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Box Jellyfish *Alatina alata* Has a Circumtropical Distribution

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Abstract

Species of the box jellyfish (Cubozoa) genus *Alatina* are notorious for their sting along the beaches of several localities of the Atlantic and Pacific. These species include *Alatina alata* on the Caribbean Island of Bonaire (the Netherlands), *A. moseri* in Hawaii, and *A. mordens* in Australia. Most cubozoans inhabit coastal waters, but *Alatina* is unusual in that specimens have also been collected in the open ocean at great depths. *Alatina* is notable in that populations form monthly aggregations for spermcast mating in conjunction with the lunar cycle. Nominal species are difficult to differentiate morphologically, and it has been unclear whether they are distinct or a single species with worldwide distribution. Here we report the results of a population genetic study, using nuclear and mitochondrial sequence data from four geographical localities. Our analyses revealed a general lack of geographic structure among *Alatina* populations, and slight though significant isolation by distance. These data corroborate morphological and behavioral similarities observed in the geographically disparate localities, and indicate the presence of a single, pan-tropically distributed species, *Alatina alata.* While repeated, human-mediated introductions of *A. alata* could explain the patterns we have observed, it seems more likely that genetic metapopulation cohesion is maintained *via* dispersal through the swimming medusa stage, and perhaps *via* dispersal of encysted planulae, which are described here for the first time in *Alatina*.

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Introduction

Life-cycle and life-history characteristics have profound impacts on the basic biology of marine species, affecting geographic ranges and population dynamics. For instance, species possessing both benthic and planktonic life stages may be expected to display lower dispersal abilities and smaller geographic ranges than species lacking benthic stages (Gibbons *et al.,* 2010). Many marine species possess pelagic larval stages that are often thought to be most responsible for dispersal (Bradbury *et al.,* 2008; Bowen *et al.,* 2013), but the mobility of all stages in a life cycle are relevant to determining species ranges (Johannesson, 1988). Species of the phylum Cnidaria exhibit a wide variety of life-cycle plasticity and dispersal capabilities (reviewed in Fautin, 2002). Jellyfish species (of the cnidarian subphylum Medusozoa) typically possess a benthic, sessile stage (polyp) that reproduces asexually by budding new polyps or medusae, the latter of which represent the sexually reproductive stage of the textbook medusozoan life cycle. Short-lived, ciliated larval forms known as planulae usually precede the benthic polyp stage. A sometimes overlooked life stage present in some medusozoans is the podocyst, a resting stage produced by polyps. Podocysts are essentially polyp-derived tissues encysted in a protective layer of perisarc, and are able to withstand extreme salinity changes and desiccation, sometimes for years (Dumont, 1994; Ikeda *et al.,* 2011; Carrette *et al.,* 2014).

For scyphozoan and cubozan species, the medusa stage is usually the life stage chosen for investigating global patterns of abundance, distribution, and diversity (Dawson *et al.,* 2014; Lucas *et al.,* 2014), because the polyp stage is often difficult to locate in the field. Recent focus has been on the sudden or periodic increases in biomass of scyphozoans that often have detrimental impacts on human activities; such increases are commonly known as “jellyfish blooms” (Condon *et al.,* 2013; Dawson *et al.,* 2014). Much debate exists surrounding the identification of the main factors driving jellyfish population dynamics, such as eutrophication, species introductions, and global climate change (for reviews of jellyfish blooms see Graham *et al.*, 2001; Graham and Bayha, 2007; Purcell *et al.*, 2007; Condon *et al.*, 2013; Dawson *et al.*, 2014; Lucas *et al.*, 2014). While “blooms” of scyphozoans have been most heavily studied, the population dynamics of box jellyfish—in spite of their potentially large public health impacts due to their potent venoms and their generally coastal, shallow water distributions—remain poorly understood (Yoshimoto and Yanagihara, 2002; Bentlage *et al.,* 2009; Gershwin *et al.,* 2009). Periodic or seasonal population dynamics of box jellyfish have long been documented in, and associated with, tropical Australia (Barnes, 1966; Fenner, 1998; Fenner and Harrison, 2000), and an apparent increase in box jellyfish abundance on the Mediterranean Coast of Spain has been reported in recent years (Bordehore *et al.*, 2011, 2015; Fontanet, 2014).

In several tropical to subtropical localities, species of the box jellyfish genus *Alatina* display monthly nearshore aggregations (Thomas *et al.*, 2001; Chiaverano *et al.*, 2013; Lewis *et al.*, 2013; Carrette *et al.*, 2014). Unlike typical jellyfish blooms, which consist of sudden, and often unpredictable, increases in single-species biomass linked to environmental conditions (Condon *et al.*, 2013; Dawson *et al.*, 2014), *Alatina* swarms are directly correlated with reproductive events related to the lunar cycle; mating aggregations occur 8 to 10 days after the full moon (*Alatina moseri* Mayer 1906 in Hawaii, *A. mordens* Gershwin 2005 in

Australia, and *A. alata* (Reynaud, 1830) in Bonaire, the Netherlands). Though the animals are present in large numbers (hundreds to thousands of individuals) during these monthly reproductive swarms, the whereabouts of *Alatina* medusae in the interim is poorly known. Further, juveniles have been reported only on few occasions (Arneson, 1976; Arneson and Cutress, 1976; Lewis *et al.*, 2013). Medusae of *Alatina alata* have been recorded swimming at depths greater than 540 m, and collected as deep as 1067 m (Morandini, 2003 as *Carybdea alata*; Lewis *et al.*, 2013). Reports of box jellyfish at great depths are unusual; cubozoans are generally not thought to disperse across the open ocean (but see Bentlage *et al.*, 2010). In addition, numerous *Alatina* specimens have been collected from surface waters in the open ocean and from depths as great as 2282 m (Lewis *et al.*, 2013).

Among the eight nominal species of *Alatina*, *A. alata* is found in the eastern Atlantic and Caribbean, and displays what appears to be an identical life history to that of two Pacific species, *A. moseri* and *A. mordens* (Carrette *et al.*, 2014). Bentlage *et al.* (2010) found that the two Pacific species share mtDNA (16S) haplotypes, and suggested that they belong to a widespread population from a single species, despite the large geographic distance separating them. In this contribution, we integrated molecular analyses and early development and morphological data to clarify the geographic distribution of *Alatina* from Bonaire, Hawaii, Saipan, and Australia. We show that *Alatina* does not follow the current paradigm, which restricts cnidarians with a bentho-pelagic life cycle to relatively narrow geographic ranges (Gibbons *et al.*, 2010). Rather, we propose that they are members of a single, widespread, possibly circumtropical species known as *Alatina alata*.

Materials and Methods

Sampling and morphology

We sampled *Alatina* specimens from four localities (Fig. 1): *Alatina alata* from Karel's Pier (Kralendijk, Bonaire, the Netherlands); *Alatina mordens* from Osprey Reef (Coral Sea, Queensland, Australia); *Alatina moseri* from Waikiki (Oahu, Hawaii), and *Alatina* sp. from Mañagaha Island (Saipan, Northern Mariana Islands). Medusae were collected individually by hand or by using dip nets at the sea surface from a boat, docks, or the beach. Specimens were preserved in 5%– 8% buffered formalin for morphological examination; a piece of tentacle was placed in 95% ethanol (EtOH) for DNA extractions. Other than two specimens from Saipan, which we tentatively identified as *Alatina grandis* (Agassiz & Mayer, 1902) (Bentlage *et al.*, 2010) (see Results section), all specimens had the same general appearance in the field. The detailed morphology of *Alatina* samples was examined using the methods outlined in Bentlage and Lewis (2012) and Lewis *et al.* (2013). Bell height and bell width were measured, and the presence of taxon-defining morphological characters was confirmed by consulting the taxonomic keys provided in Gershwin (2005) and the recent redescription of *Alatina alata* (Lewis *et al.*, 2013). A list of the museum specimens examined is provided in Appendix 1. Photography and videography were used to document the presence of *A. alata* in nearshore waters of Bonaire, the Netherlands (Fig. 2b), in connection with monthly spermcasting events (see Lewis *et al.*, 2013 and this study). Male and female adults were placed in buckets of seawater and examined over a period of 24 hours. Embryos released from females (following internal fertilization) were photographed, and videos were taken to

document developmental stages from blastulae to free-swimming planulae (following methods in Lewis *et al.*, 2013).

