHVVTAILFGANAFFVFDSGKLEDSNLQDIQGKMEAVIKKIPTVSIEGFGSVQLTDEEKSLASNLSCKF
HGDFLLESLPTTFEDAVMTYQKLPELLGEEATDAVPMKVWLVPLKKFYSQADLLVRDISQGIVRKVHSILELHEQRRRCNEAMDDRLVKQLPLIYDRVSNF
QQIFQDYILTVQQTIAKKLPLIREGTEEESSLEEIIEDRANSPFSNENVNIWLDNIEREIGSLKACAGILEKTQTKFVSNQTELDREVLAENVKHALCFVFTYMER
NDPYLRVLSQYLESPDTEITGDVAPSVENNSRMVIKMKQKALAFSEFAKKLEKSRNVHFLAAAITNGKYQGASIYHYKEGSLVTEDFTFPPLAPVKTIKDRSD
LLWYACDLTFDPNTAHQQLALSDDNKYVESGKHQKYSKHPERFDNYRQVLCKEPLRGRHYWELEWNGTVIAGVTYLSIGRKNIDIQSVIGFNETSWTFISFP
KNGYFIKGTGIELLRVGLPDPDFTRLGVYLDCHASTLSFYKVSSNVLHHFHTIEIKPKLSEPVYAIIRIGDVDRPYHGRARLL

Tx beta-subunit, *Scorpaenopsis oxycephala*

MSSDILVLAALGRPFTLGTLYDARKDKLIPGFTLWEDEVLQENTVESAQPSSVFEITASDSIEDKSSLMDIEASLKASFLGGLVEVGGSAKYLNDTKKFKNQS
RVTLQYKATTSFKQLMTNLETKHVEYSEYFQNIEATHVVIGILYGANAFFVFDSDKVDSSKVQDIQGSMEAVIKKIPSVEISGQGAVKLTSEESDITNSFSCKF
HGDFHLTSNPTTFEDAVKTYQQLPQMMGKETAVPMTVWLVPMTNFYSEAPQLMADSSTPILRKVRNTLEAMRQLDMRCNDSLEDDTVKLFPLLEKKLKT
FQKHYRDYMTNLRRTISQKLQSIRKGDEDESAMLQLFEDKLRSPFNIDSLNMWMECAEREINVLRSCIDIIEEAKHKVVLSQTKLDREVLDDSEVKHAVCYIFT
YVTDYDPYLNALSDFSKSIKPKKFSPSKKDYWYTSDDVPEMMREKAHHFYNLAKDMENRCVRFLVASIVNPKEEGAAIHYYREGIQIINDFSNPRIPPVETIQ
DRRELLWYDCELTLDPETAHPALHLSEGNKKAMSGKPQPSDNNPERFDHYQQVLCNEGLSKRHYWEVEWCGYVRVGITYKAIQRKTSDSVNAVGQNLAS
WAFDYDPKSGYCHIYGNEKTPVKVASPGFDRLGVYLDWPAGTLSFYMVTSTWVTHLHTFSAKFYEAVYPAFLIGDAQQKTNGQIKLL

