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A New Deepwater Species of Stauromedusae, *Lucernaria janetae* (Cnidaria, Staurozoa, Lucernariidae), and a Preliminary Investigation of Stauromedusan Phylogeny Based on Nuclear and Mitochondrial rDNA Data

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Abstract. The deepwater stauromedusan *Lucernaria janetae* n. sp is described from adult and juvenile specimens collected from the East Pacific Rise. *Lucernaria janetae* is the first species in the genus recorded from the Pacific Ocean, and differs from its congeners in size and morphology. Mitochondrial (16S) and nuclear (SSU) ribosomal gene sequences from *L. janetae* were analyzed with those of representative stauromedusan taxa to evaluate stauromedusan monophyly. Both genes recovered a strongly monophyletic Stauromedusae that is the sister group to all other medusozoans. Support of these hypotheses is robust to method of phylogenetic reconstruction and to outgroup selection, buttressing the argument that Stauromedusae should be recognized as the class Staurozoa. The molecular markers used here favor the same topology of relationships among our samples and clearly distinguished between two species, *Haliclystus sanjuanensis* and *H. octoradiatus,* that have been considered synonymous by many workers. A stable systematic framework for Stauromedusae appears achievable through comprehensive study of both morphological and sequence data.

Introduction

Deep-sea hydrothermal vent communities have been intensively studied since their discovery (Ballard, 1977; Lonsdale, 1977), but continue to yield major new macrofaunal taxa and kinds of communities. Among some of these novel communities associated with areas of diffuse flow near active vents are spectacular fields of "stalked jellyfish" (Stauromedusae) up to 10 cm in height (Lutz *et al.,* 1998; Halanych *et al.,* 1999). Stauromedusans are typically small and solitary, and live in shallow near-shore habitats of temperate seas, highlighting the unusual nature of this deep-sea occurrence. Despite their benthic nature, members of Stauromedusae have traditionally been grouped as an order within the cnidarian class Scyphozoa. However, recent phylogenetic analyses of Cnidaria based on morphology (Marques and Collins, 2004) and molecular data (Collins, 2002) suggest that Stauromedusae is not more closely related to the scyphozoan taxa Coronatae, Rhizostomeae, and Semaeostomeae (herein united as Scyphozoa, following Marques and Collins, 2004) than it is to Cubozoa or Hydrozoa.

Evolutionary discussions of stauromedusans have largely focused on their relationship to other groups of Cnidaria (*e.g.,* Uchida, 1929, 1972; Thiel, 1966) rather than on the relationships among its component groups (but see Thiel, 1936; Uchida, 1972). Comparatively little effort has been put into determining the systematic relationships within Stauromedusae. As a result, families and genera are recognized by a mosaic of features, many of which are not exclusive, or which suggest contradictory groupings. As an example relevant to the findings reported here, *Lucernaria* is often grouped with *Haliclystus* to the exclusion of *Lucernariopsis* because both the former have muscles in the

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peduncle. However, both *Lucernaria* and *Lucernariopsis* lack perradial anchors and have a single-chambered peduncle, whereas *Haliclystus* has anchors and a four-chambered peduncle. The taxonomy of Stauromedusae is further hindered by the fact that many species are rarely encountered. The group is in need of a thorough systematic revision.

In November 2003, a camera sled towed by the R/V *Atlantis* serendipitously captured footage of a stauromedusan aggregation near 8° 37′ North on the East Pacific Rise. This was not the first sighting of stauromedusans in the deep East Pacific (*e.g.,* Lutz *et al.,* 1998; Halanych *et al.,* 1999), but two subsequent dives in the DSV *Alvin* allowed for these animals to be collected and examined in detail. Our samples include individuals at several ontogenetic stages, allowing us to describe the morphology of both adults and juveniles. On the basis of our examinations, we find that these specimens belong to a new species, described here as *Lucernaria janetae.* This is the first species of *Lucernaria* described from the Pacific Ocean. In addition, we extracted DNA from a specimen of *L. janetae* and amplified two genes, one coding for the complete small subunit of the nuclear ribosome (SSU), the other for a region of the mitochondrial large ribosomal subunit (16S). Combining these data with data from five other species of Stauromedusae, we assess the usefulness of these markers for revealing historical relationships within Stauromedusae and present an initial investigation of stauromedusan phylogeny.

Materials and Methods

Footage from a camera sled towed by the R/V *Atlantis* of the Woods Hole Oceanographic Institution in November 2003 revealed dense aggregations of large stauromedusans in a previously undocumented area of weak hydrothermal activity at 8° 36.745′N, 104° 12.740′W. During two dives (3935, 3927) in the submersible *Alvin,* the extent of the aggregations was determined, populations were documented with still digital photography and video, and several specimens were collected by using suction samplers.

Live material was photographed and examined and then fixed in 20% formalin or 95% ethanol. Formalin-fixed material was transferred to 70% ethanol after 2 weeks. Additional material was frozen for molecular or isotopic analyses. All specimens have been deposited at the Field Museum of Natural History, Chicago, Illinois. Preserved specimens were examined whole and in dissection; some material was processed for histology with standard paraffin techniques. Histological slides were stained in Masson's trichrome (Presnell and Schreibman, 1997). Pieces of tissue from tentacles, subumbrellar vesicles, and gastric filaments were smeared on a slide; nematocysts in these smears were examined using differential interference contrast microscopy at 100× magnification. Cnidae terminology follows Mariscal (1974). Nematocyst type, size, and location are recorded because these data may be useful for future systematic studies of Stauromedusae.