DNA extraction, polymerase chain reaction (PCR), and sequencing

DNA was extracted from tentacle tissue, using DNeasy Tissue kits (Qiagen Inc., Valencia, CA), following the manufacturer's protocol for animal tissues. Cubozoans are unusual in that they have linear mitochondrial genomes consisting of eight chromosomes (Smith *et al.*, 2012). From three separate mitochondrial chromosomes, we amplified and sequenced three markers: 1) a 1005-bp fragment from the region containing two open-reading frames, ORF314 and POLB (ORF-PolB) (see Smith *et al.*, 2012); 2) a 558-bp fragment from the cytochrome c oxidase subunit I (COI); and 3) a ~545-bp fragment from the ribosomal RNA (16S). ORF314 and POLB are present in the mitochondrial genomes of several other medusozoan groups; ORF314 may have DNA-binding properties to help maintain the ends of mitochondrial telomeres, and POLB codes for a putative DNA polymerase beta (Kayal *et al.*, 2012). From the nuclear genome, we obtained a ~558-bp fragment spanning the internal transcribed spacers (ITS), ITS1 and ITS2, of the ribosomal RNA operon, including 5.8S, as well as a 710-bp fragment of the large ribosomal RNA subunit (28S) spanning the expansion regions D1–D3 (Cannone *et al.*, 2002). Polymerase chain reaction protocols followed standard procedures. Thermocycler profiles were conducted with initialization at 94–95 °C (3–5 min), followed by 36 – 40 cycles of denaturation at 94–95 °C (30 s), annealing at 52–54 °C (30 s), and extension at 72 °C (1–2 min). The final extension was further executed at 72 °C (5–10 min). Polymerase chain reaction products were purified by combining 3 μl of 0.75 units (U) of Exonuclease I and 0.5 U of Shrimp Alkaline Phosphatase (ExoSAP; USB Corp., Cleveland, OH) with 8 μl of PCR product, followed by incubation at 37 °C for 30 min and deactivation at 80 °C for 20 min. Cycle sequencing was accomplished using the same primers as those used in PCRs (Table 1). Cycle sequencing products with fluorescently labeled dideoxy terminators were visualized after clean up, using Sephadex columns on an Applied Biosystems (Thermo Fisher Scientific, Waltham, MA) 3130*xl* or 3730*xl* Genetic Analyzer at the Smithsonian's Laboratory of Analytical Biology (LAB), of the National Museum of Natural History, Washington, D.C.

Molecular genetic analyses

Sequences were assembled, trimmed, and aligned in Geneious ver. 6.1.8 (Kearse *et al.*, 2012). The number of haplotypes and haplotype and nucleotide diversity were calculated in DnaSP ver. 5.10.1 (Librado and Rozas, 2009). Allelic states of nuclear sequences with more than one heterozygous site were estimated using PHASE 2.1, as implemented in DnaSP, with three runs, each a unique random-number seed, for each dataset. Each of these runs was conducted for 1000 iterations with 1000 burn-in iterations, and all runs returned consistent allele identities. The best-fit models of DNA sequence evolution for each alignment were determined using the Akaike information criterion (AIC) with a correction for finite sample sizes (AICc), as implemented in jModelTest 2.1.7 (Guindon and Gascuel, 2003; Darriba *et al.*, 2012). To evaluate whether multiple species of *Alatina* were present in our sampling, we obtained all publicly available 16S sequences from non-*Alatina* cubozoans deposited in GenBank. These were then aligned to sequences generated for the *Alatina* specimens collected for this study (Appendix 2) using MAFFT with the E-INSi option (Katoh and

Standley, 2013). To exclude regions of uncertain homology of 16S across Cubozoa, Gblocks ver. 0.91b (Castresana, 2000; Talavera and Castresana, 2007) was run with standard parameters, except that half the taxa were allowed to be gaps for any position. The maximum likelihood topology (ML) was inferred using PHYML (Guindon *et al.*, 2010), assuming the best-fitting model for this dataset, TIM2+I+G. Node support was assessed by conducting ML searches using 1000 nonparametric bootstrap replicates in PHYML. The resulting alignments used herein, as well as the 16S phylogenetic tree, are available through Figshare (Collins *et al.,* 2016).

Because many sequences of the five markers were obtained from non-overlapping sets of specimens (see Appendix 2), we did not combine markers for analyses. Arlequin ver. 3.5.1.2 (Excoffier *et al.*, 2005) was used to perform an analysis of molecular variance (AMOVA) (Weir and Cockerham, 1984; Excoffier *et al.*, 1992; Weir, 1996) and to estimate population differentiation using pairwise FSTs, both tested with 20,000 nonparametric permutations. AMOVA was used to estimate the proportion of variation explained among groups (FCT), among localities within groups (FSC), and among all localities (FST). To determine the possibility of a correlation between pairwise FSTs and geographic distance, we used the Isolation-by-Distance Web Service with a 10,000-permutations significance test (Jensen *et al.*, 2005). Geographic distances were measured, using Google Earth, “as the crow flies” between known occurrence sites of *Alatina* worldwide (Fig. 1). For mitochondrial data, these previous analyses were performed with an analogue of Wright’s FST (ΦST), which incorporates the model of sequence evolution. The best-fitting model calculated for the alignments was not available in Arlequin; therefore, the TrN was chosen, as it was the model available with the highest best-fit score (lowest AICc). Haplotype networks were constructed in Network ver. 4.6.1.3 (Flux Technology Ltd., Suffolk, England), using the median-joining algorithm (Bandelt *et al.*, 1999). Networks were post-processed using maximum parsimony calculations (Polzin and Daneshmand, 2003) to remove unessential median vectors in the network.

Results

Morphology

We compared the morphology of the specimens in the *Alatina moseri* (Mayer, 1906) syntype (Hawaii) series in the Smithsonian’s National Museum of Natural History collections (items are denoted by catalog reference code beginning USNM; USNM 21800, 22311, 29632, 42112) with the *Alatina alata* neotype (USNM 1195802; see Lewis *et al.*, 2013). Museum specimens of *A. moseri* had gonads, and the three medusae ranged from 70 – 82 mm (bell height; BH) by 22–26 mm (bell width; BW). According to Mayer (1906), live material measured BH = 80 mm by BW = 47 mm. These measurements are consistent with those of the live, gonad-bearing *A. alata* neotype (70 mm × 40 mm) and additional nontype material from the Atlantic Ocean (see Lewis *et al.*, 2013). Mayer (1906) described the 24 velarial canals of *A. moseri* medusae as unbranched. However, we examined the syntype series and found that while some velarial canals are simple, many are split into two or three short, secondary branches (similar to *A. alata*). Further, velarial lappets and corresponding warts characteristic of *A. alata* are also present, or at least partially visible, although some have

sloughed off in the center, leaving just an outline of the wart. The lack of pit eyes noted by Gershwin (2005) is an artefact often seen in long-preserved box jellyfish material (see Bentlage *et al.*, 2010; Lewis *et al.*, 2013; Carrette *et al.*, 2014). Although Gershwin (2005) argued that, in *A. mordens*, cirri are arranged in pairs rather than in bunches, we noted no differences with the gastric phacellae of the *A. moseri* syntype series and the *A. alata* neotype. Carrette *et al.* (2014) also found no morphological differences when comparing adult medusae of *A. moseri* with those of *A. mordens* from Osprey Reef, Australia. Furthermore, the cnidome (nematocyst composition) of adult *A. alata* medusae (see Arneson, 1976; Lewis *et al.*, 2013) is indistinguishable from that of *A. mordens* and *A. moseri* (Gershwin, 2005, 2006; Yanagihara *et al.*, 2002 as *Carybdea alata*) bearing euryteles (in tentacles) and isorhizas (in bell warts, and tentacle base) (Gershwin, 2005; Lewis *et al.*, 2013). During early development, polyps of *A. mordens*, *A. moseri* (Carrette *et al.*, 2014), and *A. alata* (Arneson and Cutress, 1976 as *Carybdea alata*) bear stenoteles and ovoid, heterotrichous, microbasic euryteles.

