



Stauromedusan populations inhabiting deep-sea hydrothermal vents along the southern East Pacific Rise

Richard A. LUTZ¹, Allen G. COLLINS², Eric R. ANNIS¹, Andrew J. REED¹, Kyle F. BENNETT¹,
Kenneth M. HALANYCH³ and Robert C. VRIJENHOEK⁴

(¹) *Institute of Marine and Coastal Sciences, Rutgers University, New Brunswick, NJ 08901, USA.*
Tel. (732) 932-8959, Fax (732) 932-6557, E-mail: rlutz@imcs.rutgers.edu

(²) *National Systematics Laboratory of NOAA's Fisheries Service, National Museum of Natural History,*
MRC-153, Smithsonian Institution, Washington, DC 20013, USA

(³) *Life Sciences Department, Auburn University, Auburn, AL 36849, USA*

(⁴) *Monterey Bay Aquarium Research Institute, Moss Landing CA 95039, USA*

Abstract: Dense populations of “stalked jellyfish” (Stauromedusae) were encountered at two geographically-separated, deep-sea hydrothermal vent fields located at 7°26'S; and 20°02'S along the East Pacific Rise (EPR). DSV *Alvin* was utilized (on dives 3320 and 3334) at the sites to collect numerous stauromedusans, which were immediately frozen (at -70°C) or preserved in 70% ethanol upon arrival on board the research support vessel (*R/V Atlantis*). High resolution images were collected of the organisms *in situ*. A 530-560-bp region of mitochondrial 16S was amplified, and edited 16S sequences were aligned, along with sequences from 7 other stauromedusans. Results suggest that the Stauromedusae encountered at 7°26'S, and 20°02'S are very closely related, if not conspecific. It remains unclear whether or not these Stauromedusae are distinct from *Lucernaria janetae* (collected from 8°37'N along the EPR), which was recently described by Collins and Daly (2005). To date, dense populations of stauromedusans have been encountered at four separate deep-sea hydrothermal vent fields along the EPR located at the following latitudes: 20°50'N, 8°37'N, 7°26'S and 20°02'S. Many fascinating questions remain concerning the biogeography and mechanisms of dispersal of these cnidarians between discrete deep-sea hydrothermal systems.

Keywords: Stauromedusae • *Lucernaria* • Hydrothermal vents • East Pacific Rise • 16S sequences

Introduction

Stauromedusae (“stalked jellyfish”) are found throughout the world's oceans, but have been encountered most commonly in shallow, temperate waters of the Northern Hemisphere (Eckelbarger & Larson, 1993). Juveniles and adults are sessile and it is generally believed that dispersal is highly limited and occurs via a nonciliated, creeping

planula stage. To date, stauromedusans have been reported from only a few deep-sea hydrothermal vent sites (Lutz et al., 1998; Halanych et al., 1999; Collins & Daly, 2005). In addition, there has been only one report of Stauromedusae in a presumably non-vent, abyssal habitat (*Lucernaria bathyphila* Haeckel, 1880 at a depth 2800 m in the north-east Atlantic) (Naumov, 1961). We herein provide observations and analyses of stauromedusans encountered and

sampled at two geographically-separated, deep-sea hydrothermal vent fields located at 7°25.950'S, 104°47.171' W and 20°02.020' S, 113°41.056' W (hereafter referred to as 7°26'S and 20°02'S) along the East Pacific Rise (EPR). The observations made at the 20°02'S site represent the deepest reported occurrence of Stauromedusae in the world's oceans, as well as the most southerly extent of the range of stauromedusans encountered to date in hydrothermal vent environments.

Materials and Methods

On December 23, 1998 and January 7, 1999 the submersible *DSV Alvin* visited, respectively, two previously undocumented areas of hydrothermal activity along the crest of the East Pacific Rise at approximately 7°26'S (Alvin Dive 3320, depth 2747 m) and 20°02'S (Alvin Dive 3334, depth 3001 m). Stauromedusan populations were imaged extensively using externally-mounted, 400-watt HMI (mercury, metallic, iodide) lights and a high-resolution, 3-chip camera linked to a Beta SP video recording system on the submersible. Selected video images were frame-grabbed using a PC with a capture board and printed.

