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Consolidation of venom proteomes from major Cnidarian species (Scyphozoa and Cubozoa) obtained using liquid chromatography-tandem mass spectrometry

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ABSTRACT

Jellyfish envenomation is a significant cause of marine injuries and fatalities. Species under Scyphozoa and Cubozoa can trigger life-threatening effects upon envenomation. Despite the severity of these incidents, treatment options remain very limited. Proteomic information obtained using LC-MS/MS is crucial for identifying key toxins and elucidating the mechanisms underlying jellyfish envenomation. This review aims to provide a comprehensive list of venom proteomes from major Scyphozoans and Cubozoans. Among the ten major Scyphozoans and Cubozoans discussed in this review, the major toxin families were found to be phospholipases, proteases, metalloproteinases, serine protease inhibitors, pore-forming toxins, ion channel inhibitors, C-type lectins, and venom allergens. In addition to the relatively well-characterised venom toxins, several putative toxins were also identified. Interestingly, CfTX-like toxins, categorised as pore-forming toxins, were also homologous across most Scyphozoans apart from Cubozoans. These venom proteins are vital for developing antivenoms against various venomous jellyfish.

1. Introduction

In the animal kingdom, jellyfish are classified under the diverse Cnidaria phylum consisting of over 10,000 species. There are two major lineages from the Cnidaria phylum: (i) Anthozoans (sea anemones and corals) and (ii) Meduzoans (jellyfish and hydra). The Meduzoans can be categorised further into four classes: (i) Scyphozoans (true jellyfish), (ii) Cubozoans (box jellyfish), (iii) Staurozoans (stalked jellyfish) and (iv) Hydrozoans (hydroids) (Daly, Seymour & Wilson, 2014; Piontek et al., 2020). Depending on the species, jellyfish possess venoms with varying degrees of severity, including local and systemic symptoms (Cunha and Dinis-Oliveira, 2022). For instance, the *Lobonema smithii* and *Chrysaora chinensis* only cause swelling and numbness in the stinging area (Li et al., 2016), but others induce relatively severe symptoms, including profound anaphylactic shock and mortality due to heart failure (Li et al., 2016). The Irukandji Syndrome is the most severe envenomation symptom exclusively induced by the deadly Cubozoans (box jellyfish) (Hwang et al., 2020; Tibballs et al., 2011). It is characterised by sweating, anxiety, muscle spasms, severe hypertension and heart failure (Tibballs et al., 2011). To date, sixteen Irukandji jellyfish have been identified, including *Carukia barnesi*, *Malo kingi*, *Malo maxima*, *Malo filipina*, and *Malo bella* (Santhanam, 2020a,b). Other deadly cubozoans include *Chironex fleckeri*, *Carybdea alata, Morbakka* sp., and *Chiropsoides buitendijiki* (Tibballs et al., 2011).

Incidences of jellyfish envenomation have been increasing worldwide, especially in the tropical regions of Southeast Asia (Li et al., 2016; Hwang et al., 2020). In 2006, an estimated 150 million jellyfish envenomation cases were reported globally, with the number projected to increase annually (Cunha and Dinis-Oliveira, 2022; Dobson et al., 2024; Hwang et al., 2022). Jellyfish blooms are driven by climate change (i.e. global warming), salinity shifts, overfishing, and habitat degradation (Santhanam, 2020a,b). In 2017, envenomation by *C. alata* and *C. buitendijki* were reported involving a 12-year-old boy, who experienced a three-day coma following the sting off the coast of Penang, Malaysia (Loh, 2017). Fatal envenomation involving *C. alata* was also reported at Pulau Langkawi, Malaysia, resulting in the deaths of two teenagers (Fenner, Lippmann & Gershwin, 2010). Subsequently, a Swedish tourist in his 60s also reportedly died from a jellyfish sting at Pulau Langkawi, Malaysia. Although the exact species responsible was not identified, it was postulated at the time that the sting may have been caused by the recently discovered *C. buitendijki* or the *Morbakka* sp. (Vinther, 2018).

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The cnidocyte, which are densely packed in epithelial surfaces of jellyfish tentacles, is a unique feature of the Cnidarians (Daly, Seymour & Wilson, 2014; Tibballs et al., 2011). The Golgi apparatus which contains a collagen-walled capsule, contributes to the formation of cnidocyte organelles by producing nematoblasts, subsequently developing an eversible- or non-penetrant tubule depending on the jellyfish species (Cunha and Dinis-Oliveira, 2022). Each of these nematoblasts synthesises a single nematocyst, which comprises a spin-laden tubule of approximately 200–800 μm in length so that the capsular contents or "venom" can be pierced into the prey's epidermal tissue (Liu et al., 2018). Upon trigger of nematoblasts, the capsules of the cnidocyte organelles which are connected to the nematocysts, propel a barbed arrow-like tubule at high velocity towards the site of impact. Thousands of these tubules, carrying potent toxins, will be injected into the prey (Hwang et al., 2020; Liu et al., 2018), leading to an immediate electric shock-like sensation and intense pain (Daly, Seymour & Wilson, 2014). Death can swiftly follow, with the entire process occurring within a matter of minutes (Tibballs et al., 2011).

A study by Mustafa et al. (1995) investigated the mode of action of the *C. fleckeri* venom in cardiomyocytes by monitoring the qualitative changes of intercellular Ca^{2+} concentrations. It was found that the venom toxin induces a Ca^{2+} overload in cardiac cells by opening L-type Ca^{2+} channels, leading to spontaneous releases of more Ca^{2+} into the cell cytoplasm. These imbalances result in an overall reduction in stimulated contraction, causing heart failure (Mustafa et al., 1995). These observations were supported by another study, where a partially purified toxin (pCrTX) from *Carybdea rastonii*, another Cubozoan species, induced the contraction of aortic strips and intestinal smooth muscle by increasing cation permeability, permitting an influx of Ca^{2+} in both muscles (Azuma et al., 1986). To the best of our knowledge, the precise mechanism between cardiotoxicity and intracellular Ca^{2+} overload remains unclear (Cunha and Dinis-Oliveira, 2022).

