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Microstructure of octocoral sclerites for diagnosis of taxonomic features

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Abstract The octocoral family Xeniidae constitutes a major faunistic component of Indo-Pacific coral reefs, particularly in the Red Sea. The sclerites of several common genera, among which *Xenia*, are composed of minute platelets, or corpuscle-like forms, with a surface that appears almost smooth at light microscope magnification. Scanning electron microscopy (SEM) revealed the fine microstructure of the xeniid-sclerites, leading to the establishment of several new taxa, including the genus *Ovabunda*, whose spheroid sclerites are composed of aggregations of minute microscleres rather than the *Xenia*-style platelets. The present study applied for the first time high-resolution environmental SEM (ESEM) in order to examine the detailed structure of these octocoral sclerites. It determines whether and how the microstructural features in three type specimens of *O. biseriata*, *O. faraunensis* and *O. impulsatilla* play a role in determining their structural stability: namely, how the corpuscular microscleres adhere together and maintain the sclerites' spheroid shape. The study revealed the surface of the microscleres to be constructed of coarse granular crystals, and the adjacent microscleres to be interconnected by various microstructural means. It is suggested that these microstructural features are diagnostic for the genus. Future examination of type specimens of other *Ovabunda* species, as well as additional xeniid genera, will also enable a better evaluation of the taxonomic importance of high-resolution images in relation to other genera.

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Introduction

Soft corals (Octocorallia) are common members of the benthic communities of Indo-Pacific coral reefs (Benayahu and Loya 1981; Fabricius and Alderslade 2001). Unlike reef-building stony corals (Scleractinia), octocorals do not produce a massive skeleton but sclerites, which are internal polycrystalline aggregates of calcite-composed skeletal elements. Sclerites demonstrate a high variability in shape and size, and constitute an important trait in octocoral taxonomy (e.g., Bayer 1981; Fabricius and Alderslade 2001; Tentori and Ofwegen 2011).

Species of the octocoral family Xeniidae Wright and Studer (1889), form a major benthic component of shallow coral-reef communities throughout the tropical Indo-West Pacific (e.g., Reinicke 1997; Fabricius and Alderslade 2001), and in the Red Sea in particular (e.g., Benayahu 1985, 1990; Reinicke 1997). Xeniid colonies harbor zooxanthellae and comprise mostly small-sized and very soft colonies that are often slippery to the touch due to their mucous secretion. Furthermore, most xeniids feature several longitudinal rows of pinnules on each side of their tentacles (Reinicke 1997). The number of pinnules on the outermost row are used for identifying the species of the different genera, along with the morphological features and dimensions of their sclerites.

The sclerites of a number of xeniid genera constitute minute platelets, or corpuscle-like forms, with a surface that appears almost smooth at light microscope magnification (Fabricius and Alderslade 2001). For many decades, these sclerites had been considered as exhibiting a relatively

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simple structure, lacking the multiform variability known for other octocoral families (e.g., McFadden et al. 2009). It is thus unsurprising that most taxonomic studies on xeniids did not refer to the actual sclerites' structure but only to their shape and dimensions (e.g., Hickson 1931; Verseveldt and Cohen 1971). As the limited resolution of light microscopy has revealed relatively little variation in most of the xeniid-sclerite surface micro-architecture, such sclerites have been commonly termed "*Xenia*-like", since they are abundant in species of that common genus. This form of sclerite has also been attributed to several other genera of the family, including *Cespitularia*, *Efflatounaria*, *Funginus*, *Heteroxenia*, *Sansibia* and *Sympodium* (see Fabricius and Alderslade 2001). The dimensions of these sclerites, range 0.015–0.050 mm in diameter, have been used as an important feature for distinguishing between species within the respective genera (e.g., Reinicke 1997).

Following the introduction of scanning electron microscopy (SEM) in octocoral taxonomy, this methodology was also applied to the study of xeniid sclerites (Benayahu 1990; Reinicke 1997). Alderslade (2001) was the first to consider the relevance of sclerite surface microstructure for xeniid taxonomic diagnosis and, consequently, established several new genera, including *Bayerxenia*, *Ignotia*, *Ixion*, *Orangaslia* and *Ovabunda*. In addition, Benayahu (2010) established the genus *Yamazatum*, featuring novel types of sclerites revealed by SEM. As opposed to the *Xenia*-style platelets, which comprise a latticework of radially-arranged dendritic calcite rods, *Ovabunda* spheroids are aggregations of a mass of minute, corpuscular-shaped microscleres (Alderslade 2001). Based on this finding, several previously described *Xenia* species have now been reassigned to the genus *Ovabunda* (Alderslade 2001; Janes 2008; Aharonovich 2010).

