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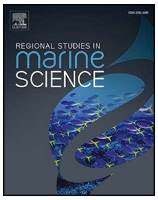

Molecular signatures reveal intra-species divergence, undetectable by traditional morphology, in the deadly box jellyfish, *Chironex yamaguchii* (Cubozoa; Chirodropidae) of Western Pacific

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ABSTRACT

Extensive systematics studies have led to remarkable advances in understanding cubozoan diversity, but identifying chirodropid species remains challenging due to morphological similarities and early taxonomic placements of many cubomedusae. Building from this state of knowledge, we investigated the identity of a venomous and deadly chirodropid box jellyfish in the Western Pacific (the Philippines and Japan) by analysing molecular and morphological features of the species. Here, we hypothesised that *Chironex yamaguchii* occurs in the Coral Triangle region of the Philippines, in addition to the records from the South Kuroshio region in Japan. After thorough morphological and molecular examinations, our findings reveal *C. yamaguchii* exists in the Coral Triangle. Traditional morphology including the pedalial canals, and microscopy of cnidocytes show nearly identical features between Japan's and the Philippines' cubomedusae. Remarkably, molecular analyses using cytochrome oxidase I (*cox1*) and ribosomal RNA (*16S*), revealed subtle divergence between Philippine and Japan's *C. yamaguchii* (p-distances: *cox1*=0.04–0.05; *16S*=0.03–0.11; patristic distances: *cox1*= 0.02–0.02; *16S*= 1.90–5.20; *p*-values for both markers and distance values = <0.01). Phylogenetic analyses also confirmed the clade for *C. yamaguchii* from Japan is distinct from the Philippine clade with moderate to strong branch supports (> 90 %) while haplotype analyses indicated mutations separating Philippine *C. yamaguchii* from Japan's specimens and other *Chironex* species. Further, the genetic markers and morphological identification were inconsistent, with *C. yamaguchii* from Japan and the Philippines appearing as distinct species in species-delimitation assessments using Assemble Species by Automatic Partitioning and Multi-rate Poisson Tree Processes. With these outcomes, we classified Philippine *C. yamaguchii* as putative cryptic species, awaiting clarification on possible reproductive isolation of this cubozoan. Our integrative approach resolved the taxonomic uncertainty, and solidified occurrence of *C. yamaguchii* in the Coral Triangle. This study emphasizes the importance of combined morphological and molecular analyses for accurate species identification in Cubozoa.

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1. Introduction

Marine species and their distributions are fundamental components of marine biodiversity since they play critical roles in ecosystem functioning such as habitat formation, and food web dynamics (Dipper, 2022). However, many marine species such as medusozoans remain understudied despite the urgency to describe them, amidst necessity in conservation and management efforts, climate emergency and crisis of biodiversity loss (Bouchet et al., 2023; Pörtner et al., 2023; Barros-Platiau et al., 2024). Currently, around 246,000 known valid marine species exist (updated from Bouchet et al., 2023; WoRMS Editorial Board, 2024). This number continues to grow with ~ 2300 new species being identified each year (Bouchet et al., 2023; WoRMS Editorial Board, 2024). Despite extensive biological and ecological studies of marine cnidarian species, including jellyfish and corals, further research on their distribution, diverse habitats, and representation in conservation efforts is needed (Goffredo and Dubinsky, 2016; Chen, 2021; Durrant, 2024). This gap in scientific information links to the challenges associated with studying these organisms such as access to their natural habitats, and funding and public interest that favour more well-known and charismatic species like bony fish and marine mammals (Collier et al., 2016; Chen, 2021). These factors leave marine cnidarians relatively underrepresented in scientific literature (Collier et al., 2016; Chen, 2021; Bouchet et al., 2023). Nevertheless, cnidarians remain vital to aquatic ecosystems (Dipper, 2022; Durrant, 2024). Thus, understanding the diversity and distribution of marine cnidarians is critical (Goffredo and Dubinsky, 2016; Durrant, 2024).

Members of the box jellyfish genus *Chironex*, a cnidarian group, exemplify profound importance in marine ecosystems (Sucharitakul et al., 2019). With their powerful venom, distinctive morphology, and complex life cycles, *Chironex* species are significant components of Indo- and Western Pacific ecosystems (Carrette et al., 2002; Sucharitakul et al., 2019; Rowley et al., 2020). These cubozoans not only influence trophic dynamics through their predatory behaviour but may also contribute to nutrient cycling and energy transfer within marine food webs (Hamner et al., 1995; Carrette et al., 2002; Morrissey et al., 2024). However, our understanding of the fundamental biology and venom biochemistry of this group largely depends on scientific studies on one species of the genus, i.e. *C. fleckeri* (Piontek et al., 2020; Rowley et al., 2020; Morrissey et al., 2024). Furthermore, the ecological roles and zoological research for *Chironex* species require comprehensive biological or systematics studies, particularly in the face of management strategies for venomous medusozoans, global change and biodiversity loss (Rowley et al., 2020; Pörtner et al., 2023). Building on this research need, we must clarify the genus' evolutionary history and population dynamics, which remain poorly understood (but see Sucharitakul et al., 2019 and Rowley et al., 2020). This knowledge gap warrants further assessments into their systematics.

Chironex members comprise some of the most venomous metazoans, causing severe envenomations and human fatalities in the Indo- and Western Pacific (OPIHE, 1999; Kitatani et al., 2015; Sucharitakul et al., 2019; Hifumi et al., 2020; see Fig. S1 and Table S1, Supplementary Materials). The cubozoan, through their tentacles, can induce extreme envenomation that may lead to severe pain, notable injury on the skin with welts, cardiac arrest and death (Sucharitakul et al., 2019; Piontek et al., 2020). In the Philippines alone, many coastal communities recognize severe envenomation and fatalities due to *Chironex* (Fig. S1a-c and Table S1, Supplementary Materials). Despite their potent venom and significant public health impact, scientific research on *Chironex* lags behind other cnidarian groups, such as scleractinian corals, which are already being explored for scientific advances such as bioprospecting and global change ecology (Cooper et al., 2014; Goffredo and Dubinsky, 2016; Klein et al., 2024, but see venom biochemistry in Piontek et al., 2020). To address this knowledge gap, a deeper understanding of *Chironex*'s ecological dynamics and public health management will be examined (Condie et al., 2018; Rowley et al., 2020). This can be initiated through accurate taxonomic examination and extensive zoological surveys of this cubozoan group (Condie et al., 2018; Sucharitakul et al., 2019).

Genus *Chironex* consists of congeners with morphological similarities among them, leading to challenges in their taxonomic identification (Jarms and Morandini, 2019; 2023). *Chironex* species may be misidentified, partly due to early taxonomic reconstructions combining disparate characteristics, which obscure their true identity (Lewis and Bentlage, 2009; Sucharitakul et al., 2017). For example, the medusae of *Chironex* contain few diagnostic characteristics such as the smooth cuboid bell and pedalial canal bend, leading to potential misidentification and the possibility of overlooking cryptic species within the group (Lewis and Bentlage, 2009; Jarms and Morandini, 2019; 2023). Despite recent advances in understanding *Chironex* spp., the need for continued research and careful identification of this group remains (Sucharitakul et al., 2019; Morrissey et al., 2024). Additional collections of specimens of this genus from a range of ecoregions, including the Coral Triangle, are warranted to address this knowledge disparity, reveal potential morphological and genetic variations within the group, and eventually shed light on the diversity of *Chironex* (Lewis and Bentlage, 2009; Sucharitakul et al., 2019).

Integrating molecular analytical tools with morphology can overcome the taxonomic limitations in traditional morphology of cubozoans, revealing biological diversity and uncovering evolutionary information undetectable by morphology alone (Lawley et al., 2016; Sucharitakul et al., 2017). This combined approach is particularly important in marine ecosystems, where vastness and complexity may lead to underestimation of biodiversity (Lawley et al., 2016; Dipper, 2022). By integrating molecular and morphological data, we can gain a more comprehensive understanding of marine species, including subtle genetic variations and potentially the evolutionary history of marine cnidarians including box jellyfish (Medina et al., 2001; Lawley et al., 2016).

Here, we aimed to analyse morphological data and molecular markers to examine the taxonomic identity and assess the relationship between genetic and morphological information from a box jellyfish species in the Coral Triangle. Given the conservative morphology of Cubozoa and the potential presence of *Chironex* species in this ecoregion (Lewis and Bentlage, 2009; Jarms and Morandini, 2019; 2023), we focused on identifying a chirodropid species from the Philippines. Cubozoan taxonomic literature indicates occurrence of *Chironex yamaguchii* in the Coral Triangle (Sabah, Malaysia in Chuan et al., 2021; in the Philippines in Lewis and Bentlage, 2009; Jarms and Morandini, 2019; 2023). However, the proposition about the cubozoan's Philippine distribution follows a clear caveat stating possible existence of species in the region, distinct from *C. yamaguchii* in South Kuroshio (Japan) due to morphological conservativeness of many cubozoans, and absence of genetic samples of *Chironex* from the Philippines (Lewis and Bentlage, 2009; Azama et al., 2023). Considering this taxonomic information, we hypothesised that the chirodropid box jellyfish *C. yamaguchii* occurs in the Coral Triangle region of the Philippines. We also hypothesised that genetic findings using mitochondrial markers would reveal molecular barcoding signatures and these signatures would be consistent with morphological information indicating a *Chironex* species.

2. Materials and methods

2.1. Specimen collection

Six specimens of cubomedusae were collected in July 2016 and additional 14 specimens were collected in 2019 in central Philippines (see Fig. 1a-c; Tables 1–2). All specimens were scooped from the water using a plastic bucket filled with ambient water, preventing damage on them. In 2019, fresh tissue from each specimen was collected in the field for molecular analyses (Table 2). Then, specimens were preserved in 4 % formalin in filtered seawater and deposited in the National Museum of the Philippines (NMP) and Invertebrate Biology Museum of the

University of the Philippines Diliman (UPDIM). See Tables 1–2.

2.2. Morphological examination

Morphological assessments were made on both fresh and preserved specimens. Diagnostic features such as bell height were described and their lengths were measured using a digital caliper (ET50) with 0.1 mm precision by following standard methods (see Fig. S2, Supplementary Materials; Lewis and Bentlage, 2009; Sucharitakul et al., 2017). Fresh weights (FW) of the specimens were obtained after removing excess water with cloth or paper towel and by using a weighing scale with 0.01 g precision, at the coast. Juvenile cubomedusae were differentiated from mature individuals which contain fully developed gastric saccules, following Southcott (1956) and Lewis and Bentlage (2009). We used stereo- and compound microscopes to observe microscopic tissues. Cnidocytes from fresh specimens were collected by scraping the surface of a tentacle or by gently pressing a plastic tape on the tentacle and transferring the tentacle cells from the tape onto a microscope slide (as in Sucharitakul et al., 2017). Based on morphology, the box jellyfish was identified using guides by Mayer (1910), Southcott (1956), Barnes (1965), Gershwin (2006), Lewis and Bentlage (2009), Sucharitakul et al. (2017) and Jarms and Morandini (2019; 2023).

Data from observations, including photos and videos of box jellyfish, posted on citizen-science platforms (https://rb.gy/77wnb; https://rb.gy/9wey1) from 2015 to 2024 were manually downloaded and recorded as part of the Philippine Jellyfish Stings Project, which systematically documents occurrences of jellyfish in the Philippine region (Boco et al., 2024; Capidos et al., 2024). Contributors in the program were contacted to gather additional details of the observations such as GPS coordinates, nearest landmark to the observation (providing up to 20 m^2 resolution of the location of medusae), observation date and time, and estimated sizes (e.g., bell height) of the box jellyfish. We sorted our citizen-science records, retaining photos and videos showing chirodropid box jellyfish. These were further examined to ascertain whether they belong to a species within the genus *Chironex* by comparing the morphology of cubozoans in the citizen-science media to the photos and descriptions of box jellyfish in taxonomic guides and from other high-resolution (>300 dots per inch) images and videos of box jellyfish, following Collins et al. (2011), Lawley et al. (2016) and Terenzini et al. (2023). These procedures were also followed in examining our preserved materials. Here, we examined and used in the comparisons, photographs of live and preserved medusae of *C. yamaguchii* from Okinawa, Japan (by Sho Toshino; see also page 779 in Jarms and Morandini, 2019; 2023; two photos of *C. yamaguchii* collected from another verified source: https://churaumi.okinawa/en/fishbook/00000405) and Kota Kinabalu, Malaysia (a video of a live medusa of the same species collected in February 2020 from 6°02'27.2" N, 116°06'32.8" E; accession number: IPMB-C 01.00080; Chuan et al., 2021). Further analyses were made using these materials: high resolution materials showing *C. fleckeri* medusae (two videos of 2 medusae observed by M. Hicks in 14 February 2021 at 20°16'21.1"S 148°43'33.7"E in the port of Airlie in Australia; two photographs of a

