***Carybdea morandinii*—New investigations on its life cycle reveal its true genus: *Carybdea morandinii* Straehler-Pohl & Jarms, 2011 becomes *Alatina morandinii* (Straehler-Pohl & Jarms, 2011)**

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Abstract: The name *Carybdea morandinii* Straehler-Pohl & Jarms, 2011 was applied to unknown cubopolyps found on dead coral rock at the Hagenbeck Zoo, Hamburg, Germany, during 1999, and a description was given of its early life history. Although included in Carybdeidae Lesson, 1843, its familial affinities remained uncertain. In 2006, a population of unidentified cubopolyps was discovered on the walls of a saltwater tank at the Umikirara Aquarium, Nagasaki, Japan. In addition, an immature cubomedusa and polyps were collected at Ginowan and Ishigaki, Okinawa, Japan, in 2002 and 2012. Genetic analysis established that these polyps and the medusa belonged to the same species as the polyps found in Nagasaki. Comparisons of the anatomy and asexual propagation methods of Japanese polyps, and their newly detached medusae, with early life stages of cubozoans cultured in Germany, revealed that the Japanese populations were likely conspecific with *Carybdea morandinii*. Subsequent genetic analysis confirmed this identification. Morphology of Japanese medusae revealed that they were referable to the family Alatinidae Gershwin, 2005, and within that family to the genus *Alatina* Gershwin, 2005, rather than to the family Carybdeidae.

Key words: Alatinidae, Cubozoa, life cycle, systematics, taxonomy

Introduction

Identification of cubozoans at ranks below the orders Carybdeida and Chirodropida is problematic because medusae within these two groups closely resemble each other, and inspections of inner body structures are needed in most cases. Particular difficulties arise in the allocation of sessile and immature stages of Cubozoa because of a profound lack of knowledge about developmental stages. Thus, problems arose during 1999 in determining the identity of cubopolyps found on a block of dead coral in the Troparium at Hagenbecks Tierpark (Zoo), Hamburg, Germany (Straehler-Pohl & Jarms 2011). The early life history of the species was traced from polyp to young medusa. Morphological characters were then compared

with the few existing accounts of polyps, young medusae, and metamorphic phases of cubozoan species (Werner et al. 1971, Studebaker 1972, Cutress & Studebaker 1973, Straehler-Pohl 2001, 2009, Straehler-Pohl & Jarms 2005, 2011, Toshino et al. 2013, 2014, 2015, Carrette et al. 2014), namely those of *Alatina moseri* (Mayer 1906), *Tripedalia cystophora* Conant, 1897, *Copula sivickisi* (Stiasny 1926), and *Carybdea* sp. (=“*Carybdea marsupialis*”) from Puerto Rico and *Morbakka virulenta* (Kishinouye 1910). The unidentified species differed in its early life history from those five. As the medusae could not be reared to maturity, Straehler-Pohl and Jarms (2011) established the binomen *Carybdea morandinii* for the species, although they were aware that the sessile stages might prove to be referable to a known species of medusa. New investigations in Japan, and a collaboration between German and Japanese laboratories working on the species, have now resolved the identity of the genus of *Carybdea morandinii*.

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This study was undertaken to reveal the identity of the unknown cubopolyps discovered in the Umikirara Aquarium in Japan, to verify the identity of *Carybdea morandinii*, and to demonstrate the importance of observations on all life history stages in the Cubozoa when making identifications.

Methods

For this study, the development of cubozoan polyps of unknown identity from a population discovered in a public Japanese aquarium was compared morphologically to the early life history of *Carybdea morandinii*, originating from the population discovered in the public German aquarium Troparium but with an unknown original origin.

As both populations showed similarities at all stages, they were also compared genetically.

Additionally, as one immature medusa sampled in the wild in Japanese waters showed similarities to the artificially-reared 2-month-old Japanese medusae, they were added to both the morphological and genetic comparisons.

Sampling locations

Polyps of "*Carybdea morandinii*":

Germany: A saltwater tank at the Troparium of Hagenbecks Tierpark (Zoo), Hamburg, Germany, during 1999. They were discovered on a block of dead coral, the origin of which is unknown. According to the staff of Hagenbecks Tierpark, it came from somewhere in east Asia.

