New records of two species of Cubozoa from Thailand

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Abstract: The stings of box jellyfishes can be fatal, so knowing the fauna of a certain area is important to save lives. Five described and two still-undescribed species of Cubozoa have been reported from Thailand: Chironex indrasaksajiae, Chironex sp., Chiropsella sp., Chiropsoides buitendijki, Copula sivickisi, Morbakka fenneri, and Tripedalia cystophora. We made detailed observations of the morphology of two of the species newly recorded in Thailand: Alatina morandinii and Tripedalia binata. The molecular phylogeny of these species is also discussed. Additional investigations are needed to understand the diversity of Cubozoa in Thailand.

Key words: Alatina morandinii, box jellyfish, species diversity, sting, Tripedalia binata

Introduction

Jellyfish in the phylum Cnidaria, class Cubozoa are called box jellyfish and their stings can be fatal (Cunningham & Goetz 1996, Fenner & Williamson 1996). Numerous severe or fatal injuries to humans caused by cubozoan stings have been reported from the Gulf of Thailand and the Andaman Sea (Aungtonya & Chanachon 2012, Thaikruea et al. 2015, Thaikruea & Siriariyaporn 2015, 2016). Consequently, it is important to understand the species diversity, biology, and ecology of cubozoans. Several studies of the distribution (Stiasny 1922, 1926, Aungtonya & Chanachon 2012, Aungtonya et al. 2018), seasonal occurrence (Sucharitakul et al. 2018), and life cycle (Toshino et al. 2016) of cubozoans have been carried out in Thailand. However, the sampling coverage remains small and their species diversity is not clear.

To date, five described and two still-undescribed cubozoan species have been reported in Thai waters: Chironex indrasaksajiae Sucharitakul et al., 2017, Chiropsella sp., Chiropsoides buitendijki (Horst, 1907), Chironex sp., Copula sivickisi (Stiasny, 1926), Morbakka fenneri Gershwin, 2008, and Tripedalia cystophora Conant, 1897 (Stiasny 1922, Kramp 1961, Aungtonya & Chanachon 2012, Sucharitakul et al. 2017, 2018). The current study performed morphological and molecular phylogenetic analyses of two cubozoans that are new records for Thailand.

Materials and Methods

Medusae of two unidentified species were collected using an underwater fish-luring lamp (YF-500, Hapyson, Japan) in a mangrove swamp in Trat Province (12.1670N, 102.5701E), the eastern Gulf of Thailand, between 21:00 and 23:30 on March 17, 2014 (water surface temperature 32.3°C, salinity 21.8). The medusae were captured with a dip net (mesh size 0.2 mm) and fixed with 5% formaldehyde solution in seawater after observing spawning (see Toshino et al. 2016). Parts of the tentacles were preserved in 99.5% ethanol for molecular analysis.

Unidentified cubopolyps were found on the walls of culture tanks in Bangsaen Aquarium, Chon Buri Province, on March 18, 2014. The polyps were transferred to Petri dishes filled with filtered seawater (1 μm filter pore size) and kept at 25°C. The polyps were fed Artemia nauplii twice a week. The culturing water was replaced completely with filtered seawater (1 μm filter pore size) about 3 hrs after
feeding. Metamorphosis was induced by raising the temperature from 25 to 30°C. During metamorphosis, the cultures were not fed and the water was not changed. Newly detached medusae were kept in a 1000-mL polypropylene beaker in filtered seawater (1 µm) at 28°C. The medusae were fed Artemia nauplii daily. The culture water was replaced with fresh seawater about 3 h after feeding.

Taxonomic observations and morphological measurements were made on live or preserved specimens. Lengths were measured using the method described in Gershwin (2005a), Straehler-Pohl & Jarms (2011) and Toshino et al. (2015) with digital calipers (CD-20CPX, Mitutoyo Corporation, Japan) and Image J (NIH, USA) to the nearest 0.1 mm.

Near-complete sequences of the nuclear 18S rDNA gene (approximately 1800 bp) were used for molecular phylogenetic analysis. Genomic DNA was extracted from the ethanol-preserved tissue of cultured specimens using the DNeasy Blood and Tissue Kit (QIAGEN, Germany) according to the manufacturer's instructions. 18S rDNA was PCR amplified and sequenced using the primers and protocols outlined in Collins et al. (2008). The new sequences were aligned using MEGA 6.06 with built-in ClustalW (Tamura et al. 2013). Phylogenetic analysis and pairwise distance measurements were determined using the maximum likelihood method with 1000 bootstrap replications in MEGA 6.06. All sequences have been deposited in DDBJ under accession numbers LC480260 and LC480261 (Table 1).