Given that each jellyfish species have distinct characteristics, treatment and management of jellyfish envenomation will vary depending on the degree of severity of envenomation. This suggests that species-specific treatments are more appropriate rather than a panacea treatment solution (Hwang et al., 2020). Unfortunately, the efficacy of most suggested treatments are lacking due to the absence of randomised controlled trials (Hwang et al., 2020). General first aid measures include preventing the victim from rubbing the stung area and calming the victim to reduce heart rate (Lakkis, Maalouf & Mahmassani, 2015). Home remedies to deactivate undischarged nematocysts are also available:Vinegar (i.e. 4 % acetic acid), ethanol, ammonia (or urea), sodium bicarbonate, papain, aluminium sulfate, and saltwater (Lakkis, Maalouf & Mahmassani, 2015; Staggs and Pay, 2022). However, it was recently discovered that 4 % acetic acid has the opposite effect by triggering nematocyst discharge for certain Scyphozoan and Cubozoan stings, particularly *Nemopilema nomurai*, *Cyanea capillata*, and *Chironex fleckeri* (Hwang et al., 2020). For anaphylaxis and cardiac arrest cases caused by life-threatening Cubozoans, prompt injection of epinephrine to increase blood pressure, followed by administration of the box jellyfish antivenom, is recommended (Lakkis, Maalouf & Mahmassani, 2015).

As of current, this box jellyfish antivenom developed in 1970 by the Commonwealth Serum Laboratories, remains the only antivenom available globally. The antibodies were produced by hyper-immunising sheep with *C. fleckeri* venom (Daly, Seymour & Wilson, 2014). However, there are doubts regarding the clinical efficacy of this antivenom, as cases of mortality among envenomed patients has been reported despite the antivenom administration (Andreosso et al., 2014). The mechanism on how the antivenom reverses the neurotoxic and myotoxic effects of the venom, is also lacking (Lakkis, Maalouf & Mahmassani, 2015). Ca^{2+} antagonists such as verapamil, diltiazem, and nifedipine, have demonstrated some cardioprotective effects against *C. fleckeri* and *Carybdea rastonii*, but with inconsistent outcomes. A study found that verapamil has no effect on Ca^{2+} influx, but instead antagonised a non-specific La^{3+} channel, another pore blocker. This suggests that the venom may have the capability to bypass the Ca^{2+} ion channel and form an alternative entry route through a non-selective pore in the presence of verapamil. This finding supports the idea that *C. fleckeri* venom contains a pore-forming toxin (Cunha and Dinis-Oliveira, 2022).

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a powerful analytical technique used for comprehensive proteomic profiling of an organism. It offers high-speed and high-throughput, integrating automated workflows with highly sensitive detection of trace protein components (Sharma et al., 2015; Malih et al., 2014). It consists of three main parts: an ion source that ionises the sample, an analyser that separates ions based on respective mass-to-charge (m/z) ratios, and a detector that identifies and measures the relative abundance of the ions (Garg and Zubair, 2024). The complex protein compositions of the venom from various venomous species can be revealed, which in turn aids in the understanding of the symptoms and physiological effects of envenomation (Sharma et al., 2015). LC-MS/MS has been successfully applied to characterise venoms from venomous snakes, such as kraits, cobras, and pit vipers (Zainal Abidin et al., 2016).

While several venom toxins have been identified across various jellyfish species, including phospholipase A_2s (PLA_2s), zinc metalloproteinases, and box jellyfish toxins, most of these proteins have not been identified using the LC-MS/MS approach. Instead, they have been primarily characterised through genomic and transcriptomic approaches (Bentlage and Lewis Ames, 2012; Brinkman et al., 2015; Fenner, Lippmann & Gershwin, 2010; Karabulut et al., 2022; Upata et al., 2022). It is important to note that not all genomic sequences translate into functional proteins due to the complexity of protein expression, which can be influenced by unpredictable temporal and environmental factors (Al-Amrani et al., 2021). Proteomics data provides insights into protein structure and functions, thereby offering a more comprehensive understanding of an organism's characteristics (Al-Amrani et al., 2021). This knowledge also facilitates deeper comprehension of the mechanisms underlying jellyfish envenomation and toxicity, and aids in the development of efficient antivenom therapies (Zainal Abidin et al., 2016; Lau et al., 2019). Additionally, it serves as a valuable resource for discovering novel proteins and peptides with potential therapeutic applications (Lau et al., 2019).

This review consolidates the proteomics information available from major Scyphozoan and Cubozoan species. Scyphozoan and Cubozoan toxins have been the most challenging to identify and characterise compared to other Cnidarian toxins due to their relatively unstable protein toxins (Santhanam, 2020a,b). The venom proteomes of jellyfish species were reported with the LC-MS/MS approach using two different ionisation techniques: (i) electrospray ionisation (ESI) (Brinkman et al., 2014, 2015; Edirisinghe et al., 2024; Li et al., 2016, 2018, 2020; Ponce et al., 2016; Riyas et al., 2021; Zhu et al., 2015) and (ii) higher-energy collisional dissociation (HCD) (Jouiaei et al., 2015; Leung et al., 2020; Li et al., 2022; Wang et al., 2019; Weston et al., 2013; Yue et al., 2021).

