

Predation on copepods by an Alaskan cladorhizid sponge

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Deep water cladorhizids are now generally thought to be carnivorous. While the methods of prey capture have been established for species of *Asbestopluma*, and perhaps *Chondrocladia*, information is sparse for species of *Cladorhiza*. The external morphology of the deep-sea Alaskan species, *Cladorhiza corona*, was examined immediately after collection, and then again later using scanning electron microscopy (SEM). All sponges examined had captured, and in most cases, fully encased, prey items which for the most part consisted of planktonic copepods. The hypothesis of this paper is that the primary process of prey capture is by means of a sticky substance and that prey stick to the sponge on contact. The abundant cladorhizid anisochelae do not seem to be arranged in a manner such that they can be used for prey capture as has been seen in *Asbestopluma*. Digestion of the prey occurs in a temporary cavity created by migrating sponge cells. Within this cavity lipid from the copepod is liberated, then absorbed and transported to the central part of the sponge. It is not known how much of the remaining tissue of the copepod is digested. Copepods have very high lipid content so their capture would provide the sponge with abundant energy from each prey item. The sponges seem to have their highest density in areas where bottom flow and migrating copepod numbers are both high.

INTRODUCTION

By now it is presumed that all members of the sponge family Cladorhizidae, and perhaps members of other families such as the Guitarridae and Mycalidae, are carnivorous (Vacelet, in press). Cladorhizids do not have the typical aquiferous system seen in most other sponges, so it is presumed that all of their nutrition comes from captured prey. For the most part these prey items are small crustaceans, but feeding on other phyla could occur opportunistically (Vacelet & Duport, 2004), and in one case nutrient needs are at least supplemented by methanotrophic bacteria (Vacelet et al., 1995).

Prey capture in cladorhizids has been described, or inferred, for only *Asbestopluma hypogea* Vacelet & Boury-Esnault, 1996 (cf. Vacelet & Duport, 2004) and *Chondrocladia gigantea* (Hansen, 1885) (cf. Kübler & Barthel, 1999). Species of *Chondrocladia* consist of a central stalk from which horizontal branches with thin-walled water-filled spheres extend (Vacelet, 2006). An aquiferous system is present only in the stalk and apparently is used to maintain the water pressure that keeps the bulbous extensions of the axis inflated. Kübler & Barthel (1999) suggest that copepod prey get caught on the spheres, which have palisades of isochelae on their surfaces. On contact the sphere deflates and the copepod is suddenly

