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Spicular analysis of surficial sediments as a supplementary tool for studies of modern sponge communities

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Abstract

The method of spicular analysis that examines surficial sediments has been used to study the sponge spicule assemblage in the lagoon reef of Bocas del Toro, Panama. The method allowed to recognize some highly diagnostic spicule morphotypes that may belong to four sponge species as yet unnoticed in this area. The presence of these sponges must have been overlooked while studying faunistic compositions within an ecosystem due to their cryptic and/or excavating nature. Despite some limitations, the method of spicular analysis may be used as a supplementary tool for the studies of modern shallow-water sponge communities.

Keywords: Cryptic sponges, Excavating sponges, *Triptolemma endolithicum*, *Alectona wallichii*, *Samus anonymus*, *Cliona mucronata*

Background

Sponges are sessile filter feeders that inhabit marine and fresh-water ecosystems all over the world. They are the main component of marine fauna and, next to corals, are one of the most important organisms inhabiting reefs and shallow-water habitats [16]. Sponges can also be the key components of deep-sea environments (e.g., [27, 54]). In the past, they usually received considerably less attention than other marine organisms. However, there has been an increase of interest in sponges over the last decades [16, 28, 66].

In spite of the fact that marine sponge fauna is now under intensive studies, in some, even well-studied regions, sponges are still poorly known. The most investigated areas with the best known sponge fauna include Mediterranean, Caribbean, and Australia (e.g., [63]). Still, there are new sponge species described from these sponge-rich regions every year (e.g., [18, 51, 64, 67, 68]), and it is presumed that there is still about twice as many species unknown to scientists [66]; particularly small, cryptic, or excavating species are difficult to observe and sometimes are overlooked. Thus, sponge inventory lists are incomplete.

The main characters used in sponge identification for ecological/general field studies include: shape, color, and consistency of a specimen (e.g., see [13]). However, it is especially the type and the arrangement of the internal mineral skeleton consisting of spicules that is crucial for sponge taxonomical assignment (e.g., see [29]).

Hexactinosan hexactinellid sponges may have a fused skeleton derived from secondary deposition of silica over spicules originally secreted loosely, while lithistid demosponges may have articulated spicules. Spicules can also appear free in the sponge body (as in the case of some hexactinellids) [35]. In the class Demospongiae, the spicules are frequently joined by spongin. The diversity of spicule morphotypes in demosponges is great as there are several hundred spicule morphotypes distinguished in this group (e.g., [8]).

Moreover, the spicule combination within a sponge skeleton is an important feature for sponge classification. Some sponges possess spicules of only one type, while others are characterized by a set of several morphotypes. The spicule set of each species is genetically controlled. However, under unfavorable conditions such as low silica levels in the seawater, not all morphotypes proper to the

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sponge are produced [39]. Some morphologies are widespread across the classification, while others are much more restricted in occurrence and thus play an important diagnostic role.

The spicular analysis method

Sponge spicules may be incorporated in the sediment in several ways. For example, they may become part of the sediment after the sponge's death when the body disintegrates and the mineral spicules remain. They are then the only proof of the sponge's former existence. Heavy storms usually cause detachment of sponge body parts, which may disintegrate and contribute isolated spicules to the sediment. They can also be torn off by various sponge-feeding animals [72]. Thus, under favorable preservation conditions, spicules may give information about the sponges that live or had lived recently in the studied area (see, for example, [6]).

The method of spicular analysis investigates isolated sponge spicules from the sediment and identifies them by comparing them with the spicules of living sponges. This method is usually used to reconstruct fossil and subfossil sponge communities. It has become popular in the middle of the twentieth century when it has been applied to reconstruct ancient marine sponge communities (e.g., [32, 33]). Later, it was used for reconstructing both fossil marine (e.g., [5–7, 30, 31, 37, 46]) and fresh-water sponge associations from all over the world [24–26, 44, 45, 69, 70]. However, the spicular analysis technique can also be used to supplement studies of modern sponge communities by indicating the presence of some difficult-to-track sponge species.

Methods

The surficial sediment samples were collected in June 2011 and July 2014 by SCUBA surveys in patch reef environments of the Bocas del Toro archipelago, on the northwestern coast of Panama (Fig. 1). The studied area lies within the Almirante Bay and represents a diverse, well-developed patch reef with a well-studied coral–sponge community (e.g., [13, 15, 17, 19, 21, 22]). Two surface sediment samples, down to a depth of ~1 cm, were recovered in plain patches of shallow-water (~8 m) reef sand (9°21′38.6″N/82°16′40.9″W and 9°21′42.98″N/82°16′47.6″W).

The obtained sediments were processed in the Bocas del Toro Research Station laboratory. The samples were subsequently dried and then treated with hydrogen peroxide (30 %) in order to remove small particles of organic matter. Additionally, a small amount of sediment was treated by acetic acid (60 %) to remove carbonates and thus concentrating siliceous spicules in order to make the rare spicule morphotypes easier to find. The sponge spicules were then transported to the Institute of Paleobiology, Polish Academy of Sciences, Poland, for the further analysis. The spicules were then handpicked from the residue under a binocular microscope NIKON SMZ1270. All representatives of morphological spicule types found in the sediment were mounted on SEM stubs and identified using the SEM. The photographed spicule