Numerous stauromedusans were collected from each site and were immediately frozen (at -70° C) or preserved in 70% ethanol upon arrival on board the research support vessel (*R/V Atlantis*). Voucher material representing the two populations have been deposited in the National Museum of Natural History under numbers 1086349-1086351. In the preliminary analyses described herein, the methodology was virtually identical to that described by Collins & Daly (2005) and may be summarized as follows. The DNeasy extraction kit (Qiagen GmbH, Hilden, Germany) was used to obtain DNA from one frozen specimen from each site. A 530-560-bp region of mitochondrial 16S (mt16S) was amplified, using the forward primer from Cunningham & Buss (1993) combined with the reverse primer from Schroth et al. (2002). Products were purified and sequenced in both directions. 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 7 other stauromedusans: *Haliclystus octoradiatus* (Lamarck, 1816); *Haliclystus sanjuanensis* Hyman, 1940; *Haliclystus* sp. (California); *Haliclystus* sp. (Chile); *Depastromorpha africana* Carlgren, 1935; *Lucernaria janetae* Collins & Daly, 2005 and *Craterolophus convolvulus* (Johnston, 1835). New sequences have been assigned Genbank accession numbers DQ465035-DQ465037. For each dataset, we searched for optimal trees by using the criteria of maximum parsimony (MP) and maximum 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 the Akaike information criterion (AIC) employed by ModelTest ver.3.7 (Posada & 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. For further methodological details, see Collins & Daly (2005).

Results

Dense aggregations of Stauromedusae were clustered on bare basalt amidst shimmering water emanating at deep-sea hydrothermal vents located at both 7°26'S and 20°02'S along the East Pacific Rise (Fig. 1). A wide size spectrum of individual polyps was present at each site, with the maximum dimension (proximal end of stalk to tip of tentacles) of the largest individuals measuring approximately 10 cm. Distinct morphological characteristics, such as broad concave shape of the subumbrella, the lack of rhopaloids, paired gonads, and the arrangement of eight adradial lobes, each tipped with a cluster of short capitate tentacles, suggest a close affinity with previously described species of the genus *Lucernaria*.

The uncorrected pair-wise p-differences of mt16S from the various representatives of the analyzed Stauromedusae are summarized in Table 1. The resulting, single-most, parsimonious phylogenetic tree inferred from mt16S sequences, which is identical to the ML topology, is depicted in Figure 2. There are no differences in the mt16S sequences derived from single individuals taken from populations at 7°26'S and 20°02'S. A modest divergence (1.8%) was observed between the individuals sampled at these two sites and an individual of *Lucernaria janetae* from 8°37'N along the EPR. To date, no studies documenting intraspecific variability for any molecular markers in Stauromedusae are available. The most detailed study of variation in this region of mitochondrial 16S and its relationship to species boundaries in a reasonably closely related taxon, the hydrozoan genus *Coryne*, documented minimal interspecific divergences of 3.7%, but maximal intraspecific divergences of 5.5% (Schuchert, 2005). Therefore, while it is possible that individuals from all three sites are conspecific, one cannot make a strong conclusion for or against this hypothesis given the data presented here.

Discussion

To date, dense populations of stauromedusans have been encountered at four separate deep-sea hydrothermal vent fields along the EPR located at the following latitudes:

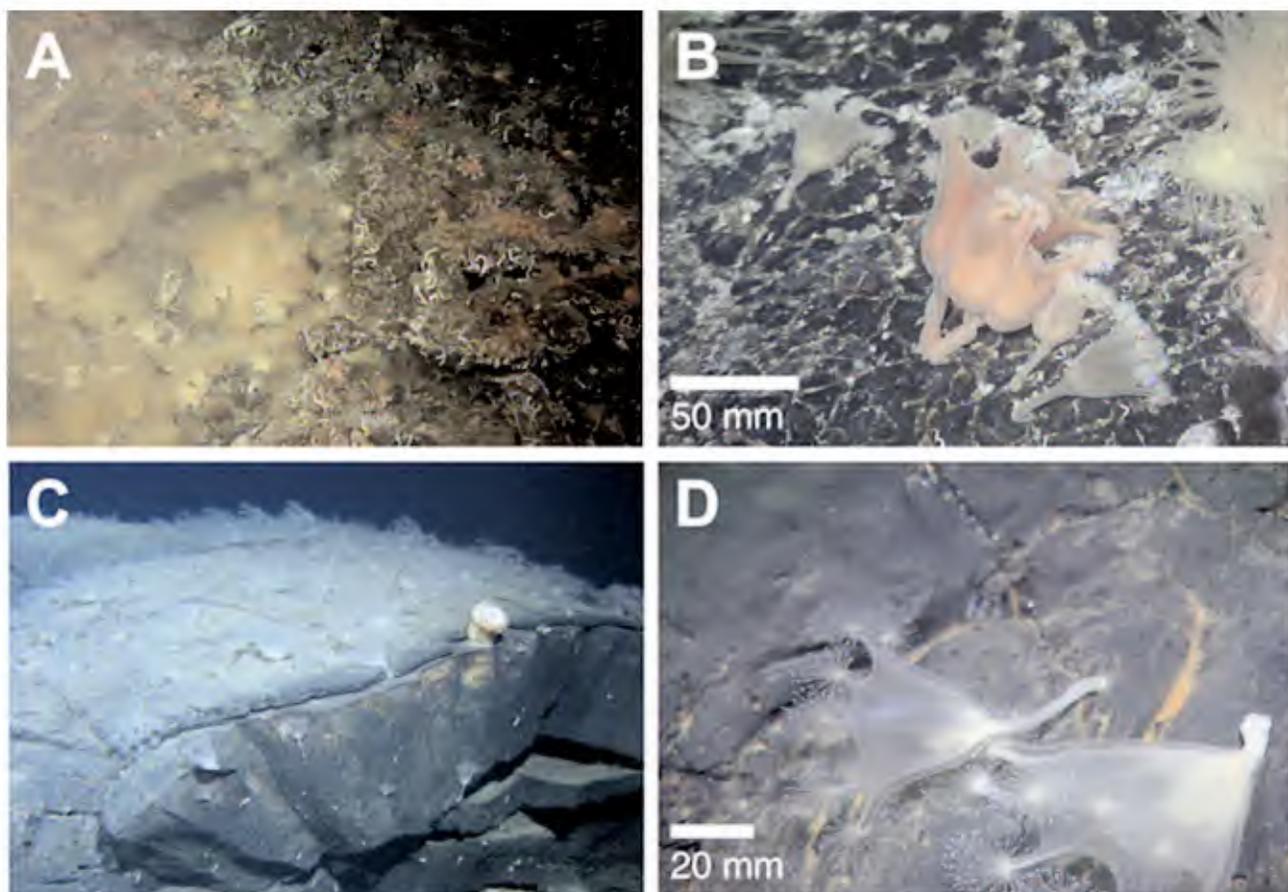


Figure 1. **A.** A dense aggregation of stauromedusans clustered on bare basalt amidst shimmering water emanating at a deep-sea hydrothermal vent located at approximately 7°26'S along the East Pacific Rise (EPR). **B.** Close-up of individual stauromedusan polyps from the 7°26'S site depicted in (A). **C.** A population of stauromedusans inhabiting bare basalt amidst shimmering water emanating at a deep-sea hydrothermal vent located at approximately 20°02'S along the EPR. **D.** Close-up of individual stauromedusan polyps from the 20°02'S site depicted in (C).

Figure 1. **A.** Agrégation dense de stauroméduses fixées sur du basalte nu, dans l'eau miroitante émanant de la source hydrothermale localisée approximativement à 7°26'S sur la Ride Pacifique Est (EPR). **B.** Gros plan de polypes de stauroméduses du site représenté en (A). **C.** Une population de stauroméduses fixées sur du basalte, dans l'eau miroitante de la source hydrothermale localisée approximativement à 20°02'S sur EPR. **D.** Gros plan de polypes de stauroméduses du site représenté en (C).

Table 1. Uncorrected pair-wise p-differences of mitochondrial 16S from representatives of Stauromedusae.

Tableau 1. Différences non corrigées relatives (%) appariées des séquences de 16S mitochondrial des différents représentants de Stauroméduses.