In the course of our collections, using pelagic, tethered drift SCUBA dives at night in the Philippine Sea off the west coast of Saipan, we obtained two exemplars of a more distinctive form of *Alatina*, which we tentatively identified as ripe females of *Alatina grandis*. Specimens of *Carybdea grandis* were collected off Fakarava and Anaa Island, in the Tuamotu Archipelago (formerly Paumotu Islands) and described by Agassiz and Mayer (1902). There were no subsequent reports for over a century (see Bentlage, 2010). Measuring 180 mm (bell height) by 46 mm (bell width), approximately twice the size of the average *Alatina* specimens in our study, these new exemplars share many characters with the original species description, but additional samples (with key morphological characters preserved) are needed to firmly establish its identity. Our phylogenetic analysis confirms our conclusion, that these large specimens are distinct from the remainder of the collected *Alatina* specimens (see *Phylogenetics and population genetics* below).

Embryonic development

In this study, we documented that the early ontogeny of Bonaire *Alatina alata* embryos to the planula stage (Fig. 2, c–e) matched the findings of Arneson (1976) and Arneson and Cutress (1976) for the same species (as *Carybdea alata*) in Puerto Rico. The subsequent ontogenetic changes of *A. alata* from polyp to medusa stage (Arneson and Cutress, 1976) are also known to be identical in *A. moseri* from Hawaii and *A. mordens* from Australia (Carrette *et al.*, 2014). Additionally, for all putative *Alatina* species, polyps can revert to a resting podocyst stage under adverse conditions (Carrette *et al.*, 2014). During this study, while examining developing embryos in the lab approximately 24 h after their release into the aquarium water, we discovered multiple cysts (~150 μm in diameter), each containing a single planula bearing characteristic equatorial eye-spots, rotating on its longitudinal axis. During the course of our microscopic examination, one planula successfully bored through the outer, “shell-like” perisarc (within 5–50 min), emerging as swimming planula (Fig. 2, c–e). To our knowledge, this is the first documented occurrence of a planula hatching in a cnidarian. Whether this encysting stage is a form of diapause (*i.e.,* a dormancy stage or delay in development in response to adverse environmental conditions) remains to be examined. Another open question is whether all or just some of maturing embryos develop a perisarc.

The mechanisms involved in piercing the membrane were not investigated here, nor was the composition of the perisarc. Our findings resemble, to a certain extent, the “blastocysts” reported in *Morbakka virulenta* by Toshino *et al.* (2013), but in the case of *M. virulenta,* polyps (not zygotes) formed cysts that endured adverse conditions for many months, reemerging as polyps (not planulae).

Phylogenetics and population genetics

The 16S-ML phylogeny shows that specimens of *Alatina alata* from Bonaire, *A. mordens* from Australia, *A. moseri* from Hawaii, and *Alatina* sp. from Saipan fall into a single, well-supported clade with little differentiation among species and no apparent geographic structuring (Fig. 3). In contrast, the two large specimens that we tentatively identified as *Alatina grandis* were highly divergent from them (Fig. 3). While the placement of *Alatina grandis* is ambiguous due to low bootstrap support, it presents >20% sequence divergence from the remainder of *Alatina* spp., which in turn have <1.31% average sequence divergence in 16S (Table 2).

In light of the 16S-based phylogenetic results, we removed *A. grandis* and all other cubozoans from further analysis, leaving just the representatives of *Alatina alata* from Bonaire, *A. mordens* from Australia, *A. moseri* from Hawaii, and *Alatina* sp. from Saipan, for which we realigned full-length sequences to preserve the largest amount of sequence information possible for further analysis. Table 3 provides the number of specimens sequenced per geographic region (N), number of haplotypes (Nh), haplotype diversity (h), and nucleotide diversity (π) for all markers. All mitochondrial markers and ITS had high overall haplotype diversity (h = 0.96 – 0.99; Table 3), with ORF-PolB and COI being the most diverse (0 – 6.47% and 0 – 4.48%, respectively; Table 2), 16S and ITS with intermediate divergence (0 –2.02% and 0 –2.15%, respectively; Table 2), and 28S the most conserved (0 – 0.28%; Table 2).

Overall ΦSTs and FSTs from the mitochondrial markers and nuclear ITS, respectively, indicated significant structure between sampled localities (ΦST = 0.086 – 0.17, FST = 0.088; Table 4). However, the majority of the overall variance was found within (>80%) rather than among geographic locations (Table 4). Pairwise comparisons across markers (measured by ΦST and FST; Table 5) showed that most of the Pacific sites were significantly different from Bonaire, but not significantly different from each other (samples were grouped by ocean basin: *i.e.*, Pacific containing Saipan, Australia, and Hawaii *vs.* Atlantic containing Bonaire; see Materials and Methods). In this case, ΦST and FSTs were larger (ΦST = 0.25–0.35, FST = 0.11; Table 4), and variation within populations still explained the majority of the overall variance observed (>60%). ITS was the only marker to demonstrate significant structure within basins (FSC = 0.075; Table 4). We did not find significant evidence for isolation by distance among regions, other than for COI (Table 4).

Haplotype networks (Fig. 4) show no well-defined geographic structure and each locality shares haplotypes with at least one other locality for one or more markers. Even though some specimens from Bonaire cluster together, others from this locality are more closely related to Pacific specimens. Nevertheless, pairwise ΦST and FST values involving Bonaire were mostly significant (at $P < 0.05$), with its lower range in the nuclear ITS (FST = 0.075–

0.153) and upper range in the mitochondrial COI (ΦST = 0.294 – 0.403) (Table 5). Conversely, comparisons within the Pacific were primarily not significant, other than Hawaii–Saipan for ITS (FST = 0.186) (Table 5).

Revised systematics

***Alatina alata* diagnosis (from Lewis *et al.*, 2013, table 1 and figs. 1– 6)—**"*Alatina* with tall, narrow bell, flared at base, tapering into truncated pyramid at apex; 4 crescentric gastric phacellae at interradial corners of stomach; 3 simple to palmate branching velarial canals per octant, each with a velarial lappet bearing a row of 3 to 4 nematocyst warts; 4 long, wing-like (*sensu* Reynaud, 1830) pedalia, each with a pink tentacle. Cnidome: heterotrichous microbasic p– euryteles and small birhaploids in tentacles, and large isorhizas in nematocyst warts." For more details on morphology and taxonomic history.

Neotype locality—Bonaire, the Netherlands (Atlantic Ocean).

Neotype specimen—National Museum of Natural History, Smithsonian Institution, Washington D.C.: USNM 1195802, 1 ind, female, BW 40 mm, BH 70 mm (live), BW 30 mm, BH 69 mm (8% formalin-preserved), 24 June 2011, Karel's Pier, Kralendijk, Bonaire, the Netherlands, 12°09′06.37″ N, 68°16′06.37″ W; depth = surface.

Systematics

Phylum Cnidaria Verrill, 1865

Subphylum Medusozoa Peterson, 1979

Class Cubozoa Werner, 1973

Order Carybdeida Gegenbaur, 1857

Family Alatinidae Gershwin, 2005

Genus *Alatina* Gershwin, 2005

Species *Alatina alata* (Reynaud, 1830)

Synonymy list

Carybdea (medusa) alata

Reynaud, 1830 (in Lesson, 1830, pl. 33, fig. 1a)

La Marsupiale ailé

Lesson, 1837, p. 9, n. 26

Marsupialis alata

Lesson, 1843, p. 278

Charybdea alata

Haeckel, 1880, p. 441; 1940a, p. 5

Tamoya alata

Agassiz, 1862, p. 174

Carybdea alata

Mayer 1910, p. 508 –510; Mayer, 1915, p. 171; Bigelow, 1918, p. 400; 1938, pp. 144 –151, text–figs. 11–16; Kramp, 1961, p. 304; Arneson 1976, pp. 36, figs. 1, 2, table 1, 2, pls. I–V; Arneson and Cutress, 1976, pp. 227–236, table 1, pl. I A–G; Cutress, 1971, p. 19, pl. 1; Larson, 1976, pp. 242; Larson *et al.,* 1991, p. 313, table 2; Thomas *et al.*, 2001; Yoshimoto and Yanagihara, 2002; Humann and Deloach, 2002; Morandini, 2003, p. 15–17, fig. 2; Gershwin, 2005, pp. 501–523; Calder, 2009, pp. 12, 13, fig. 1; Bentlage, 2010, p. 52; Bentlage *et al.,* 2010, p. 498; Bentlage and Lewis, 2012, p. 2602; Yanagihara and Shohet, 2012, pp. 1–2

