NOTE

A Positive Chitosan Test for Spicules in the Anthozoan Order, Pennatulacea

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ABSTRACT: In the coelenterate seapen colony, *Leioptilus guerneyi,* two types of minute spicules have been found; these give the characteristic chitosan tests indicative of chitin. Chitin is essentially unknown in the Anthozoa. The definition of the order Pennatulacea requires the addition of chitinous spicules to the calcareous spicules already described for the group.

CHITIN OCCURS WIDELY but irregularly distributed throughout the animal phyla. In a comprehensive report, Jeuniaux (1963) reviewed and tabulated its distribution. Additional occurrences found later have been reported in: Pogonophora tubes (Blackwell et al., 1965); a protozoan (Krishnan and Rajulu, 1965); the phylum priapulida (Shapeero, 1962); the cuttlefish, *Sepia officinalis* (Okafor, 1965); and coelenterates, brachiopods, solenogasters, chitons, and gastropods (Hyman, 1966).

Chitin has been found in some members of the coelenterate classes Hydrozoa and Scyphozoa. Wainwright (1962) reported its presence in the anthozoan coral, *Pocillopora damicornis.* The skeleton of this species yields upon demineralization an organic residue of 0.1 per cent, the major part of which is chitin. Chitin is unreported elsewhere from the Anthozoa.

The fleshy, feather-shaped, orange seapen, *Leioptilus guerneyi* (= *L.,* or *Ptilosarcus quadrangulare*) (Fig. 1) occurs from high subtidal to some depth along the Pacific Coast. The animal is anchored in the substratum by its peduncle and may stand in excess of 2 feet above the bottom when fully expanded. According to Hyman (1940), the animal is, in fact, a colony of numerous individuals consisting of three forms. The main fleshy stalk, with its peduncle embedded in the sand, represents the original individual. Budding off this stalk continuously with time are thin leaves that bear at their edges eight-tentacled, food-gathering autozoids. On the "ventral" surface of the colony along the long axis are two rows of clusters of moundlike, tentacleless siphonozoids through which water enters and leaves the center of the colony.

The endoskeleton consists of two types of minute, amber-colored spicules plus a long supporting rod that runs down the center of the primary individual. In the superficial tissue of the peduncle and tissue not occupied by autozoids and siphonozoids, the spicules are rice-shaped (Fig. 2). Elongated spicules are found interlaced and partially exposed among the siphonozoids (Fig. 3). Elongated spicules are also found embedded in the flesh around the autozoids and form a collar of two conspicuous projections, into which the autozoids can withdraw (Fig. 4).

The simplest tests for the presence of chitin are based on the chitosan method published by Campbell (cited by Richards, 1951). Hyman (1966) considers these tests to be qualitative but not quantitative for chitin.

Both types of spicules and the supporting rod survived treatment with hot saturated potassium hydroxide, but only the two types of spicules gave the characteristic tests indicative of chitin. The spicules appear to be unchanged by treatment with potassium hydroxide or 2 per cent hydrochloric acid; this suggests they are pure chitin.

The residue of the supporting rod following KOH treatment is inorganic in composition,

