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Spicule network patterns of *Phyllidia varicosa*

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ABSTRACT: Spicule network patterns inside the body of a nudibranch play an important role in supporting the soft body of the nudibranch. These patterns can also be one of the indicators for prediction of the phylogenetic affinity of the nudibranch. Specimens of the nudibranch (*Phyllidia varicosa*) were collected from Koh Phi Phi and neighbouring islands in Krabi. The spicule network in the mantle of the central notum looked like a net, whilst at the edge of the mantle it appeared as a radial line crossing the body. The spicules in the nudibranch foot were interlaced and were perpendicular to the body length. A study of the spicule contents by indirect examination indicated that the $CaCO_3$ content in the central notum, mantle edge, and foot was 460 ± 20, 462 ± 20, and 469 ± 20 mg/g dry weight, respectively. The spicule content in the various body regions did not differ significantly ($p = 0.7$). The relationship between total weight of the spicule network (TW) and whole body dry weight (WB) was estimated as $\mathrm{TW} = 0.446\mathrm{WB}$ ($R^2 = 0.9994$).

KEYWORDS: opisthobranch, nudibranch spicule, $CaCO_3$

INTRODUCTION

Spicules in various invertebrates are important as a supporting structure and for self protection[1–3]. Nudibranchs are known to have reduced or absent shells[4]. Many species of nudibranchs have developed spicules which co-function with the body tissue, supporting the body[5–7], and may co-function with chemical defenses to protect the body from predators[8,9]. Spicules comprise a large percentage of the total dry weight[8–10]. However, research is still lacking on spicules, and especially on their function in nudibranchs living in the marine ecosystem. In addition, knowledge on spicule networks has been used to construct an evolutionary diagram of nudibranchs[11]. Because of the limited amount and lack of clarity of information on each species, it is very hard to classify nudibranchs[11–13].

Fusiform spicules in nudibranchs are composed of calcite ($CaCO_3$), brucite ($Mg(OH)_2$), and fluorite (CaF_2), whereas spherular spicules are composed of pure calcite. Some authors have suggested that the pH inside the organales could have an effect on the uptake rate of calcium and magnesium that is reflected as well in differences in the spicule content between mantle and foot[14].

Phyllidia varicosa Lamarck, 1801 (Nudibranchia: Phyllidiidae) is widely distributed in the Indo-West Pacific Oceans, the central Pacific, and the Red Sea[11]. Although this nudibranch species is common, there appears to be limited knowledge of its ecology. Most previous studies[15–17] were concerned with the transfer mechanism of secondary metabolites, such as isonitriles, from the nudibranch's diet that includes species such as *Hymeniacidon* sponges.

The current study focuses on the spiculation patterns and content, and includes a detailed description of the function of various organs that contain spicules. In addition, an attempt is made to clarify the relationship between spicule content and the growth of *Phyllidia varicosa*.

MATERIALS AND METHODS

Spicule network pattern

Five specimens of *Phyllidia varicosa* used for the experiment were collected at depths of 5–10 m on coral reefs in the Andaman Sea, near Koh Phi Phi in Krabi province (7° 41′ 07.82″ N, 98° 45′ 53.27″ E). The samples were collected by scuba diving during the dry season (December to April). The body length of all specimens was measured before washing with filtered seawater (through GF/F) and stored at −20 °C until analysis. Each sample was cut (at least 4 mm^2 of each body region) to separate the foot, central notum, and mantle edge after removing the visceral organs and then the spicule network pattern was analysed

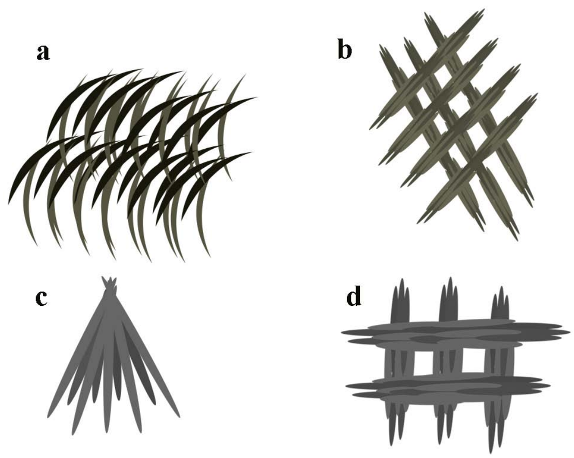

Fig. 1 Spicule network in different body regions under a light microscope and naked eye: (a) horizontal arrangement, cross-hatch perpendicular to the body length; (b) horizontal arrangement, cross alternately; (c) vertical arrangement, one end of the spicule bunched like a dome; (d) horizontal arrangement, lattice-like, circumferential tracts intersect with radial tracts, creating a grid.

under a light microscope (Olympus CX41RF).