Tx alpha-subunit, *Sebastapistes strongia*

MSSDIMVMAGLGRPFTLGMLYDARREKLIPGFSLFGDETLQQYATSTPQRSSEFQIVASDSVESKSNLMDIEASLGVSFLGGLVEVGGSAKYLNNTKKYQNQS
RVTLKYKVTTTYQQFKAPPGKVNVQQTAISDKNVATHVVTAILFGANAFFVFDSDKLEDSNLQDIQGKMEAAIKKIPTVTIEGSGSVQLTDEEKSLTSNLSCKF
HGDFLLESLPTTFEDAVTTYKTLPELLGEEATDAVPMKVWLVPLRKFYSQADLLVRDISQGIVRKVQSILEDLIIEQRRRCNEAIDDRLVKQLPLIIIDRVSNFQQ
LFQDYILTVQQTIAKKLPLIRDGTEEESSLEEITEDRANSPFSNENVNIWLDNIETEIGSLKACAGILEKTQTKFVSNQTELDREVLAENVKHALCFVFTYMERN
DPYLRVLAQYLESPDAEIPGNVAPSVENNSRMVIKMKQKALDFSEFAKKLEKSRNVHFLAAAITNGKYQGASIYHYKEGSLVTEDFTFPPMAPAKTIKDRSDL
LWYACDLTFDPNTAHQVLALSDDNKYVESGKQQNYSKHPERFENHRQVLCKEPLRRRHYWEVEWNGFVIVGVTYSSVGRKNLDIQSVIGFNETSWTLMSF
PRNGFFVKGADMSPVRIGLPDPDFTRLGVFLDHHASTLSFYKVSSNVLHHFHTIEIKPQLSEPVYAIIRIGDVDVPLHGSVRLL

Tx beta-subunit, *Sebastapistes strongia*

MSSDILVVAALGRPFTLGTLYDARKDKLIPGFTLWEDEVLQENTVESAQPSSVFEITASDSIEDKSSLMDIEASLKASFLGGLVEVGGSAKYLNDTKKFKNQS
RVTLQYKATTSFKQLMTNLETKHIVEYSEYFQNIEATHVVIGILYGANAFFVFDSDKVDSSNVQDIQGSMEAVIKKIPSVEISGQGSVKLTSEDSEVTNSFSCKF
HGDFHLPSNPTTFEDAVKTYHQLPQMMGKETAVPMTVWLVPMTNFYSEAPQLMADSSTPVFRKVRNTLEAMRTLDMRCNDSLEDDTVKLFPLLEKKLKT
FQKHYRDYMTNFRRTISKKLQSIRKGDEDESAMLQLFEDKLRSPFNTDSLNMWMECAEREINVLRSCIDIIEEAKHKVVLSQSKMVRELLDSEVKHAVCYVF
TYVTDYDPFLKALSDFSKSIKPKKYRPSKKDYWYTSDDVPEMMRGKAIIIIFYNLAKDMDNRCVRFLVASIINPKEEGAAIIYYRFGIQIINDFSKPYIPPVETI
QDRRELLWYDCELTLDPETAHPALHLSEGNKKAVSGKPQPSDNNPERFDHYQQVLCNKGLSKRHYWELEWCGYVRVGITYKGMRRKTSDSVNAVGQNL
GSWAFDYNPKSGYSHIYGNEKSSVKVASPGFDQLGVYLDWPAGTLSFYMVTSTWVTHLHTFSAKFIEAVYPAILIGDGQQKTNGQIKLL

FIGURE 5 Fish cytolysin amino acid sequences identified by shotgun mass spectrometry (MS) in *S. mystes* scorpionfish venomous spine extracts. Higher sequence coverage was observed for the homologues (α- and β-cytolytic toxins) from *Scorpaena plumieri*, *Scorpaenopsis oxycephala* and *Sebastapistes strongia* toxins. The MS-identified unique peptides are highlighted in light blue and those present in more than one database-deposited protein are highlighted in grey (non-unique peptides). Peptide identifications in *Sebasticus marmoratus* and *Dendrochirus zebra* cytolysin sequences are not shown in the figure.

proteins. All identified putative cytolysins showed less than 40% of coverage, suggesting they differ significantly from the database-reported proteins. The peptide sequences obtained for the α- and β-subunits of *S. plumieri* display about 24% and 36% coverage, respectively. Similar values were seen for *Scorpaenopsis* and *Sebastapistes* cytolysins (Figure 5). The identified sequences with the least coverage obtained in our analysis were those matching lionfish *D. zebra* subunits (α and β) and the α-subunit of *Sebasticus marmoratus*

(not shown). Despite the low coverage, unique peptides were recovered in all the sequences.