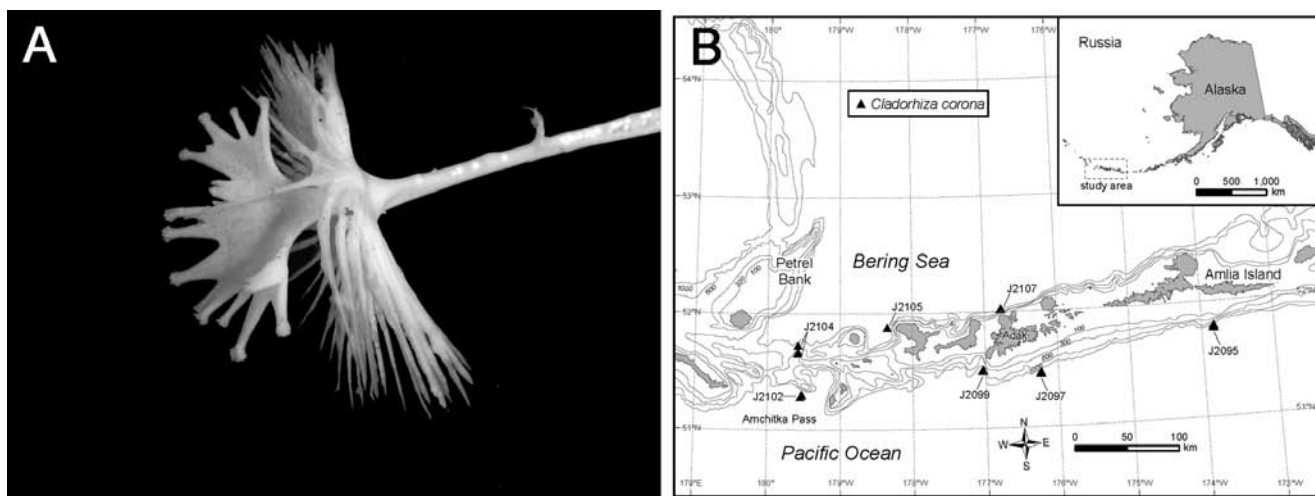


Figure 1. (A) Upper portion of a live individual of *Cladorhiza corona*, photographed in the laboratory on the ship; note long stalk leading to radiating appendages which are used to catch prey items, above which is the crown of club-tipped appendages; white objects in the stalk may be embryos; (B) map of the central Aleutian Islands showing stations at which *C. corona* was collected.

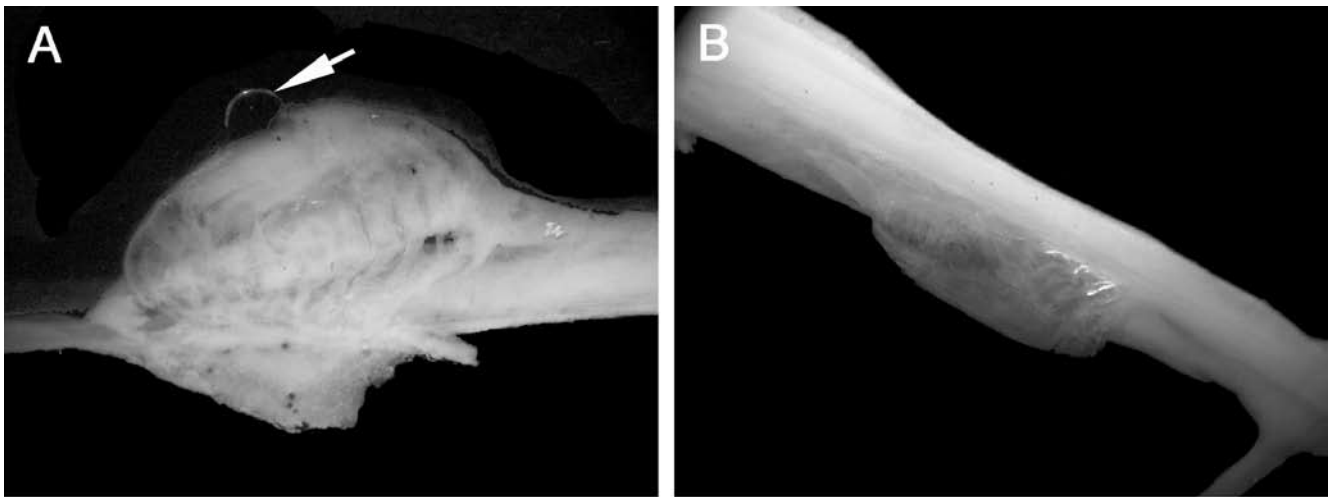


Figure 2. Copepods captured and encapsulated, along two of the radiating appendages of the sponge, photographed aboard ship immediately after retrieval from the ROV biobox. (A) Copepod length, 5 mm; (B) copepod length, 3 mm. The sponge tissue in (A) has been pulled away, revealing the copepod body and an oil droplet (arrow), most likely released from the copepod by the actions of the cells making up this temporary digestive cavity (originals in colour).

surrounded by sponge tissue. Archaeocytes migrate toward the prey and begin the digestion process, breaking the prey into small pieces which can be phagocytized and carried to sites of nutritional need.

In *Asbestopluma hypogea*, prey are caught by becoming entangled in filaments lined with palmate anisochelae (Vacelet & Dupont, 2004). They propose that the 2.5 μm spacing of the anisochelae on the filaments is exactly what is needed to snag the finer parts (setules) of the crustacean setae. As the animal struggles, more setae are likely to be caught. After about 12 hours, the prey is completely engulfed by migrating elongated cells that seem to be similar

to pinacocytes. The prey is eventually surrounded by these cells, which form a temporary 'digestive cavity'. Electron microscopy showed that archaeocytes and one type of bacteriocyte were strongly implicated in the extracellular and intracellular digestion of the prey.

