RAFFLES BULLETIN OF ZOOLOGY **74**: 383–402
Date of publication: 15 May 2026
DOI: 10.26107/RBZ-2026-0026
https://zoobank.org/urn:lsid:zoobank.org:pub:32F28DF5-5B2A-4166-AE81-5284847C9C45

Chironex box jellyfishes (Cnidaria: Cubozoa: Chirodropida) in Singapore: *Chironex blakangmati*, new species, and range extension of *C. indrasaksajiae*

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Abstract. Two venomous box jellyfish species of the genus *Chironex* Southcott, 1956 (Cnidaria: Cubozoa: Chirodropida) were collected from Singapore's coastal waters: *Chironex indrasaksajiae* Sucharitakul, 2017 and a novel species described herein as *Chironex blakangmati*, new species. *Chironex indrasaksajiae* was collected from both the Johor and Singapore Straits around mainland Singapore. *Chironex blakangmati*, new species, was collected from Sentosa Island along the Singapore Strait and is the fourth species described in the genus. While *C. blakangmati*'s volcano-shaped pedalial canal and tentacle number are similar to *C. yamaguchii* Lewis & Bentlage, 2009, its elongated, sharp-tipped velarial canals and DNA sequences distinguish it from other *Chironex* species. Comparisons of *Chironex blakangmati*, new species, with *C. yamaguchii* and *C. fleckeri* reveal novel morphological differences at the terminal end of the perradial lappet along the velarium edge, where *C. blakangmati*, new species, lacks velarial canals extending from the perradial lappet terminus. Juvenile *Chironex yamaguchii* specimens were examined and ontogenetic variations of velarial canals are herein reported. Preliminary cnidome analysis reveals eight types of nematocysts observed in *C. blakangmati*, new species, five types in *C. indrasaksajiae*, and five types in *C. yamaguchii*. Molecular phylogenetic reconstruction places *C. blakangmati*, new species, in a clade distinct from its congeners, as sister group to *C. yamaguchii* based on 16S rRNA gene analysis but diverging earlier than the clade comprising *C. yamaguchii* and *C. indrasaksajiae* based on cytochrome c oxidase subunit I gene analysis for which sequence data are comparatively limited. Understanding the biodiversity and seasonality of venomous cubomedusae will help mitigate the risk they pose to human health and safety during maritime activities.

Key words. 16S rRNA, COI barcoding, marine biodiversity, phylogenetic analysis, Southeast Asia, venomous

INTRODUCTION

Medusozoans in the class Cubozoa, or box jellyfish, are fascinating animals with highly evolved complex eye structures and mating behaviour compared to their scyphozoan and hydrozoan pelagic counterparts (Lewis & Long, 2005; Nilsson et al., 2005; Kingsford & Mooney, 2014). Comprising approximately 50 species classified into two orders (Carybdeida and Chirodropida), eight families and 19 genera, cubozoans are distinguished by the presence of several unique structures including pedalia located at each corner of their cuboidal bell, presence or absence of gastric saccules suspended in the bell cavity, and presence or absence of pinnate glands (see Jarms & Morandini, 2019). The overturned portion at the base of the bell, called the velarium, is a powerful muscular flap that contracts to allow cubomedusae to actively swim against currents while capturing prey with their stinging cellular structures called nematocysts, dodging obstacles with eyes exhibiting the most advanced vision among early metazoans (Nilsson et al., 2005; Garm et al., 2007). Therein, the arrangement of velarial canals, functioning in nutrient exchange, is a diagnostic trait (see Bentlage & Lewis, 2012).

Multi-tentacled box jellyfishes (Cnidaria: Cubozoa: Chirodropida) generally fall into one of three chirodropid families and seven genera of cubomedusae (but see Toshino et al., 2015, describing *Meteorona kishinouyei* as a single tentaculate chirodropid). Collectively, some species in the order Chirodropida, primarily of the genus *Chironex* Southcott, 1956 (Fig. 1), have been responsible for serious envenomation cases and even fatalities in the tropics, from the Indo-Pacific and Indian Oceans, possibly as far up to the Gulf of Oman (Williamson et al., 1996). Generally, distributions

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ISSN 2345-7600 (electronic) | ISSN 0217-2445 (print)

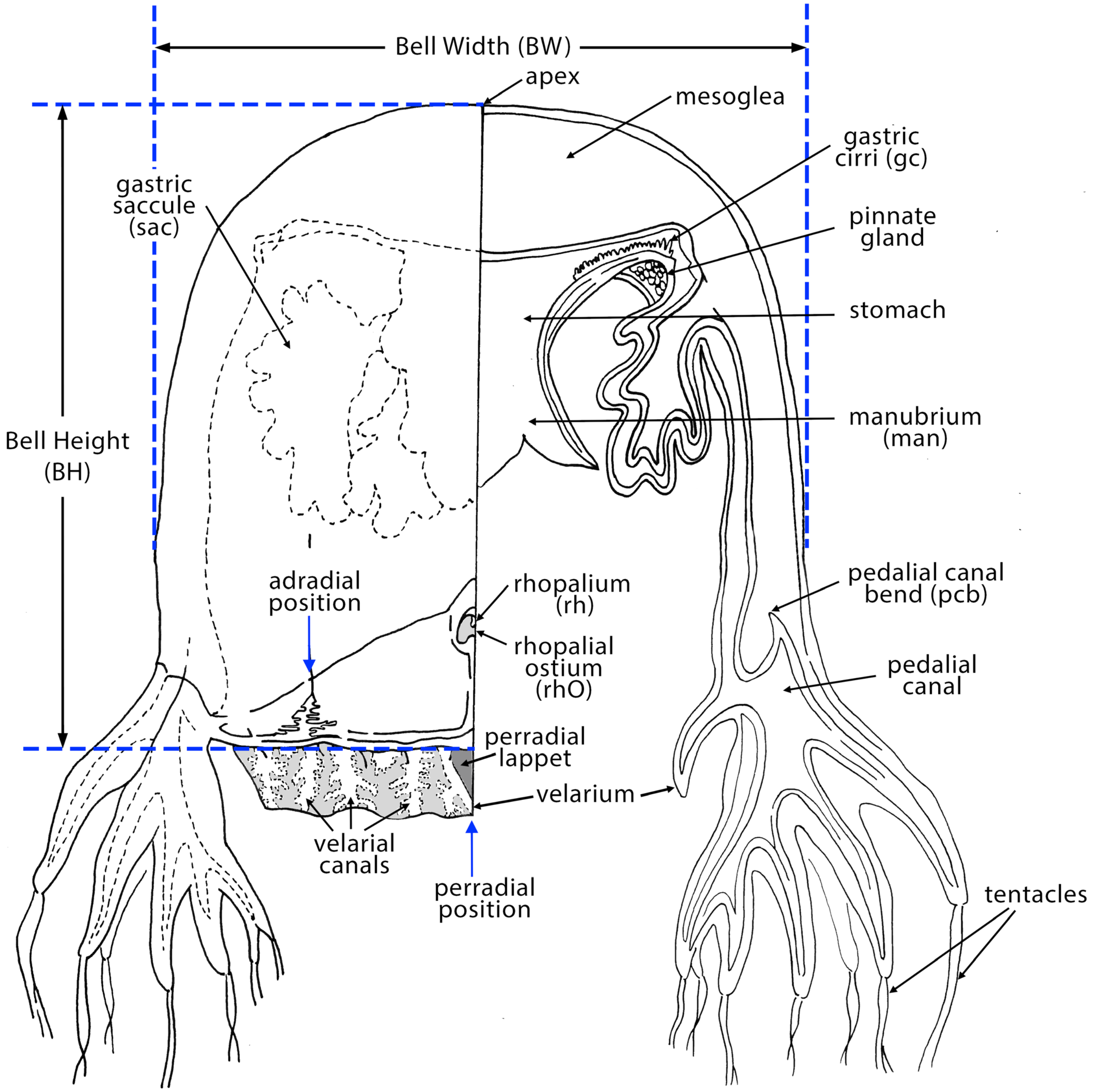

Fig. 1. Schematic diagram of *Chironex* medusa anatomy, perradial view. Modified from generic chirodropid diagram in Gibbons et al. (2022).

of cubozoan species are not well understood due largely to inadequate sampling and difficulty in distinguishing species owing to limited morphological variation among them. A study of large-scale patterns of cubozoan occurrence and reported sting characteristics reported *Chironex* species as being exclusively Indo-Pacific in distribution (Bentlage et al., 2010) and recent studies corroborate these earlier findings (e.g., Keesing et al., 2016; Sucharitakul et al., 2019; Sathirapongsasuti et al., 2021; Boco et al., 2025).

In the last 15 years, several new genera and species of box jellyfish have been described, with even a new family established (Chiropsellidae Toshino, Miyake & Shibata, 2015). Furthermore, the combination of molecular and morphological methods has served to clarify differences between and among box jellyfish congeners sometimes exhibiting minimal phenotypic plasticity (Bentlage & Lewis, 2012), yielding more robust and reliable taxonomic and molecular reference databases for chirodropid species (e.g., Gershwin, 2006a; Lewis & Bentlage, 2009; Bentlage et al., 2010) several of which are venomous or even deadly. While taxonomic progress is being made, substantial gaps remain in understanding ecological variations (both temporally and spatially), changes occurring in morphology and sting potency from development to moribund, reproductive behaviour, distribution of larvae and recruitment into polyps, and influence of physical forces on occurrences of chirodropid box jellyfishes (Kingsford & Mooney, 2014).

Previously in Singapore, multi-tentacled species of cubomedusae incorrectly categorised as *Chiropsalmus quadrigatus* Haeckel, 1880, observed in October 1956 and another time "about a year earlier" by Searle (1957), were suspected to have inflicted harm on people around the island and the region (Searle, 1957; Sharma, 1973). More recently, *Chiropsalmus quadrigatus* was reassigned to the genus *Chiropsoides* Southcott, 1956 (as *Chiropsoides buitendijki* (van der Horst, 1907)), since the holotype morphology was found to be inconsistent with the original description of the genus *Chiropsalmus* Agassiz, 1862 (see Gershwin, 2006a). Other cubozoans reported from Singapore include a yet unidentified species of *Carybdea* Péron & Lesueur, 1810 (Searle, 1957) and *Morbakka*, a larger Irukandji-causing box jellyfish (Iesa & Yap, 2018).

One documented fatality of a child stung by "sea-wasp" or "fire medusa" in Singapore waters occurred in 1974, and "(other) serious cases of injury from these (medusae)" were noted by Sharma (1973). A recent spike in reports of box jellyfish observations occurred from 2017 to 2022, after some 40 years since the last known fatal incident (Iesa, 2024). Two local serious sting incidents suspected to be caused by box jellyfishes occurred at East Coast Park in March 2020 and at Sentosa Island in July 2020 (Tan & Smruthi, 2020). Other cubozoan sightings occurred at Lazarus Island, Pulau Seringat, Tuas, Sembawang Park, and Punggol (Iesa, 2024).