Spicule content

Measurement of the spicule content was conducted using the modified method of Penney[9]. Each piece of nudibranch organ was put on a small pre-weighed aluminium tray. Thereafter, the sample was dried in the oven at 56 °C for 24 h. The sample was then kept in a desiccator. Each piece of nudibranch organ was re-weighed before being placed in a muffle furnace at 500 °C for 24 h to disintegrate the organic tissue. The samples were taken out of the furnace and kept in the desiccator before the spicule weight was determined and the sample examined.

Data analysis

Differences between spicule weights of organs from nudibranchs of various size were examined by one-way ANOVA. Linear regression analysis was used to examine the relationship between total spicule weight and whole body dry weight.

RESULTS

Spicule network patterns

Based on observations of the spicule network under a light microscope, it was concluded that there were some differences among the spicule networks in each body region. The networks could be grouped into four general patterns (Fig. 1).

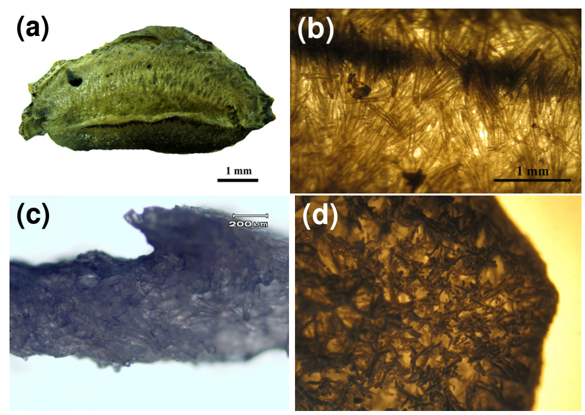

Fig. 2 Spicule network of foot. Macroscopic view: (a) inside foot and outside foot edge. Under a light microscope: (b) inside and central foot; (c) cross-section of foot; (d) outside foot edge.

Foot

Spicules of the foot inside the body are arranged in a cross-hatch pattern perpendicular to the body length (Figs. 1a, 2a,b). The cross-sectional view shows the horizontal tract arrangement of the spicules (Fig. 2c). At the outside (foot edge), which was exposed to the substrate, spicules are arranged in vertical tracts, with one end of the spicule bunched like a dome (Fig. 2d).

Central notum

Spicules inside the central notum are intertwined as lines and crossed alternately with lines meeting at angles of 35° and 125°. This arrangement forms a lattice-like pattern (Figs.1b, 3b–d). Almost all spicules are similar in size and have a fusiform shape with a horizontal tract arrangement. The dorsal surface of the mantle is supported by vertical tracts with papillae. One end of the spicules is bunched like a dome (Figs. 1c, 3e). A cross-section of this organ shows a haphazard arrangement that creates space within the tissue (Fig. 3f).

Mantle edge

Spicules in the mantle edge are firstly intertwined as lines and then arranged in a lattice-like pattern, with the circumference crossed with radial lines of the body at an angle of 90°. Almost all spicules are in horizontal tracts (Figs. 1d, 4a–c). The cross-section of this organ shows a non-compact arrangement of spicules that cause space within the tissue. Moreover, a mixture of small and large fusiform spicules are found in this organ and some are arranged at an angle of 45° to the body (Fig. 4d).

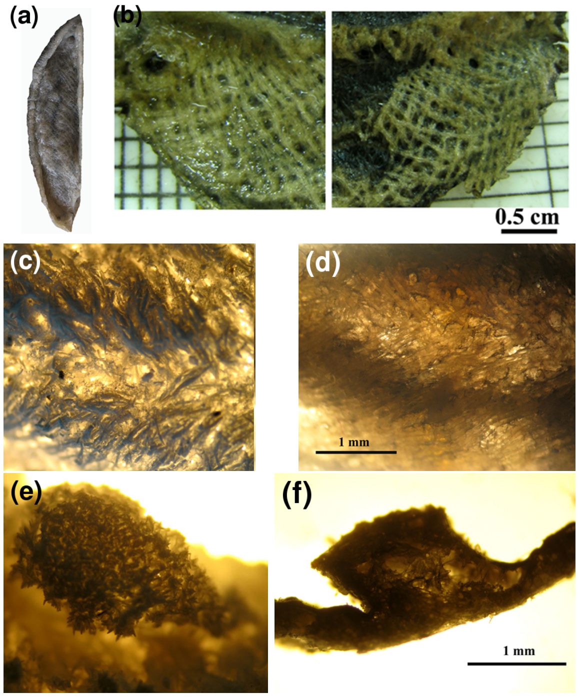

Fig. 3 Spicule network of central notum. Macroscopic view: (a–b) inside central notum. Under light microscope: (c–d) inside central notum; (e) outside papillae; (d) cross-section of papillae.