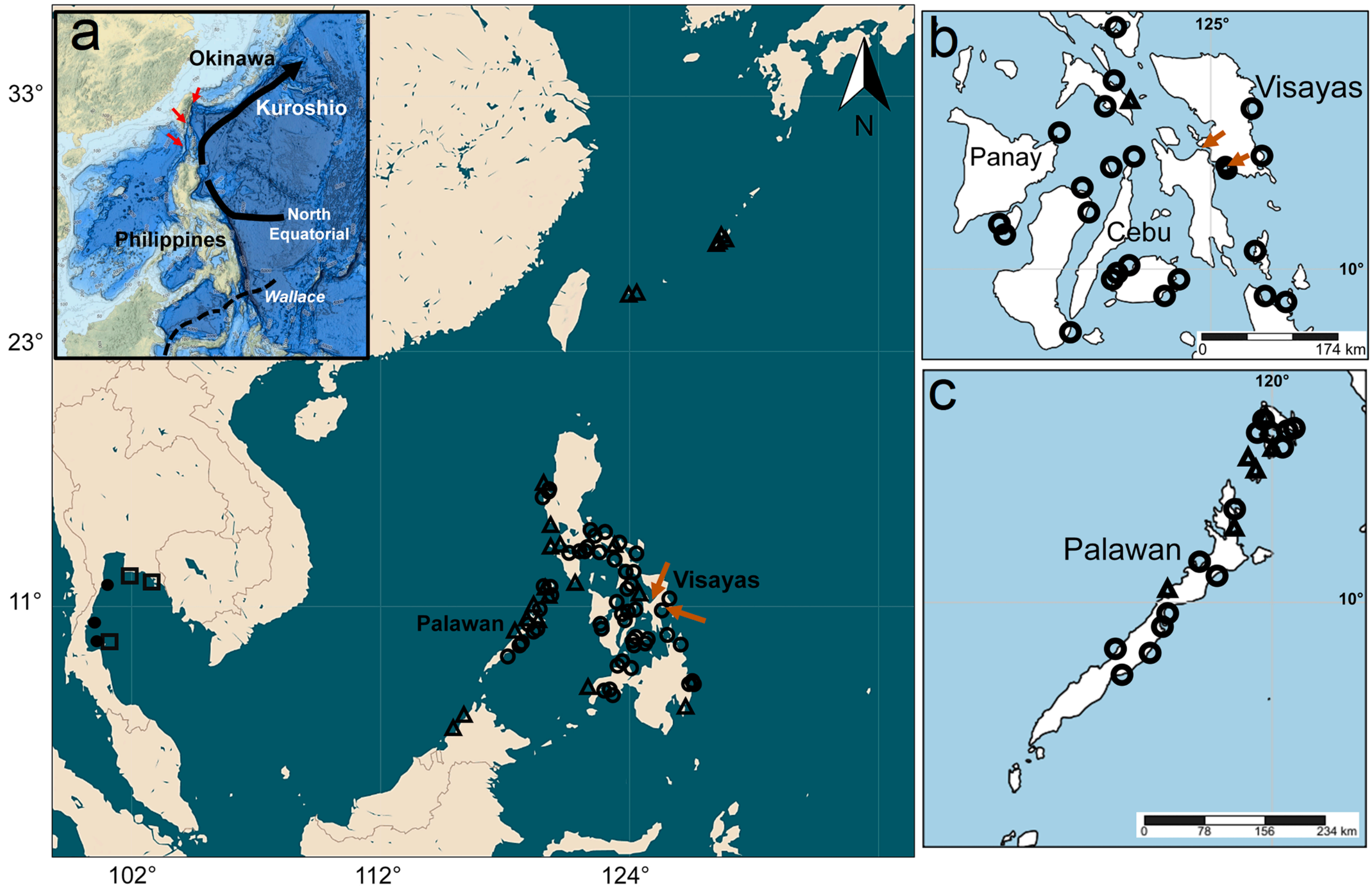

Fig. 1. Distribution of *Chironex* spp. in the northwestern Pacific. **a.** *Chironex yamaguchii* occurs in the Coral Triangle (the Philippines; Malaysia) and Okinawa, Japan whereas *C. indrasaksajiae* and *Chironex* sp. A. exist in Thailand waters. Inset bathymetric map of the Philippines and Okinawa, Japan shows major ocean currents (North Equatorial and Kuroshio), shallow depths (50–100 m) in light blue shades and shallow zones between Okinawa and the Coral Triangle are pointed by red arrows (a); **b-c.** distribution of *C. yamaguchii* in central (**b**) and western (**c**) Philippines. Triangles are locations of *C. yamaguchii* based on museum specimens while circles are locations of this species based on citizen science. Squares = *C. indrasaksajiae* and dot = *Chironex* sp. A in Thailand. Orange arrows = locations of new specimens. Base maps = main from ESRI (a-c) and inset (a) from NOAA's NCEI.

Table 1
Records of *Chironex yamaguchii* medusae in western Pacific (Malaysia, Philippines and Japan). Countries where the cubomedusae occur are in bold and italic. Biogeographic zones (i.e., ecoregion)[Ŧ] for locations and depth of each record were identified. WPS = West Philippine Sea, N = Northern Philippine Sea, and SPS = Southern Philippine Sea ecoregions.

Locality	Latitude	Longitude	Ecoregion	Depth (m)	Date	Reference
Japan						
Ishigaki Island (Is)	24°24’34.6"N	124°08’12.5"E	South Kuroshio	-	24 Sep 2007	USNM 1121554–5
Horseshoe Cliffs	26°30’06.3"N	127°50’29.0"E	South Kuroshio	34	19 Sept 1988	USNM 1121556
Nakagusuku Bay, Okinawa Is	26°25’50.0"N	127°50’53.2"E	South Kuroshio	-	July 1992	Lewis and Bentlage (2009)
Kana Beach, Okinawa Is	26°28’07.1"N	127°58’13.4"E	South Kuroshio	-	-	Lewis and Bentlage (2009)
Ginowan, Okinawa Island	26°16’48.0"N	127°43’12.0"E	South Kuroshio	-	-	Lewis and Bentlage (2009)
Chatan Beach, Okinawa Is	26°18’00.0"N	127°43’48.0"E	South Kuroshio	-	-	Lewis and Bentlage (2009)
Motobu Port, Okinawa Is	26°39’00.0"N	127°52’48.0"E	South Kuroshio	-	-	Lewis and Bentlage (2009)
Sukuji Beach, Ishigaki Is	24°27’56.1"N	124°07’24.8"E	South Kuroshio	-	-	Lewis and Bentlage (2009)
Ginowan Port, Okinawa Is	26°16′38″N	127°43′48″E	South Kuroshio	-	15 July 2012	Kondo et al. (2018)
Chatan, Okinawa Is	26°18’00.0"N	127°43’48.0"E	South Kuroshio	-	19 Aug 1998	RUMF-ZG−04366
Urasoe, Okinawa Is	26°13’56.1"N	127°40’46.7"E	South Kuroshio	-	10 Sept 2013	Azama et al. (2023)
Urasoe, Okinawa Is	26°13’57.1"N	127°40’46.0"E	South Kuroshio	-	28 Aug 2019	Azama et al. (2023)
Ginowan, Okinawa Is	26°16’28.2"N	127°42’35.2"E	South Kuroshio	-	30 June 2011	Azama et al. (2023)
Nago, Okinawa Is	26°31’00.0"N	128°02’00.0"E	South Kuroshio	-	22 July 2022	Azama et al. (2023)
Yagaji, Okinawa Is	26°40’11.8"N	128°01’24.5"E	South Kuroshio	-	22 Aug 2019	Azama et al. (2023)
Ishigaki port	24°20’00.0"N	124°08’00.0"E	South Kuroshio	-	14 July 2020	Azama et al. (2023)
Philippines						
Bolinao Bay, Pangasinan	16°33’00.0"N	119°52’12.0"E	WPS	2.9–4.0	10 May 1909	USNM 28694
Bolinao Bay, Pangasinan	16°33’00.0"N	119°52’12.0"E	WPS	2.9–4.0	10 May 1909	USNM 28697; Lewis and Bentlage (2009)
Subic Bay	14°45’00.0"N	120°12’00.0"E	WPS	-	7 Jan 1908	USNM 27911; 27915
Hamilo Cove, Batangas	14°10’10.8"N	120°34’38.9"E	WPS	2.9	13 July 1908	USNM 28700
San Miguel Bay, Luzon	13°57’42.1"N	123°06’43.2"E	N Philippine Sea	20	14 June 1909	USNM 28701
San Miguel Bay, Luzon	13°57’42.1"N	123°06’43.2"E	N Philippine Sea	20	14 June 1909	USNM 28695
Tilik Bay, Lubang Is	13°48’56.0"N	120°12’09.4"E	WPS	39	14 July 1908	USNM 28698; Lewis and Bentlage (2009)
Mansalay Bay, Mindoro Is	12°31’07.3"N	121°26’48.0"E	WPS	2.0–2.9	4 June 1908	USNM 27917–8; Lewis and Bentlage (2009)
Busuanga, Palawan	12°07’07.6"N	119°55’50.4"E	WPS	-	1913	Light (1914)
Culion Is, Palawan	11°45’24.7"N	120°02’27.1"E	Sulu Sea	-	1913	Light (1914)
Linapacan Strait, Palawan	11°37’14.9"N	119°48’43.2"E	WPS	84	18 Dec 1908	USNM 28696; Lewis and Bentlage (2009)
Linapacan Is, Palawan	11°27’19.6"N	119°48’36.0"E	WPS	2.9–6.0	19 Dec 1908	USNM 28692
Taytay, Palawan	10°49’40.6"N	119°30’43.7"E	Sulu Sea	-	1913	USNM 38019; Lewis and Bentlage (2009)
Ulugan Bay, Palawan	10°04’48.0"N	118°46’48.0"E	WPS	0.5–2.0	28 Dec 1908	USNM 28691
Cataingan Bay, Masbate	11°59’58.0"N	124°00’04.5"E	Visayan	2.0–2.9	April 1908	USNM 27913; Lewis and Bentlage (2009)
Opong Bay, Leyte	**11°25’35.5"N**	**124°50’04.7"E**	Visayan	1.5	16 July 2016	This study; see Table 2
Opong Bay, Leyte	**11°25’37.2"N**	**124°50’32.6"E**	Visayan	2.3	29 May 2019	This study; see Table 2
Sangputan Bay, Babatngon	**11°25’09.0"N**	**124°54’03.0"E**	Visayan	2.0	29 May 2019	This study; see Table 2
Marabut, Samar	**11°11’50.7"N**	**125°10’54.6"E**	SPS	1.8	17 July 2016	This study; see Table 2
Pujada Bay, Mindanao	6°55’39.7"N	126°14’24.1"E[A]	SPS	2.9	15 May 1908	USNM 27914; Lewis and Bentlage (2009)
Zamboanga del Sur	7°40’48.0"N	122°01’12.0"E	Sulu Sea	-	6 Feb 1908	USNM 27916
Malaysia						
Kota Kinabalu, Malaysia	6°02’27.2"N	116°06’32.8"E	West Coral Triangle	-	14 Feb 2020	Chuan et al. (2021)

Locations in bold are collection areas of new specimens in this study. Note: Light 1914 specimens cannot be recovered.

- = no data

[Ŧ] Based on Spalding et al. (2007) and Nañola et al. (2011).

[A] Location predicted using geographic references of the record.

medusa observed by C. Hedges at 12°38’30.5"S 141°50’35.9"E in Albatross Bay, off the town of Weipa in Australia; nine photographs of the same species observed in Northern Australia by G. Bell, deposited in the website Oceanwide Images at http://www.oceanwideimages.com/species.asp?s = Chironex+fleckeri); two photos of *C. indrasaksajiae* medusae observed in 12 September 2018 from Koh Pha Ngan province in Thailand by a team of the Coastal Marine Research Centre, Thailand; photos (n = 2) of *Chiropsalmus quadrumanus* captured on September 2015 from northwest Atlantic (Florida, USA: 29°40’09.2"N 81°12’52.5"W) through observer Jose Nuñez; and a photo of a specimen of *Chiropsoides buitendijki* observed by A. Waharak in Phang-Nga Province of Thailand. Finally, we compared our box jellyfish to the images and descriptions of *Chiropsella* spp., e.g., *C. bronzie,* from Barnes (1965), as the synonym Chiropsalmus quadrigatus), Gershwin (2006) and Jarms and Morandini (2019; 2023) to distinguish our box jellyfish from *Chiropsella* which can possibly coexist with other cubozoans in the Philippines, akin to the coexistence with *C. fleckeri* in Australia (Gershwin, 2006).

2.3. Molecular analyses

2.3.1. DNA extraction, amplification and sequencing

Tissue samples (size: 1 cm^2) were collected from the bell margin from each of 14 specimens collected in 2019 (see Table 2). They were fixed in 96 % ethanol and stored in −20 °C. DNA was extracted through the following proteinase-phenol-chloroform protocol, appropriate for extracting genetic material of *Chironex* spp. (Licandro et al., 2015; Sucharitakul et al., 2017). Tissues were placed in microcentrifuge tubes (1 tube per specimen) containing lysis buffer and proteinase K (1 mg/ml solution) and incubated in 60 °C for 48 hours. Phenol was then added, and samples were centrifuged at 13,500 rpm for 15 minutes. The supernatant solution was transferred to a new microcentrifuge tube. New solutions of phenol, and chloroform were added and between additions of these solutions, the resulting mixtures were centrifuged at 13,500 rpm for 15 minutes. The supernatant was gently mixed with 6 M NaCl in a new microcentrifuge tube. To precipitate the DNA, the mixture was mixed with cold (-2 °C) absolute ethanol and kept overnight in −20 °C, Then, the samples were centrifuged at 13,000 rpm for 10 minutes, the supernatant was discarded, 70 % ethanol was added and again centrifuged at 13,000 rpm for 5 minutes. After air-drying the

Table 2
Details of whole medusae of *Chironex yamaguchii* collected from several localities in the Philippines in this study (see also Fig. 1).

Locality[a]	Collection date	Bell height (mm)	Interperradial Distance (mm)	Fresh weight (g)	No. tentacles per pedalium (range)	Museum codes
Opong Bay	16 July 2016	91.2	120.0	325.2	8–9	NMP2017C−05
Opong Bay	16 July 2016	87.0	110.0	310.8	8–8	UPDIM-CUB004
Marabut, Samar	17 July 2016	63.0	75.3	153.0	7–9	UPDIM-CUB007–0
Marabut, Samar	17 July 2016	70.3	85.0	191.4	7–8	UPDIM-CUB007–11
Marabut, Samar	17 July 2016	53.6	55.8	125.0	8–9	UPDIM-CUB007–12
Marabut, Samar	17 July 2016	64.0	80.0	177.0	7–9	UPDIM-CUB005
Opong Bay	29 May 2019	45.1	64.0	143.0	8–8	NMPZC−2021.01
Opong Bay	29 May 2019	59.0	75.0	150.0	8–9	NMPZC−2021.02
Opong Bay	29 May 2019	67.0	82.2	184.6	7–7	NMPZC−2021.03
Opong Bay	29 May 2019	63.0	79.0	162.0	6–8	NMPZC−2021.04
Sangputan Bay	29 May 2019	54.0	68.6	147.0	7–7	NMPZC−2021.05
Sangputan Bay	29 May 2019	48.2	43.0	118.3	7–8	NMPZC−2021.06
Sangputan Bay	29 May 2019	46.0	57.0	136.5	7–8	NMPZC−2021.07
Sangputan Bay	29 May 2019	40.6	40.2	140.7	8–8	NMPZC−2021.08
Sangputan Bay	29 May 2019	50.4	40.4	149.0	7–8	NMPZC−2021.09
Sangputan Bay	29 May 2019	50.4	45.6	147.1	8–9	NMPZC−2021.10
Sangputan Bay	29 May 2019	41.2	40.6	171.0	8–8	NMPZC−2021.11
Sangputan Bay	29 May 2019	40.5	42.0	135.0	8–9	NMPZC−2021.12
Sangputan Bay	29 May 2019	46.5	44.9	182.6	7–8	NMPZC−2021.13
Sangputan Bay	29 May 2019	44.0	48.3	147.2	7–8	NMPZC−2021.14

[a] Refer to Table 1 for the geographic coordinates of these samples.

supernatant, Tris-EDTA buffer was added to obtain the purified nucleic acids.