The mechanism of prey capture has not yet been determined for species of *Cladorhiza*, although inferences have been made based on morphology of the microscleres and the lack of aquiferous system (Vacelet, in press). In this paper it is proposed that the Alaskan deep-sea species, *Cladorhiza corona* Lehnert et al., 2005, is a predator primarily of copepods using a somewhat different mechanism to catch its prey.

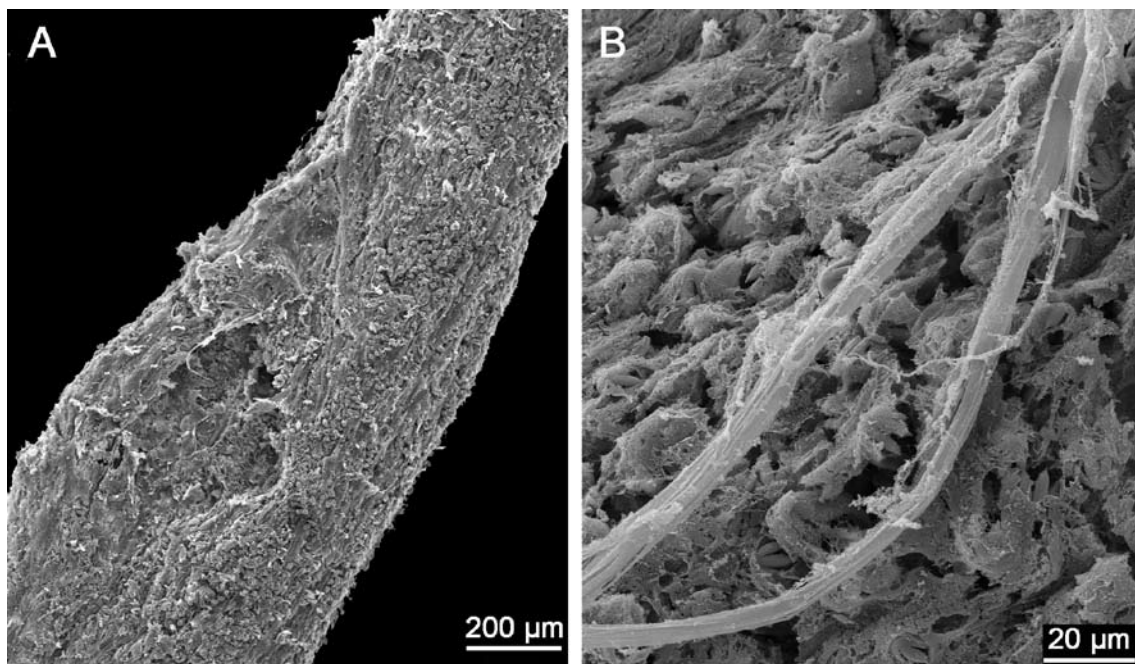


Figure 3. Scanning electron microscopy view of one of the radiating appendages of *Cladorhiza corona*. (A) Low magnification view showing diversity of cells at the surface of the appendage; (B) close-up of a part of the radiating appendage. Here the anisochelae can be seen to be covered with a coating of what is hypothesized to be a sticky substance used to catch the crustacean prey items. Note the crustacean setae lying on the surface of this material.