Hitherto, three species of *Chironex* have been formally described: *Chironex fleckeri* Southcott, 1956 (Australia), *C. yamaguchii* Lewis & Bentlage, 2009 (Japan, the Philippines, and East Malaysia [Sabah]) and *C. indrasaksajiae* Sucharitakul, 2017 (Thailand) (for generic morphology, see Fig. 1). This work reports a fourth species, *Chironex blakangmati*, new species, as well as a range extension for the Thailand chirodropid *Chironex indrasaksajiae* into Singapore waters. The presence of only minor distinctions among the morphological characteristics of *Chironex* species has delayed new species descriptions within this genus (see Lewis & Bentlage, 2009). In the original description of *C. yamaguchii*, Lewis & Bentlage (2009) stated that morphological data supported a single species shared between Japan and the Philippines and that differences seen in museum vouchers were likely attributable to a developmental series. Corroborating those findings was a recent comparative molecular and citizen science study of *C. yamaguchii* samples from the western Pacific (i.e., the Philippines, Malaysia, Thailand, and Okinawa) that corresponded to a single species, albeit with intraspecific variation, based on biogeography (Boco et al., 2025).

In this study, we aim to better resolve the species taxonomy within *Chironex* based on morphological comparisons (including nematocyst examination) among various collections, and molecular phylogenetic analyses with the 16S (large subunit) rRNA and cytochrome c oxidase subunit I (COI) genes. Despite all works since Mayer (1910) suggesting a broad distribution of *Chironex yamaguchii* (as *Chiropsalmus quadrigatus*), our molecular phylogenetic findings show that newly obtained Singapore samples differ from all other *Chironex* species reported to date, meriting a new species description which we provide herein for *Chironex blakangmati*, new species. The complete diversity of *Chironex* remains to be discovered, with suggestions of possible undescribed species distributed in Thailand (see Marsh & Slack-Smith, 2010; Keesing et al., 2016; Sucharitakul et al., 2019; Sathirapongsasuti et al., 2021). With the newest *Chironex* species name now stabilised, further filling the knowledge gap in the distribution and seasonality of venomous box jellyfishes in Southeast Asia, we have crossed a crucial milestone towards mitigating harmful and potentially fatal box jellyfish envenomation.

MATERIAL AND METHODS

Collection. Specimens were collected in Singapore opportunistically upon reports of beached individuals, by staff of Sentosa Beach Patrol, members of the public, or staff of National Parks Board Singapore (NParks), from 2020 to 2021 (Fig. 2). The specimens collected were preserved in the laboratory and deposited in the Zoological Reference Collection (ZRC), Lee Kong Chian Natural History Museum, National University of Singapore. Additionally, museum vouchers in formalin solution were examined and photographed at the ZRC, National History Museum, United Kingdom (NHMUK), and Tohoku University Museum, Japan (TUM).

Morphological observation. Freshly collected specimens were first examined in the laboratory, with details of living external appearance noted and photographed. Tissue from the tentacles and velarium were subsampled and preserved in molecular-grade absolute ethanol for further molecular genetic analyses. Thereafter, the animal was fixed in 3–4% freshwater formaldehyde or 5% formalin in filtered seawater. The fixed specimen was further observed and dissected so that the internal morphology could be examined. Measurements were made using a pair of Vernier callipers. Gross morphological observations followed Lewis & Bentlage (2009), Jarms & Morandini (2019), and Gibbons et al. (2022). Nematocysts of the cubozoans were characterised from squash preparations made from subsampled tissue of the tentacles. Where possible, at least ten unfired nematocyst capsules were visualised and measured at 100× magnification oil immersion on an Olympus CX41RF compound microscope or Olympus BX50 compound microscope. Fired nematocysts were also observed and recorded. Nematocyst nomenclature followed that of Mariscal (1974) and Östman (2000).

Phylogeny reconstruction. Genomic DNA was extracted from the preserved tissue using DNeasy Blood and Tissue Kit (Qiagen). Two molecular genetic markers were targeted: 16S rRNA was amplified using the primers med-rnl-F and med-rnl-R with conditions following Lawley et al. (2016), and COI amplified using the primers mlCOIintF and HCO2198 with conditions following Leray et al. (2013). All PCR products obtained were purified with either SureClean Plus (Bioline) or Ampure (Beckman Coulter Inc.). Thereafter, cycle sequencing was conducted with BigDye Terminator

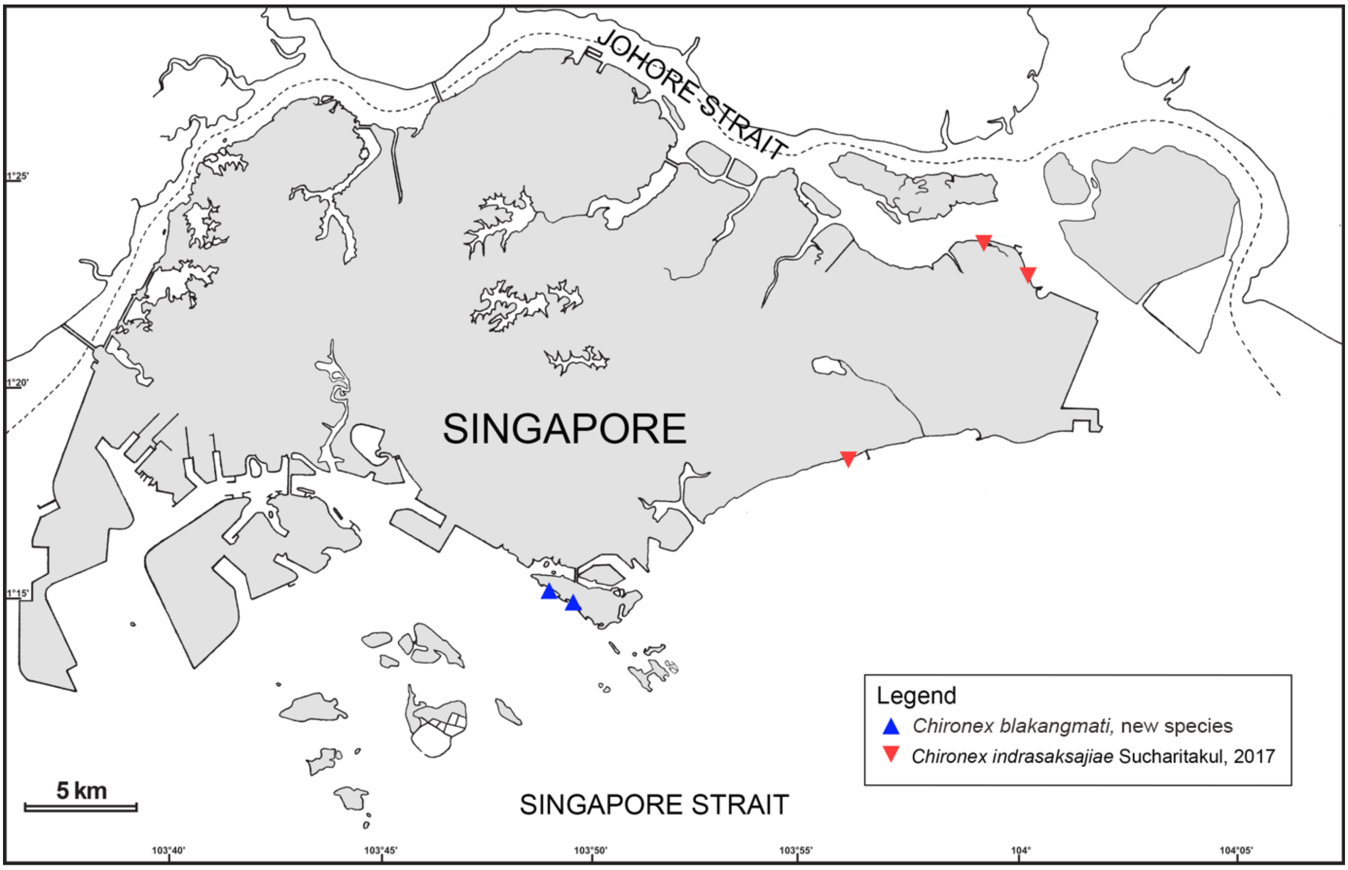

Fig. 2. Map of Singapore indicating locations where cubozoan medusae *Chironex blakangmati*, new species, and *Chironex indrasaksajiae* were collected for this study. *Chironex indrasaksajiae* is also known from the Gulf of Thailand (Sucharitakul et al., 2017, 2019) and, tentatively, Palk Bay along the southeast coast of India (Saravanan et al., 2024).

v3.1 (Applied Biosystems), following the manufacturer's protocols. Amplicons were sequenced on ABI 3130 XL Genetic Analyzer (Thermo Scientific).

Sequences obtained in this study were assembled in Geneious v11.1 (Dotmatics) and contigs were BLAST-searched against GenBank based on sequence similarity to cubozoans in the NCBI nucleotide core database. All sequences obtained in this study were subsequently deposited into GenBank (accession numbers PZ055904, PZ056183–PZ056187). Our sequences and previously published 16S and COI data obtained from GenBank of Chirodropida plus two outgroup Carybdeida species (*Morbakka virulenta* and *Tamoya* cf. *haplonema*) were consolidated and then aligned using MAFFT v.7.313 under –auto setting (Katoh et al., 2002; Katoh & Standley, 2013). The 16S dataset comprised 44 sequences (3 new from this study and 41 published) with a length of 667 bp, while the COI dataset comprised 41 sequences (1 new from this study and 40 published) with a length of 761 bp. Data matrices are available at Zenodo (http://dx.doi.org/10.5281/zenodo.18790140).

Analysis of maximum likelihood was performed in RAxML v.8.2.11 (Stamatakis, 2014) with 50 random starting trees and 1000 bootstrap pseudoreplicates, under the GTRGAMMA model. Further, Bayesian inference was conducted in MrBayes v3.2.6 (Ronquist et al., 2012), based on the best-fit model deduced under Akaike information criterion implemented in jModelTest2 (Darriba et al., 2012). Two parallel runs with four chains of Markov chain Monte Carlo (MCMC) were performed for 12 million generations, recording one tree per 100 generations. MCMC convergence between runs was assessed using Tracer 1.7 (Rambaut et al., 2018), and the first 20,001 trees were discarded as burn-in.

Abbreviations. ZRC – Zoological Reference Collection, Lee Kong Chian Natural History Museum, Singapore. NHMUK – National History Museum, United Kingdom. TUM – Tohoku University Museum, Japan. fwf – freshwater formalin. swf – seawater formalin. BH – Bell height in mm: measured from velarial turn-over to the furthest point of the bell. DBW – Diagonal bell width. IRW – Interradial width in mm. NT – Number of tentacles per pedalia. RhO – Rhopalial ostium. PW – Pedalial width. PL – Pedalial length.