Two primer sets for two genes, *cox1*(Geller et al., 2013) and *16S* (Sucharitakul et al., 2017), were used in the polymerase chain reactions (PCR) for the specimens. *Cox1* was amplified using these primers: jgHCO2198 (5’ TAN ACY TCN GGR TGN CCR AAR AAY CA 3’) and jgLCO1490 (5’ TNT CNA CNA AYC AYA ARG AYA TTG G 3’). We used these *16S* primers which were designed for molecular identification of *Chironex* spp., i.e. P16sf (5’AAGGGCCGCGGTAACTCTG 3’) and S16sr (5’ACCCTGTTATCCCCGTGGT 3’; see Sucharitakul et al., 2017). DNA amplification of the genes was conducted using a thermal cycler. The reaction mixture (total volume: 25 μL) consisted of PCR-grade water, Hotstart PCR buffer (2.5 μL), $MgCl_2$ (2 μL), 2.5 mM of dTNP mixture (1.875 μL), the forward and reverse primers, Taq® Hotstart DNA Polymerase (0.125 μL) and a DNA template. Thermal cycling was run in temperature and duration of cycles similar for *16S* and *cox1*, i.e., 95 °C for 5 min. pre-denaturation, 95 °C of denaturation (30 sec), annealing for 30 s at 50 °C for *cox1* and 64 °C for *16S*, and infinite final extension at 4 °C. A 1 % agarose gel (Vivantis) was used to visualise the PCR products. Genetic sequences were obtained through the Sanger sequencing protocol and they were deposited to GenBank (accession codes: *cox1:* PQ780731–44, *16S:* PQ788837–50). Sequenced nucleotide-chromatograms were inspected and aligned using CLUSTAL Omega algorithm in Geneious Prime 2024 (https://www.geneious.com).

2.3.2. Genetic divergence

To assess extent of genetic differences between our sequences for this species and other *Chironex* spp., we calculated uncorrected pairwise (p) and patristic distances between them in MEGA 11 and Geneious Prime 2024, respectively. In this study, patristic distances reveal genetic change by summing branch lengths between two nodes in a phylogenetic tree, complementing our p-distance values (Ogedengbe et al., 2018). Sequences from GenBank in the analyses (code, c.: *cox1*; *16S*) include those from Japan’s *C. yamaguchii* (c: FJ665180; AGC0592), *C. fleckeri* (c: FJ665181; GQ849103), *C. indrasaksajiae* (c: KU646841; KX090147) and the cubozoan outgroup *Carybdea prototypus* (synonym: *C. brevipedalia*; c: OM108433; KT288256). The representative GenBank sequence was chosen based on its closest identity to our sequences as determined using percent identity in BLAST and their relatively low p-distances from our sequences.

We fitted the patristic and p-distances (i.e., response variables) in generalised linear mixed model (GLMM) and generalised linear model (GLM) analyses (Agresti, 2015), to test the differences of the means of the genetic distances across *Chironex* species (i.e., the fixed predictor). These model approaches were chosen due to the non-normal and slightly skewed nature of our data in Shapiro-Wilk test, q-q plot visualisation, and Levene’s test. Here, both analyses included Gamma distribution family and identity link function while the response data include continuous values in decimal, fit for Gamma-based models (Agresti, 2015). Gamma distribution and identity link are appropriate choices because we maintained the original scale of the response values without assuming a specific relationship between the mean and variance as the data represent evolutionary characteristics such as the differences of nucleotide sequences (Agresti, 2015; see Liu et al., 2019; Lozano-Fernandez, 2022). The GLMMs were initially performed to explore potential effects of four random factors such as the individual sequences and, for each sample, the number of base pairs, time the PCR reaction was completed and the DNA-sequencing end time (derived from Lozano-Fernandez, 2022), on the response data with the fixed predictor (species). The random factors did not exhibit significant effects; thus, the data were re-analysed in GLM without the random factors. Significant results were obtained at 95 % confidence interval from the best model based on the goodness-of-fit test, Akaike information criterion (AIC; Agresti, 2015). To determine which means from the predictors differed in the GLM, pairwise analyses were performed using the *emmeans* and *contrast* functions, with adjustments for multiple comparisons through the false discovery rate (*fdr*) method (Lenth, 2024). These model analyses were conducted using the functions *glmer* (GLMM) from *lme4* package and *glm* (GLM) from *stats* package in R version 4.4.1 (R Core Team, 2024).

We interpreted within-species divergence here based on these metrics: ≤ 0.01 (Sucharitakul et al., 2017) to ≤ 0.02 (Azama et al., 2023) p-distances in *Chironex* spp. and patristic distances of $\cong 0.5$ (for *cox1* in marine cephalopods, decapods and foraminiferans; Girard et al., 2022) to ≤ 6.7 (*16S* in marine ghost shrimps; Schnabel and Peart, 2024). However, the exact thresholds for genetic divergence that indicate distinct species are not universally defined for cubozoans (Osathanunkul et al., 2022). With this consideration, we also assessed divergence in the context of other available data including the phylogenetic trees and morphology of our species (Lozano-Fernandez, 2022).

2.3.3. Phylogenetic analyses

The phylogenetic trees were constructed for all sequences of *Chironex* in GenBank including our sequences from both markers using Maximum Likelihood (ML) under the Tamura-Nei model through the PhyML plugin (Guindon et al., 2010) in Geneious Prime 2024. Branch stability in the tree was assessed using bootstrap indices from 1000 replicates and values from Shimodaira-Hasegawa (SH)-like approximate likelihood ratio test (aLRT; Medina et al., 2001; Gamero-Mora et al., 2022). SH-like aLRT was used because it offers a consistent, computationally efficient approach with high statistical power while maintaining the ability to detect "true clades" even in subtle genetic differences (Anisimova et al., 2011; Gamero-Mora et al., 2022). The index values here were interpreted using these metrics for medusozoans: $\leq 70\ \% =$ low, 71–94 % $=$ moderate, $\geq 95\ \% =$ high support value (Medina et al., 2001; Gamero-Mora et al., 2022).

2.3.4. Haplotype network

We used PopART (http://popart.otago.ac.nz/index.shtml) to generate Templeton–Crandall–Sing (TCS) networks of haplotypes from sequences of *cox1* and *16S* genes to visualize the haplotypes and mutation frequencies of *Chironex* spp. with our sequences (as in Gamero-Mora et al., 2022). These network analyses include sequences from *C. yamaguchii* of Japan (GenBank code, *cox1*: FJ665180; *16S*: AGC0592), *C. indrasaksajiae* (*cox1*: KU646841, KT223648; *16S*: KX065449, KX090147), *C. fleckeri* (*cox1*: FJ665181; *16S*: GQ849101) and the outgroup *Carybdea prototypus* (*cox1*: OM108432–3; *16S*: KT288255–6).

2.3.5. Species delimitation

We used Assemble Species by Automatic Partitioning (ASAP) server (https://bioinfo.mnhn.fr/abi/public/asap/#; Puillandre et al., 2020) to determine whether the Philippine samples belong to a likely known or new species. Like Automatic Barcode Gap Discovery (ABGD), ASAP employs genetic distances to indicate intra- and inter-specific variation, but ASAP operates with a scoring system (asap score) that enables partitioning of candidate species without the need of *a priori* information about the morphological species with the target genetic sequences (Puillandre et al., 2020). We used the *p*-distance model in ASAP for barcode-based analyses of species partitions (Young et al., 2021). To complement ASAP results, we analysed the Maximum Likelihood's phylogenetic tree in Multi-rate Poisson tree processes (mPTP) server (http://mptp.h-its.org; Kapli et al., 2017). Our study requires species delimitation while considering evolutionary relationships within a monophyletic group, such as *Chironex*, making mPTP the suitable choice for this complementary procedure (Kapli et al., 2017; Wu et al., 2023). Candidate species results were visualised with the phylogenetic trees in this paper.

2.4. Envenomation

Using the citizen-science program and procedures described above (2.2 Morphological examination), we recorded cases of envenomation by the chirodropid box jellyfish and their locations in our study (adapted from Thaikruea et al., 2012). See sting case validation in Supplementary Table 1. Direct envenomation from *C. yamaguchii* was confirmed through capture of the species alongside the victim, and record of the morphology of this box jellyfish in the absence of nematocyst examination (developed through Tibballs, 2006 and Thaikruea et al., 2012). In addition, the envenomation diagnosis is supported by stings of this jellyfish which leave a cross-hatched tentacle imprint on the skin, cause intense pain (i.e., pain scores of ≥7 / 10), and induce multiple wheals from this multi-tentacled species (referenced from Williamson and Burnett, 1995 and Nagai et al., 2002). Sting cases were linked to *C. yamaguchii*, rather than other chirodropid species, based on a combination of citizen-science photos and videos of the species, eyewitness description of the box jellyfish involved in the case such as the number and shape of tentacles, shape of bell typical of *Chironex* and occurrence of the case in a location (i.e., village or town's coast or ecoregion) where *C. yamaguchii* exists (as in Thaikruea et al., 2012).

2.5. Ecology

Records of box jellyfish occurrences from various localities and biogeographic regions (i.e., ecoregions) were compiled using peer-reviewed literature and records in museums (see Table 1). Additional records from the Philippines were gathered through citizen-science observations, detailed in the Morphological Examination section here. Locations and names of these ecoregions such as Sulu Sea, West Philippine Sea, Visayan Sea, Celebes Sea, Northern and Southern Philippine Sea were based on Spalding et al. (2007) and Nañola et al. (2011). Maps illustrating the geographic distributions of the cubomedusae were generated to visualize the box jellyfish's spatial occurrence, enhancing the potential for species discovery (see DeSalle and Goldstein, 2019).

3. Results

3.1. Systematics

Phylum CNIDARIA Verrill, 1865
Subphylum MEDUSOZOA Peterson, 1979
Class CUBOZOA Werner, 1973
Order CHIRODROPIDA Haeckel, 1880
Family CHIRODROPIDAE Haeckel, 1880
Genus CHIRONEX Southcott, (1956)
Chironex yamaguchii Lewis and Bentlage, (2009)

3.2. Diagnosis

Chironex species with volcano-shaped pedalial canal bend, varying from pointed to blunt-tipped, and a cube-shaped bell with smooth exumbrella.

3.3. Materials examined

Preserved specimens: four mature medusae (accession codes, a.c.: NMP-2017C05, UPDIM-CUB004, UPDIM-CUB007–0, UPDIM-CUB007–11 in Table 2) and 14 juvenile medusae with developing gastric saccules (a.c.: UPDIM-CUB007–12, UPDIM-CUB005, NMPZC-2021.01–2021.14, Table 2). Digital materials: photographs (n = 259) and videos (n = 98) identifying 87 medusae of *C. yamaguchii* (see Table A.1 in the Appendix for their dates of occurrence and geographic locations; Fig. 1a-c).

3.4. Description

Live and preserved chirodropid medusae with smooth exumbrella and semi-transparent to transparent bell, (Fig. 2a-f), typical of some cubomedusae (Fig. 2f-h). Two bean-shaped concavities, that mirror each other, on the upper exumbrella, prominent among preserved specimens placed against black background (Fig. 2a-e). Appearance of concavities consistent with their appearance in records of *C. yamaguchii* (see page 511 in Mayer, 1910; page 779 in Jarms and Morandini, 2019). Size and weight ranges of mature medusae are 70–91 mm BH, 85–120 mm IBW, 191–325 g FW (Table 2). Juvenile medusae size-weight ranges are 38–70 mm BH, 40–82 mm IBW, 118–184 g FW (Table 2). Claw-like pedalia, 4, and each pedalium has up to 9 tentacles (Fig. 2a-f; Table 2). Pedalial canal bend volcano-shaped with blunt or sharp tips (Fig. 3a-e) unlike in congeners with corniculum tip (*C. fleckeri*: Fig. 3f) or bulbous edge (*C. indrasaksajiae*: Fig. 3g). Gastric phacellae V-shaped in each corner of the stomach, prominent among live (Fig. 2d) and preserved medusae (Fig. 2e; Fig. 4a). They hold numerous, unbranched gastric cirri (Fig. 4a-b). Gastric saccules grape-shaped forming clusters and opaque, formed as series of multiple swellings, occupying the