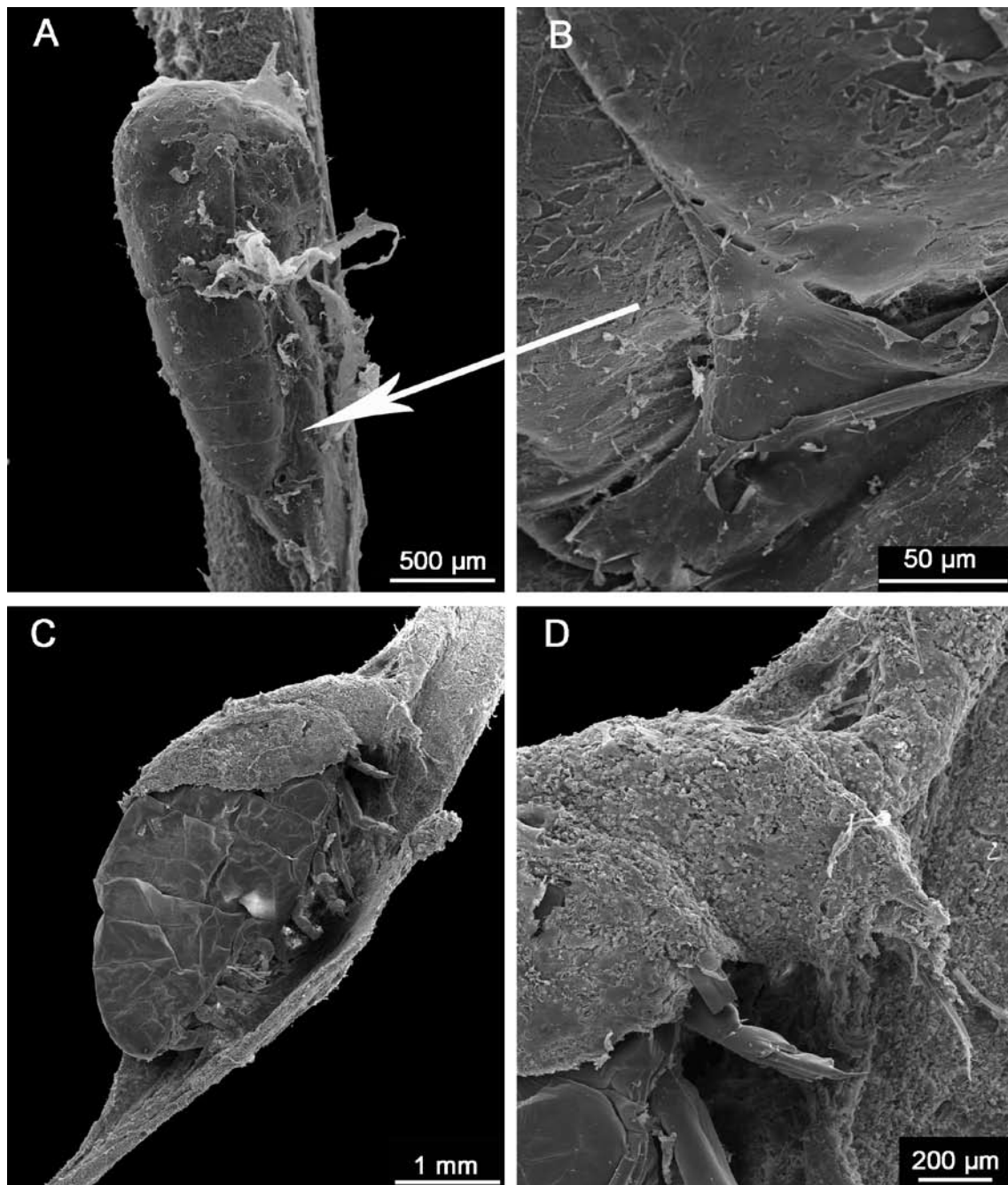


Figure 4. Scanning electron microscopy examination of the copepods in Figure 2. (A,B) Smaller copepod with only a thin sheet of sponge cells beginning to cover the copepod body; note in (B) the encroachment of the sponge between the thoracic somites of the copepod; (C,D) larger copepod with most of the fully developed sponge tissue making up the temporary digestive cavity removed. Note in (D) the presence of large numbers of anisochelae in the sponge tissue covering the copepod. It can also be seen that most of the setae and appendage extremities of the copepod have been removed.

MATERIALS AND METHODS

Specimens of *Cladorhiza corona* were collected from several sites along the central Aleutian Ridge using the ROV *Jason II* (Figure 1B). A description of the sponge and station details is in Lehnert et al. (2005). Three specimens were used to determine the method of feeding in this species; two were from Station J-2097, 51.462°N 176.240°W, 1720 m, 28 July 2004 and one from Station J-2104, 51.731°N 179.585°W, 1010 m, 5 August 2004.

