Zootaxa 3786 (2): 101–123
www.mapress.com/zootaxa/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

http://dx.doi.org/10.11646/zootaxa.3786.2.1
http://zoobank.org/urn:lsid:zoobank.org:pub:9C3B70D0-4092-4ACC-A134-1CEC31E232C7

Four new species of Cladorhizidae (Porifera, Demospongiae, Poecilosclerida) from the Northeast Pacific

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Abstract

Interest in cladorhizid sponges has grown rapidly in the past 19 years since a unique feeding strategy, carnivory, was described by Vacelet and Boury-Esnault in 1995. Since that time, 31% of the 133 extant cladorhizids have been described. Previously, seven species of cladorhizid sponges were known from the Northeast Pacific. Here we describe four additional species, including two species of *Asbestopluma* and two species of *Cladorhiza.* We report on species ranges, habitat, and ecology, including one from a chemosynthetic environment that appears to be using methane-oxidizing bacteria as a nutrient source. In fact, three of the four species described here were found in chemosynthetic habitats. The presence of small crustacean prey was also documented for three of these species.

Key words: Porifera, Cladorhizidae, Deep Sea Ecology, Deep Sea Biology, taxonomy

Introduction

Interest in carnivorous sponge biology has grown considerably since carnivory was first described in a population of deep-sea sponges, *Asbestopluma hypogea*, that were discovered inhabiting a shallow marine cave in the Mediterranean by Vacelet and Boury-Esnault (1995). Carnivory in sponges is understood to be an adaptation to living in the food-poor, deep-sea environment where, presumably, the cost of filter feeding outweighs the benefit; most carnivorous sponges are deep-sea dwellers, although a few have been described from shallow water (Lambe, 1893; Vacelet and Boury-Esnault, 1995; van Soest & Baker, 2011). Recent molecular analysis (Vargas et al., 2012) agrees with a previous hypothesis that *Abyssocladia* belongs within the Cladorhizidae, yet the evolutionary lineage of carnivory is not certain. A description of two new *Chondrocladia* species revealed a new spicule type, a trochirhabd (Vacelet *et al.*, 2009). These spicule types were previously known only from early Jurassic and Miocene sediments, suggesting that *Chondrocladia* and, therefore, carnivory in sponges, is ancient.

There are currently 133 species recognized from seven cladorhizid genera (van Soest *et al.*, 2013). However, it is uncertain that carnivory is monophyletic as several species within two other Poecilosclerida families (Guitarridae and Esperiopsidae) appear to be carnivorous as well (Vacelet, 2007). Interpretations of this information are 1) recent independent development of carnivory along several lines of evolution within the Poecilosclerida and 2) carnivory developed early within the Poecilosclerida and is a symplesiomorphic trait (Vacelet, 2007).

Of the 133 extant cladorhizids, 41 species have been described since 2002, nearly 31% of recognized species. The occurrence of methane oxidizing symbionts has been described in one species, *Cladorhiza methanophila* (Vacelet *et al.*, 1996), however, carnivorous sponges have often been collected in the vicinity of chemosynthetic communities (Vacelet, 2007). Common among carnivorous sponges is a lack of aquiferous system and choanocyte chambers except for species of *Chondrocladia*.

Accepted by E. Hajdu: 19 Feb. 2014; published: 9 Apr. 2014

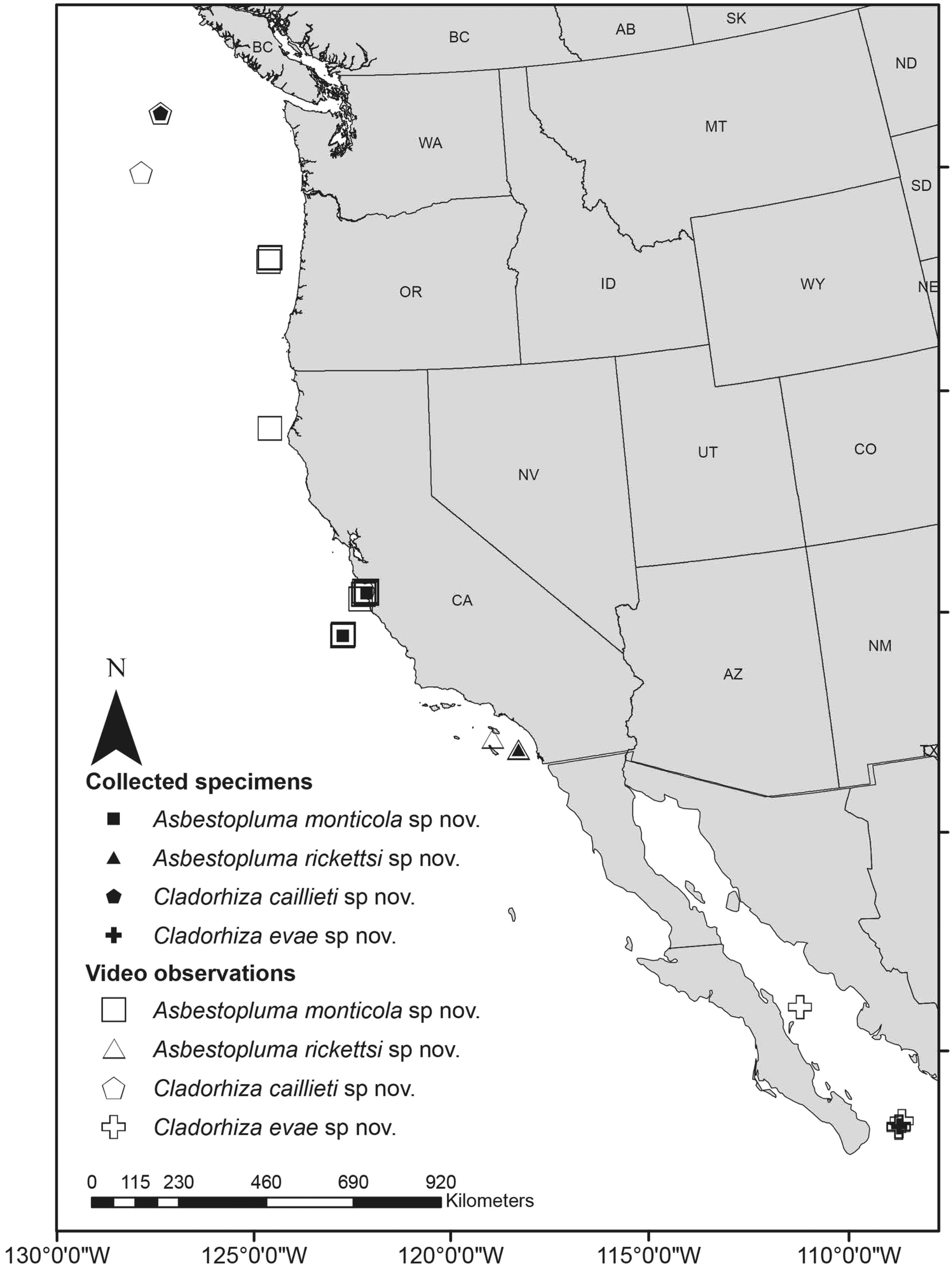

FIGURE 1. Map showing location of specimen collections and video observations.

TABLE 1. Spicule measurements for four new species of Cladorhidae from the NE Pacific. Number of spicules measured (n), average length (L) and width (W, where available) in µm ± standard deviation are shown.

Specimen	Large style 1	Large style 2	Large style 3	Microacanthoylostrongyles
Asbestopluma monticola sp. nov.	L 751 ± 46 µm (n=53)	L 687±69 µm (n=103)	L 462.1±79.7 µm (n=85)	L 98.1 ±10.7 µm (n=50)
	W 25.5 ±1.4 µm (n=53)	W 17.5 ±2.8 µm (n=66)	W 39.97 ±7.7 µm (n=67)	W 1.7 ±0.4 µm (n=50)
Asbestopluma rickettsi sp. nov.	L 956 ± 50 µm (n=50)	L 642.6 ± 62.6 µm (n=50)	L 555 ± 53 µm (n=50)	L 102.6 ± 9.8 µm (n=50)
	W 19.8 ± 1.8 µm (n=50)	W 14.0 ±2.5 µm (n=66)	W 26.6 ± 6.4 µm (n=50)	W 1.5 ± 0.4 µm (n=50)
Cladorhiza caillieti sp. nov.	L 1371.58 ± 104.91 µm (n=155)	L 807.22 ± 174.02 µm (n=237)	L 381.9 ± 87.04 µm	
	W 34.38 ± 6.92 µm (n=155)	W 18.04 ± 5.62	W 10.74 ± 2.14 µm (n=103)	
Cladorhiza evae sp. nov.	L 2243 ± 460 µm (n=13)	L 1224.36 ± 432.3 µm (n=263)	L 825 ± 132.7 µm (n=21)	
	W 29.26 ± 11.00 µm (n=22)	W 26.13 ± 11.07 µm (n=50)	W 23.67 ± 10.68 µm (n=28)	

TABLE 1. (Continued)

Specimen	Sigma 1	Sigma 2	Sigma 3	Sigmancistra	Anisochelae 1	Anisochelae 2
Asbestopluma monticola sp. nov.	L 22.9 ± 1.5 µm (n=50)				L 11.8 ± 0.5 µm (n=50)	
Asbestopluma rickettsi sp. nov.	L 17.06 ± 1.3 µm (n=50)				L 53.5 ± 5.3 µm (n=50)	L 9.3 ± 0.7 µm (n=50)
Cladorhiza caillieti sp. nov.	L 160.1 ± 11.87 (n=105)	L 95.58 ± 18.55 µm (n=89)	L 96.17 ± 16.61 (n=60)	L 44.05 ± 2.28 µm (n=150)	L 33.98 ± 2.24 (n=150)	L 18.88 ± 1.67 (n=150)
Cladorhiza evae sp. nov.	L 170.35 ± 9.7 µm (n=170)	L 72.08 ± 11.76 µm (n=111)		L 42.3 ± 2.3 µm (n=50)	L 22.6 ± 1.6 µm (n=50)	

Recent surveys of seamounts, chemosynthetic biological communities, and canyons by the Monterey Bay Aquarium Research Institute have yielded numerous publications describing biological communities (Paull *et al.*, 2005; Lundsten *et al.*, 2009a,b), deep-sea ecology (McClain *et al.*, 2009; McClain *et al.*, 2010; Duffy *et al.*, 2013), new species (Cairns, 2007; Williams & Lundsten, 2009; Mah *et al.*, 2010), novel observations of organisms (Lundsten *et al.*, 2012), geology (Clague *et al.*, 2010), and marine debris (Schlining *et al.*, 2013). The use of remotely operated vehicles (ROVs) with deep-diving (4000 m) capabilities has increased scientist's abilities to study deep-water habitats.

