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Evolution of box jellyfish (Cnidaria: Cubozoa), a group of highly toxic invertebrates

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Cubozoa (Cnidaria: Medusozoa) represents a small clade of approximately 50 described species, some of which cause serious human envenomations. Our understanding of the evolutionary history of Cubozoa has been limited by the lack of a sound phylogenetic hypothesis for the group. Here, we present a comprehensive cubozoan phylogeny based on ribosomal genes coding for near-complete nuclear 18S (small subunit) and 28S (large subunit) and partial mitochondrial 16S. We discuss the implications of this phylogeny for our understanding of cubozoan venom evolution, biogeography and life-history evolution. Our phylogenetic hypothesis suggests that: (i) the last common ancestor of Carybdeida probably possessed the mechanism(s) underlying Irukandji syndrome, (ii) deep divergences between Atlantic and Indo-Pacific clades may be explained by ancient vicariant events, and (iii) sexual dimorphism evolved a single time in concert with complex sexual behaviour. Furthermore, several cubozoan taxa are either para- or polyphyletic, and we address some of these taxonomic issues by designating a new family, Carukiidae, a new genus, *Copula*, and by redefining the families Tamoyidae and Tripedaliidae. Lastly, cubozoan species identities have long been misunderstood and the data presented here support many of the recent scientific descriptions of cubozoan species. However, the results of a phylogeographic analysis of *Alatina moseri* from Hawai'i and *Alatina mordens* from Australia indicate that these two nominal species represent a single species that has maintained metapopulation cohesion by natural or anthropogenic dispersal.

Keywords: Cubozoa; box jellyfish; Irukandji; systematics; biogeography; phylogeography

1. INTRODUCTION

Although Cubozoa is the smallest class of Cnidaria, comprising some 50 described box jellyfish species, it is well known for several remarkable attributes. From the possession of complex eyes and associated visual capabilities (e.g. Nilsson *et al.* 2005), to extraordinary courtship and mating behaviour (e.g. Lewis & Long 2005), to extreme toxicity (e.g. Brinkman & Burnell 2009), there are many reasons why cubozoans catch the attention of the scientific community and public. Despite this interest, studies of cubozoan evolution have been hampered by a paucity of specimens in natural history museums preserved for both morphological and molecular investigation, as well as by their perceived lack of diversity. The last decade has seen more than a doubling in recognized cubozoan species, but so far a robust phylogenetic framework for investigating the evolution of cubozoan diversity has been missing. We present a comprehensive phylogeny for Cubozoa and use it to discuss the evolution of venom, life history and biogeography.

2. MATERIAL AND METHODS

(a) *Phylogenetic inference*

A list of specimens used for this study is provided in the electronic supplementary material, table S1, including museum catalogue numbers where vouchers exist. Tentacle tissue was preserved in pure EtOH or saturated salt DMSO buffer (Dawson *et al.* 1998), from which DNA was extracted using organic phenol–chloroform extraction protocols according to the procedure outlined in Collins *et al.* (2008) or using the automated DNA isolation system AutoGenPrep 965 (AutoGen Inc., Holliston, MA, USA) following the manufacturer's protocol. Ribosomal genes coding for partial mitochondrial 16S (16S) and near-complete nuclear 18S (small subunit; SSU) and 28S (large subunit; LSU) were amplified using the primers and protocols outlined in Cartwright *et al.* (2008) and Collins *et al.* (2008). PCR products were either sequenced by Cogenics (Houston, TX, USA) or at the Laboratory of Analytical Biology, Smithsonian Institution (Suitland, MD, USA). Trace files were assembled in SEQUENCHER (v. 4.8; Gene Codes, MI) and subsequently aligned using MUSCLE (v. 3.7; Edgar 2004). Highly variable, poorly aligned regions were

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removed from the final alignments using Gblocks (v. 0.91b; Castresana 2000) with the default parameters except that allowed gap positions were set to half. Nucleotide sequences were deposited in NCBI GenBank (electronic supplementary material, table S1) and alignments used for analyses were deposited in TreeBASE (treebase.org).

In addition to the alignments for each gene, all three were combined into a concatenated alignment. These four alignments were analysed using maximum parsimony (MP) in PAUP* (Swofford 2003) and maximum likelihood (ML) in RaxML (v. 7.0.3; Stamatakis 2006). MrModelTest (v. 2.3; Nylander 2004) was used to evaluate nucleotide substitution models for ML analyses. The concatenated dataset was partitioned by gene for analyses and number of invariant sites and gamma shape parameters were calculated separately for each partition in RaxML.

To establish the root of the cubozoan phylogeny, SSU and LSU sequences of both cubozoan taxa and outgroup taxa were aligned using Muscle, subsequently pruned using Gblocks and analysed with ML and MP using the criteria described above. Since the large divergence between ingroup and outgroup for the 16S gene does not allow for reliable alignment, we decided *a priori* not to analyse this marker using an outgroup.

In order to investigate the possibility of strongly supported character conflict among partitions in the combined datasets, we performed an incongruence length difference (ILD) test (Farris *et al.* 1995*a*,*b*) as implemented in PAUP*. The ILD test has often been used as a test of combinability of datasets for phylogenetic analyses (e.g. Cunningham 1997), but interpretation of ILD test results has been the subject of debate (e.g. Barker & Lutzoni 2002).

(b) Alatina *phylogeography*

Mitochondrial 16S of seven specimens of *Alatina mordens* from Osprey Reef (Coral Sea, Queensland, Australia) and 19 specimens of *Alatina moseri* from Waikiki (O'ahu, HI, USA) were amplified and sequenced using the same techniques as above (GenBank nos. GQ506980–GQ507005 associated with USNM voucher specimens). All sequences were aligned using Muscle and the beginning and end of the alignment were trimmed to the position at which the nucleotides for every specimen are known. A statistical parsimony haplotype network was calculated in TCS (v. 1.21; Clement *et al.* 2000) using the 95 per cent connection limit criterion and gaps treated as a fifth character state.

3. RESULTS

(a) *Phylogeny of Cubozoa*

The partition-homogeneity test, with 100 replicates, could not refute the null hypotheses of congruence among partitions in the combined datasets LSU/SSU with outgroup ($p = 0.38$; electronic supplementary material, figure S1) and LSU/SSU/16S without outgroup ($p = 0.56$; figure 1), suggesting the absence of strong conflict among partitions.

Phylogenetic analyses under both MP (not shown) and ML lead to highly congruent results, and node support is similar under both ML and MP (figure 1; electronic supplementary material, figures S1–S6). Both Chirodropida and Carybdeida are monophyletic clades with the root of Cubozoa falling in between the two in the SSU and combined LSU/SSU datasets with outgroup (electronic supplementary material, figures S1 and S3). Monophyly of Carybdeida is weakly contradicted in the LSU analysis (electronic supplementary material, figure S2); several deep nodes receive lower support when an outgroup is included in LSU analyses (compare figures S2 and S4, electronic supplementary material). In general, LSU and SSU analyses do not strongly disagree with one another; contradictory relationships are weakly supported (compare figures S2–S5, electronic supplementary material).