While fresh, the sponges were photographed and examined for evidence of prey capture. Lumps along the

basal appendages (Figure 1A; see Lehnert et al., 2005, figure 1F) were dissected and photographed. The entire sponge body was then immersed in a buffered 4% formalin solution for 48 hours, after which it was transferred to 70% ethanol for storage.

For SEM studies, pieces of the preserved sponge with prey items were dehydrated to 100% ethanol and dried using a Samdri critical point dryer. Specimens were mounted on oversize stubs, coated with gold-palladium and examined with a Hitachi S-800 fitted with a digital capture board.

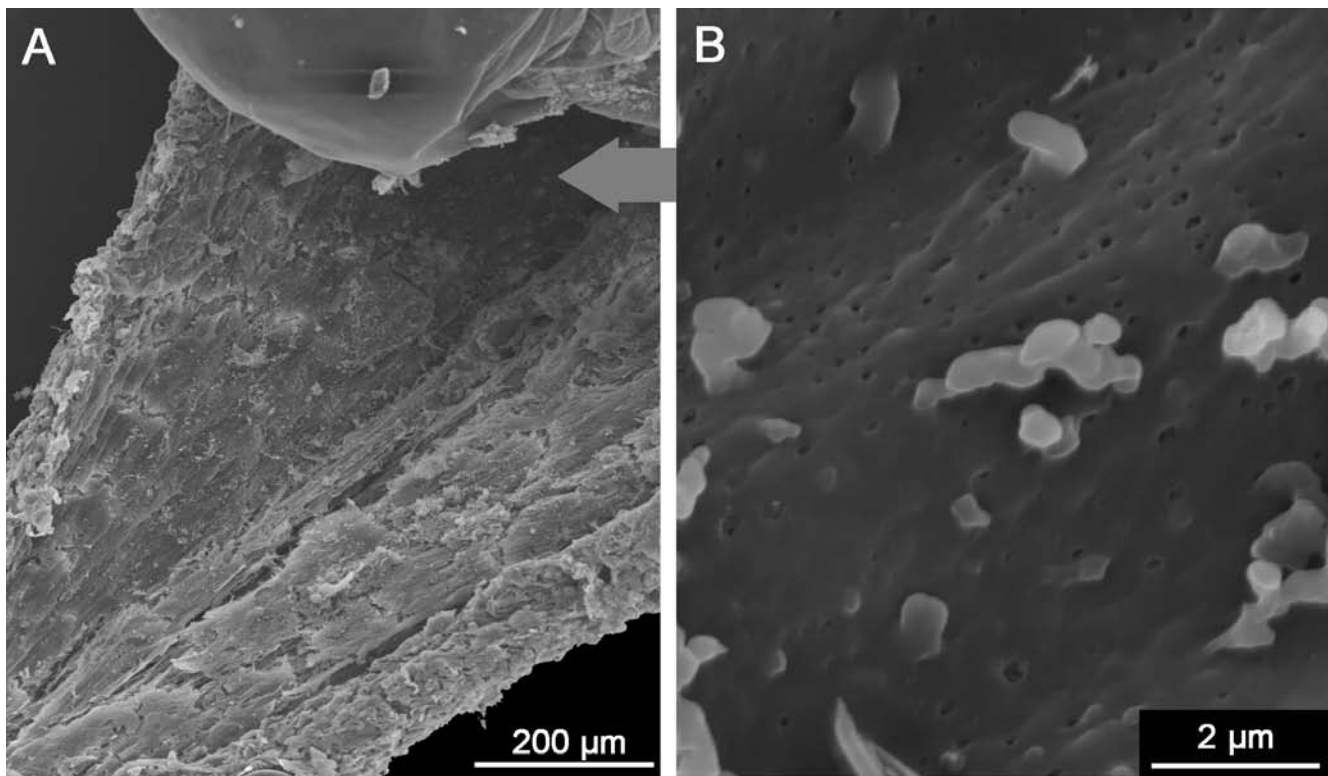


Figure 5. (A,B) Close-up of Figure 4C, showing details of the interior of the temporary digestive cavity established by the sponge. Note the very fine pores and small ($0.5 \times 2.0 \mu\text{m}$) processes extending from the surface of the cells.