2. Biology of the Cnidarian jellyfish (Scyphozoa and Cubozoa)

There are 187 and 46 verified species from the Scyphozoans and Cubozoans, respectively, as detailed in the World Register of Marine Species (http://www.marinespecies.org). The Scyphozoa comprises of four orders: (i) Coronatae (crown jellyfish), (ii) Rhizostomeae, (true jellyfish), (iii) Stauromedusae (stalked jellyfish) and (iv) Semaestomeae (sea nettle). In contrast, the Cubozoa only comprises of two orders: (i) Carybdeida and (ii) Chirodropida (Rizman-Idid et al., 2016).

According to Santhanam (2020a,b), the four main orders of the Scyphozoa are further divided into the following family-level classification: (i) Coronatae consists of five families (Atollidae, Linuchidae, Nausithoidae, Paraphyllinidae, and Periphllidae), (ii) Rhizostomeae consists of ten families (Catosylidae, Lobonematidae, Lychnorhizidae, Rhizostomatidae, Stomolophidae, Cassiopeidae, Cepheidae, Mastigiidae, Thysanostomatidae, and Versurigidae), (iii) Stauromedusaae consists of one family (Stauromedisidae) and (iv) Semaestomeae consists of

five families (Cyaneidae, Drymonematidae, Pelagiidae, Phacellophoridae, and Ulmaridae). Similarly, the two major orders of the Cubozoa are further divided into their respective families as follows: (i) Carybdeida consists of five families (Carybdeidae, Tripedaliidae, Tamoyidae, Carukiidae, and Alatinidae) and (ii) Chirodropida consists of two families (Chirodropidae and Chiropsalmidae).

One of the distinct physical characteristics between the two Cubozoan orders are the number of tentacles per pedalium: the Carybdeida only has one tentacle per pedalium, while the Chirodropida has multiple tentacles per pedalium (Bentlage and Lewis Ames, 2012; Santhanam, 2020a). Additionally, the Cubozoa can also be distinguished based from the box-like shape of their bell (Santhanam, 2020a). On the other hand, the Scyphozoa generally exhibits a four-part symmetry and possess an internal jelly-like substance called the mesoglea. They are also lacking in general organs, including a head, skeleton, or any specialised organs for breathing or excretion. Their mouths are connected to a central stomach, which contains four interconnected diverticula that spread outwards (Santhanam, 2020b).

Both the Cubozoa and Scyphozoa display the Metagenetic Life Cycle, which involves a bottom-swelling polyp stage and a free-swimming medusa stage (Bentlage and Lewis Ames, 2012). When resources and environmental conditions are scarce and harsh, the life cycle shifts to the formal, given that the polyp form is more adapted to survival. Conversely, when resources and environmental conditions are plentiful and optimal, the latter life cycle is favoured (Ceh et al., 2015). Species diversity within the Cubozoa are relatively low compared to the Scyphozoa, likely due to limited taxonomic studies and allopatric speciation (Bentlage and Lewis Ames, 2012). This observation is supported in Table 1, which showcased a list of Scyphozoan and Cubozoan species recorded in Malaysian waters as of 2021, as documented in the Field Guide to the Jellyfish of Western Pacific (Venmathi Maran et al., 2021a, b). There are a total of eighteen species under the Scyphozoa class, and only three species under the Cubozoa class (Table 1).

While all Scyphozoans induce hyperalgesia, smaller species or those with shorter tentacles are less severe. Species belonging to the genera *Chrysaora* and *Cyanea* induce the most painful, though not lethal, stings (Santhanam, 2020b). Cubozoans, however, pose a more significant threat to humans. Fatalities from the highly lethal *C. fleckeri* are reported almost annually, and the number of cases is likely underestimated since many go unreported. Moreover, *Carukia barnesi*, another Cubozoan jellyfish, was recently revealed to induce Irukandji syndrome as well (Santhanam, 2020a).

Table 1
List of Scyphozoan and Cubozoan jellyfish from Malaysia consolidated from the Field Guide to the Jellyfish of Western Pacific (Venmathi Maran et al., 2021a,b).

Class	Family	Species
Scyphozoan	Linuchidae	*Linuche aquila*
	Cyaneidae	*Cyanea* sp.
	Pelagiidae	*Chrysaora chinensis*
	Pelagiidae	*Pelagia noctiluca*
	Ulmaridae	*Aurelia* sp.
	Catostylidae	*Acromitus flagellatus*
	Catostylidae	*Acromitus hardenbergi*
	Catostylidae	*Acromitus maculosus*
	Catostylidae	*Catostylus townsendi*
	Cepheidae	*Cephea cephea*
	Cepheidae	*Netrostoma dumokuroa*
	Lobonemidae	*Lobonemoides robustus*
	Lychnorhizidae	*Anomalorhiza shawi*
	Lychnorhizidae	*Lychnorhiza malayensis*
	Mastigiidae	*Mastigias papua*
	Mastigiidae	*Mastigas* sp.
	Mastigiidae	*Phyllorhiza punctata*
	Rhizostomatidae	*Rhopilema esculentum*
	Rhizostomatidae	*Rhopilema hispidium*
Cubozoan	Carukiidae	*Morbakka* sp.
	Chiropsalmidae	*Chiropsoides buitendijiki*
	Chirodropidae	*Chironex yamaguchii*

3. Venom proteome of Cnidarian jellyfish (Scyphozoa and Cubozoa)

The proteomic information from nine Scyphozoans representing five protein families: (i) *Cyanea nozakii* (Table 2) and *Cyanea capillata* (Table 3) under the Cyaneidae, (ii) *Aurelia coerulea* (Table 4) under the

Table 2
List of detected toxic proteins in the venom of *C. nozakii* using LC-MS/MS from various sources associated with its specific clinical symptoms/toxicity.