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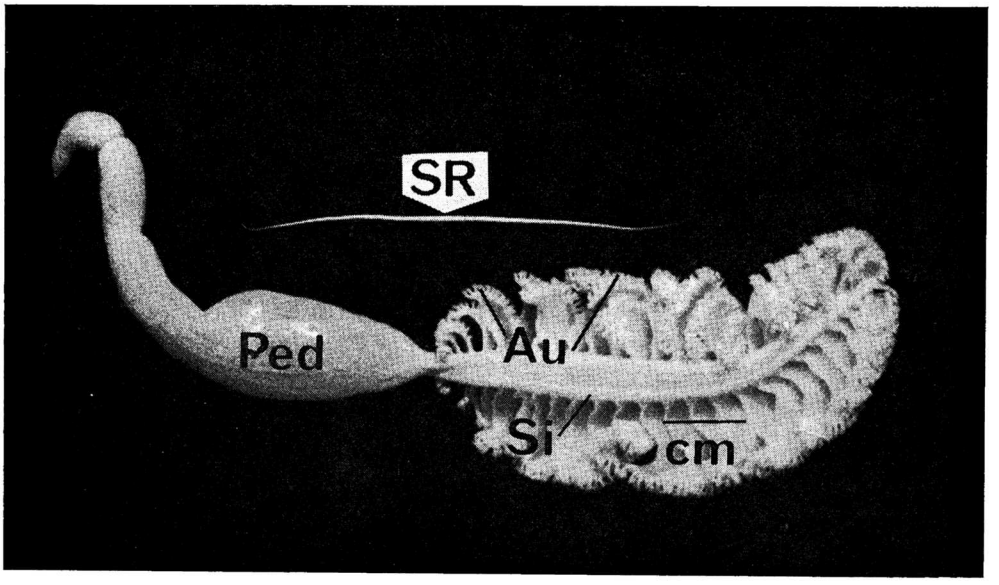

FIG. 1. *Leioptilus guerneyi.* Entire colony with supporting rod (*SR*) removed; *Ped,* peduncle; *Si,* siphonozoids; *Au,* autozoids. × 1.

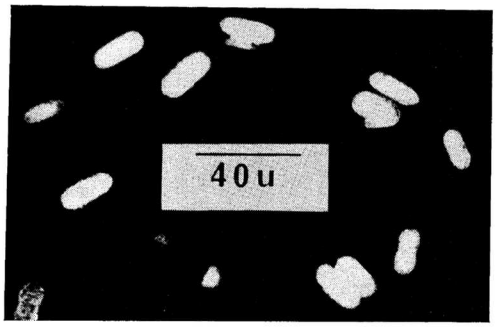

FIG. 2. *Leioptilus guerneyi.* Rice-shaped spicules from the peduncle.

consisting mainly of calcium and magnesium. Dry combustion of an untreated rod at 600°C results in a brittle inorganic rod and a weight loss of about 43 per cent, presumably a non-chitinous organic material.

The presence of discrete chitosan-positive spicules in *L. guerneyi* is the first report of chitin in the order Pennatulacea and the second report for the class Anthozoa. Other species should be examined. Hyman (1966) defined the Pennatulacea in part as possessing a "skeleton of separate calcareous spicules." In view of the present study it seems reasonable that this

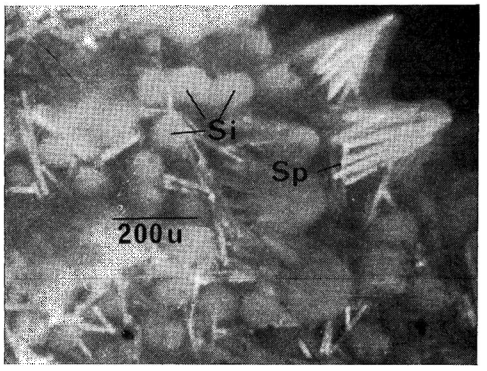

FIG. 3. *Leioptilus guerneyi.* Elongated spicules (*Sp*) among the siphonozoids (*Si*).

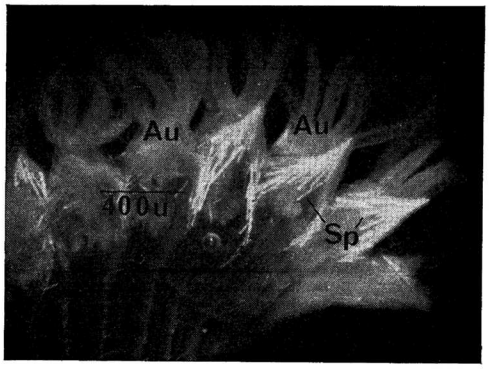

FIG. 4. *Leioptilus guerneyi.* Collars of elongated spicules (*Sp*) around the autozoids.

definition should be modified to include chitinous spicules.

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