The fastest evolving marker, 16S (electronic supplementary material, figure S6), shows much congruence with both SSU and LSU (electronic supplementary material, figures S4 and S5). One point of difference involves Alatinidae and Tripedaliidae. These families group together in the 16S-based phylogeny (electronic supplementary material, figure S6), as well as in SSU-based phylogeny without outgroup (albeit without support; electronic supplementary material, figure S5). This putative clade uniting Alatinidae and Tripedaliidae seems surprising, but may be explained by nucleotide saturation. 16S evolves much more rapidly than both LSU and SSU, thus leading to higher degrees of nucleotide saturation that may confound signal at the deeper nodes. The addition of LSU data appears to overcome signal, artificial or otherwise, from 16S and SSU data. Indeed, a combined analysis of all three genes (figure 1) leads to a phylogenetic hypothesis that is most congruent with the LSU dataset. Despite the incongruence in the placement of Tripedaliidae between markers, the strongest support for its placement occurs in the combined analysis, where it is recovered as sister to Carybdeidae.

(b) Alatina *phylogeography*

The final alignment of *Alatina* 16S sequences contained 545 sites; of the 28 variable characters, 10 were parsimony informative. We found 20 unique haplotypes and uncorrected pairwise distances among haplotypes did not exceed 1.84 per cent. The haplotype network (figure 2) shows that haplotypes of both *A. moseri* and *A. mordens* are not reciprocally monophyletic and appear inter-digitated. The most common mt16S haplotype ($n = 4$) was found in two specimens of each *A. moseri* and *A. mordens*.

4. DISCUSSION

(a) *Phylogenetic analyses and signal*

Since evolutionary rates differ from gene to gene, some incongruence among topologies using different gene trees is not surprising. Combined analysis of all genes should lead to a better estimate of the evolutionary relationships of taxa compared with single-gene analyses (Gadagkar *et al.* 2005). We find alignment quality to be much improved for the ingroup when excluding highly divergent outgroup taxa. Thus, combined analysis excluding outgroup taxa (figure 1) should represent the best estimate of evolutionary relationships in Cubozoa to date.

Previous phylogenetic analyses of Medusozoa (Collins 2002; Collins *et al.* 2006) were limited in their sampling within Cubozoa. Increased taxon sampling lends itself to begin investigating several questions concerning the evolution of cubozoan toxicity, behaviour and biogeography. Further, it becomes clear that the taxonomic framework at the family level *sensu* Daly *et al.* (2007) is inconsistent with a phylogenetic approach to taxonomy.

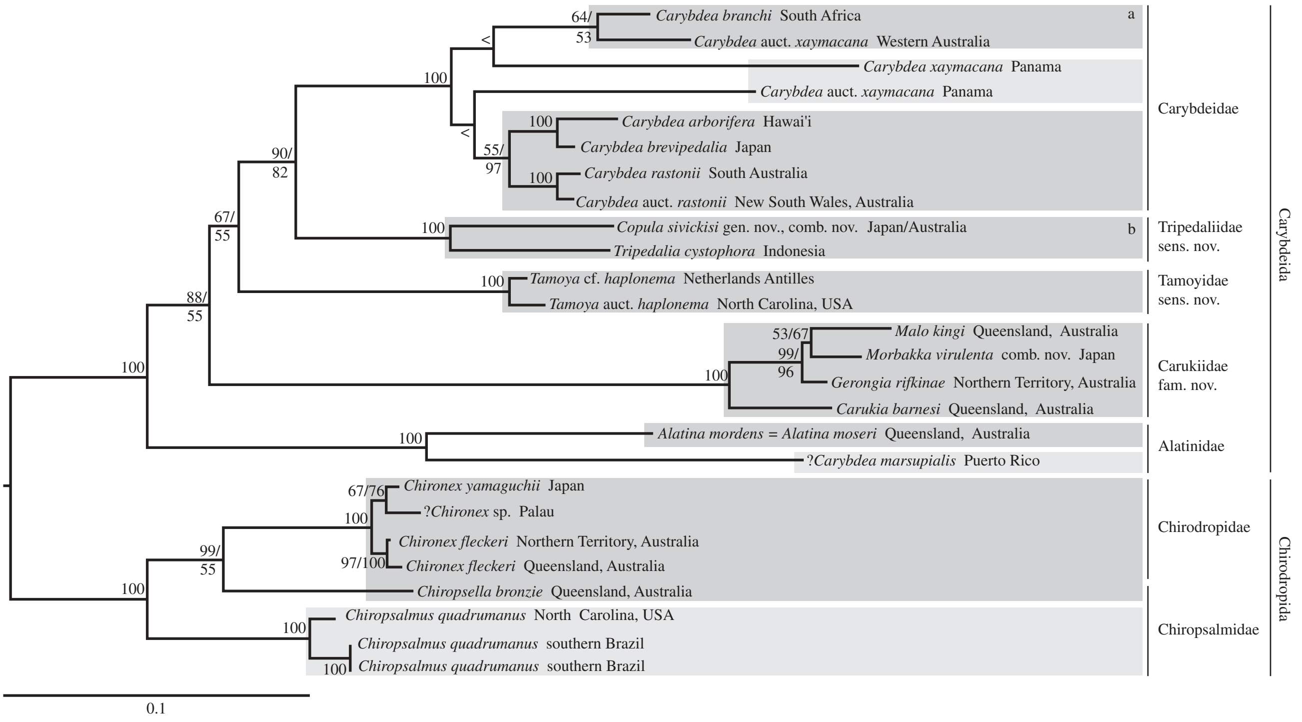

Figure 1. Maximum likelihood topology (under GTR + I + G) of the combined nuclear LSU, SSU and mitochondrial 16S dataset. The alignment contains 5546 characters (LSU, 3292 characters; SSU, 1777 characters and 16S: 486 characters), of which 4369 are invariant and 936 parsimony informative. ML/MP parametric bootstrap support values (1000 replicates) are indicated on each node; if only one value is given it applies to both ML and MP. Less than symbol, bootstrap support less than 50; dark grey, Indo-Pacific; light grey, Atlantic/Caribbean; a, includes SE Atlantic and b, both nominal species also occur in the Caribbean.

Chiropsalmidae, Tamoyidae and Carybdeidae are probably para- or polyphyletic. Consequently, we amend the diagnoses of several taxa or designate new taxa to establish monophyly (changes reflected in figures and table). However, we choose to leave Chiropsalmidae unchanged, as we are missing several chirodropid genera in our analyses.