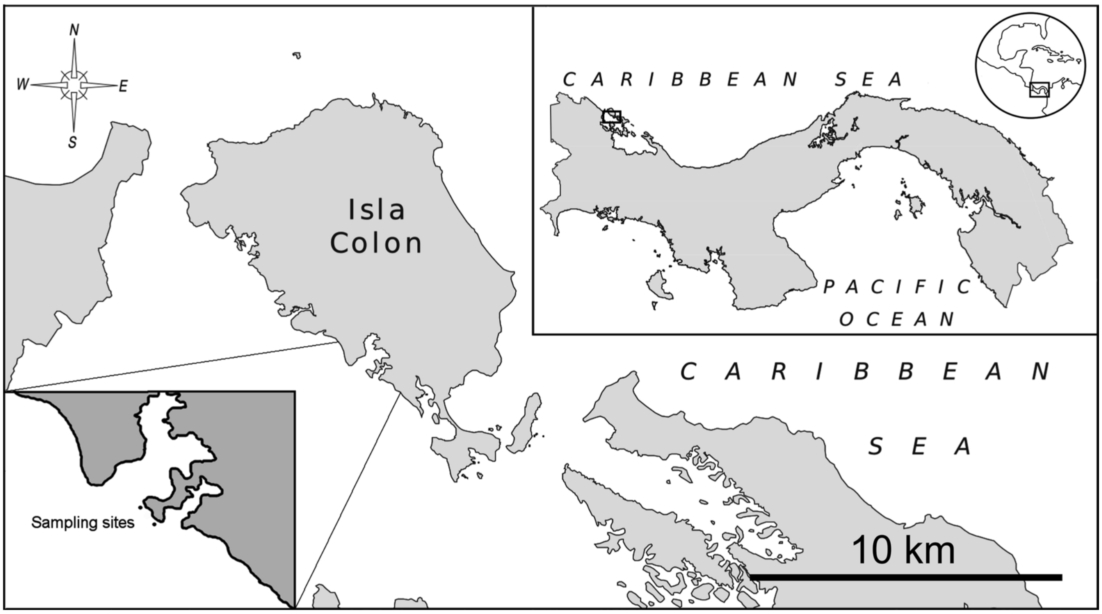

Fig. 1 Map of the sampling area after Łukowiak et al. [36] (modified)

morphotypes were compared to spicules that belong to sponges listed from the Bocas del Toro region (Table 1). The sponge list was compiled from published data, e.g., [10, 13, 15, 17, 19, 21, 22] and the STRI webpage database. Next, the studied spicules were compared with the spicules of sponges noted from the wider Caribbean area [e.g., [1, 23, 61, 62, 64, 73, 74]; World Porifera Database (WPD)]. For details of the terminology used in this paper, see the Thesaurus of Sponge Morphology [8] and Systema Porifera [29].

The spicule assemblages are deposited in the Institute of Paleobiology, Polish Academy of Sciences, Warszawa, Poland, under ZPAL Pf.24 and ZPAL Pf.25.

Results

Among numerous spicules that are present in the sediment, some can be assigned to sponges already known from the investigated area (for the full list of sponges of Bocas del Toro, see Table 1), and some are noted from the study area for the first time. The spicule morphotypes belonging to species as yet unnoted from Bocas del Toro are described, and their taxonomical position is discussed below.

Amphitriaenes of *Samus anonymus* Gray, [20]

The spicules found in the surface sediments of the Bocas del Toro lagoon are short-shafted amphiprotriaenes with clads directed forward, away from the rhabd. The cladi are always divided three times. The amphitriaenes vary from slender with well-developed rhabd and thin cladi (Fig. 2c, e) to stout ones with short rhabd and cladi (Fig. 2d, f). The rhabds vary from 30 to 50 μm in length and 20–75 μm in thickness. The total length of the spicule may exceed 400 μm. They are identical with spicules of *Samus anonymus.*

Remarks

Samus anonymus is characterized by small amphitriaenes (Fig. 2a, b) and sigmaspire microscleres. The megascleres may be short-shafted amphiprotriaenes with clads directed forward, away from the rhabd, or amphiplagiotriaenes with the forward-directed clads forming with the rhabd an angle of about 45° [8]. However, the cladi do not always divide three times and some are only divided dichotomously [65]. The rhabds vary from 20 to 80 μm in length and by up to 38 μm in thickness. The cladome may be up to 160 μm, and the total length of an amphitriaene including cladi may exceed 150 μm [65]. *Samus anonymus* occupies cavities of the porous concretion and the chambers previously excavated by boring sponges [6]. This tetractinellid was first considered to belong to Hadromerida [20, 56, 71] and Homosclerophorida [14]; its lithistid affinity was also considered [65]. Currently, it is regarded as the only species within family Samidae (suborder Spirophorina; [42]).

This cosmopolitan species inhabits shallow-water habitats of the Pacific and Atlantic Oceans, Brazil, Indian Ocean, Mediterranean, and Australia. It was also reported from Cuba, Colombia, Gulf of Mexico, and the Caribbean Sea ([51], WPD). So far, however, it was not reported in Bocas del Toro.

Mucronate tylostyles of *Cliona mucronata* Sollas, [52]

The spicules found in the surface sediments of Bocas del Toro are short, stout tylostyles with spherical heads and with a well-defined mucronate tip (Fig. 3c–f). The length of the spicules varies from 120–150 μm × 18–22 μm. The head is 16–19 μm × 17–21 μm. Even though the straight, thin tylostyles of *Cliona mucronata* are similar to tylostyles of some other clionaid spicules (e.g., *Cliona ampliclavata*, *C. aprica*, *C. tenuis*; for more details, see [74], the mucronate tylostyles do not resemble spicules of any other clionaid including species inhabiting this and adjacent areas (e.g., *Cliona aprica, C. caribbea, C. delitrix, C. langae*; compare also with [43, 74]).

Remarks

Cliona mucronata is characterized by 2 kinds of tylostyles: straight, short, stout tylostyles with spherical heads (sometimes trilobed) and mucronate tips (Fig. 3a, b); and straight, thin tylostyles with oval or spherical heads and hastate tips. In addition to macroscleres, *C. mucronata* also possess spiraster microscleres that are sometimes transformed into branched amphiasters [9]. The mucronate tylostyles are of about 64–89 μm × 11.5–12.5 μm [9, 59] and with a head of 6.6–14 × 8–11.5 μm [59].

These clionaids (order Clionaida) are excavating, mostly cryptic sponges. They occupy small holes and chambers excavated in a solid calcareous base [49, 52]. They often live, overgrow, and bore in other sponges or corals (see [9]).