Not surprisingly, recent ROV surveys have also yielded unexpected and truly extraordinary species of Cladorhizidae from the Northeast Pacific Ocean including *Cladorhiza corona* (Lehnert *et al.*, 2005), *Cladorhiza pteron* (Reiswig and Lee, 2007), *Lollipocladia tiburoni* (Vacelet, 2008), and *Chondrocladia lyra* (Lee *et al.*, 2012). Precise collection of specimens using robotic manipulator arms and other instruments allows for the retrieval of mostly undamaged specimens, with extremely accurate location information, environmental data, and high-resolution imagery available for each. These technologies greatly increase our ability to document species ranges, habitat preferences, and community characteristics.

Here we describe four new species of Cladorhizidae from the Northeast Pacific Ocean that were collected using ROVs (Fig. 1). Two arborescent species of *Asbestopluma* were collected off California, USA. One species of *Cladorhiza* was collected at a newly discovered hydrothermal vent field in the Gulf of California, Mexico. A second species of *Cladorhiza* was collected from the Endeavor Segment of the Juan de Fuca Ridge, Canada.

Interestingly, three of these four specimens come from sites characterized as supporting chemosynthetic biological communities (hydrothermal vents and seeps). In addition to collected specimens, video observations were used to better characterize habitat and geographic distribution of these new species.

Methods

Specimens. Remotely operated vehicles (ROVs) were used to video tape and collect specimens. Specimens were collected using an ROV manipulator arm or suction sampler, and fixed in 70% ethanol upon retrieval at the surface. Prior to collection, specimens were video taped in situ using an Ikegami HDL-40 high definition video camera. At MBARI these video observations were merged with ancillary data so that latitude, longitude, depth, and CTD information is known for every observation. Using MBARI's Video Annotation and Reference System database (VARS, Schlining & Jacobsen Stout, 2006), we queried for additional observations of specimens that may be these species based on limited macroscopic characters.

Spicule examination. Spicules from fragments of each body part were disassociated in concentrated nitric acid, rinsed, filtered, and mounted on glass microscope slides using the methods of Reiswig and Browman (1987). A compound microscope fitted with a computer digitizer was used to measure the length and, where appropriate, the width of ~50 spicules of each spicule type within each body part of each species. Anomalies were noted, but not necessarily enumerated. Immature spicules were deemed to be those of similar or lesser length than the mean length, but considerably thinner than the mean width.

For scanning electron microscopy (SEM), cleaned spicules were deposited onto membrane filters that were then taped to SEM stubs. Preparations were coated with gold-palladium and imaged in either a Hitachi S-3500N microscope (Hitachi High-Technologies Corp., Tokyo, Japan) at the University of Victoria or a Hitachi S-3400N microscope (Hitachi High-Technologies Corp., Tokyo, Japan) at Moss Landing Marine Laboratories.

Systematics

Class Demospongiae Sollas, 1885

Order Poecilosclerida Topsent, 1928

Suborder Mycalina Hajdu, Van Soest & Hooper, 1994

Family Cladorhizidae Dendy, 1922

Diagnosis. Sponges, typically small, symmetrical, usually from deep water, with diagonal, radiating supporting processes and basal root adaptations for those living in soft sediments. Axial skeleton composed of monactinal or diactinal megascleres, from which radiating extra-axial tracts diverge to lateral processes. Microscleres include (an)isochelae, sigmas, forceps or micro(subtylo)styles (microspined, spear-shaped in a few cases). Considerable reduction to complete loss of the choanocytes being associated with an adaptation to carnivory (Hajdu & Vacelet, 2002).

Genus *Asbestopluma* Topsent, 1901: 23

Type species. *Cladorhiza pennatula* Schmidt, 1875 (by subsequent designation).

Diagnosis. Cladorhizidae with palmate and/or arcuate (an)isochelae and, in some cases, the latter in combination with tridentate anchorate anisochelae (Lopes & Hajdu, 2013).

Asbestopluma monticola sp. nov.

Figs. 2 & 3

Type material. Holotype: CASIZ 192095; MBARI 941-A1; January 27, 2006, Davidson Seamount off central California, USA; latitude: 35.722787, longitude: -122.722553, depth: 1280 m. The holotype was recovered from the summit of Davidson Seamount using the MBARI's ROV *Tiburon*.

Paratype: CASIZ 194901; MBARI V3745-A1, Monterey Canyon, November 14, 2013; latitude: 36.72559, longitude: -122.01423, depth: 1323.28 m. The paratype was recovered from steep rock outcrop of the Monterey Canyon.

Type locality. Holotype: Davidson Seamount, California, USA. **Paratype:** Monterey Canyon, USA.

Etymology. The species name (Latin *mont* = mountain + *-cola* = dweller), mountain dweller, is descriptive of the type locality, where dense stands of this beautiful sponge thrive.

Diagnosis. Branching Cladorhizidae with three size classes of megasclere styles and three microsclere types of a single size class including acanthose tylostyles, sigmas, and palmate anisochelae.

Description. Holotype: an arborescent, dichotomously-branching sponge with bottle-brush arrangement of filaments 19.37 cm tall and 13 cm wide, but was likely wider as several branches were broken off before measuring (Fig. 2A–E). Filaments are 1–9 mm in length (Fig. 4A). At the base, the stalk is 7 mm wide and branches all taper to approximately 1 mm width distally. Attached to hard substrate via conic holdfast disk, 1.3 cm in width. Sponge is white in situ and in preserved state. **Paratype:** matching description above for holotype, 28 cm tall, 9 mm wide at conical base, 6 mm stalk width, and 2 mm branch tip width, tapering distally.

Spicules. Large styles 1 (Fig. 3A, Table 1), fusiform, straight, often with pointed end rounded, in axes of branches and stem: L 751 ± 46 µm (n=53), W 25.5 ±1.4 µm (n=53). Large styles 2 (Fig. 3B), fusiform, straight or slightly curved, in filaments and their inserts in branch axes: L 687±69 µm (n=103), W 17.5 ±2.8 µm (n=66). Large styles 3 (Fig. 3C), fusiform, thick, strongly bent, mainly in basal cone: L 462.1±79.7 µm (n=85), W 39.97 ±7.7 µm (n=67). Microacanthotylostrongyle (Fig. 3D) thin, rough, mostly curved, occurs in basal cone and sparsely throughout branch axes: L 98.1 ±10.7 µm (n=50), W 1.7 ±0.4 µm (n=50). Sigma (Fig. 3E) robust, contort, without profile discontinuity near ends (not clearly sigmancistroid), occurs throughout specimen: L 22.9 ± 1.5 µm (n=50). Palmate anisochelae (Fig. 3F) foot with frontal tooth bearing two broad lateral flukes and distal medial spine extending toward spicule center, lateral wings short and never meet the frontal tooth; without spurs; occurs throughout the specimen: L 11.8 ± 0.5 µm (n=50).

Habitat and associated fauna. *Asbestopluma monticola* was first observed while conducting ROV dives at Davidson Seamount off central California in 2002. A single specimen was collected in 2006 while surveying Davidson Seamount once more. A dense population of *A. monticola* was observed there and they were noted as living attached to both the seafloor and, also, dead hexactinellid sponges. Since 2006, hundreds of additional observations of this species have been made. They are abundant in Monterey Canyon off northern California and central Oregon, a range of ~1000 km. They co-occur with numerous species of sponges (*Staurocalyptus* sp., *Farrea* sp., *Chonelasma* sp.), corals (*Anthomastus ritteri* Verrill, *Paragorgia arborea* Linnaeus, *Keratosis* sp., *Corallium* sp., *Clavularia* sp.), crustaceans (lithodid crabs, pandalid shrimps, amphipod), echinoderms (comatulid crinoids, *Gorgonocephalus* sp. ophiuroids, *Hippasteria* sp. asteroids), and vertebrates (*Careproctus* sp., egg case of Rajiformes, and *Psychrolutes phrictus* Stein & Bond). Small crustaceans like the pandalid shrimp were observed to be actively climbing upon and around the branches of *A. monticola*. The average depth of observation was 1236 m (±211; n=428). Oxygen concentration is low (0.85 ±0.3 ml/L; n=428) and temperature averages 3.18 °C (±0.54; n=428). Small crustacean prey were observed in various states of decomposition on *A. monticola* (Fig. 4A–D).

Remarks. Among the 42 known species of *Asbestopluma*, eight species branch. These are compared with *A. monticola* below. *Asbestopluma monticola* differs from *A. formosa* (Vacelet 2006), in that it lacks the characteristic embryo-containing branching enlargements, they differ in branching patterns—*A. formosa* has fan shaped-branches that divide dichotomously three or four times in a single plane with long, thin, and parallel terminal branches, and *A. monticola* also lacks microstrongyles. *Asbestopluma monticola* resembles *A. desmophora* (Kelly and Vacelet, 2011), however, it lacks desmas and sigmancistras and has bent fusiform styles in its basal cone. *Asbestopluma monticola* differs from *A. bitrichela* (Lopes *et al.*, 2011) in a lack of desmas and anchorate/unguiforate anisochelae. This new species differs from *A. delicata* (Lopes *et al.*, 2011) in the absence of microstrongyles and palmate isochelae. *Asbestopluma monticola* differs from *A. magnifica* (Lopes *et al.*, 2011) in