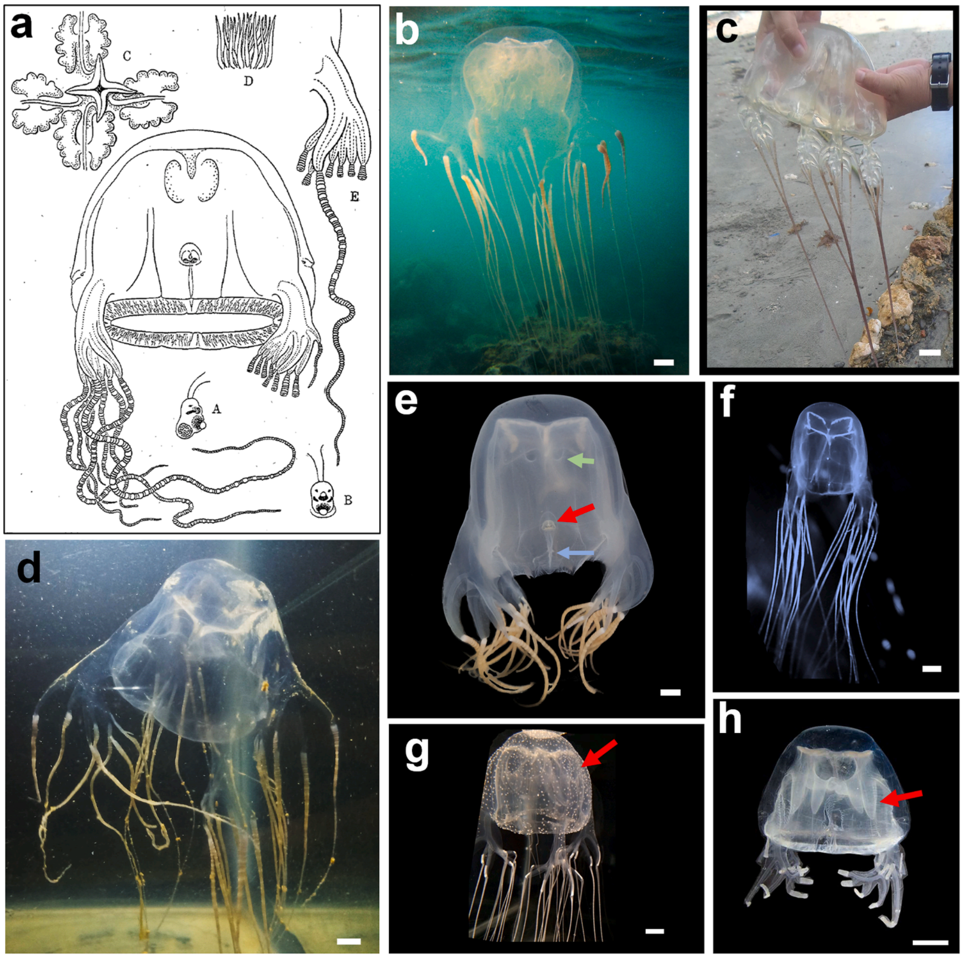

Fig. 2. Cubomedusae including *C. yamaguchii*. **a.** Morpho-anatomical structures of *C. yamaguchii* specimen collected in 1908 in the Philippines and misidentified as *Chiropsalmus quadrigatus* (synonym) in Mayer (1910) with original labels: A = rhopalium, originally called the "sense-club", B = anterior view of rhopalium, C = stomach with four lips and gastric saccules, D = gastric cirri, E = Pedalium (line drawing in Mayer, 1910); **b.** *C. yamaguchii* recorded *in situ* in June 2015 in Guiuan, eastern Philippines; **c.** medusa out of water in Osmeña, Marabut in Samar, Philippines; **d.** live medusa of *C. yamaguchii* from Eastern Philippines in aquarium; **e.** newly preserved medusa with visible upper exumbrellar concavity (green arrow), rhopalium in rhopalial niche (red arrow), and frenulum (blue arrow); **f.** *C. yamaguchii* from Okinawa, Japan in aquarium; **g.** medusa of *Chiropsalmus quadrumanus* from Florida, United States of America; **h.** specimen of *Chiropsoides buitendijki* from Phang-nga province in southern Thailand. Scale bars: b-f: 30 mm, g-h: 20 mm. Photos courtesy of Hirofumi Hirayama (f), Jose Nuñez (g) and Ananya Waharak (h).

subumbrellar cavity, prominent in mature specimens (Fig. 4c). This is consistent with observations for Okinawa specimens (C. Lewis Ames; B. Bentlage). Crucifurm manubrium about 2/3 as long as bell height. Manubrium ends with four narrow lanceolate lips (Fig. 4d). Perradial lappets smooth, lacking nematocyst warts, triangular with frenulum (muscular brackets) on each side of the bell (Fig. 2e; Fig. 4e). Frenulae extend from the tip of rhopalial niche to the velarium (Fig. 2e; Fig. 4e). Velarial canals numerous, highly branched as in *C. fleckeri* of Australia and *C. indrasaksajiae* from Thailand (Fig. 4e). Rhopalia bearing six eyes (Fig. 4f-g). Statolith oval, attached at the base of the rhopalium (Fig. 4f-g). Rhopalial niche ostia dome-shaped with single, transparent upper cover and under leaf-tip-shaped depression of the exumbrella (Fig. 2e; Fig. 4f). Broad trailing tentacles are flat in life (Fig. 2b-c). When contracting or among preserved specimens, tentacles rounded like stacks of dish plates forming striations or bands (Fig. 4h). Tentacle section proximal to pedalium lavender-coloured to gray in life, yellowish when contracted (live) and preserved (Fig. 2b-c; Fig. 4h). Tentacle bands appear lavender, black, and gray and these colors alternate with transparent bands in some specimens (Fig. 4h-i). Nematocysts are cucumber-shaped microbasic mastigophore, oval trirhopaloid, and small trirhopaloid (Fig. 4j-k).

3.5. Remarks

Morphological structures including the shape of pedalial canal bend and types of cnidocytes of cubomedusae reveal structures almost identical to those in *C. yamaguchii* medusae of Japan and in previous records of *Chironex* sp. from the Philippines (Oba et al., 2004; see Lewis and Bentlage, 2009). Their smooth exumbrella features bean-shaped concavities, mirroring each other on the upper bell half (Fig. 2a-e), similar to those in drawings in early specimens of *C. yamaguchii* of the Philippines (Fig. 2a; see also pages 516–517 in Mayer, 1910) and *C. fleckeri* (Fig. 1 of Southcott, 1956). These concavities were not mentioned in Mayer (1910) and Southcott (1956) for the descriptions of their specimens. In addition, the pedalial canal bend exhibits a volcanic shape with blunt or pointed tips in both Japanese and Philippine specimens (Fig. 3a-e). These findings support *C. yamaguchii*'s occurrence in the Coral Triangle (the Philippines).

Compared to other chirodropid medusae, *C. yamaguchii* stands out with smooth exumbrella, prominent pedalial canal bend and flat-shaped tentacles among fresh and preserved specimens (Fig. 2a-f). Our extensive data collection did not reveal *Chiropsella* which notably exhibits knob-like gastric saccules and tentacles with round shape in cross section (Gershwin, 2006) while *Chiropsalmus* usually contains exumbrellar

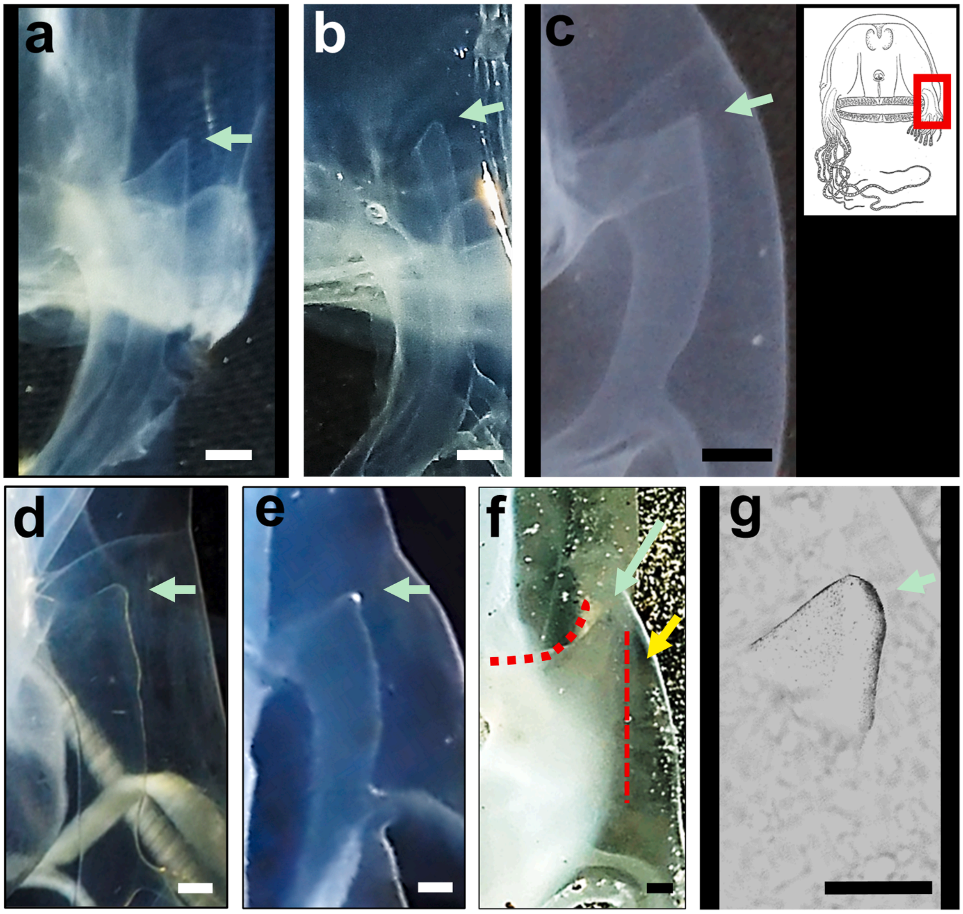

Fig. 3. Pedalial canals with "knee" bend (green arrow) of *Chironex* species. **a-b.** Pedalial canal of a fresh specimen of *C. yamaguchii* with both pointed (a) and blunt-tipped (b) canal bend from Marabut town in eastern Philippines; **c.** pedalial canal of preserved specimen of *C. yamaguchii* from Guiuan, Eastern Samar in the Philippines; **d-e.** blunt (d) and pointed (e) canal bends within a live specimen of *C. yamaguchii* from Okinawa, Japan; **f.** knee bend of pedalial canal of fresh *C. fleckeri* from Albatross Bay in Queensland, Australia (broken lines emphasize the corniculum- or hook-shaped knee bend, yellow arrow = pedalium); **g.** sketch rendering the blunt-shaped pedalial canal bend of *C. indrasaksajiae* of Thailand. Inset sketch has rectangle indicating the position of pedalial canal on the cubomedusa. Scale bars: 5 mm length. Photos courtesy of Sho Toshino (d-e) and Cemone Hedges (d). Sketch modified from Mayer (1910) indicates morphological part shown in this figure.

nematocysts and smooth pedalial canal bend (e.g., *Chiropsalmus quadrumanus*, Fig. 2g). Like the absence of *Chiropsalmus,* we did not observe medusae of *Chiropsoides buitendijki* which appear with single-branched pedalia with notable "long" finger-shaped gastric saccules, absent in *Chironex* (Fig. 2h). These comparisons and descriptions reveal distinct morphology of *C. yamaguchii.*

Cubomedusae in our digital materials revealed *Chironex* cf. *yamaguchii* (Table A.1, Appendix). Despite appearing as a species of *Chironex*, their species-level identity was not confirmed due to ambiguous pedalial canal bend in the images or videos. However, their morphology such as the shape of umbrella and the number (and range) of tentacles does not match members of *C. indrasaksajiae, C. fleckeri* or a potentially undescribed *Chironex.*

3.6. Molecular analyses results

3.6.1. Genetic divergence

Philippine specimens exhibit close genetic distances among them (p distances = *cox1*: 0.00–0.01; *16S*: 0.00–0.02 and patristic distances = *cox1*: 0.00–0.00; *16S*: 0.01–2.51). *C. fleckeri* and *C. indrasaksajiae* exhibit large average p-distances (> 0.15) from Philippine specimens (see Table 3; Fig. 5a-b). Specifically, they were 0.16 (*C. fleckeri*) and 0.13 units (*C. indrasaksajiae*) larger than those from Japan's *C. yamaguchii* for the *cox1* marker (Fig. 5a; see Supplementary Table 3). Mean *16S* p-distances of *C. fleckeri* and *C. indrasaksajiae* from Philippine sequences were 0.4 and 0.42 units larger, respectively, than the distance from Japan's *C. yamaguchii* (Fig. 5b; Supplementary Table 3). Similarly, patristic distances from Philippine sequences exhibit significant differences across *Chironex* species for both markers (Table 3). Japan's sample showed the smallest average patristic distances (*cox1:* 0.02; *16S:* 4.49) from Philippine specimens (Fig. 5c-d; Supplementary Table 3), placed within intra-species distance of some marine non-cnidarian invertebrates (<6.00). The patristic distance from *C. fleckeri* exceeds that from Japan's *C. yamaguchii* by averages of 0.15 (*cox1*) and 5.81 (*16S*) units (Fig. 5c-d; Supplementary Table 3). In contrast, *C. indrasaksajiae* largely surpasses the average patristic distance from Japan's *Chironex* by 0.26 units for *cox1* and 8.35 units for *16S* (Fig. 5c-d; Supplementary Table 3).

3.6.2. Phylogeny

Chironex spp. appear in geographically separated clades except for the unique clade of *Chironex* sp. C from Thailand (*cox1*; Fig. 6a) and Palau's *Chironex* sp. joining Thailand's *C. indrasaksajiae* (*16S* marker; Fig. 6b). However, the 2020 samples of *C. yamaguchii* from Japan formed a distinct clade (98 % bootstrap support and 92.6 % SH-aLRT value), separate from the clade consisting the 2007 specimens of this species from Japan and the Philippines's 2019 samples for *cox1* (Fig. 6a). For both markers, the 2019 and 2007 samples of *C. yamaguchii* are sister groups with moderate to strong branch supports: 95 % bootstrap index and 89.7 % SH-aLRT value for *cox1* and 99 % bootstrap index and SH-

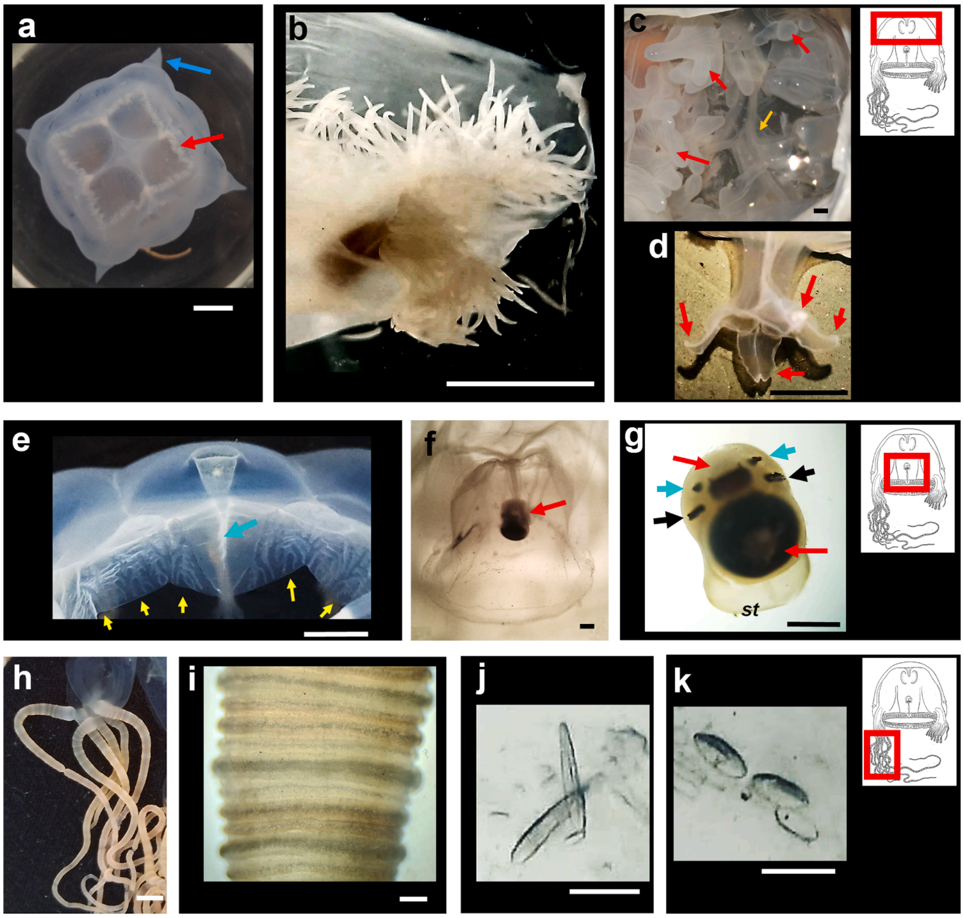

Fig. 4. Morpho-anatomical parts of *C. yamaguchii* specimens from the Philippines. **a.** apex view of medusa with gastric cirri, forming a “square” (red arrow), and cruciform-shaped gastric area; **b.** gastric cirri appear as hair-like filaments; **c.** chicken comb-shaped gastric saccules (red arrow). Note the position of manubrium (yellow arrow); **d.** manubrium with lanceolate lips (red arrows point tips); **e.** complex branches of velarial canals (yellow arrow) with frenulum (blue arrow) in the middle of the velaria; **f.** rhopalium (red arrow) in its niche; **g.** rhopalium with lens eyes (red arrows), pit eyes (blue arrows), slit eyes (black arrows) and statocyst (*st*); **h.** tentacles; **i.** tentacle segment with bands of cnidocytes that appear like “stacks of pancakes;” **j-k.** mastigophore (j) and microbasic eurytele (k) nematocysts in the cubozoan tentacles. Inset line drawings with rectangle indicating position of body parts shown in each figure row (e.g., a-d). Scale bars: a = 4 cm, b-d = 4 mm, e = 10 mm, f-g: = 2 mm, h = 10 mm, i = 1 mm, j-k = 20 *μ*m. Right-hand sketches modified from Mayer (1910) indicate morphological parts shown in this figure.