Alderslade (2001) noted that some sclerites of *Ovabunda* began to fall apart shortly after exposure to sodium hypochlorite, which is routinely used for removal of the organic tissue components, while others only disintegrated following application of a certain physical force. As our recent SEM images of sclerites of several Red Sea *Ovabunda* species had mostly yielded intact sclerites (Aharonovich 2010), we were intrigued to know how these *Ovabunda* microscleres maintain their structural stability. In the present study, we applied high resolution environmental SEM (ESEM; e.g., Hayat 2000; Muscariello et al. 2005) to examine sclerites from type material of three common Red Sea species: *O. biseriata* (Verseveldt and Cohen 1971), *O. faraunensis* (Verseveldt and Cohen 1971), and *O. impulsatilla* (Verseveldt and Cohen 1971), in order to determine whether and how the corpuscular microscleres adhere together in order to maintain the sclerites' typical spheroid-shape.

Materials and methods

In the present study, we examined three types, obtained as a loan from the Natural History collection of the The Hebrew University of Jerusalem (HUJ): *O. biseriata* (HUJ I Co. 72), *O. faraunensis* (HUJ I Co. 140) and *O. impulsatilla* (HUJ I Co. 84). These species were originally classified as *Xenia* by Verseveldt and Cohen (1971) and had since been reassigned to *Ovabunda,* as detailed: *O. biseriata* and *O. faraunensis* by Alderslade (2001) and *O. impulsatilla* by Janes (2008). Notably, the decisions of the latter studies were not based on examination of the respective type material. In the present study, two or three polyps were removed from each type colony of *O. biseriata, O. faraunensis* and *O. impulsatilla*, placed in separate Eppendorf tubes, and 10% sodium hypochlorite was added to dissolve the tissues. After 20–30 min, the supernatant and organic debris were discarded and the sclerites were carefully and gently rinsed 4–5 times in distilled water to remove excess bleach and debris. Wet preparations of the sclerites were made and examined under an Optihot Nikon light microscope at ×400 magnification to ensure that the preparation was indeed free of organic remains. For SEM examination, the sclerites were filtered through a 0.45-µm Millipore filter; the filters were dried at room temperature, glued to stubs, and gold-coated. The sclerites were then viewed under a JEOL 840A SEM at 15 kV (×3,000 and 9,000) and a Quanta 200 FEG ESEM at 5–20 kV (>×60,000). Although gold-coating is not obligatory for ESEM (Muscariello et al. 2005), in our experience it yields superior images and, therefore, in the current study all samples were routinely coated.

Results

SEM examination of sclerites from the type colonies of *O. biseriata*, *O. faraunensis* and *O. impulsatilla* confirmed that they indeed belong to the genus *Ovabunda*. Their sclerites are spheroid, distinctly constructed of juxtaposed corpuscular-shaped microscleres (Fig. 1a–c). The majority of the microscleres are viewed from their flat plane, and some from their circumferential rim (Fig. 1d–f). The latter microscleres appear as if wedged among the former and appear perpendicular to the surface of the spheroid sclerite. These features present even higher clarity in all three species at higher ESEM magnification (×60,000) (Fig. 1g–i). The diameter of a microsclere is 400–700 nm. Its surface, which appears rather smooth under lower magnification (Fig. 1a–f), is in fact constructed of coarse granular crystals (Fig. 1g–i). This microstructural feature is also confirmed at higher magnifications (×150,000). The high-resolution images further demonstrate that