TAXONOMY

Order Chirodropida Haeckel, 1880

Family Chirodropidae Haeckel, 1880

Genus *Chironex* Southcott, 1956

Chironex blakangmati, new species

(Figs. 3, 4, 11D & 12D; Table 1)

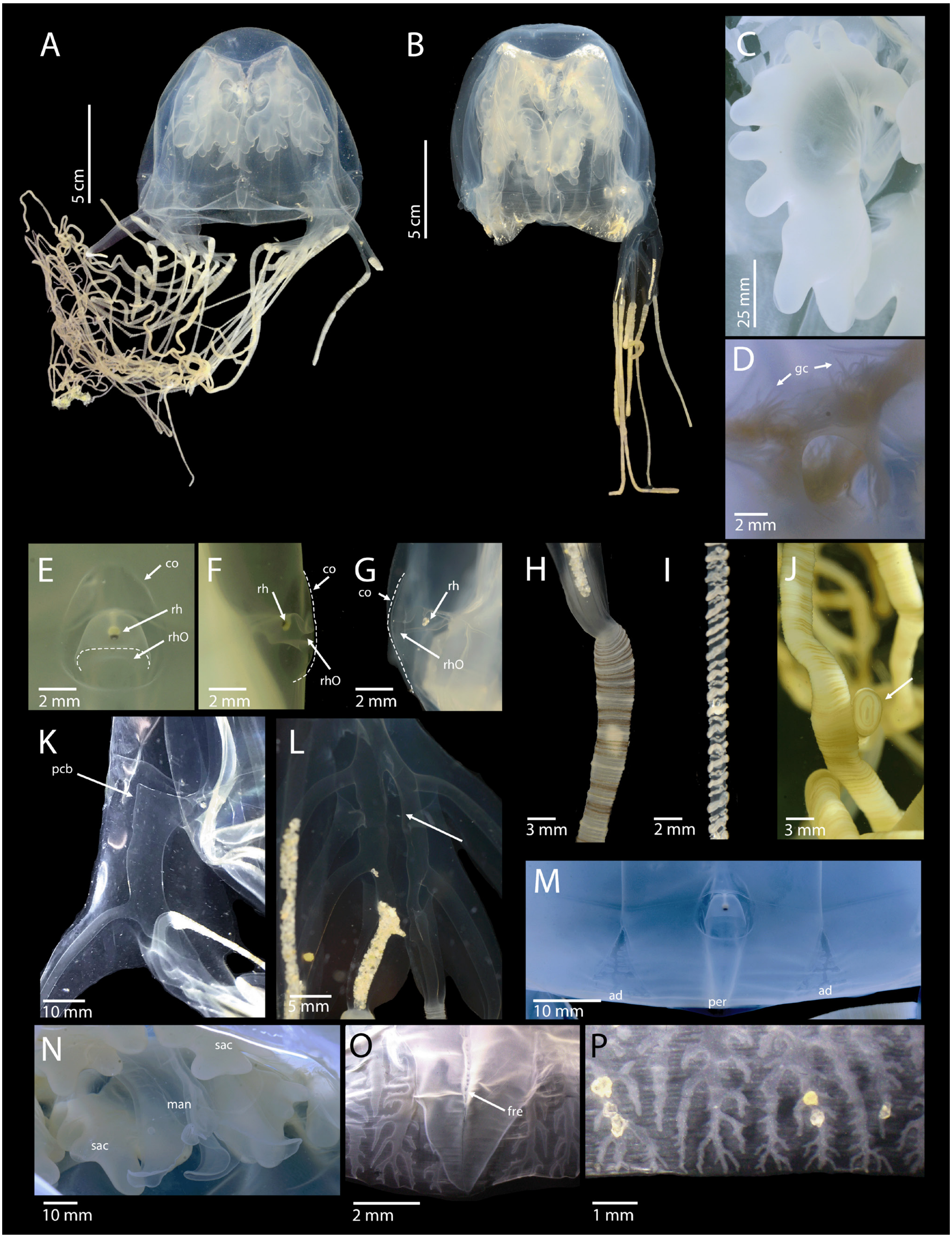

Fig. 3. *Chironex blakangmati*, new species, from Singapore. A, live individual (paratype, ZRC.CNI.1462), lateral perradius view; B, live individual (holotype, ZRC.CNI.3014), lateral perradius view; C, cockscomb gastric saccule (sac); D, gastric cirri (gc); E, rhopalial niche front view; F, rhopalial niche side view (paratype, ZRC.CNI.1462); G, rhopalial niche side view (holotype, ZRC.CNI.3014); H, tentacle contracted with alternating brown dark bands; I, tentacle stretched; J, preserved tentacle with hollow cross section (arrow); K, pedalium with volcano pedalial canal bend (pcb) marked by arrow; L, U-shaped alternating tentacle pattern with gap marked by an arrow; M, adradial positions (ad) marked by arrows, and rhopalial niche at perradial position (per); N, gastric saccules (sac) surrounding cruciform manubrium (man); O, velarium at perradial position with frenulum (fre) arrowed, lappet terminating in simple triangular tip; P, velarium with candelabrum velarial canal pattern (holotype, ZRC.CNI.3014). rh = rhopalium, rhO = rhopalial ostium, co = convex boundary.

Table 1. Cnidae measurements from tentacles of *Chironex blakangmati*, new species (paratype, ZRC.CNI.1462). Six types of undischarged nematocysts (A–G, according to Fig. 5) with corresponding lengths and widths.

Type	Description	n	Length (µm)	Avg. length (µm)	Width (µm)	Avg. width (µm)
A	Giant microbasic p-mastigophore	12	52.53–79.44 ±7.12	61.98	10.25–13.08 ±0.89	11.34
B	Medium microbasic p-mastigophore	13	36.04–62.72 ±7.97	46.15	7.93–11.99 ±1.38	9.99
C	Large lemon-shaped heterotrichous microbasic eurytele	2	35.72–36.87 ±0.81	36.29	19.18–20.85 ±1.18	20.01
E	Round heterotrichous microbasic eurytele	2	14.30–15.22 ±0.65	14.76	10.28–11.59 ±0.93	10.94
F	Small ellipsoidal isorhiza	55	12.30–16.59 ±0.92	14.55	9.19–12.17 ±0.63	10.57
G	Small holotrichous isorhiza	13	12.05–16.03 ±1.03	13.88	4.15–6.21 ±0.56	5.65

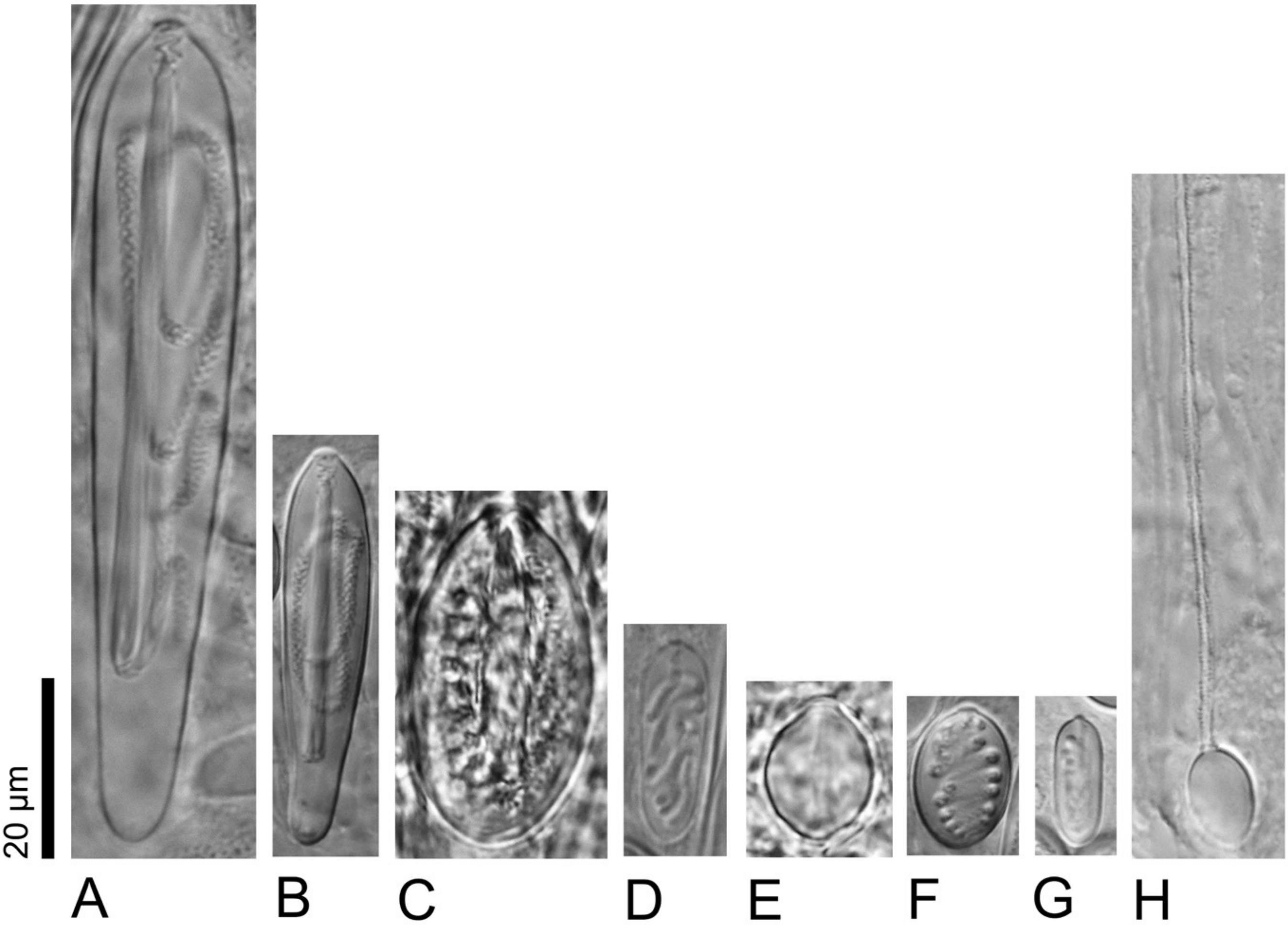

Fig. 4. *Chironex blakangmati*, new species (paratype, ZRC.CNI.1462) tentacle cnidome. A, giant microbasic p-mastigophore (type 1); B, medium microbasic p-mastigophore (type 2); C, large hetrotrichous microbasic eurytele; D, medium ellipsoidal isorhiza (type 1); E, medium heterotrichous microbasic eurytele; F, medium holotrichous isorhiza; G, small ellipsoidal isorhiza (type 2); H, discharged small holotrichous isorhiza.

Material examined. Holotype (Fig. 3B): ZRC.CNI.3014 (preserved in 4% fwf), SS, Sentosa Island, Palawan Beach, 1°14′54.2″N, 103°49′19.8″E, coll. Chan Tak Mun, Sentosa Beach Patrol, 18 November 2022; dissected individual (undetermined sex) cut into two pieces, with grains of sand sticking to edge of velarial lappet and within pedalia canal lumen. Within lot, fragments of gastric saccules and tentacles also present. Material inadvertently stained pink due to residual dye left on dissecting mat cover. NT 7, BH 97.75 mm, IRW 90.54 mm, RhO width 5.22 mm, RhO height 1.09 mm, RhO dome (h) 8.32 mm × (w) 9.34 mm, PL 48.89 mm, PW 18.73 mm, Velarium 19.78 mm, Manubrium 50.99 mm, gastric filaments height 4.18 mm, tentacle width 4.19 mm.

Paratype (Fig. 3A): ZRC.CNI.1462 (1 ex. preserved in 4% fwf), SS, Sentosa Island, Siloso Beach, 1°15′12.6″N, 103°48′53.8″E, coll. Sentosa Beach Patrol, morning of 9 October 2020; dissected individual (undetermined sex) that was cut into four pieces; small fragments of tentacles present in the lot; one piece of the material accidentally stained pink by leeching of coloured cloth during its study. NT 7, BH 78.81 mm, IRW 91.97 mm, RhO width 4.16 mm, RhO height 1.08 mm, RhO dome (h) 7.29 mm × (w) 5.25 mm, PW 15.65 mm, Velarium 10.40 mm, Manubrium 26.05 mm, gastric filaments height 2.12 mm, tentacle width 3.14 mm.

Type locality. Sentosa Island, Singapore, Singapore Strait.

Etymology. This species is named using Bahasa Melayu, the Austronesian language spoken in the region and the national language of Singapore, for Sentosa Island from which the animal was collected. Sentosa Island was historically referred to as "Pulau Blakang Mati", meaning "Island of Death Behind" and, as such, "blakangmati" in denoting the geographic location is a noun in apposition.