Other proteins identified in this study that are present in the skin mucus of several fish include putative proteases (serine and metalloproteases) and protease inhibitors (Borges et al., 2018; Zhang et al., 2024). We also identified several other proteins from the mucus of venomous and non-venomous fish (Table 1) as putative hemopexins (Ramos et al., 2012), peroxiredoxins, glyceraldehyde-3-phosphate dehydrogenase, and phosphatidylethanolamine- and fatty acid-binding proteins (Zhang et al., 2024; Ziegman et al., 2019). Regarding other enzymes, a putative antimicrobial lysozyme was identified, along with mucus-characteristic antioxidant components such as superoxide dismutase and glutathione peroxidase (Díaz-Puertas et al., 2023).

Regarding the identified lectins, we observed the two main types in *S. plumieri* venom, B-type and C-type (Andrich et al., 2015; de Santana Evangelista et al., 2009). For the plumieribetin (B-type lectin), the coverage percentage was about 70% and for the C-type lectins, we recovered ~55% of the deposited protein sequence (Sp-CL4). Other sequences identified with lower coverage corresponded to putative bulb-type lectins and galectins. The same labelling of unique and non-unique peptides used for cytolysin sequences (highlighted in blue and grey) was displayed for the lectins (Figure 6).

We also identified several peptides corresponding to a putative porin with multiple database matches, showing similarity to a bryoporin from the three-spined stickleback, *Gasterosteus aculeatus* (Table 1). Since the samples were spine-macerated tissues, several housekeeping and tissue proteins were identified, but these components were excluded from the reported proteomics results in Table 1.

3.4 | Size exclusion chromatographic analysis of *S. mystes* venom

By SEC-chromatography, the anal spine extract was separated and the main proteins were analysed by SDS-PAGE (Figure 7). After minute 10 of the method, no proteins were eluting, indicating that the peaks would correspond to other small components present in the extract. In the fraction at minute 7.6, a clear band with a corresponding mass to the cytolysin subunits (~80 kDa) was observed. MS analysis confirmed their presence in that fraction.

In the fraction eluting at minute 8.7, we identified a protein that could be part of the venom or mucus. It was a cysteine-rich secretory (SCP) domain-containing protein, a member of the CAP superfamily, a type of protein similar to those previously shown in the venoms of *Pterois volitans* lionfish (Díaz et al., 2025) and *Synanceia horrida* stonefish (Ziegman et al., 2019). Fractions eluting at minute 9.2 and 9.6 contained the C-type isolectins Sp-CL4 and plumieribetin (Figure 7).

4 | DISCUSSION

Scorpaena is a genus of marine fish distributed in all the oceans (Wibowo & Motomura, 2021), with 65 identified species. They are known for their potential danger to humans due to their sharp spines, which in some cases display anterolateral grooves and associated venom glands (Smith & Wheeler, 2006). However, only a few venoms from Scorpaenoid fish have been characterized. For instance, there are proteomics studies on the venom of the spotted scorpionfish *S. plumieri* from the Western Atlantic coasts of Brazil (Borges et al., 2018; Campos et al., 2016) and transcriptomics of the Indo-Pacific scorpionfish species *Scorpaenopsis cirrosa*, *Scorpaenopsis neglecta* and *Scorpaenopsis possi* (Xie et al., 2019).

The most representative toxins in Scorpaeniformes fish venoms, the cytolysins (Ziegman & Alewood, 2015), are not seemingly present in all *Scorpaena* species. Xie et al. (2019) identified their protein transcripts in *Scorpaenopsis possi* but not in two other scorpionfish species, *S. neglecta* and *S. cirrosa*. This finding suggests that even when all scorpionfish possess spines, not all may produce venom, at least not cytolysins.