RESULTS

The body of *Cladorhiza corona* consists of three main parts (Figure 1A): an elongated stalk about 25–30 cm tall that extends from the basal plate to a radiating series of 60+ appendages, immediately above which is a perpendicular structure termed the crown. During collection the sponge was often referred to as a ‘flower sponge’ because of its superficial resemblance to the common daisy. The radiating appendages of the ‘flower’ are connected about halfway along their length by an ectosomal membrane.

In all the photographs the radiating appendages showed slightly differing morphologies, that is, the tissue adhering to the supporting polyspicular styles was of varying thickness ranging from uniform to thin or blob-like. On inspection, the ‘blobs’ usually consisted of lumps of tissue, occasionally with encased copepods (Figure 2), but on a few sponges amphipods were also found (it was later presumed that the amphipods originated on octocorals that were collected during the same ROV dive, that the amphipods eventually left their host, and were trapped by the sponge and encased during the 12–17 hours that the specimens were in the ROV collection box). Three sponges examined had captured and were in the process of digesting six, eight, and nine copepods, respectively. Two of the temporary ‘digestive cavities’ were opened to reveal the encased copepods. In both cases large orange-yellow lipid droplets were lying next to the copepod within the cavity (e.g. Figure 2A). Otherwise the muscle material of the copepods looked to still be present. Near the base of 2–5 of the radiating appendages orange spots were noted on all sponges. These spots were the colour of the lipid next to the partially digested copepods so were presumed to

be remnants of prey in the final stages of transport to the centre of the sponge.

The SEM examination of the radiating appendages shows the surface to be made up of a loose association of cells (?pinacocytes and archaeocytes) covering the polyspicular tracts of styles (Figure 3A). Amongst (or within?) these cells are very large numbers of anchorate anisochelae, about 80% of which have their alae oriented toward the surface of the sponge (Figure 3B). A rough estimate suggests that there can be as many as 85–100 anisochelae 0.04 mm^{-2} .

Two copepods caught on the radiating appendages (Figure 2) were examined with SEM (Figure 4). One appeared to have been recently trapped and was mostly visible while the second was fully encased in sponge tissue which was subsequently partially dissected away. In the first case (Figure 4A) most of the copepod body is visible, but there are sheet-like cells from the sponge creeping up the front and sides of the prey (Figure 4B). Later stages of this digestive process probably involves the full-scale development of what Vacelet & Duport (2004) termed a ‘temporary gut’ as seen in the second copepod examined. This copepod is fully encased in a thick outer layer of cells (Figure 4C,D), containing very large numbers of anisochelae, and an inner layer of much smoother-looking cells (Figure 5). The latter bear a myriad number of small pores $0.01 \mu\text{m}$ in diameter, and processes 0.5 to $1.5 \mu\text{m}$ in length and $0.5 \mu\text{m}$ in diameter. Below this copepod, on the outside of the sponge, are a series of crustacean setae, most likely from this prey animal, which may be left over from the initial entrapment, or have broken off and are being discarded by the sponge. In either case, none of the setules are entangled in the alae of the anisochelae (Figure 3B).