Polyps of unknown identity (Japanese population):

Nagasaki: A saltwater tank at the Saikai Pearlsea Resort, Umikirara Aquarium, Sasebo, Nagasaki, during 2006. Polyps were discovered on the wall of the tank. According to husbandry specialist Mr. Hitoshi Akiyama, the water in this tank came directly from the sea near the aquarium, and may have contained either planulae or creeping polyps.

Ishigaki: A piece of dead coral at Nagura Bay, Ishigaki Is., Okinawa, on March 26, 2006. One polyp was discovered on the dead coral.

Ginowan: A glass plate at Ginowan Marina, Okinawa, on November 27, 2006. One polyp was discovered on the plate.

Medusa of *Alatina* sp. from the wild:

Japan: Ginowan Marina, Okinawa, Japan. One immature medusa was collected using light traps during the night between 19:30 and 21:00 on July 5, 2012.

Lab cultures

"*Carybdea morandinii*":

Laboratory in Stade-Hagen, Germany: Living polyps of all known stages in the private "Medusa('s) nursery" laboratory of the first author in Stade-Hagen; specimens were donated by Prof. Dr. André C. Morandini (Universidade de São Paulo) and had the same origin as type series ZMH

No. C.11999 of *Carybdea morandinii* in Hamburg, Germany.

The cubopolyps and young medusae were cultured as described by Jarms et al. (2002) and by Straehler-Pohl & Jarms (2011). Metamorphosis could not be induced artificially, but it occurred during the spring and summer period (May to September) when room temperature rose above 24°C and direct sunlight penetrated the room at least 4–5 hours a day.

Polyps of unknown identity:

Laboratory in Kitasato University, Japan: Polyps were incubated in petri dishes (diameter 75 mm, height 45 mm) with filtered seawater (1 µm) at 23°C. *Artemia* sp. nauplii were fed to the polyps twice or thrice a week. A complete rearing water change with filtered seawater (1 µm) followed about 3 hours after feeding. Metamorphosis was induced by temperatures between 25°C to 30°C. Newly detached medusae were kept in a separate polypropylene beaker (volume 1000 mL) with filtered seawater (1 µm) at 30°C. *Artemia* nauplii were fed to the medusae on a daily basis. Culture water was replaced with filtered seawater about three hours after feeding.

Measurements and abbreviations

Medusae: Standard measurements were used (Gershwin 2005a, b, 2006, Straehler-Pohl 2014): bell height (BH)=length between bell turn-over (velarium excluded from measurement) and top of apex; interpedalial diameter (IPD)=distance between opposite pedalia (outer pedalial wing edges) at the level of the bell turn-over; interrhopalial diameter (IRD)=distance between opposite sense niches. Nematocyst distribution: ">"=more than; ">>"=a lot more than.

Polyps: Standard measurements of the polyps were adapted from Straehler-Pohl et al. (2011) and Straehler-Pohl & Jarms (2011): Hypostome Length (HL)=length from tentacle crown base to hypostome tip; Mouth Disc Diameter (MDD)=widest diameter of mouth disc; Stalk Length (StL)=length from basal disc to gastric cavity base; Total Body Length (TBL)=refers to the maximal length of the completely relaxed polyp body from basal disc to tip of hypostome.

To compare body dimensions and proportions, the following proportion quotients (%) were used: measurements compared to body length (HL/TBL×100; StL/TBL×100).

Photographs were taken with the following digital cameras Canon Powershot G12; Canon Eos 550D; Canon EOS Kiss X5; OLYMPUS E-330.

Identification of characteristics

Zooxanthellae in polyps and newly detached medusae (Figs. 1B, I) were identified by their location lining the gastric tissue (on the inside of the endoderm), the nematocyst warts on the exumbrella and the velarium, their distinctive granular shape and their brownish-green colour in

tissue squashes and histological sections (Fig. 1) according to descriptions and images by Muscatine (1974), Schlichter & Fricke (1990), Arai (1997) and Straehler-Pohl & Jarms (2011).