The Invisorb extraction kit (Invitek GmbH, Berlin) was used to obtain DNA from one specimen each of *Lucernaria janetae* (FMNH 10329) preserved in 95% ethanol, *Craterolophus convolvulus* (Johnston, 1835), *Depastromorpha africana* Carlgren, 1935, and an undescribed species of *Haliclystus.* From these DNA preparations, as well as those from *Haliclystus octoradiatus* Clark, 1863, and *Haliclystus sanjuanensis* Hyman, 1940 (see Table 1 for locality data for all samples), a 530–560-bp region of mitochondrial 16S was amplified, using the forward primer from Cunningham and Buss (1993) combined with the reverse primer from Schroth *et al.* (2002). Products of the polymerase chain reaction (PCR) were purified and sequenced in both directions by using a Megabace 500 automated sequencer. Similarly, nearly complete sequences of the gene coding for SSU (or 18S) were obtained (except for *H. sanjuanensis,* which had already been sequenced for a prior study; Collins, 2002) by using standard PCR and sequencing primers (Medlin *et al.,* 1988). Edited 16S sequences were aligned by using ClustalW and then improved by eye with the software SeaView (Galtier *et al.,* 1996) along with sequences from two stauromedusans and six representatives of outgroup taxa obtained from GenBank; edited SSU sequences were aligned by eye into a dataset (derived from that used in Collins, 2002) comprising more than 150 other cnidarian species. All alignments used in this study are available upon request.

Phylogenetic analyses were carried out on three datasets using PAUP* 4.0 (Swofford, 2002). The first data set contains 230 characters of 16S that are hypothesized to be homologous across our stauromedusan samples and the six outgroup taxa representing Anthozoa, Cubozoa, Hydrozoa, and Scyphozoa. For the SSU sequences, we excluded regions that could not be reliably aligned across Stauromedusae and the eight outgroup taxa; the resulting alignment is 1746 bases. The third dataset comprises both 16S and SSU data from Stauromedusae; narrowing the taxonomic focus allowed us to include an additional 233 characters from 16S rDNA. For each dataset, we searched for optimal trees by using the criteria of maximum parsimony (MP) and maxi-

Table 1

Stauromedusans sampled for molecular data

Species	Locality of collection
Craterolophus convolvulus	Helgoland, Germany
Depastromorpha africana	False Bay, South Africa
Haliclystus octoradiatus	Northern Germany
Haliclystus sanjuanensis	Washington State, USA
Haliclystus sp.	Los Molinos (near Valdivia), Chile
Lucernaria janetae	8° 37′ North on the East Pacific Rise

mum likelihood (ML), with 500 and 100 replicate searches, respectively, and with sequences added randomly to the starting topology. Gaps were treated as missing data. We used likelihood ratio tests employed by ModelTest ver. 3.6 (Posada and Crandall, 1998) to determine an appropriate model of nucleotide evolution assumed for the ML searches. We assessed node support with bootstrap analyses of 500 and 200 pseudo-replicate data sets under MP and ML. In addition, we calculated decay indices (Bremer, 1988) by using constrained tree searches to measure the extent to which the parsimony criterion must be relaxed to compromise clades present in the most parsimonious topology. Finally, for the two data sets containing outgroup taxa, we conducted a series of MP analyses with all combinations of outgroups to determine their impact on rooting the portion of the topology containing Stauromedusae.

Results

Lucernaria janetae, Collins and Daly, new species

Lucernaria sp., Lutz *et al.,* 1998

Differential diagnosis. Exceptionally large, cream-colored stauromedusan with 8 adradial clusters of about 100 tentacles. Adults lack primary tentacles; small juveniles may bear small, ovate primary tentacles. Calyx goblet-shaped, equal in height to peduncle; peduncle monocameral and muscular. Gonads lanceolate, extending from base of calyx to base of arms.

Material examined. Holotype (FMNH 12492) 1 adult, East Pacific Rise, −2538 m, 8° 36.745′N, 104° 12.740′W, 6 Nov. 2003. Paratypes (FMNH 10328) 4 adults, 3 juveniles, East Pacific Rise, −2538 m, 8° 36.745′N, 104° 12.740′W, 6 Nov. 2003. Additional specimens (FMNH 10327) 8 adults, East Pacific Rise, −2553 m, 8°36.578′N, 104° 12.623′W, 8 Nov. 2003.

Adult external anatomy. Calyx goblet-shaped, creamy white with faint greenish or orange cast in life; all preserved specimens creamy white (Fig. 1). Calyx of live specimens to 100 mm wide, 50 mm deep; calyx width in preserved specimens to 30 mm, depth to 15 mm. Exumbrella smooth, without ridges or visible clusters of nematocysts. Mouth rectangular, slightly elongated at corners, opaque and lighter in color than calyx in life and in preservation. Inter- and per-radial notches approximately equal. Arms equidistant, identical in size and morphology, each with rounded cluster of about 100 monomorphic, capitate secondary tentacles. No anchors or primary tentacles. Rounded head of each secondary tentacle opaque cream, sharply demarcated from stalk. Secondary tentacles in center of cluster slightly longer than those on periphery.

Peduncle same color as calyx, tubular, length approximately equal to depth of calyx. Junction between peduncle and calyx abrupt rather than smoothly tapering (Fig. 1). Peduncle monocameral, divided by four septal cords (Fig. 2A) that may extend only midway down its length; each cord bears a pinnately branched longitudinal muscle (Fig. 2B). Basal disc not distinct; peduncle does not flare proximally. In one specimen, small juvenile attached to basal end (Fig. 3B).