Charybdea moseri

Mayer, 1906, pp. 1135–1136, pl. 1, fig. 2–2c; *n. sp.*, description and illustrations; Bigelow, 1909, pp. 19–20, young stage of *C. grandis*; Bigelow, 1938, p. 144, junior synonym of *C. alata*; Chu and Cutress, 1954, p. 9, cause of dermatitis; Kramp, 1961, p. 304, in synonymy of *C. alata*

Carybdea moseri

Mayer, 1915, p. 171, probably young of *C. alata* var. *grandis*; Mayer, 1917, p. 189 [in part], fig. 3, only half-grown stage of *C. alata*

Carybdea alata var. moseri

Mayer, 1910, p. 512, probably a variety or young stage of *C. grandis*, probably identical with *C. philippina*; Light, 1914, p. 196 = *Charybdea philippina* [Semper 1860]; Mayer, 1915, p. 171, *C. moseri* is probably only a young of this medusa; Mayer, 1917, p. 189 [in part], fig. 3, only half-grown stage of *C. alata*; Stiasny, 1919, pp. 34, 37–38, fig. 5; Stiasny, 1940, pp. 5–6; Bigelow, 1938, p. 144, in synonymy of *C. alata*

Alatina alata

Gershwin, 2005; Gershwin and Gibbons, 2009; Lewis *et al.*, 2013; Yanagihara *et al.*, 2016

Alatina moseri

Gershwin, 2005, 2006; Gershwin *et al.,* 2009, 2013; Bentlage, 2010; Bentlage *et al.,* 2010; Straehler-Pohl, 2011; Straehler-Pohl and Jarms, 2011; Kayal *et al.*, 2012, 2013; Smith *et al.,* 2012; Bentlage and Lewis, 2012; Kingsford *et al.*, 2012; Yanagihara and Shohet, 2012; Chiaverano *et al.,* 2013; Toshino *et al.,* 2013, 2015; Carrette *et al.,* 2014; Crow *et al.,* 2015; Straehler-Pohl and Toshino, 2015

Alatina cf. moseri

Carrette *et al.,* 2014

Alatina mordens

Gershwin, 2005, 2006; Gershwin *et al.,* 2009, 2013; Bentlage, 2010; Bentlage *et al.,* 2010; Straehler-Pohl, 2011; Straehler-Pohl and Jarms, 2011; Bentlage and Lewis, 2012; Chiaverano *et al.,* 2013; Toshino *et al.,* 2013, 2015; Courtney and Seymour, 2013; Carrette *et al.,* 2014; Crow *et al.,* 2015

Alatina *nr* mordens

Courtney and Seymour, 2013

Alatina *sp*

Carrette *et al.,* 2014

Discussion

Historical background of the *Alata* species group

By the turn of the 19th century, 10 nominal species of the *"alata"* species group had been described for cubozoans from disparate geographic areas. These were eventually all united under the species name *Carybdea alata* (Bigelow, 1938; Kramp, 1961; Arneson, 1976). However, more recently Gershwin (2005) established the genus *Alatina* for all such cubomedusae, characterized by a tall and narrow bell, four crescentric, gastric phacellae, four "wing-like" pedalia, and three to four bifurcating to palmate velarial canals per octant. The taxonomic revision (Gershwin, 2005) resurrected five species of box jellyfish from the Indo-Pacific, previously synonymized under the name *Carybdea alata*, using the new genus-species combinations *Alatina moseri*, *A. grandis*, *A. madraspatana*, *A. pyramis*, and *A. tetraptera*. The revision also described two new *Alatina* species from Australia, *A. mordens* and *A. rainensis*, the former based mainly on medusa size, bell wart size, and an apparent reduced number of eyes per rhopalium (compared with other cubozoans). Lewis *et al.* (2013) established a neotype for the oldest species in the group, *A. alata* (Reynaud, 1830), bringing the number of currently recognized species to six. As body size is a factor of development influenced by environmental conditions, rhopalia eye spots are known to fade, and bell warts rub off following specimen preservation, doubts have been raised as to the validity of some of the nominal species, some of which have a single mention in the literature.

Alatina alata species complex

Bentlage *et al.* (2010) suggested that *Alatina mordens* from Australia and *A. moseri* from Hawaii likely represent a single species, and speculated about whether the two populations were connected at present, or were the result of human-mediated introductions. By increasing taxon and marker sampling from different ocean basins, we show that *A. moseri* and *A. mordens* from the Pacific, *A.* sp. from Saipan, as well as *A. alata* from its neotype locality (Bonaire, the Netherlands, Atlantic), are the same species. All nominal species sampled in this study (excluding *A. grandis*) form a single clade in our maximum likelihood

(ML) analysis, with little divergence among geographic locations and lack of apparent geographic structuring (Fig. 3). Indeed, using several nuclear and mitochondrial markers we found that populations share haplotypes for all markers and lack a well-defined geographic clustering. All of the markers exhibit variability, particularly ORF-PolB and COI, but there are no clear divisions that would suggest the existence of multiple species among our samples (Fig. 4). Nevertheless, more comprehensive sampling of molecular data, especially of specimens from Bonaire and other localities in the Caribbean, could clarify population-level relationships among the distinct localities, to further investigate the possibility of a recent separation between populations in the different ocean basins in contrast to ongoing circumtropical gene flow.

Using a reverse taxonomic approach, we reevaluated the morphology of each of the nominal species and realized that they cannot be reliably delineated. Thus, in light of the genetic patterns presented and the uniformity in morphology, we conclude that the nominal species investigated herein all correspond to a single species, in spite of the large geographic distances among the populations sampled. Given that *A. alata* (Reynaud, 1830) is the oldest species described within the genus *Alatina*, *A. moseri* and *A. mordens* are to be considered junior synonyms of *A. alata*.

At this point, the status of the other nominal *Alatina* species (see Gershwin, 2005 for an overview) remains uncertain, as type material exists only for *A. rainensis*, which we have yet to examine or sample. However, we have tentatively identified the two large alatinid specimens from Saipan (USNM 1296954 and USNM 1296955) as *A. grandis*, a species that had been described from the Tuamotu Archipelago, French Polynesia, more than a century ago (Agassiz and Mayer, 1902), and whose type is badly damaged, making species identification difficult (Bentlage *et al.*, 2010). Molecular data show that the specimens that we identified as *A. grandis* are distinct from *A. alata* within the family Alatinidae, and we expect that morphological examination of better-preserved samples will provide clear distinction between this species and *A. alata*.

Population structure and historical demography

Even though specimens from the different localities sampled share haplotypes in each of the markers analyzed, we detected some geographic structure, as measured by pairwise ΦST and FST. The Atlantic population shows the greatest separation from those of the Pacific localities. Nevertheless, even though Isolation by Distance (IBD) was significant and high for COI, it was not detected for any other marker, and no significant difference was revealed between the ocean basins in our AMOVA. Overall, there is no well-defined geographic structure, although some degree of divergence exists between Atlantic and Pacific specimens. This divergence could be due to lower rates of gene flow among distant localities or incomplete sorting of a large ancestral population.

Based on our findings, we suggest that *Alatina alata* is a single widespread species, found in several tropical and subtropical locations in the Pacific and Atlantic Oceans. Even though our findings are contrary to other studies of widespread marine invertebrates (Dawson and Jacobs, 2001; Goetze, 2011), similar results have been reported in fishes (Theisen *et al.*, 2008; Lewallen, 2012). Numerous other cnidarian species with a bentho-pelagic life cycle

are globally distributed, a phenomenon that is frequently attributed to repeated species introductions, often through commercial shipping activities (Bayha and Graham, 2013). The most likely means of cnidarian species introductions is *via* transport of polyps or cysts in ballast water or attachment to the hull of ships (Bayha and Graham, 2013). Under a scenario of species introductions, one would assume reduced haplotype diversities in the regions where it occurred due to the bottleneck created by the introduction of a few propagules into a locality. However, we observed very high haplotype diversities in all sampled localities and across markers. Thus, either there have been multiple separate introductions of *Alatina alata* from unknown source populations or *A. alata* is indeed capable of maintaining population cohesion across ocean basins. Of note is the observation of Smith *et al.* (2012), that *Alatina alata* (as *A. moseri*) mitochondrial haplotype diversity is nearly the highest of any metazoan species ever measured to date, which could be the result of extremely large, effective population size. What is clear from the literature is that *A. alata* has been present both in the Atlantic (Lewis *et al.*, 2013) and at Hawaii for more than a century (Chiaverano *et al.*, 2013). This indicates that putative introductions from one ocean basin to the other would have taken place prior to present-day commercial shipping activities. Unfortunately, the history of *A. alata* in other localities is not well documented, although large aggregations have been reported in Australia since 1999 (see Carrette *et al.*, 2014, as *A. mordens*).