#	Species	1	2	3	4	5	6	7	8	9
1	<i>Halicylistus octoradiatus</i>	-								
2	<i>Halicylistus sanjuanensis</i>	13.0%	-							
3	<i>Halicylistus</i> sp. (California)	13.0%	0.4%	-						
4	<i>Halicylistus</i> sp. (Chile)	12.8%	4.1%	4.1%	-					
5	<i>Depastromorpha africana</i>	18.9%	18.8%	18.8%	19.4%	-				
6	<i>Lucernaria</i> from 7 S	23.9%	23.9%	24.3%	24.1%	25.7%	-			
7	<i>Lucernaria</i> from 20 S	23.9%	23.9%	24.3%	24.1%	25.7%	0.0%	-		
8	<i>Lucernaria janetae</i>	24.2%	23.5%	23.9%	23.7%	26.5%	1.8%	1.8%	-	
9	<i>Craterolophus convolvulus</i>	26.1%	22.1%	22.4%	22.2%	23.0%	21.9%	21.9%	21.9%	-

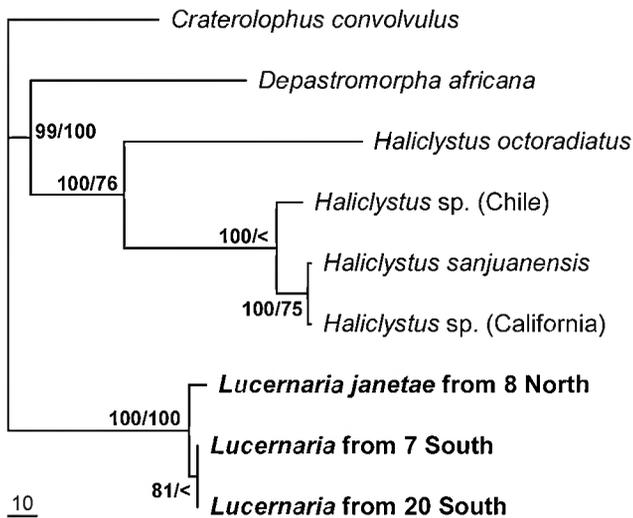


Figure 2. Phylogram of single-most, parsimonious tree inferred from mitochondrial 16S sequences of representatives of Stauromedusae, rooted as in Daly & Collins (2005). Scale bar represents ten character changes. Bootstrap values under parsimony and maximum likelihood are shown at each node.

Figure 2. Phylogramme de l'arbre de maximum de parcimonie construit à partir des séquences 16S mitochondriales de représentants des Stauroméduses, enracinées de la même façon que Daly & Collins (2005). L'échelle représente 10 changements de caractères. Les valeurs de bootstrap sous critère de parcimonie et maximum de vraisemblance sont données à chaque nœud.

20°50'N, 8°37'N, 7°26'S and 20°02'S. The presence of Stauromedusae at a depth of 3001 m at the 20°02'S site represents the deepest reported occurrence of stauromedusans in the world's oceans. Specimens were not collected at the 20°50'N site (Lutz et al., 1998), so genetic analyses could not be conducted on individuals from this population.

The Stauromedusae encountered at 7°26'S and 20°02'S along the southern EPR are very closely related, if not conspecific, because sampled individuals were invariant (Table 1). Of course, additional sampling of specimens collected from these two sites and studies investigating within and between species variation of mitochondrial 16S, and other markers, are necessary before one can conclude strongly that the two populations belong to the same species. The divergence between individuals sampled from the two southern EPR sites and one individual of *Lucernaria janetae* (collected from 8°37'N along the EPR) is slight (Table 1). *L. janetae* was recently described by Collins & Daly (2005) and it remains unclear whether or not *L. janetae* is distinct from the Stauromedusae observed along the southern EPR. Further morphological and molecular analyses are necessary to answer this question. If all the observed EPR Stauromedusae represent a single species, then its range would extend from 8°37'N to 20°02'S, the

most southerly extent of the range of Stauromedusae encountered to date in hydrothermal vent environments. Such a broad latitudinal range would be intriguing, given that the only dispersive stage known in Stauromedusae is a nonciliated, creeping planula larval form (Otto, 1976 & 1978). Such a form has not been documented for many species of Stauromedusae, so it is unclear how general the character is. Indeed, no such low-dispersal planula stage has been observed in stauromedusans encountered from the various EPR hydrothermal vent sites. If these stages do exist in Stauromedusae of the EPR, it raises the question of how gene flow is maintained over such large distances. In summary, many fascinating questions remain concerning the biogeography and mechanisms of dispersal of these cnidarians between discrete deep-sea hydrothermal systems.

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