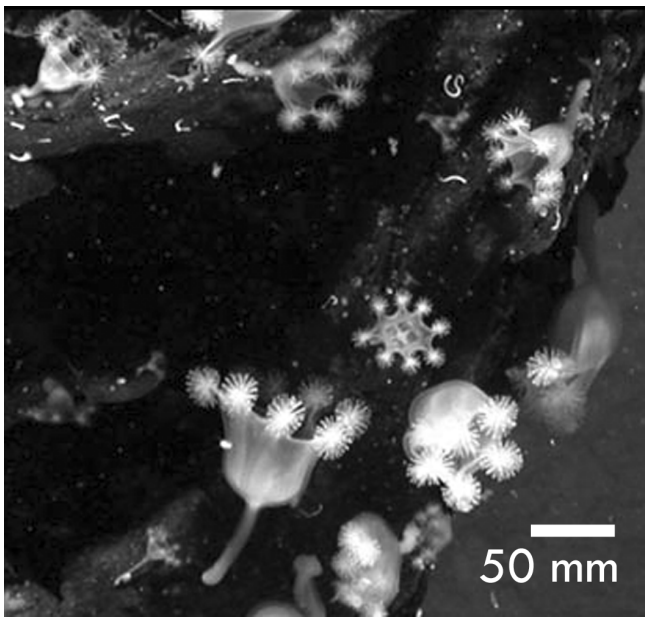

Figure 1. Living specimens of *Lucernaria janetae,* n. sp., *in situ.* Note variety of sizes represented in population.

Internal anatomy. Gamete-bearing tissue in 8 large, paired, lanceolate pads densely covered with bilobed, often U-shaped, vesicles that contain nematocysts and gametes (Fig. 2C). Vesicles on a single pad vary in size and shape, and are not arranged in rows. Coronal muscle separates paired sets of pads from one another. Each gametogenic pad extends from the base of the calyx into the base of the arms; proximal portion of pads separated by gastric filaments. Gastric filaments opaque cream, long, slender, bluntly pointed, restricted to base of calyx, between gametogenic pads.

Four equally developed, Y-shaped coronal muscles separate adjacent arms of calyx: stem of each Y runs between adjacent arms, arms of each Y belong to adjacent calyx arms. Radial muscles strong, discontinuous between arms.

Cnidom. Euryteles and holotrichs (Fig. 4). See Table 2 for size and distribution.

Morphology of juveniles. Smallest juvenile attached to underside of basal end of large adult (Fig. 3B); total height 2 mm, calyx width 1 mm, color uniformly white. Compared to adult or larger juvenile, calyx relatively tall and narrow,

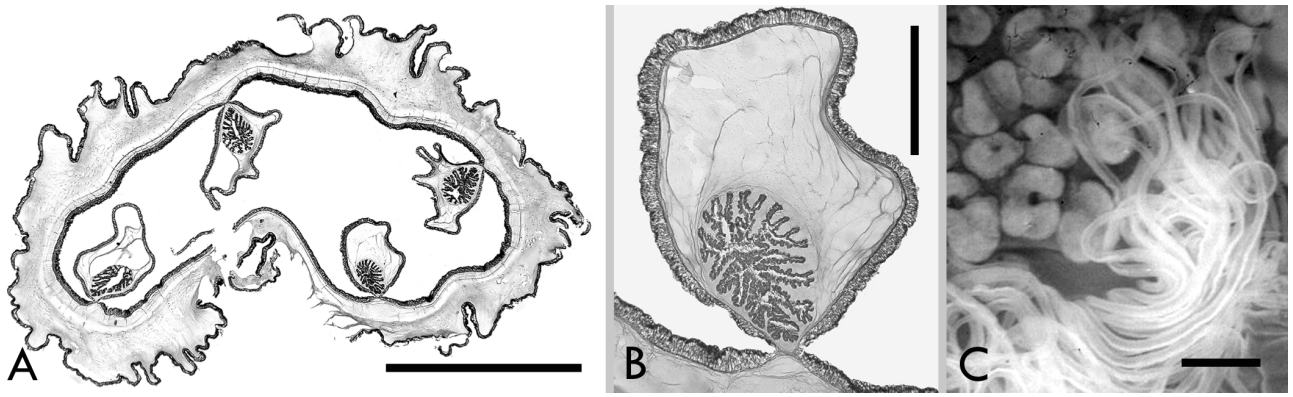

Figure 2. Internal anatomy of *Lucernaria janetae,* n. sp. (A) Transverse section through peduncle, showing four septal muscles. (B) Detail of a septal muscle. (C) Subumbrellar surface, showing U-shaped vesicles and slender gastric filaments. Scale bars: A = 2 mm; B, C = 0.5 mm.

more wedge- than goblet-shaped. Eight clusters of secondary tentacles; calyx not notched between each cluster. Secondary tentacle clusters with fewer members; tentacles relatively thicker, shorter, not capitate; opaque, round head at distal end not demarcated from stalk. Primary tentacles not visible.

Larger juveniles not attached to adult. Calyx width of larger of two specimens 3 mm, depth 3 mm; peduncle length 4 mm; smaller specimen calyx width 3 mm, depth 4 mm, peduncle length 2 mm. Color of both uniformly white. Calyx shape and proportions similar to those of adults (Fig. 3A): calyx goblet-shaped with rounded proximal end. Inter- and per-radial notches equal in depth, relatively shallower than in adults but clearly divide calyx into eight arms. Eight clusters of capitate secondary tentacles (Fig. 3A, C); compared to adults, clusters with fewer members. Small, oval, opaque primary tentacles (Fig. 3C); primary tentacles nodule-like, raised between secondary tentacles. No nematocysts found in primary tentacles; secondary tentacles with sparse, relatively