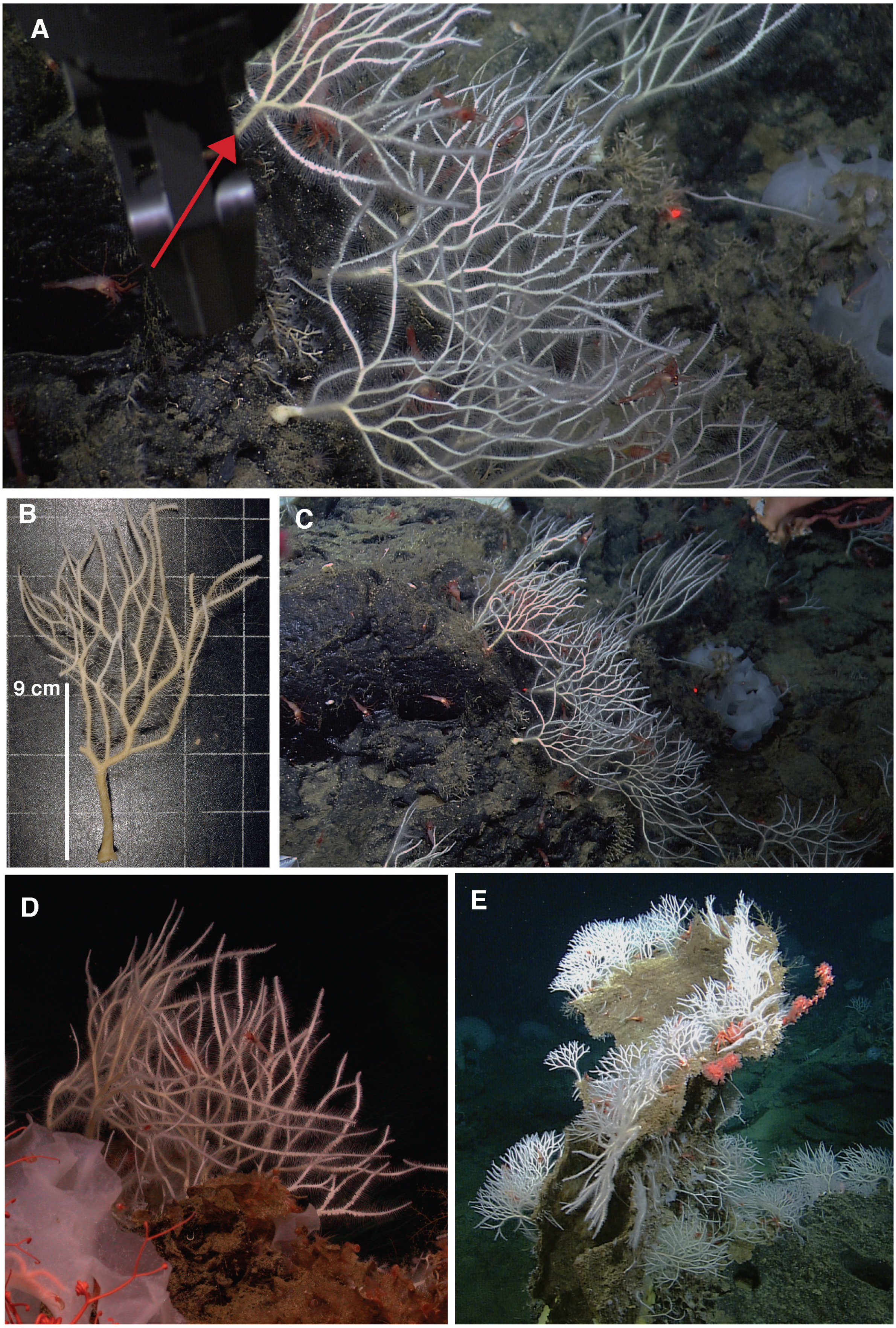

FIGURE 2. *Asbestopluma monticola* **sp. nov.**: (A) red arrow indicating collection of holotype from Davidson Seamount, (B) lab image of holotype, C–E additional specimens showing density, habitat, and community at Davidson Seamount.

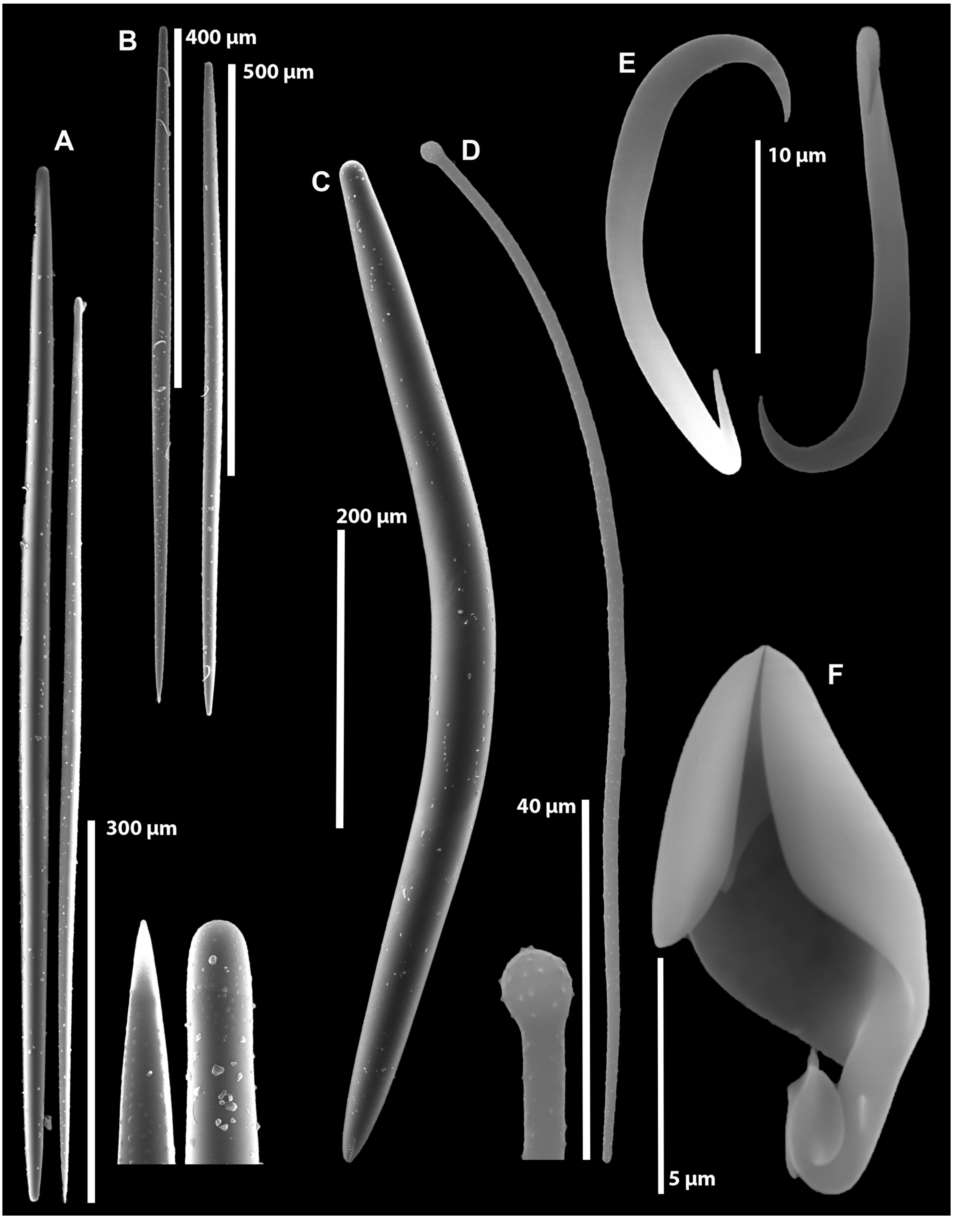

FIGURE 3. *Asbestopluma monticola* **sp. nov.** spicules: (A) large styles 1, (B) large styles 2, (C) large styles 3, (D) microacanthotylostrongyle, (E) sigma, (F) palmate anisochela.

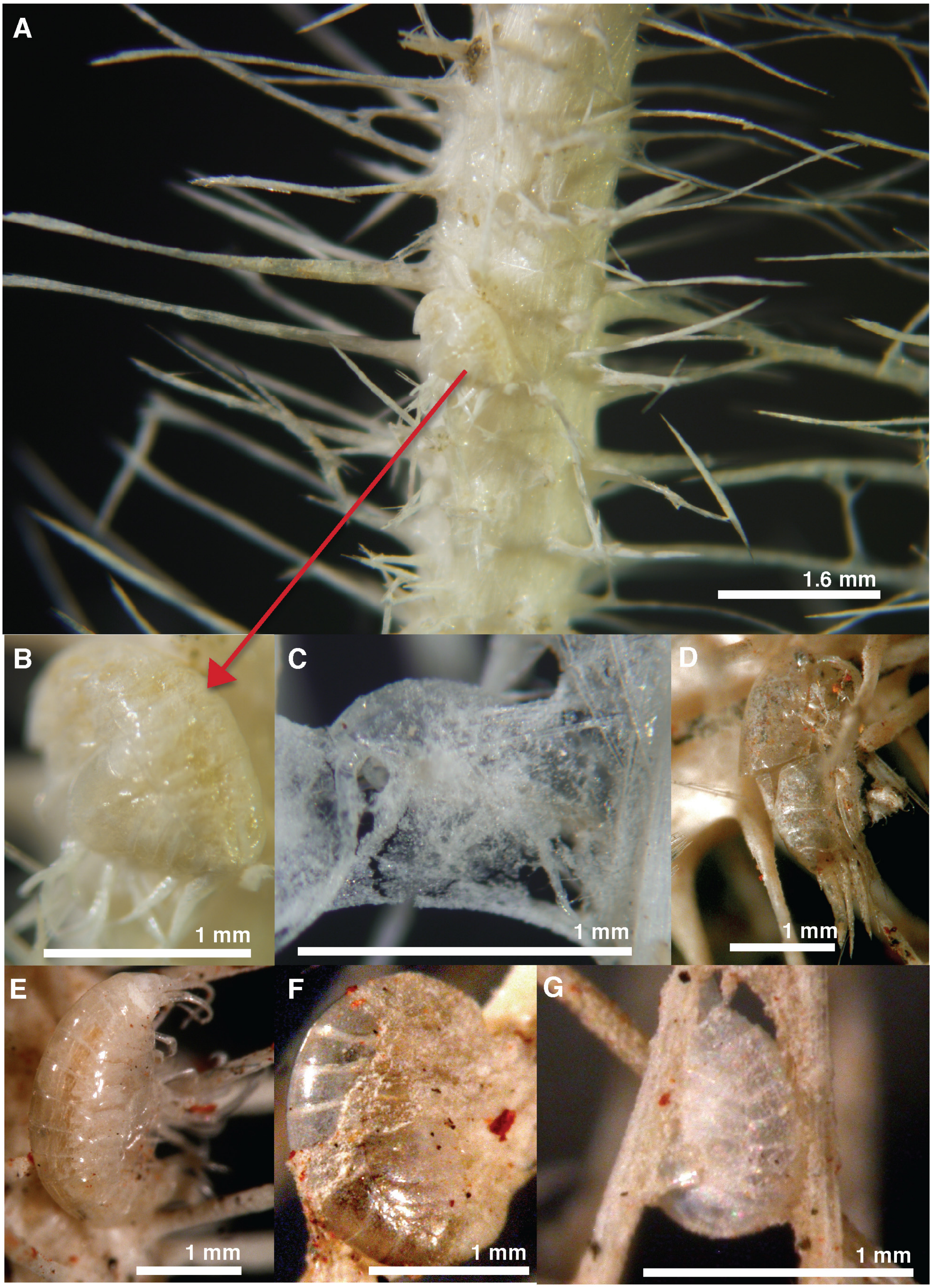

FIGURE 4. (A) Filaments of *Asbestopluma monticola* **sp. nov.** Images of prey in various states of decomposition on *A. monticola* **sp. nov.** holotype (A–C), *Cladorhiza caillieti* **sp. nov.** paratype (D), and *Cladorhiza evae* **sp. nov.** (E–G).

being much smaller (~50% less) in total length, in having a single size class of anisochelae, and having a microtylostrongyle. When compared to *A. furcata* (Lundbeck, 1905), *A. monticola* has larger megascleres and only one size class of anisochelae (*A. furcata* has two size classes of anisochelae). *Asbestopluma monticola* is very similar to *A. ramosa* Koltun, however, *A. ramosa* has a flabelliform branching pattern, with vertical branches emanating from a single point and much thicker branches averaging 1.2 cm. *Asbestopluma ramosa* has larger spicules, especially when comparing anisochelae (Koltun, 1959; Stone *et al.*, 2011). *Asbestopluma ramosa* also does not appear to have the robust, bent fusiform styles of *A. monticola*. *Asbestopluma monticola* superficially resembles *A. rickettsi* (described below), however, *A. monticola* differs in having only one size class of anisochelae. In situ, *A. rickettsi* is more transparent and much more delicate looking than *A. monticola.* A comparison of spicule data for all known *Asbestopluma* species through 2011 is published in Lopes *et al.* (2011).