Do life-history characteristics favor dispersal abilities in *Alatina alata*?

Box jellyfish differ from the majority of scyphozoan jellyfish that possess a bentho-pelagic life cycle, which is defined by a sessile polyp stage that metamorphoses into one or more free-swimming medusae. In box jellyfish the sessile, asexually reproducing polyp (cubopolyp) generates either a single medusa through complete metamorphosis, or multiple medusae *via* metamorphosis coupled with transverse fission at the apical end of the polyp (Straehler-Pohl and Jarms, 2005). Conversely, in both scyphozoans and cubozoans sexual reproduction occurs exclusively during the adult medusa stage. In the case of *A. alata*, reproductive success is achieved by the formation of highly synchronized, monthly inshore spermcasting aggregations, occurring 8 –10 days after the full moon, during which males release sperm that is taken up by females for internal fertilization. These aggregations have been documented in several Atlantic and Pacific localities, including Bonaire, Hawaii, and Australia (Bentlage *et al.*, 2010; Chiaverano *et al.*, 2013; Lewis *et al.*, 2013; Carrette *et al.*, 2014). Reports of live *A. alata* medusae in the interim are rare, limited to a few documented cases in which a remotely operated vehicle (ROV) at ~100 m (USNM 1005621) and a manned submersible at ~540 m (USNM 1195809) were used (Lewis *et al.*, 2013; Fig. 2a); therefore, *A. alata* is considered a deep-sea box jellyfish species (see Bentlage *et al.*, 2010).

In the present study, we observed free-floating, encysted planulae (Fig. 2, c–e) with a morphology different from previously reported encysted life stages in box jellyfish, such as podocysts, which are sessile, encysted polyps (see Carrette *et al.*, 2014). While the latter provides a good means of protection during bad conditions, and could potentially foul the hull of ships, the inherent ability of free-floating, encysted planulae to be immediately dispersed to the open ocean (by tides and currents) could ensure effective and broad distribution of planulae before their imminent settlement as polyps. That said, planula larvae themselves are short-lived in *A. alata*, from 2–3 days (Carrette *et al.*, 2014), to 5–6 days

(Arneson, 1976), while the lifespan of the encysted planulae newly described herein is unknown. Since the dispersal potential of larvae is generally highly correlated with the duration of its pelagic stage (Scheltema, 1971; Grantham *et al.*, 2003), planulae are unlikely vectors for long-distance dispersal in *A. alata*.

Medusae of *Alatina alata* are strong swimmers (Chiaverano *et al.*, 2013) with a potentially long lifespan (~1 year; see Arneson, 1976), and have been reported swimming at great depths and in the open ocean (see Lewis *et al.*, 2013 and Fig. 1). This suggests that adults might contribute to dispersal, such as has been hypothesized for other marine organisms (see Kinlan *et al.*, 2005). The deepwater habit of *A. alata* appears to be uncommon for box jellyfish, as most are reported, and predicted, to inhabit shallow, nearshore waters (Yoshimoto and Yanagihara, 2002; Bentlage *et al.,* 2009; Gershwin *et al.*, 2009). Recently, a *Chironex* box jellyfish was reported in waters at depths of around 50 m, which, although comparatively much shallower than the depths at which *A. alata* has been documented (Lewis *et al.*, 2013), is much deeper than previously documented for *Chironex* (Keesing *et al.*, 2016). Whether adult medusae, cysts, or a combination of the two provide a natural means for maintaining global population cohesion in *A. alata* is unclear at this point, although the deep-sea tendencies of *A. alata* medusae may partly explain how these widespread populations maintain genetic connectivity. Indeed, several deep-sea hydrozoan jellyfish appear to have distributions spanning ocean basins (Collins *et al.*, 2008). In addition, jellyfish species living in the deep sea may also be globally distributed by natural means due to habitat homogeneity (Bentlage *et al.*, 2013). However, these additional examples of widespread species distributions pertain specifically to cnidarians with holopelagic development, in which the benthic polyp stage has been lost.

Concluding Remarks

We conclude that *Alatina alata* is a single species, comprising multiple nominal species, with a wide geographical distribution. *A. mordens* and *A. moseri* should be regarded as junior synonyms of *A. alata*. Currently, it is impossible to determine with certainty whether the observed widespread distribution of *A. alata* is a result of natural dispersal mechanisms or repeated anthropogenic introductions occurring as long as 100 years ago. Future studies with increased locus sampling allowed by current sequencing technologies such as the ezRAD (Toonen *et al.*, 2013), are needed to properly address this question. Further, expanding the geographic range to include *Alatina* samples from intermediate ocean basins, that is, the Indian and eastern Atlantic Oceans, should lead to a better understanding of the dispersal patterns and historical demography of this apparently cosmopolitan species.

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Appendix 1. Alatina alata records from museum collections or the literature

Species	Described as	Locality	Latitude	Longitude	Depth (m)	Reference (museum collection code no. or literature)
Alatina alata	*Charybdea alata*	Samoa	−13.83	−171.76	16	Stiasny, 1940
Alatina alata	*Alatina moseri*	Hawaii	25.12	−170.83	–	USNM 22311
Alatina alata	*Charybdea alata*	South Pacific Ocean	−7.77	−167.17	3800	Stiasny, 1940
Alatina alata	*Carybdea moseri*	Hawaii	21.38	−158.32	–	USNM 22309
Alatina alata	*Alatina alata*	Hawaii	21.59	−158.11	–	USNM 1245460
Alatina alata	*Alatina moseri*	Hawaii	21.28	−157.84	–	USNM 1124426–1124451, 1245923, 1245925, 1155723, 42112, 51962
Alatina alata	*Alatina moseri*	Hawaii	20.94	−157.08	–	USNM 21800, 29632
Alatina alata	*Alatina moseri*	South Pacific Ocean	−12.18	−150.00	860	USNM 1195808
Alatina alata	*Carybdea alata*	French Polynesia	−17.48	−149.84	0	FLMNH 8380
Alatina alata	*Alatina alata*	Mississippi (Gulf of Mexico)	27.64	−88.35	0	USNM 1131245
Alatina alata	*Carybdea alata*	Belize	16.79	−88.08	–	USNM 58207–58211
Alatina alata	*Alatina alata*	Mississippi (Gulf of Mexico)	29.16	−88.02	98–133	USNM 1131246
Alatina alata	*Alatina alata*	Mississippi (Gulf of Mexico)	29.32	−87.76	96.5–108.7	USNM 1005621
Alatina alata	*Charybdea alata*	Cuba	22.10	−84.97	–	Stiasny, 1940
Alatina alata	*Charybdea alata*	Cuba	20.13	−82.98	–	Stiasny, 1940
Alatina alata	*Charybdea alata*	Cuba	23.22	−82.35	–	Stiasny, 1940
Alatina alata	*Carybdea alata*	Cuba	23.53	−81.80	–	USNM 41920
Alatina alata	*Carybdea alata*	Bahamas	27.77	−78.77	–	USNM 41921