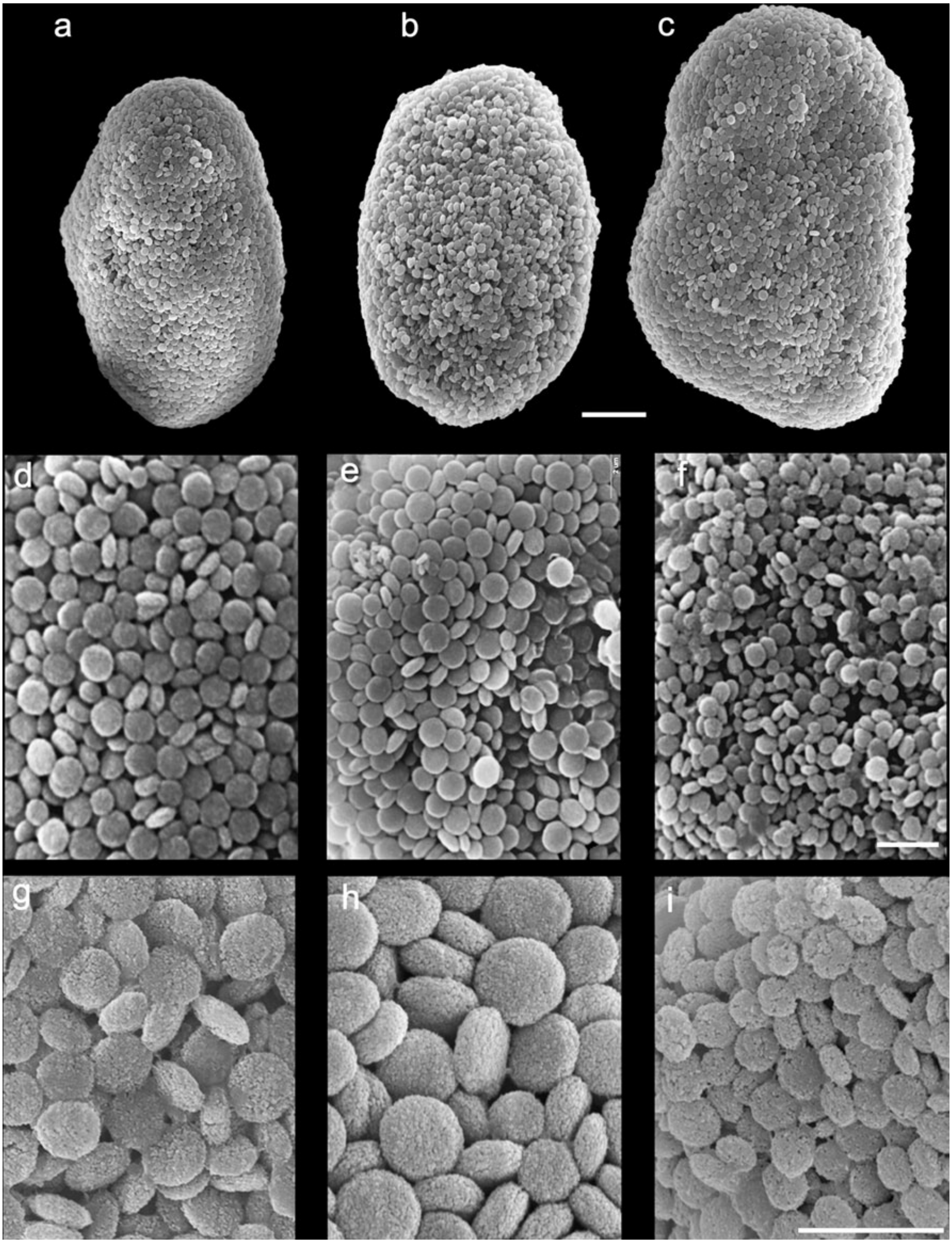

Fig. 1 Images of sclerites from type colonies of *Ovabunda*. SEM of whole sclerite: **a** *O. biseriata*; **b** *O. farauensis*; **c** *O. impulsatilla*. SEM of sclerite surface of **d** *O. biseriata*; **e** *O. farauensis*; **f** *O. impulsatilla*. ESEM of microscleres surface: **g** *O. biseriata*; **h** *O. farauensis*; **i** *O. impulsatilla*. *Scale* at (**c**) 10 μm also applies to (**a**, **b**); at (**f**) 2 μm also applies to (**d**, **e**); at (**i**) 2 μm also applies to (**g**, **h**)

adjacent microscleres are interconnected by various microstructural means (Fig. 2a–d). In *O. biseriata,* partial calcite cementation takes place between the rims of adjacent microscleres (Fig. 2a). In *O. faraunensis,* fine columnar calcite deposition can be observed between juxtaposed microscleres (Fig. 2b). In *O. impulsatilla*, a similar