In the current venomics study of *S. mystes*, we identified several putative venom cytolysins. The sequences we identified have unique peptides corresponding to the proteins from several scorpionfish species, including the Western Atlantic sister species *S. plumieri*. We also identified the two representative lectins from that species, plumieribetin and isolectin Sp-CL4. The recovered sequence coverage was much higher for the two lectins (~55%–70%) than that for the putative pore-forming toxins (25%–40%), suggesting that *S. mystes* lectins could be more conserved (from a potential common ancestor) than the pore-forming toxins, probably having undergone fewer genetic changes. However, this conclusion should be confirmed by further analyses.

Skin lectins of *S. mystes*, as in *S. plumieri*, are likely produced by mucus glands (Borges et al., 2018). Regarding their function, de Soto-Luna et al. (2018) demonstrated that the *S. mystes* spine extract has antimicrobial activity against gram-positive bacteria and yeast. Skin mucus lectins could facilitate this effect, since it has been shown that fish galectins and mannan-binding lectins, for instance, participate in several immune processes against pathogens (Rajan et al., 2011). Lysozyme is another mucus component produced by fish skin leukocytes with antibacterial activities (Ghafoori et al., 2014) that was identified in *S. mystes* spine extracts.

Regarding *S. mystes* spine proteases, we recovered the same classes present in *S. plumieri*. There were cathepsin B (cysteine protease), aspartyl aminopeptidase and peptidase M14 (metalloproteinases), peptidase S1 and prolyl endopeptidase (serine proteases), among others. A putative thimet oligopeptidase was also present, an enzyme previously associated with angiotensin-processing activities (Tenorio et al., 2016). Their activities have also been linked to epidermal mucus defences against pathogens (Díaz-Puertas et al., 2023).

All scorpaenoid fish express hyaluronidases in their venoms (Harris, 2023; Saggiomo et al., 2021), including scorpionfish (Borges et al., 2018). Although our laboratory has identified hyaluronidases in other fish venoms (Díaz et al., 2025), we were unable to recover the sequences corresponding to this enzyme in the current analysis, probably because they differ from those deposited in databases. Borges et al. (2018) and, more recently, Pirovani et al. (2026) partially

B-type lectin plumieribetin, *Scorpaena plumieri*

NYLSKNDELRKGDSLVSNNGEFKAVFQDDANFVIYGWQPLWASDTYGSDAIRLCMQAD
CNLVMYNGSGQARWHTNTSKGDCNMCRLFLTNEGKLVLNKESTEIWNSDKNKGSK

C-type isolectin Sp-CL4, *Scorpaena plumieri*

DAEPPSPAEECAAVKMESCGDDWLYIDENRKVKYFETPKTFQEAQDHCESEDGNLVAM
HTEFQKYVVACLSWIYNHKLHRMWIGAGRSEGETQNIDGSDFDYAKWKGGQPDNFGG
NEDCIEANFIDWGYLNDVECSEKLPFMCA

Bulb-type lectin domain containing protein, *Gasterosteus aculeatus*

MSKNYLSKNDELRKGDFLISNNREWKAVFQEDGNFVIYGWKPFWASDTSGSDGMRVC
MQEDCNLVMYNKSDQPKWHTNSSKPKSNMCRLRLTDDGKLVVSRESEELWNSDKNK
GMK

Bulb-type lectin domain-containing protein, *Amphiprion percula*

MSRNYLSKNDELRKGDSLFSNNREWKAVFQDDGNFVIYGWKPTWASDTYGSDAVRLC
MQADCNLVMYNTCDQPRWHTNSAKGSCNMCRLQLTDDGKLVVYRESQEIWSSANSR
GMK

Galectin, *Triplophysa rosa*

MAIGNPKQPILNPAIPFATTILDGLHCGTMVLIQGKVPSKTDRFQVDLTCGNSITPRADVA
FHFNPRFKSTPHIVCNTLKQECWEKEEHHYQMPFKHNEAFEIIILVEEDLFKVAVNGSHL
LAYKHRLELKKIDTLSISGKVEIRAIAFIPGSSAIKSPNKDEKSQTTSHKTIKSESGDLKLPF
KSQFDKGLRPGDTIIVKGQISNSSNRYRRINIINPGSHSCFIQ