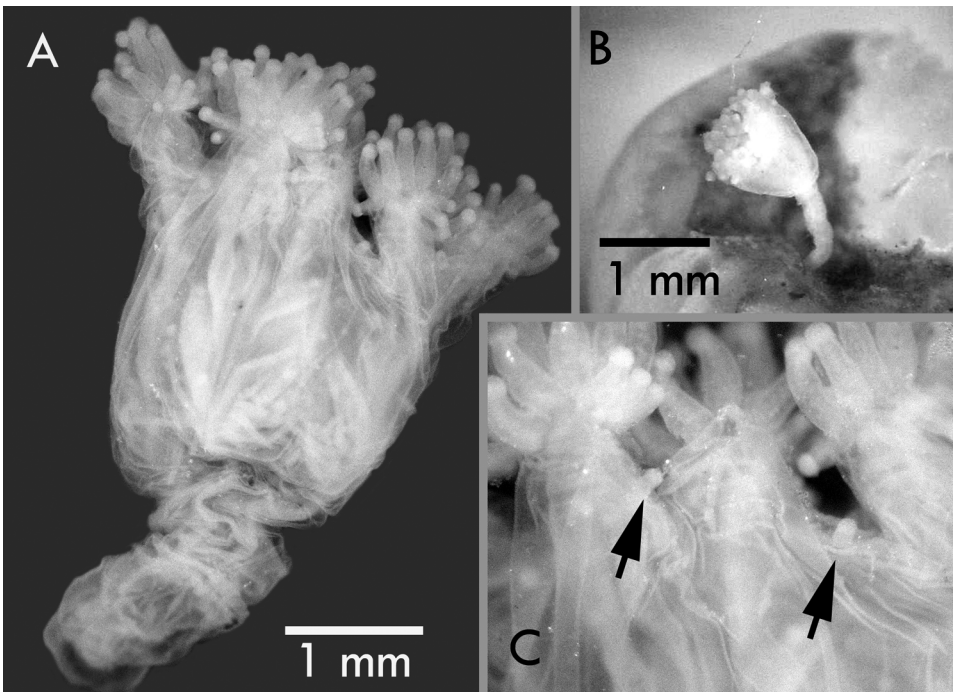

Figure 3. Morphology of juvenile specimens of *Lucernaria janetae,* n. sp. (A) One of the two larger juveniles; general shape and proportions as in adults. Scale bar = 1 mm. (B) Smallest juvenile, attached to basal end of an adult. Scale bar = 1 mm. (C) Primary tentacles (arrows) on specimen in A.

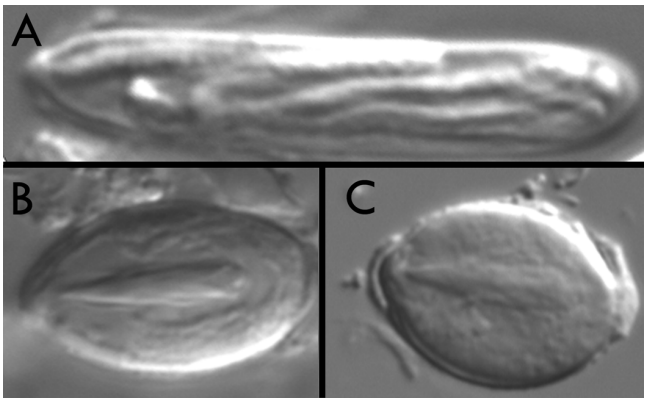

Figure 4. Representative cnidae from *Lucernaria janetae,* n. sp. (A) Holotrich. (B) Eurytele. (C) Eurytele.

smaller euryteles (5.4–9.5 × 3.3–5.6 μm, $n = 10$) and no holotrichs.

Etymology. Named for Dr. Janet Voight, The Field Museum, Chicago, in recognition of her commitment to discovering and describing deep-sea invertebrates.

Natural history and distribution. In terms of number of individuals and biomass, *Lucernaria janetae* is the dominant macrofaunal organism where it occurs. Asexual propagation is not known in Stauromedusae, but observations of dense aggregations and a juvenile attached to the base of the peduncle raise the question of whether *L. janetae* might be able to proliferate in this manner. Several specimens contained small pieces of crustacean legs and antennae, suggesting that *L. janetae* eats small pelagic crustaceans. The high density is likely the result of limited dispersal; most stauromedusans have nonciliated, creeping planulae (Otto, 1976, 1978). In the intertidal species *Haliclystus octoradiatus,* young settle together (Wietrzykowski, 1912); in *L. janetae,* the smallest specimen was associated with a large adult.

Lutz *et al.* (1998) and Halanych *et al.* (1999) observed *Lucernaria*-dominated communities at 20°50.304′N 109°05.422′W and 7°25.23′S 107°47.72′W, respectively. We tentatively identify these as *L. janetae,* but their identity cannot be definitely determined in the absence of specimens. Sea anemones (*Cynanthea* sp.) were noted by Lutz *et al.* (1998) and Halanych *et al.* (1999), but we did not observe any. Like Halanych *et al.* (1999), we found a few specimens of the tubeworm *Tevnia jerichonana* in the stauromedusan communities we sampled. A few specimens of mobile, vent-associated invertebrates were seen at the type locality, including galatheid (*Munidopsis squamosa*) and bythograeid (*Bythograea thermydon*) crabs.

Similar species. Most species of Stauromedusae are small (< 40 mm width or height); *Lucernaria* comprises all described species of Stauromedusae having an adult calyx diameter larger than 50 mm (Kramp, 1961). All species of *Lucernaria* except *L. janetae* are known only from the Atlantic Ocean. *L. janetae* is easily distinguished from *Lucernaria quadricornis* Müller, 1776, on the basis of habitat: *L. quadricornis* occurs in the shallow North Atlantic (*e.g.,* Hargitt, 1904; Mayer, 1910; Kramp, 1961; Cornelius *et al.,* 1990). Furthermore, the two differ noticeably in the shape of the gonads and the depth of the perradial notches. *Lucernaria bathyphilia* Haeckel 1880, the only other large, deep-water species in the genus, differs from *L. janetae* in the length of the peduncle and pairing of the arms. In *L. bathyphilia,* the peduncle is about one- tenth as long as the calyx; in *L. janetae,* the peduncle and calyx are about equal in length. Haeckel's (1881) drawings indicate that the arms of *L. bathyphilia* are extremely short—just barely separated from the margin of the bell; *L. janetae* has 8 distinct arms.

