Documentation of Three Sponge Species Belong to the Family of Petrosiidae

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Abstract: Taxonomic identification of organisms is the first and the most critical step in any research involving organisms. Four marine sponges collected from two different sites were identified based on their morphology, skeleton, and spicules characteristics. The description of each species was compared with the source description of sponges to identify the species. One of the sponge species investigated was identified as Neopetrosia exigua and two species were found to be Xestospongia testudinaria. Final identification of the species was assigned by experts. This simple identification may be useful to non-specialists as it is based on external or internal morphological characteristics.

Key words: Sponge identification; Neopetrosia exigua, Xestospongia testudinaria

INTRODUCTION

The seas surrounding Malaysia are one of the largest areas in the tropical region that contain an exceptionally high diversity of sponges and corals. The documentation of the marine organisms, especially in the Indomalayan region, is still the major task in modern biology (Lim et al., 2009). More than 1500 sponge species have been reported from Southeast Asia (Hooper et al., 2000). The numbers are growing and many new species are being discovered every year. However, researches related to sponges are frequently being avoided due to the difficulties in taxonomy and lack of expertise.

Sponges (Phylum Porifera) are the most primitive and ancient metazoans (Muller et al., 1999). These organisms have neither tissues nor organs. They have different colours, sizes, and shapes. Sponges are divided into three classes according to the composition of the skeleton. The first class, Calcarea, is the only class that has spicules composed of calcium carbonate. The second class, Hexactinellida, has siliceous spicules while the third class, Demospongiae, has anhydrous siliceous spicules and proteinaceous fibres (Bergquist 2001). On the other hand, sponges are filter feeders; water passes through pores present on the outer wall called ostia in to the canals and then to the chamber called spongocoel, and finally exits through large openings called oscula at the end of the body cavity. Therefore, sponges are described as asconoid, syconoid, or leuconoid according to their body plans and the complexity of the canal system that connects between the ostia and the oscula. All species under Demospongiae are leuconoid because they possess a reticulated canal system (Bergquist 2001).

In general, a sponge’s wall consists of two layers; an ectodermal layer, which is composed of a group of cells called pinacocytes, and an endodermal layer, which is composed of a group of flagellated cells called choanocyte (Figure 1). These layers are separated by a gelatinous matrix called mesohyl or mesenchyme where in there are either spicules or spongine fibres. Spicules, which are the elementary structure support of the skeleton, are mainly made of calcium carbonate (Bergquist 2001; Campbell et al., 1999). The arrangement of these spicules in the skeleton is remarkably different in the euctosomal skeleton and the choanosomal skeleton. The morphology of the spicules as well as their arrangement in the euctosomal skeleton and choanosomal skeleton are critical features in sponge identification.

The present study provides a preliminary identification of three sponge species collected from two different sites from seas surrounding Malaysia. The species were identified based on the external morphological characteristics and spicules and skeleton characteristics. The standard description of each species was illustrated and documented.

MATERIALS AND METHODS

Sampling:
Three sponge species were collected from Langkawi Island (6°12’47.01 N 99°44’39.32 E) and Snake Island, South China Sea (4°03’16.78 N 103°24’29.33 E) in 2008 with the use of SCUBA diving equipment (Figure 2). Information about the organisms, place of collection, date of collection, and depth were recorded.

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The vouchers of the specimens were deposited in the Department of Biomedical Science, IIUM, Malaysia to facilitate subsequent taxonomic identification.

Fig. 1: Sponge body structure (Campbell et al., 1999).

Fig. 2: Collection sites of sponge species investigated in this study; Malaysian map (Google Maps). Site A: Langkawi Island (4°03'16.78"N 103°24'29.33"E); Site B: Snake Island (6°12'47.01"N 99°44'39.32"E).
**Taxonomic Identification:**

**Sponge Morphology:**

General morphological characteristics of the sponge species were recorded with reference to the photographs taken the underwater pictures (Figure 3), *in situ*, or in the lab (Figures 4 and 5).