Diagnosis. *Chironex* with conical to cuboidal bell. Seven tentacles per pedalium, branching U-shaped alternating. Pedalial canal bend volcano shaped. Tips of velarial canals sharp towards velarial margin (Fig. 11D), with simple triangular tip at edge of velarium in perradial position (Fig. 3O & Fig. 12). Absence of velarial canals at perradial position where frenulum tapers off (Fig. 12D).

Description. Eight cockscomb shaped gastric saccules, each gastric saccule with fat, blunt-edged finger-like projections 5–14, no additional gonads. Pedalia claw-like, each bearing 7 tentacles, arrayed with topmost tentacle perpendicular to lower 3 pairs; lower pairs symmetrically opposite branching (Fig. 3L). Rhopalial niche ostium dumb-bell shaped (Fig. 3E–G), single upper and lower scales, no additional extensions. Trailing tentacles flat and broad in live state as in *Chironex fleckeri* and *C. yamaguchii* (Lewis & Bentlage, 2009). Velarial canals branch with terminal branching pattern resembling menorah-like candelabrum, with tips of velarial canals sharp close to edge of velarium (see Figs. 3O & 3P). Perradial lappet at frenulum position terminates simply with triangular tip (Fig. 3O).

Transparent bell, yellowish with slightly opaque inner structures with cockscomb gastric saccules visible through bell (Fig. 3A). Tentacles transparent to khaki-coloured, with darker shades depending on degree of contraction (Fig. 3H–J). Bell white to creamy in formalin. Tentacles khaki with alternating darker nematocyst bands (Fig. 3H). In holotype, gastric saccules are differentially preserved: some saccules are completely transparent, while some are opaque (Fig. 3C).

Eight nematocyst types found in the tentacles (Fig. 4; Table 1): microbasic p-mastigophore (two size ranges: giant (L 52.53–79.44 µm, W 10.25–13.08 µm, n = 12) and medium (L 36.04–62.72 µm, W 7.93–11.99 µm, n = 13)), large heterotrichous microbasic eurytele (L 35.72–36.87 µm, W 19.18–20.85 µm, n = 2, heterotrichous microbasic eurytele (L 14.30–15.22 µm, W 10.28–11.59 µm, n = 2) and two isorhizas (ellipsoidal (L 12.30–16.59 µm, W 9.19–12.17 µm, n = 55) and round (L 12.05–16.03 µm, W 4.15–6.21 µm, n = 13)). The most abundantly sampled is the small ellipsoidal isorhiza (57% of observed nematocysts).

Remarks. The volcano-shaped pedalial canal bend of *Chironex blakangmati*, new species, is also present in both *C. yamaguchii* and *C. indrasaksijiae*. The specimens, however, do not display the range of shapes of pedalial canal bend (spike, volcano and bulbous) reported in *Chironex indrasaksajiae* (Sucharitakul et al., 2017)— in *C. blakangmati*, all pedalial canal bends are volcano shaped. The number of tentacles of *Chironex blakangmati* falls within the range of *C. yamaguchii*, however, scrutiny of velarial canals show differences in branching and overall shape of canal tips (Fig. 12), with *Chironex blakangmati* having the most sharp-tipped canals toward the distal portion of the velarial margin (Fig. 11D). The absence of velarial canals at perradial lappet tip, where frenulum tapers off, could be an ontogenetic characteristic of an immature individual (see Fig. 9 for variations in preserved juveniles of *C. yamaguchii*). Further examination with more specimens where available in the future would clarify this trait.

Chironex indrasaksajiae Sucharitakul, 2017
(Figs. 5, 6 & 11C; Table 2)

Chironex indrasaksajiae Sucharitakul, 2017 (in Sucharitakul et al., 2017): 36, Figs. 1A–D, 2A–D, 3A–B, 4A–B & 5A–F, Table 2.

Material examined. ZRC.CNI.2819 (1 ex. preserved in 4% fwf); JS, Changi Beach (near Carpark 2), 1°23′29.5″N, 103°59′29.6″E, coll. Muhammad Abu Dzar Al-Ghifari bin Muhammad Norman and staff of NParks, 12 December 2020; NT12, BH 66.61 mm, IRW 69.73 mm, RhO width 3.18 mm, RhO height 1.04 mm, dome RhO (h) 7.29 mm × (w) 7.29 mm, PL 41.63 mm, PW 14.56 mm, Velarium 14.58 mm.

ZRC.CNI. 2821 (1 ex. preserved in 4% fwf); SS, East Coast Park (near BBQ pit 6B), 1°17′49.2″N, 103°54′13.8″E, coll. member of public and staff of NParks, 9 January 2021; NT15, BH 152.97 mm, IRW 119.69 mm, RhO width 9.38 mm, RhO height 2.12 mm, RhO dome (h) 17.69 mm × (w) 15.60 mm, PL 92.64 mm, PW 33.30 mm, Velarium 38.13 mm.

ZRC.CNI.2822 (1 ex. preserved in 4% fwf); JS, Changi Beach (near Carpark 7), 1°22′27.2″N, 104°00′23.2″E, coll. staff of NParks, 13 January 2021, NT12–15, BH 121.75 mm, IRW 107.22 mm, RhO width 7.32 mm, RhO height 1.08 mm, RhO dome (h) 9.38 mm × (w) 13.50 mm, PL 63.49 mm, PW 17.73 mm, Velarium 22.90 mm, Manubrium 50.00 mm.

Type locality. Chao Lao Beach, Chanthaburi Province, Thailand.

Diagnosis. Maximum known height of *Chironex indrasaksajiae* 170 mm (Fig. 5A), smaller than *C. fleckeri* but larger than *C. yamaguchii.* Number of tentacles per pedalium, 10–12. Pedalial canal bend shapes can vary from bulbous to concave, occasionally with secondary feature(s)

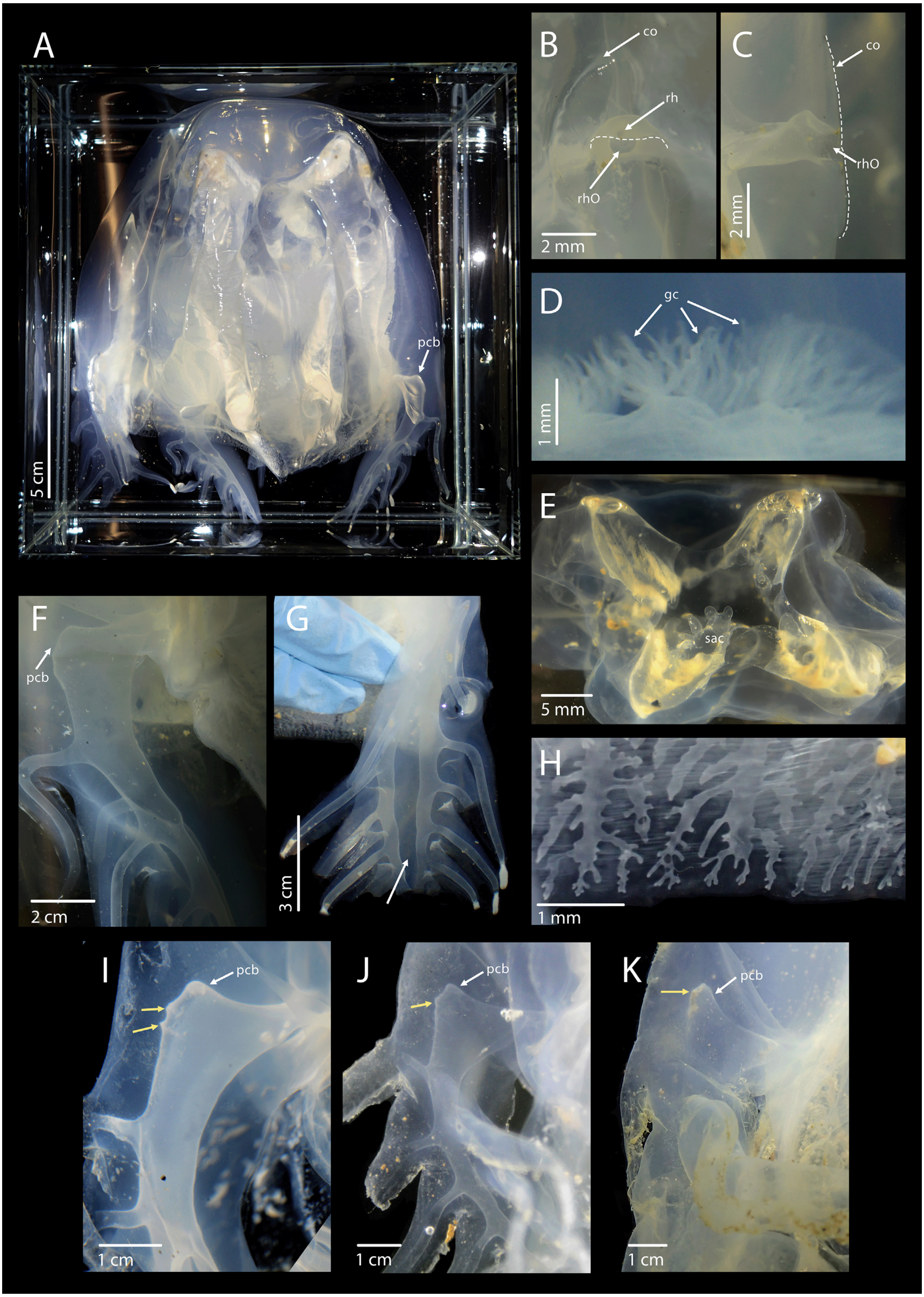

Fig. 5. *Chironex indrasaksajiae* from Singapore (ZRC.CNI.2821). A, whole, freshly collected individual; B, rhopalial niche front view; C, rhopalial niche side view; D, gastric cirri (gc); E, cockscomb gastric saccule; F, rounded pedalial canal bend (pcb); G, alternate arrangement of tentacles, with gap (arrow); H, velarial canals with rounded tips at velarium edge.; I–K: variations in pedalial canal bends, secondary structures indicated with yellow arrows. rh = rhopalium, rhO = rhopalial ostium, co = convex boundary.

Table 2. Cnidae measurements from tentacles of *Chironex indrasaksajiae* (ZRC.CNI.2821). Five types of undischarged nematocysts (A–E, according to Fig. 8) with corresponding lengths and widths.