Asbestopluma rickettsi sp. nov.

Figs. 5 & 6

Type material. Holotype: CASIZ 192771; MBARI specimen D472-A13b; collected by ROV *Doc Ricketts* May 19, 2013, northwest of La Jolla, California, USA; latitude: 32.90433, longitude: -117.78224, depth 1020 m. **Paratype:** CASIZ 192772; MBARI specimen D472-A13a; collected by ROV *Doc Ricketts* May 19, 2013, northwest of La Jolla, California, USA; latitude: 32.90433, longitude: -117.78224, depth 1020 m.

Type locality. Northwest of La Jolla, California, USA.

Etymology. Named in honor of Edward F. Ricketts, marine biologist and ecologist made popular as 'Doc Ricketts' in John Steinbeck's Cannery Row. He is best known as co-author of *Between Pacific Tides,* a pioneering book on intertidal ecology. Coincidentally, the type specimens were collected by MBARI's ROV *Doc Ricketts*.

Diagnosis. Branching Cladorhizidae with three size classes of megascleres and three microscleres including an acanthose tylostyle, and sigma of one size class, and palmate anisochelae of two size classes.

Description. An arborescent, dichotomously-branching sponge with bottle-brush arrangement of filaments (Fig. 5A–C). **Holotype**: Sponge is 21.78 cm tall and 12.38 cm wide (Fig. 5D). At the base, the stalk is 4.5 mm wide and branches all taper to approximately 1 mm in width distally. Filaments are 0.9–1.2 mm in length (Fig. 5E–F). Attached to hard substrate via conic holdfast disk, 1.26 cm in width. **Paratype**: Filaments are 1.5 – 5 mm in length. Sponge is 21.78 cm tall and 12.38 cm wide (Fig. 5G). At the base, the stalk is 3.4 mm wide and branches all taper to approximately 1 mm width distally. Sponge is white in situ and in preserved state.

Spicules. Large styles 1 (Fig. 6A, Table 1) fusiform, straight, often with pointed end rounded, in axes of branches and stem: L 956 ± 50 µm (n=50), W 19.8 ± 1.8 µm (n=50). Large styles 2 (Fig. 6B), fusiform, straight or slightly curved, in filaments and their inserts in branch axes: L 642.6 ± 62.6 µm (n=50), W 14.0 ±2.5 µm (n=66). Large styles to anisostrongyles 3 (Fig. 6C), fusiform, thick, strongly bent, mainly in basal cone: L 555 ± 53 µm (n=50), W 26.6 ± 6.4 µm (n=50). Microacanthotylostrongyle (Fig. 6D) thin, rough, mostly curved, occurs in basal cone and sparsely throughout branch axes: L 102.6 ± 9.8 µm (n=50), W 1.5 ± 0.4 µm (n=50). Sigma (Fig. 6E) without profile discontinuity near ends (not clearly sigmancistroid), rare throughout specimen: L 17.06 ± 1.3 µm (n=50). Anisochelae 1 (Fig 6F) robust, palmate with wide lateral wings and narrow tooth slightly wider than shaft: L 53.5 ± 5.3 µm (n=50), occurs rarely throughout. Anisochelae 2 (Fig. 6G) palmate head, foot with frontal tooth bearing two broad lateral flukes, lateral wings short and never meet the frontal tooth; narrow lower foot shaft looks like a short blunt spur but true spur is lacking; occurs abundantly throughout the specimen: L 9.3 ± 0.7 µm (n=50).

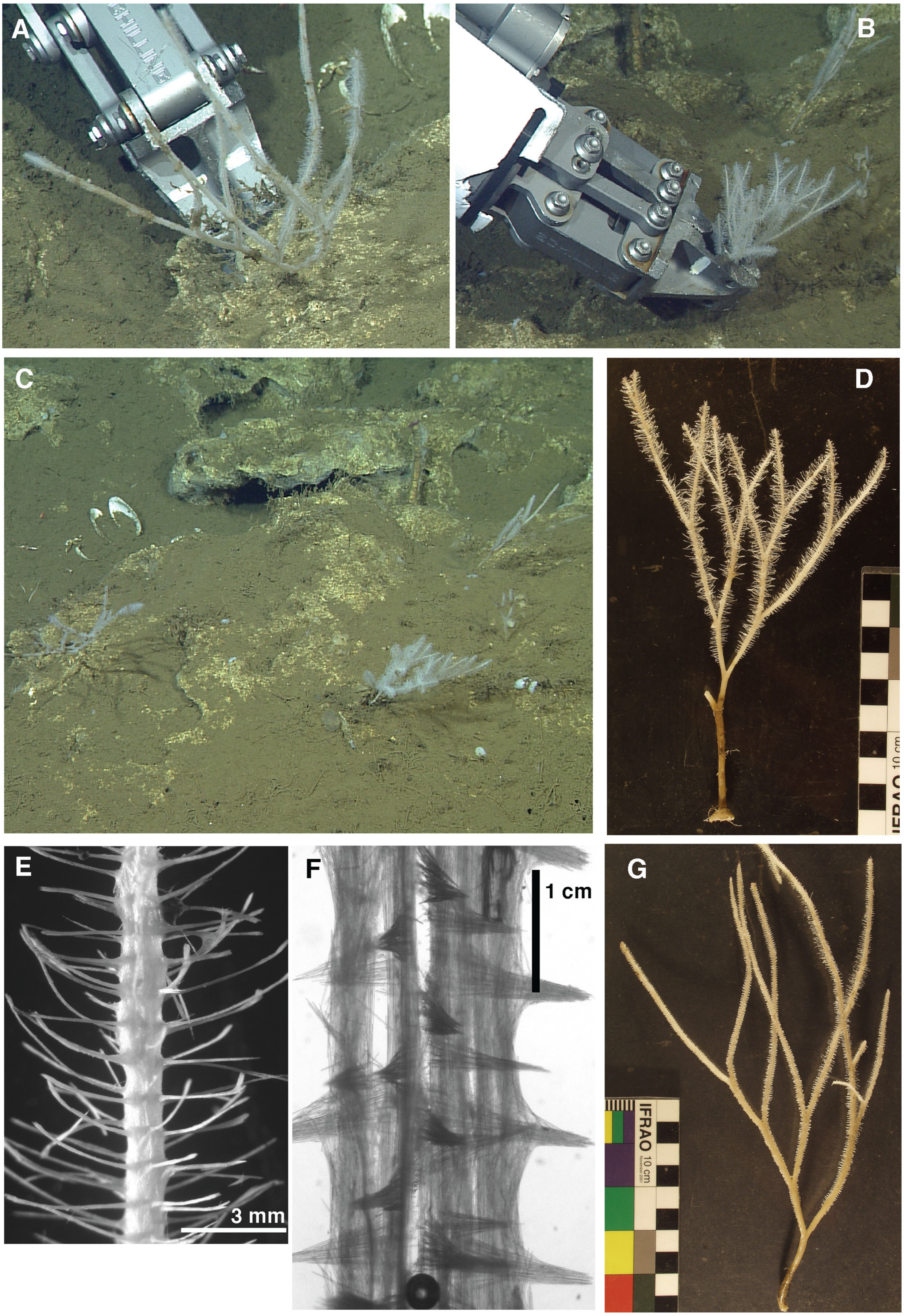

FIGURE 5. *Asbestopluma rickettsi* **sp. nov.**: type specimens being collected (A–B) and prior to collection showing attachment to authigenic carbonate (C). Also seen are vesicomyid shells and a siboglinid tube worm tube. Lab image of holotype, scale is 10 cm (D). Lab image of holotype branch axis and filaments (E). Light microscopy image of showing filaments embedded in axis (F). Lab image of paratype, scale is 10 cm (G).