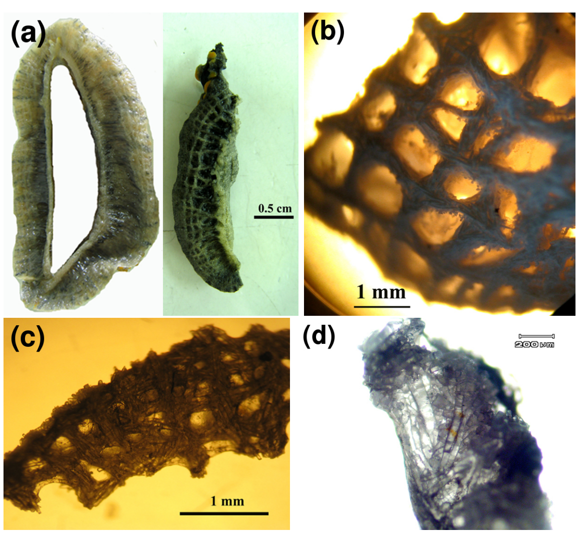

Fig. 4 Spicule network of mantle edge. Macroscopic view: (a) ventral side of mantle edge. Under light microscope: (b–c) ventral side of mantle edge; (d) cross-section of mantle edge.

Spicule content

The average spicule content ($n = 5$) of the foot, central notum and mantle edge were 469 ± 20, 460 ± 20, and 462 ± 20 mg/g dry weight, respectively. Statistical analysis (ANOVA) suggested that there were no significant ($p = 0.709$) differences in the spicule content among the various body regions, but there were significant ($p = 0.0004$) differences in the spicule content among various lengths (2.0–7.5 cm). In addition, spicule weight showed a positive linear relationship ($R^2 = 0.999$) with whole body dry weight (Fig. 5).

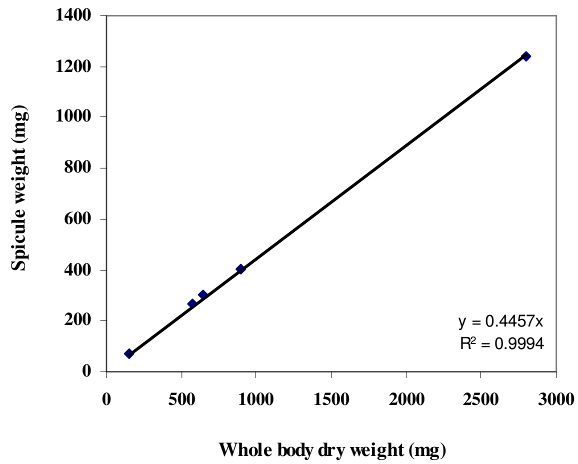

Fig. 5 Linear relationship between spicule weight and whole body dry weight.

DISCUSSION

The spicule networks reported here demonstrate clearly that the spicule arrangement in each organ depends on the function of the organ. Organs requiring flexibility have spicules in horizontal tracts that are intertwined in various patterns to support movement of the nudibranch body. Flexibility of the foot and mantle is important for gastropod movement, e.g., muscular waves in the foot of caenogastropods and the rolling of mantle tissue over the dorsum in dorid nudibranchs[4]. Moreover, spicules in a sheath of connective tissue are better suited to resist tensile (tearing or pulling) or torsional (twisting) stresses[9,18]. Thus the loose arrangement of spicules may play an important role in supporting nudibranch locomotion. The arrangement of spicules in a cross-hatch pattern perpendicular to the body length (Fig. 1a) may support the elasticity of the foot during movement. The horizontal tract arrangement in the central notum assists with support and elasticity.

The spicules inside the organs that are in contact with substrates or other environments, such as the foot edge, papillae, and dorsal surface of the mantle, have vertical tracts perpendicular to the longitudinal body axis (Fig. 1c) in order to strengthen the soft mantle tissue and resist predators. The arrangement of spicules inside the papillae of the mantle tissue (Fig. 3f) increases the body cavity size of the dorsal mantle that could be used as storage for many chemical defenses[4,19].

At the mantle edge, the loose lattice-like arrangement of spicules and the circumference crossed with the radial lines of the body results in an increased body cavity that may allow for more haemolymph to circulate through this space which would allow more flow to the gills. This characteristic is different from *Cadlina luteomarginata* and other dorids which do have an anal gill and for which the greatest density of spicules occurs at the mantle edge[9]. Hence the function of the mantle edge of *C. luteomarginata* and other dorids may not be related to respiration.

The difference in spicule networks in each body region indicates substantial evolutionary development. Nudibranchs in the group Porostomata, members of which have no radula, are classified as highly evolved. Valdés[20] suggested also that *P. varicosa* was highly evolved. *P. varicosa* has a quite complex spicule network in each body region. According to Penney[11], spicule network characters of porostomata species are similar in the central notum with spicule tracts arranged at 45° to the longitudinal body axis as in the present study. Moreover, the lattice-like network at mantle edge is a specific character in the Phyllidiidae[11], which may relate to the position of the lateral gill of phyllidiid nudibranchs.

Spicule contents (mg/g dry weight) did not differ among body regions of *P. varicosa* ($p = 0.709$) in contrast with the pattern previously found in *C. luteomarginata*[9]. Isometric investment was not found in *P. varicosa* although it has been found in some body regions of *C. luteomarginata*[9]. The mantle and foot of *C. luteomarginata* showed isometric investment but rhinophores showed negative allometric investment[9].

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