Type	Description	n	Length (µm)	Avg. length (µm)	Width (µm)	Avg. width (µm)
A	Giant microbasic p-mastigophore	8	78.62–109.47 ±11.83	90.94	12.71–17.35 ±1.76	14.74
B	Medium microbasic p-mastigophore	15	69.83–81.42 ±3.28	77.32	11.51–14.24 ±0.69	12.98
C	Large lemon-shaped heterotrichous microbasic eurytele	1	41.42	–	22.86	–
D	Medium round heterotrichous microbasic eurytele	1	16.80	–	15.45	–
E	Small ellipsoidal isorhiza	13	13.74–18.93 ±1.49	16.53	5.62–7.63 ±0.63	6.53

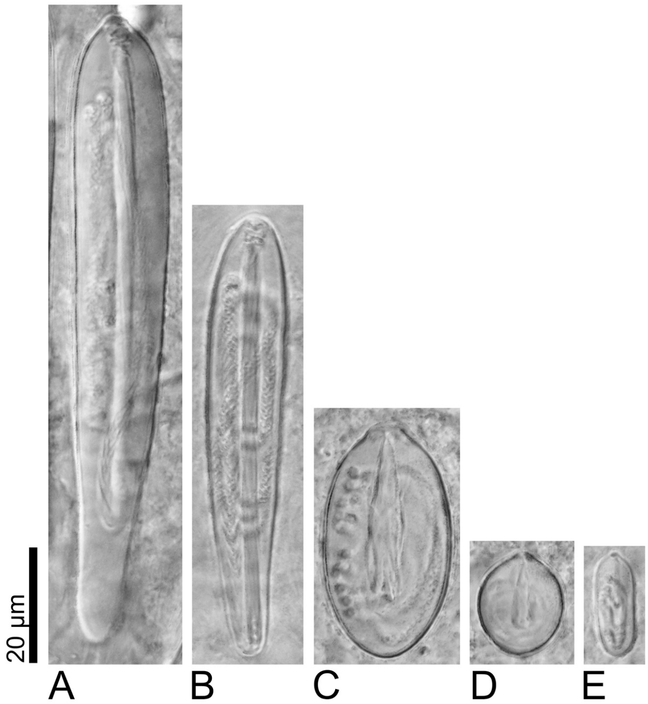

Fig. 6. *Chironex indrasaksajiae* (ZRC.CNI.2821) tentacle cnidome. A, giant microbasic p-mastigophore; B, medium microbasic p-mastigophore; C, large hetrotrichous microbasic eurytele; D, medium heterotrichous microbasic eurytele; E, small ellipsoidal isorhiza.

across canal (Fig. 5I–K). Tips of velarial canals round towards velarial margin (Fig. 11C).

Description. All specimens were found damaged to varying degrees; lengths and full extent of tentacles not observed due to most being truncated upon collection (Fig. 5A & G). Number of tentacles can reach up to 15 per pedalia. U-shaped alternating branching of pedalia (Fig. 5G).

Robust and hefty bell, translucent to whitish. Volcano-shaped pedalial canal bend (Fig. 5F). Gastric saccules were either damaged or small relative to bell size (e.g., ZRC. CNI.2821 with gastric saccules less than a quarter of bell height; Fig. 5E).

Five types of nematocysts were observed from tentacles of *Chironex indrasaksajiae* from Singapore (Fig. 6; Table 2): giant microbasic p-mastigophores (L 78.62–109.47 µm, W 12.71–17.35 µm, n = 8), medium microbasic p-mastigophores (L 69.83–81.82 µm, W 11.51–14.24 µm, n = 15), large lemon-shaped heterotrichous microbasic eurytele (L 41.42 µm, W 22.86 µm, n = 1), medium round heterotrichous microbasic eurytele (L 16.80 µm, W 15.45 µm, n =1) and small ellipsoidal isorhiza (L 13.74–18.93 µm, W 5.62–7.63 µm, n =13). Two out of five of the nematocysts found in Singapore examples were reported in the original description: medium p-mastigophore (ca. L 81–82 µm, W 12–14 µm, n =2) and large heterotrichous microbasic eurytele (ca. L 33 µm, W 17 µm, n =1) (see Fig. 4 in Sucharitakul et al., 2017).

Remarks. Gastric saccules, while cockscomb in shape, do not appear to fill up the bell cavity in some cases. They can appear as reduced digitate appendages (gross size <5 cm; Fig. 5E). However, due to the degree of damage sustained by the specimens prior to collection, extensive observation of the finer features was not possible.

Chironex fleckeri Southcott, 1956
(Figs. 7, 11B & 12A)

Chironex fleckeri Southcott, 1956: 254–280, Figs. 1–3, Plates 1–3 (except Plate 2, Fig. 2), Table 1.
Chiropsalmus quadrumanus Stiasny, 1926: 250, Fig. 1.

Material examined. NHMUK 1983.4.25.5 (1 ex. preserved in swf), Australia, north Queensland, Cairns, coll. K. de Witte, December 1960. NT13–16, BH 64 mm, IRW 76 mm, RhO width 3 mm, RhO height 1 mm, dome RhO (h) 8 mm × (w) 6 mm, PL 39 mm, Velarium 23 mm.

NHMUK 1987.7.9.3 (1 ex. preserved in swf), Australia, Northern Territory, Darwin, Vestey's beach, 1 m, 12°26.3′S, 130°49.8′E, coll. P. Alderslade, 14 April 1987. NT12, BH 65 mm, IRW 71 mm, RhO width 8 mm, RhO height 2 mm, dome RhO (h) 10 mm × (w) 10 mm, PL 84 mm.

Type locality. Cairns and Cardwell regions, north Queensland and Darwin, Northern Territory, Australia.

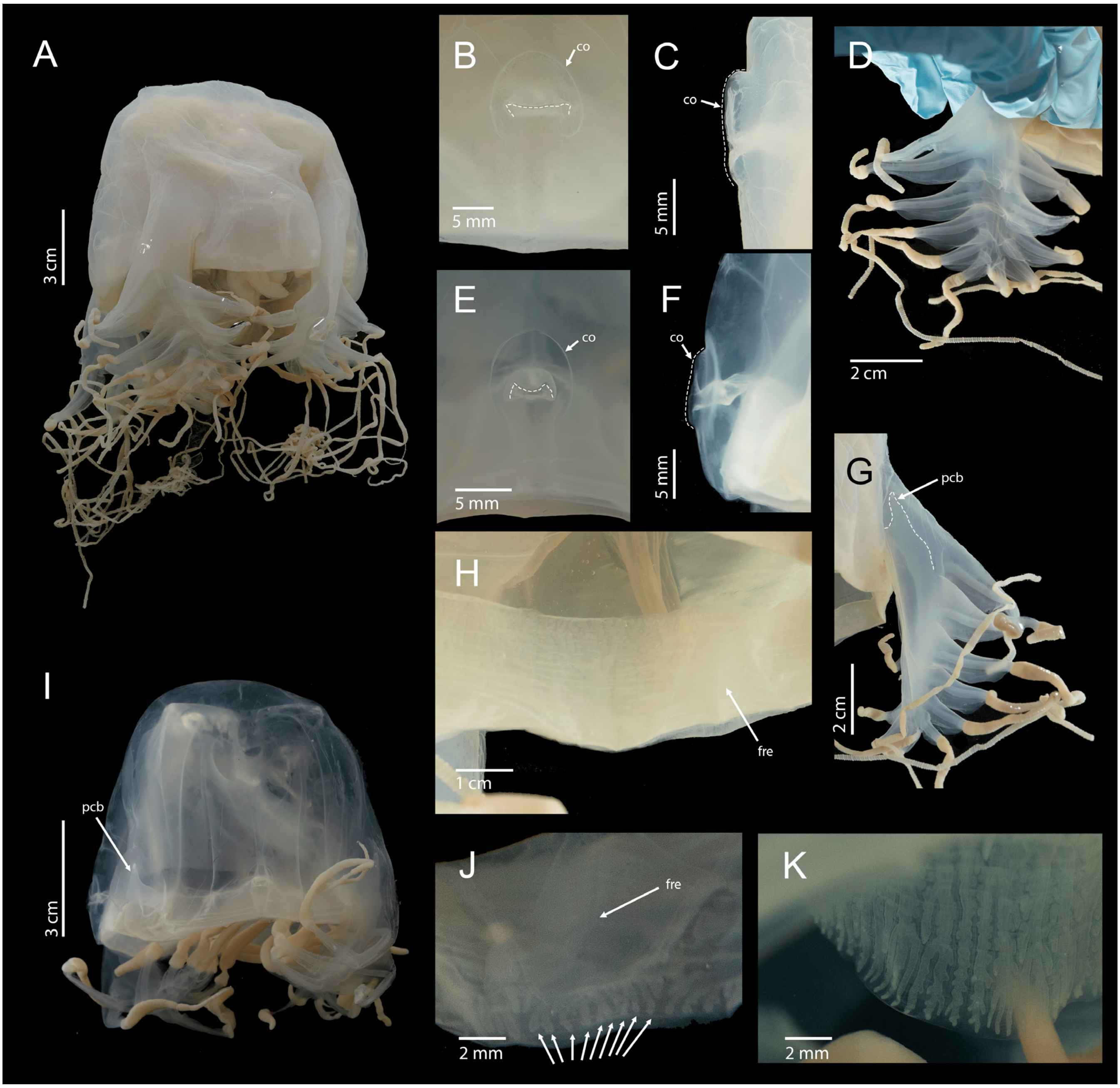

Fig. 7. *Chironex fleckeri* from Australia, preserved individuals. A, whole individual (NHMUK 1987.7.9.3); B–C, rhopalial niche views of NHMUK 1987.7.9.3 (B, front; C, side); D, alternate branching pedalia with small gap (arrow); E–F, rhopalial niche views of NHMUK 1983.4.25; G, thorn-shaped pedalial canal bend (pcb) of NHMUK 1987.7.9.3; H, velarium with frenulum (fre) indicating perradial position; I, lateral perradius view of NHMUK 1983.4.25.5; J, velarial canals at terminal end of perradial lappet; K, velarium at non-perradial position. co = convex boundary.

Diagnosis. Bell height reaching 250 mm and bell width reaching 300 mm, with cuboidal bell shape wider than high. Number of tentacles up to 15 per pedalium. Pedalial canal bend with upwards thorn shape (Fig. 7G). Rhopalial niche dumbbell shape, framed by 1 upper and 1 lower scale. U-shaped alternating branching of pedalia (Fig. 7D).

Remarks. Robust bell, translucent to pinkish in preserved state (Fig. 7I and 7A respectively). Number of tentacles can reach up to 16. Rhopalial niche dumbbell shape, framed by 1 upper and 1 lower scale but observed to also have extension on upper scale (Fig. 7E). Thorn-shaped pedalial canal bend (Fig. 7G & 7I). Velarial canal tips rounded (Figs. 7J–K & 11B), with multiple continuous velarial canal extensions at perradial lappet tip where frenulum terminates; at least 9 extensions observed in NHMUK 1987.7.9.3 (Fig. 7J) and 4 extensions were sketched in Southcott, 1956 (Fig. 12A).

Chironex yamaguchii Lewis & Bentlage 2009
(Figs. 8–10, 11A & 12B–C)

Chironex yamaguchii, Lewis & Bentlage, 2009: Figs 1–2.