Galectin, *Salarias fasciatus*

MQKGMIVKNMSFKVGQTLTVVGVPTPDSTNFAINIGNDDQEITMHINPRFNAHGDENTV
VCNSYQGGNWCEEHREGGFPFRQGEEFTVIIEFTPAEFVVTLSDGSAIHFPNRIGAEKYSV
LNFDGDVRITSLQIK

FIGURE 6 Fish lectin amino acid sequences identified by shotgun mass spectrometry (MS) in *S. mystes* scorpionfish venomous spines' extracts. The two main types of *Scorpaena plumieri* lectins (B- and C-type) were present in *S. mystes* spine extracts with high sequence coverage. Other ubiquitous fish mannose-binding lectins were also identified and are shown in the figure. The MS-identified unique peptides are highlighted in light blue and those present in more than one database-deposited protein are highlighted in grey (non-unique peptides).

identified (∼14%) the *S. plumieri* venom hyaluronidase primary structure. Interestingly, most of these fragments were obtained by de novo sequencing and differ, in some amino acid residues, from those from *Pterois* spp. and *Synanceia* spp. hyaluronidases. These findings suggest variations in the characteristics of scorpionfish hyaluronidases that make MS identification more difficult.

The presence of functional cytolysins in *S. mystes* was confirmed by the ability of the venom to lyse rabbit erythrocytes and induce plasma membrane permeabilization of primary neurons. Interestingly, venom-treated neurons did not die after treatment. Their neurites, even when having signs of alteration, continued to grow several days after the treatment. Propidium iodide has a molar mass of about 668 Da and the relevant ions for excitable cells (Ca^{+2}, Na^{+} and K^{+}) have molar masses of about 23–40 Da. Consequently, if propidium iodide penetrates the cells and stains the nucleic acids, this would suggest that ion influx (Ca^{+2}, Na^{+}) and efflux (K^{+}) occur as well, and these movements may activate ganglia cells. The effect is consistent with cytolysin pore-forming activities, which alter several types of cells, including neurons, as part of nociception-triggered mechanisms (Gomes et al., 2013; Kiriake et al., 2013; Ouanounou et al., 2002).

In addition to the *S. plumieri* cytolysins, in the venom of this Atlantic scorpionfish Borges and collaborators (2018) reported the presence of several other α- and β-cytolysin subunits, including those from *Synanceia horrida*, *Scorpaenopsis oxycephala*, *Sebastapistes strongia*, *Dendrochirus zebra* and *Pterois antennata*, all members of the suborder Scorpaenoidei. In *S. mystes*, we identified cytolysin peptide sequences previously reported only for *S. plumieri*, *S. oxycephala*, *S. strongia*, *D. zebra* and *Sebasticus marmoratus*. The ones with higher coverage were the β-subunits of *S. oxycephala* and *S. plumieri*, but as mentioned, we could only recover ∼30%–35% of the sequences' length. Not identifying all the peptides present in the trypsin digestion for MS analysis is common, and it does not mean they are not there. However, in previous studies from our proteomics laboratory, such as those performed in red lionfish spines, we were able to obtain ∼75%–85% of *Pterois volitans'* cytolysin subunits coverage of the previously reported sequences, and 60%–70% from those of other lionfish species (Díaz et al., 2025). These findings suggest that although *S. mystes* toxins show certain similarity to the sequences of other scorpionfish species, they may have also diversified to some extent.