(b) *Toxicity*

The evolution of venom in Cubozoa is of significant interest, as many cubozoans are known to be highly toxic (e.g. Williamson *et al.* 1996), resulting in major costs to public health and the tourism industry, particularly in Australia (e.g. Bailey *et al.* 2003). Efforts have led to the characterization of some venom components of a few cubozoan species (see Brinkman & Burnell 2009 for a review) and the development of an antivenom for the deadly cubozoan *Chironex fleckeri* (see Currie 2003). In order to enhance data interpretation and risk management, a historical framework providing a clear understanding of species identities and systematics is vital. In addition to retrospective interpretation of venom data, the phylogenetic framework we present here is relevant for phylogenetic forecasting. That is, close relatives of a highly toxic species are more likely than not highly toxic as well.

Toxicity varies from species to species with some being completely harmless to humans while others can cause death within minutes. The chirodropid *C. fleckeri* is considered the most lethal jellyfish known (Wiltshire *et al.* 2000). Not surprisingly, its close relative *Chironex yamaguchii* has caused human fatalities in Japan and the Philippines (Fenner & Williamson 1996; Fenner 1997 (both as *Chiropsalmus quadrigatus*); Lewis & Bentlage 2009). By contrast, *Chiropsalmus* and *Chiropsella* species are considered much less dangerous (but see Bengtson *et al.* 1991). Differences in toxicity among chirodropids may be explained by differences in the amount of tentacle surface area, and consequently, the amount of venom that can be delivered (see Nagai 2003). Interestingly, an unvouchered tissue specimen from Palau appears to be closely related to *C. yamaguchii* from Japan, raising questions about the toxicity and identity of this chirodropid.

In contrast to the notion that chirodropids represent the most lethal box jellyfishes, haemolytic activity of purified toxin proteins appears lower in *C. yamaguchii* (Nagai *et al.* 2002; as *C. quadrigatus*) than in *Alatina* sp. (Nagai *et al.* 2000*a*; as *Carybdea alata*) and highest in *Carybdea brevipedalia* (Nagai *et al.* 2000*b*; as *C. rastoni* [*sic*]). Similarly, lethal doses of venom appear much lower in *C. brevipedalia* when compared with *Alatina* sp. and *C. yamaguchii* (Nagai 2003). Note, however, that these haemolytic assays do not appear to have been standardized among treatments, potentially making direct comparisons unreliable. Nonetheless, Bailey *et al.* (2005) also reported higher haemolytic activities in a species of *Carybdea* compared with two chirodropid species. However, haemolytic activity does not appear to be the lethal factor in the venoms investigated (Bailey *et al.* 2005), and haemolytic proteins represent only a fraction of the proteins present in cubozoan venom (Chung *et al.* 2001).

Sequencing of haemolytic proteins demonstrated two carybdeid and three chirodropid protein toxins to display

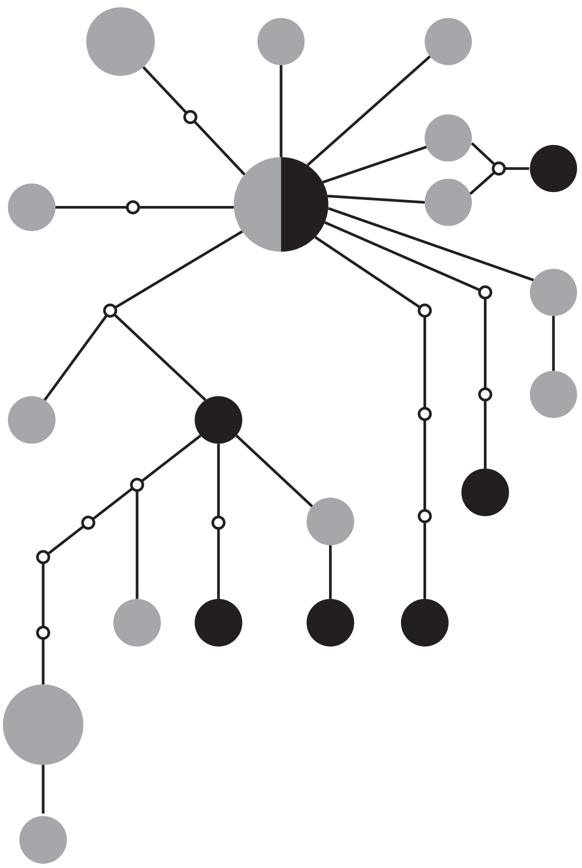

Figure 2. Statistical parsimony network for mitochondrial 16S sequences of *Alatina* spp. from Hawai'i (*A. moseri*) and Australia (*A. mordens*) as calculated by TCS under the 95% connection limit criterion. Gaps were treated as a fifth character state. *Lines* represent one mutational step; s*mall hollow circles* correspond to inferred alleles that have not been sampled. The area of each respective solid circle reflects the number of alleles represented; the smallest solid circles represent a single allele. Grey circle, Waikiki, Honolulu, Hawai'i; black circle, Osprey Reef, Coral Sea, Australia.

a moderate amount of divergence (Nagai 2003; Brinkman & Burnell 2009). Despite reported differences among toxin protein sequences within Cubozoa, secondary structure models suggest at least two shared structural motifs that may be related to cytolytic activity (Brinkman & Burnell 2009 and references cited therein). Thus far no homologous protein outside of Cubozoa has been identified, suggesting that cubozoan venoms may contain a novel and unique family of proteins (Brinkman & Burnell 2009).

Several cubozoan species are known to cause a set of symptoms called Irukandji syndrome. Initially, Irukandji syndrome was attributed to *Carukia barnesi* whose sting causes a sharp prickling sensation without visible injury (Barnes 1964). Systemic effects are delayed by minutes to hours and include severe low back pain, progressing to limb cramping, nausea, vomiting, headache, restlessness and 'a feeling of impending doom' (Barnes 1964; Fenner 2006). Despite strong systemic effects Irukandji syndrome caused by *C. barnesi* is not considered life-threatening (Barnes 1964). Since its original description, the syndrome has been reported from, or attributed to, other cubozoans: *Morbakka* (Fenner *et al.* 1985), *Tamoya* (Morandini & Marques 1997), *Malo* (Gershwin 2005*a*, 2007), *Alatina* (Yoshimoto & Yanagihara 2002 (as *C. alata*); Gershwin 2005*b*; Little *et al.* 2006) and *Gerongia* (Gershwin & Alderslade 2005).

Usually Irukandji syndrome in cubozoans other than *Carukia* is referred to as Irukandji-like syndrome (or as Morbakka syndrome by *Morbakka*; Fenner *et al.* 1985). Irukandji-like syndrome shares the same basic symptoms of classic Irukandji syndrome, but may be less severe in some species or even more severe in others. For example, a more severe case causing a fatality off North Queensland, Australia, was attributed to *C. barnesi*'s close relative *Malo kingi* (Fenner & Hadok 2002; Gershwin 2007; but see Bailey 2003). Thus far, very little is known about the mechanism(s) underlying Irukandji syndrome. Thorough toxicological studies of Irukandji-causing species from disparate clades should clarify the function and nature of the syndrome.