Family	Genus/ Species	Clinical Symptoms/ Toxicity	Toxic proteins in the nematocyst of jellyfish species (species with the closest homology)	References
Cyaneidae	*C. nozakii*	cardiotoxicity: acute myocardial infarction and a significant decrease in both heart rate and blood pressure leading to mortality	Phospholipase A_2 ● Phospholipase A_2 (*Rhopilema esculentum*) (*Nematostella vectensis*) ● PLA_2G12 (*N. vectensis*) Metalloproteinase ● zinc metalloproteinase nas-4/13/14/15-like (*H. vulgaris*) ● putative zinc metalloproteinase C607.06c-like (*H. vulgaris*) ● A disintegrin and metalloproteinase with thrombospondin motifs 20-like (*H. vulgaris*) ● A disintegrin and metalloproteinase with thrombospondin motifs 9-like (*H. vulgaris*) ● Disintegrin and metalloproteinase domain-containing protein 17-like (*H. vulgaris*) ● Matrix metalloproteinase (*H. vulgaris*) ● Matrix metalloproteinase-24-like (*H. vulgaris*) Serine protease inhibitor ● Serine protease inhibitor (*Cyanea capillata*) Others ● Toxin (*Actineria villosa*) ● Toxin TX2, partial (*Aurelia aurita*) ● RACK-like protein (*Carukia barnesi*) ● Calmodulin (*Rhizostoma octopus*) ● peroxiredoxin 4 (*Cyanea capillata*) ● Pp3 (*Clytia hemisphaerica*)	(Li et al., 2016, 2018; Riyas et al., 2021)

Table 3
List of detected toxic proteins in the venom of *C. capillata* using LC-MS/MS from various sources associated with its specific clinical symptoms/toxicity.

Family	Genus/ Species	Clinical Symptoms/ Toxicity	Toxic proteins in the nematocyst of jellyfish species (species with the closest homology)	References
Cyaneidae	*Cyanea capillata*	Skin swelling, erythematous stripes Starts of with a burning feeling, followed by more severe pain or itching	Protease • Chymotrypsinogen B-like (*H. vulgaris*) • Mitogen-activated protein kinase 1-like (*H. vulgaris*) • Serine protease 1, partial (*A. aurita*) Metalloproteinase • Zinc metalloproteinase nas-15 (*Exaiptasia pallida*) • Zinc metalloproteinase nas-13-like (*Acropora digitifera*) • A disintegrin and metalloproteinase with thrombospondin motifs 6-like (*H. vulgaris*) Other putative toxins • U-actitoxin-Avd9b (*Anemonia viridis*)	(Wang et al., 2019)

Table 4
List of detected toxic proteins in the venom of *A. coerulea* using LC-MS/MS from various sources associated with its specific clinical symptoms/toxicity.

Family	Genus/ Species	Clinical Symptoms/ Toxicity	Toxic protein in the nematocyst of jellyfish species (species with the closest homology)	References
Ulmaridae	*A. coerulea*	non-lethal, mainly itchiness and pain in localised regions	serine protease inhibitors ● Serine protease inhibitor (*C. capillata*)	(Li et al., 2022; Liu et al., 2018)

Table 5
List of detected toxic proteins in the venom of *Rhopilema esculentum* using LC-MS/MS associated with its specific clinical symptoms/toxicity.

Family	Genus/ Species	Clinical Symptoms/ Toxicity	Toxic proteins in the nematocyst of jellyfish species (species with the closest homology)	References
Rhizosomatidae	*Rhopilema esculentum*	None reported to date, but it is postulated that symptoms and toxicity may be non-lethal and minimal, given that it is marketed as an edible jellyfish	Protease ● Nematocyst-expressed protein 6 (*N. vectensis*) Other toxin ● U-actitoxin-Avd3j (*A. viridis*)	(Leung et al., 2020; Zhu et al., 2015)

Ulmaridae, (iii) *Rhopilema esculentum* (Table 5), *Lychnorhiza malayensis* (Table 6) and *Nemopilema nomurai* (Table 7) under the Rhizostomatidae, (iv) *Chrysaora caliparea* (Table 8) and *Chrysaora fuscescens* (Table 9) under the Pelagiidae, and (v) *Catostylus* sp. (Table 10) under the Catostylidae, along with one Cubozoan: *C. fleckeri* (Table 11) under the Chirodropidae, were consolidated.

Not all Scyphozoans and Cubozoans discovered to date have been the subject of proteomic research. Given the likelihood that jellyfish species within the same family share similar protein and toxin profiles, this review serves as a valuable resource to elucidate the proteomic information available for other jellyfish species within the same family, for which proteomic data is still unavailable.

Venom proteins exhibiting sequence homology to the Cnidaria were included for analysis in this review, while those with homologs in other taxa (e.g. snakes, insects, and ants), were excluded. Additionally, only articles involving venom isolated specifically from nematocysts were included, while those involving extracts obtained from crushing whole jellyfish tissues, were excluded. This decision is based on the principle that homologous proteins can serve different functions across taxa, and even within different tissues of the same organism. For instance, PLA_2 enzymes found in snake venoms are known to be neurotoxic or myotoxic in humans (Tonello and Rigoni, 2017), while homologous PLA_2 enzymes in humans do not induce toxicity. Instead, these proteins function as regulators in inflammatory responses (Hidalgo et al., 2024). Similarly, PLA_2 located in jellyfish nematocysts may exhibit toxicity, while those located in other tissues are involved in essential physiological functions (Millar et al., 2009). Finally, studies that did not report the source species of the identified putative toxins and venom components in their LC-MS/MS analyses, were also excluded. Accurate taxonomic attribution is essential for reliable interpretation of sequence homology and functional annotation.