Species	Described as	Locality	Latitude	Longitude	Depth (m)	Reference (museum collection code no. or literature)
Alatina alata	*Carybdea alata*	Bahamas	26.06	−77.55	457–610	USNM 1195809
Alatina alata	*Carybdea alata*	Bahamas	25.45	−77.27	–	USNM 41919
Alatina alata	*Carybdea alata*	Cuba	20.00	−75.12	–	USNM 94780
Alatina alata	*Carybdea alata*	North Carolina	35.05	−74.68	204–228	USNM 53694
Alatina alata	*Carybdea alata*	South Carolina	32.55	−72.23	0–100	USNM 42017
Alatina alata	*Carybdea alata*	Northwest Atlantic	37.35	−69.17	0–90	USNM 56737
Alatina alata	*Alatina alata*	Bonaire	12.19	−68.30	–	USNM 1248604, 1248677
Alatina alata	*Alatina alata*	Bonaire	12.15	−68.28	–	USNM 1195801–1195907, 1205447–1205450, 1156074, 1156075
Alatina alata	*Carybdea alata*	Venezuela	10.90	−67.97	–	USNM 53659
Alatina alata	*Carybdea alata*	Puerto Rico	18.07	−67.88	–	USNM 54398, 54472
Alatina alata	*Carybdea alata*	Northwest Atlantic	37.83	−67.42	0–150	USNM 56735
Alatina alata	*Carybdea alata*	Northwest Atlantic	38.31	−66.86	0–50	USNM 56736
Alatina alata	*Charybdea alata*	Virgin Islands	17.72	−64.93	–	Stiasny, 1940
Alatina alata	*Charybdea alata*	Virgin Islands	17.75	−64.92	950	Stiasny, 1940
Alatina alata	*Carybdea alata*	Bermuda	31.88	−64.45	327–335	USNM 58691
Alatina alata	*Carybdea alata*	Bermuda	31.93	−64.42	55	USNM 58692, 58316
Alatina alata	*Carybdea alata*	Bermuda	32.58	−63.97	550–675	USNM 58655
Alatina alata	*Carybdea alata*	Bermuda	31.92	−63.95	0–300	USNM 54367
Alatina alata	*Carybdea alata*	Bermuda	31.93	−63.77	350	USNM 54366
Alatina alata	*Carybdea alata*	Antigua and Barbuda	17.00	−61.76	–	USNM 54385
Alatina alata	*Charybdea alata*	North Atlantic Ocean	27.03	−53.65	–	Stiasny, 1940
Alatina alata	*Carybdea alata*	Brazil	−14.62	−38.83	1067	Morandini, 2003
Alatina alata	*Charybdea alata*	Seychelles	−5.02	54.77	1880	Stiasny, 1940
Alatina alata	*Charybdea alata*	Sri Lanka	6.60	79.10	2530	Stiasny, 1940
Alatina alata	*Charybdea alata*	Sri Lanka	5.47	80.00	4000	Stiasny, 1940
Alatina alata	*Carybdea alata*	Indonesia	−2.33	118.83	–	USNM 42094
Alatina alata	*Charybdea alata*	Papua	−1.33	138.70	3450	Stiasny, 1940
Alatina alata	*Alatina alata*	Papua New Guinea	−3.38	143.53	1–2	USNM 1296950, 1296951
Alatina alata	*Alatina alata*	Saipan	15.24	145.71	–	USNM 1296956–1296960
Alatina alata	*Alatina mordens*	Australia	−13.90	146.63	–	USNM 1124410–1124425
Alatina alata	*Charybdea alata*	New Caledonia	−23.53	167.60	1060	Stiasny, 1940

(USNM, collection catalog coding of the National Museum of Natural History, Smithsonian Institution, Washington, D.C.; FLMNH, Florida Museum of Natural History, Gainesville, FL). Latitude and longitude are presented in decimal degrees.

Appendix 2. Sequences from Alatina alata and Alatina grandis specimens collected in this study and from other cubozoans available in GenBank

					GenBank Accession Numbers				
Order	**Family**	**Species**	**Isolate**	**Geographic location**	**ORF-PolB**	**COI**	**16S**	**ITS**	**28S**
Carybdeida	Alatinidae	*Alatina alata*	AGC519	Kralendijk, Bonaire	KU707221	–	–	–	KU707403
			AGC520		KU707222	KU707303	KU707330	–	KU707404
			TF1		KU707223	KU707304	KU707329	KU707358	KU707405
			TF2		KU707224	KU707305	–	KU707359	KU707406
			TM1		KU707225	KU707306	KU707331	KU707360	KU707407
			TM2		KU707226	KU707307	KU707332	KU707361	KU707408
			TM3		KU707227	KU707308	KU707333	KU707362	KU707409
			QLD28	Osprey Reef, Australia	KU707228	KU707292	KU707321	–	KU707390
			QLD29		KU707229	KU707293	KU707322	–	–
			QLD31A		–	–	–	–	KU707391
			QLD31B		–	–	–	–	KU707392
			QLD32A		KU707230	KU707294	KU707323	KU707349	KU707393
			QLD32B		KU707231	KU707295	KU707324	KU707350	KU707394
			QLD32C		KU707232	KU707296	KU707325	KU707351	KU707395
			QLD32D		KU707233	KU707297	KU707326	–	KU707396
			QLD32E		KU707234	KU707298	KU707327	KU707352	KU707397
			QLD32F		KU707235	KU707299	–	KU707353	KU707398
			QLD32G		KU707236	KU707300	KU707328	KU707354	KU707399
			QLD32H		KU707237	KU707301	–	KU707355	KU707400
			QLD32I		KU707238	KU707302	–	KU707356	KU707401
			QLD32J		KU707239	–	–	KU707357	KU707402
			CU01	Waikiki, Hawaii	–	KU707276	–	KU707334	KU707367
			CU02		KU707240	–	–	KU707335	KU707368
			CU03		–	–	GQ506987	–	–
			CU04		KU707241	–	GQ506988	KU707336	KU707369
			CU05		KU707242	KU707277	GQ506989	–	KU707370
			CU06		KU707243	KU707278	GQ506990	KU707337	KU707371
			CU07		KU707244	KU707279	GQ506991	KU707338	KU707372
			CU08		KU707245	KU707280	GQ506992	KU707339	KU707373
			CU09		–	–	GQ506993	–	–
			CU10		–	–	GQ506994	–	–
			CU11		KU707246	–	GQ506995	KU707340	KU707374
			CU12		KU707247	KU707281	GQ506996	–	KU707375
			CU13		KU707248	KU707282	KU707316	KU707341	KU707376
			CU14		KU707249	KU707283	GQ506997	KU707342	KU707377
			CU15		KU707250	KU707284	KU707317	KU707343	KU707378
			CU16		KU707251	KU707285	KU707318	–	KU707379
			CU17		KU707252	KU707286	KU707319	–	KU707380
			CU18		KU707253	–	KU707320	–	KU707381
			CU19		KU707254	–	–	–	KU707382
			CU20		KU707255	KU707287	GQ507000	KU707344	KU707383
			CU21		KU707256	–	GQ507001	–	KU707384
			CU22		KU707257	–	GQ507002	–	KU707385
			CU23		KU707258	KU707288	–	KU707345	KU707386
			CU24		KU707259	KU707289	GQ507003	KU707346	KU707387
			CU25		KU707260	KU707290	GQ507004	KU707347	KU707388
			CU27		KU707261	KU707291	GQ507005	KU707348	KU707389
			GS		KU707262	KU707274	KU707314	–	–
			isoB		KU707263	KU707275	KU707315	–	–
			Saipan31	Saipan, Northern Mariana Islands	KU707264	KU707269	KU707309	–	–

					GenBank Accession Numbers				
Order	**Family**	**Species**	**Isolate**	**Geographic location**	**ORF-PolB**	**COI**	**16S**	**ITS**	**28S**
			Saipan32		KU707265	KU707270	KU707310	KU707363	KU707410
			Saipan33		KU707266	KU707271	KU707311	KU707364	–
			Saipan34		KU707267	KU707272	KU707312	KU707365	–
			Saipan36		KU707268	KU707273	KU707313	KU707366	–
		non-*Carybdea marsupialis*		–	–	–	AF360118	–	–
				–	–	–	GQ849105	–	–
		Alatina grandis	Saipan38	Saipan, Northern Mariana Islands	–	–	KU707411	–	–
			Saipan39		–	–	KU707412	–	–
	Tripedaliidae	*Tripedalia cystophora*		–	–	–	GQ849123	–	–
				–	–	–	GQ849124	–	–
				–	–	–	KM200334	–	–
		Copula sivickisi		–	–	–	GQ849113	–	–
	Tamoyidae	*Tamoya haplonema*		–	–	–	HQ824526	–	–
				–	–	–	HQ824527	–	–
				–	–	–	HQ824529	–	–
		Tamoya ohboya		–	–	–	GQ849095	–	–
				–	–	–	HQ824528	–	–
		Tamoya sp.		–	–	–	GQ849122	–	–
	Carybdeidae	*Carybdea arborifera*		–	–	–	GQ849096	–	–
				–	–	–	KP053889	–	–
				–	–	–	KP053890	–	–
				–	–	–	KM200331	–	–
		Carybdea mora		–	–	–	AB720900	–	–
				–	–	–	GQ849106	–	–
				–	–	–	GQ849108	–	–
				–	–	–	GQ849125	–	–
				–	–	–	GQ849107	–	–
		Carybdea cf. *rastonii*		–	–	–	GQ849116	–	–
				–	–	–	GQ849117	–	–
		Carybdea rastonii		–	–	–	GQ849112	–	–
		Carybdea xaymacana		–	–	–	GQ849115	–	–
				–	–	–	GQ849114	–	–
				–	–	–	GQ849118	–	–
	Carukiidae	*Carukia barnesi*		–	–	–	GQ849097	–	–
				–	–	–	GQ849098	–	–
		Gerongia rifkinae		–	–	–	GQ849119	–	–
		Morbakka virulenta		–	–	–	GQ849120	–	–
				–	–	–	GQ849121	–	–
		Cubozoa sp.		–	–	–	JN184782	–	–
Chirodropida		*Chirodropidae* sp.		–	–	–	GQ849104	–	–
		Chironex fleckeri		–	–	–	GQ849101	–	–
				–	–	–	GQ849102	–	–
				–	–	–	GQ849103	–	–
		Chiropsella bronzie		–	–	–	GQ849099	–	–
				–	–	–	GQ849100	–	–
		Chiropsalmus quadrumanus		–	–	–	GQ849109	–	–
				–	–	–	GQ849110	–	–
				–	–	–	GQ849111	–	–

