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A New Species of Box Jellyfish (Cnidaria: Tripedaliidae: *Tripedalia*) from Hong Kong, China

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We describe a box jellyfish, *Tripedalia maipoensis* sp. nov., based on samples collected from a shrimp pond in Hong Kong. This new species is morphologically distinct from other species of the family Tripedaliidae by the following combination of characters: (1) three pedalia at each bell corner; (2) each pedalium with one tentacle; and (3) velarium with forked canals. Phylogenetic analyses based on a concatenated dataset of the *16S*, *18S* and *28S* rRNA genes show that *T. maipoensis* sp. nov. is sister to the morphologically similar species *T. cystophora*, but the two species exhibit 17.4% divergence in the *16S* rRNA gene, supporting *T. maipoensis* sp. nov. as a distinct species. This new species represents the fourth described species of Tripedaliidae, and the first record of the family in Chinese coastal waters.

Key words: Box jellyfish, *Tripedalia*, Morphology, Phylogeny, Hong Kong.

BACKGROUND

The class Cubozoa, commonly known as box jellyfish due to the cube-shaped bells, consists of a small group of cnidarians with approximately 50 described species and is well-known to the public for containing some of the most venomous marine animals in the world (Bentlage et al. 2010; Collins and Jarms 2022). Cubozoa is divided into two monophyletic orders, the Carybdeida and Chirodropida (Bentlage et al. 2010). The two orders can be easily distinguished, as carybdeids possess only one tentacle per pedalium, whereas chirodropids possess multiple tentacles per pedalium.

Tripedaliidae is a small family of Carybdeida, with only three described species in two genera (*Copula* and *Tripedalia*). *Tripedalia* possesses two to three pedalia per bell corner, which differs from *Copula*, which has one pedalium per bell corner (Collins et al. 2006; Bentlage and Lewis 2012). Currently *Tripedalia* is represented by only two species: *T. binata* Moore, 1988 which has two pedalia at each bell corner, and *T. cystophora* Conant, 1897 which has three pedalia at each bell corner. Although both species inhabit estuarine waters in mangrove areas, *T. cystophora* has been reported from a much wider geographic range than *T. binata*. Following its first discovery in Jamaica in 1857, *T. cystophora* has been widely reported around the tropics and subtropics, including the United States (Orellana and Collins 2011), Japan (Uchida 1970), Singapore (Kong and Iesa 2021), Mexico (Lasley et al. 2016), Australia (Ekins and Gershwin 2014) and India (Riyas and Kumar 2020). In contrast, *T. binata* is only known from Australia (Underwood et al. 2013) and Thailand (Toshino et al. 2019).

Although box jellyfish are often common animals in coastal habitats, their diversity in the Chinese

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coastal waters is poorly known, with only three species reported, all from the family Carybdeidae (Liu 2008). In the summer of three consecutive years from 2020 to 2022, we found box jellyfish from the mangrove habitat of intertidal shrimp ponds (locally known as *gei wai*) in Mai Po Nature Reserve, Hong Kong (Fig. S1A), located on the eastern shore of the Pearl River Estuary. Here we report the box jellyfish as a new species, *Tripedalia maipoensis* sp. nov. (Tripedaliidae). We describe the morphology of this new species and determine its phylogenetic placement based on three gene markers.

MATERIALS AND METHODS

We collected the samples using a plankton net of 30 cm mouth diameter and 100 μm mesh size (Fig. S1B). In the laboratory, we attempted to rear the box jellyfish and observed their mating behaviour and early development (Fig. S2; Supplementary Note). We fixed adults for morphological description in 4% formalin solution for three days, then transferred them to 90% ethanol for long-term preservation. We preserved adults for molecular studies in 95% ethanol. All specimens were deposited into the Tropical Marine Biodiversity Collections of the South China Sea (TMBC), Chinese Academy of Sciences, Guangzhou. We took photographs of live and preserved specimens using either a Canon EOS5D Mark IV camera with a 100 mm macro lens, or a Canon 700 camera attached to a Motic BA 210 microscope through a phototube. We measured the following characters of the formalin fixed specimens as in Straehler-Pohl (2014) and Acevedo et al. (2019): bell height (BH): distance from apex of the bell to the velarial turnover; diagonal bell width (DBW): distance between opposite pedalia at level of pedalia joining bell; interrhopalial width (IRW): distance between adjacent rhopalia; DRT: distance from rhopalium to turnover of bell; pedalia width (PW): distance at the widest part of the pedalia; and pedalia length (PL): distance at the longest part of the pedalia.