Gene sequences. New sequences generated for this study have been assigned GenBank accession numbers AY845338–AY845348. The region of mitochondrial 16S amplified from our stauromedusan samples is roughly 545 bases long. A number of insertion and deletion events (indels) were inferred during the alignment of stauromedusan 16S sequences, but none of these indels were longer than two bases. The near-complete SSU sequences from stauromedusans vary between 1750 and 1754 bases in length, and there are few indels. Not surprisingly, the mitochondrial 16S gene appears to evolve considerably faster than the nuclear SSU gene in Stauromedusae. For example, the uncorrected p-distance between the 16S of *L. janetae* and *Craterolophus convolvulus* is 21.4%, whereas that between their SSU is 1.39%. Similarly, our sampled representatives of *L. janetae* and *Haliclystus octoradiatus* differ by 24.0% and 1.33% for 16S and SSU, respectively. Within the genus *Haliclystus, H. sanjuanensis* and *H.* sp. from Chile have the least-diverged sequences (4.02% for 16S and identical for SSU). By these measures, both *H. sanjuanensis* and

Table 2

Size and distribution of nematocysts of Lucernaria janetae

Tissue	Nematocyst	*n*	*N*	Range
Subumbrellar vesicle	Eurytele B, C	34	3/3	19.3–12.2×6.2–8.9
	Holotrich A	34	3/3	20.4–16.4×2.2–4.3
Tentacle	Eurytele B, C	34	3/3	15.5–12.6×6.4–7.7
	Holotrich A	34	3/3	21.8–18.3×2.9–4.5
Gastric filament	Eurytele B, C	31	3/3	12.1–10.8×8–8.8

Letters refer to Figure 4; "*N*" is the proportion of examined specimens that had a particular type of nematocyst; "*n*" is the number of capsules measured; size presented as range of lengths by widths, in micrometers, for undischarged capsules.

H. sp. from Chile differ from *H. octoradiatus* by roughly 12% (16S) and 0.5% (SSU).

GenBank contains sequences of 16S and SSU for Stauromedusae that we infer to be erroneous. For 16S, the GenBank sequence U19376, identified as *Haliclystus* sp., is identical to the one we obtained from *C. convolvulus.* Our 16S sequence for *C. convolvulus* differs from a sequence in GenBank identified as *C. convolvulus* (U19375) by a single nucleotide change. The SSU sequence in GenBank purporting to be *Haliclystus* sp. (AF099103) is identical to our SSU sequence from *C. convolvulus,* whereas another GenBank sequence (AF099104) for *C. convolvulus* is highly similar to sequences from *Haliclystus.* The SSU sequences were generated as part of the same study (Kim *et al.,* 1999), and evidently the species names attached to them were inadvertently reversed at some point.

Phylogenetic relationships. Mitochondrial 16S and nuclear SSU data indicate identical sets of relationships among the stauromedusans sampled here (Figs. 5, 6). Both 16S and SSU sequences recover a monophyletic Stauromedusae. *Craterolophus convolvulus* is the sister taxon to a clade containing all other species. Within this clade, *L. janetae* appears at the base, and *D. africana* is sister to the three species of *Haliclystus. H. sanjuanensis* from the Northwest Pacific and *Haliclystus* sp. from Chile are more closely related to each other than either is to *H. octoradiatus* from northern Europe.

Inferred relationships among our samples are robust to the method used to reconstruct them. The topology of the ingroup based on 16S data does not change whether the optimality criterion is MP (Fig. 5) or ML (not shown). Similarly, the SSU-based MP topology (not shown) perfectly mirrors the topology for which our data are most likely (Fig. 6). In both the 16S and SSU analyses, the positions of all taxa are supported with bootstrap values greater than 75%, with the exception of *L. janetae* (Figs. 5, 6). Although its placement is unequivocal in all analyses, the position of *L. janetae* receives only limited support from both molecular markers. However, the placement of the root between *Craterolophus convolvulus* and the clade containing *L. janetae* at its base is remarkably stable to the use of different combinations of outgroup taxa. When 16S data are used, the root position shown in Figure 5 is found in all sets of most-parsimonious trees obtained using any combination of the outgroups as well as any used alone. Similarly, for SSU data, all possible combinations of outgroups, with the exception of hydrozoans used alone, yield a topology containing a root as shown in Figure 6.

Discussion

Diversity of Stauromedusae

We have added one to the total of roughly 50 known species of Stauromedusae (Mills, 2004). Stauromedusans form an easily distinguished group that is potentially united