![Image A](image1.png)

![Image B](image2.png)

![Image C](image3.png)

**Fig. 3:** Sponge 1L82008. A: Under water photo. B: Choanosomal skeleton (100×). C: Spicules morphology (100×). Species name: *Neopetrosia exigua*.

**Nitric Acid Spicule Preparation:**

Nitric acid spicule preparation was performed according to Kelly-Borges and Pomponi (1992) with some modifications. Briefly, the tissue samples were placed into a solution of concentrated nitric acid at room temperature for one day. Then, the mixture was centrifuged (5 min, 3000x g) and the acid was discarded. The pellet was washed with distilled water followed by ethanol. The precipitated spicules were then pipetted onto warmed slides. After drying (3 h), the morphology of spicules was analysed using light microscopy (Nikon, series 901) at different magnification.

**Thick Sectioning:**

Thick sectioning preparation was performed according to Ackers *et al.* (1985) with some modifications. Briefly, the sponge specimens were soaked in 70% ethanol for 24 h in order to remove water. After that, slices (1 to 2 mm³) were made from the whole sponge body by free-hand sectioning. Then, the slices were soaked in absolute ethanol until they were free of water (48 h). The water-free slices were soaked in xylol and left until they look translucent (3 weeks). Then, the translucent slices were transferred to slides and they were left to dry for several days. The prepared slides were then examined using light microscope at different magnifications.

**Final Identification:**

Final identification of sponge and coral species was done by an expert according to the descriptions of each species. All the sponges were identified by Mr. Lim Swee Cheng (Tropical Marine Science Institute, National University of Singapore, Singapore).
Results:

Sponge Identification:

Three sponge species were investigated. All species examined showed similar spicules morphology (oxeas) but the dimension of the spicules was different. Spicules in sponge 1L82008 (Figure 3C) were smaller (mean 130 µm) than spicules in other species (mean 393 µm) (Figures 4C and 5C). Furthermore, denser and regular choanosomal skeletons in sponges 7S82008 (Figure 4B) and 8S82008 (Figure 5B) were clearly different from the irregular choanosomal skeleton in sponge 1L82008 (Figure 3B). Obviously, 1L82008 (Figure 5A) appears with lamellae erected from a relatively dense reticulate skeleton, while sponges 7S82008 and 8S82008 look barrel in shape (Figures 4A and 5A). Besides, both sponges 7S82008 and 8S82008 appear to be similar except in their external morphology.

Fig. 4: Sponge 7S82008. A: Out of the water photo. B: Choanosomal skeleton (40×). C: Spicules morphology (100×). Species name: Xestospongia testudinaria.

The described species were compared with the literatures, and the taxonomic identification of these species were found to be consistent with Neopetrosia exigua and Xestospongia testudinaria (Cheng et al., 2008; Hooper et al., 2002; Hooper 2010; World Porifera database).

Taxonomy:

Phylum Porifera Grant, 1836
Class Demospongiae Sollas, 1885
Order Haplosclerida Topsent, 1928
Petrosina Boury-Esnault & Van Beveren, 1982
Family Petrosiidae Van Soest, 1980
\'Genus Neopetrosia de Laubenfels, 1949
Neopetrosia exigua kirkpatrick, 1900
**Fig. 5**: Sponge 8S82008. A: Out of the water photo. B: Choanosomal skeleton (40×). C: Spicules morphology (100×). Species name: *Xestospongia testudinaria*.

**Material Examined:**
Malaysia, Langkawi Island (Figure 2, site A), 1-5 m depth; 6°12′47.01 N 99°44′39.32 E; 24/8/2008, voucher specimen number 1L82008.

**Description:**
Shape encrusted with erected lamella (15-20 cm length, 4 cm width). The surface appears as a crumble with plenty of oscules (Figure 3A).

**Colour:**
Brown to red. Out of the water, the specimens turn very dark brown.

**Skeleton:**
Ectosomal skeleton appears multispicular where the oxeas penetrated through a multispicular tangential layer. Choanosomal skeleton (Figure 3B) shows irregular multi-spicular tracts of oxeas forming irregular oval meshes.