We collected tissues of the bell corner from three individuals, and extracted genomic DNA using the PureLinkTM Genomic DNA Mini Kit (Invitrogen, Germany) according to the manufacturer's protocol. We amplified two nuclear genes (18S ribosomal RNA [*18S*] and 28S ribosomal RNA [*28S*]) and one mitochondrial gene (16S ribosomal RNA [*16S*]) as in Cartwright et al. (2008) and Collins et al. (2008), and sequenced the products bi-directionally at BGI, Shenzhen. We deposited the nucleotide sequences into NCBI GenBank (accession numbers listed in Table S1). We retrieved sequences of Tripedaliidae, Carybdeida and Chirodropida from GenBank. Since sequences of the three genes were not available in GenBank for a *T. cystophora* specimen from the type location in Costa Rica, we re-assembled the transcriptome using the raw reads (accession number SRR8101518) downloaded from GenBank, and obtained the sequences of these genes from the assembled contigs. We filtered the reads using Trimmomatic v.0.38 (Bolger et al. 2014) to remove adapters and low-quality sequences, then assembled the transcriptome using Trinity v2.8.6 (Grabherr et al. 2011). We identified sequences of *16S*, *18S* and *28S* using the BLASTn option of BLAST$^{®}$+ v.2.10.1+ (Camacho et al. 2009) to search against those from other specimens of *T. cystophora* retrieved from NCBI. We aligned the multiple sequences using ClustalW (Thompson et al. 1994) under the default settings for each gene and combined after trimming poorly aligned sequences by trimAl (Capella-Gutiérrez et al. 2009). We then conducted phylogenetic analysis using the Maximum-Likelihood (ML) method based on the concatenated dataset of three genes. We partitioned the concatenated dataset by gene and applied ModelFinder (Kalyaanamoorthy et al. 2017), which indicated GTR+F+I to be the most appropriate model for all three genes. We performed ML analysis using IQ-TREE v1.4.4 (Nguyen et al. 2015) with 1000 ultrafast bootstrap (UFBoot) replicates. For *16S*, we also calculated *p*-distances – a measure of genetic distance – between the new species and other species, as well as between the different specimens of the new species using MEGA v.11 (Tamura et al. 2021).

RESULTS

TAXONOMY

Family Tripedaliidae Conant, 1897
Genus *Tripedalia* Conant, 1897

Tripedalia maipoensis sp. nov.

(Figs. 1, 2, Fig. S1)
urn:lsid:zoobank.org:act:68BA7074-70C9-400A-9F49-572E6DC3622B

Material examined: TMBC030991 (holotype): adult medusa, female (Fig. 1A–B), collected 21 April 2022, preserved in 4% formalin, stored in 90% ethanol. TMBC030992–TMBC031003 (paratypes): adult medusa, one individual each, same sampling location as holotype, TMBC030992 (male), TMBC030993 (female), TMBC030994 (male), TMBC030995 (female), TMBC030996 (sex cannot be determined), TMBC030997 (male), TMBC030998 (female), TMBC030999 (female), TMBC031000 (male), TMBC031001 (male), TMBC031002 (female), TMBC031003 (female), TMBC030992–TMBC030995 collected on 21 April 2022, TMBC030996–TMBC031003 collected in August 2020, TMBC030992–TMBC030997 preserved in 4% formalin and stored in 90% ethanol, TMBC030998–TMBC031003 preserved in 95% ethanol.