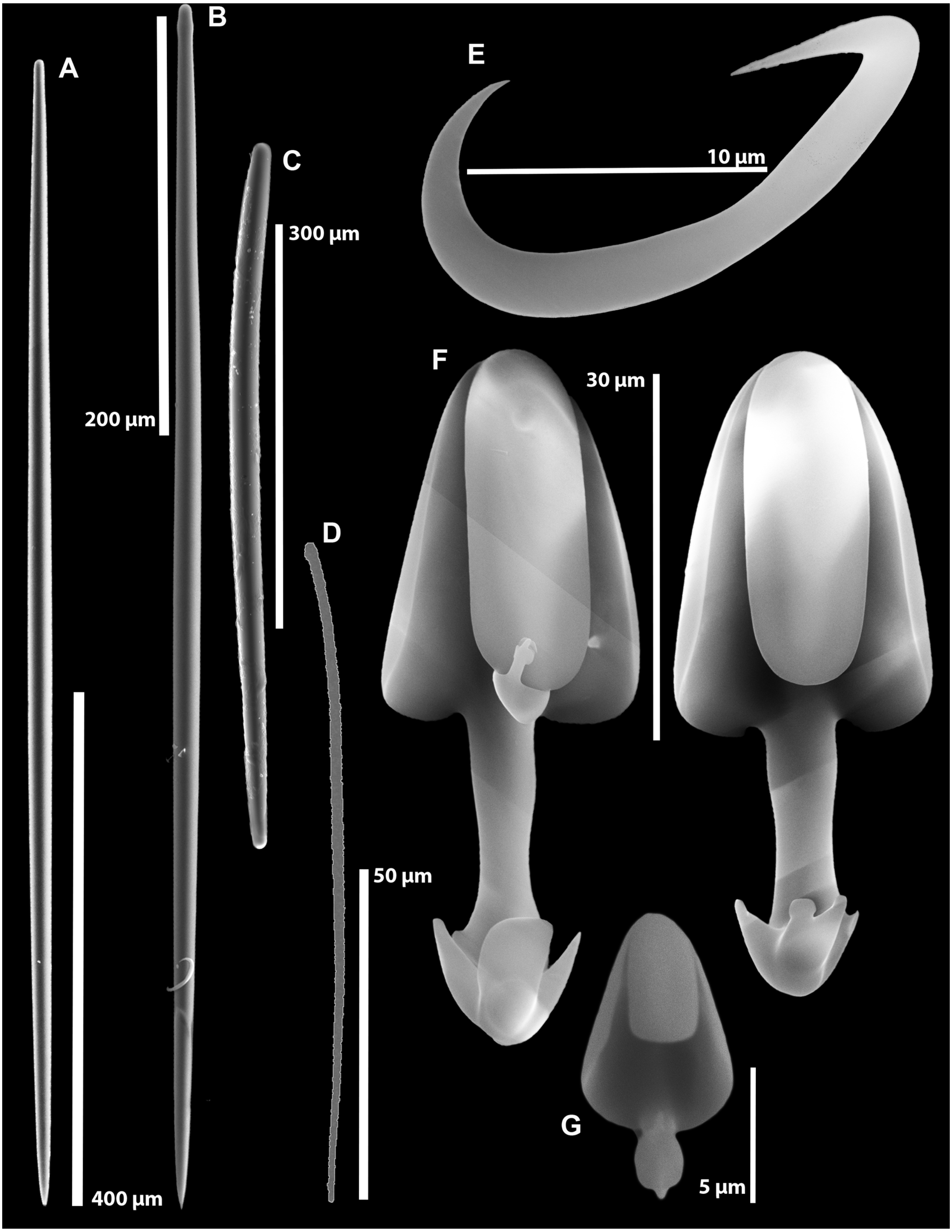

FIGURE 6. *Asbestopluma rickettsi* **sp. nov.** spicules: (A) large styles 1, (B) large styles 2, (C) large styles 3, microacanthotylostrongyle (D), sigma (E), palmate anisochelae 1 (F), palmate anisochelae 2 (G).

Habitat and associated fauna. *Asbestopluma rickettsi* was observed and collected while surveying a chemosynthetic community in a low-oxygen basin off southern California, northwest of La Jolla. Twenty-one individuals were observed in an area of active fluid flow. The substrate was composed of outcrops of authigenic carbonate with a thin sediment veneer. Other organisms observed include vesicomyid clams, siboglinid tube worms, and mats of flocculent bacteria. Average depth of observation was 1031 m (±48.5; n=21), oxygen concentration was low at 0.33 ml/L (±0.001; n=21), and temperature averaged 3.93 °C (±0.02; n=21). No evidence of crustacean prey capture was observed in *A. rickettsi.* This specimen was collected in an area with an active chemosynthetic community and was found to be utilizing methane-oxidizing bacteria as a food source (V. Orphan, California Institute of Technology, pers. comm.). It remains to be seen whether these bacteria are true symbionts, as has been demonstrated in one other species of Cladorhizidae.

Remarks. *Asbestopluma rickettsi* differs from *A. formosa* (Vacelet, 2006), in that it lacks the characteristic embryo-containing branching enlargements, it does not have fan shaped branches divided dichotomously three or four times in a single plane with terminal branches being long, thin, and parallel, and it does not have microstrongyles. It differs from *A. desmophora* (Kelly and Vacelet, 2011) as it lacks both desmas and sigmancistras. This new species differs from *A. bitrichela* (Lopes *et al.*, 2011) in a lack of desmas and anchorate/ unguiferate anisochelae. *Asbestopluma rickettsi* differs from *A. delicata* (Lopes *et al.*, 2011) in absence of microstrongyles and palmate isochelae. It differs from *A. magnifica* (Lopes *et al.*, 2011) considerably in size (*A. magnifica* is ~50% longer), in size classes of megascleres (*A. rickettsi* has larger styles), a larger anisochelae 1 size (~34 µm vs. 52 µm) and presence of large alae of large anisochelae. *Asbestopluma rickettsi* differs from *A. furcata* (Lundbeck, 1905) in having larger megasclere style sizes. A comparison of spicule data for all known *Asbestopluma* species through 2011 is published in Lopes *et al.*, (2011). *A. rickettsi* differs from *A. monticola* in that it has two size classes of anisochelae.

Genus *Cladorhiza* Sars

Cladorhiza Sars, 1872

Type species. *Cladorhiza abyssicola* Sars, 1872.

Diagnosis. Cladorhizidae with only anchorate unguiferate anisochelae (Lopes & Hajdu, 2013).

Cladorhiza caillieti sp. nov.

Figs. 7–9

Type material. Holotype: CASIZ 194449; MBARI specimen D266-A1d; collected by ROV *Doc Ricketts* August 1, 2011, at Endeavor Segment, Juan de Fuca Ridge hydrothermal vent field, Canada; latitude: 47.95685, longitude: -129.08485, depth 2071 m. **Paratypes:** MBARI specimen D266-A1a (CASIZ 192776),b (CASIZ 192777),c (CASIZ 192778); collected by ROV *Doc Ricketts* August 1, 2011, at Endeavor Segment, Juan de Fuca Ridge hydrothermal vent field, Canada; latitude: 47.95685, longitude: -129.08485, depth 2071 m.

Type locality. Endeavor Segment, Juan de Fuca Ridge hydrothermal vent field, Canada.

Etymology. Named in honor of Gregor M. Cailliet, Ph.D., Professor Emeritus, Moss Landing Marine Laboratories for his contributions to ichthyology and deep-sea biology and for providing mentorship and inspiration to graduates of Moss Landing Marine Laboratories, including the first author.

Diagnosis. Cladorhizidae unbranched, with bottle-brush filament arrangement, two size classes of fusiform megasleres, four microsclere types including flat sigmas in two size classes, a third, thin, contort sigma, a sigmancistra, and two size classes of unguiferate anisochelae.

Description. A stipitate sponge with bottle-brush filament arrangement (Fig. 7A–C). One collected specimen with partial rhizoid (Fig. 7D); three others broken but assumed to have had rhizoid as well. **Holotype:** 7 cm long, 2.1 mm wide, filaments up to 1.72 cm long (Fig. 7C left & D). **Paratypes** (a) 9.13 cm long, 2.6 mm wide at base, (b) 8.7 cm long, 2.3 mm wide at base; specimen, (c)5.4 cm long (appears broken), 3.6 mm wide at base. Long, fragile filaments, up to 1.72 cm long, which break off easily. White in situ and in preserved state.

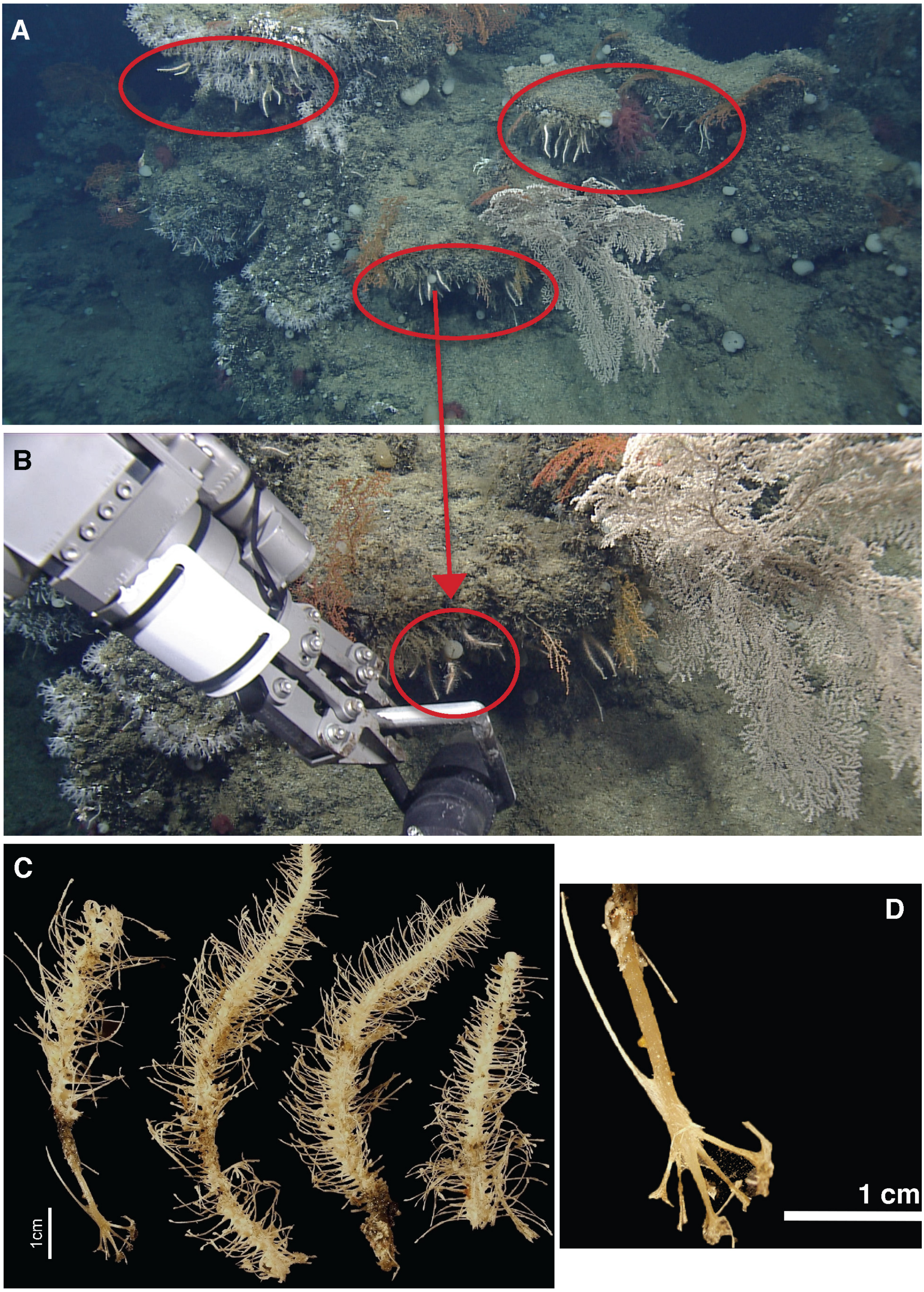

FIGURE 7. *Cladorhiza caillieti* **sp. nov.**: *in situ* image of numerous specimens attached to the underside of overhanging ledges (A), collection of type specimens (B), lab image of type specimens, holotype is on the left (C), close-up of rhizoid from holotype (D).