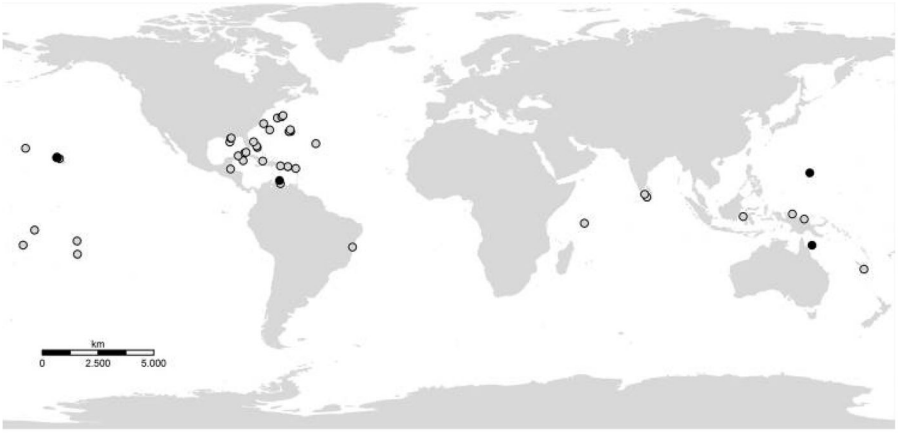

Figure 1.

Localities where *Alatina* medusae have been recorded in the literature or in museum collections (gray dots) and sampled for genetic analysis (black dots). Collection data and museum catalogue numbers, if applicable, are provided in Appendix 1.

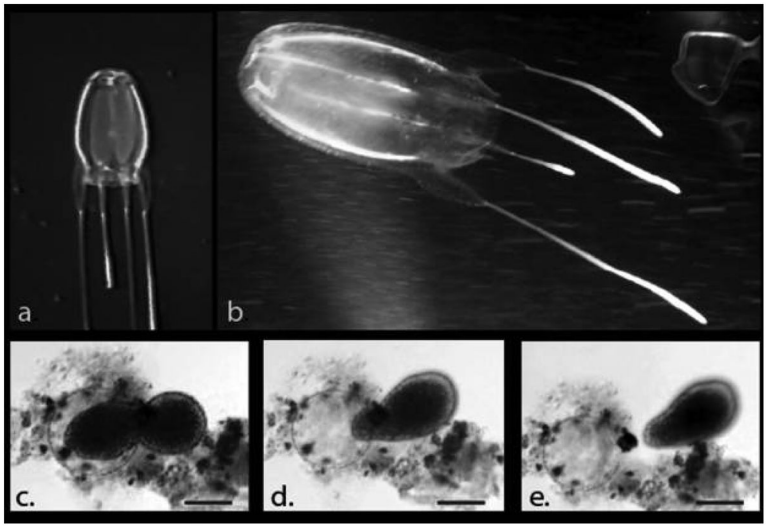

Figure 2.

Live *Alatina alata* medusae and encysted planula larva. (a) *A. alata* medusa recorded at a depth of 500–540 m, west off Gorda Cay, Bahamas, from the Johnson Sea Link I manned submersible (frame grab from video voucher USNM 1195809); (b) *A. alata* medusa next to diver 10–20 cm below the surface off Kralendijk, Bonaire, the Netherlands (Photograph courtesy of Jennifer Collins); (c–e) series of digital frame grabs taken from video footage of *A. alata* planula emerging from a cyst (scale bar = 50 μm).

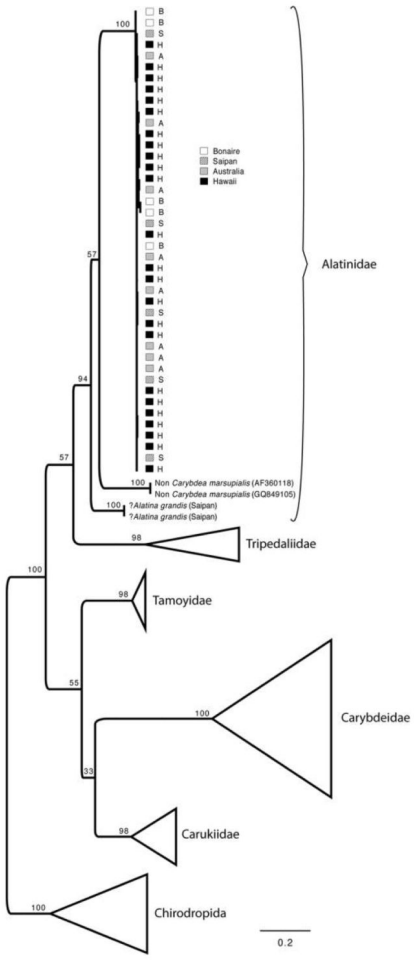

Figure 3.

Maximum-likelihood (ML) topology of the mitochondrial 16S of sampled *Alatina* specimens clustered within a cubozoan phylogeny, assuming the TIM2+I+G model of nucleotide evolution. ML nonpara-metric bootstrap support values are indicated for each node. Squares following legend indicate geographical origin of sampled specimens.

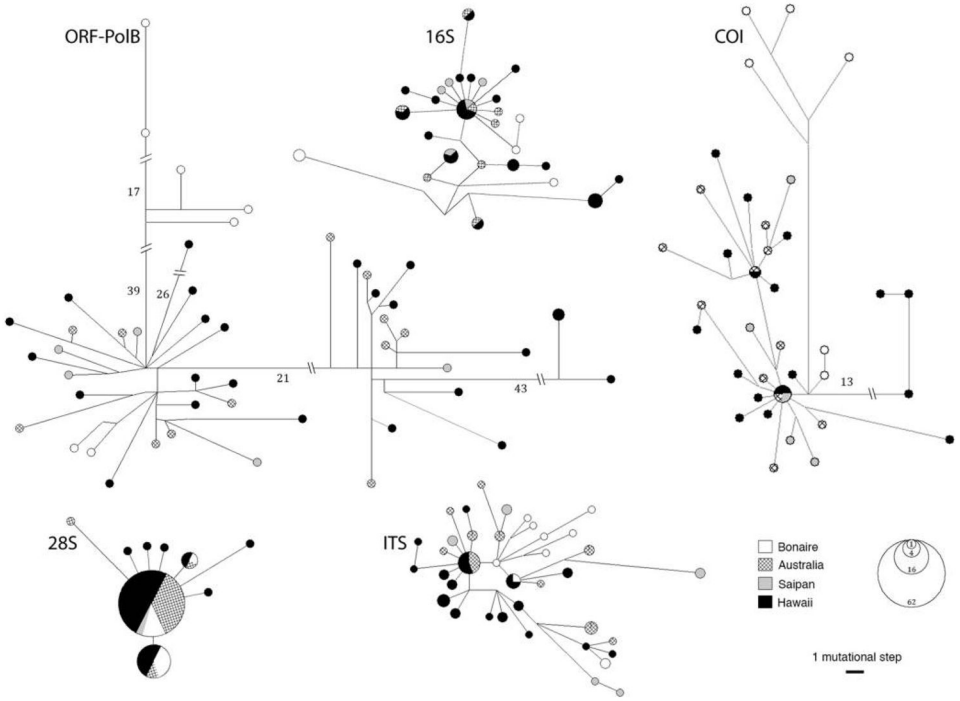

Figure 4.