Type locality: Mai Po Nature Reserve, Hong

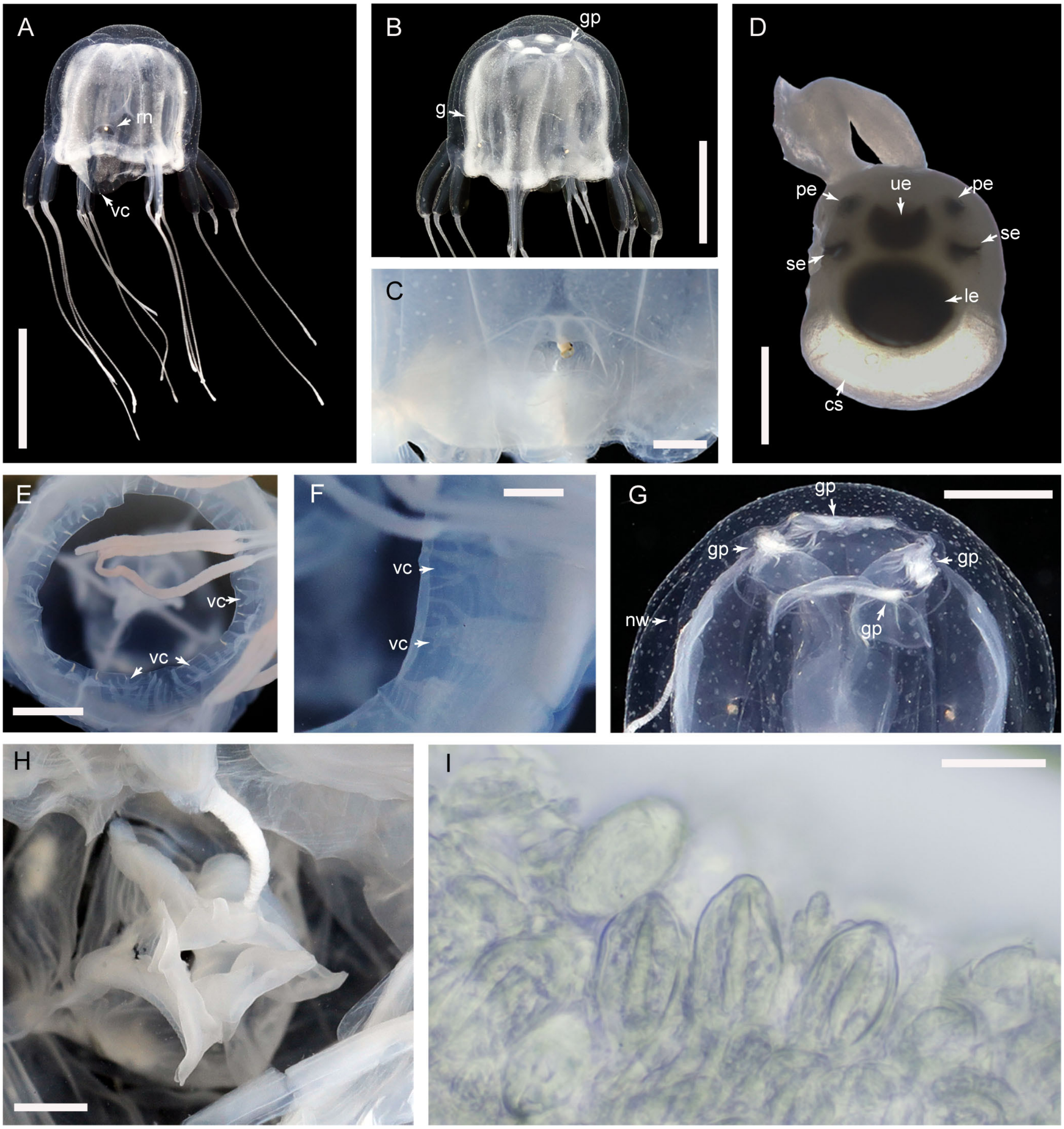

Fig. 1. Morphological characteristics and phylogenetic position of *Tripedalia maipoensis* sp. nov. (A–B) holotype (TMBC030991), female, living specimen, lateral view showing the gross morphology (A), and oblique top view showing gastric phacellus (B); (C) Rhopalial niche, preserved specimen, paratype (TMBC030992); (D) Rhopalium, showing six eyes and a statolith; (E) Upside down view of the bell showing the velarial canals, preserved specimen, paratype (TMBC030992), male; (F) details of the velarial canals, preserved specimen, holotype (TMBC030991), female; (G) oblique top view, showing nematocyst warts and gastric phacellus, living specimen, paratype TMBC030992; (H) manubrium, preserved, paratype (TMBC030997); (I) Nematocyst batteries from tentacle, paratype (TMBC030997). Scale bars: A–B = 1 cm; C, F, H = 1 mm; D = 0.3 mm; E, G = 5 mm; I = 10 µm. Abbreviations: cs, crystalline statolith; g, gonad; gp, gastric phacellae; le, lower lens eye; nw, nematocyst wart; pe, pit eye; rn, rhopalial niche; se, slit eye; ue, upper lens eye; vc, velarial canal.