3.1. Scyphozoa class

3.1.1. Cyaneidae family

3.1.1.1. Cyanea nozakii *venom.* Venom proteins in *C. nozakii* homologous to the Cnidarians were successfully identified using LC-MS/MS, revealing proteins such as serine protease inhibitors, RACK-like proteins, calmodulin, and peroxiredoxin 4 (Table 2) (Riyas et al., 2021). Serine protease inhibitors impede blood clot formation, while calmodulin, a signalling protein, regulates various immunological processes, including apoptosis, autophagy, immune responses, and inflammation (Riyas et al., 2021). Peroxiredoxin 4 acts as an antioxidant by decomposing hydrogen peroxide, a reactive oxygen species, into water and oxygen, providing protection towards tissue oxidative damage in prokaryotes and eukaryotes (Liu et al., 2018). The precise function of RACK-like protein remains elusive (Riyas et al., 2021).

In a separate investigation by Li et al. (2016), the crude venom of *C. nozakii* yielded a total of 174 potential venom proteins from LC-MS/MS analysis, some of which demonstrated similarity to organisms under the Cnidaria. Some of which included the PLA_2, PLA_2G12, zinc metalloproteinase nas-4/6/13/15, A disintegrin and metalloproteinase with thrombospondin, A disintegrin and metalloproteinase, and matrix metalloproteinase (Table 2). PLA_2, as well as A disintegrin and metalloproteinase with thrombospondin induce inflammatory symptoms such as swelling and wheals. PLA_2 additionally exhibits myotoxic, neurotoxic, haemolytic, pro-inflammatory, hypotensive, platelet-aggregating, and cytotoxic activities. A disintegrin and metalloproteinase inhibit ADP-induced human platelet aggregation and bovine aortic endothelial cell migration (Li et al., 2016). Assays targeting metalloproteinase, PLA_2, and haemolytic activities on the identified lethal toxins confirmed that the toxicity of *C. nozakii* predominantly originated from metalloproteinase activity alone (Li et al., 2018).

Table 6
List of detected toxic proteins in the venom of *L. malayensis* using LC-MS/MS associated with its specific clinical symptoms/toxicity.

Family	Genus/Species	Clinical Symptoms/Toxicity	Toxic proteins in the nematocyst of jellyfish species (species with the closest homology)	References
Rhizostomeae	*L. malayensis*	neurotoxic effects	Propeptide ● 332-1 secreted propeptide (*M. kingi*) Neuropeptide ● pp3 (*C. hemisphaerica*) ● pp11 (*C. hemisphaerica*)	Riyas et al. (2021)

Table 7
List of detected toxic proteins in the venom of *N. nomurai* using LC-MS/MS associated with its specific clinical symptoms/toxicity.

Family	Genus/ Species	Clinical Symptoms/Toxicity	Toxic proteins in the nematocyst of jellyfish species (species with the closest homology)	References
Rhizostomeae	*N. nomurai*	significant oedema at region of sting, increase in vascular permeability in dorsal skin and kidney haemorrhage, abnormal swimming behaviour, and pathological changes in heart, gill, and brain tissues observed in zebrafish model blistering and wheal eruption	Voltage-gated potassium ion channel inhibitor • kappa-stichotoxin-Shd1a/kappa-stichotoxin-Shd1b (*Stichodactyla haddoni*) Metalloproteinase • A disintegrin and metalloproteinase with thrombospondim motifs 6/9 (*H. vulgaris*) • Zinc metalloproteinase nas-8-like (*H. vulgaris*) Protease • Serine/threonine-protein kinase dyrk2 isoform X1 (*H. vulgaris*) • Carboxypeptidase D-like (*H. vulgaris*) • Aminopeptidase 2 (*H. vulgaris*) Pore-forming toxins • MAC/Perforin domain containing protein (*Rhopilema esculentum*) • Toxin CqTX-A (*Chiropsalmus quadrigatus*) • Toxin CrTX-A (*Carybdea rastonii*) Phospholipase • Acidic PLA_2 (*H. vulgaris*) • PLA_2 (*H. vulgaris*) Protease inhibitors • Serine protease inhibitor (*H. vulgaris*) Lectins • L-rhamnose-binding lectin CSL2 (*H. vulgaris*) Other putative toxins • PI-acitoxin-Aeq3b (*Actinia equina*)	(Li et al., 2020; Wang et al., 2019; Yue et al., 2021)

Table 8
List of detected toxic proteins in the venom of *Chrysaora caliparea* using LC-MS/MS associated with its specific clinical symptoms/toxicity.

Family	Genus/ Species	Clinical Symptoms/ Toxicity	Toxic proteins in the nematocyst of jellyfish species (species with the closest homology)	References
Pelagiidae	*Chrysaora caliparea*	disrupt cell membrane permeability leading to cell death	Pore forming toxins ● TX-1 toxin (*C. fuscescens*) ● Chrysaoralin (*C. quinquecirrha*)	Riyas et al. (2021)

3.1.1.2. Cyanea capillata *venom.* A total of fifty-three putative *C. capillata* venom toxins were identified using LC-MS/MS. Among these, seven of these toxins are homologous to the Cnidarian species (Table 3). The functions of these seven putative toxins in the Cnidarian species are unknown. It was suggested that PLA_2s and neurotoxins are responsible for symptoms like swelling and erythematous stripes, as well as severe pain and itching sensation, respectively. However, none of the PLA_2s and neurotoxins detected are homologous to the Cnidarian species (Wang et al., 2019).