While disparate clades in Cubozoa contain Irukandji-causing species, all are part of Carybdeida. Irukandji syndrome is particularly well documented for species of Tamoyidae *sensu* Daly *et al.* (2007). Interestingly, *Tamoya* consistently falls outside Tamoyidae (figure 1; electronic supplementary material, figures S1–S6) and is easily distinguishable from its other genera, *Carukia*, *Malo*, *Gerongia* and *Morbakka*. Hence, we amend the meaning of Tamoyidae Haeckel, 1880 to contain all those carybdeid medusae that possess frown-shaped rhopaliar niche ostia lacking rhopaliar horns (type genus *Tamoya* Müller, 1859). We propose the new family Carukiidae, with type genus *Carukia* Southcott, 1967, to contain those carybdeids that lack gastric filaments and possess frown-shaped rhopaliar niche ostia with rhopaliar horns (genera *Carukia*, *Malo* Gershwin, 2005, *Gerongia* Gershwin & Alderslade, 2005 and *Morbakka* Gershwin, 2008). Both Tamoyidae sens. nov. and Carukiidae branch before Carybdeidae and Tripedaliidae, while Alatinidae represents the earliest diverging carybdeid clade (?*Carybdea marsupialis* may be misidentified; see below). This topology suggests that the last common ancestor of Carybdeida probably possessed the mechanism(s) underlying Irukandji syndrome (figure 3). Further, the ability to cause Irukandji syndrome may have been lost in the lineage leading to Carybdeidae and Tripedaliidae (figure 3; a species of *Carybdea* was linked to Irukandji syndrome (Little *et al.* 2006), but this attribution appears unconfirmed (Gershwin 2006*a*)). A syndrome described as Irukandji-like may be caused by a couple of non-cubozoan species (e.g. Fenner *et al.* 1996 (*Stomolophus nomurai*); Fenner 1998 (*Gonionemus* and *Physalia*)), but homology will remain obscure until the mechanism(s) underlying the syndrome are clarified.

Even with a robust phylogeny, several problems hamper cubozoan venom studies. Difficulties in extracting venom, the use of whole tentacle tissue instead of isolated nematocysts and contradictory results among research groups (Brinkman & Burnell 2009 and references cited therein) need to be addressed. Furthermore, toxins have been reported to differ among different body parts of specimens and possibly different ontogenetic stages (Brinkman & Burnell 2009 and references cited therein). Finally, taxonomic uncertainties and resulting misidentification may impede toxicological research. For example, the number of bioactive proteins isolated from *C. marsupialis* from the Mediterranean

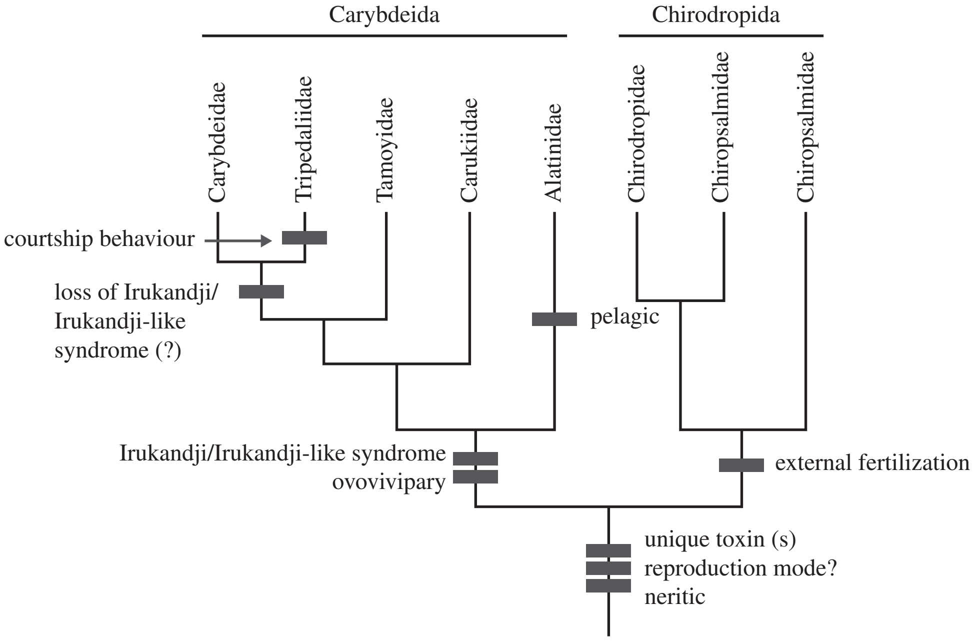

Figure 3. Trends in toxicity and life-history evolution; phylogenetic relationships follow figure 1.

(Rottini *et al.* 1995) differs from that of the same nominal species from the Caribbean (Sanchez-Rodriguez *et al.* 2006) leading to the interpretation of intraspecific venom variation. However, true *C. marsupialis* from the Mediterranean is easily distinguishable from its congeners in the Caribbean (i.e. *Carybdea xaymacana* and *C.* auct. *xaymacana*) by their gastric phacellae. The existence of *C. marsupialis* in the Caribbean is most probably an example of taxonomic confusion.

(c) *Courtship behaviour*

Our working hypothesis with increased taxon sampling supports the preliminary finding that *Carybdea sivickisi* is more closely related to *Tripedalia cystophora* than it is to any species of *Carybdea* (Collins 2002; Collins *et al.* 2006). To retain monophyly of *Carybdea*, we designate the new genus *Copula* to accommodate *Carybdea sivickisi* Stiasny, 1926; the name is in reference to the well-documented courtship behaviour and sexual dimorphism (see below). We amend the meaning of Tripedaliidae Conant, 1897 to contain all carybdeids that display sexual dimorphism of the gonads, produce spermatophores and in which at least the males possess subgastral sacs/seminal vesicles (see Hartwick 1991). Species of *Carybdea* Péron & Lesuer, 1810, the sole genus within Carybdeidae Gegenbauer, 1857, can readily be differentiated from all other cubozoans by their possession of heart-shaped rhopaliar niche ostia (see Gershwin 2005*b* for diagnoses of *Carybdea* and Carybdeidae). The new genus *Copula* is defined to contain tripedaliids that possess adhesive pads on the exumbrellar apex with which they attach themselves to substrates when resting (see Hartwick 1991); its type species is *Copula sivickisi* (Stiasny, 1926).

Tripedaliid life histories are unique among Cubozoa and Cnidaria. In *Copula sivickisi*, a mature male and female engage in sexual activity by entangling their tentacles. While swimming as a couple, the male brings its oral opening close to that of the female and produces a spermatophore that is ingested by the female (Lewis & Long 2005; Lewis *et al.* 2008). The subsequent gestation period spans some 2–3 days after which an embryo strand is released into the water column (Lewis & Long 2005; Lewis *et al.* 2008). Sexual dimorphism of medusae and similar courtship behaviour were documented by Werner (1973) for *T. cystophora*, but he did not observe fertilization, gestation or embryo release. However, in contrast to the production of an embryo strand, *T. cystophora* seems to release free-swimming planulae (Conant 1898).