### Results

**Class Cubozoa Werner, 1973**

**Family Carybdeidae Gegenbaur, 1857**

**Table 1.** Taxa included in the phylogenetic analyses and GenBank accession numbers for the sequences. Sequences obtained in this study are in **bold**. a) Bentlage et al. (2010); b) Chae et al. (2017); c) Collins (2002); d) Straehler-Pohl & Toshino (2015); and e) Toshino et al. (2015).

| Species               | Accession No. | Locality                         | Reference |
|-----------------------|---------------|----------------------------------|-----------|
| Carybdea branchi      | GQ849089      | Cape town, South Africa,         | a         |
| Carybdea brevipesdalia| KY212121      | Jeju Island, South Korea         | b         |
| Copula stiviciski     | AF358110      | Unknown                          | c         |
| Tripedalia cystophora| GQ849088      | Kakaban, Derawan Island marine lake, Indonesia | a |
| **Tripedalia binata** | **LC480260**  | Trat, Thailand                   | **This study** |
| Tamoya haplonema      | GQ849085      | North Carolina, Oak Island, USA  | a         |
| Tamoya ohboya         | GQ849086      | Bonaire, No Name Beach, Netherlands Antilles | a |
| Malo kingi            | GQ849084      | Queensland, Port Douglas, Australia, | a |
| Morbakka virulentia   | GQ849083      | Hiroshima, Japan                 | a         |
| Alatina alata         | GQ849082      | Queensland, Osprey Reef, Australia, | a |
| Alatina morandini     | LC047803      | Nagasaki, Japan                  | d         |
| Alatina morandini     | LC047805      | Hamburg, Germany                 | d         |
| Alatina morandini     | LC047802      | Ishigaki Island, Ryukyu Islands, Japan | d |
| Alatina morandini     | **LC480261**  | Bangsaen Aquarium, Chon Buri, Thailand | **This study** |
| Chironex flecteri     | GQ849073      | Darwin, Northern Territory, Australia | a |
| Chironex yamaguchii   | GQ849076      | Ishigaki Island, Ryukyu Islands, Japan | a |
| Chiropsella bart      | AF358103      | Unknown                          | c         |
| Meteorona kishinouyei| LC033480      | Matsukawa-ura, Souma, Fukushima, Japan | e |
| Chiropsalmus quadrumanus | GQ849078   | Macae, Rio de Janeiro state, Brazil | a |

| Table 2. **Morphometrics (mm) of Tripedalia binata** after Toshino et al. (2015). BH, bell height; DBW, diagonal bell width; DEW, diagonal exumbrella width; DSW, diagonal subumbrella width; ML, manubrium length; IKL, inner keel length; IKW, inner keel width; IRW, interrhopalial width; OKL, outer keel length; OKW, outer keel width; RH, rhopalium height; PCW, pedalial canal width; PP, primary pedalium; PW, pedalial width; SP, secondary pedalium; TBW, tentacle base width; and VW, velarial width. |

| BH  | DBW | DEW | DSW | IRW | PW (PP/SP) | PCW (PP/SP) | OKW (PP/SP) |
|-----|-----|-----|-----|-----|-----------|-------------|-------------|
| 8.0 | 14.2| 13.2| 11.9| 6.3 | 1.1/1.1   | 0.3/0.3     | 0.3/0.4     |
| IKW (PP/SP) | OKL (PP/SP) | IKL (PP/SP) | TBW (PP/SP) | RH | VW | ML | SEX |
| 0.5/0.4 | 4.0/4.6 | 4.2/3.9 | 0.3/0.3 | 1.4 | 1.8 | 4.5 | Female |

Class Cubozoa Werner, 1973
Order Carybdeida Gegenbaur, 1857
New records of two cubozoans from Thailand

Fig. 1. Preserved mature female medusae of *Tripedalia binata*: A) lateral, B) apical, and C) oral views. Scale bar = 1 cm.

Fig. 2. Preserved mature female medusae of *Tripedalia binata*: A) gonad, B) manubrium, C) phacellae, D) rhopalial niche ostium, E) pedalium (lateral view), F) pedalium (dorsal view), G) tentacle, H) velarium, and I) pedalial canal bend. Scale bars = 0.5 mm (A, D, I) and 1 mm (B, C, E–H). po, stomach pocket; pu, stomach purse.
Fig. 3. Live mature polyps of *Alatina morandinii*: A) lateral view, B) oral view, and C) creeping polyp, lateral view. Scale bars = 0.2 mm.

Fig. 4. Live young medusae of *Alatina morandinii*: A, B) lateral, C) apical, and D) oral views, E, F) exumbrella, G) gastric filaments, H) rhopalium, I) pedalium, and J) tentacle structure. Scale bars = 1 mm (A–F), 0.2 mm (G), and 0.1 mm (H–J).
Family Tripedaliidae Conant, 1897
Genus Tripedalia Conant, 1897
Tripedalia binata Moore, 1988