3.1.2. Ulmaridae family

3.1.2.1. Aurelia coerulea *venom.* The serine protease inhibitor homologous to *C. capillata* was detected within *A. coerulea* venom from the LC-MS/MS analysis (Table 4) (Liu et al., 2018). Among the marine jellyfish, *A. coerulea* has relatively low toxicity and can be successfully bred in controlled environments (Liu et al., 2018). Individuals stung by *Aurelia* sp. generally experience mild localised symptoms such as itchiness and pain, with only occasional systemic symptoms like low blood pressure and muscle weakness (Li et al., 2022).

3.1.3. Rhizostomatidae family

3.1.3.1. Rhopilema esculentum *venom.* Using a nano-LC-MS/MS approach, a total of forty putative venom proteins were characterised in *R. esculentum*, and categorised into various functional types, including proteases, phospholipases, protease inhibitors, pore-forming toxins, cysteine-rich proteins, neurotoxins, and other toxins (Table 5) (Leung et al., 2020). Among these, the nematocyst-expressed protein 6, and U-actitoxin-Avd3j demonstrated similarity to *Nematostella vectensis*, and *Anemonia viridis*, respectively (Leung et al., 2020). The functions of these two putative toxins in Cnidarians remain unclear. Although PLA_2 or PLA_2-like activity has been reported in the oral arms of *R. esculentum* by Zhu et al. (2015), no PLA_2 venom proteins or its isoforms homologous to the Cnidarian species were detected from the LC-MS/MS study by Leung et al. (2020), prompting a possibility of venom protein degradation.

3.1.3.2. Lychnorhiza malayensis *venom.* LC-MS/MS analysis of *L. malayensis* venom revealed the presence of seventeen proteins, including the 332-1 secreted propeptide, as well as the pp3 and pp11 neuropeptides which demonstrated similarity to *Malo kingi* and *Clytia hemisphartica*, respectively (Table 6). Pp3 and pp11 are endogenous neuropeptides known to promote the maturation of follicles, ovulation,

Table 9
List of detected toxic proteins in the venom of *C. fuscescens* using LC-MS/MS associated with its specific clinical symptoms/toxicity.

Family	Genus/ Species	Clinical Symptoms/ Toxicity	Toxic proteins in the nematocyst of jellyfish species (species with the closest homology)	References
Pelagiidae	*Chrysaora fuscescens*	N.A.	Metalloproteinase • Endothelin-converting enzyme 1-like (*H. vulgaris*) • Endothelin-converting enzyme 2-like (*H. vulgaris*) • Endothelin-converting enzyme 1-like (*H. vulgaris*) • Endothelin-converting enzyme 1-like (*H. vulgaris*) • Endothelin-converting enzyme 1-like (*N. vectensis*) Pore-forming toxins • Toxin TX2 (*A. aurita*) • Uncharacterised protein (*H. vulgaris*) Venom allergens • Cell wall protein PRY3-like (*H. vulgaris*) • Cell wall protein PRY3-like (*H. vulgaris*) • Cell wall protein PRY3-like (*H. vulgaris*) • Cell wall protein PRY3-like (*H. vulgaris*) • Cell wall protein PRY3-like (*H. vulgaris*) C-type lectin • Golgi-associated plant pathogenesis-related protein 1 (*H. vulgaris*) • Golgi-associated plant pathogenesis-related protein 1 (*H. vulgaris*) • Golgi-associated plant pathogenesis-related protein 1 (*H. vulgaris*) Glycoside hydrolase • beta-hexosaminidase subunit alpha-like isoform X1 (*H. vulgaris*) Enzyme inhibitor • tripeptidyl-peptidase 1-like (*H. vulgaris*)	Ponce et al. (2016)

and release of sperm. However, its exact toxicity in Scyphozoan venoms remains elusive.

The 332-1 secreted propeptide belongs to the ShK toxic domain family. Calitoxin, a protein homologous to the 332-1 secreted propeptide, has been detected in *Calliactis parasitica* (sea anemone) in previous studies (Riyas et al., 2021). ShT is a K^+ channel blocker that belongs to the ShKT domains. Proteins having ShKT domains are prevalent across the Cnidarian species, including the Anthozoans, Hydrozoans, Scyphozoans, and Cubozoans. Most of these proteins also contain other domains apart from the ShKT, such as the zinc- and astacin-metalloproteinases.

Table 10
List of detected toxic proteins in the venom of *Catostylus* sp. using LC-MS/MS associated with its specific clinical symptoms/toxicity.

Family	Genus/ Species	Clinical Symptoms/ Toxicity	Toxic proteins in the nematocyst of jellyfish species (species with the closest homology)	References
Caatostylidae	*Catostylus* sp.	hemolysis, cytotoxic effects, dermonecrosis, inflammation, and pain	Pore forming toxin • Toxin CrTX-A (*C. rastonii*)	Edirisinghe et al. (2024)

This may explain the dual functionality of some venom toxins, where they could both immobilise prey through ShKT-mediated ion channel blockade and contribute to digestion via proteinase activity (Ponce et al., 2016).

3.1.3.3. Nemopilema nomurai *venom.* The lethal fraction of *N. nomurai* was subjected to LC-MS/MS analysis, and thirteen venom toxins matched in the Tox-Prot database was revealed (Table 7). Among these, the kappa-stichotoxin-Shd1a/kappa-stichotoxin-Shd1b is one of the venom toxins homologous to *Stichodactyla haddoni* (sea anemone). This venom toxin is a voltage-gated potassium ion channel inhibitor, which prompts its potential role in lethality. It is postulated that it could increase membrane permeability, disrupt osmotic pressure, and eventually lead to pulmonary oedema, infection, and mortality (Li et al., 2020).