Kong. 22. 4887°N, 114.0417°E.

Etymology: *maipoensis* is the Latinized form of the type locality Mai Po.

Diagnosis: Medusa with three pedalia per bell corner, each pedalium with one tentacle, velarium with forked canals.

Description: The bell is transparent, colourless, cuboidal-shaped with smooth, edges rounded, roof slightly arched, with white nematocyst warts of different shapes and sizes densely scattered from apex to bell margin (Fig. 1A–B, G; Fig. S1C–E). There is a pair of gonads at each corner of the bell, extending from stomach rim to velarium in both sexes (Fig. 1A, Fig. S1C). There are four frown-shaped rhopalial niches, each located between two bell corners. Each rhopalial niche ostium has a prominent upper covering scale, and a small lower scale (Fig. 1A, C). Each ropalial niche has a rhopalium suspended from the roof with a stalk. Each of the four rhopalia bears six eyes: two lateral pit eyes, two lateral slit eyes, a smaller upper lens eye, and a larger lower lens eye (Fig. 1D). Each rhopalium also bears a large crystalline statolith below the lower lens eye. The velarium is smooth, without nematocyst warts. The velarial canals are biforked to multi-branched with 3–6 branches, with sharp tips (Fig. 1E–F). The velarial canal tips are white in mature specimens (Fig. 1E). The manubrium extends from the bell roof to 1/2–2/3 bell height (Fig. S1C, E); they are cruciform with four terminal lobes that are free of nematocyst warts (Fig. 1H). The stomach is flat, with its four corners each connected to a brush-like gastric phacella (Fig. 1G, Fig. S1F). The pedalia are flat and unbended, each with one pedalial canal inside (Fig. 1A–B, Fig. S1C–D) and nematocyst warts on the outer surface (Fig. 1B, Fig. S1D). Pedalial canal straight, extending through pedalium, slightly tapering at distal end, knee bend rounded without appendage. Each pedalium bears a single unbranched tentacle. Nematocysts from tentacles are oval-shaped (Fig. 1I). Juvenile medusae are similar with adults in gross morphology. At ~BH 3.2 mm, they developed from two to three pedalia per bell corner (Fig. S1G–H).

Sexual Dimorphism: Female gonad ‘wings’ are slender, extending from the stomach to the velarium;