Tissue squashes from the tentacles, calices and umbrellas of polyps and/or medusae, respectively, of different stages were observed under 400x magnification and basic forms of nematocysts identified according to Östman (2000) and Gershwin (2006).

“Gonads” in Cubozoa

Campbell (1974: 142) stated that “gonads” in most cnidarians are not separate organs, as found in higher animals, because germ cells are generally found in the interstitial portions of the body tissue which, prior to the

formation of germ cells, exhibit no reproductive specialization. However, we follow Campbell (1974), Marques & Collins (2004), Benthage et al. (2010), Morandini & Marques (2010) and Straehler-Pohl et al. (2014) in using the term gonad to refer to areas where gametes are formed.

Molecular methods

Sequences of the nuclear 18S rDNA genes (approximately 1800 bp) were used for molecular phylogenetic analyses. Genomic DNA was extracted from fresh or 99.5% ethanol-preserved polyps and 1 young medusa of unknown identity from Okinawa (Ishigaki, Ginowan) and Nagasaki and polyps of the German *Carybdea morandinii* culture using Chelex 100 (Bio-Rad, Japan) or DNeasy Blood and Tissue Kits (Qiagen, Germany). 18S rDNA was PCR am-

“*Carybdea morandinii*”: A species with a curious polyp morphology

Fig. 1. Anatomical features of the polyp (A–C) and life cycle stages (D–I) of *C. morandinii* from German cultures (images A to C, E, G, H were altered and newly composed from Straehler-Pohl & Jarms (2011): A: Budding polyp with inflated, dome shaped hypostome, shutter-lens lamella with slit-like opening faintly visible through transparent hypostome tissue; B: a) longitudinal section through hypostome, shutter lens lamella and upper calyx, b) close up of longitudinal section of hypostome and shutter-lens lamella (bracket), note mesoglea axis (fat black arrow) in lamella and zooxanthellae (white arrow) in hypostome at mouth opening; C: Inner and outer polyp anatomy—left: inner anatomy showing ectoderm (ecD), mesoglea (Mes) and entoderm (enD), the zooxanthellae stored in the tissue surrounding the mouth opening (white arrow), the position of the shutter-lens lamella (ShLL) and the two-chambered digestion system with antechamber (AC) in the hypostome and main gastric cavity (GC) inside the calyx; right: outer anatomy showing tentacles (T), hypostome (H), calyx (C) and stalk (St). D: polyp with inflated hypostome; E–G: metamorphosis type 2 complete): E: phase 2; F: phase 3; G: phase 4; H: 1-day old medusa; I: 4-week old medusa with zooxanthellae (white arrows) inside nematocyst warts and velarium. AC: antechamber; C: calyx; ecD: ectoderm; enD: endoderm; GC: gastric cavity; H: hypostome; Mes: mesoglea; St: stalk; T: tentacle; TB: tentacle base.

plified and sequenced using primers and protocols outlined in Collins et al. (2008). Phylogenetic analysis (maximum likelihood and Minimum Evolution methods) and pairwise distance measurements were determined using the maximum likelihood method with 1000 bootstrap replications in MEGA 6.06. All of these sequences have been deposited in the DNA Data Bank of Japan (DDBJ). Those sequences were compared to 18S rDNA sequences of 17 cubozoan species of 6 families and two scyphozoan species as outgroups as listed in Table 1.

Results and Discussion

In 1999, an unknown cubozoan polyp was found on a block of dead coral in the Troparium of Hagenbecks Tierpark (Zoo), Hamburg, Germany. The provenance of the coral rock substrate was unknown, although Tierpark staff thought it might have come from East Asia (Straehler-Pohl & Jarms 2011). On testing temperature range tolerances of the polyps in the laboratory, and noting that the metamorphosis season was in the summer of the northern hemisphere, it was thought the origin might have been Japan (Straehler-Pohl 2001). The life cycle was followed from polyp to young medusa, including descriptions of asexual reproduction methods and observations of a resting stage. As the medusa could not be reared to maturity, Straehler-Pohl and Jarms (2011) decided to describe the species un-

der the binomen *Carybdea morandinii*, although they were aware that the generic or specific names might change in the future if the sessile stages were found to belong to a known species of medusa.