Cliona mucronata inhabits the Indian Ocean (including Indo-Pacific Ocean), Indonesia, Madagascar, and Australia (WPD; [9, 58]). This species is not listed for Bocas del Toro in any available source. The nearest locality to Bocas del Toro from which it was noted is the Mexican Tropical Pacific [2] but with some doubts (for more details see WPD).

Tuberculate diactines of *Alectona wallichii* [11]

The spicules found in the sediment of Bocas del Toro lagoon are very rare and are always broken. However, despite the fragmentation, the mushroom-shaped knobs that cover (most likely) the diactinal spicule forming the longitudinal rows of tubercles are well defined (Fig. 4c).

Table 1 Sponge list noted from Bocas del Toro, Panama

Order	Family	Species
Verongiida	Aplysinidae	*Aiolocroia crassa* (Hyatt, 1875)
		Aplysina lacunosa (Lamarck, 1814)
		Aplysina archeri (Higgin, 1875)
		Aplysina fulva (Pallas, 1776)
		Aplysina cauliformis (Carter, 1882)
		Aplysina insulares Duchassaing & Michelotti, 1864
		Aplysina fistularis (Pallas, 1766)
		Verongula rigida Esper, 1794
		Verongula reiswigi Alcolado, 1984
Dictyoceratida	Irciniidae	*Ircinia* sp.
		Ircinia strobilina (Lamarck, 1816)
		Ircinia felix (Duchassaing & Michelotti, 1864)
		Ircinia campana (Lamarck, 1814)
		Dysidea etheria de Laubenfels, 1936
	Spongiidae	*Spongia* sp.
		Spongia (Spongia) pertusa Hyatt, 1877
		Spongia (Spongia) tubulifera Lamarck, 1814
		Hyattella cavernosa (Pallas, 1766)
		Hyattella intestinalis (Lamarck, 1814)
	Thorectidae	*Hyrtios* sp.
		Hyrtios proteus Duchassaing & Michelotti, 1864
Haplosclerida	Petrosiidae	*Xestospongia* sp.
		Xestospongia muta (Schmidt, 1870)
		Xestospongia bocatorensis Díaz, Thacker, Rützler & Piantoni, 2007
		Neopetrosia rosariensis (Zea & Rützler, 1983)
		Neopetrosia proxima (Duchassaing & Michelotti, 1864)
		Neopetrosia subtriangularis (Duchassaing, 1850)
		Neopetrosia carbonaria (Lamarck, 1814)
		Petrosia sp.
		Petrosia (Petrosia) pellasarca (de Laubenfels, 1934)
		Petrosia weinbergi van Soest, 1980
	Phloeodictyidae	*Oceanapia peltata* (Schmidt, 1870)
		Oceanapia nodosa (George & Wilson, 1919)
		Oceanapia oleracea (Schmidt, 1870)
		Oceanapia bartschi (de Laubenfels, 1934)
		Aka coralliphaga (Rützler, 1971)
		Siphonodictyon brevitubulatum Pang, 1973
		Calyx podatypa (de Laubenfels, 1934)
	Chalinidae	*Chalinula molitba* (de Laubenfels, 1949)
		Chalinula zeae de Weerdt, 2000
		Haliclona sp.
		Haliclona (Rhizoniera) curacaoensis (van Soest, 1980)
		Haliclona (Reniera) implexiformis (Hechtel, 1965)
		Haliclona (Reniera) manglaris Alcolado, 1984
		Haliclona (Reniera) mucifibrosa de Weerdt et al., 1991
		Haliclona (Reniera) tubifera (George & Wilson, 1919)
		Haliclona (Haliclona) vansoesti de Weerdt, de Kluijver & Gomez, 1999
		Haliclona (Haliclona) hogarthi (Hechtel, 1965)
		Haliclona (Halichoclona) albifragilis (Hechtel, 1965)

Table 1 continued

Order	Family	Species
		Haliclona (Soestella) twincayensis de Weerdt et al., 1991
		Haliclona (S.) vermeuleni de Weerdt, 2000
		Haliclona (S.) caerulea (Hechtel, 1965)
		Haliclona (S.) piscaderaensis van Soest, 1980
		Haliclona (Soestella) walentinae Díaz, Thacker, Rützler & Piantoni, 2007
		Haliclona (Halichoclona) magnifica de Weerdt, Rützler & Smith, 1991
		Haliclona (Gellius) megasclera Lehnert & van Soest, 1996
		Haliclona (Soestella) melana Muricy & Ribeiro, 1999
	Niphatidae	*Amphimedon compressa* Duchassaing & Michelotti, 1864
		Amphimedon virdis Duchassaing & Michelotti, 1864
		Amphimedon erina (de Laubenfels, 1936)
		Amphimedon complanata (Duchassaing, 1850)
		Niphates sp.
		Niphates caycedoi (Zea & van Soest, 1986)
		Niphates erecta Duchassaing & Michelotti, 1864
		Niphates amorpha Wiedenmayer, 1977
		Cribrochalina vascum (Lamarck, 1814)
		Niphates ramosa Gammill, 1997
	Callyspongiidae	*Callyspongia vaginalis* (Duchassaing & Michelotti, 1864)
		Callyspongia armigera Duchassaing & Michelotti, 1864
		Callyspongia fallax Duchassaing & Michelotti, 1864
		Callyspongia pallida (Hechtel, 1965)
Suberitida	Suberitidae	*Terpios* sp.
		Terpios manglaris Rützler & Smith, 1993
		Prosuberites sp.
		Prosuberites laughlini Díaz et al., 1987
		Suberites aurantiacus (Duchassaing & Michelotti, 1864)
Clionaida	Spirastrelliadae	*Spirastrella* sp.
		Spirastrella coccinea Schmidt, 1868
		Spirastrella hartmani Boury-Esnault, Klautau, Bézac, Wulff & Solé-Cava, 1999
		Spirastrella cf. *mollis* Verill, 1907
		Dipastrella megastelata Hechtel, 1965
	Placospongiidae	*Placospongia intermedia/melobdeoides* Sollas, 1888
	Clionaidae	*Cliona* sp.
		Cliona delitrix Pang, 1973
		Cliona varians (Duchassaing & Michelotti, 1864)
		Cliona caribbaea Carter, 1882
		Cliona tenuis Carter, 1882
		Cliona aprica Pang, 1973
		***Cliona mucronata* Sollas, 1878**
		Spheciospongia vesparium (Lamarck, 1815)
		Cervicornia cuspidifera (Lamarck, 1815)
Tethyida	Tethyidae	*Tethya* sp.1
		Tethya sp.2
		Tethya aff. *seychellensis* (Wright, 1881)
		Tethya actinea de Laubenfels, 1950
		Tectitethya crypta (de Laubenfels, 1949)
		Tectitethya keyensis Sara & Bavestrello, 1996
Polymastida	Polymastiidae	*Polymastia* sp.
Poecilosclerida	Crambeidae	*Monanchora arbuscula* (Duchassaing & Michelotti, 1864)