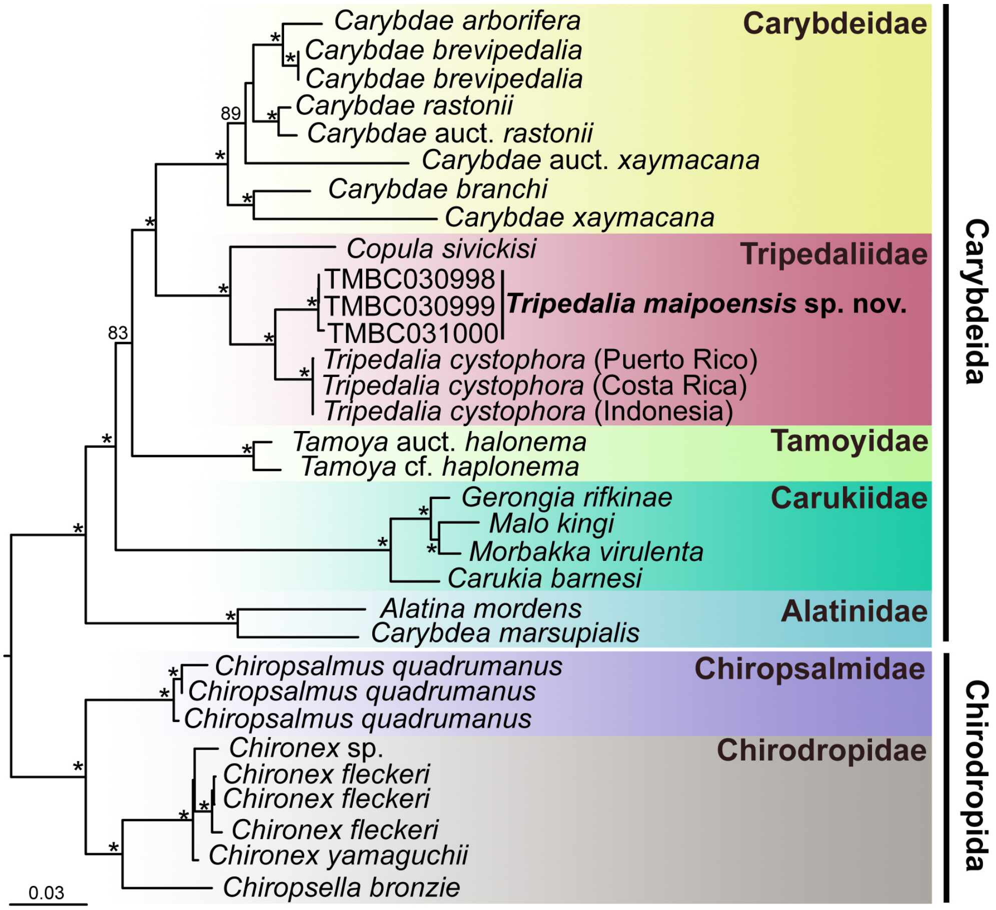

Fig. 2. Maximum-likelihood tree of cubozoans based on a concatenated dataset of *16S*, *18S* and *28S*. Ultrafast bootstrap (UFBoot) values higher than 75 are shown on nodes. Asterisks indicate UFBoot values > 90.

whitish to light yellow in live specimens (Fig. 1A–B). Male gonad 'wings' are broad with a rounded top, extending from the stomach to the velarium; whitish in live specimen (Fig. S1C).

Measurements: *n* = 8 adults. Mean BH 12.5 (± 3.3) mm, mean DBW 15.2 (± 6.9) mm, mean IRW 1.4 (± 0.7) mm, mean DRT 2.7 (± 1.3) mm, mean PL 4.9 (± 1.1) mm, mean PW 1.5 (± 0.5) mm.

Distribution and ecology: The new species is currently known only from the type locality, but may also occur in the adjacent waters of the Pearl River Estuary as the ponds are connected to the estuary through a tidal channel. The new species was found every year since 2020 in some of the brackish water intertidal shrimp ponds of Mai Po Nature Reserve during April and May, and also extending to June in 2021 when water temperature ranged from 20°C to 29°C, and salinity from 5.8 psu to 18 psu. The new species was recorded in water channels flanked by Common Reed (*Phragmites australis*) or mangroves of mainly *Kandelia obovata* mixed with the golden leather fern *Acrostichum aureum*.

Genetic analyses: A 567 base pair (bp) fragment of *16S*, 1734 bp fragment of *18S* and 1809 bp fragment of *28S* was used in the genetic analyses. Phylogenetic analyses based on the combined dataset of three genes strongly supported the monophyly of *Tripedalia* (Fig. 2). Specimens of *T. maipoensis* sp. nov. were grouped to a distinctive clade and sister to the *T. cystophora* group comprising specimens from Costa Rica and Indonesia with strong support (UFBoot = 100, Fig. 2). The *Tripedalia* clade is then sister to *Copula sivickisi* (Fig. 2). For *16S*, the intraspecific *p*-distance of *T. maipoensis* sp. nov. for *16S* is 0.1%, while the interspecific *p*-distance between *T. maipoensis* sp. nov. and *T. cystophora* is 17.5%. The percentage of genetic distance for *16S* between *T. maipoensis* sp. nov. and *Copula sivickisi* is 26.6%, while the value between *T. cystophora* and *C. sivickisi* is 28.3%.

Key to genera and species of Tripedallidae Conant, 1897 with the new species

1. One pedalium per bell corner .... *Copula sivickisi*
\- More than one pedalium per bell corner 2
2. Two pedalia per bell corner .... *Tripedalia binata*
\- Three pedalia per bell corner 3
3. Velarial canals simple, unforked .... *Tripedalia cystophora*
\- Velarial canals forked .... *Tripedalia maipoensis* sp. nov.

DISCUSSION

Tripedalia maipoensis sp. nov. and *T. cystophora* can be easily distinguished from *T. binata*, which bears two pedalia per bell corner. The new species differs from *T. cystophora* by having forked velarial canals, which are unforked in *T. cystophora*. At ~2.3 mm bell height, some juvenile medusae bear two pedalia per bell corner while others bear three, indicating that the third pedalium emerged in a narrow time window during the development.