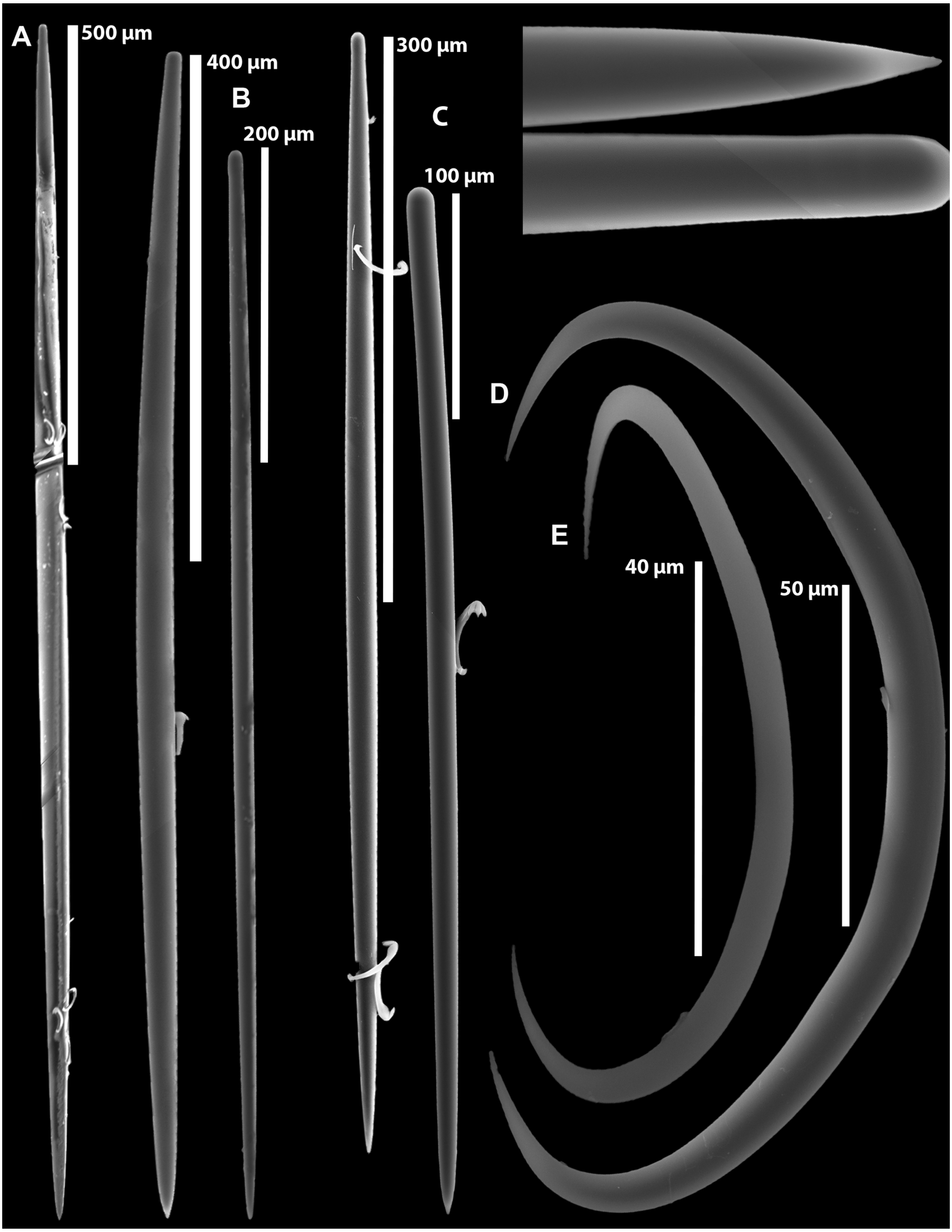

FIGURE 8. *Cladorhiza caillieti* **sp. nov.** spicules: large styles 1 (A), 2 (B) and 3 (C), sigma 1 (D) and 2 (E).

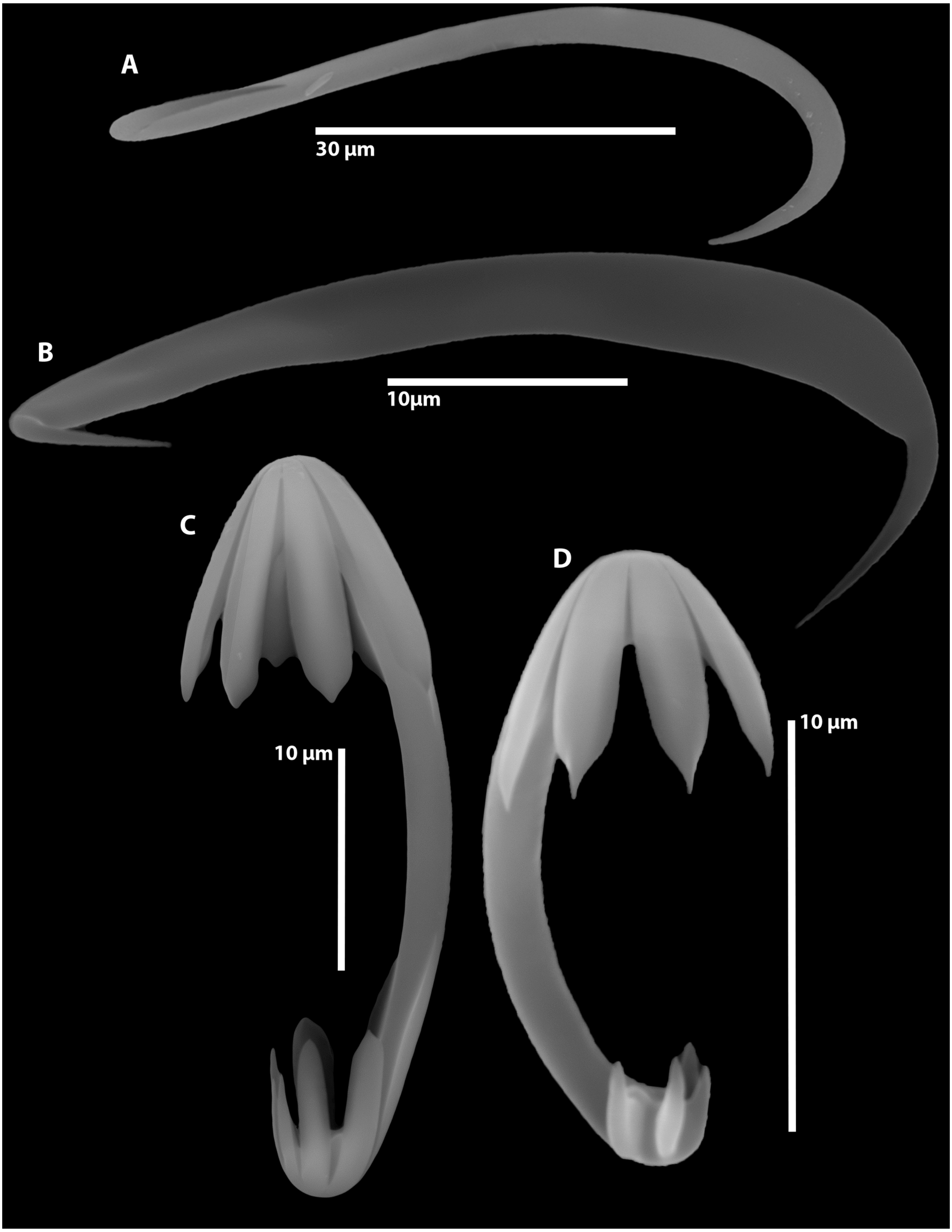

FIGURE 9. *Cladorhiza caillieti* **sp. nov.** spicules: sigma 3 (A), sigmancistra (B), unguiferate anisochelae 1 (C) and 2 (D).

Spicules. Large styles 1 (Figs. 8A, Table 1) fusiform, straight, often with pointed end rounded common in axis and filament: L 1371.58 ± 104.91 µm (n=155), W 34.38 ± 6.92 µm (n=155). Large style 2 (Fig. 8B) fusiform, straight more abundant in filament and rhizoid: L 807.22 ± 174.02 µm (n=237), W 18.04 ± 5.62. Large style 3 (Fig. 8C) non-fusiform, straight common throughout but more abundant in filaments and rhizoid: L 381.9 ± 87.04 µm, W 10.74 ± 2.14 µm (n=103). Sigma 1 (Fig. 8D) robust, "flat back", common in filaments, rare in axis: L 160.1 ± 11.87 (n=105). Sigma 2 (Fig. 8E) flat, small abundant in filaments but rare in axis: L 95.58 ± 18.55 µm (n=89). Sigma 3: (Fig. 9A) thin, contort common in axis, filaments, and rhizoid: L 96.17 ± 16.61 (n=60). Sigmancistras (Fig. 9B) small, contort abundant in filaments and axis: L 44.05 ± 2.28 µm (n=150). Multidentate unguiferate anisochelae typically five teeth on head, 3–4 teeth on foot, abundant on filaments and axis. Anisochelae in two size classes: anisochelae 1 (Fig. 9C): L 33.98 ± 2.24 (n=150); anisochelae 2 (Fig. 9D): L 18.88 ± 1.67 (n=150).

Habitat and associated fauna. *Cladorhiza caillieti* was observed and collected on the Endeavor Segment of the Juan de Fuca Ridge where lava flows had very little sediment cover, if at all. They were often observed in a downward facing position, hanging from the underside of overhanging ledges of basalt (Fig. 7A–B). Other organisms observed in this community included Primnoidae and *Swiftia* sp. of Gorgonacea, *Anthomastus* sp. of Alcyonacea, serpulid polychaete worms, comatulid crinoids, and numerous unidentified species of sponges. Average depth of observation was 2149 m (±172; n=5), oxygen concentration was 1.46 ml/L (±0.11; n=5), and temperature averaged 1.87 °C (±0.05; n=5). Numerous crustacean prey were observed in various states of decomposition on *C. caillieti* (Fig. 4E).

Remarks. Thirty-six other species of *Cladorhiza* are currently recognized (Lopes and Hajdu, 2013; van Soest *et al.*, 2013) from sublittoral (110 m) to hadal (7295 m) depths. *Cladorhiza caillieti* differs from all other *Cladorhiza* in several ways including having different shape and two size classes of anisochelae, three different sigmas (including large flat-backed sigmas, smaller smooth sigmas, and even smaller thin, contort sigmas), and one size of sigmancistra. *Cladorhiza caillieti* differs from *C. evae* **sp. nov.** (described below) in spicule size classes and types. *Cladorhiza caillieti* has two size classes of anisochelae, the presence of a small, contort sigma, and non-fusiform small styles.

Cladorhiza evae sp. nov.