Species of both Carybdeidae and Alatinidae appear to be ovoviviparous and eggs are fertilized internally after female medusae have taken up sperm released into the water column by males during spawning aggregations (Studebaker 1972; Arneson 1976). Neither courtship behaviour nor sexual dimorphism appears to be present in these two families. Further, embryos are released within minutes to hours after fertilization (Studebaker 1972; Arneson 1976). To our knowledge, reproductive strategies of both Tamoyidae sens. nov. and Carukiidae remain undocumented. In Chirodropida, Yamaguchi & Hartwick (1980) reported external fertilization for medusae of both *C. fleckeri* and *Chiropsella bronzie* (as *C. quadrigatus*). While information from Carukiidae and Tamoyidae are needed, it appears that internal fertilization is a synapomorphy of Carybdeida. Further, we suggest that sexual dimorphism evolved a single time concomitant with complex sexual behaviour (figure 3).

(d) *Biogeography*

In general, cubozoan distributions are not well documented on intermediate geographical scales (e.g. provinces, states or countries) owing to a lack of sampling, which hampers biogeographic enquiries at this scale (see Bentlage *et al.* 2009 for a possible strategy to address this issue). However, on larger scales (e.g. ocean basins) several patterns emerge in light of our results. In particular, we uncovered numerous deep divergences among Indo-Pacific and Atlantic clades (figure 1).

In Chirodropida, the genus *Chiropsalmus* is exclusively Atlantic, whereas the confamilial *Chiropsella* is from the Indo-Pacific groups with the exclusively Indo-Pacific *Chironex* (family Chirodropidae). Similarly, what had been recognized as Tamoyidae (Tamoyidae sens. nov. plus Carukiidae) can also be divided geographically: Tamoyidae is restricted to the Atlantic and the described species of Carukiidae are known from Australia and Japan, but probably range throughout the Indo-Pacific (Cleland & Southcott 1965; B. Bentlage 2009, unpublished notes). The pattern within Alatinidae and Tripedaliidae is unclear owing to limited taxon sampling. Both sampled species of Tripedaliidae, *Copula sivickisi* and *T. cystophora*, can be found in all three oceans and future studies should seek to determine if these species are truly circumtropical or flocks of regional species.

Our densest sampling is in *Carybdea*, but unfortunately, relationships among Indo-Pacific and Atlantic/Caribbean taxa lack support, so it is unclear whether there are deep divergences separating lineages into exclusively Atlantic/Caribbean and Indo-Pacific clades (figure 1). Nonetheless, integrating phylogeny and taxonomic investigations suggests that *Carybdea* spp. are more restricted in their geographical distributions than has been recognized by most workers. For example, *C. xaymacana* has been sampled from both the Caribbean and Western Australia, but deep divergence indicates crypticism in this nominal species. Similarly, *Carybdea rastonii* has traditionally been viewed as having a wide distribution with occurrence records from South Australia, Hawai'i and Japan among others. Our sampling shows that this is also a case of numerous species being united under the same name. Examination of the specimens suggests that *C. rastonii* can be distinguished morphologically (Gershwin & Gibbons 2009; B. Bentlage 2009, unpublished notes). Rather than having cryptic species in the sense that they are indistinguishable morphologically, this appears to be a case in which species have been proposed historically (*C. rastonii* Haacke, 1886 (South Australia), *C. brevipedalia*[1] Kishinouye, 1891 (Japan) and *Carybdea arborifera* Maas, 1897 (Hawai'i)) but subsequently synonymized and/or disregarded.

Discovering that widespread nominal *Carybdea* spp. represent geographically isolated species assemblages indicates that these medusae do not exchange genetic material across large bodies of open water. Hence, we suppose that speciation in the genus is largely driven by vicariance. Dispersal events, however, cannot be ruled out as a source to account for diversification. For instance, *C. arborifera* probably arose in Hawai'i after long-range dispersal; islands have existed at the present position of the Hawaiian Islands from at least the late Paleocene onwards, but were always remote (Carson & Clague 1995).

The inability to cross open ocean habitats is most likely a widespread phenomenon in Cubozoa, as most species appear to inhabit near shore habitats above the continental shelves (i.e. the neritic zone). Considering this, deep divergences between Atlantic and Indo-Pacific clades of Cubozoa may be explained by ancient vicariant events. Unfortunately fossil jellyfishes are rare, leading to uncertainty in dating cladogenetic events (Cartwright & Collins 2007).

However, fossils that possibly represent cubozoans have been discovered from the upper Jurassic (*Quadrumedusina quadrata* Haeckel, 1869), upper Carboniferous (*Anthracomedusa turnbulli* Johnson & Richardson 1968) and the middle Cambrian (Cartwright *et al.* 2007). Given their neritic habitat, cubozoans probably diversified as a result of plate movements in concert with eustatic sea-level fluctuations; the splitting of Pangea could have provided the setting for this.

In contrast to the general pattern described above, we have evidence for a pelagic cubozoan. The genus *Alatina* is represented with several nominal species in the Pacific (see Gershwin 2005*b*) from which we sampled *A. moseri* Mayer, 1906 and *A. mordens* Gershwin, 2005 from or nearby their type localities (Hawai'i and the Coral Sea, respectively). We found no genetic divergences corresponding to geographical locality; in fact both 'species' share at least one 16S haplotype (figure 2). Additionally, no clear pattern differentiating the two populations exists. Rather, the haplotype network reflects a well-mixed population with regular gene flow. Inspection of specimens (including Hawaiian-type material USNM 22308, 22311 and 29632) and study of its original description (Mayer 1906) demonstrates that *A. moseri* has been present in Hawai'i at least since the beginning of the twentieth century. The initial discovery of *Alatina* spp. in Australia seems not as well documented as is true for many marine invertebrates from this continent.

In contrast to other cubozoans, *Alatina* spp. live at or close to the edge of the continental shelf (Arneson & Cutress 1976; as *C. alata*) and have been obtained from great water depths before (e.g. Morandini 2003; as *C. alata*). It seems that *A. moseri* can only be encountered in shallow waters several days after the full moon (e.g. Thomas *et al.* 2001; Yanagihara *et al.* 2002; both as *C. alata*) when individuals congregate to spawn; the same is true for *A. mordens* (T. Carrette & J. Seymour 2008, personal communication). Furthermore, it has been suggested that individuals of *Alatina* spp. live up to 12 months (Arneson & Cutress 1976). Hence, it seems quite possible that *Alatina* spp. have an oceanic lifestyle and are able to maintain cohesive metapopulations across ocean basins.