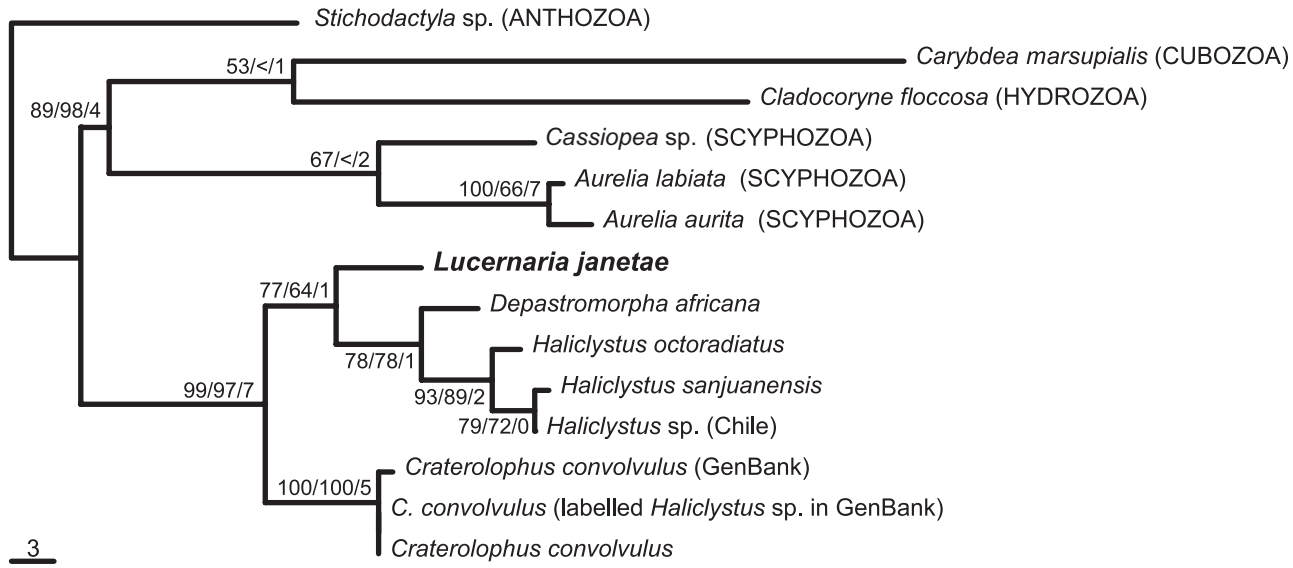

Figure 5. Maximum parsimony (MP) phylogram of relationships among sampled species of Stauromedusae based on mitochondrial 16S data, with MP and maximum likelihood (ML) bootstrap and decay indices shown at the nodes. "<" indicates a bootstrap index less than 50. The assumed model (TrNef+G) of nucleotide evolution for ML tree searches has one rate for transversions (1.0000), two rates for transitions (A–G, 1.6893; C–T, 3.3596), a gamma shape parameter (0.4330), and equal base frequencies. Listed in the order they appear in the figure, GenBank accession numbers for outgroups are as follows: Cm, AF360118; Cf, AY512535; Cs, U19374; Al, AF461401; Aa, U19373; and Ss, AY345874.

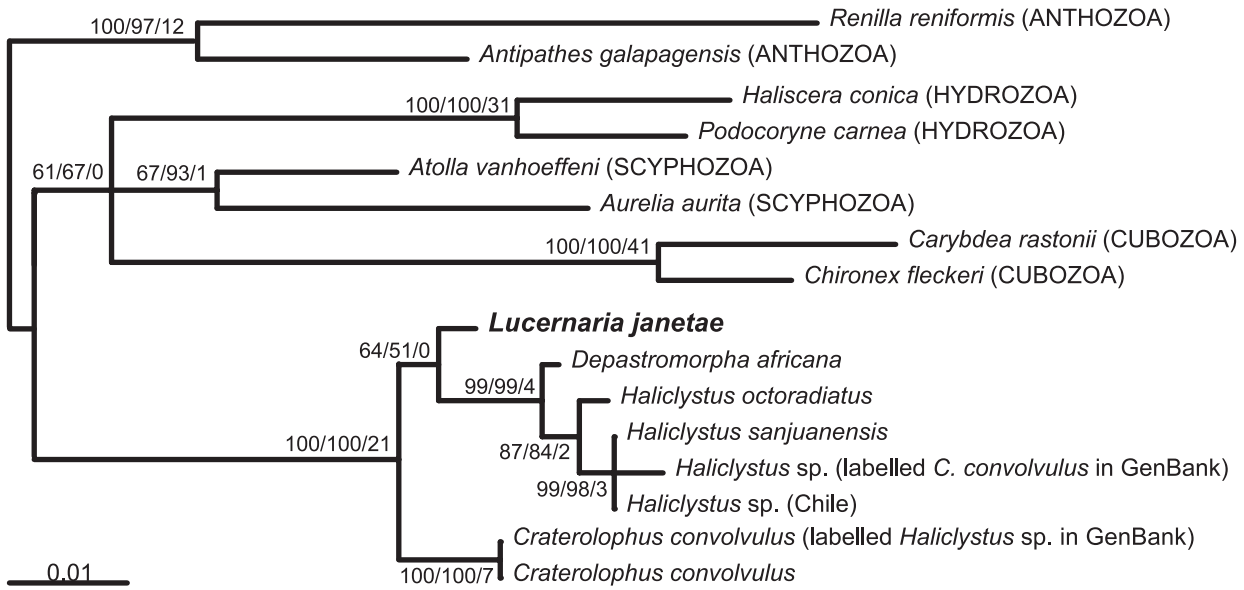

Figure 6. Maximum likelihood (ML) phylogram of relationships among sampled species of Stauromedusae based on nuclear SSU data, with maximum parsimony (MP) and ML bootstrap and decay indices shown at the nodes. "<" indicates a bootstrap index less than 50. The assumed model (TrN+I+G) of nucleotide evolution for ML tree searches has one rate for transversions (1.0000), two rates for transitions (A–G, 2.5175; C–T, 4.6618), an assumed proportion of invariant sites (0.5139), a gamma shape parameter (0.6443), and unequal base frequencies. Listed in the order they appear in the figure, GenBank accession numbers for outgroups are as follows: Hc, AF358064; Pc, AF358092; Av, AF100942; Aa, AY039208; Cr, AF358108; Cf, AF358104; Rr, AF052581; and Ag, AF100943.

by a nonciliated creeping planula with 16 rectangular endodermal cells (Otto, 1976, 1978), a four-chambered peduncle with an adhesive basal disk, eight clusters of capitate tentacles, and perhaps complex ovaries involving follicle cells. The generality of this last feature is somewhat tentative as it has been studied in only a single species, but it is dramatically different from what has been observed in coronates, rhizostomes, and semaeostomes (Eckelbarger and Larson, 1993). The molecular data presented here, though limited in terms of taxon sampling, indicate that Stauromedusae is indeed a clade.