Table 3
Results from the generalised linear model analyses for the differences of uncorrected pairwise p- and patristic distances of cytochrome oxidase I (*cox1*) and ribosomal RNA (*16S*) between sequences (n = 14 for each marker) in this study and available *Chironex* spp. sequences with species as the independent factor.

Source	AIC	df	Deviance	*F*	*P*
p-distance					
cox1	−23.059	2	5.680	7.579	**< 0.001**
16S	−11.175	2	2.861	5.823	**0.002**
Patristic					
cox1	−402.154	2	37.42	10,076	**< 0.001**
16S	150.891	2	5.668	125.7	**< 0.001**

AIC = Akaike information criterion for the best model. df = degrees of freedom
P values in bold are significant

aLRT value of 98.0 % for *16S* (see Fig. 6a-b).

3.6.3. Haplotypes

Philippine *C. yamaguchii* has two *cox1* haplotypes with 17 mutations separating them from Japan’s *C. yamaguchii* (Fig. 7a). Here, there are 74 mutations between haplotypes of the Philippine species and Australia’s *C. fleckeri*, and 271–276 mutations exist between the Philippine cubomedusae and the two haplotypes of Thailand’s *C. indrasaksajiae* (*cox1*; Fig. 7a). Moreover, the *16S* marker reveals one haplotype each for *C. yamaguchii* from the Philippines and Japan (Fig. 7b). A few (= 8) *16S* mutations separate the Philippine species from the one from Japan (Fig. 7b). However, the Philippine species separates from *C. fleckeri* and *C. indrasaksajiae* through 49 and 42 *16S*-based mutations, respectively (Fig. 7b). Overall, haplotypes of *C. fleckeri* and *C. indrasaksajiae* had the greatest number of mutations between those of the Philippine specimens (Fig. 7a-b).

3.6.4. Species delimitation

Delimited species do not entirely match morphology-based identification of *Chironex* spp. (see Fig. 6). Specifically, *cox1* samples of *C. yamaguchii* from Japan (2007, 2020) and the Philippines (2019) form groups of candidate species in ASAP and mPTP analyses (Fig. 6a). Analyses for *16S* also revealed the sample in Japan as species distinct from the Philippine specimens (Fig. 6b). Similarly, *C. fleckeri* samples appear as distinct species for both delimitation analyses. *C. indrasaksajiae* (separate from *C. yamaguchii* clades) joined *Chironex* sp. A in ASAP (*cox1*) but grouped with *Chironex* species A, B, D, and E for *cox1* in mPTP, similar to its grouping with Palau’s *Chironex* in mPTP (*16S*) (Fig. 6a-b).

3.7. Envenomation

Frequent records of *Chironex* (including *Chironex yamaguchii*)

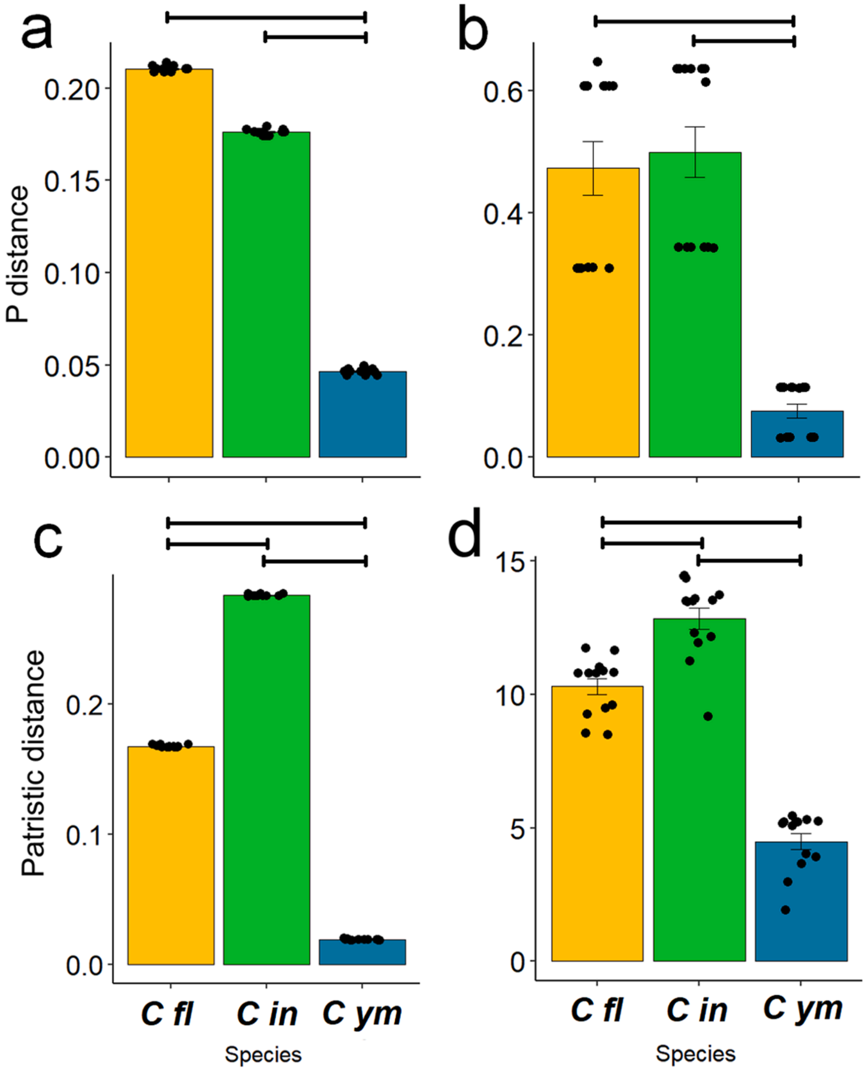

Fig. 5. Averages and standard deviation (error bar) of the p- and patristic distances between each genetic sequence obtained from this study and available sequences from *Chironex* spp., such as *C. fleckeri* (*C fl*), *C. indrasaksajiae* (*C in*) and Japan's *C. yamaguchii* (*C ym*). P- and patristic distances for cytochrome oxidase I (**a, b**) and ribosomal RNA 16S (**c, d**) in this study (n = 14 for each marker). Black dots are raw data points. Horizontal bars show significant differences ($p < 0.05$) between means based on estimated marginal means analyses.

envenomation incidents in the Philippines were reported primarily in warm months of April (13 %), May (7 %), and June (10 %) across various years and locations based on citizen-science reporting (Table 4). All sting incidents happened within 100 km^2 area and ecoregions where *Chironex* species exist (see Tables 1 and 4; Appendix Table A.1; Fig. 1). April reports reveal incidents in both the northern and central Philippines, consisting of sting encounters directly from and linked to *C. yamaguchii* (Table 4). May included sting incidents in northern Philippines (Padre Burgos and Tagkawayan Bay, Quezon) down to southern regions of Zamboanga, while June saw incidents in northern Philippine areas such as Dalahican in Quezon and Siruma in Camarines Sur (see Table 4).

Direct envenomation cases and those attributed to *C. yamaguchii* manifest varied clinical presentations and outcomes in our records (Fig. 8a-j Table 4; Table S4, Supplementary Materials). Common immediate (~10 minutes post-sting) symptoms include severe pain (pain score: ≥7/10), localized erythema, and distinctive imprint with cross-hatch patterns on the skin, consistent with stings of *Chironex* (Fig. 8a-i; Table 4; Table S4, Supplementary Materials). Prompt medical intervention is often necessary, with hospitalization required in severe cases presenting with systemic effects such as syncope and dermal swelling (see Table S4, Supplementary Materials). Despite attempts to administer basic treatments like seawater or vinegar during tentacle removal, outcomes range from localized discomfort and gradual recovery (~4 days) to fatal consequences, revealing diverse and extreme envenomations of *Chironex* medusae (Fig. 8a-j; Table 4; Table S4, Supplementary Materials).

3.8. Ecology

Chironex yamaguchii occurs in various ecoregions of western Pacific such as South Kuroshio with Okinawa (Japan) coasts, West Philippine Sea, Visayan Sea, the eastern Philippine Seas and the West Coral Triangle with Malaysia (Fig. 1a-c; Table 1). Records span locations on coastal areas and coral reef ecosystems in localities such as northern (Quezon, Mindoro), western (Palawan) and south–southeastern (Mindanao), Philippines (Fig. 1a-c; Table 1). Our records for this species at surface waters (~0–5 m) fall within 0–84 m depth range of the water column (Table 1). Verified citizen-science reports show species records in ecoregions that match museum-based locations, except for *Chironex* species (one *C. yamaguchii* and 4 *C.* cf. *yamaguchii*) in the Celebes Sea ecoregion (Fig. 1a-c; Table 1 and Table A.1, Appendix).

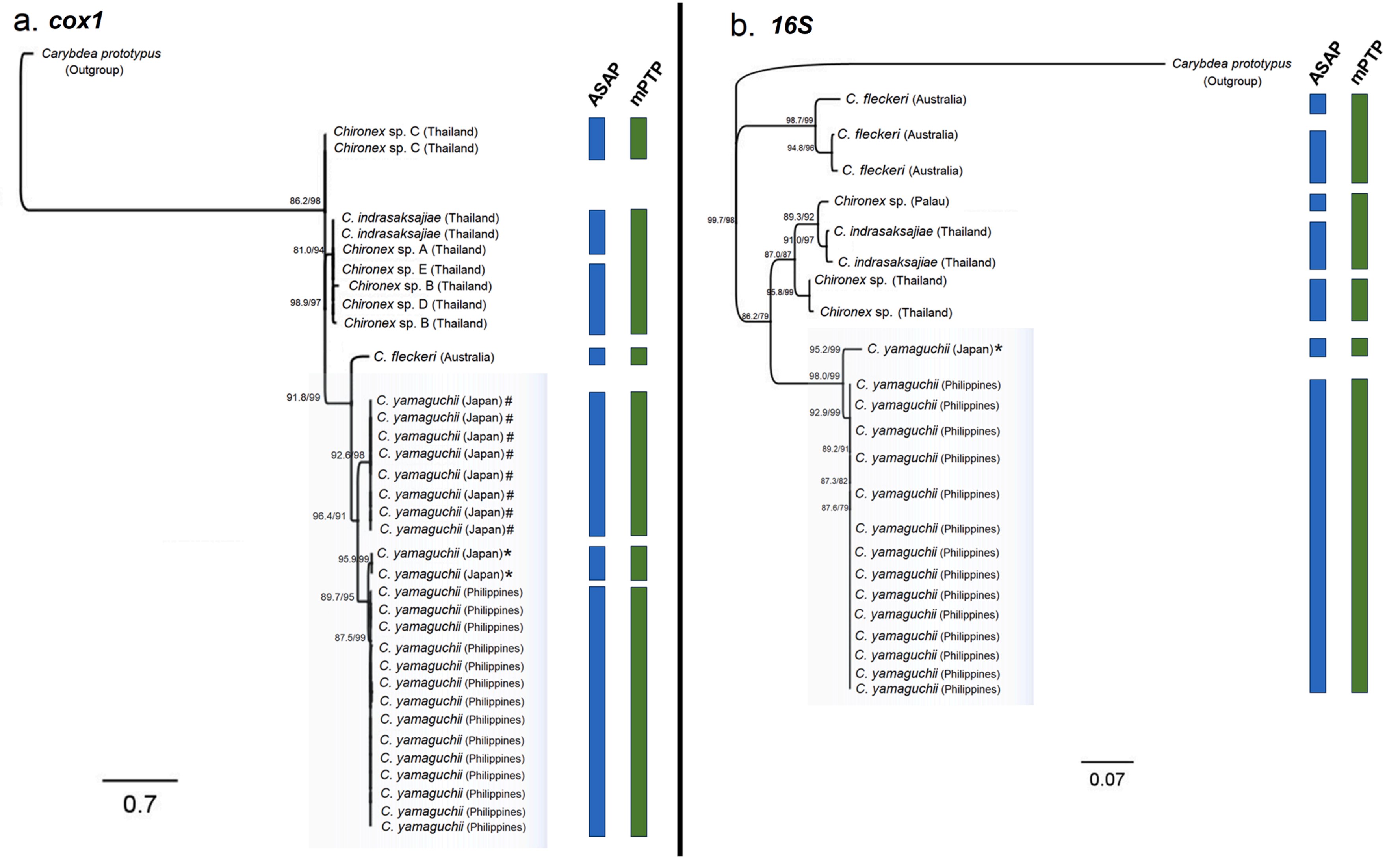

Fig. 6. Phylogenetic trees for the sequences of cytochrome oxidase I (a: *cox1*) and ribosomal RNA gene (b: *16S*) from *Chironex* spp. including *C. yamaguchii* in this study, those from Japan sampled in years 2007 (*) and 2020 (#) and *Chironex* sp.'s *16S* sequence from Palau (GenBank code: GQ849104). *Carybdea prototypus* is the outgroup. Each coloured vertical bar represents one species based on ASAP and mPTP analyses results.