Table 1 continued

Order	Family	Species
		Monanchora unguifera (Dendy, 1922)
	Microcionidae	*Clathria* sp.
		Clathria (Thalysias) venosa (Alcolado, 1984)
		Clathria (Thalysias) microchela (Stephens, 1916)
		Clathria (Thalysias) schoenus (de Laubenfels, 1936)
		Clathria (Thalysias) echinata (Alcolado, 1984)
		Clathria ferrea (de Laubenfels, 1936)
		Clathria aspera Gammill, 1998
		Artemisina melana van Soest, 1984
		Holopsama helwigi (de Laubenfels, 1936)
		Pandaros acanthifolium Duchassaing & Michelotti, 1864
	Acarnidae	*Acarnus nicoleae* van Soest, Hooper & Hiemstra, 1991
		Acarnus souriei (Lévi, 1952)
	Mycalidae	*Mycale* sp.
		Mycale (Paresperella) sp. nov.
		Mycale (Mycale) laevis (Carter, 1882)
		Mycale (Arenochalina) laxissima (Duchassaing & Michelotti, 1864)
		Mycale (Carmia) microsigmatosa Arndt, 1927
		Mycale magnidarapihidiphera van Soest, 1984
		Mycale carmigropila Hajdu & Rützler, 1998
		Mycale citrina Hajdu & Rützler, 1998
		Mycale arndti van Soest, 1984
		Mycale cf. *americana* van Soest, 1984
		Mycale angulosa (Duchassaing & Michelotti, 1864)
		Mycale (Mycale) arenaria Hajdu & Desqueyroux-Faúndez, 1994
		Mycale cf. *diversisigmata* (van Soest, 1984)
	Coelosphaeridae	*Lyssodendoryx* sp.
		Lissodendoryx issodictyalis (Carter, 1882)
		Lissodendoryx (Lissodendoryx) colombiensis Zea & van Soest, 1986
		Forcepia (Forcepia) colonensis Carter, 1874
	Tedaniidae	*Tedania (Tedania) ignis* (Duchassaing & Michelotti, 1864)
		Tedania klausi Wulff, 2006
	Desmacididae	*Desmapsamma anchorata* (Carter, 1882)
	Iotrochotidae	*Iotrochota* sp.
		Iotrochota birotulata (Higgin, 1877)
Merliida	Merliidae	*Merlia normani* Kirkpatrick, 1908
Desmacellida	Desmacellidae	*Biemna* sp.
		Biemna caribea Pulitzer-Finali, 1986
		Biemna tubulata (Dendy, 1905)
		Neofibularia nolitangere (Duchassaing & Michelotti, 1864)
Chondrillida	Chondrillidae	*Chondrilla caribensis* f. *hermatypica* Duran & Piantoni, 2007
		Chondrilla caribensis f. *caribensis* Rützler, Duran & Piantoni, 2007
		Chondrosia sp.
		Chondrosia collectrix Schmidt, 1862
		Chondrosia reniformis Nardo, 1847
	Halisacridae	*Halisacra* sp.
		Halisacra caerulea Vacelet & Donadey, 1987
Axinellida	Raspaillidae	*Ectyoplasia ferox* (Duchassaing & Michelotti, 1864)
	Axinelliade	*Dragmacidon reticulatum* (Ridley & Dendy, 1886)
		Dragmacidon lunaecharta (Ridley & Dendy, 1886)

Table 1 continued

Order	Family	Species
		Ptilocaulis walpersi (Duchassaing & Michelotti, 1864)
		Axinellidae sp.
		Axinella corrugata (George & Wilson, 1919)
		Dragmaxia cf. *undata* Alvarez, van Soest & Rützler, 1998
	Heteroxyidae	*Myrmekioderma* sp.
		Myrmekioderma rea (de Laubenfels, 1934)
		Myrmekioderma gyroderma (Alcolado, 1984)
Suberitida	Halichondriidae	*Halichondria* sp.
		Halichondria lutea Alcolado, 1984
		Halichondria magniconulosa Hechtel, 1965
		Halichondria melanadocia (de Laubenfels, 1936)
		Hymeniacidon caerulea Pulitzer-Finali, 1986
Bubarida	Desmanthidae	*Petromica ciocalyptoides* van Soest & Zea, 1986
	Dictyonellidae	*Svenzea zeai* (Alvarez, van Soest & Rützler, 1998)
Scopalinida	Scopalinidae	*Scopalina ruetzleri* (Wiedenmayer, 1977)
Agelasida	Agelasidae	*Agelas* sp.
		Agelas dispar (Duchassaing & Michelotti, 1864)
		Agelas clathrodes (Schmidt, 1870)
		Agelas conifera (Schmidt, 1870)
Tetractinellida	Theonellidae	*Discodermia dissoluta* Schmidt, 1870
	Samidae	*Cinachyra* sp.
		***Samus anonymus* Gray, 1867**
	Tetillidae	*Cinachyrella alloclada* (Uliczka, 1929)
		Cinachyrella apion (Uliczka, 1929)
		Cinachyrella kuekenthali (Uliczka, 1929)
	Geodiidae	*Geodia* sp.
		Geodia papyracea Hechtel, 1965
		Geodia giberosa Lamarck, 1815
		Erylus formosus Sollas, 1886
	Ancorinidae	*Stellettinopsis megastylifera* (Wintermann-Kilian & Kilian, 1984)
		Asteropus sp.
		Penares sp.
		Penares schulzei (Dendy, 1905)
		Stelletta sp.1
		Stelletta sp.2
		Stelletta fibrosa Schmidt, 1862
		Stryphnus raratriaenus Cárdenas, Menegola, Rapp & Díaz, 2009
	Thoosidae	***Alectona wallichii* (Carter, 1874)**
	Thrombidae	*Thrombus kittonii* Carter, 1874
	Pachastrellidae	***Triptolemma endolithicum* van Soest, 2009**
Dendroceratida	Darwinellidae	*Aplysilla gracilis* (Merejkowsky, 1878)
		Chellonaplysilla erecta (Row, 1911)
	Dictyodendrillidae	*Igernella notabilis* (Duchassaing & Michelotti, 1864)
		Spongionella (Megalopastas) sp.
Homosclerophorida	Plakinidae	*Plakortis angulospiculatus* (Carter, 1879)
		Plakortis halichondrioides (Wilson, 1902)
		Plakortis simplex species complex Lehnert & van Soest, 1998
		Plakinastrella onkodes Uliczka, 1929
		Oscarella sp.