Our investigation of historic specimens demonstrates that *A. moseri* was present in Hawai'i more than a century ago. An early introduction of *A. moseri* into Hawai'i is possible, but it seems unlikely that this would have occurred from the Coral Sea, given that ship traffic from Australia to Hawai'i was probably low at the time and the observed genetic signal would suggest multiple introductions rather than a single one. Considering the possible effect World War II naval traffic had on the spread of marine organisms (e.g. Coles *et al.* 1999), *A. moseri* may conversely have been introduced into the Coral Sea. Indeed, it is conceivable that *A. moseri* was introduced into the Coral Sea from Hawai'i and prior to that into Hawaiian waters from yet another location. However, considering the life cycle of *A. moseri* (and the synonymous *A. mordens*), we find dispersal by natural means a more viable explanation of the pattern we observe. Investigation of additional *Alatina* spp. may show that some of these also represent artificial taxonomic units.

(e) **Carybdea marsupialis***: a model organism misidentified?*

We recover *C. marsupialis* together with *A. moseri* as the sister group to the remaining carybdeids. This placement appears surprising considering the stark morphological differences between *Carybdea* spp. and *Alatina* spp. (compare Gershwin & Gibbons 2009 with Gershwin 2005*b*). Specimens of *C. marsupialis* for this and other studies (Collins 2002; Collins *et al.* 2006) are derived from a polyp culture at the museum of the University of Hamburg, Germany. To our knowledge these polyps were originally obtained by B. Werner some 40 years ago in La Paguera, Puerto Rico (A. C. Arneson 2008, personal communication) and used for life cycle studies (e.g. Werner *et al.* 1971; Straehler-Pohl & Jarms 2005). *Alatina* spp. can be found in Puerto Rico, and considering the placement of *C. marsupialis* as a close relative of a member of Alatinidae, it is possible that the culture in Hamburg actually contains the polyp stage of a species of *Alatina* rather than *Carybdea*. Since this particular culture has served as the stock for several important experiments on *Carybdea* development (e.g. Stangl *et al.* 2002; Fisher & Hofmann 2004; Straehler-Pohl & Jarms 2005), it is vital to confirm the identification of the polyps by either rearing medusae to adulthood or collecting fresh material from the Caribbean for genetic comparisons. Inclusion of *C. marsupialis* from close to its type locality in Italy in future phylogenetic studies should also help shed light on this issue.

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ENDNOTE

[1] The name *C. brevipedalia* is not in widespread usage, but its original description and type locality (Kishinouye 1891) demonstrate that it is the senior synonym of the name *C. mora* Kishinoye, 1910, recently used as valid in Gershwin (2006*b*) and Gershwin & Gibbons (2009).

REFERENCES

Arneson, A. C. 1976 Life history of *Carybdea alata* Reynaud, 1830 (Cubomedusae). M.S. thesis, University of Puerto Rico, Mayagüez, Commonwealth of Puerto Rico, USA.

Arneson, A. C. & Cutress, C. E. 1976 Life history of *Carybdea alata* Reynaud, 1830 (Cubomedusae). In *Coelenterate ecology and behavior* (ed. G. O. Mackie), pp. 227–236. New York, NY: Plenum Press.

Bailey, P. M. 2003 Fatal envenomation by jellyfish causing Irukandji syndrome. *Med. J. Aust.* **178**, 139–140.

Bailey, P. M., Little, M., Jelinek, G. A. & Wilce, J. A. 2003 Jellyfish envenoming syndromes: unknown toxic mechanisms and unproven therapies. *Med. J. Aust.* **178**, 34–37.

Bailey, P. M., Bakker, A. J., Seymour, J. E. & Wilee, J. A. 2005 A functional comparison of the venom of three Australian jellyfish—*Chironex fleckeri*, *Chiropsalmus* sp., and *Carybdea xaymacana*—on cytosolic Ca^{2+}, haemolysis and *Artemia* sp. lethality. *Toxicon* **45**, 233–242. (doi:10.1016/j.toxicon.2004.10.013)

Barker, F. K. & Lutzoni, F. M. 2002 The utility of the incongruence length difference test. *Syst. Biol.* **51**, 625–637. (doi:10.1080/10635150290102302)

Barnes, J. H. 1964 Cause and effect in Irukandji stingings. *Med. J. Aust.* **1**, 897–904.

Bengtson, K., Nichols, M. M., Schnadig, V. & Ellis, M. D. 1991 Sudden death in a child following jellyfish envenomation by *Chiropsalmus quadrumanus*. Case report and autopsy findings. *J. Am. Med. Assoc.* **266**, 1404–1406. (doi:10.1001/jama.266.10.1404)

Bentlage, B., Peterson, A. T. & Cartwright, P. 2009 Inferring distributions of chirodropid box-jellyfishes (Cnidaria: Cubozoa) in geographic and ecological space using ecological niche modeling. *Mar. Ecol. Prog. Ser.* **384**, 121–133. (doi:10.3354/meps08012)

Brinkman, D. L. & Burnell, J. N. 2009 Biochemical and molecular characterisation of cubozoan protein toxins. *Toxicon* **54**, 1162–1173. (doi:10.1016/j.toxicon.2009.02.006)

Carson, H. L. & Clague, D. A. 1995 Geology and biogeography of the Hawaiian Islands. In *Hawaiian biogeography—evolution on a hot spot archipelago* (eds W. L. Warren & V. A. Funk), pp. 14–29. Washington, DC: Smithsonian Institution Press.

Cartwright, P. & Collins, A. G. 2007 Fossils and phylogenies: integrating multiple lines of evidence to investigate the origin of early major metazoan lineages. *Integr. Comp. Biol.* **47**, 744–751. (doi:10.1093/icb/icm071)

Cartwright, P., Halgedahl, S. L., Hendricks, J. R., Jarrard, R. D., Marques, A. C., Collins, A. G. & Lieberman, B. S. 2007 Exceptionally preserved jellyfishes from the middle Cambrian. *PLoS ONE* **2**, e1121. (doi:10.1371%2Fjournal.pone.0001121)

Cartwright, P., Evans, N. M., Dunn, C. W., Marques, A. C., Miglietta, M. P., Schuchert, P. & Collins, A. G. 2008 Phylogenetics of Hydroidolina (Cnidaria: Hydrozoa). *J. Mar. Biol. Assoc. UK* **88**, 1663–1672. (doi:10.1017/S0025315408002257)

Castresana, J. 2000 Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol. Biol. Evol.* **17**, 540–552.

Chung, J. L., Ratnapala, L. A., Cooke, I. M. & Yanagihara, A. A. 2001 Partial purification and characterization of a hemolysin (CAH1) from Hawaiian box jellyfish (*Carybdea alata*) venom. *Toxicon* **39**, 981–990. (doi:10.1016/S0041-0101(00)00237-3)

Cleland, J. B. & Southcott, R. V. 1965 Injuries to man from marine invertebrates in the Australian region. National Health and Research Council, Special Report Series 12, 282 p., Canberra, Australia.