Chiropsalmus quadrigatus: Mayer (1910: 516–517, Philippine records); Light (1914a: 291–295); Light (1914b: 197); Mayer (1915: 171); Mayer (1917: 190; Fig. 4); Light (1921); Thiel (1928: 16, remarks, Fig. 6); Stiasny (1931: 139); Stiasny (1937: 213–217, Philippine records); Yamaguchi (1982); Fenner (1997:

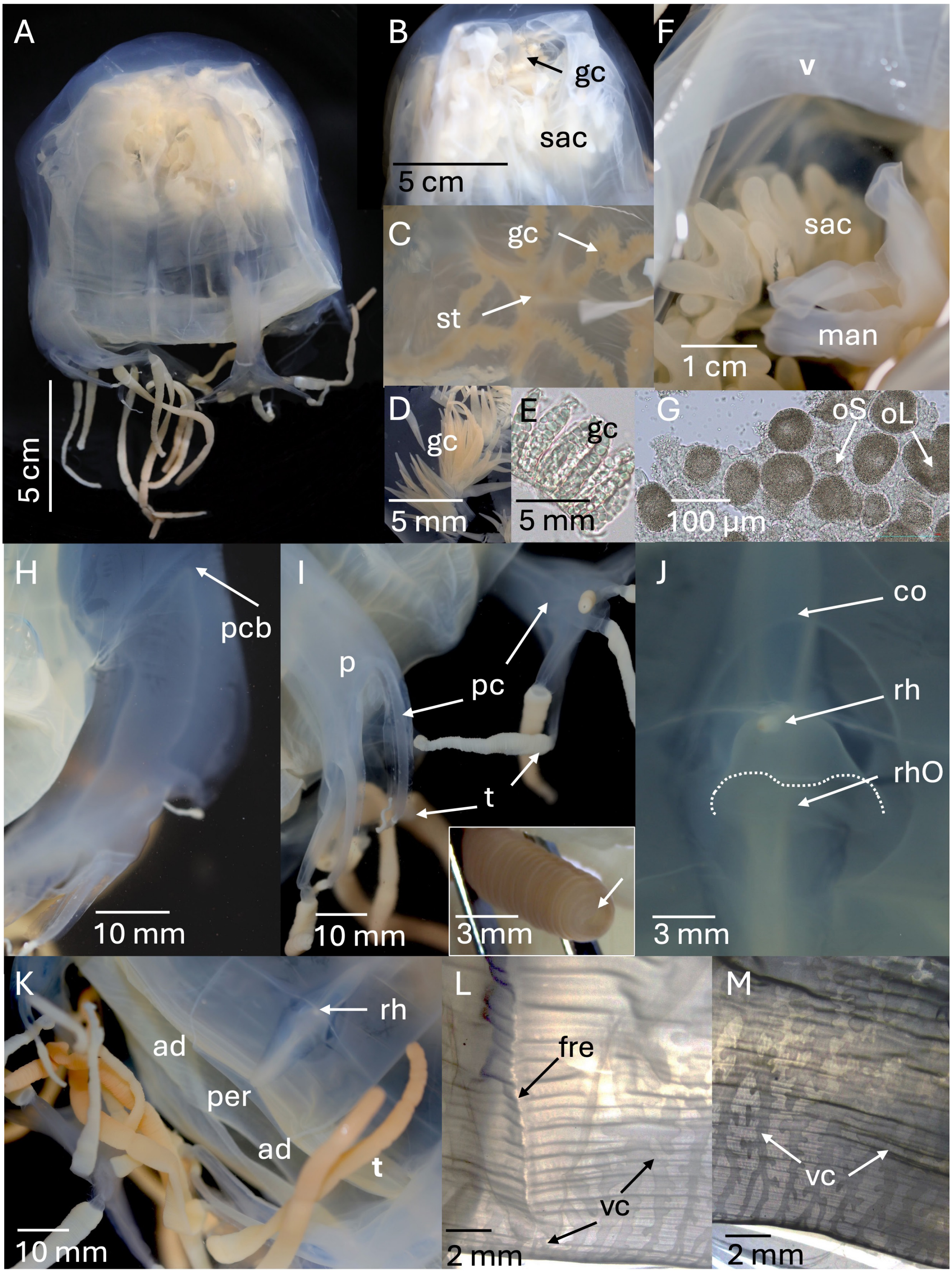

Fig. 8. *Chironex yamaguchii* from Okinawa, preserved individual (TUM 112967). A, lateral perradial view; B, upper portion of the bell showing gastric sacs (sac) and gastric cirri (gc) near apex; C, bell apex showing stomach (st) giving rise to four interradial canals (appearing as apical cross) lined with gastric cirri (gc); D, dissected gastric cirri (gc) viewed under a dissecting microscope; E, dissected gastric cirri (gc) viewed under a stereo microscope and photographed with revealing multiple tiny vesicles comprising each digit; F, cockscomb gastric saccule (sac) within subumbrella surrounding cruciform manubrium (man); three of the four lips visible in foreground with velarium (v) visible in background; G, dissected gastric sac viewed under stereo microscope revealing oocytes of two main size classes large (oL) and small (oS); H, pedalium with volcano pedalial canal bend (pcb); I, U-shaped alternating tentacle pattern; inset shows tentacle contracted with alternating brown dark bands and tip (arrow); J, rhopalial niche front view (dumbbell shaped outlined with dotted line), showing suspended rhopalium (rh) within the rhopalial ostium (rhO) convex boundary (co); K, adradial positions (ad) marked by arrows, and rhopalial niche at perradial position (per); L, velarium at perradial position with frenulum (fre); perradial lappet with simple rounded triangular terminus giving rise to 3–4 short velarial extensions; M, velarium with candelabrum velarial canal pattern reaching the edge of the velarium. A–C, F, H–K photographed with Canon EOS SD Mark IV camera; D, L, M viewed with Nikon SMZ745T (Japan) microscope and photographed with the Nikon Digital Sight 1000, Japan; E, G viewed with Nikon Eclipse Ni (Japan) and photographed with the Nikon Digital Sight 1000.

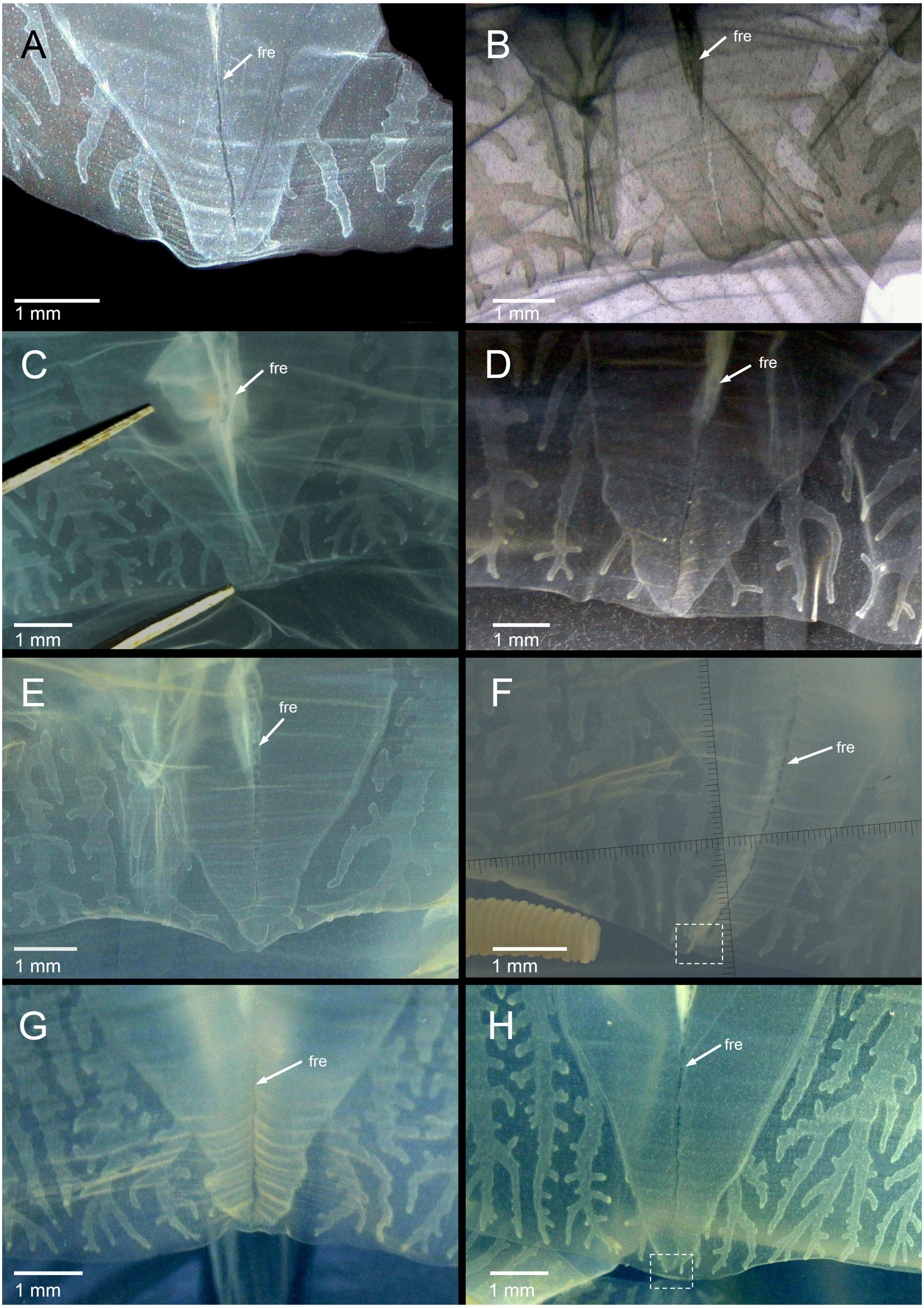

Fig. 9. Perradial positions of preserved juvenile *Chironex yamaguchii* from Okinawa, showing ontogenetic variation in perradial lappet terminal extensions. A–B, TUM 1129968 (IRW 50 mm, BH 30); C, TUM 112969, IRW 60.0 mm, BH 40.0 mm; D, TUM 112970 (IRW 70 mm, BH 50 mm); E, F, TUM 112971 (IRW 80 mm, BH 60 mm); G, TUM 112972 (IRW 80 mm, BH 60 mm); H, TUM 112973 (IRW 75 mm, BH 55 mm). Dashed bound boxes indicate terminal extensions of the perradial lappet. fre = frenulum.

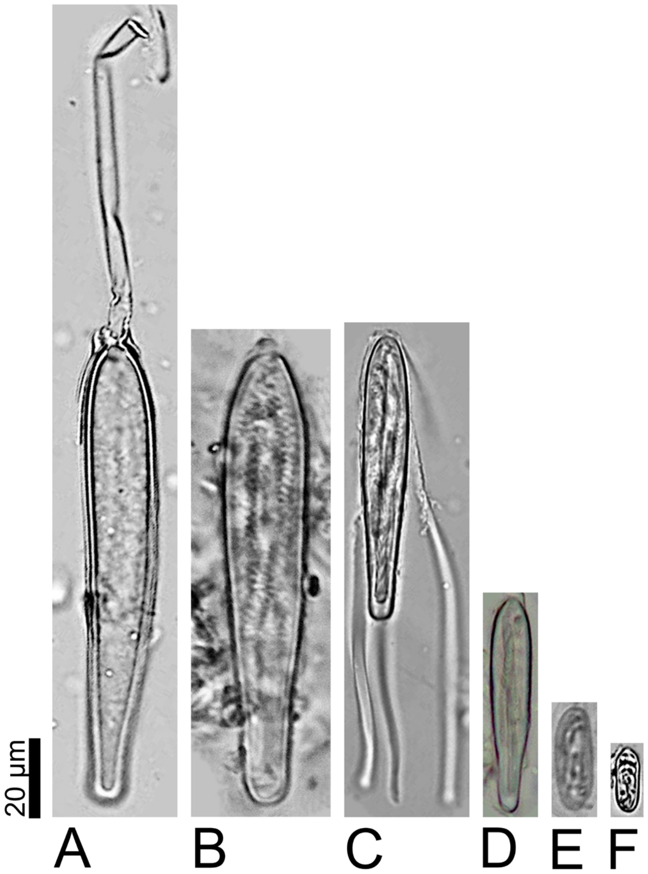

Fig. 10. *Chironex yamaguchii*. from Okinawa, preserved individual (TUM 112967) tentacle cnidome. A, giant microbasic p-mastigophore (type 1) discharged with broken tubule; B, giant microbasic p-mastigophore (type 1) intact; C, medium microbasic p-mastigophore (type 2) intact bearing filaments; D, small microbasic p-mastigophore (type 3) intact; E, medium ellipsoidal isorhiza (type 1); F, small ellipsoidal isorhiza (type 2). All viewed with Nikon Eclipse Ni (Japan) and photographed with the Nikon Digital Sight 1000.