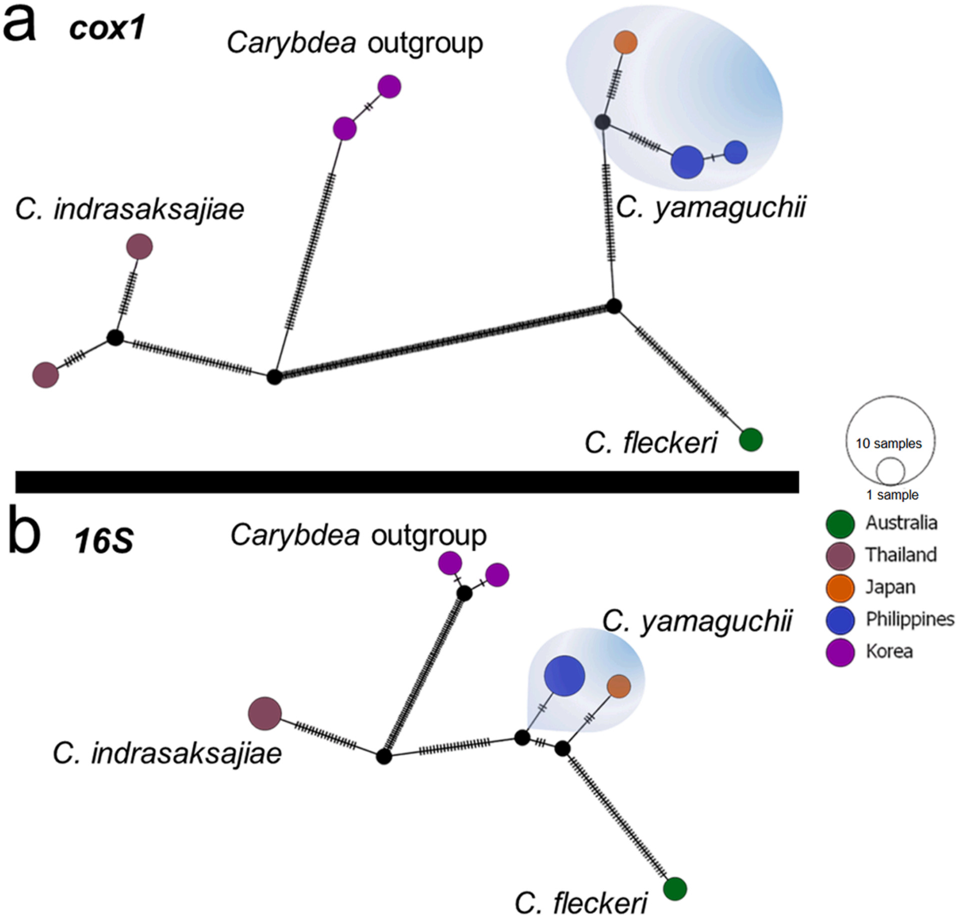

Fig. 7. Haplotype networks of *Chironex* species including *C. yamaguchii*, based on mitochondrial *cox1* (a) and ribosomal *16S* (b) sequences.

Probable bloom. Large aggregation of *C. yamaguchii* medusae were reported in northern Philippines (Patnanungan port; 14°45’02.8"N 122°13’02.0"E) in May 2024 (J. Israel obs., Video S1, Supplementary Material). An estimated density of 14.33 individuals m^{-2} of the species occurred, as recorded in the video.

Supplementary material related to this article can be found online at doi:10.1016/j.rsma.2025.104033.

Predation. C. yamaguchii actively preyed upon eel catfish species in two reported predation events. One event occurred in Capalong Camarines, Norte (14°19’41.9"N, 122°30’29.3"E) which contained the catfish, *Plotosus* sp. inside the stomach of this cubozoan in June 2023 (one video, three photos; J. Katigbak obs., see Fig. S3, Supplementary Material). In May 2023, the cubomedusa “wrapped” around the eel catfish, *Plotosus lineatus* with tentacles in northern Philippines (Catanauan, Quezon: 13°35’58.7" N, 122°13’41.5" E; Yachie obs.; two videos, one photo; Video S2, Supplementary Material).

Supplementary material related to this article can be found online at doi:10.1016/j.rsma.2025.104033.

3.9. Ethnozoology

Local names. The local names of this box jellyfish include *dikya* (Tagalog), *salabay* (Tagalog, Cebuano, Bicolano, Aklanon, Waray), *kurominas, kuriminas* (Ilocano) and *sallabay* (Zamboangueño) based on citizen-science and validated news reporting and *habu-kurage* in Okinawan (Lewis and Bentlage and Azama *et al.*). Here, we establish these common names for scientific reporting of this species in English: “the Philippine box jellyfish”, “the Okinawan box jellyfish” and “viper box jellyfish”.

Diet. Citizen-science reports show that many locals consume cubomedusae of *C. yamaguchii* collected from the coasts along their residential areas: Looc, Romblon (12°15’12.7"N, 121°59’50.3"E; B. Madrazo observations, obs.); El Nido, Palawan (11°08’46.9"N 119°23’46.9"E; D. Dioso and C. Torio obs.); Aborlan, Palawan (9°21’34.3"N 118°32’01.7"E; K. Dagz obs., May 2024); Pagbilao, Quezon (13°54’33.6"N, 121°47’00.8"E; J Lonto and Featrmedia obs., see also tinyurl.com/4crh4j9x) and Lingig, Surigao del Sur (8°09’27.4"N, 126°26’06.0"E; M. Piodo obs.; see tinyurl.com/yrsdsxxx). See also Supplementary Table S5. In these observations, the cubomedusae are captured using either a scoop net or by carefully holding the top of the exumbrella from a boat or by slowly wading in the water, ensuring the tentacles do not come into contact with the harvester (Video S3, Supplementary Material).

Supplementary material related to this article can be found online at doi:10.1016/j.rsma.2025.104033.

4. Discussion

In this study, we leveraged an integrative approach, combining morphological and molecular analyses to test the hypothesis that *Chironex yamaguchii* occurs in the Coral Triangle. Our findings support this hypothesis. With the confirmed distribution of *C. yamaguchii*, molecular signatures revealed genetic divergence within this species, which contradicts the hypothesis that molecular findings would be absolutely consistent with morphological information from the box jellyfish. Considering all evidence here including morphology, and outcomes from the phylogenetic tree and species delimitations based on multiple indicators such as *cox1* and *16S* markers, we classify *C. yamaguchii* as putative cryptic species. We follow this classification as direct evidence of reproductive isolation within populations of this species is absent

Table 4
Occurrences of envenomation directly due (i.e., direct) to the box jellyfish, *C. yamaguchii* and those stings attributed (i.e., linked) to this species in various locations in this study. Superscript F = resulted to fatality of the victim. Date is the day of sting incident. N/D = not disclosed here to comply for regulations on personal information.

Locality	Latitude	Longitude	Date	Direct/ Linked
Tagkawayan Bay, Quezon^{F}	13°56’47.6"N,	122°32’56.9"E	22 May 2015	Linked
Tagkawayan Bay, Quezon^{F}	13°56’46.7"N,	122°32’59.6"E	28 May 2016	Linked
Northern Philippines	N/D	N/D	April 2019	Direct
Dalahican, Quezon	13°54’28.9"N,	121°38’10.6"E	23 June 2018	Direct
Padre Burgos, Quezon	13°53’27.2"N,	121°52’11.1"E	26 May 2018	Direct
Padre Burgos, Quezon	N/D	N/D	March 2019	Linked
Atimonan, Quezon	13°58’47.0"N	121°58’46.8"E	20 April 2023	Linked
San Andres, Quezon	13°26’10.7"N,	122°39’06.5"E	14 May 2023	Linked
Sabang, Puerto Galera	13°31’32.1"N	120°58’04.0"E	30 June 2023	Linked
Northern Philippines	N/D	N/D	March 2024	Linked
Central Philippines	N/D	N/D	April 2019	Direct
Central Philippines	N/D	N/D	April 2023	Linked
Matalom, Leyte	10°14’54.9"N,	124°46’10.4"E	20 May 2023	Linked
Hinoba-an, Negros Occidental	9°38’53.2"N,	122°27’24.7"E	8 Feb 2021	Linked
Calatrava, Negros Occidental	10°33’16.7"N	123°28’16.6"E	13 June 2021	Direct
Tuburan, Cebu	10°47’10.7"N	123°50’46.1"E	16 June 2021	Linked
Ave Maria Island, Guimaras	10°32’42.4"N	122°31’06.5"E	7 July 2022	Direct
Western Philippines	N/D	N/D	March 2022	Direct
Western Philippines^{F}	N/D	N/D	May 2024	Linked
Siruma, Camarines Sur	14°01’38.5"N	123°15’38.0"E	18 April 2017	Direct
Siruma, Camarines Sur	14°01’32.8"N	123°15’25.1"E	29 July 2022	Direct
Northern Philippines	N/D	N/D	April 2019	Direct
Siruma, Camarines Sur	14°01’35.2"N	123°15’31.8"E	10 June 2023	Linked
Santa Fe, Cebu^{F}	11°08’51.2"N	123°47’34.4"E	25 June 2023	Linked
Placer, Surigao del Norte^{F}	9°38’52.5"N	125°36’16.2"E	11 June 2023	Direct
Coron, Palawan	11°57’01.4"N	120°11’34.8"E	22 July 2023	Direct
Lopez Jaena, Misamis Occidental	8°32’41.0"N	123°46’34.4"E	6 June 2023	Linked
Buug, Zamboanga Sibugay^{F}	7°39’37.4"N	123°03’31.3"E	20 Aug 2023	Linked
Bolong, Zamboanga	7°05’17.2"N	122°14’09.5"E	4 May 2018	Linked
Bolong, Zamboanga	7°05’17.4"N	122°14’09.4"E	6 May 2018	Linked

(Monro and Mayo, 2022; see discussion below). Yet, our study confirmed this species’ range within South Kuroshio ecoregion with Okinawa’s coastal waters in Japan and the Coral Triangle areas of the Philippines and Sabah, Malaysia. Our findings are vital given convergent morphological features, such as the similar appearance of tentacles among multiple taxa of cubozoans that can challenge taxonomic identification of chirodropid box jellyfish (as in Veron, 2000; Lawley et al., 2016; Jarms and Morandini, 2023). These features led taxonomists to encounter taxonomic ambiguity and obscured species distinctions when relying solely on morphology (Lewis and Bentlage, 2009; Lawley et al., 2016). However, integrating molecular data and morphological information, such as the methodology used in our study, provides crucial insights that bolster species identification of cubozoans.

Thorough examinations of preserved specimens here confirmed the taxonomic identity of this species with the binomen *C. yamaguchii* consistent with morphological findings for this species in Japan (see Lewis and Bentlage, 2009). These examinations were crucial because morphological similarities in many chirodropid species, such as *C. fleckeri*, and early taxonomic placements of this species hindered the identification of *C. yamaguchii* (Lewis and Bentlage, 2009). Indeed, early descriptions of the Philippine species as *Chiropsalmus quadrigatus* (synonym) in Mayer (1910) led to early misidentification of *C. yamaguchii* (Lewis and Bentlage, 2009). This misidentification perpetuated when Stiasny (1937) reconstructed this species using Haeckel (1880)’s and Mayer (1910)’s descriptions, and morphology of the specimen from the Maldives, thereby combining disparate features and obscuring the identity of this cubozoan species (see Stiasny, 1937; Lewis and Bentlage, 2009). Building on our findings, Lewis and Bentlage (2009)’s re-examination, and latest knowledge on the systematics of *Chironex* (Jarms and Morandini, 2023; Collins, 2024), this study highlights the need for continued taxonomic research and careful identification of box jellyfish to fully understand species diversity of Cubozoa.

Challenges in identifying cubozoans partly stem from subtle macro-level (morphological) differences that may require a combination of morphological characters and microscopy for accurate species identification (Straehler-Pohl and Gul, 2017; Jarms and Morandini, 2019; 2023) as demonstrated in this study. For example, the cubozoans *Alatina alata* and *A. grandis* exhibit moderate differences in bell shape, and the number of tentacles (Gershwin, 2005; Larson, 1976). *A. grandis* has larger torpedo-shaped bell (up to 240 mm bell height) with velarial canals that are complexly branched, and the canal margins are jagged compared to *A. alata* with bell height of up to ~70 mm and showing simple to bifurcated velarial canals with smooth margins (Lawley et al., 2016; Straehler-Pohl and Gul, 2017). Like these carybdeids (order Carybdeida), chirodropid box jellyfish species exhibit moderate morphological similarities among them (Gershwin, 2006; Jarms and Morandini, 2023). For instance, *Chiropsella bronzie* appears nearly identical in bell shape, and form of pedalia to *Chironex* spp., however, the two differs because the former prominently displays “spaghetti-like” to cylindrical tentacles whereas the latter contains flat tentacles, particularly those proximal to the pedalia (Southcott, 1956; Gershwin, 2006; Jarms and Morandini, 2023). Moreover, by morphology alone, *C indrasaksajiae* appears notably similar to *C. yamaguchii* but the Thailand species has knob-like to bulbous tip of the pedalial canal bend, which also enables this species to be distinguished from Australia’s *C. fleckeri* (Sucharitakul et al., 2017). These few morphological differences such as those in velarial canal morphology, and tentacle shape between cubozoan sister species necessitate a comprehensive approach to species identification, incorporating multiple morphological characters (Lawley et al., 2016) such as those successfully employed in this study.

