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FROM CLINICAL TOXINOLOGY TO PROTEOMIC DISCOVERY: DECODING THE VENOM PROTEIN COMPOSITION OF *CHIRONEX YAMAGUCHII* FROM SABAH

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INTRODUCTION

Multi-tentacled box jellyfish endemic to Sabah, Malaysia, have been implicated in at least six pediatric fatalities between 2006 and 2022.[1] An unpublished local investigation confirmed the species responsible as *Chironex yamaguchii*, one of the most venomous jellyfish globally. Clinically, stings can lead to rapid cardiorespiratory collapse and death within minutes[2], highlighting the urgency of understanding its venom composition. Currently, only one available box jellyfish antivenom targets *Chironex fleckeri*, with unproven efficacy against *C. yamaguchii*. Despite its clinical importance, no proteomic profiling has been conducted to identify the specific toxins and underlying mechanisms responsible for its lethality

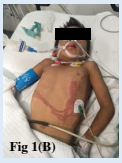
METHODOLOGY

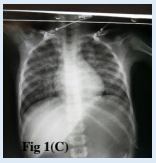
Specimens of *C. yamaguchii* were collected from Lahad Datu's coastal waters, where prior severe envenomation was reported. Identification followed established morphological criteria.[2] Venom was extracted via nematocyst autolysis, sonication, and centrifugation. Protein concentrations were measured using a Nanodrop spectrophotometer. Proteomic analysis included sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE) and reverse-phase high-performance liquid chromatography (rpHPLC). Tandem mass spectrometry (LC-MS/MS) was used for protein identification and toxin profiling.

RESULT

LC-MS/MS analysis identified 25 unique protein groups in the venom of *C. yamaguchii*. Among these, six toxins—CqTX-A, CfTX-1, CfTX-2, CfTX-A, CfTX-B, and a TX-like toxin fragment—were detected. These are associated with membrane pore formation, hemolysis, and cardiovascular toxicity, aligning with clinical features of rapid systemic collapse.

Stings from *C. yamaguchii* typically produce multiple whip-like lash marks with deep linear imprints on the skin. The pattern often resembles shoelaces or a ladder-like configuration. Central areas may show early necrosis, while the surrounding skin is inflamed, erythematous, and oedematous due to severe local envenomation. These distinctive lesions aid in clinical identification and differentiation from other jellyfish species.



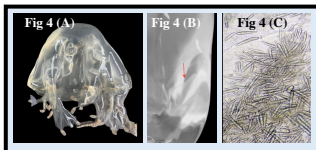


Apart from severe pain and marked local skin reactions, patients stung by *C. yamaguchii* may develop life-threatening systemic envenomation. This includes rapid onset of cardiorespiratory dysfunction, often requiring mechanical ventilation. The chest X-ray above demonstrates non-cardiogenic pulmonary edema that developed within two hours post-sting, consistent with venom-induced increased pulmonary capillary permeability and systemic inflammatory response.

Jellyfish sampling was conducted at the coastal waters of Pulau Sakar, Lahad Datu. Specimens were collected by boat using a modified scoop net or by bare hand, grasping the non-venomous bell area to ensure safe handling.

The tentacle was excised and soaked in seawater at 4°C for five days to facilitate nematocyst separation from the tissue. Each day, an aliquot of the soaking water was collected and examined under a microscope to monitor nematocyst release. Upon completion of the process, the water was filtered using a mesh net to concentrate the nematocysts, which were then frozen for transport.

The filtered sample was centrifuged to isolate intact nematocysts, which were then freeze-dried for preservation. For venom extraction, the dried nematocysts were rehydrated in purified water and subjected to sonication to rupture the capsules and release crude venom for further analysis.



Characteristic features (Fig 4A) of *C. yamaguchii*: inter-radial width of bell 7.9-14cm, bell height 7.5-12cm, maximum of 9 tentacles emerges from claw-like pedalia, flat and broad tentacles, upswept 'spike' to the pedalia canal with volcanic in shape (Red arrow, Fig 4B).