Clement, M., Posada, D. & Crandall, K. 2000 TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* **9**, 1657–1660. (doi:10.1046/j.1365-294x.2000.01020.x)

Coles, S. L., DeFelice, R. C., Eldredge, L. G. & Carlton, J. T. 1999 Historical and recent introductions of non-indigenous marine species into Pearl Harbor, Oahu, Hawaiian Islands. *Mar. Biol.* **135**, 147–158. (doi:10.1007/s002270050612)

Collins, A. G. 2002 Phylogeny of Medusozoa and the evolution of cnidarian life cycles. *J. Evol. Biol.* **15**, 418–432. (doi:10.1046/j.1420-9101.2002.00403.x)

Collins, A. G., Schuchert, P., Marques, A. C., Jankowski, T., Medina, M. & Schierwater, B. 2006 Medusozoan phylogeny and character evolution clarified by new large and small subunit rDNA data and an assessment of the utility of phylogenetic mixture models. *Syst. Biol.* **55**, 97–115. (doi:10.1080/10635150500433615)

Collins, A. G., Bentlage, B., Lindner, A., Lindsay, D., Haddock, S. H. D., Jarms, G., Norenburg, J. L., 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)

Conant, F. S. 1898 The Cubomedusae. *Mem. Biol. Lab. Johns Hopkins Univ.* **4**, 1–61.

Cunningham, C. W. 1997 Can three incongruence tests predict when data should be combined? *Mol. Biol. Evol.* **14**, 733–740.

Currie, B. J. 2003 Marine antivenoms. *J. Toxicol. Clin. Toxicol.* **41**, 301–308. (doi:10.1081/CLT-120021115)

Daly, M. *et al.* 2007 The phylum Cnidaria: a review of phylogenetic patterns and diversity three hundred years after Linneaeus. *Zootaxa* **1668**, 127–182.

Dawson, M. N., Raskoff, K. A. & Jakobs, D. A. 1998 Field preservation of marine invertebrate tissue for DNA analyses. *Mol. Mar. Biol. Biotechnol.* **7**, 145–152.

Edgar, R. C. 2004 MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucl. Acids Res.* **32**, 1792–1797. (doi:10.1093/nar/gkh340)

Farris, J. S., Källersjö, M., Kluge, A. G. & Bult, C. 1995*a* Constructing a significance test for incongruence. *Syst. Biol.* **44**, 570–572.

Farris, J. S., Källersjö, M., Kluge, A. G. & Bult, C. 1995*b* Testing significance of incongruence. *Cladistics* **10**, 315–319. (doi:10.1111/j.1096-0031.1994.tb00181.x)

Fenner, P. J. 1997 The global problem of cnidarian (jellyfish) stinging. M.D. thesis, University of London, London, UK.

Fenner, P. J. 1998 Dangers in the ocean: the traveller and marine envenomation. *J. Travel Med.* **5**, 135–141. (doi:10.1111/j.1708-8305.1998.tb00487.x)

Fenner, P. J. 2006 Jellyfish responsible for Irukandji syndrome. *Q. J. Med.* **99**, 802–803. (doi:10.1093/qjmed/hcl104)

Fenner, P. J. & Hadok, J. C. 2002 Fatal envenomation by jellyfish causing Irukandji syndrome. *Med. J. Aust.* **177**, 362–363.

Fenner, P. J. & Williamson, J. A. 1996 Worldwide deaths and severe envenomation from jellyfish stings. *Med. J. Aust.* **165**, 658.

Fenner, P. J., Fitzpatrick, P. F., Hartwick, R. J. & Skinner, R. 1985 'Morbakka', another cubomedusan. *Med. J. Aust.* **143**, 550–555.

Fenner, P. J., Williamson, J. A. & Burnett, J. W. 1996 Clinical aspects of envenomation by marine animals (Abstract). *Toxicon* **34**, 145 (full article available from www.marine-medic.com.au/pages/articles/pdf/parisArticle.pdf). (doi:10.1016/0041-0101(96)83656-7)

Fischer, A. B. & Hofmann, D. K. 2004 Budding, bud morphogenesis, and regeneration in *Carybdea marsupialis* Linnaeus, 1758 (Cnidaria: Cubozoa). In *Developments in hydrobiology—coelenterate biology 2003* (eds D. G. Fautin, J. A. Westfall, P. Cartwright, M. Daly & C. R. Wyttenbach), pp. 331–337. Dordrecht, The Netherlands: Springer.

Gadagkar, S. R., Rosenberg, M. S. & Kumar, S. 2005 Inferring species phylogenies from multiple genes: concatenated sequence tree versus consensus gene tree. *J. Exp. Zool.* **304B**, 64–74. (doi:10.1002/jez.b.21026)

Gershwin, L. 2005*a* Two new species of jellyfishes (Cnidaria: Cubozoa: Carybdeida) from tropical Western Australia, presumed to cause Irukandji Syndrome. *Zootaxa* **1084**, 1–30.

Gershwin, L. 2005*b* *Carybdea alata* auct. and *Manokia stiasnyi*, reclassification to a new family with description of a new genus and two new species. *Mem. Qld Mus.* **51**, 501–523.

Gershwin, L. 2006*a* Jellyfish responsible for Irukandji syndrome. *Q. J. Med.* **99**, 801–802. (doi:10.1093/qjmed/hcl105)

Gershwin, L. 2006*b* Nematocysts of the Cubozoa. *Zootaxa* **1232**, 1–57.

Gershwin, L. 2007 *Malo kingi*: a new species of Irukandji jellyfish (Cnidaria: Cubozoa: Carybdeida), possibly lethal to humans, from Queensland, Australia. *Zootaxa* **1659**, 55–68.

Gershwin, L. & Alderslade, P. 2005 A new genus and species of box jellyfish (Cubozoa: Carybdeidae [*sic*]) from tropical Australian waters. *Beagle, Rec. Mus. Art Galler. North. Territ.* **21**, 27–36.

Gershwin, L. & Gibbons, M. J. 2009 *Carybdea branchi*, sp. nov., a new box jellyfish (Cnidaria: Cubozoa) from South Africa. *Zootaxa* **2088**, 41–50.

Hartwick, R. F. 1991 Observations on the anatomy, behaviour, reproduction and life cycle of the cubozoan *Carybdea sivickisi*. *Hydrobiologia* **216/217**, 171–179. (doi:10.1007/BF00026459)

Kishinouye, K. 1891 Zwei neue Medusen von *Charybdea* (*Ch. brevipedalia* n. sp., *Ch. latigenitalia* n. sp.). *Dobutsugaku Zasshi* **3**, 437–440 (in Japanese with German diagnoses).

Lewis, C. & Bentlage, B. 2009 Clarifying the identity of the Japanese Habu-kurage, *Chironex yamaguchii*, sp. nov. (Cnidaria: Cubozoa: Chirodropida). *Zootaxa* **2030**, 59–65.

Lewis, C. & Long, T. A. F. 2005 Courtship and reproduction in *Carybdea sivickisi* (Cnidaria: Cubozoa). *Mar. Biol.* **147**, 477–483. (doi:10.1007/s00227-005-1602-0)

Lewis, C., Kubota, S., Migotto, A. E. & Collins, A. G. 2008 Sexually dimorphic cubomedusa *Carybdea sivickisi* (Cnidaria: Cubozoa) in Seto, Wakayama, Japan. *Publ. Seto Mar. Biol. Lab.* **40**, 1–8.