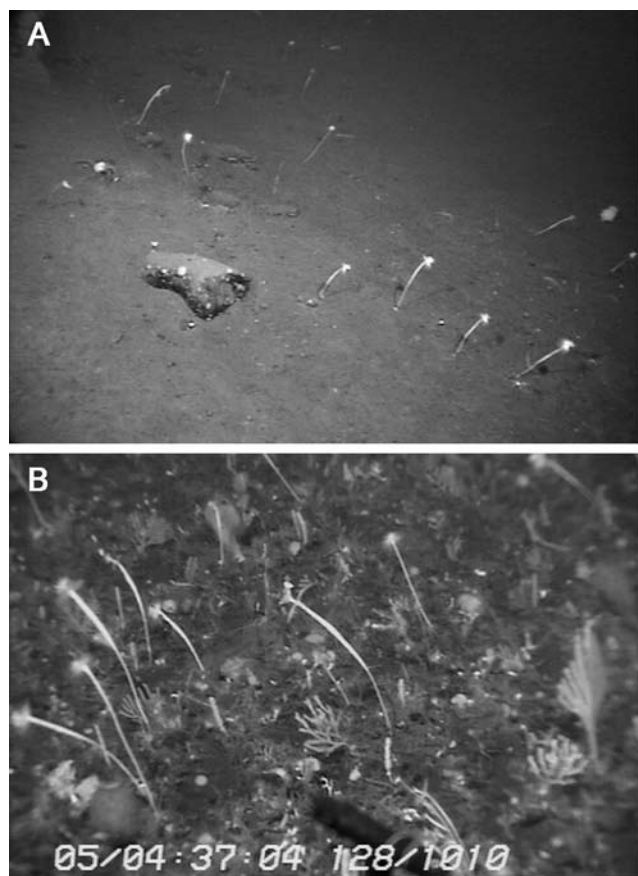


Figure 6. *In situ* frame grabs from: (A) Dive J-2097, on the Pacific Ocean side of the Aleutian Ridge, depth 1718 m; and (B) dive J-2104, on the north side of Amchitka Pass, depth 1010 m. *Cladorhiza corona* are the large white stalked structures in each image. The field of view is about 1.5 m across the central part of the image in (A) and about 90 cm in (B).

DISCUSSION

It is clear that this sponge is a predator in the manner of other cladorhizids. Prey capture in this family is so far known to be effected through at least two mechanisms: *Chondrocladia* species are presumed to engulf their prey by sudden collapse of water-filled spheroidal appendages (Kübler & Barthel, 1999; but see also Vacelet, 2006 for contradictory observations) while *Asbestophuma* species use an entanglement strategy coupled with a sticky substance (Vacelet & Dupont, 2004). Neither strategy seems to apply to this species of *Cladorhiza*. There are no water filled appendages, no long filaments in which crustacean setae can become entangled, and the anisochelae are not spaced with any regularity and are often very close together, if not immediately adjacent (Figure 3B). It may be, then, that each genus in the family has developed its own mechanism of prey capture, and that genera in related families will have developed other, novel, methods to obtain their nutrition in the absence of a water-flow based system.

For *Cladorhiza corona* I hypothesize that the mechanism of capture involves primarily the use of some sort of sticky substance, at least sticky with respect to the physical properties of the crustacean cuticle. An examination of the sponge surface at high magnification reveals a layer of

crossed thread-like features, which could be remnants of a sticky material, perhaps mucus-based (Figure 3B). This idea was discussed at length with my ship-board colleagues, and an attempt to experimentally test the idea was made. Three additional specimens were collected at Station J-2104, Amchitka Pass, 1010 m (station details in Lehnert et al., 2005). These were in the best condition of all collected, so two were set aside as holotype and paratype, and the third was put into a test tube rack in an aquarium in a walk-in cold room. An amphipod that had most likely been collected with one of the octocorals was selected to be the prey item. The amphipod was launched toward the sponge with a pipette and when it touched the sponge it stuck fast. Interestingly it landed on the sponge 'backwards', that is, it was stuck by the dorsal surface of the urosome. The amphipod remained stuck to the sponge for about 15 hours, and then escaped. The sponges were in the biological collection box on the ROV for about 12 hours before being landed on the ship, so they may not have been in the best condition when the experiment was started. Since they are without an aquiferous system it was not possible to tell how healthy the sponges might have been. Consequently, it cannot be determined whether the amphipod escaped because it was not entangled in any way, or whether the sponge was incapable of encasing it.

Our earliest collected specimens were put in the collection box with octocorals and rocks with sponges. They were difficult to work with because hundreds of sediment grains were stuck to the main body of the sponge and the radiating appendages (but not the stalk). The last three specimens collected, including the one used in the feeding experiment, were isolated in their own collection container and they came to the surface clean and shiny (Figure 1A, and figure 1F in Lehnert et al., 2005). These observations also suggest that the surface of the sponge was 'sticky', and that some substance was involved in the capture of prey items.