After Guzmán and Guevara [21, 22], Collin et al. [13], Díaz [15], Díaz et al. [17], Gochfeld et al. [19] and Cárdenas et al. [10]; http://biogeodb.stri.si.edu/bocas_database/search/class/1/?page=2; compiled; with sponge species described in this study in bold

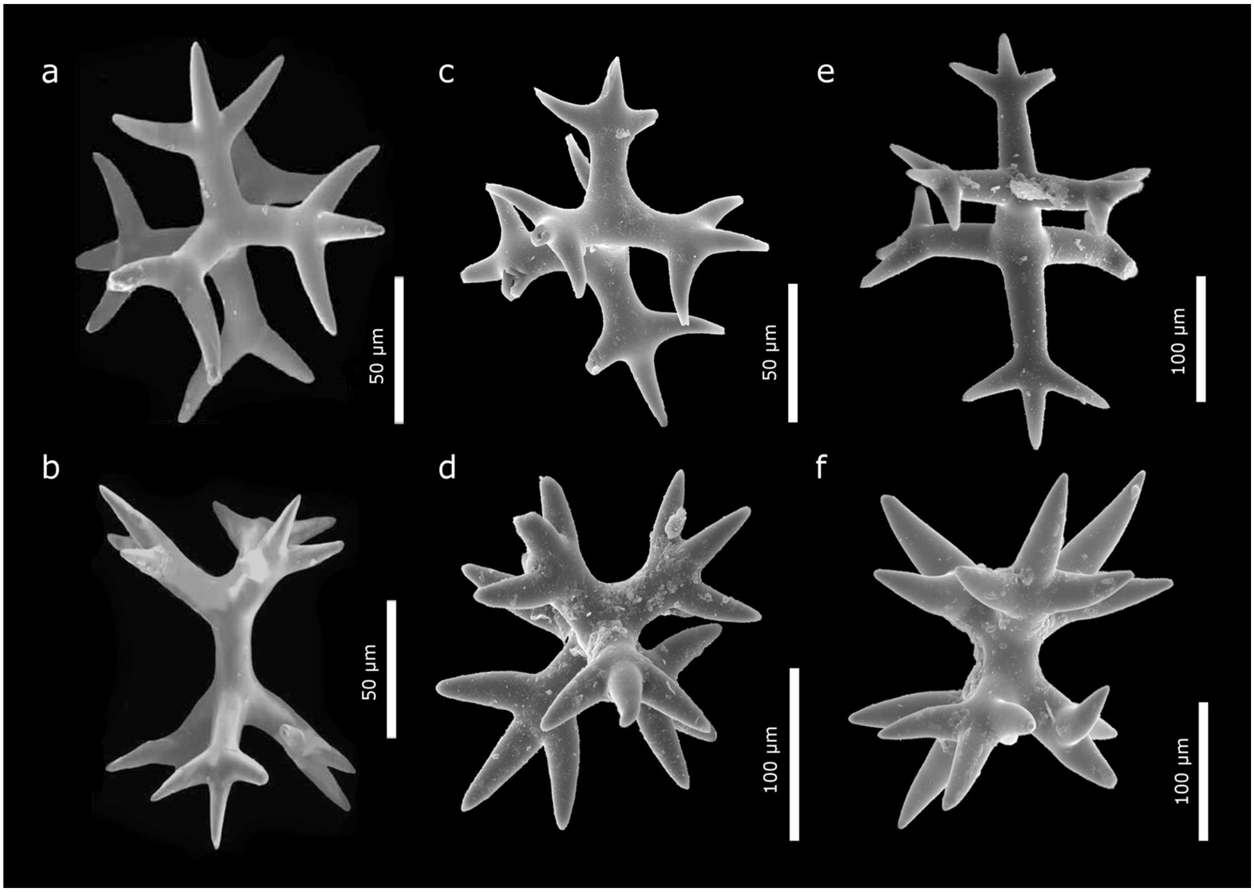

Fig. 2 *Samus anonymus* Gray, [20] spicules; **a**, **b** amphitriaenes of *S. anonymus*; from van Soest and Hooper [65], **c**–**f** sediment spicules

The thickness of the spicule exceeds 50 µm in the thickest place and 30 µm in the thinnest place, and the knobs are about 12 µm in diameter. The tuberculate diactines of *Alectona wallichii* are very characteristic and do not resemble spicules of any other sponge, including thoosid species inhabiting this and adjacent areas (e.g., *Alectona jamaicensis*; [43]). In fact, the only other species of *Alectona* possessing morphologically similar acanthoxeas (but only those with spine-like tubercles, not the mushroom-like ones) is *A. millari* [12]. *Alectona wallichii* was synonymized by De Laubenfels [14] with *Alectona millari* [11]. However, Bavestrello et al. [3] demonstrated that these two species are distinct [48].