Little, M., Pereira, P., Carrette, T. & Seymour, J. 2006 Jellyfish responsible for Irukandji syndrome. *Q. J. Med.* **99**, 425–427. (doi:10.1093/qjmed/hcl057)

Mayer, A. G. 1906 Medusae collected in the Hawaiian Islands by the steamer Albatross in 1902. *Bull. US Fish Comm. 1903* **23**, 1131–1143.

Morandini, A. C. 2003 Deep-Sea medusae (Cnidaria: Cubozoa, Hydrozoa and Scyphozoa) from the coast of Bahia (western south Atlantic, Brazil). *Mitt. Hamb. Zool. Mus. Inst.* **100**, 3–25.

Morandini, A. C. & Marques, A. C. 1997 'Morbakka' syndrome: first report of envenomation by Cubozoa (Cnidaria) in Brazil. In *VII Congreso Latino-Americano sobre Ciencias do Mar, Resumos Expandidos*, vol. 2, pp. 188–189, Oceanographic Institute of the University of São Paulo (IO-USP), Santos, Brazil.

Nagai, H. 2003 Recent progress in jellyfish toxin study. *J. Health Sci.* **49**, 337–340. (doi:10.1248/jhs.49.337)

Nagai, H., Takuwa-Kuroda, K., Nakao, M., Sakamoto, B., Crow, G. L. & Nakajima, T. 2000*a* Isolation and characterization of a novel protein toxin from the Hawaiian box jellyfish (sea wasp) *Carybdea alata*. *Biochem. Biophys. Res. Commun.* **275**, 589–594. (doi:10.1006/bbrc.2000.3352)

Nagai, H., Takuwa-Kuroda, K., Nakao, M., Ito, E., Miyake, M., Noda, M. & Nakajima, T. 2000*b* Novel proteinaceous toxins from the box jellyfish (sea wasp) *Carybdea rastoni*. *Biochem. Biophys. Res. Commun.* **275**, 582–588. (doi:10.1006/bbrc.2000.3353)

Nagai, H., Takuwa-Kuroda, K., Nakao, M., Oshiro, N., Iwanga, S. & Nakajima, T. 2002 A novel protein toxin from the deadly box jellyfish (sea wasp, Habu-kurage) *Chiropsalmus quadrigatus*. *Biosci. Biotechnol. Biochem.* **66**, 97–102. (doi:10.1271/bbb.66.97)

Nilsson, D., Gislen, L., Coates, M. M., Skogh, C. & Garm, A. 2005 Advanced optics in a jellyfish eye. *Nature* **435**, 201–205. (doi:10.1038/nature03484)

Nylander, J. A. A. 2004 *MrModeltest* v. 2. Uppsala University. Program distributed by the author.

Rottini, D., Gusmani, L., Parovel, E., Avian, M. & Patriarca, P. 1995 Purification and properties of a cytolytic toxin in venom of the jellyfish *Carybdea marsupialis*. *Toxicon* **33**, 315–326. (doi:10.1016/0041-0101(94)00174-7)

Sanchez-Rodriguez, J., Torrense, E. & Segura-Puertas, L. 2006 Partial purification and characterization of a novel neurotoxin and three cytolysins from box jellyfish (*Carybdea marsupialis*) nematocyst venom. *Arch. Toxicol.* **80**, 163–168. (doi:10.1007/s00204-005-0023-7)

Stamatakis, A. 2006 RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690. (doi:10.1093/bioinformatics/btl446)

Stangl, K., Salvini-Plawen, L. V. & Holstein, T. W. 2002 Staging and induction of medusa metamorphosis in *Carybdea marsupialis* (Cnidaria, Cubozoa). *Vie Milieu* **52**, 131–140.

Straehler-Pohl, I. & Jarms, G. 2005 Life cycle of *Carybdea marsupialis* Linnaeus, 1758 (Cubozoa, Carybdeidae) reveals metamorphosis to be a modified strobilation. *Mar. Biol.* **147**, 1271–1277. (doi:10.1007/s00227-005-0031-4)

Studebaker, J. P. 1972 Development of the cubomedusa, *Carybdea marsupialis*. M.S. thesis, University of Puerto Rico, Mayagüez, Commonwealth of Puerto Rico, USA.

Swofford, D. L. 2003 *PAUP**. *Phylogenetic analysis using parsimony* (**and other methods*), version 4. Sunderland, MA: Sinauer Associates.

Thomas, C. S., Scott, S. A., Galanis, D. J. & Goto, R. S. 2001 Box jellyfish (*Carybdea alata*) in Waikiki: their influx cycle plus the analgesic effect of hot and cold packs on their stings to swimmers at the beach: a randomized, placebo-controlled, clinical trial. *Hawaii Med. J.* **60**, 278.

Werner, B. 1973 Spermatozeugmen und Paarungsverhalten bei *Tripedalia cystophora* (Cubomedusae). *Mar. Biol.* **18**, 212–217. (doi:10.1007/BF00367987)

Werner, B., Cutress, C. E. & Studebaker, J. P. 1971 Life cycle of *Tripedalia cystophora* Conant (Cubomedusae). *Nature* **232**, 582–583. (doi:10.1038/232582a0)

Williamson, J., Fenner, P., Burnett, J. & Rifkin, J. 1996 *Venomous and poisonous marine animals: a medical and biological handbook*. Sydney, Australia: NSW University Press.

Wiltshire, C. J., Sutherland, S. K., Fenner, P. J. & Young, A. R. 2000 Optimization and preliminary characterization of venom isolated from 3 medically important jellyfish: the box (*Chironex fleckeri*), Irukandji (*Carukia barnesi*), and blubber (*Catostylus mosaicus*) jellyfish. *Wilderness Environ. Med.* **11**, 241–250.

Yamaguchi, M. & Hartwick, R. 1980 Early life history of the sea wasp, *Chironex fleckeri* (class Cubozoa). In *Development and cellular biology of coelenterates* (eds P. Tardent & R. Tardent), pp. 11–16. Amsterdam, The Netherlands: Elsevier/North-Holland Biomedical Press.

Yanagihara, A. A., Kuroiwa, J., Oliver, L., Chung, J. & Kunkel, D. 2002 Ultrastructure of a novel eurytele nematocyst of *Carybdea alata* Reynaud (Cubozoa, Cnidaria). *Cell Tissue Res.* **308**, 307–318. (doi:10.1007/s00441-002-0545-8)

Yoshimoto, C. M. & Yanagihara, A. A. 2002 Cnidarian (coelenterate) envenomations in Hawai'i improve following heat application. *Trans. R. Soc. Trop. Med. Hyg.* **96**, 300–303. (doi:10.1016/S0035-9203(02)90105-7)