Median-joining networks for mitochondrial (ORF-PolB, COI, and 16S) and nuclear (ITS and 28S) genes of *Alatina* specimens sampled. Each circle indicates one mitochondrial haplotype or nuclear allele, and symbols within indicate collection location (see key). The area of circles and symbols is proportional to its frequency in the dataset, according to circle size scale. Lines connecting haplotypes or alleles are proportional to the number of hypothesized mutational steps (see scale). Longer lines were reduced (indicated by parallel bars), and the number of mutational steps between closest nodes is indicated (not proportional to scale).

Table 1

Primers used for sequencing

Marker	Primer name	Primer	Reference
ORF-PolB	AM_ORF314-F1	AGCGCTATGATTAGAGTATTTAAGG	This study
	AM_ORF314-R1	TCAATTCTAGTTTAGAGCTTCCTC	This study
	AM_polB-F1	ATCCTGTACTAAGCCAAATCATC	This study
	AM_polB-R1	ATATAATCGGTCGTTAGTCGGC	This study
COI	med-cox1-F	ACNAAYCAYAAAGATATHGG	This study
	med-cox1-R	TGGTGNGCYCANACNATRAANCC	This study
16S	med-rnl-F	GACTGTTTACCAAAGACATAGC	This study
	med-rnl-R	AAGATAGAAACCTTCCTGTC	This study
ITS	C2	GAAAAGAACTTTGRARAGAGAGT	Chombard *et al.*, 1997
	D2	TCCGTGTTTCAAGACGGG	Chombard *et al.*, 1997
28S	28S-F63	AATAAGCGGAGGAAAAGAAAC	Medina *et al.*, 2001
	28S-R635	GGTCCGTGTTTCAAGACGG	Medina *et al.*, 2001

ORF-PolB primers were designed based on Kayal *et al.* (2012). COI and 16S were designed based on conserved regions among medusozoan cnidarians that overlap well with the commonly used Folmer *et al.* (1994) and Palumbi (1996) fragments, respectively.

Table 2

Percentage pairwise difference between sampling localities

		Bonaire	Australia	Hawaii	Saipan
ORF-PolB (0–6.47)	Bonaire	1.90 / 3.57	1.42	1.47	1.58
	Australia	4.47	1.26 / 2.55	1.55	1.27
	Hawaii	4.7	2.98	1.68 / 3.42	1.58
	Saipan	4.26	2.43	2.88	1.21 / 2.22
COI (0–4.48)	Bonaire	0.84 / 1.93	1.02	1.06	1.14
	Australia	2.52	0.59 / 1.23	1.06	0.59
	Hawaii	2.66	1.57	1.17 / 1.86	1.03
	Saipan	2.51	1.18	1.57	0.68 / 1.15
16S (0–2.02)	Bonaire	0.64 / 1.30	0.36	0.42	0.38
	Australia	1.15	0.27 / 0.69	0.38	0.26
	Hawaii	1.27	0.72	0.43 / 0.80	0.42
	Saipan	1.1	0.56	0.67	0.21 / 0.45
ITS (0–2.15)	Bonaire	0.35 / 0.92	0.35	0.31	0.49
	Australia	0.88	0.42 / 0.82	0.36	0.5
	Hawaii	0.81	0.72	0.30 / 0.64	0.52
	Saipan	1.19	1.05	1.05	0.48 / 1.35
28S (0–0.28)	Bonaire	0.06 / 0.09	0.07	0.07	0.05
	Australia	0.08	0.06 / 0.06	0.06	0.05
	Hawaii	0.09	0.07	0.07 / 0.09	0.05
	Saipan	0.06	0.03	0.05	

Values below diagonal are the averages; values above diagonal are the standard deviations. Ranges below marker names are the minimum and maximum percentage pairwise differences recorded for the marker. Absent values indicate lack of sampling for average and standard deviation calculations.

Table 3

Molecular diversity indices

	Indices	Bonaire	Saipan	Australia	Hawaii	All
ORF-PolB	*N*	7	5	12	24	48
	Nh	7	5	12	23	47
	h	1 ± 0.08	1 ± 0.13	1 ± 0.03	0.99 ± 0.01	0.99 ± 0
	π	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0	0.03 ± 0	0.03 ± 0
COI	*N*	6	5	11	18	40
	Nh	6	5	11	17	36
	h	1 ± 0.09	1 ± 0.13	1 ± 0.04	0.99 ± 0.02	0.99 ± 0.01
	π	0.02 ± 0	0.01 ± 0	0.01 ± 0	0.02 ± 0	0.02 ± 0
16S	*N*	5	5	8	24	42
	Nh	4	5	8	15	26
	h	0.9 ± 0.16	1 ± 0.13	1 ± 0.06	0.94 ± 0.03	0.96 ± 0.02
	π	0.01 ± 0	0.01 ± 0	0.01 ± 0	0.01 ± 0	0.01 ± 0
ITS	*N*	5	4	9	15	33
	Nh	9	5	10	16	19
	h	0.98 ± 0.05	0.89 ± 0.09	0.92 ± 0.04	0.95 ± 0.02	0.97 ± 0.01
	π	0.01 ± 0	0.01 ± 0	0.01 ± 0	0.01 ± 0	0.01 ± 0
28S	*N*	7	1	13	23	44
	Nh	3	1	4	8	9
	h	0.6 ± 0.08	0 ± 0	0.29 ± 0.11	0.52 ± 0.08	0.47 ± 0.06
	π	0.001 ± 0	0 ± 0	0.001 ± 0	0.001 ± 0	0 ± 0

Haplotype and nucleotide diversity are represented as mean ± standard deviation.

N, number of individuals sequenced; Nh, number of haplotypes or alleles; h, haplotype diversity; π, nucleotide diversity.

Table 4

AMOVA and Isolation by Distance (IBD) analyses

	ORF-PolB	COI	16S	ITS	28S
All four sampled localities					
Among population variation	14%	17%	9%	9%	3%
Within population variation	86%	83%	91%	91%	97%
ΦST/FST	**0.137**	**0.170**	**0.086**	**0.088**	0.032
Atlantic *vs.* Pacific					
Among group variation	31%	35%	27%	4%	12%
Within group variation	−1%	0%	−2%	7%	−2%
Within population variation	70%	65%	75%	89%	90%
ΦST/FST	**0.302**	**0.354**	**0.249**	**0.110**	0.099
ΦSC/FSC	−0.010	0.001	−0.031	**0.075**	−0.027
ΦCT/FCT	0.309	0.353	0.272	0.038	0.122
Isolation by distance (r)	0.791	**0.968**	0.850	0.260	0.955
P-value	0.208	0.041	0.129	0.417	0.121

Analysis of molecular variance (AMOVA) is represented in the first part of the table with only one group encompassing the entire dataset including all four populations; then with two groups divided by ocean basin (*i.e.,* Pacific containing Saipan, Australia, and Hawaii *vs.* Atlantic containing Bonaire). The mitochondrial AMOVA was calculated assuming the TrN model of nucleotide evolution. Molecular variance is divided into components of among groups (ΦCT and FCT), among localities within groups (ΦSC and FSC), and among all localities (ΦST and FST). Values in bold are significant ($P < 0.05$).

Table 5

Pairwise ΦST and FST between sampling localities

		Bonaire	Australia	Hawaii
ORF-PolB	Bonaire			
	Australia	**0.33856**		
	Hawaii	**0.26632**	−0.00804	
	Saipan	**0.30904**	0.0088	−0.01313
COI	Bonaire			
	Australia	**0.40264**		
	Hawaii	**0.2944**	0.00702	
	Saipan	**0.37697**	−0.00964	0.00402
16S	Bonaire			
	Australia	**0.15865**		
	Hawaii	**0.24323**	−0.04435	
	Saipan	0.19975	−0.05031	0.01688
ITS	Bonaire			
	Australia	**0.0751**		
	Hawaii	**0.10827**	0.02792	
	Saipan	**0.15351**	0.06368	**0.18581**
28S	Bonaire			
	Australia	**0.17493**		
	Hawaii	0.05603	−0.00225	
	Saipan	0.01712	−0.31725	−0.25806

Values in bold are significant ($P < 0.05$). The Pairwise ΦSTs (mitochondrial) were calculated assuming the TrN model of nucleotide evolution.