Remarks

The spicules of *A. wallichii* include robust tuberculate and smooth diactines (or derived polyactines) and microsclere amphiasters. The tuberculate diactines are acerate ([48]; Fig. 4a, b). These fusiform, slightly curved spicules are covered by rows ([11] describes twelve rows) of tubercles. The tubercles are alternately situated along the longitudinal lines placed along the spicule [11]. The tubercles may be of spine to mushroom shape [48]. The sizes of diactines vary from 22 to 805 µm × 16 to 125 µm (for more details, see Table 2 in [48, 57]).

Alectona wallichii is an excavating thoosid that occupies chambers of coral skeletons [48] and molluscan shells [55].

Alectona wallichii is recorded from North Atlantic Ocean, Indian Ocean [55], Japan Sea, South Africa (Tuléar; WPD; [11], and Hawaii [57]).

Short-shafted mesotriaenes of *Triptolemma endolithicum* van Soest, [64]

The spicules described here extracted from the bottom sediments of the studied area are various types of mesodichotriaenes. Some of them possess short to moderately long, conically ended rhabds (Fig. 5c–e, g, h), and some others have blunt rhabds (Fig. 5f). The cladomes may be branched only on the very end of the cladi, or the cladi may divide very near the rhabd. The clads are irregularly trifurcated or tetrafurcated (Fig. 5d–g), with desmoid appearance in several different planes. The length of the rhabd varies from 40 to 65 µm. The cladomes are up to

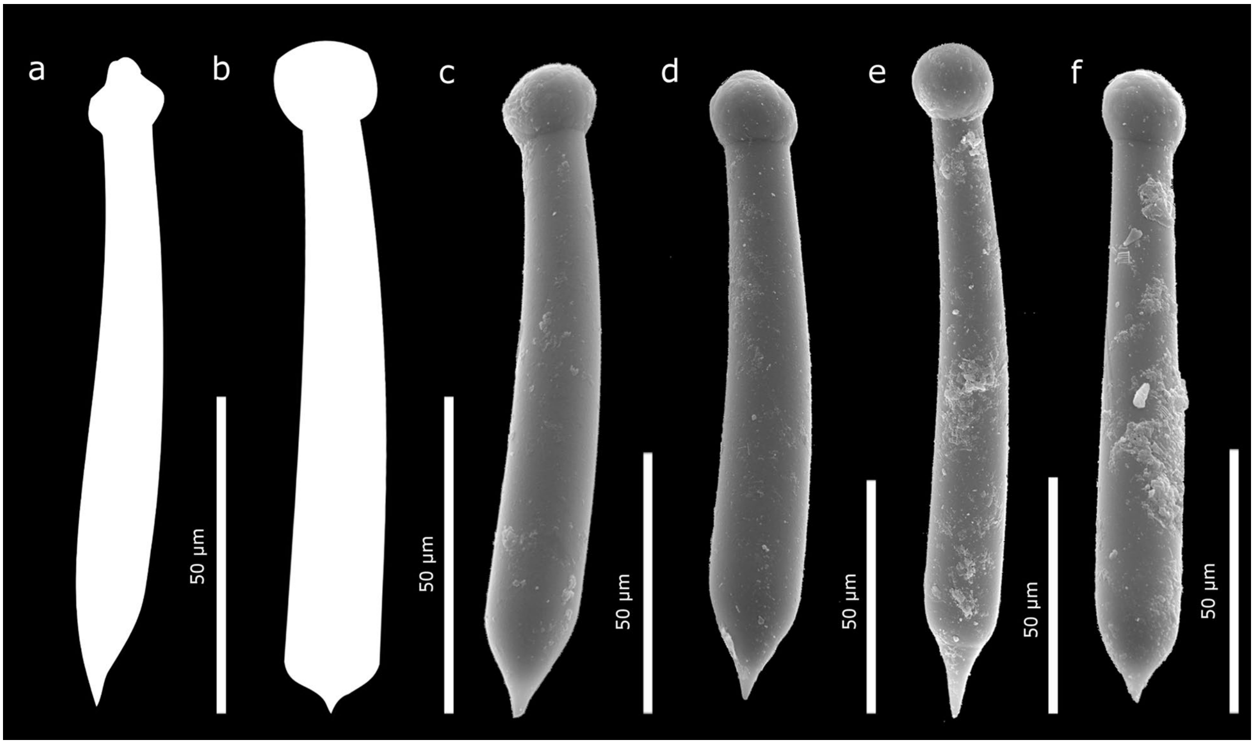

Fig. 3 *Cliona mucronata* Sollas, [52] spicules; **a** *C. mucronata* subtylostyle; redrawn from Vacelet and Vasseur [58], **b** *C. mucronata* subtylostyle; redrawn from Calcinai et al. [9], **c**–**f** sediment spicules