(Fig 4C) The nematocysts of *C. yamaguchii* consist primarily of **mastigophores (eurytales)**, which are penetrating nematocysts responsible for delivering venom deep into tissues. These capsules are equipped with spiny tubules capable of piercing skin and blood vessels, contributing to the potent cardiotoxic and dermonecrotic effects observed in envenomation. Minor types such as **atrichous isorhizas** may also be present, though their role in toxicity is less significant.

Fig 5

SDS-PAGE electrophoresis of the *C. yamaguchii* crude venom at varying dilutions revealed multiple distinct protein bands, indicating the presence of a complex mixture of proteins with diverse molecular weights.

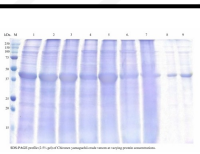
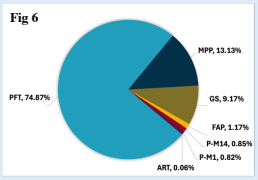


Table 1

Toxins	Abbreviation		
Pore forming toxin	PFT	38.55%	74.87%
M13 metallopeptidase	MPP	6.76%	13.13%
Gelsolin	GS	4.72%	9.17%
Flagellar-associated proteins	FAP	0.60%	1.17%
Peptidase M14	P-M14	0.44%	0.85%
Peptidase M1	P-M1	0.42%	0.82%
ADP-ribosyltransferase	ART	0.03%	0.06%
		51.49%	100.00%

Fig 6



Out of the 25 protein groups identified, seven were classified as toxin-related. Among these, six major toxins—**CqTX-A, CfTX-1, CfTX-2, CfTX-A, CfTX-B**, and a **TX-like toxin fragment** were detected. Together, these constituted **74.87%** of the total pore-forming toxin content, highlighting their dominant role in the venom's cytolytic and cardiotoxic effects.

DISCUSSION

This is the first venom proteomic profile of *C. yamaguchii* in Malaysia. The identification of cardiotoxic and hemolytic proteins reinforces clinical observations of sudden cardiac arrest. These findings contribute critical insights for translational research, with implications for species-specific antivenom development, diagnostic innovation, and emergency response protocols.

The detection of CfTX family toxins, which have previously been characterized in *Chironex fleckeri*[3], suggests a conserved venom architecture across Chirodropid species. This supports the hypothesis of functional homology in cardiotoxic and cytolytic components, potentially explaining the overlapping clinical manifestations observed in different geographic regions. Such molecular parallels also highlight the feasibility of developing cross-reactive antivenoms, while emphasizing the need for localized venom profiling to capture interspecies variations that may affect therapeutic efficacy.

CONCLUSIONS

Proteomic analysis offers vital understanding of *C. yamaguchii* envenomation. Future studies should explore the functional and lethal properties of these toxins to guide evidence-based clinical management and improve survival outcomes in jellyfish sting cases.

REFERENCE

1. Muhamad Na'im Bin Ab Razak, Ahmad Khaldun Ismail, Praneetha Palasuberniam, Fairrul Kadir, Balu Alagar Venmathi Maran. "Jellyfish Sting Related Mortalities in Malaysia (1990-2022)". Presented at the 22nd Scientific Congress of The Asia Pacific Association of Medical Toxicology. 2024.
2. Lewis, C. & Bentlage, B. (2009). Clarifying the identity of the Japanese *Habu-kurage*, *Chironex yamaguchii*, sp. nov. (Cnidaria: Cubozoa: Chirodropida). *Zootaxa*, 2030, 59–65.
3. Piontek, M., Seymour, J. E., Wong, Y., Gilstrom, T., Potriquet, J., Jennings, E., Nimmo, A., & Miles, J. J. (2020).The pathology of *Chironex fleckeri* venom and known biological mechanisms. *Toxicon: X*, 6, 100026

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