In 2012, the second author received and maintained unidentified cubopolyps detected in a saltwater tank at the Umikirara Aquarium, Nagasaki, Japan in 2006.

To reveal the identity of the unknown cubopolyps discovered in the Umikirara Aquarium in Japan a collaboration between our German and Japanese laboratories was set up. The unknown Japanese polyps and their further developmental stages were compared with those of the four cubozoan species maintained in Germany (*Alatina moseri*, *Tripedalia cystophora*, snf *Carybdea* sp. (=“*Carybdea marsupialis*” from Puerto Rico), *Carybdea morandinii*) and later on with species maintained in Japan: *Copula sivickisi* (Toshino et al. 2014) and *Morbakka virulenta* (Toshino et al. 2015a). The cubopolyps from Japan (Fig. 2A) differed from all others except those of *Carybdea morandinii* (Straehler-Pohl & Jarms 2011) in characters such as colour, body size and gross morphology, the existence of zooanthellae in tentacles, hypostome and calyx lining the gastric cavity, and especially the horizontal twin-chamber system of the gastric cavity (Straehler-Pohl & Jarms 2011).

Table 1. List of species used for genetic analysis.

Species	GenBank No.	Reference
<i>Alatina moseri</i> (Australia)	GQ849082	Bentlage et al. 2010
<i>Alatina</i> sp. (Ishigaki Is., Okinawa)	LC047802 (as <i>Alatina morandinii</i>)	this study
<i>Alatina</i> sp. (Ginowan, Okinawa)	LC047804 (as <i>Alatina morandinii</i>)	this study
<i>Alatina</i> sp. (Nagasaki)	LC047803 (as <i>Alatina morandinii</i>)	this study
<i>Carybdea morandinii</i> (Germany)	LC047805 (as <i>Alatina morandinii</i>)	this study
<i>Carybdea branchi</i> (South Africa)	GQ849089	Bentlage et al. 2010
<i>Carybdea brevipedalia</i> (Japan)	GQ849092	Bentlage et al. 2010
<i>Carybdea rastonii</i>	AF358108	Collins et al. 2006
<i>Carybdea xaymacana</i>	AF358109	Collins et al. 2006
<i>Carybdea xaymacana</i> (Panama)	GQ849090	Bentlage et al. 2010
<i>Carukia barnesi</i>	AF358107	Collins et al. 2006
<i>Copula sivickisi</i>	AF358110	Collins et al. 2006
<i>Gerorgia rifkinae</i>	AF358105	Collins et al. 2006
<i>Meteorona kishinouyei</i> (Fukushima, Japan)	LC033480	Toshino et al. 2015b
<i>Morbakka virulenta</i> (Japan)	GQ849083	Bentlage et al. 2010
<i>Malo maxima</i> (Australia)	GQ849084 (as <i>Malo kingi</i>)	Bentlage et al. 2010
<i>Tripedalia cystophora</i> (Indonesia)	GQ849088	Bentlage et al. 2010
<i>Chironex fleckeri</i> (Australia)	GQ849073	Bentlage et al. 2010
<i>Chironex yamaguchii</i> (Japan)	GQ849076	Bentlage et al. 2010
<i>Chiropsalmus quadrumanus</i> (Brazil)	GQ849078	Bentlage et al. 2010
<i>Chiropsella bronzie</i>	AF358103 (as <i>Chirodropida</i> sp.)	Collins et al. 2006
<i>Atolla wyvillei</i> (Monterey Bay)	HM194788	Collins et al. 2006
<i>Haliclystus sanjuanensis</i>	AF358102	Bayha et al. 2010