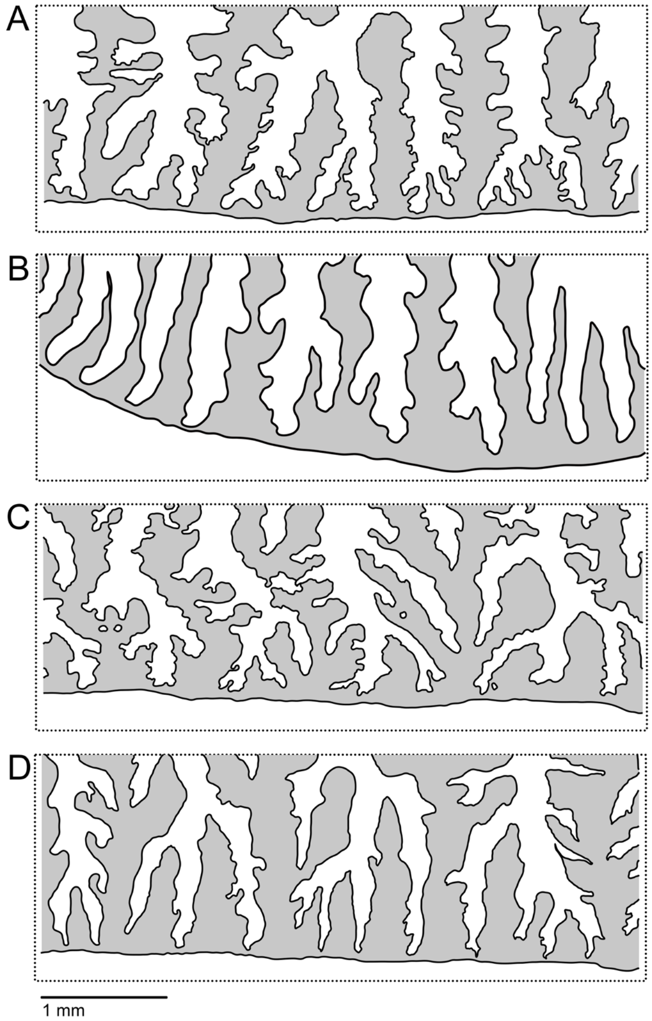

Fig. 11. Velarial canal patterns and their respective terminal position at the velarial margin of: A, *Chironex yamaguchii* (redrawn from Lewis & Bentlage, 2009, of paratype USNM 1121555); B, *C. fleckeri*; C, *C. indrasaksajiae*, D, *Chironex blakangmati*, new species.

Okinawa and Philippine records); Nagai et al. (2002); Sakanashi et al. (2002); Kawamura et al. (2003); Koyama et al. (2003); Nagai (2003); Oba et al. (2004); Noguchi et al. (2005).
Chironex n. sp. B: Gershwin (2006b).

Material examined. TUM 112967 (1 ex. preserved in swf), Japan, Okinawa, Ginowan, coll. C. Lewis, July 1996. NT 8, BH 109 mm, BW 105 mm, IRW 100 mm, dome RhO (h) 9 mm × (w) 5.5 mm. Mature female ripe with oocytes in varying degrees of maturity (Fig. 8G).

TUM 112968–112973 (6 juvenile ex. preserved in swf), Japan, Okinawa, Ginowan, coll. C. Lewis, July 1996. TUM 112968, IRW 50 mm, BH 30 mm; TUM 112969, IRW 60 mm, BH 40 mm, TUM 112970, IRW 70 mm, BH 50 mm (late juvenile); TUM 112971, IRW 80 mm, BH 60 mm (late juvenile); TUM 112972, IRW 80 mm, BH 60 mm (late juvenile); TUM 112973, IRW 75 mm, BH 55 mm (late juvenile).

Type locality. Ishigaki Island, Ryukyu Archipelago, Okinawa Prefecture, Japan.

Diagnosis. Bell height reaching 110 mm; cuboid bell; up to nine tentacles per pedalium; proximal pedalial canal bend volcano-shaped; velarial canals do not reach the edge except those extending from distal tip of perradial lappet (a new character referred to herein as perradial lappet terminal canals; Fig. 8L).

Remarks. Velarial canal tips rounded (Figs. 8L, 8M, 9 & 11A), with multiple continuous velarial canal extensions at perradial lappet tip; 2–3 extensions observed in TUM 112967 (Fig. 8L). Juveniles TUM 112971–112973 with emerging bulges in pairs interradially corresponding to developing gonads, while TUM 112968–112970 lack cockscomb structures. Gastric cirri present along the perimeter of the stomach in all except TUM 112968. Variations in velarial canal extensions observed in juvenile specimens (Fig. 9), with both presence and absence of extensions at tip of perradial lappet. Velarial canal extensions at perradial lappet terminus are seen in late juveniles with bell heights 55 mm (Fig. 9H) and 60 mm (Fig. 9F) (see also Lewis & Bentlage, 2009, Fig. 2F, G).

Table 3. Comparison of characters within the family Chirodropidae (adapted from Gershwin, 2006; Lewis & Bentlage, 2009; Gibbons et al., 2022).

Genus	Saccule shape	No. of tentacles per pedalia	Pedalial canal bend	Pedalial arrangement	Remarks
Chirodectes	Absent (filamentous gonads)	12–14	Rose thorn-shaped bend (Volcano-shaped with huge upturned spike)	Y-branched pedalia with reduced to almost absent palm	See Jarms & Morandini, 2019
Chirodropus	Elongate, tapered, with numerous axial processes, or absent	5–9	Volcano canal bend	Undivided, opposite or alternate branching	See Gershwin, 2006, and Gibbons et al., 2022
Chironex	Cock's comb	5–16	Spike, volcano or bulbous canal bend	Divided U-shaped branching	

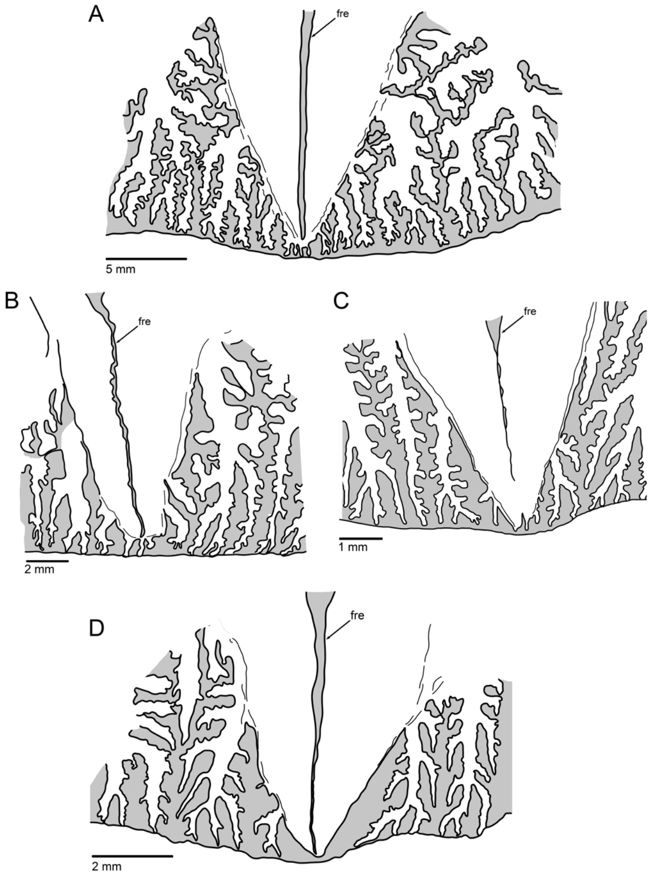

Fig. 12. Velarium at perradial position showing presence or absence of velarial canals extending from perradial lappet tip where frenulum (fre) terminates. A, present in *Chironex fleckeri* (drawn based on Southcott, 1956); B, present in adult *C. yamaguchii* (drawn from TUM 112967); C, present in juvenile *C. yamaguchii* (drawn from TUM 112973); D, absent in *C. blakangmati*, new species (drawn from ZRC.CNI.1462).

Cnidome. In this study we examined the tentacle cnidome of *C. yamaguchii* from Okinawa (TUM 112967) to characterise the main types of nematocysts directly involved in prey capture and human envenomation in a medusae of the largest reported bell size. Previously, Oba et al. (2004) categorised the cnidome of *C. yamaguchii* (as *Chiropsalmus quadrigatus*) across all body parts of juveniles and larger medusae to reveal variation in cnidome composition based on bell size, likely due to a change in target prey items, from small crustacean zooplankton to medium-sized fish, during development. Of the eight nematocyst types identified, the most abundant type found in large *C. yamaguchii* medusae (>70 mm in bell height) were microbasic p-mastigophore (MM) and ellipsoidal isorhiza (Oba et al., 2004). Our investigation of the tentacle cnidome of a large *C. yamaguchii* medusa (109 mm bell height) from Okinawa corroborated those findings revealing only MM and ellipsoidal isorhiza in the absence of any other nematocyst type (Fig. 9). Oba et al. (2004) did not provide size ranges for the various types of nematocysts they reported, but lengths for MM were estimated at 40 µm and ellipsoidal isorhiza at 11 µm. Herein, we report three distinct size categories of mastigophores, type 1 (large: avg 69.6 µm, range 64–75 µm), type 2 (medium: avg 55 µm, range 54–56 µm) and type 3 (small: avg 39.4 µm, range 37–46 µm); as well as two categories of ellipsoid isorhizas, type 1 (large:

Table 4. Comparison of characters within the genus *Chironex* (adapted from Gershwin, 2005; Lewis & Bentlage, 2009; Sucharitakul et al., 2017, 2019: Jarms & Morandini, 2019; Chuan et al., 2021).

Species	**Bell**	**No. of tentacles per pedalia**	**Pedalial canal bend**	**Velarial canals**	**Rhopalial niche**	**Geographic location**	**Reference**
Chironex fleckeri	Box shape bell, flat apex	12–16	Rose thorn-shaped canal bend	>3 canal extensions present at velarium edge of perradial lappet. Rounded tips.	Opening dumbbell shape, framed by 1 upper and 1 lower scale; upper covering scale, with or without additional extension ('M' shape if with extension)	Australia	Southcott, 1956; Jarms & Morandini, 2019; this study
Chironex indrasaksijiae	Box shape bell, flat apex	10–15	Bulbous, concave or volcano canal bend, may have secondary features across pedalial canal	No information on edge of perradial lappet. Rounded tips.	Opening frown-shaped or dumbbell shape, framed by 1 upper and 1 lower scale	Thailand, India & Singapore	Sucharitakul et al., 2017; Saravanan et al., 2024; this study
Chironex sp.	Box shape bell, flat apex	9–11	Spike canal bend	No information	No information	Thailand	Sucharitakul et al., 2019
Chironex yamaguchii	Box shape bell, flat apex	5–9	Volcano canal bend	2–3 short velarial canal extensions at velarium edge of perradial lappet. More rounded than sharp tips.	Opening dumbbell shape, framed by 1 upper and 1 lower scale; upper scale slightly 'M' shape	Japan, the Philippines & East Malaysia (Sabah)	Lewis & Bentlage, 2009; Jarms & Morandini, 2019; Chuan et al., 2021; Boco et al., 2025
Chironex blakangmati, new species	Conical to box shape bell, pointed apex	7	Volcano canal bend	Absent at velarium edge of perradial lappet (likely immature). Sharp tips.	Opening frown-shaped, framed by 1 upper and 1 lower scale	Singapore	This study