As mentioned in the introduction, most discussions of the evolution of Stauromedusae have aimed to determine its phylogenetic position within Cnidaria. Members of Stauromedusae possess features that appear to be homologous with those of Cubozoa and Scyphozoa, such as intramesogleal muscles of the polyp, gastric filaments, hollow structures ontogenetically derived from primary polyp tentacles known as anchors or rhopalioids, and a metamorphosis from juvenile to adult morphology concentrated at their oral ends (Uchida, 1929; Hirano, 1986; Kikinger and Salvini-Plawen, 1995). Because of these similarities, Stauromedusae, Cubozoa, and Scyphozoa have classically been treated as a natural group. Indeed, a recent cladistic analysis of morphological and life-history characters (Marques and Collins, 2004) favored the recognition of this clade. In contrast, molecular data raise the possibility that these groups form a paraphyletic assemblage whose members share a set of characters that were lost in the lineage leading to Hydrozoa (Collins, 2002; Collins, unpubl data). Our 16S and SSU data add to the accumulating evidence that this is the case.

Specifically, accepting that Anthozoa is the sister group of Medusozoa (Haeckel, 1879; Werner, 1973; Salvini-Plawen, 1978; Schuchert 1993; Bridge *et al.,* 1995; Collins, 2002), Figures 5 and 6 reveal that Stauromedusae is the sister taxon to all other medusozoans. By comparison to medusozoan outgroups—particularly Cubozoa and the scyphozoan taxa Coronatae, Rhizostomeae, and Semaeostomeae—characters by which we recognize Stauromedusae can be sorted into likely synaporphies and symplesiomorphies (Fig. 7). For example, because four intramesogleal muscles associated with peristomial pits are characteristic of most species of Stauromedusae as well as of the polyps of scyphozoans, it seems likely that these characters are symplesiomorphies that have been lost in both Cubozoa and Hydrozoa. In the case of Cubozoa, polyps still possess intramesogleal muscles, but they are not united in four muscle bundles (Chapman, 1974). By similar reasoning, gastric filaments and a coronal muscle are features that were likely lost in the ancestry of Hydrozoa.

The relationship between the metamorphosis of primary tentacles into anchors or rhopalioids in Stauromedusae and the metamorphosis of primary tentacles into the rhopalia in

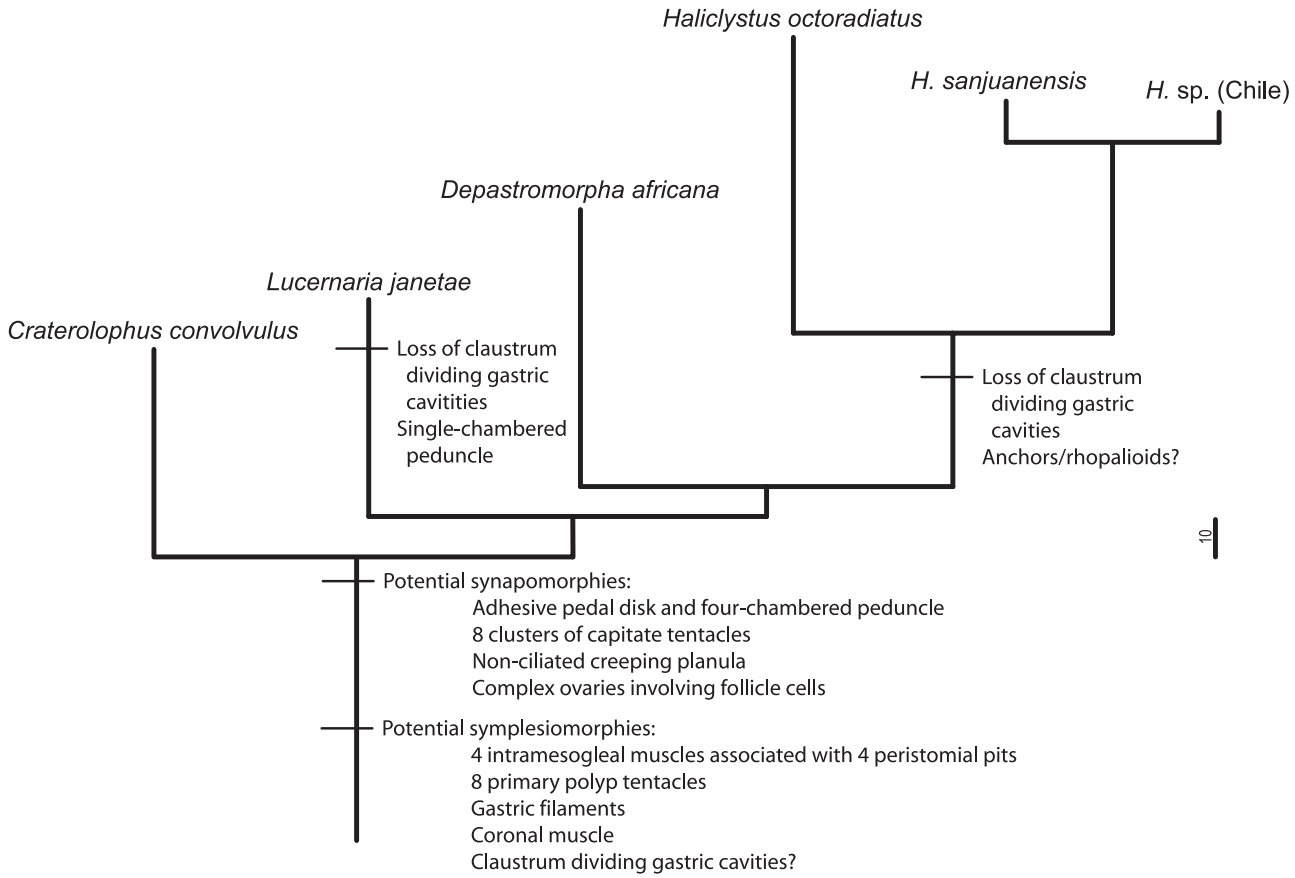

Figure 7. Maximum parsimony (MP) phylogram of relationships among sampled species of Stauromedusae based on combined 16S and SSU data, rooted as in Figures 5 and 6. Scale bar indicates 10 nucleotide character changes. Selected morphological and life-history features are mapped at various nodes. It is stressed that greater taxon sampling is a must to more fully understand the true history of character evolution within Stauromedusae.