Figs. 10–12

Type material. Holotype: CASIZ 192773; MBARI sample D399-A4a; collected by ROV *Doc Ricketts* April 28, 2012, in the newly found Alarcon Rise hydrothermal vent field, east of Cabo Pulmo, BCS, Mexico; latitude: 23.37753, longitude: -108.53125, depth: 2299 m. **Paratypes:** MBARI sample D399-A4b (CASIZ 192774) and c (CASIZ 192775); collected by ROV *Doc Ricketts* April 28, 2012, in the newly found Alarcon Rise hydrothermal vent field, east of Cabo Pulmo, BCS, Mexico; latitude: 23.37753, longitude: -108.53125 bottle-brush filament arrangement, depth: 2299 m.

Type locality. Alarcon Rise hydrothermal vent field, east of Cabo Pulmo, BCS, Mexico.

Etymology. Named in honor of Eve Lundsten, beautiful wife of the first author whose commitment and support have endured through the years. Eve's love for the Gulf of California also inspired this naming as the type specimen was collected in the deep sea, east Cabo Pulmo, Baja California Sur, Mexico, near where we honeymooned in 2006.

Diagnosis. Cladorhizidae unbranched, with three size classes of megaslere styles and four microsclere categories including sigmas of two size classes, contort sigmancistra, and unguiferate anisochelae.

Description. A stipitate sponge with filaments arranged in four or five discreet longitudinal rows, with valleys or depressions between rows (Fig. 10A–E). All three specimens with partial rhizoids (Fig. 10D); filaments long and fragile on specimens a (holotype) and b (paratype), shorter on the smaller and, presumably, younger, c (paratype). **Holotype:** 18.7 cm long, 3.2 mm wide at base, filaments up to 1.97 cm long. **Paratypes:** (b) 17.9 cm long, 3.4 mm wide at base, filaments up to 1.8 cm long, (c)13.7 cm long, 1.7 mm wide at base, filaments up to 5.4 mm long. White in situ and in preserved state.

Spicules. Large styles 1 (Fig. 11A, Table 1) fusiform, straight, often with pointed end rounded, found throughout: L 2243 ± 460 µm (n=13). Large style 2 (Fig. 11B) fusiform, straight, often with pointed end rounded, found throughout: L 1224.36 ± 432.3 µm (n=263), W 26.13 ± 11.07 µm (n=50). Large style 3 (Fig. 11C) fusiform, straight, often with pointed end rounded, found throughout, however, smaller styles more abundant in filaments: L 825 ± 132.7 µm (n=21). Sigma 1 (Fig. 11D) robust, not contort, nor sigmancistroid; abundant in filament and axis:

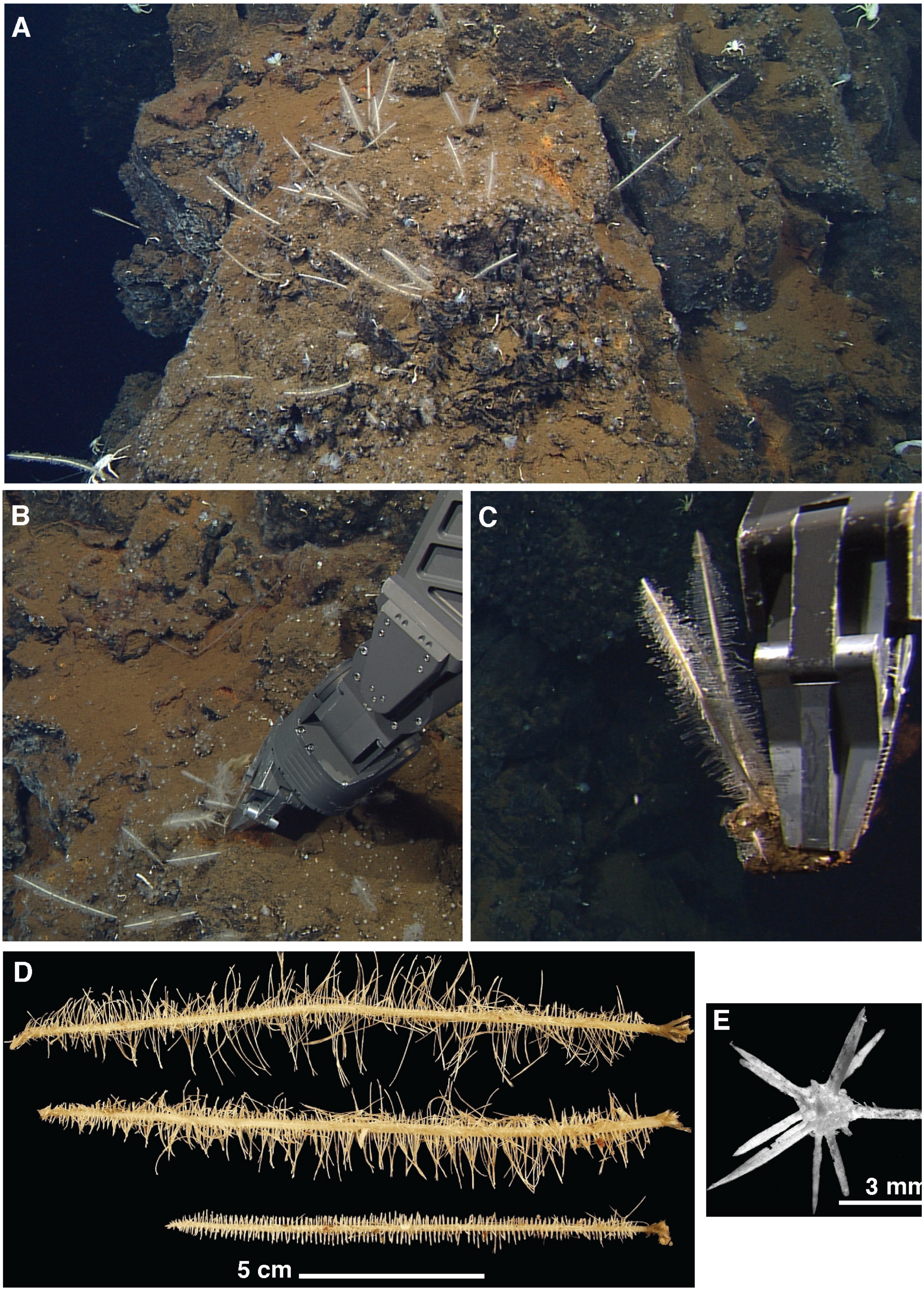

FIGURE 10. *Cladorhiza evae* **sp. nov.**: group of >20 individuals in situ (A), collection of type specimens (B–C), lab image of type specimens, holotype is on top (D), light microscopy image showing cross section of axis and filaments (E).

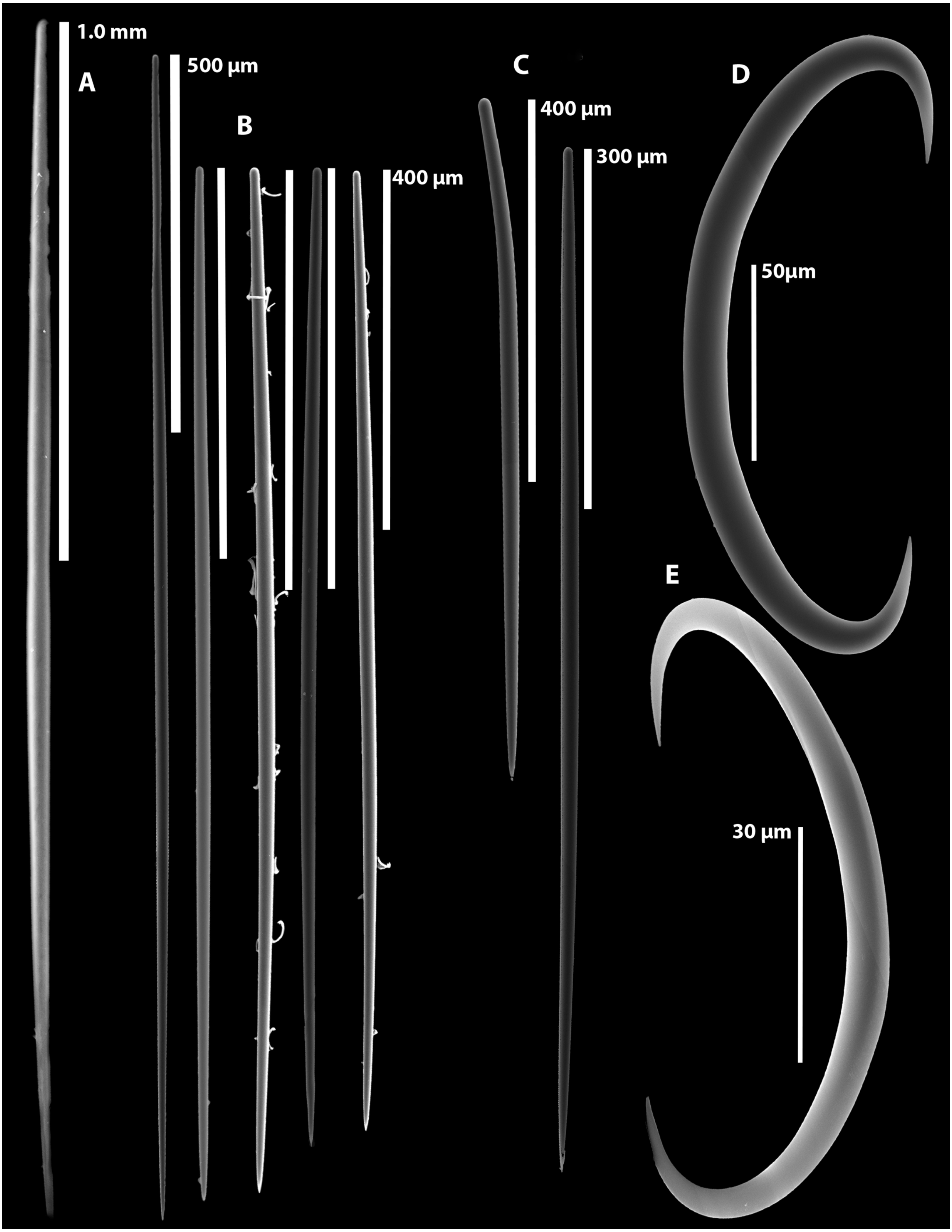

FIGURE 11. *Cladorhiza evae* **sp. nov.** spicules: large styles 1 (A), 2 (B), and 3 (C), sigma 1 (D) and 2 (E).