If *Cladorhiza corona* does not snare its prey with the small microscleres, what then are all those anisochelae used for? Ridley & Dendy (1887) suggested the layer of microscleres could offer protection against parasites or perhaps carnivores. Since this spicule type is widespread among the poecilosclerid demosponges, such a possibility has to be considered. One wonders, as well, if they could not also be of some structural advantage, in this case holding together the highly mobile tissue of cladorhizid sponges, analogous perhaps to the pieces of iron bars encased in concrete for the purpose of adding strength and rigidity.

Copepods seem to be especially valued prey items of all the carnivorous sponges studied so far. Lee et al. (1971) examined the lipid content of copepods collected at a variety of depths in the Pacific and Arctic basins. Copepods from deeper waters, 750–1600 m, were generally very high in lipids, from 32–61% dry weight. Most (61–82%) of the lipids present were in the form of wax esters rather than triglycerides. Lee et al. (1970) suggested that triglycerides and wax esters function as energy reserves in deep dwelling zooplankton. Since lipids generally have the highest caloric output compared to protein and carbohydrates (Paine, 1971), it seems likely that copepods are food packets from which the sponges would gain the greatest amount of energy. Catching copepods using a passive intercept mechanism would offer

the sponge the greatest return for energy spent. Other planktonic organisms, such as diatoms, have also been seen in the SEM images, either on the surface of the appendages or surrounded by sponge cells. It is not known whether any of these were 'digested' by the sponge or were merely empty frustules stuck to the sponge.

Cladorhizids are usually thought to be rare in deep sea communities, with many species being known from one or two specimens. However, there are areas where cladorhizids are moderately abundant. Vacelet (2006) notes that *Chondrocladia lampadiglobus* Vacelet, 2006 was moderately abundant a short distance away from active hydrothermal vents, perhaps taking advantage of the higher abundances of zooplankton in these areas. *Cladorhiza corona* was also occasionally seen in patches of 10–100 individuals covering an area of 25 m² or so. Exact densities are difficult to obtain because of the angle of the camera on the ROV and variable height of the ROV above the sea-floor. As a proxy, an average sponge height of 25 cm was used to gauge horizontal distances in the video. Two such fields of view are given in Figure 6.

Cladorhiza corona most likely will always be found in high abundance where copepod density is high. The highest abundances seen to date are on the north side of Amchitka Pass at about 1000 m (Station J-2104, Lehnert et al., 2005). This is an area of high water flow between the Bering Sea and the North Pacific. Recent studies on circulation through Amchitka Pass suggest that at 1000 m current velocities are routinely between 10 and 20 cm s⁻¹. Coyle (2005) showed that the central Aleutian passes had higher abundances of copepods than passes toward the eastern end of the Aleutian chain. Some of these, such as species of the copepod genus, *Neocalanus*, undergo ontogenetic vertical migrations. Vertically migrating zooplankton, coupled with the moderately strong current, would assure the sponges of a regular supply of food, and probably explains the high number of prey items on each sponge. This carnivorous sponge, and probably a few others, such as *Chondrocladia lampadiglobus*, live in food rich environments rather than the food poor environment documented for *Asbestopluma hypogea*. Other presumed carnivorous sponges, *Euchelipluma elongata* Lehnert et al., 2006 and *Esperiopsis flagrum* Lehnert et al., 2006, have also been recently described from the Amchitka Pass area (Lehnert et al., 2006).

The serendipitous discovery of this sponge and the brief examination of its biology is all due to the generosity of Dr Robert Stone, who kindly invited me to participate in the Aleutians cruise so that I could work on deep water octocorals. I would also like to thank the members of the geological team on this cruise, who became sufficiently curious about this unusual animal that they were very helpful in spotting individuals at the edge of the view of the ROV camera. The success of this cruise was due entirely to the captain and crew of the RV 'Revelle', and the pilots and technicians responsible for the operation of the deep-diving ROV 'Jason'. My former student, S. Gerken, helped with fixation and other tasks on the ship and my student, Anne Simpson did the same at the laboratory in Maine. In Hawaii, the SEM images were produced with the very knowledgeable help of T. Carvalho. J. Vacelet kindly brought me up-to-date on the literature of this fascinating group and his sending of his in-press manuscript is greatly appreciated.

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