The ML analysis based on a concatenated dataset of the *16S*, *18S* and *28S* reveals an overall tree topology that is consistent with the cubozoan phylogenetic relationships revealed in a previous study (Bentlage et al. 2010). Members of the family Tripedaliidae form a well-supported clade and is sister to Carybedeidae (UFBoot = 100, Fig. 2). The monophyly of *Tripedalia* is strongly supported. Acevedo et al. (2019) has suggested that *16S* sequences can be useful in determing the genetic distances among members of Carybedeidae. The interspecific genetic distance threshold of *16S* ranges from 10.8% to 33.9% among species (Acevedo et al. 2019). Our results show a *p*-distance of 17.5% between the two species in *16S*, compared to a much lower intra-specific *p*-distance in both *T. maipoensis* sp. nov. (0.1%) and *T. cystophora* (1.1%), supporting *T. maipoensis* sp. nov. as a distinct species. Unfortunately, there is no genetic data available for *T. binata*, nor any available specimen of *T. binata* for molecular work in the current study, therefore the relationships between this species and the other two species remains unclear.

CONCLUSIONS

In this study, we reported the new species *Tripedalia maipoensis* sp. nov. collected from an intertidal shrimp pond in the Mai Po Nature Reserve, Hong Kong. Although the box jellyfish family Tripedaliidae has been widely reported from tropical and subtropical waters, this is the first record of it in Chinese coastal waters. We described the species with both morphological and molecular data. Future investigations along Chinese coasts are warranted to improve our understanding of the distribution of this new species and the diversity of Cubozoa.

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Authors' contributions: JWQ designed the study. CKMO, RNCC, MKPN and YS collected the samples. YS, JHYT and RTHW performed morphological observation, photography and molecular work. YS performed the phylogenetic analysis. RNCC and MKPN cultured the jellyfish. YS, JHYT, RTHW, CKMO and JWQ drafted the manuscript. All authors read and approved the final version of the manuscript.

Competing interests: The authors declare that they have no competing interests.

Availability of data and materials: All specimens are deposited at the Tropical Marine Biodiversity Collections of the South China Sea (TMBC), Chinese Academy of Sciences, Guangzhou. The DNA sequences have been deposited into the GenBank database.

Consent for publication: All of the authors agreed to publish the paper.

Ethics approval consent to participate: Not applicable.

REFERENCES

Acevedo MJA, Straehler-Pohl I, Morandini AC, Stampar SN, Bentlage B, Matsumoto GI. 2019. Revision of the genus *Carybdea* (Cnidaria: Cubozoa: Carybdeidae): clarifying the identity of its type species *Carybdea marsupialis*. Zootaxa **4543(4):**515–548. doi:10.11646/zootaxa.4543.4.3.

Bentlage B, Cartwright P, Yanagihara AA, Lewis C, Richards GS, Collins AG. 2010. Evolution of box jellyfish (Cnidaria: Cubozoa), a group of highly toxic invertebrates. Proc Royal Soc B **277:**493–501. doi:10.1098/rspb.2009.1707.

Bentlage B, Lewis C. 2012. An illustrated key and synopsis of the families and genera of carybdeid box jellyfishes (Cnidaria: Cubozoa: Carybdeida), with emphasis on the "Irukandji family" (Carukiidae). J Nat Hist **46:**2595–2620. doi:10.1080/00222933.2012.717645.

Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics **30(15):**2114–2120. doi:10.1093/bioinformatics/btu170.

Camacho C, Couloris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. BMC Bioinform **10:**421. doi:10.1186/1471-2105-10-421.

Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T. 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics **25(15):** 1972–1973. doi:10.1093/bioinformatics/btp348.

Cartwright P, Evans NM, Dunn CW, Marques AC, Miglietta MP, Schuchert P, Collins AG. 2008. Phylogenetics of *Hydroidolina* (Cnidaria: Hydrozoa). J Mar Biolog Assoc UK **88(8):**1663–1672. doi:10.1017/S0025315408002257.

Collins AG, Bentlage B, Lindner A, Lindsay D, Haddock SHD, Jarms G, Norenburg JL, Jankowski T, Cartwright P. 2008. Phylogenetics of Trachylina (Cnidaria: Hydrozoa) with new insights on the evolution of some problematical taxa. J Mar Biol Assoc UK **88:**1673–1685. doi:10.1017/S0025315408001732.

Collins AG, Jarms G. 2022. World List of Cubozoa. Cubozoa. Accessed through: World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=135219 on 2022-05-11.