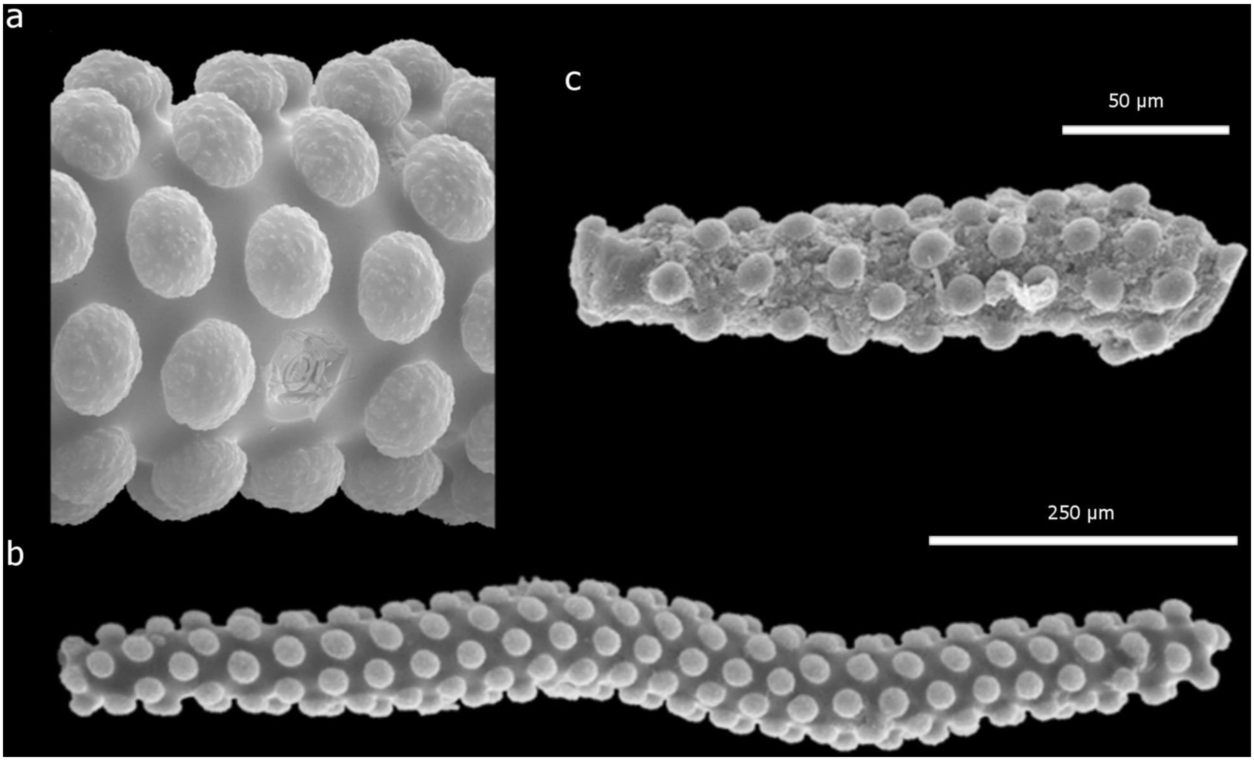

Fig. 4 *Alectona wallichii* [11] spicules; **a** mucronate diactine—fragment [57], **b** mucronate diactine; from Vacelet [57], **c** sediment spicule (fragment)

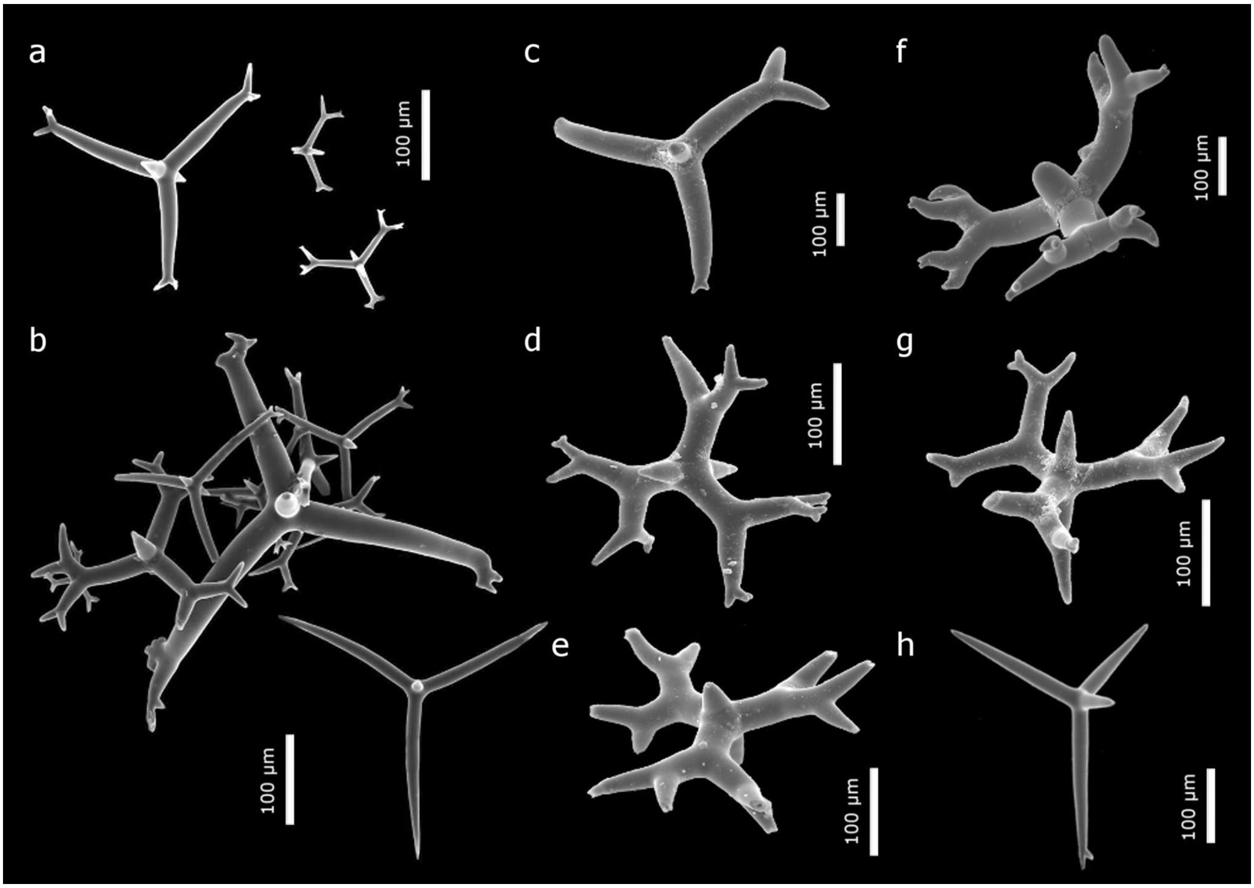

Fig. 5 *Triptolemma endolithicum* van Soest, [64] spicules; **a**, **b** triaene and mesodichotriaenes of *T. endolithicum*; from van Soest [64], **c**–**h** sediment spicules

300 µm. The protoclads are 30–180 µm, deuteroclads are 25–100 µm long, trichoclads are 20–55 µm long, and tetraclads are up to 10 µm long. The mesodichotriaene megascleres of *T. endholithicum* resemble those of *T. cladosum* [53], but *T. cladosum* is known only in Indonesia [53].