Description. Bell cubic 8 mm high and 13 mm in diameter (Table 2, Fig. 1A–C). Rounded nematocyst clusters scattered over entire exumbrella (Fig. 1A, B). Gonads leaf-shaped (Fig. 2A), but butterfly-shaped in female and stick-shaped in male before spawning. Manubrium long, about 60% of bell height (Fig. 2B). Manubrium cruciform with four narrow, lanceolate lips (Fig. 2B). Phacellae epaullette-shaped comprised of single-rooted simple gastric filaments (Fig. 2C). Four stomach pockets located beneath each gastric phacella (Fig. 2C). Four stomach purses located above the perradial mesenteries (Fig. 2C). Rhopaliar niche ovum-shaped without rhopalial horns (Fig. 2D). Pedalia knife-shaped, two per corner of the umbrella (Fig. 2E, F). Pedalial canal bend rounded without appendage (Fig. 2I). Tentacles eight, with one per pedalium, base width up to 0.3 mm thick, round in cross-section, perl-string-like composed of nematocyst rings (Fig. 2G). Velarium with 3 velarial roots, 1 velarial canal root per octant, pointed digit-like shape (Fig. 2H). Velarial width about 20% of diagonal subumbrella width.

Nematocysts
Not examined in this study.

Family Alatinidae Gershwin, 2005
Genus Alatina Gershwin, 2005
Alatina morandinii Straehler-Pohl & Jarms, 2011

Description (cubopolyp). Polyp solitary, with amphora-shaped calyx and short stalk (Fig. 3A, B). The expanded body about 1.0 mm in length. Zooxanthellae (Fig. 5B) in the body, except stalk. Tentacles 10 to 19 (mean: 13, n= 20), bearing a single stenotele at the tip of each tentacle (Fig. 5B, C). Polyps asexually produce daughter polyps by lateral budding. The buds develop into worm-shaped creeping polyps (Fig. 3C), which bear 4 to 6 tentacles.

Nematocysts
Two different types of nematocyst were found in the entire polyp: stenoteles and heterotrichous microbasic euryteles (Fig. 5A).

Molecular analysis
The maximum likelihood tree (Fig. 6) revealed that Tripedalia binata from Trat Province and Tripedalia cystophora were in a monophyletic group, the Tripedaliidae. The polyps from Bangsaen Aquarium were identical to Alatina morandinii from Germany and Japan.

Discussion
Tripedalia binata was described by Moore (1988) based on specimens from Australia and India. The morphological inspection of T. binata from Thailand agrees well with the morphological descriptions of Moore (1988) and Underwood et al. (2013). Straehler-Pohl et al. (2014) reported that rhopalial horns could be observed above the top of the rhopalial niches in T. binata from Australia. However, rhopalial horns were not present in the Thai specimen. Additional specimens collected from Thailand are needed for comparison and clarification as to whether this is intraspecific variation or not. Tripedalia binata is a rare species that has been recorded only from eastern India (Moore 1988) and northern Australia (Underwood et al. 2013). The medusae appear in creeks, aquaculture ponds, and sandy beaches near mangroves during the rainy season (Moore 1988, Underwood...
et al. 2013). Our report is the third official record after the report of Toshino et al. (2016) for this species since its discovery. Our sampling site was a semi-closed mangrove swamp leading to the sea, with seawater flowing into the swamp during rising tides (Toshino et al. 2016).

The family Alatinidae Gershwin, 2005 comprises 13 species in three genera: *Alatina* Gershwin, 2005, *Keesingia* Gershwin, 2014, and *Manokia* Southcott, 1967 (Southcott 1967, Gershwin 2005b, 2014). *Alatina morandinii* was described as *Carybdea morandinii* (Straehler-Pohl & Jarms 2011). However, the species was recently moved to the genus *Alatina* based on its morphology and molecular phylogenetic analyses (Straehler-Pohl & Toshino 2015). *Alatina morandinii* is a mysterious species because mature medusae have not yet been found in the wild. Cubopolyps of the species have been reported in tanks from aquariums in Germany and Japan and on dead coral near the shore in Okinawa, southern Japan (Straehler-Pohl & Jarms 2011). In this study, the polyps were found in tanks at the Bangsaen Aquarium, Thailand. According to husbandry specialist Nattawut Luangoon, the water in this tank came directly from the sea near the aquarium, and may have contained either embryos or polyps of this species. The creatures and substrates in the tank all originated from Thailand, and never from other places. This study is the first record of an Alatinidae species in Thailand though *Alatina alata* (Reynaud, 1830) has been reported from “Indochina”, the Malayan Archipelago, the Philippines and other tropical regions (Kramp 1961, Lawley et al. 2016).

Three species belonging to the order Carybdeida and four species belonging to the order Chirodropida have previously been reported from Thai waters (Stiasny 1922, 1926, Aungtonya & Chanachon 2012, Sucharitakul et al. 2017, 2018). In addition, we recorded two more species of the order Carybdeida. However, more unrecorded or undescribed species like *Chiropsella* sp. and *Chironex* sp. (Sucharitakul et al. 2018) are likely to inhabit Thailand waters. Intensive sampling and further investigations are needed to understand the diversity of Cubozoa in Thai waters, which may also help to prevent sting injuries in humans.

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