Genetic markers, including p-distance and patristic values from *cox1* and *16S* markers here, indicate that the Philippine specimens clearly belong to a species of *Chironex*. Using these genetic markers demonstrates molecular tools can validate morphological evidence from marine species like box jellyfish (Lawley et al., 2016). Here, genetic distances enabled distinction between species with similar morphological traits such as *Chironex indrasaksajiae* from Thailand and *C. yamaguchii* from the Philippines and Japan. Despite their nearly identical morphology, the genetic difference between the Philippine specimens and their counterparts in Japan, reveal their intra-specific variations (see also Fig. 5) that could not have been detected by morphological information alone. These findings emphasize the critical role of molecular tools in accurately identifying and confirming our

Fig. 8. Envenomation by a chirodropid jellyfish. **a-c.** confirmed stings on adult legs by the box jellyfish *Chironex yamaguchii* in central (a), northern (b) and western (c) Philippines, **d-i.** envenomation indirectly linked to *C. yamaguchii*: stings on the legs (d) and an arm (e) in a child victim of envenomation in northern Philippines, lesions due to stings on the thigh (**f**) and legs (**g**) of an adult from an envenomation incident in central Philippines, **h-i.** stings resulting to death of a child with erythematous to urticarial cross-hatch lesions on the face (h) and left arm (i), **j.** *C. yamaguchii* medusa collected immediately after a jellyfish envenomation incident with the victim, confirming stings from this box jellyfish species. Photos courtesy of anonymous participants of the citizen-science platform, the Philippine Jellyfish Stings Project, in this study.

species, providing a clearer understanding of the biodiversity of *Chironex* in Western Pacific.

Phylogenetic findings for *C. yamaguchii* specimens from Japan and the Philippines provide robust evidence for the occurrence and genetic differentiation of this species from the Coral Triangle (as in Dawson and Jacobs, 2001). Specifically, high bootstrap and SH-aLRT values support the phylogenetic tree, which clearly delineates *C. yamaguchii* between South Kuroshio (Japan) and Coral Triangle (Philippines) bioregions. This phylogenetic divergence between the Philippine and Japanese specimens aligns with genetic patterns seen in other marine zooplankton and jellyfish species, e.g. *Cassiopea* spp., in the Western Pacific (Taniguchi et al., 2004; Holland et al., 2004). For example, the copepod *Neocalanus flemingeri* exhibits distinct genetic populations across the Northwestern Pacific, driven by oceanographic barriers and historical climatic events (Taniguchi et al., 2004). As in the copepod, the jellyfish *Mastigias papua* shows significant genetic differentiation among populations in various parts of the Western Pacific, such as Palau (De Souza and Dawson, 2018). This pattern of intra-species genetic divergence extends to jellyfish with oceanic medusae such as genus *Aurelia* (Lawley et al., 2021). Indeed, multiple cryptic species exist within this genus, with distinct genetic lineages in different geographic regions, including the Western Pacific (Lawley et al., 2021). These examples across taxa existing in Western Pacific clearly point to how geographic factors are relevant to genetic divergence within species. Furthermore, considering that some cubozoan species (e.g. *Copula sivickisi*) are part of unique genetic stocks within limited geographic areas, and that the North Equatorial and Kuroshio Currents act as oceanographic barriers regulating the distribution of many species such as zooplankton (see Fig. 1a;

Kingsford et al., 2021; Hsiung et al., 2022), *C. yamaguchii* may belong to local genetic stocks. This could explain their genetic divergence, similar to the distinct genetic stocks of its congener *C. fleckeri* within < 100 km range in northern Australia (Kingsford et al., 2021; Morrissey et al., 2024). Thus, oceanographic barriers like ocean currents and ecological factors such as local adaptation and habitat specificity may potentially influence the genetic variation documented here (De Souza and Dawson, 2018; see also paragraph 11 below of the Discussion). The degree to which these factors influence genetic divergence within marine species with planktonic life stage including cubomedusae and the species here will require further assessment in future systematics and evolutionary studies.

Philippine *C. yamaguchii* specimens possess unique haplotypes with several mutations separating them from their Japanese conspecifics. These genetic mutations resonate with genetic structuring across geographic locations in many marine zooplankton species, including the copepod *Calanus sinicus*, which exhibits genetic distinctions between populations from the East China Sea and the Yellow Sea (Huang et al., 2014) and in other medusozoan species, such as *Cassiopea ornata*, where distinct haplotypes exist in different regions within the Western Pacific (Holland et al., 2004). Further, species delimitation findings here support this haplotype separation, identifying potential population units within *C. yamaguchii*. These findings suggest that *C. yamaguchii* from these biogeographic regions represent distinct genetic entities, warranting further investigation into their taxonomic status. This genetic structuring across zooplankton species including the cubomedusae here underscores complementary support of outputs across molecular methods (with haplotype networks and species-delimitation algorithms) and complexity of genetic differentiation in many cnidarian taxa such as cubozoans.

We classify *C. yamaguchii* from the Coral Triangle as putative cryptic species following criteria in Chenuil et al. (2019) and Shin and Allmon (2023) for marine invertebrates (see support by Monro and Mayo, 2022). The criteria dictate that a molecular signature exhibits cryptic speciation if substantial genetic divergence relevant to reproductive isolation is detected (Monro and Mayo, 2022; Shin and Allmon, 2023). These criteria and our putative classification align with the lens of both phylogenetic and morphological species concepts used to interpret our systematics findings (Wheeler, 1999; Monro and Mayo, 2022; Shin and Allmon, 2023). Related to this, *C. yamaguchii* shows notable genetic divergence between the Japanese and Philippine populations as indicated by the p- and patristic distances for mitochondrial markers exceeding within-species distances for *Chironex* spp. Here, the Philippine clade diverged from the samples in Japan regardless of sampling time of Japan's specimens (*cox1 and 16S* samples collected in 2007 and recent *cox1* samples collected in 2020). Distinct haplotypes in the Philippine specimens, separated by mutations from Japan's haplotypes, support this molecular divergence. This indicates independent evolutionary paths, despite morphological findings suggesting otherwise (Monro and Mayo, 2022). However, we lack a number of samples from nuclear markers such as Internal Transcribed Spacer regions or direct evidence from cross-breeding experiments to clearly describe level of reproductive isolation (or its absence) between Japan and Philippine samples and eventually declare whether the box jellyfish populations exhibit cryptic speciation (see Monro and Mayo, 2022; Shin and Allmon, 2023). These outputs were beyond the scope of our study and now we inspire future assessments of possible crypsis of the Philippine cubomedusae.

The lack of samples for the *16S* marker from Japan could have influenced the phylogenetic trajectory of this chirodropid in our study (as in Huang et al., 2021). However, the reanalyses of our genetic data with sample sizes balanced for each *Chironex* species, maintained the noticeable divergence between the Philippine specimens of *C. yamaguchii* against the samples from Japan (see Supplementary Table S2 and S6). Both genetic markers also moderately support the independent clade of Philippine samples, with the phylogeny diverging from those in Japan with moderate to strong support (see Fig. S4, Supplementary Material). Aside from individual markers, the phylogenetic tree with concatenated sequences also delineated *C. yamaguchii* specimens from the two geographic locations (Fig. S5, Supplementary Material). This consistent divergence between the populations of *C. yamaguchii* in contrasting ecoregions suggests distinct evolutionary routes of this cubozoan. Nonetheless, we recommend future studies of phylogenetics of this species with aim to include balanced sample sizes, nuclear markers and more *16S* samples from South Kuroshio to further validate our findings here.

Integration of morphological data and outputs from species delimitation algorithms, as attempted in ASAP and mPTP here, emphasizes the challenges and complexities inherent in marine zooplankton and invertebrate systematics (Kapli et al., 2017; Wu et al., 2023). The discrepancies between genetic and morphology-based species designations point to the need for a multi-faceted approach, incorporating multiple molecular sources and morphological findings to accurately resolve evolutionary relationships (Dellicour and Flot, 2018). However, despite their benefits in our integrative approach, these species delimitation algorithms (ASAP and mPTP) produced outputs not entirely consistent to each other. For example, ASAP separated *16S* samples of *Chironex* from Palau against Thailand's *C. indrasaksajiae* but mPTP indicated these samples as one species (Fig. 6b). In our analyses with balanced sample sizes, ASAP grouped *C. fleckeri* and *C. yamaguchii* (for *cox1*) as one species unlike mPTP that separated Japan and Philippine specimens and *C. fleckeri* as three distinct species (Fig. S4, Supplementary Material). Inconsistent outputs between ASAP and mPTP also manifested in systematics studies for other marine metazoans (Bañón et al., 2024). In light of these outcomes, we caution dependence on species-delimitation algorithms as "panacea" to species designations and, if accessible, include phylogenetic tree findings from multiple molecular markers in examining cubozoan systematics as in this study.

Related to integrative approaches, citizen science proved instrumental in this systematics study of *C. yamaguchii*, contributing a diverse range of images and videos of the box jellyfish, crucial in assessing and comparing their appearance to preserved materials and museum records (Earp and Liconti, 2020; as in Boco et al., 2024). When combined with molecular and morphological analyses, these citizen-science contributions may aid identification of genetic divergence and phylogeographic patterns, detect cryptic species and understand evolutionary relationships and species distributions (Anthony et al., 2023; Pocock et al., 2024). We harnessed these benefits except for phylogeography. Notably, we would have not recorded potential bloom formation, characteristics and occurrence of envenomation (Fig. 8a-j; Fig. S5, Supplementary Material) and dietary use of cubomedusae of *C. yamaguchii* without coverage by citizen science. Certainly, citizen science also elucidated the widespread distribution of *C. yamaguchii* and it even revealed peaks of the quantity of observations (not the peaks of occurrences themselves) on the cubozoans and their stings around warm months of April to June in the Philippines (Fig. S5, Supplementary Material). These outputs, with the other taxonomic evidence such as the molecular signatures, prove the presence of a *Chironex* species in the Coral Triangle. These benefits of citizen science underscore the importance of this method in marine biodiversity research (Terenzini et al., 2023; Boco et al., 2024; Pocock et al., 2024).

In marine ecosystems, the complex interplay of biological, oceanographic, and geographic factors may influence the occurrence of *C. yamaguchii*. For instance, warm sea surface temperatures (~28–30 °C) and varying salinity levels (29–32 PSU) may support their occurrence while adequate light penetration in shallow waters such as coral reefs enhance the growth of prey species, like juvenile reef fish, providing a robust ecosystem for cubozoans in the Western Pacific (Przeslawski et al., 2008; Podbielski et al., 2022). Though our findings only confirm the existence of this species in shallow zones such as coastal reefs, the possible presence of this species in deep habitats like the mesophotic zone remains because direct evidence of the presence of the congener

Chironex sp. at depths of 39–56 m exists (see Keesing et al., 2016). These deep areas link to ocean currents, including the Kuroshio and North Equatorial Currents, which govern biophysical conditions including the distribution and dispersal of larvae of cubozoans and their prey in Western Pacific (see Fig. 1a; Hsiung et al., 2022; inferred from Terenzini et al., 2023). Regarding biophysical influence, our haplotype analyses revealed mutation differences among populations of *Chironex* spp., which suggest that *C. yamaguchii* can adapt to different local environments such as ecoregions of South Kuroshio and the Coral Triangle (as in Sjöstrand et al., 2014 and Haasl and Payseur, 2016). These haplotypes may indicate either the influence of genetic profiles on the box jellyfish's ecological distribution by allowing populations to thrive in varied environmental conditions, or they reflect inherent adaptations in bioregions such as the Coral Triangle (Sjöstrand et al., 2014 and Haasl and Payseur, 2016). For instance, genetic adaptations might enable different populations to survive at specific temperature ranges, or prey availability, thereby maintaining their wide range of potential habitats (Jahnke et al., 2022). Understanding these genetic differences may explain why *C. yamaguchii* occurs in limited yet diverse ecoregions. While we maintain above as speculations, we recommend further assessments of biophysical factors regulating the existence of *C. yamaguchii*.

Classifying the Philippine cubozoan as a putative cryptic species implies a need for tailored management strategies regarding human-cubozoan interactions, focusing on the species both as a whole and population units (Chenuil et al., 2019; as in Mukherjee and Mackessy, 2021). These strategies emphasize continuous scientific research and regional cooperation required to understand *C. yamaguchii*'s occurrences and manage our interactions with this species (as in Condie et al., 2018 and Verdadero et al., 2022). This is crucial due to the cubozoan's confirmed distribution and involvement in envenomation and human fatalities in the South Kuroshio and Coral Triangle regions (see Fig. 8; Fig. S6, Supplementary Materials; Condie et al., 2018; Hifumi et al., 2020). This regional cooperation is further enhanced by incorporating local knowledge and citizen science contributions themselves which play a valuable role in informing distribution of this species and their envenomation (Tengö et al., 2021; Pocock et al., 2024). Together, these collaborative and integrative efforts may ensure a more comprehensive understanding of the presence of *C. yamaguchii* (Condie et al., 2018; Tengö et al., 2021). Ultimately, our findings must inform the development of effective management strategies to mitigate *C. yamaguchii*'s impacts on public health and to understand their ecological roles, which require sustained scientific experiments and international cooperation (Condie et al., 2018; Mukherjee and Mackessy, 2021).

4.1. Conclusion

Our integrative approach through a combination of morphological and molecular techniques elucidated the taxonomic status of the box jellyfish species *Chironex yamaguchii* and confirmed its presence in the Coral Triangle region of the Philippines. Using whole animal specimens, multiple mitochondrial markers and species-delimitation algorithms, we detected genetic divergence within this box jellyfish species, enabling classification of the Philippine cubozoan as putative cryptic species. This notable advancement in our understanding of cubozoan diversity reveals the imperative of integrating multiple lines of evidence in species identification and indicates the potential for uncovering further insights into the evolutionary history and diversification of this medusozoan group. Our findings underscore the necessity for continued systematics research and accurate identification of box jellyfish as they relate to important implications of their ecological roles and in managing interactions of humans with venomous medusozoan species.

Ethical standards

Specimen collections comply with local government permits, and genetic sequencing adheres to permits from the Philippine Department of Environment and Natural Resources, the Department of Agriculture and agreements in the Access and Benefit Sharing of Genetic Resources of the Pacific (Nagoya Protocol). Local interviews with informed consent are approved by Velez College's ethics review board and adhere to the Declaration of Helsinki. Citizen science procedures adhere to the Philippines's Republic Act on Data Privacy (10173) and provisions of the United Nations Principles of Personal Data Protection and Privacy.

CRediT authorship contribution statement

Phuping Sucharitakul: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Raffy Jay Fornillos:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Christine Gloria Grace Capidos:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Sheldon Rey Boco:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ian Kendrich Fontanilla:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Allen G. Collins:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Dennis Talacay:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jonathan W Lawley:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Facundo Rey M Ladiao:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation. **Joseph Elliz Comendador:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors do not have any conflict of interest.