A total of sixty-nine putative *N. nomurai* venom toxins were identified using LC-MS/MS. Among these, there were three metalloproteinases homologous to *H. vulgaris*: (i) A disintegrin and metalloproteinase with thrombospondin motifs 6, (ii) A disintegrin and metalloproteinase with thrombospondin motifs 9 and (iii) zinc metalloproteinase nas-8-like (Table 7) (Wang et al., 2019). The presence of blistering and wheal eruption is likely mediated by metalloproteinases, which induce tissue damage by degrading the extracellular matrix, ultimately leading to necrosis, oedema, and haemorrhage (Leung et al., 2020; Wang et al., 2019). This indicates that metalloproteinase inhibitors could potentially serve as effective treatments for *N. nomurai* stings (Wang et al., 2019).

A partially purified *N. nomurai* fraction was subjected to LC-MS/MS to identify potential enzymatic toxins which may contribute to its proteolytic effects. It was postulated that metalloproteinases would be present in the fraction, but the top hits revealed three transcription factors, a retinoid X receptor, and a pp4 protein. None of the proteins homologous to the Cnidarian species are characterised venom toxins. Nonetheless, several observations have highlighted the oedematogenic and lethal properties of *N. nomurai* venom: (i) 15–75 μg of *N. nomurai* venom was enough to induce significant oedema within 0.5–1 h in mouse models, and (ii) the evidence of increased vascular permeability and proteolytic activity from both *in vivo* and *in vitro* assays (Yue et al., 2021).

Another study was able to identify twenty-three toxin homologs from a partially purified *N. nomurai* fraction using LC-MS/MS, but none of the venom toxins were homologous to the Cnidarian species. Similarly, around 30 and 100 μg/g of partially purified *N. nomurai* fraction was subjected to zebrafish model, subsequently leading to toxic effects like haemorrhage, abnormal swimming behaviour, as well as pathological changes in the heart, gill, and brain tissues. These pharmacological effects are likely induced by cardiotoxins and neurotoxins (Mohan Prakash et al., 2023).

3.1.4. Pelagiidae family

3.1.4.1. Chrysaora caliparea *venom.* Out of the eighteen proteins

Table 11
List of detected toxic proteins in the venom of *C. fleckeri* using LC-MS/MS from various sources associated with its specific clinical symptoms/toxicity.

Family	Genus/ Species	Clinical Symptoms/Toxicity	Toxic proteins in the nematocyst of jellyfish species (species with the closest homology)	References
Chirodropidae	*C. fleckeri*	severe localised and systemic effects that are potentially life-threatening: pain, inflammation, dermonecrosis, cardiovascular collapse, and death in experimental animals due to high haemolytic activity	Box jellyfish toxins/known cubozoan toxins ● Cysteine-rich venom protein pseudotoxin-like ● Turripeptide ● Astacin-like metalloproteinase toxin ● Cysteine-rich venom protein latisemin ● Toxin CfTX-A/B ● Toxin CfTX-1/2-like ● Peroxiredoxin ● Toxin CqTX-A-like ● Peroxiredoxin-4 ● Blarina toxin ● Toxin AaTX-1-like ● Venom dipeptidyl peptidase 4 ● Toxin CfTX-1/2 ● Zinc metalloproteinase nas-4/15 ● Venom factor (*Crotalus adamanteus*) ● Venom protease (*Megabombus pennsylvanicus*) ● Toxin A/B precursor ● Toxin CfTX 1/2 precursor Metalloproteinases ● Predicted protein (*Nematostella vectensis*) ● Metalloendopeptidase (*N. vectensis*) Serine Protease Inhibitors ● Predicted protein (*N. vectensis*)	(Brinkman et al., 2014, 2015; Jouiaei et al., 2015)

identified from *C. caliparea* venom using LC-MS/MS, the two pore-forming toxins, namely TX-1 toxin and Chrysaoralin, are homologous to *Chrysaora fuscescens* and *Chrysaora quinquecirrha*, respectively (Table 8). Pore-forming toxin induces transmembrane pores in the cell membrane, leading to disruption in permeability barriers and ultimately, cell death. TX-1, a toxin homologous to CfusTX-1 from *C. fuscescens*, is also a jellyfish toxin. Chrysaoralin contributes to the immobilisation of prey and assists in defence mechanisms. It exhibited close homology to *C. quinquecirrha* toxins and a hemolytic lectin from *Cucumaria echinata* (sea cucumber) (Riyas et al., 2021).

3.1.4.2. Chrysaora fuscescens *venom.* A total of twenty-seven putative toxins and venom-related proteins were identified in *C. fuscescens* venom using LC-MS/MS, among which include fourteen proteases (metalloproteinases, aspartyl proteases and serine protease), two pore-forming toxins, five venom allergens, two C-type lectins, one glycoside hydrolase, and one enzyme inhibitor. All these venom proteins are homologous to the Cnidarian species (Table 9). Some of the proteins are likely associated with essential cellular processes, including cell regulation and transport, as well as transcription and translation. Notably, among the metalloproteinases, five endothelin-converting enzyme 1-like and 2-like proteins were uncovered, which demonstrated homology with *H. vulgaris* and *N. vectensis*. While these metalloproteinases have potential roles in toxin maturation in wasps, or augmenting venom concentration in cone snails, further research is necessary to determine whether they exhibit similar toxic effects in the cnidarian species. Among the two pore-forming toxins, one is an uncharacterised protein homologous to *H. vulgaris*, while the other is a TX2 homologous to *A. aurita*. Box jellyfish toxins are known to display a wide range of toxicity, including pore formation, haemolysis, and cytotoxicity *in vitro*, as well as dermonecrosis, inflammation, pain, cardiovascular collapse, and death in animal models. The precise functions for the remaining identified proteins remain unclear, particularly in the context of human envenomation (Ponce et al., 2016).