**Spicules:**
Only oxeas, relatively small (130 µm). They are curved with pointed endings (Figure 3C).

**Xestospongia testudinaria Lamarck, 1815:**
**Material Examined:**
Two specimens (Figure 2, site B); Malaysia; South China Sea, Snake Island, 5-10 m depth; 4°03′16.78 N 103°24′29.33 E, 5/9/2008. Voucher specimen numbers 7S82008 and 8S82008.
Description:
Barrel shaped (25 cm length and 15 cm diameter) with large cavity in the centre. Sponge 8S82008 (Figure 5A) is characterised by the presence of finger-like structures on the outside surface. These structures are absent in sponge 7S82008 (Figure 4A).

Colour:
Maroon to pink. Out of the water it appears brown or light pink.

Skeleton:
Tangential layer of single spicules in ectosomal skeleton. Choanosomal skeleton (Figures 4B and 5B) shows multispiicular reticulation forming regular tracts.

Spicules:
Curved oxeas with sharp ends (Figures 4C and 5C).

Remarks:
All species described in this study belong to Petrosiidae family and belong to two different genera; Neopetrosia and Xestospongia (Li et al., 1981; World Porifera database). Members of Neopetrosia genus were previously placed under genus Xestospongia. These two genera are highly similar. Comparison between Xestospongia and Neopetrosia genera is shown in Table 1 (Cheng et al., 2008). Clearly, they differ especially in their skeleton and the size of their spicules. Neopetrosia exigua and Xestospongia testudinaria have been reported previously from South China Sea (George et al., 1987; Pattanyak 1997; Riguera 1997).

Table 1: External morphology, spicule morphology and spicule size of Neopetrosia and Xestospongia genera (Cheng et al., 2008).

<table>
<thead>
<tr>
<th>Genus</th>
<th>Morphology</th>
<th>Choanosomal skeleton</th>
<th>Spicules*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neopetrosia de Laubenfels, 1949b</td>
<td>Ramose, lobulated, consistently stiff, surface finely hispid, single isodictyal network of small spicules</td>
<td>Irregular multi-spiicular tracts, rounded meshes</td>
<td>Oxeas 104-120-144×2-3-4</td>
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<tr>
<td>Xestospongia de Laubenfels, 1932</td>
<td>Lamellar, massive, surface strongly hispid with abundant brushes of larger spicules</td>
<td>Dense, short discontinuous tracts (6-10 sps)</td>
<td>Oxeas 205-450×8-30</td>
</tr>
</tbody>
</table>

*Measurement in µm taken from type material, represented as minimum-mean- and maximum dimensions)

Discussion:
Collecting marine organisms is much more difficult than terrestrial organisms. The reason is related to the difficulties to collect them due to the marine environment. It is also due to the problems associated with taxonomy and lack of sufficient biological material (Houssen and Jaspars, 2005). Therefore, information about the organism such as the place of collection, date of collection, and depth must be carefully recorded (Collin et al., 2010). Equally important is to get in situ underwater photographs of the specimen.

It is well-known that microscopic structures including spicule morphology in sponges, or internal soft anatomy, are often necessary for accurate identification of marine invertebrates. However, photographs are useful in identification of marine organisms. Detailed examination in the laboratory is necessary for the identification of certain species. Furthermore, due to shape, colour, and size variability between organisms from the same genus, or due to the similarities of these features within a single species, thus identification by an expert or by using more advanced methods such as phylogenetic analysis are beneficial in sponge identification (Evans and Kitting, 2010).

Of course, the taxonomic identification of the specimens is the first and most critical step in any research. However, sponges are among the most difficult organisms to identify. Misidentification of this organism is common (Hooper et al., 2000). Besides, misidentification of sponges may lead to failure in the prediction of the chemical compositions.

For all species identified in this study, the descriptions of each species were compared with the original description of the same species and the taxonomic tree for each species was listed. The current study is preliminary, simple, and may be useful to non-specialists since common and standard methods of identification were used.

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