Remarks

The skeleton of *Triptolemma* consists of short-shafted mesotriaenes (predominantly mesodichotriaenes) (Fig. 5a, b), frequently complemented by monaxonic megascleres (oxeas or strongyles), amphiasters, metasters transitional to spirasters, and spiny microrhabds which may be partially absent [4]. The mesotriaenes have symmetrical, short conical rhabdomes that can be blunt [38] or sharp pointed [64]. The clads may be irregularly trifurcated or tetrafurcated, with desmoid appearance. The cladome branches out in several different planes. Also the level of division varies within a spicule with dichotomous and tetrafurcated clads co-occurring [38]. Mesotriaenes with diversely branched clads may differ according to size within one specimen [64]. The rhabdomes are 62–21 × 20–25 µm each. The cladomes are up to 400 µm [64] and include protoclads 21–302 × 12–48 µm, deuteroclads 12–72 × 6–20 µm, trichoclads 6–60 × 5–7 µm, and tetraclads up to 27.6 µm long.

Sponges of the genus *Triptolemma* are cryptic and penetrate other sponges or coral tissue [38], or crevices of the coralligenous concretions [4]. *Triptolemma endolithicum* is an encrusting species, growing on and in corals. It is usually not visible on the outer surface of the coral, but there are some cavities and corridors filled with *Triptolemma* in the coral inner part [64].

Triptolemma endolithicum was so far noted only in the Southern Caribbean (i.e., Colombia) by van Soest [64].

Discussion

The surficial sediment deposited in the areas inhabited by sponges is usually loaded with their spicules. Usually, when the sponge skeleton becomes incorporated into the sediment, it disintegrates rapidly to isolated spicules. However, under favorable conditions, the spicules may stay connected together with the collagenous fibrils forming small skeletal clusters. Nevertheless, the latter situation is rather rare and after some time, due to early diagenesis and bioturbation, the spicules become separated, broken, and sometimes selectively segregated (for more details see [36, 50]).

The spicules may be delivered to the bottom sediments in various ways. Some could have belonged to sponge specimens that were mechanically damaged (e.g., during the storms) but not killed. The pieces of the torn sponge tissue settle on the sea bottom and then become incorporated to the sediment either as clusters of sponge spicules or as loose spicules. Also, sponge-eating carnivores, such as some opisthobranches, sea stars, fishes, and turtles, can deliver sponge spicules to the sediment. As the predators consume only small parts rather than whole individuals [72], the sponges may remain alive as well, and only their fragments are detached. In both cases mentioned above, the sponge species might still be present in this area.

By contrast, when a sponge dies, its spicule complement becomes incorporated to the sediment. The spicules are thus the proof of the former presence of the sponge individual and do not necessarily indicate that the species occurs in that area.

It was already suggested that some spicule morphotypes have a greater chance of being preserved in the sediment than others (see [50]), e.g., those of spherical morphology (e.g., spherasters and selenasters) are quite resistant to mechanical destruction. Thus, they might be a dominant component of sponge spicule assemblages (for more details, see [36, 50]) only for taphonomic reasons.

The additional factor is spicule transport. The sediment containing spicules might be carried from other, more or less distant areas e.g., by currents or hurricanes [40].

Finally, the activity of bioturbating organisms has to be considered. Organisms responsible for bioturbation are mostly oligochaetes, polychaetes, bivalves, echinoids, and holothurians [34, 41]. The disturbance and homogenization of the deposits can reach down to about 3–4 cm [34], but in extreme cases, it can exceed even 20–40 cm [47, 60]. Such activity causes reworking and mixing of the sediment that could have been deposited during the last few weeks to tens of years (depending on sedimentation rate).

One has to be aware that spicules in the sediment are a mixture possibly resulting from these processes. Thus, finding spicules that belong to certain sponge species in the surficial sediment is a proof of one or more of the three following processes: (1) the presence of living individuals bearing this type of spicule in this, or nearby areas, (2) the former presence of the sponge bearing this spicule type, or (3) the transport of this spicule morphotype from other (more or less distant) areas.

In the case of the studied sponge spicules, the transport seems not to have played a significant role as most of the species recognized on the basis of the spicules are characteristic for this environment being shallow-water inhabitants. Moreover, this area is not influenced by hurricanes (Aaron O'Dea personal communication). The transport from deeper water is less likely, too. Moreover, some of these spicule morphotypes were quite numerous in the studied sediment (amphitriaenes) which also suggests the autochthonous character of the studied spicule assemblage. Their absence from the sponge list from this area is caused probably by the fact that all of them are cryptic or excavating. It is thus highly plausible that they represent species that still live in the investigated area, but were just overlooked.

Conclusions

The spicular analysis method may be used as an additional tool for tracing sponge species in marine and fresh-water environments all over the world.

Nevertheless, usage of this method is burdened by some limitations:

1. The method only suggests, does not prove, the presence of species in the studied area (conventional taxonomic studies are necessary to confirm the presence of the species).
2. It is suitable only for tracking sponges with very characteristic, highly diagnostic spicule types.
3. It is best applied in areas with no significant sediment transport and mixing.

In Bocas del Toro lagoon, the method of spicular analysis has suggested the presence of four cryptic and/or excavating sponge species unknown in this area before. Those were *Samus anonymus*, *Alectona wallichii*, *Triptolemma endolithicum*, and *Cliona mucronata*.

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Competing interests

The authors declare that they have no competing interests.

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