Fig. 2. Nagasaki population of *Alatina morandinii*: Early life stages – from cubopolyp through complete metamorphosis to newly detached medusa: A: Adult polyp; B: creeping polyp (secondary polyp produced by lateral budding); Metamorphosis—C: Phase 1, clustering of tentacles at four points around hypostome; D: Phase 2, fusion of tentacle bases, reabsorption of tentacles, formation of rhopalium out of tentacle bases; E: Phase 3 (early), development of 4 medusa tentacle buds, transformation of hypostome to manubrium; F: Phase 4, growth of developing medusa, transformation of remaining polyp tissue, absorption of stalk; G: close up of developing rhopalium, note 6 eyes – 2 median lens eyes (black arrows), 4 lateral eyes (blue arrows); H: Phase 5 (early), detachment of medusa, reabsorption of stalk tissue remains; H: newly detached medusa; I: young medusa, 1-day old; J: young medusa, 7 days old, St: statolith. Scale bars: 1 mm.

Comparison of morphology of polyp cultures from Germany and Nagasaki

Cubopolyps amphora-shaped in both cultures (German culture (Fig. 1A, D): TBL: 0.81–1.8 mm (mean: 1.12 mm, $n=56$), MDD: 0.34–0.74 mm (mean: 0.50 mm, $n=56$); Nagasaki culture (Fig. 2A): TBL: 1.46–3.36 mm (mean: 2.22 mm; $n=20$), MDD: 0.38–0.64 mm (mean: 0.51 mm; $n=20$)); dome of hypostome functioning as antechamber for main gastric tract of calyx (Fig. 1A), gastric cavity without septae but horizontally two-chambered, with shutter-lens-like diaphragm separating upper chamber in hypostome, from lower chamber inside calyx (Figs. 1B–C).

Diaphragm, originating from endoderm (Figs. 1Ba, b), with a mesogloal axis (Figs. 1Bb, black arrow), located directly above tentacular circlet. Tentacles 18–24 (mean: 22, $n=50$) when newly discovered in German culture, 8–22 (mean: 14, $n=42$) in Nagasaki culture, solid, capitate with single stenotele nematocyst in tips, arranged in a single circle around mouth disc. Hypostome dome to balloon-shaped ($HL \approx 21\%$ of TBL in both cultures). Stalk distinct ($StL \approx 31\%$ of TBL in both cultures), basal region covered by thin periderm cuticle. Colour in both cultures creamy-white with a tinge of beige when held in darkness; beige with tinge of greenish-brown due to zooxanthellae when kept in daylight. Mouth opening characteristically lined

Fig. 3. Medusa of *Alatina morandinii* 1 day after detachment from Japanese and German cultures: Nagasaki culture specimen (A, E–I): A: lateral view; E: apical view; F: oral view; G: single gastric filaments (arrows), note greenish brown coloured nematocyst warts infused with zooxanthellae; H: pedalium; I: tentacle structure; German culture specimen: B: medusa, lateral view; C: rhopalium, front view, note 2 median eyes, 4 lateral eyes (arrows); D: rhopalium, lateral view, note 2 median eyes, 2 lateral eyes (arrows); Scale bars: 1 mm (A–B, E–F), 0.5 mm (C, D, G–I).

with zooxanthellae, the dark circle of the mouth opening giving a sharp contrast to the otherwise zooxanthellae-free, colourless hypostome (Straehler-Pohl & Jarms 2011, this study: Fig. 1Bb, white arrow)

Asexual reproduction methods including lateral budding (Fig. 1A) of creeping polyps (buds with 4–6 tentacles; Fig. 2B), and the stages of complete metamorphosis (Figs. 1D–

G, 2C–H) were also identical in both cultures.

The successfully induced metamorphosis resulted in medusae (Figs. 1I, 2H–J, 3, 4) that could be reared in the Japanese cultures to a stage (Fig. 6A) in which key characters were developed so that its generic identity could be determined. The crescent shape of the gastric phacellae (Fig. 6B) revealed that it was referable to the family Alatinidae

Fig. 4. Three-week-old medusa of *Alatina morandinii* from Japanese cultures: A: lateral view; B: apical view; C: oral view; D: gastric phacellae with 5–10 filaments inside the stomach arranged around the manubrium base, note greenish brown spots marking nettle warts infused with zooxanthellae; E: growing pedalium. Scale bars: 2 mm (A–C), 1 mm (D), 0.5 mm (E).