3.1.5. Catostylidae family

3.1.5.1. Catostylus *sp. venom.* A total of ten putative toxins from the *Calostylus* sp. venom have been identified from LC-MS/MS, including a pore-forming toxin CrTX-A homologous to *Carybdea rastonii* (Table 10). Among the Scyphozoans, few research has investigated the venom composition of *Calostylus* spp. Morphological differences between the *Calostylus* spp. have been challenging due to the lack of unique traits in each species. This limitation has led to the generalisation of the genus in this study, rather than the precise identification of the *Calostylus* species. Pore-forming toxins are serine proteases, which can induce various symptoms like hemolysis, cytotoxic effects, dermonecrosis, inflammation, and pain (Edirisinghe et al., 2024).

3.2. Cubozoa class

3.2.1. Chirodropidae family

3.2.1.1. Chironex fleckeri *venom.* A total of twenty-six putative toxins were identified in *C. fleckeri* venom using LC-MS/MS. Most of these toxins belong to the CfTX toxin family, while the remaining thirteen putative toxins include seven proteases (Table 11) (Brinkman et al., 2015). Among them, four were identified as metalloproteinases, one as an alpha-macroglobulin domain-containing protein, two as cysteine-rich secretory proteins (CRiSP), and one as a turripeptide-like protease inhibitor. Members of the CfTX toxin family are highly hemolytic, and have been linked to various symptoms, including pain, inflammation, dermonecrosis, and cardiovascular collapse. These suggests the pivotal role of CFTXs in *C. fleckeri* envenomation (Brinkman et al., 2015).

Subsequent characterisation of two proteins, *C. fleckeri* toxin A and *C. fleckeri* toxin B (CfTX-A and -B), was conducted using size exclusion and cation exchange chromatography, followed by LC-MS/MS analysis. CfTX-A and -B belong to *C. fleckeri* toxin 1 and *C. fleckeri* toxin 2 (CfTX-1 and -2) family, respectively (Brinkman et al., 2014). These box jellyfish toxins (CfTX-A/B and CfTX1/2) act as pore-forming toxins, with CfTX-A/B exhibiting higher potency in *in vitro* hemolytic assays (Brinkman et al., 2014). *In vivo* studies on rodents exposed to CfTX-A, CfTX-B, CfTX-1, and CfTX-2 revealed that CfTX-A and -B induced only minor cardiovascular changes. Conversely, CfTX-1 and -2 induced cardiovascular collapse within a minute. This underscores the higher specificity of CfTX-1 and -2 towards vertebrate cardiac cells relative to

CfTX-A and -B, suggesting a more prominent role in human envenomation (Brinkman et al., 2014).

A study by Jouiaei et al. (2015) utilised a transcriptomic approach, followed by protein profiling using LC-MS/MS to identify several key venom proteins from *C. fleckeri*, including CfTX-A/B, CfTX-1/2 precursor, metalloproteinases with ShKT and peptidase M12A astacin domains, and a novel serine protease inhibitor with a Kazal domain (Table 11). These venom proteins exhibited homology to organisms within the Cnidaria phylum. Metalloproteinases, integral to prey capture, digestion, and defence mechanisms, play crucial roles during envenomation (Jouiaei et al., 2015). Specifically, the metalloproteinase M12A family is postulated to involve in extracellular matrix degradation, subsequently aiding in diffusion of other venom components to their molecular targets (Jouiaei et al., 2015).

4. Future directions and conclusion

Jellyfish envenomation remains a significant global health concern. However, current treatments for jellyfish stings are inadequate, highlighting the urgent need for effective and accessible therapies. This review emphasises the crucial role of proteins in mediating jellyfish venom toxicity and its clinical manifestations. Consequently, conducting comprehensive proteomic studies on major Scyphozoans and Cubozoans, along with their venom components, is imperative. Advancements in proteomics technology, particularly LC-MS/MS, have enabled precise protein profiling of organisms. Proteomic data on these jellyfish are vital for developing effective antivenoms, yet such information is currently lacking. Therefore, expanding proteomic research on major Scyphozoans and Cubozoans is essential for understanding the mechanisms of venom toxicity and facilitating the development of targeted antivenom therapies.

Moreover, proteomic profiling of jellyfish venom can identify novel therapeutic agents with potential analgesic or anti-inflammatory properties. Purified proteins of interest can also be tested *in vivo* to raise antibodies, contributing further to antivenom development. Common venom toxins identified across most major Scyphozoans and Cubozoans in this review include proteases, metalloproteinases, phospholipases, venom allergens, C-type lectins, serine protease inhibitors, pore-forming toxins, and ion channel inhibitors. Interestingly, CfTX-like toxins, also categorised as pore-forming toxins, are homologous across most Scyphozoans apart from Cubozoans. These proteins hold significant promise for developing antivenoms against various jellyfish species. In conclusion, enhancing our understanding of jellyfish venom through proteomic studies is critical for advancing therapeutic interventions and mitigating the health impacts of jellyfish envenomation.

CRediT authorship contribution statement

Shi Yuin Chong: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Rakesh Naidu:** Writing – review & editing, Validation, Supervision. **Iekhsan Othman:** Writing – review & editing, Supervision. **Syafiq Asnawi Zainal Abidin:** Writing – review & editing, Validation, Supervision, Project administration, Data curation, Conceptualization.

Ethics Statement

This is a review paper, so no type of experimentation was done, nor use of animals or biological material of any kind. The sources of information were cited accordingly.

Declaration of generative AI in scientific writing

During the preparation of this work, Chong Shi Yuin used ChatGPT (https://chatgpt.com) in order to explore alternative styles and approaches for writing scientific content, ensuring that all the facts and ideas are presented clearly in a straightforward manner. After using this tool, Chong Shi Yuin reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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Data availability

No data was used for the research described in the article.

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