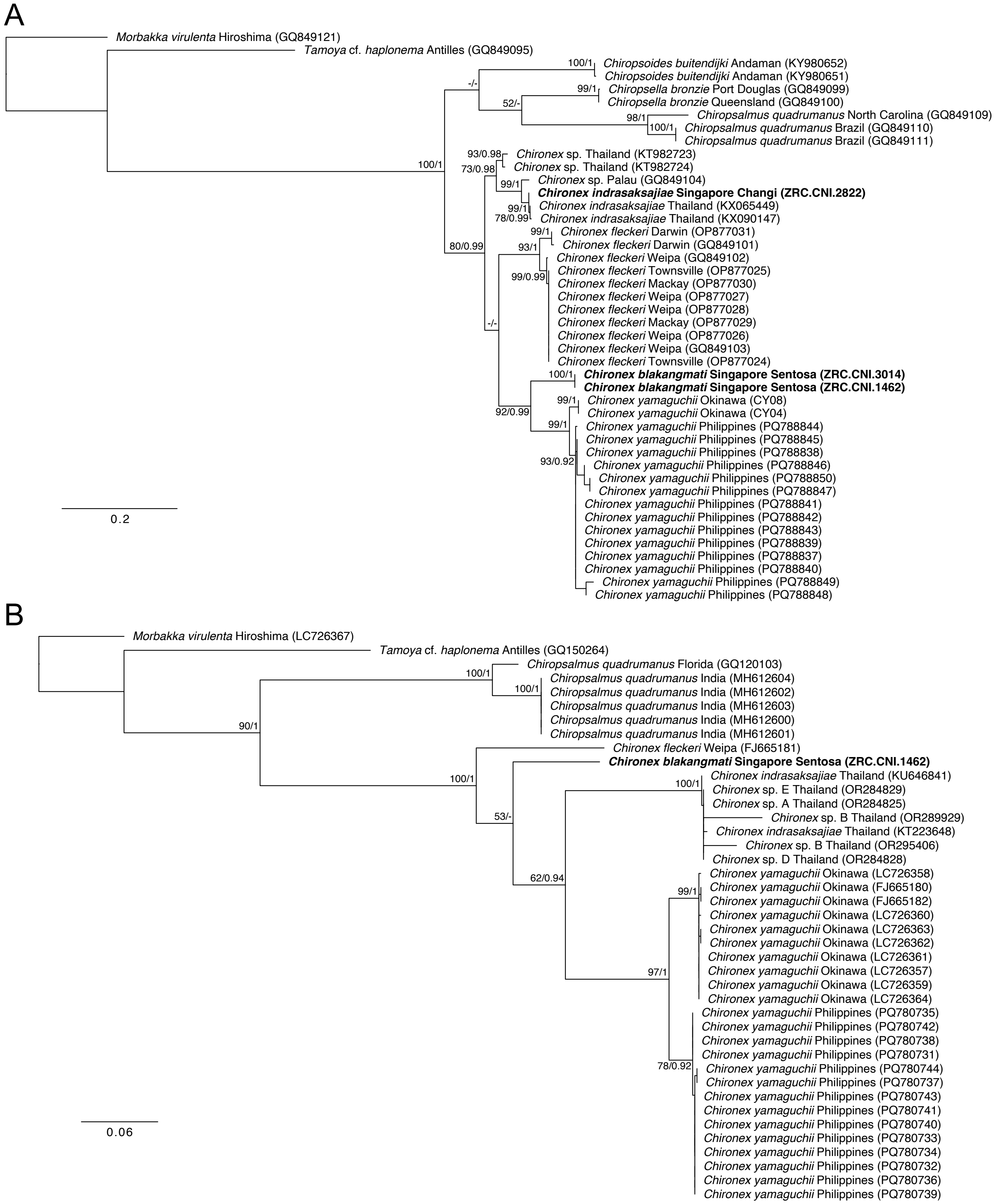

Fig. 13. Phylogenetic trees of Chirodropida with two Carybdeida species (*Morbakka virulenta* and *Tamoya* cf. *haplonema*) as outgroups. Taxa in bold denote *Chironex* sequences generated in this study. Numbers adjacent to nodes represent maximum likelihood bootstrap values (≥50) and Bayesian posterior probabilities (≥0.9). A, maximum likelihood tree based on 16S rRNA gene; B, maximum likelihood tree based on cytochrome c oxidase subunit I gene.

avg 23.7 um, range 23.8–28.8 um), and type 2 (small: avg 12.1 μm, range 10.8–14.4 μm) (Fig. 10). Mastigophores are long, have penetrating and injecting functions, and are the cause of immediate pain experienced by *C. yamaguchii* sting victims. Kitatani et al. (2015) showed that longer nematocyst tubules penetrate the subepidermal nerve plexus (located 100–200 μm from the surface of human skin) stimulating pain receptor neurons. That study also showed that the tentacle of *C. yamaguchii* from Okinawa is 99% composed of MM (primarily type 1, but bearing all three types) with an average tubule length of about four times that of the capsule (avg 600 μm, range, 20–600 μm) and, therefore, sufficient to penetrate the subepidermal nerve plexus and cause immediate pain in sting victims.

RESULTS AND DISCUSSION

The reconstructed phylogenetic tree of all known chirodropid species revealed that the specimens collected from Singapore of *Chironex blakangmati*, new species, and *C. indrasaksajiae* were nested within the *Chironex* clade based on both 16S and COI markers (Fig. 13). Maximum likelihood and Bayesian analyses produced consistent topologies within *Chironex* (phylogenetic trees are available at Zenodo; http://dx.doi.org/10.5281/zenodo.18790140).

On the 16S tree, *C. blakangmati* specimens formed a distinct, monophyletic and well-supported species clade (maximum likelihood bootstrap = 100; Bayesian posterior probability = 1), which was the sister group (bootstrap = 92; posterior probability = 0.99) to *C. yamaguchii* (bootstrap = 99; posterior probability = 1). *Chironex indrasaksajiae* collected from Changi Beach, Singapore, formed a monophyletic group with *C. indrasaksajiae* from Thailand (bootstrap = 99; posterior probability = 1), and together were the sister group to an unknown *Chironex* sp. from Palau (GQ849104; bootstrap = 99; posterior probability = 1). These sister relationships were well supported, but their interrelationships with *C. fleckeri* were unresolved. On the COI maximum likelihood tree, *C. blakangmati* is sister group to the clade comprising *C. indrasaksajiae* and *C. yamaguchii* (bootstrap = 53; posterior probability = 0.50), though relationships between the four species are not well supported (possibly due to low coverage for some taxa), all analyses support *C. blakangmati* as a species distinct from the other three congenerics.

Previously, Searle (1957) reported collecting two adult chirodropid medusae at Labrador Beach (Labrador Nature Reserve, Singapore) in shallow waters amidst dense *Sargassum* "in about three feet of water" (one caught in October 1956 and another approximately a year earlier, i.e., 1955). A sting incident attributed to the multi-tentacled cubozoan was also reported in waters near Pasir Panjang (Searle, 1957); however, the identity of the sting culprit remains unknown as the box jellyfish involved in these incidences were apparently not deposited into any zoological repository. Scrutiny of the drawing in Searle (1957) labelled "*Chiropsalmus quadrigatus*" collected from Singapore suggests that although it was assigned to *Chiropsoides* by Gershwin (2006a), the pedalial branching arrangement is closer to palmate or claw-like as in *Chironex*, rather than unilateral (as per Searle, 1957) as is characteristic of the genus *Chiropsoides.* The drawing outlines cockscomb-like gastric saccules resembling that of *Chironex*; gastric saccules of *Chiropsoides*, in contrast, are distinctly finger-like (Gershwin, 2006a; Sucharitakul et al., 2017, 2019). It is therefore likely that the chirodropid collected from along the Singapore Strait at that time belonged to the genus *Chironex*. Seven tentacles per pedalium illustrated in the historical account suggests its identity was either *Chironex yamaguchii* or *C. blakangmati*. Vouchers of *Chironex yamaguchii* in Japan and East Malaysia (Sabah) were also misidentified as *Chiropsalmus quadrigatus* (mentioned in Lewis & Bentlage, 2009; Chuan et al., 2021). However, the drawing of the box jellyfish by Searle (1957) lacks crucial morphological details such as the shape of the pedalial canal bend and interradial lappets, making conclusions about the identity of the above presumptuous in the absence of morphological or molecular examination of the original, collected specimens.

In recent years, motivation to understand the biodiversity and seasonality of cubomedusae has gained momentum primarily because of the high risk to public health and pressure on management of public beaches. Many envenomation cases require hospitalisation (Winkel et al., 2003), and in Australia, *Chironex fleckeri* can kill a healthy adult in minutes (Currie & Jacups, 2005; Gershwin et al., 2010; Thaikruea & Siriariyaporn, 2016). However, public campaigns on beach safety and intermittent beach closures have proven to be crucial countermeasures to jellyfish stings (Ang, 2020). Accordingly, in Singapore, local efforts in management of box jellyfish envenomation risks now include setting up signs and real-time media updates. Closure of public beaches at Sentosa Island occurred once on 9 October 2020 (Ang, 2020) following sightings of cubozoans along the Singapore coast.

Ultimately, local spatial and temporal distributions of cubomedusae remain poorly understood, and scant collection and study of specimens leave many unknowns about the true biodiversity of this group of venomous jellyfishes. The gap of roughly 40 years between initial and contemporary reports of cubozoan occurrences in Singapore suggests either an influx of species from other waters due to anomalies in environmental factors (e.g., larger-scale hydrodynamic patterns or interannual/multi-decadal climate variabilities), or increased beach recreation coupled with increased tendencies to report jellyfishes. Overall, this study has shed light on two *Chironex* species inhabiting a hyper-urbanised marine coastline of Singapore—*C. blakangmati,* new species, and *C. indrasaksajiae*. We also provide the first comparative report of all known *Chironex* species and present a new morphological character—perradial lappet terminal canals—useful in delineation of species in this genus. Continued sampling efforts combined with morphological validation and molecular phylogenetic studies are required to establish a more comprehensive picture of the box jellyfish diversity around Southeast Asian waters.

ACKNOWLEDGEMENTS

We are grateful to members of the Environmental Management Unit and Beach Patrol Officers of the Sentosa Development Corporation, in particular Leanne Tan, Grace Lee, Tammy Lim, Quek Chee Boon ('Uncle Quek'), Chan Tak Mun, and Ahmad Lutfi Saimin, who kept us informed of the medusae encountered along the beaches of Sentosa Island. Karenne Tun and Jonathan Tan of NParks also directed cubomedusae observed on public beaches to us. Peter Ng and Tan Koh Siang provided constructive comments that improved the manuscript, and Martyn Low advised on etymology. Hugh Carter and Jeffrey Streicher facilitated access to specimens at the Natural History Museum, London. We are grateful to the following sponsors for their generous contributions in support of the Comprehensive Marine Biodiversity Survey II: Dalio Philanthropies, GSK-EDB Trust Fund, HSBC, ExxonMobil Asia Pacific, and Shell Eastern Trading (Pte) Ltd. We also acknowledge funding from WPI-AIMEC and Sasakawa Peace Foundation's Ocean Shot Project. Part of the study was conducted at the facilities of St John's Island National Marine Laboratory, a National Research Infrastructure under the National Research Foundation (NRF), Prime Minister's Office, Singapore. Examination of specimens in the Natural History Museum, London was supported by SIGNIFY (Singapore in Global Natural History Museums Information Facility) project under the Lee Kong Chian Natural History Museum. Lastly, we thank the reviewers for assessment of the manuscript and valuable comments.

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