Cubozoa and Scyphozoa is somewhat less clear, because anchors have a relatively limited distribution among stauromedusan species. Although the growth of eight perradial and interradial primary tentacles during ontogeny appears to be nearly universal within the group, species of just three genera, *Haliclystus, Stenoscyphus,* and *Halimocyathus,* have primary tentacles that are modified into rhopalioids (Kramp, 1961). It seems possible that anchors have been derived one or more times within Stauromedusae and that their evolutionary origin (or origins) is independent from that of cubozoan and scyphozoan rhopalia. That said, the presence of primary tentacles is likely to be a feature that is shared by cubozoans, scyphozoans, and stauromedusans as a result of their common history. If so, this character was lost in the ancestry of Hydrozoa.

A final character of considerable interest is the claustrum. As in adults of Cubozoa and Scyphozoa, in stauromedusans the gastrovascular chamber is divided by four interradial septa that separate a central gut from four radial gastric pockets. In some members of Stauromedusae, the gastrovascular system has an added level of complexity because the four gastric pockets are divided transversely by a piece of tissue, the claustrum. Just such an arrangement is also seen in adult cubozoans (Uchida, 1929), which raises the possibility that this character was present in the ancestral medusozoan and subsequently lost independently in lineages leading to Hydrozoa and Scyphozoa.

The claustrum has played a fundamental role in the systematics of Stauromedusae. The group has long been divided into two primary groups, Cleistocarpida and Eleutherocarpida (Clark, 1863), on the basis of its presence or absence, respectively. We have sampled two cleistocarpid species, *C. convolvulus* and *D. africana,* and found that not only do these species not form a clade, they also do not form a paraphyletic assemblage with respect to the remaining eleutherocarpid species. The species we describe here, *L. janetae,* does not possess a claustrum, and both molecular markers indicate that this species falls between *C. convolvulus* and *D. africana* (Figs. 5 and 6). Although more species of Stauromedusae need to be sampled for molecular data before the evolution of the claustrum can be settled conclusively, at this point it seems likely that the claustrum

is a more labile feature than suspected and that it may have been lost on more than one occasion (Fig. 7). No matter what the specific history of the evolution of the claustrum within Stauromedusae, it may turn out not to be a useful character for diagnosing subgroups within the clade.

From a molecular perspective, the mitochondrial 16S marker may be useful for determining species boundaries in future studies of Stauromedusae. For instance, species of *Haliclystus* have been difficult to distinguish. Kramp (1961) considered all three of the *Haliclystus* species sampled here to be synonyms, under the name *H. auricula* (Rathke, 1806). However, Hirano (1997) recently demonstrated that circumboreal representatives of *H. auricula* can be separated, on the basis of a set of morphological characters, into four types with differing, though somewhat overlapping, distributions. As names were available for each of these distinct types, she recommended the resurrection of *H. sanjuanensis* from the eastern North Pacific and *H. octoradiatus* from northern Europe and Iceland (in addition to *H. tenuis* Kishinouye, 1910, from the western North Pacific, not sampled here) as species separate from *H. auricula.* The significant divergences in both 16S and SSU data between our samples of *H. sanjuanensis* and *H. octoradiatus* support Hirano's assertion that the different morphotypes of circumboreal *Haliclystus* represent discrete species. Several important studies of stauromedusan features that used specimens referred to as *H. octoradiatus* from the northeastern Pacific probably present observations on *H. sanjuanensis* (*e.g.,* Otto, 1976, 1978; Eckelbarger and Larson, 1993). The name *H. auricula* has also been applied to all South American specimens of *Haliclystus* observed (Kramp, 1952; Grohman *et al.,* 1999; Zagal, 2004). Our data show that our specimens of *H.* sp. from Chile and *H. sanjuanensis* are relatively closely related, though more samples are of course necessary to determine whether they represent separate species. The molecular markers we have used to begin investigating stauromedusan phylogeny should prove helpful in moving toward a stable systematic framework for Stauromedusae based upon comprehensive study of both morphological and sequence data.

Finally, the evolution of mitochondrial genes has been observed to be notably slow in anthozoans (*e.g.,* Romano and Palumbi, 1997; Shearer *et al.,* 2002; Hebert *et al.,* 2003), raising the possibility that slow mitochondrial DNA evolution might be a widespread phenomenon within Cnidaria (Hebert *et al.,* 2003) or other early-diverging metazoan lineages (Shearer *et al.,* 2002). The data derived here, however, suggest that the mitochondrial 16S gene evolves rapidly enough in Stauromedusae to differentiate between relatively closely related species. Furthermore, other recent studies of non-anthozoan cnidarians (Schroth *et al.,* 2002; Collins *et al.,* 2005; Govindarajan *et al.,* 2005), and even placozoans (Voigt *et al.,* 2004), have used mitochondrial 16S data to distinguish among closely related lineages. Therefore, it appears more likely that slow mitochondrial DNA evolution is limited to Anthozoa, rather than being the general condition for early diverging metazoans. That said, investigating this question with additional data, especially from Ctenophora and Porifera, is certainly warranted.

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