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Appendix

Table A.1
Locations of *Chironex yamaguchii* and *C.* cf. *yamaguchii* based on validated citizen-science reports in this study. Biogeographic (i.e., biogeography) locations of these records matching the ecoregions of museum-preserved specimens of *C. yamaguchii* (see Table 1) were identified. WPS = West Philippine Sea, N = Northern Philippine Sea, and SPS = Southern Philippine Sea ecoregions. Refer to Ecological analyses section of the methodology

Locality	Biogeography	Latitude	Longitude	*N*	Date	Identification
Caniogan reef, Pangasinan	WPS	16°17’34.0"N	120°00’52.8"E	1	April 2018	*C. yamaguchii*
Hundred Islands, Pangasinan	WPS	16°12’50.7"N	120°02’14.6"E	1	3 Nov 2023	*C.* cf. *yamaguchii*
Candelaria, Zambales	WPS	15°40’18.5"N	119°55’53.2"E	1	9 May 2019	*C.* cf. *yamaguchii*
Subic Bay	WPS	14°45’53.1"N	120°15’21.0"E	2	19 Dec 2023	*C. yamaguchii*
Patnanungan, Quezon	N Philippine Sea	14°45’03.2"N	122°13’01.3"E	> 20	24 May 2024	*C. yamaguchii*
Padre Burgos, Quezon	Visayan	13°53’27.2"N	121°52’11.1"E	1	26 May 2018	*C. yamaguchii*
Padre Burgos, Quezon	Visayan	13°54’15.5"N	121°49’19.3"E	1	14 May 2023	*C.* cf. *yamaguchii*
Pagbilao, Quezon	Visayan	13°54’14.2"N	121°46’40.0"E	2	7 July 2022	*C. yamaguchii*
Agdangan, Quezon	Visayan	13°50’49.5"N	121°57’24.2"E	1	18 May 2024	*C. yamaguchii*
Catanauan, Quezon	Visayan	13°35’14.7"N	122°17’03.2"E	1	9 April 2022	*C. yamaguchii*
Catanauan, Quezon	Visayan	13°35’58.7"N	122°13’41.5"E	4	8 May 2023	*C.* cf. *yamaguchii*
Mulanay, Quezon	Visayan	13°25’18.1"N	122°28’16.0"E	1	4 May 2024	*C. yamaguchii*
Calauag, Quezon	N Philippine Sea	14°11’25.8"N	122°14’38.8"E	2	26 Sept 2023	*C. yamaguchii*
Hook Bay, Quezon	N Philippine Sea	14°56’47.5"N	121°50’13.9"E	6	7 May 2023	*C. yamaguchii*
Hook Bay, Quezon	N Philippine Sea	14°56’47.5"N	121°50’13.9"E	1	4 July 2023	*C. yamaguchii*
Panukalan port, Quezon	N Philippine Sea	14°55’53.8"N	121°48’59.1"E	1	1 June 2023	*C. yamaguchii*
Basud, Camarines Norte	N Philippine Sea	14°03’24.3"N	123°01’48.1"E	1	19 April 2019	*C. yamaguchii*
Capalonga, Camarines Norte	N Philippine Sea	14°19’41.9"N	122°30’29.3"E	1	7 June 2023	*C. yamaguchii*
Balicyao Bay, Camarines Norte	N Philippine Sea	14°12’36.8"N	122°20’43.6"E	3	7 June 2023	*C. yamaguchii*
Siruma, Camarines Sur	N Philippine Sea	14°01’38.5"N	123°15’38.0"E	1	18 April 2017	*C. yamaguchii*
Siruma, Camarines Sur	N Philippine Sea	14°01’38.5"N	123°15’39.0"E	1	28 May 2017	*C. yamaguchii*
Siruma, Camarines Sur	N Philippine Sea	14°01’35.3"N	123°15’31.3"E	4	17 June 2018	*C.* cf. *yamaguchii*
Siruma, Camarines Sur	N Philippine Sea	14°01’32.8"N	123°15’25.1"E	3	29 July 2022	*C. yamaguchii*
Siruma, Camarines Sur	N Philippine Sea	14°01’38.8"N	123°15’39.9"E	1	9 May 2023	*C. yamaguchii*
Siruma, Camarines Sur	N Philippine Sea	14°01’38.8"N	123°15’39.9"E	1	1 June 2023	*C.* cf. *yamaguchii*
Sab’itan Laya Island, Caramoan	N Philippine Sea	13°51’34.5"N	123°51’19.3"E	2	4 Aug 2018	*C. yamaguchii*
Caramoan, Camarines Sur	N Philippine Sea	13°57’02.3"N	123°51’10.7"E	1	2 Oct 2019	*C.* cf. *yamaguchii*
Caramoan, Camarines Sur	N Philippine Sea	13°49’06.3"N	123°50’52.1"E	1	16 March 2023	*C. yamaguchii*
Puerto Galera, Mindoro	WPS	13°31’32.1"N	120°58’04.0"E	1	30 June 2023	*C.* cf. *yamaguchii*
Donsol, Sorsogon	Visayan	12°55’34.1"N	123°34’22.8"E	3	5 May 2019	*C. yamaguchii*
Donsol, Sorsogon	Visayan	12°56’43.1"N	123°32’04.6"E	1	5 May 2019	*C. yamaguchii*
Donsol, Sorsogon	Visayan	12°56’40.5"N	123°31’33.6"E	1	7 May 2019	*C. yamaguchii*
Castilla, Sorsogon	Visayan	12°54’29.6"N	123°51’25.0"E	1	28 April 2019	*C. yamaguchii*
Busuanga, Palawan	WPS	12°01’12.9"N	119°58’49.2"E	1	21 April 2019	*C. yamaguchii*
Busuanga, Palawan	WPS	12°09’10.3"N	119°55’05.6"E	1	13 March 2016	*C. yamaguchii*
Busuanga, Palawan	WPS	11°59’45.0"N	119°51’33.1"E	1	12 May 2023	*C. yamaguchii*
Coron, Palawan	Sulu Sea	12°00’07.2"N	120°11’30.6"E	1	14 April 2023	*C. yamaguchii*
Coron, Palawan	Sulu Sea	11°59’06.6"N	120°04’47.4"E	1	21 March 2022	*C. yamaguchii*
Coron, Palawan	Sulu Sea	11°56’41.5"N	120°11’36.7"E	1	16 July 2023	*C. yamaguchii*
Coron, Palawan	Sulu Sea	11°57’01.4"N	120°11’34.8"E	1	22 July 2023	*C. yamaguchii*
Bulalacao Island, Palawan	Sulu Sea	11°44’54.0"N	120°09’24.9"E	1	8 April 2017	*C. yamaguchii*
Bulalacao Island, Palawan	Sulu Sea	11°44’22.3"N	120°10’19.1"E	1	19 March 2022	*C. yamaguchii*
Culion, Palawan	Sulu Sea	11°53’46.0"N	120°01’00.3"E	4	1 Aug 2023	*C.* cf. *yamaguchii*
Pangatalan, Palawan	Sulu Sea	11°05’07.6"N	119°33’47.6"E	2	30 July 2017	*C.* cf. *yamaguchii*
Port Barton, Palawan	WPS	10°27’19.4"N	119°11’43.8"E	1	30 March 2021	*C. yamaguchii*
Port Barton, Palawan	WPS	10°24’39.4"N	119°09’41.2"E	1	23 Aug 2023	*C. yamaguchii*
Roxas, Palawan	Sulu Sea	10°19’23.5"N	119°21’17.6"E	1	19 April 2019	*C. yamaguchii*
Honda Bay, Palawan	Sulu Sea	9°54’17.7"N	118°48’03.4"E	1	12 April 2022	*C. yamaguchii*
Honda Bay, Palawan	Sulu Sea	9°52’25.7"N	118°45’59.9"E	3	22 April 2023	*C. yamaguchii*
Honda Bay, Palawan	Sulu Sea	9°50’34.5"N	118°44’46.3"E	1	6 May 2018	*C. yamaguchii*
Turtle Bay, Palawan	Sulu Sea	9°38’56.3"N	118°43’47.0"E	1	7 June 2015	*C. yamaguchii*
Aborlan, Palawan	Sulu Sea	9°21’33.5"N	118°32’00.7"E	1	22 May 2022	*C. yamaguchii*
Aborlan, Palawan	Sulu Sea	9°21’32.6"N	118°31’55.7"E	1	14 May 2023	*C. yamaguchii*
Quezon, Palawan	WPS	9°20’52.4"N	118°07’29.1"E	2	24 July 2021	*C. yamaguchii*
Inogbong, Palawan	Sulu Sea	8°40’00.5"N	117°39’18.9"E	34	2 July 2023	*C. yamaguchii*
Dimasalang, Masbate	Visayan	12°12’07.3"N	123°50’55.0"E	1	2 June 2018	*C. yamaguchii*
Cawayan, Masbate	Visayan	11°57’57.2"N	123°42’60.0"E	1	3 June 2018	*C. yamaguchii*
San Pascual, Masbate	Visayan	13°07’34.3"N	122°58’17.7"E	1	30 April 2022	*C. yamaguchii*
Isla de Gigantes, Iloilo	Visayan	11°35’19.2"N	123°21’06.5"E	1	4 Nov 2017	*C. yamaguchii*
Borongan city, Eastern Samar	SPS	11°40’24.0"N	125°27’52.2"E	3	9 July 2022	*C. yamaguchii*
Hernani, Eastern Samar	SPS	11°16’55.3"N	125°34’15.0"E	6	18 July 2024	*C. yamaguchii*
Marabut, Samar	SPS	11°11’30.9"N	125°11’20.9"E	1	22 March 2019	*C. yamaguchii*
Marabut, Samar	SPS	11°13’01.1"N	125°10’32.3"E	7	12 June 2021	*C. yamaguchii*
Marabut, Samar	SPS	11°11’27.5"N	125°10’43.5"E	1	16 April 2023	*C. yamaguchii*
Suyac Island, Negros Occidental	Visayan	10°56’53.1"N	123°27’20.2"E	1	11 June 2023	*C. yamaguchii*
Calatrava town, Negros	Visayan	10°33’16.7"N	123°28’16.6"E	1	13 June 2021	*C.* cf. *yamaguchii*
Ave Maria Is, Guimaras	Visayan	10°32’42.4"N	122°31’06.5"E	1	7 July 2022	*C.* cf. *yamaguchii*
Guimaras, Philippines	Visayan	10°31’59.3"N	122°31’34.4"E	1	12 Jan 2019	*C. yamaguchii*
Guimaras, Philippines	Visayan	10°31’59.3"N	122°31’34.4"E	1	17 May 2019	*C. yamaguchii*

(*continued on next page*)

Table A.1 (*continued*)

Locality	Biogeography	Latitude	Longitude	*N*	Date	Identification
Taklong Island Marine Reserve, Guimaras.	Visayan	10°24'11.7"N	122°30'40.5"E	2	14 July 2022	*C.* cf. *yamaguchii*
Malapascua Is., Cebu	Visayan	11°19'41.6"N	124°06'42.3"E	1	24 June 2023	*C.* cf. *yamaguchii*
Santa Fe, Cebu	Visayan	11°08'50.6"N	123°47'36.7"E	2	8 June 2023	*C.* cf. *yamaguchii*
Clarin, Bohol	Visayan	9°58'01.5"N	124°01'24.5"E	1	25 June 2023	*C.* cf. *yamaguchii*
Tubigon Port, Bohol	Visayan	9°57'17.5"N	123°57'29.0"E	1	13 May 2018	*C. yamaguchii*
Tubigon, Bohol	Visayan	9°57'32.9"N	123°58'22.9"E	6	4 May 2021	*C. yamaguchii*
Calape Bohol	Visayan	9°56'14.6"N	123°51'22.6"E	1	1 June 2015	*C. yamaguchii*
Calape Bohol	Visayan	9°53'11.2"N	123°49'32.9"E	2	10 May 2018	*C. yamaguchii*
Calape, Bohol	Visayan	9°53'14.5"N	123°49'38.5"E	1	27 April 2019	*C. yamaguchii*
Mabini, Bohol	Visayan	9°52'08.0"N	124°33'48.6"E	1	28 Nov 2020	*C. yamaguchii*
Town of Duero, Bohol	Visayan	9°41'31.8"N	124°24'04.5"E	2	6 May 2023	*C. yamaguchii*
Libjo, Dinagat Is.	WPS	10°10'23.6"N	125°30'02.8"E	1	29 Aug 2022	*C. yamaguchii*
Sohoton Cove, Bucas Grande	WPS	9°35'52.7"N	125°54'56.6"E	2	20 Aug 2022	*C. yamaguchii*
Placer, Surigao del Norte	WPS	9°38'52.5"N	125°36'16.2"E	1	11 June 2023	*C. yamaguchii*
Lipayo Beach, Dauin Dumaguete	Visayan	9°11'57.3"N	123°16'37.9"E	2	30 April 2023	*C. yamaguchii*
Lingig, Surigao del Sur	WPS	8°00'48.6"N	126°26'43.9"E	1	30 Nov 2019	*C.* cf. *yamaguchii*
Lingig, Surigao del Sur	WPS	7°59'56.0"N	126°24'30.9"E	1	20 Nov 2022	*C. yamaguchii*
Lingig, Surigao del Sur	WPS	8°09'42.3"N	126°26'21.6"E	5	8 Nov 2023	*C. yamaguchii*
Lopez Jaena port, Misamis Occidental	Visayan	8°33'28.4"N	123°45'51.8"E	1	30 May 2023	*C. yamaguchii*
Dakak beach, Zamboanga del Norte	Visayan	8°41'39.7"N	123°23'35.4"E	1	1 May 2023	*C. yamaguchii*
Naga, Zamboanga Sibugay	Celebes	7°46'47.3"N	122°41'09.7"E	13	10 May 2021	*C.* cf. *yamaguchii*
Alicia, Zamboanga Sibugay	Celebes	7°28'53.9"N	122°56'56.5"E	1	12 Dec 2021	*C. yamaguchii*
Buluan Island, Zamboanga Sibugay	Celebes	7°41'16.0"N	122°32'24.0"E	2	1 April 2023	*C.* cf. *yamaguchii*
Buug town, Zamboanga Sibugay	Celebes	7°40'36.4"N	123°04'56.4"E	1	25 Oct 2023	*C.* cf. *yamaguchii*
Buug town, Zamboanga Sibugay	Celebes	7°39'38.0"N	123°03'32.5"E	1	26 Aug 2023	*C.* cf. *yamaguchii*

N = number of individuals observed on a specific date of observation.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rsma.2025.104033.

Data availability

Data are available in the paper. See also new GenBank sequences.

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