Another interesting sequence we identified in the *S. mystes* pelvic spine that could contribute to haemolytic activity was a porin, whose peptides match a bryoporin from *Gasterosteus aculeatus*. Actinoporin-like proteins have been identified in several Teleostei fish and other aquatic organisms (Gutiérrez-Aguirre et al., 2006). In the red lionfish, we have identified sequences similar to sagatoxin/stichotoxin in both venom and skin mucus (unpublished results). However, there is also a possibility that some of the components found in spine extracts are not produced by these fish but by other skin parasitic or

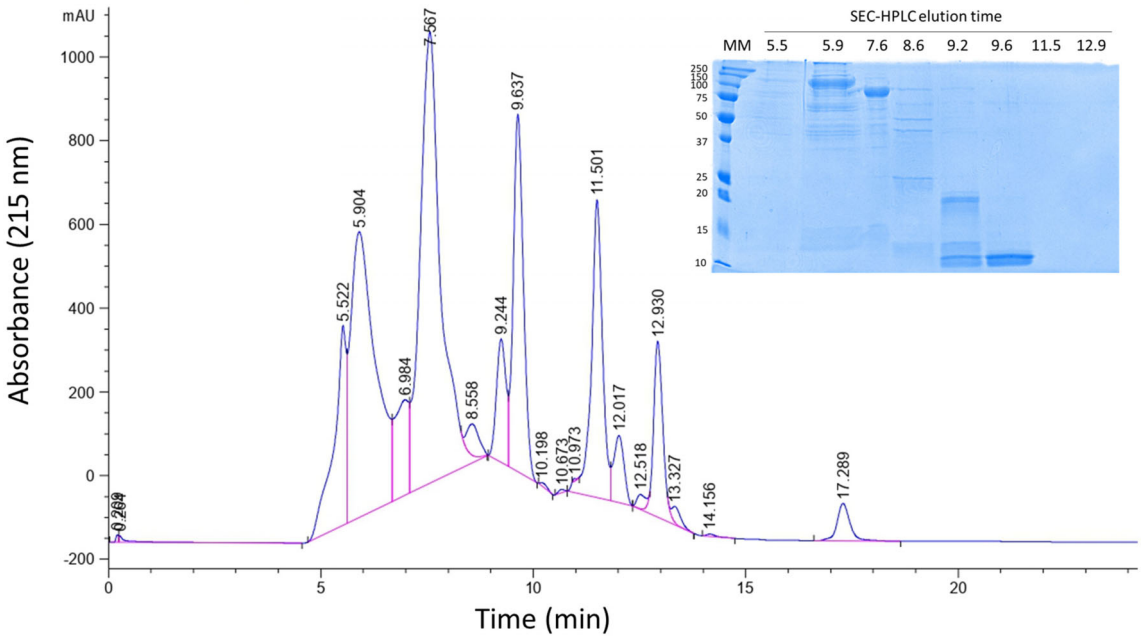


FIGURE 7 Size exclusion high-performance liquid chromatography of *Scorpaena mystes* spine extract and SDS-PAGE of the main fractions. Chromatogram of the *S. mystes* separation of the venom and mucus components in the anal spine extract. In the gel insert (SDS-PAGE under reducing conditions), the main proteins of the extract are displayed. After minute 11, components cannot be detected in the SDS-PAGE, indicating that these peaks are not proteins but other small molecules present in the extract. The blue line shows the absorbance and represents the approximate concentration of the different proteins and other components present in the extract that absorb at the indicated wavelength. The pink line represents the baseline and is extended to mark the fractions (peaks) when they are not completely separated by the chromatographic method. This graph was drawn using the Agilent 1220 HPLC equipment software.

endosymbiotic organisms. For instance, Caribbean scorpionfish species *Scorpaena grandicornis* and *S. plumieri* display several algae and some hydroid species growing on their skin (Ballantine et al., 2001). Other organisms, such as diatoms, have been identified in the venom glands of *S. mystes* (López-Fuerte et al., 2019), as well as the presence of caprellid amphipods living on their skin (Fernández-Del Valle et al., 2017). All these organisms could contribute to different components discovered in the spine extract-containing mucus.