Gershwin, 2005 and the non-branched tentacles (Fig. 6E) hinted to the genus *Alatina* Gershwin, 2005.

Newly detached to 1-day-old medusae of German and Nagasaki cultures

Bell tetradial, spheroidally-pyramidal shaped when newly detached in both cultures (German culture: BH: 0.6–0.9 mm (mean: 0.7 mm, $n=12$); IRD: 0.4–0.6 mm (mean: 0.5 mm, $n=12$)), spheroidal shaped when 1-day old (Nagasaki culture (Figs. 3A, 4): BH: 1.1–1.3 mm (mean: 1.1 mm, $n=10$), IRD: 0.8–1.1 mm (mean: 1.0 mm, $n=10$); German culture (Fig. 3B): BH: 0.9–1.1 mm (mean: 1.0 mm, $n=12$), IRD: 0.9–1.2 mm (mean: 1.1 mm, $n=12$)); exumbrella with very small, round (0.03–0.04 mm) nematocyst

warts regularly dispersed along radial furrows in Nagasaki culture (Fig. 3A) and irregularly dispersed over entire exumbrella in medusae from German culture (Fig. 3B); In both cultures, rhopalia 4, extending from bell sides, no rhopalial niche yet developed, eyes 6 (2 median lens eyes, 2 pit eyes, 2 slit eyes; Figs. 3C, D). Manubrium short (approx. 30% of bell height; Figs. 3A, B), four-lipped; gastric filaments 4 (Fig. 3G). Tentacles 4, without pedalia, filiform, white with thin, light brown annuli (Fig. 3H). Bell, yellowish to yellowish-brown due to zooxanthellae in the exumbrella (mainly in warts) and velarium.

Nematocysts (both cultures):

Tentacles: holotrichous isorhizas, atrichous isorhizas,

Fig. 5. Nematocysts of 3-week old medusa of *Alatina morandinii* (bell height 4.4 mm): A, B: Microbasic euryteles: intact (A), discharged (B); C, D: Ovoid isorhizas: intact (C), discharged (D); E, F: Spherical isorhizas: intact (E), discharged (F). All bars represent 10 μ m.

Fig. 6. Anatomical features of medusae of *Alatina morandinii* reared from polyp cultures from Nagasaki and medusae caught in the wild in Okinawa: Nagasaki culture specimen (Figs. A–E), photos by courtesy of Mr. Hitoshi Akiyama: A: medusa, lateral view; B: apex, note small crescentic gastric phacellae (arrow) arranged around manubrium base (m); C: quadrant of velarium, note 2–3 developing velarial canals (arrows) per octant flanking frenulum (f); D: rhopalium inside developing rhopalium niche without covering scales; E: developing pedalium, note developing inner wing in lower third; specimen from Okinawa, Japan (Figs. F–J): F: medusa, lateral view; G: apex, note crescentic gastric phacellae (gp) arranged around manubrium base (m); H: quadrant of velarium, note 3 developing velarial canals (arrows) per octant flanking frenulum (f); I: rhopalium (rh) inside heart-shaped rhopalium niche with two developing lower covering scales (arrows); J: developing pedalium, note slim scalpel shape of developing inner wing.

single ovoid heterotrichous microbasic euryteles.

Bell mammilation: ovoid heterotrichous microbasic euryteles >>> round holotrichous isorhizas.

Zooxanthellae were detectable in manubrium lips, velarium, and nematocyst clusters on the exumbrella in both cultures.

Later development was noted in detail only in Nagasaki specimens. German cultures died after two to four weeks due to ruptured tentacles, followed by degeneration and death by starvation.

Three-week-old medusae (Figs. 4A–E, Nagasaki culture specimens)

Bell spheroidal (BH: 2.4–3.0 mm (mean: 2.8 mm, n=10), IRD: 1.8–2.5 mm (mean: 2.2 mm, n=10) (Fig. 4A); rhopalia 4, settled inside shallow cavity of developing rhopalium niche (Fig. 4A); eyes 6 (2 median, 4 lateral); velarial canals not yet developed (4C); gastric filaments 5–10 (Fig. 4D); pedalia development commencing, growing into thin “stalks” (Fig. 4E).