Collins AG, Schuchert P, Marques AC, Jankowski T, Medina M, Schierwater B. 2006. Medusozoan phylogeny and character evolution clarified by new large and small subunit rDNA data and utility of phylogenetic mixture models. Syst Biol **55:**97–115. doi:10.1080/10635150500433615.

Conant FS. 1897. Notes on the cubomedusae. Johns Hopkins University Circular **132:**8–10.

Ekins M, Gershwin L. 2014. First record of the Caribbean box jellyfish *Tripedalia cystophora* in Australian waters. Mar Biodivers Rec **7:**1–5. doi:10.1017/S1755267214001183.

Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA et al. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. Nat Biotechnol **29:**644–652. doi:10.1038/nbt.1883.

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. Nat Methods **14:**587–589. doi:10.1038/nmeth.4285.

Kong PL, Iesa I. 2021. Biodiversity record: Mangrove box jellyfish at Sungei Simpang. Nis **14:**ze2021018. doi:10.26107/NIS-2021-0018.

Lasley RM, Ames CL, Erdman R, Parks S, Collins AG. 2016. First record of the box jellyfish *Tripedalia cystophora* (Cnidaria: Cubozoa: Tripedaliidae) in the Gulf of Mexico. Proc Biol Soc Wash **129(1):**164–172. doi:10.2988/0006-324X-129.Q2.164.

Liu RY. 2008. Checklist of marine biota of China seas. Science Press.

Nguyen LT, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol **32(1):**268–274. doi:10.1093/molbev/msu300.

Orellana ER, Collins AG. 2011. First report of the box jellyfish *Tripedalia cystophora* (Cubozoa: Tripedaliidae) in the continental USA, from Lake Wyman, Boca Raton, Florida. Mar Biodivers Rec **4:**1–3. doi:10.1017/S1755267211000133.

Riyas A, Kumar AB. 2020. First record of box Jellyfish *Tripedalia cystophora* Conant, 1897 (Cubozoa: Tripedaliidae) from India. Thalassas **36:**297–302. doi:10.1007/s41208-020-00242-8.

Straehler-Pohl I. 2014. Critical evaluation of characters for species identification in the cubomedusa genus *Malo* (Cnidaria, Cubozoa, Carybdeida, Carukiidae). Plankton Benthos Res **9(2):**83–98. doi:10.3800/pbr.9.83.

Tamura K, Stecher G, Kumar S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol **38(7):**3022–3027. doi:10.1093/molbev/msab120.

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res **22:**4673–4680. doi:10.1093/nar/22.22.4673.

Toshino S, Nishikawa J, Srinui K, Taleb S, Miyake H. 2019. New records of two species of Cubozoa from Thailand. Plankton Benthos Res **14(3):**143–149. doi:10.3800/pbr.14.143.

Uchida T. 1970. Revision of Japanese Cubomedusae. Publ Seto Mar Biol Lab **17:**289–297. doi:10.5134/175610.

Underwood AH, Taylor S, Seymour JE. 2013. Range extension of the Cubozoan, *Tripedalia binata* Moore (Cnideria: Carybdeida: Carybdeidae) from far north Queensland, Australia. Mem Queensl Mus **56(2):**607–613.

Supplementary Materials

Supplementary Note. Laboratory culture and observation of *Tripedalia maipoensis* n. sp. (download)

Fig. S1. Habitat and morphology of *Tripedalia maipoensis* sp. nov. A, A brackish water shrimp pond in Mai Po where the samples were collected. B, Sampling using a plankton net from the shrimp pond. C–D, Paratype TMBC030992, living specimen, lateral view, male. E, Paratype TMBC030992, fixed in formalin and later transferred to ethanol for preservation. F, Paratype TMBC030992, preserved, showing gastric phacellae, bottom up view. G–H, Juveniles, living specimens. Scale bars: C–E = 1 cm; F–H = 1 mm. Abbreviation: g, gonad; m, manubrium. (download)

Fig. S2. Laboratory culture of *Tripedalia maipoensis* sp. nov. A, Polyps found on a scallop shell. B, Polyps viewed under a microscope. C, A planula viewed under a microscope. D, Two suspected mating pairs: both show a female located inside the bell of a male. Scale bar: A, D = 1 cm; B–C = 1 mm. (download)

Table S1. Information on box jellyfish sequences used in phylogenetic analysis, including sample origins and GenBank accession numbers. (download)