In general, defensive venoms function by inducing pain, scaring predators and competitors away, and it has been shown that their composition is not as complex as that of predatory venom (Harris & Jenner, 2019; Magalhães et al., 2006; Ziegman et al., 2019). Due to the importance of these protective mechanisms, it would be expected that genes encoding defensive toxins would be highly conserved during evolution (Ellisdon et al., 2015). On the other hand, some variations that may have occurred over millions of years would also be expected. Adaptive learning behaviour associated with venom-induced injury has not been established for fish's natural predators (which are mostly also fish). Thus, it is not conclusive whether they can perceive pain similarly to other vertebrates and there is also no evidence that they can learn from previous encounters with venomous fish (Harris & Jenner, 2019), as has been demonstrated for certain terrestrial animals (Skelhorn et al., 2016).

Since we identified only partial protein sequences, we could not determine the exact closeness of evolutionary relationships. However, in terms of functionality, we recovered homologues of the two main isoforms of *S. plumieri* cytolysins, identifying unique conserved sequences present in the four toxin domains: the MACPF/CDC, the focal adhesion-targeting (FAT), the thioredoxin (THX) and the B30.2/SPRY (Costa et al., 2018).

Except for *S. plumieri*, no other *Scorpaena* species has been characterized regarding venom composition; however, transcriptomics analysis of the close genus *Scorpaenopsis* revealed the presence of cytolysin transcripts corresponding to *Synanceia verrucosa* neoverrucotoxin and *Pterois volitans* toxin-b in *Scorpaenopsis possi*. Cytolysin-encoding transcripts of *S. plumieri* were not identified in this venom gland (Xie et al., 2019). In the Scorpaenidae family, the genera *Scorpaenopsis* and *Scorpaena* are phylogenetically closer to each other than to *Pterois* and *Dendrochirus*, whereas stonefish is classified in a different family (Smith et al., 2018). It is intriguing, though, that the only *S. possi*-recovered toxins were similar to those from stonefish and lionfish but not to the previously identified scorpionfish sequences.

In the analysis of the other representative *S. plumieri* proteins, the lectins, we based our comparison on the most prominent ones: C-type isolectin Sp-CL4 and plumieribetin. Both were recovered in *S. mystes* venom spines with very significant coverage percentages. Although these proteins are probably not produced by venom but by mucus glands, their specific sequences have not been discovered in any other fish, indicating that these two scorpionfish species living in different oceans have a unique common evolutionary trait. Also, the fact that these lectins are conserved supports their functional relevance, which is probably not limited to innate immunity defence mechanisms or their ability to agglutinate pathogens. For instance, plumberibetin has

homology with mannose-binding plant lectins and interferes with the interaction of integrins and tissue collagen (de Santana Evangelista et al., 2009). This effect could be part of the envenomation symptomatology caused in humans and the potential defensive toxicity signs produced in potential predators. Some alternative functions of these lectins are yet to be discovered.

5 | CONCLUSIONS

The goal of the current proteomics analysis was to determine whether *S. mystes*, from the Eastern Pacific coast of Costa Rica, displays similarities in venom composition to that of the Western Atlantic scorpionfish *S. plumieri*. We demonstrated that *S. mystes* shares the representative cytolytic toxins and specific B- and C-type lectins with *S. plumieri*, as well as other proteins present in the skin mucus. Our analysis aligns with the cytogenetic studies that have established these as two geminate species, phylogenetically close and likely derived from a common ancestor, whose populations separated into different environments 3–15 million years ago during the rise of the Central American Isthmus (Lessios, 2008; Marko, 2002; Vawter et al., 1980).

AUTHOR CONTRIBUTIONS

C.D. conceptualized the study. A.A., N.O., O.B. and C.D. did the experimental work. N.O. and C.D. analysed the data and wrote the manuscript. All the authors participated in the review and editing, and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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