Nematocysts (Figs. 5A–F):

Tentacles: Ovoid isorhizas (mean: $12.39 \times 10.31 \mu\text{m}$, range: $10.13\text{--}13.86 \mu\text{m}$ long, $8.02\text{--}10.44 \mu\text{m}$ wide).

Bell: Spherical isorhizas (mean: $18.00 \times 18.09 \mu\text{m}$, range: $14.26\text{--}20.70 \mu\text{m}$ long, $14.67\text{--}20.64 \mu\text{m}$ wide).

Gastric filaments: Microbasic euryteles (mean: $9.05 \times 6.49 \mu\text{m}$, range: $7.55\text{--}11.92 \mu\text{m}$ long, $5.04\text{--}9.54 \mu\text{m}$ wide).

Manubrium: Microbasic euryteles (mean: $11.94 \times 9.04 \mu\text{m}$, range: $9.91\text{--}13.81 \mu\text{m}$ long, $8.02\text{--}10.44 \mu\text{m}$ wide).

No additional tentacles were added during development; bell becoming lighter in colour and more transparent, medusae nearly invisible in rearing tanks reaching 4–5 mm in bell height. Tentacles of medusae at that size hair-thin, nearly invisible when completely extended, up to 50 mm in length.

Two-month-old medusae (Figs. 6A–E, Nagasaki culture specimens)

Bell pyramidal to cuboidal, higher than wide (BH: 6.8–7.9 mm (mean: 7.4 mm, $n=2$), IPD: 4.4–4.9 mm (mean: 4.7 mm, $n=2$); Fig. 6A); phacellae 4, crescent-shaped, one per interradial corner of stomach (Fig. 6B), number of gastric filaments uncountable in living specimens; velarium narrow; velarial canals developing, 2–3 per octant, simple, non-branching (Fig. 6C); rhopalia 4, settled inside roundish cavity of developing rhopalian niche (Fig. 6D); covering scales still undeveloped; eyes 6 (2 median, 4 lateral). Pedalium stalked, development of inner wing commencing in lower third (Fig. 6E). Manubrium very short ($\approx 1/7$ of bell height). Gonads undeveloped.

Nematocysts:

Tentacles: isorhizas > medium-sized microbasic euryteles > ovoid isorhizas > microbasic amastigophores.

Bell: mammillation: 20–30 small spherical isorhizas per cluster.

Gastric filaments: small euryteles.

In 2000 and 2012, polyps and a small, immature *Alatina* medusa (Fig. 6F) identifiable by the crescentic shaped gastric phacellae, the “T”-shaped rhopalian niche opening with clearly separated lower covering scales and the non-branched tentacles were collected in Okinawa, Japan (1 polyp and 1 medusa in Ginowan and 1 polyp in Ishigaki). As this still immature medusa showed alatinid characters, and showed similarities in development to the young medusae of the Nagasaki culture, it was speculated that it might represent a further developmental stage. Genetic analysis of specimens from the cultures of Nagasaki and the Okinawa specimens confirmed that they belonged to the same species (Fig. 7).

Fig. 7. Maximum-likelihood tree for 18 cubozoan taxa based on the nuclear 18S rDNA data set. Scale bars indicate branch length in substitutions per site. Nodal support values are presented as the ML bootstrap value; only values >50% are shown. Morphological analysis revealed that medusa *Alatina* sp. from Japan and *Carybdea morandinii* from Germany are identical.