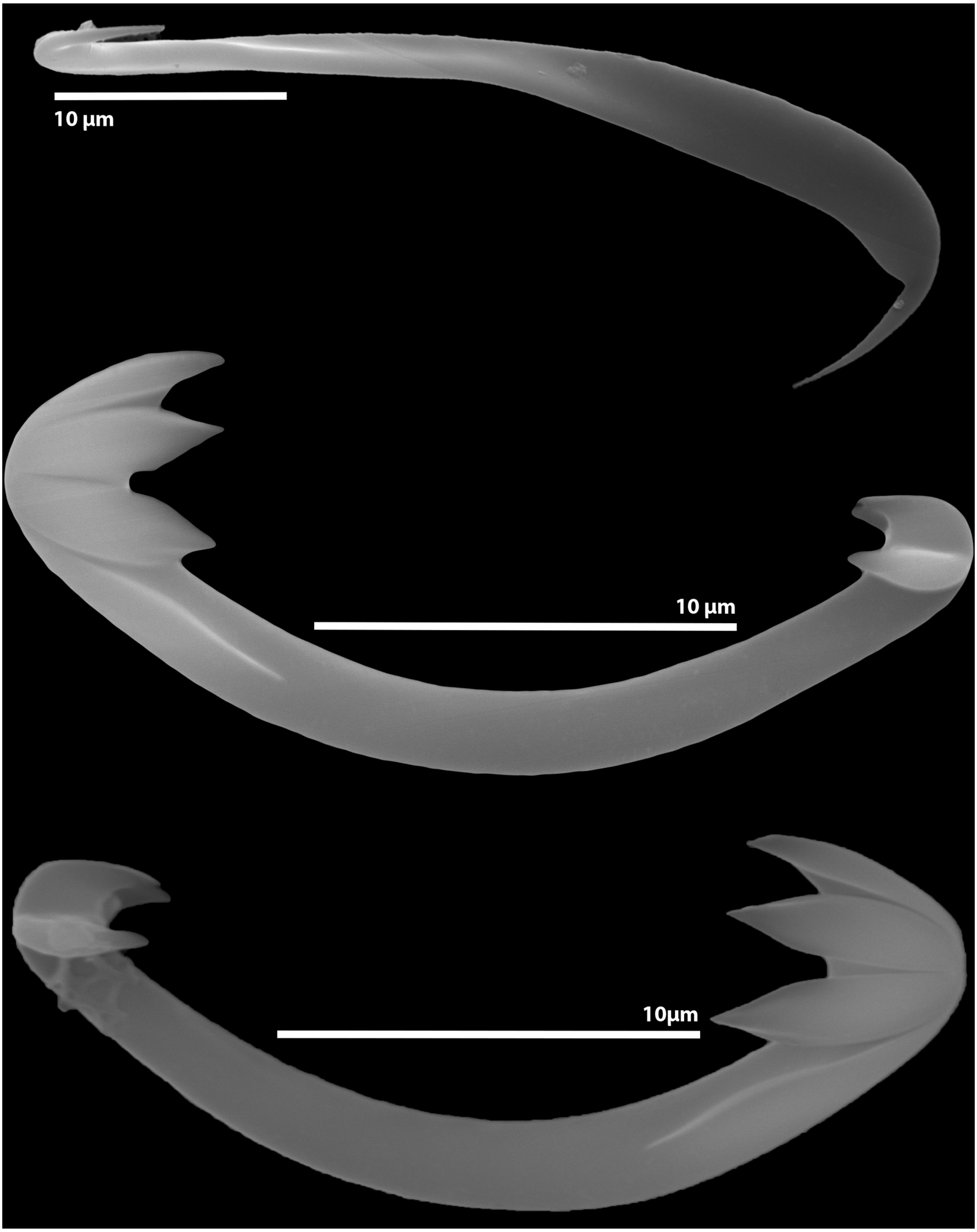

FIGURE 12. *Cladorhiza evae* **sp. nov.** spicules: sigmancistra (A), unguiferate anisochelae (B).

L 170.35 ± 9.7 µm (n=170). Sigma 2 (Fig. 11E) most 15° contort, some 90° contort, few flat, abundant in filament and axis: L 72.08 ± 11.76 µm (n=111): Sigmancistras (Fig. 12A) 90° contort, abundant in filament and axis: L 42.3 ± 2.3 µm (n=50). Multidentate unguiferate anisochelae (Fig. 12B) five teeth on head and three on foot, abundant in filaments and axis: L 22.6 ± 1.6 µm (n=50).

Habitat and associated fauna. *Cladorhiza evae* was collected from an inactive hydrothermal chimney that was covered in hydrothermally altered sediment. Galatheid and bythograeid crabs were observed in close proximity to *C. evae* on this inactive chimney. Nearby active chimneys had much richer communities of organisms with dense populations of siboglinid worms, galatheid and bythograeid crabs, and *Thermarces* sp., a zoarcid fish. Average depth of observation was 2373 m (±154; n=8), oxygen concentration was 1.54 ml/L (±0.27; n=8), and temperature averaged 2.02 °C (±0.23; n=8). Numerous crustacean prey were observed in various states of decomposition on *C. evae* (Fig. 4F–G).

Remarks. Of the thirty-six other species of *Cladorhiza* currently recognized (Lopes and Hajdu, 2013; van Soest *et al.*, 2013), *C. evae* differs from even the most similar in spicule size classes and suites. For example, *C. evae* differs from *C. rectangularis* (Ridley and Dendy, 1887) in having greater style width, larger sigmas of two size classes, and the presence of a sigmancistra. *Cladorhiza linearis* (Ridley and Dendy, 1887) differs from *C. evae* in having larger styles (to 3000 µm), small, non-contort sigmas of one size class, and larger anisochelae. *Cladorhiza septemdentalis* (Koltun, 1972) has smaller styles, larger anisochelae, and smaller sigmancistras than *C. evae*. Similarly, *C. thompsoni* (Topsent, 1909) has smaller styles, larger anisochelae, and no sigmancistras. *Cladorhiza segonzaci* (Vacelet, 2006) has smaller styles, sigmas, and sigmancistras. *Cladorhiza evae* differs from *C. caillieti* in the presence of a large (~2500 µm) size class of megascleres, a single size class of anisochelae, and no small, thin, contort sigmas.

Discussion

The four species described here add to the growing number of cladorhizid species known in the NE Pacific. Only one of these species, *Asbestopluma monticola* sp. nov., was found in a non-chemosynthetic habitat. It was observed living among a dense community of corals, sponges, and echinoderms attached to the hard rock substrate at the summit of Davidson Seamount, on steep walls in the Monterey Canyon, and similar habitat off northern California and central Oregon. It was also observed living upon dead hexactinellid sponges. *Asbestopluma monticola* had numerous crustacean prey in various stages of decomposition attached to filaments and axis spicules. *Asbestopluma rickettsi* sp. nov. was found in an active and rich chemosynethic community within a low-oxygen basin off Southern California. It was attached to outcrops of authigenic carbonate and nearby megafauna included vesicomyid clams and siboglinid polychaete worms, both of which are known to harbor methane-oxidizing bacterial symbionts. Initial investigations demonstrated active consumption of 13C-labeled methane, as well as the presence of bacterial particulate methane monooxygenase, both of which are indicative of a close association between *A. rickettsi* sp nov. and methane-oxidizing bacteria (V. Orphan, California Institute of Technology, pers. comm.). Interestingly, no crustacean prey were observed on either of the type specimens of *A. rickettsi*. Both species of *Cladorhiza* described herein were found with numerous crustacean prey in various states of digestion. Further investigation of consumption by methane-oxidizing bacteria is in process (S. Goffredi, Occidental College, pers. comm.). *Cladorhiza caillieti* was observed on older basalt ~500 m from active hydrothermal venting. The community members where *C. caillieti* was observed and collected was quite similar to the suspension feeding community at Davidson Seamount, with numerous corals, echinoderms, and other sponges observed. Both *Cladorhiza* species were found in deep water, with higher oxygen concentrations than the shallower *Asbestopluma* species. *Cladorhiza evae* was found on an inactive hydrothermal chimney with rhizoids rooted in hydrothermally altered sediment. Only tens of meters away, active hydrothermal venting was observed. Other organisms observed on this chimney included Bythograeidae and Galatheidae crabs.

Only seven other species of Cladorhizidae are recorded from the Northeast (NE) Pacific—see Lopes and Hajdu (2013) or van Soest *et al.* (2013) for global species ranges. Four *Asbestopluma* species are known from the NE Pacific including *A. biserialis californiana* De Laubenfels, 1935; from the Baja California Sur, Mexico, *A. globularis* Lévi, 1964 off Mexico and Central America, *A. occidentalis* (Lambe, 1893) from shallow water (73–120m), off British Columbia, Canada and Southeast Alaska. Koltun (1959) synonomized *A. occidentalis* with *A. lycopodium* but this is not generally accepted. *Asbestopluma ramosa* Koltun 1958 was described from specimens collected in the Sea of Okhotsk, however, it has also been reported in the Aleutian Islands and the Eastern Gulf of Alaska (Stone *et al.*, 2011). Only one other *Cladorhiza* has been described for the NE Pacific, *C. pteron* (Reiswig and Lee, 2007). Three other species of *Cladorhiza* have been described from regions just beyond the boundaries of what is considered the Northeast Pacific, including *C. corona* (Lehnert, Watling & Stone, 2005) from the Aleutian Islands, *C. linearis* (Ridley & Dendy, 1886) Panama to central South Pacific, and *C. rectangularis* (Ridley & Dendy, 1886) north central Pacific, south of Aleutian Islands.

Although these new species don't present novel morphologies or spicules, they do increase the number of known cladorhizids in the Northeast Pacific considerably. Certainly *A. rickettsi* is notable in being only the second species of cladorhizid identified as consuming methane-oxidizing bacteria. *Asbestopluma monticola* is notable for its large size and abundance at both Davidson Seamount and in Monterey Canyon, where it is a successful member of a suspension feeding guild at these locations. The presence of numerous prey on *C. caillieti,* in close proximity to hydrothermal venting indicate that these sponges may be able to utilize both methane-oxidizing bacteria and small crustacean prey as nutrient sources. The same may be true for *C. evae* and, perhaps, all cladorhizids. Numerous additional cladorhizid sponges from the Northeast Pacific await description (seen and collected by the authors), and many more, likely, await discovery. Future work should include better understanding of species geographic and depth distribution, food sources and preferences, and observation of food capture in situ for deep-sea species. Additional surveying, collecting, and identification of species from various locales will aid in these discoveries.

Acknowledgments

We are grateful for support from the pilots of the ROVs *Doc Ricketts* and *Ventana* and the crews of the R/Vs *Western Flyer* and *Rachel Carson*. We thank J. Barry, D. Clague, A. DeVogelaere, and R. Vrijenhoek for use of video observations and collected specimens. We thank K. Buck, C. Piotrowski, S. Tanner, and P. Whaling for technical support as well as S. Goffredi and V. Orphan for information on methane-oxidizing bacteria. We appreciate the comments from anonymous reviewers who greatly improved this manuscript. This work was partially supported by the David and Lucile Packard Foundation's funding of MBARI.

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