Anatomical characters of Okinawa specimen from nature (Figs. 6F–J, Table 2)

Medusa estimated to be 2–3 months old: Bell pyramidal to cuboidal, higher than wide (BH: 8.0 mm ($n=1$); IPD: 6.7 mm ($n=1$); Fig. 6F); phacellae 4, crescent-shaped, one per interradial corner of stomach; gastric filaments approx. 30 per phacellum, long, simple, rooted singly or in pairs (Fig. 6G); velarium narrow, velarial canals lengthening, 2–3 per octant, simple, non-branching. Manubrium very short ($\approx 1/7$ of bell height) (Fig. 6H); rhopalia 4, settled inside heart-shaped cavity of rhopalian niche; one upper and two lower covering scales developing (Fig. 6I); eyes 6 (2 median lens eyes, 2 lateral pit eyes, 2 lateral slit eyes). Pedalium long ($\geq 1/2$ BH), long-stalked ($\geq 1/3$ PL), with no distinct outer wing, only broad outer keel; inner wing (lower 2/3 of pedalium) narrow, pointed scalpel-shaped, broadening below stalk, tapering from first half towards distal end (Fig. 6J); gonads developing as narrow stripes, attached at centre of septum. Other morphological characters agreeing with Nagasaki specimens.

Nematocysts: the same as in the specimens reared from the Nagasaki polyp culture.

Genetic analysis of specimens from the cultures of Germany and Nagasaki and the Okinawa specimens confirmed that they belonged to the same species (Fig. 7).

Comparison of *Carybdea morandinii* with the specimens from Okinawa and Nagasaki based on molecular data

In the resulting ML tree (Fig. 7), four major monophyletic clades were formed in the order Carybdeida: 1) Carybdeidae (*Carybdea branchi*, *Carybdea brevipedala*,

lia, *Carybdea rastonii* and *Carybdea xaymacana*); 2) Tripedaliidae (*Copula sivickisi* and *Tripedalia cystophora*); 3) Carukiidae (*Carukia barnesi*, *Morbakka virulenta* and *Malo maxima*); 4) Alatinidae (*Alatina moseri*, *Alatina* sp. and *Carybdea morandinii*). The monophyly of the Alatinidae was evident in the 18S phylogenetic tree with high bootstrap values, well supporting the validity of the species. According to this analysis *Carybdea morandinii* and *Alatina* sp. from Japan are the same species (Fig. 1). Therefore, *Carybdea morandinii* is assigned here to the genus *Alatina* (as *Alatina morandinii*).

But even if the molecular analysis confirmed that all cultures and populations represented the same species, it did not reveal if this species was new to science or if it belonged to an already known, valid alatinid species as there is not enough molecular data from all valid alatinid species available. All characters in the reared and collected medusae of *A. morandinii* were still at an immature state of development and were mostly limited to genus-specific characters: (1) a transparent, cuboidal to pyramidal bell (Fig. 6F) covered with minute nematocyst warts, (2) crescent-shaped gastric phacellae (Fig. 6G), (3) rhopalial niche openings with three small covering scales, giving the orifice a faint "T"-shape appearance (Fig. 6I), and (4) long ($\geq 1/2$ of BH), long-stalked ($\geq 1/3$ of PL) pedalia with slim, pointed scalpel-shaped inner wings with straight pedalial canals showing rounded knee bends without any appendages (Fig. 6J).

In conclusion

Carybdea morandinii was found in Japan, and morphological and molecular data indicate that it belongs to the genus *Alatina*. Insufficient homology with morphological early life cycle data and molecular data for *A. moseri* indicates that *A. morandinii* is not this species but as these data are lacking for *A. grandis* and *A. rainensis*, it is not yet possible to confirm that *A. morandinii* is not one of these species. In the future, when additional molecular data are available for all valid alatinid species, and mature medusae of *A. morandinii* are collected or reared, we plan to test the hypothesis of *A. morandinii*'s synonymy with either of these valid *Alatina* species. But until then, the taxonomic status of *Carybdea morandinii* Straehler-Pohl & Jarms, 2011 is changed at a generic level to *Alatina morandinii* (Straehler-Pohl & Jarms 2011) and the species name still considered as valid.

Systematics

Phylum Cnidaria Verrill, 1865

Subphylum Medusozoa Petersen, 1979

Class Cubozoa Werner, 1973

Order Carybdeida (Lesson 1843)

Family Alatinidae Gershwin, 2005

Genus *Alatina* Gershwin, 2005

Alatina morandinii (Straehler-Pohl & Jarms 2011)

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