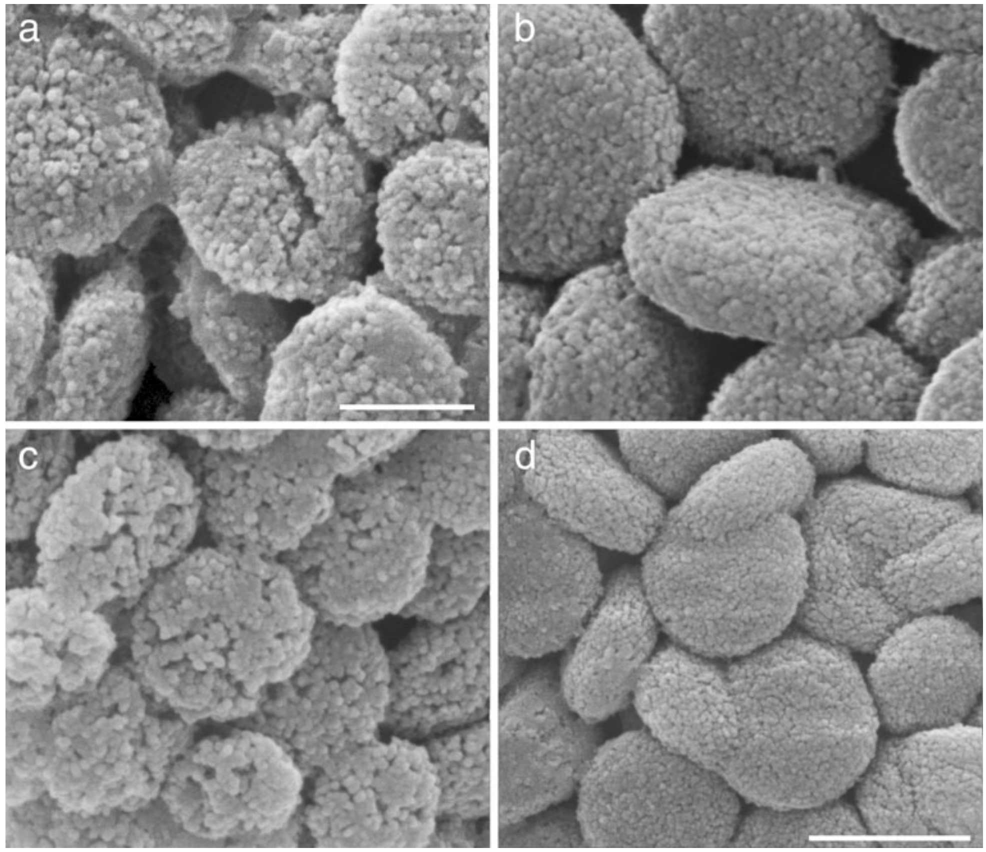

Fig. 2 ESEM images of microscleres from type colonies of **a** *Ovabunda biseriata*; **b** *O. farauensis*; **c**, **d** *O. impulsatilla. Scale* at (a) 500 nm also applies to (**b**, **c**); at (**d**) 1 µm

cementation of adjacent rims is visible (Fig. 2c), along with the integration of microscleres, positioned in different planes (Fig. 2d).

Discussion

The current study confirms that the three type specimens should indeed be assigned to the genus *Ovabunda*. It demonstrates for the first time the microstructural features of octocoral sclerites following the application of high-resolution SEM, up to ×150,000. Since we examined dehydrated, gold-coated sclerites, rather than exploiting the ESEM ability to work with wet samples, the highly detailed images nonetheless disclosed microstructural features of *Ovabunda* sclerites that had not been described previously. Prior to this study, SEM findings (Benayahu 1990; Reinicke 1997; Alderslade 2001) had failed to disclose the finer details of the structure of *Ovabunda*'s microscleres, or to relate to the question of how these corpuscular-microscleres adhere together and maintain the sclerites' spheroid shape. The coarse granular crystalline nature of the microscleres is revealed here for the first time (Figs. 1g–i and 2a–d). In addition, most of the hypochlorite-treated sclerites remained intact after dissolution of the organic material, as confirmed by the ESEM images (Figs. 1 and 2), suggesting the suitability of the protocol used by us for the preparation of sclerites.

The fine structure of the microscleres (Figs. 1g–i and 2a–d), including their crystal structure, is distinct among octocorals. The latter most commonly features elongated, columnar or needle-shaped crystals, which are typically oriented parallel to the axis of the sclerites (see Tentori and Ofwegen 2011 and references therein). Other, albeit less common, reported types are granular, hound's tooth and scale-like crystals. The uniquely structured *Ovabunda* spheroids and their round microscleres feature granular crystals were previously also found in an isidid gorgonian (Noe and Dullo 2006). It is suggested here that this microstructure may provide a suitable surface area for attachment of the coenenchyme in the live state of the colonies, and consequently facilitate the microscleres' function as a reinforcing internal skeleton (Koehl 1982; Lewis and Wallis 1991).

In recent years, ESEM has been successfully employed in taxonomy, exploiting its advantage for examining wet or uncoated samples e.g., for Crustacea (Valdecasas and Camacho 2005), Foraminifera (Georgescu 2009) and Coleoptera (Liu et al. 2009). Those studies have demonstrated that ESEM facilitates systematic work without damaging the specimens. In a study of modular organisms, octocorals included, removal of a few polyps (see "Materials and Methods") in order to isolate sclerites does not substantially harm the type material, and is advantageous for obtaining the ESEM high-resolution images of dry sclerites, which are superior to the images previously obtained by SEM (e.g., Benayahu 1990; Alderslade 2001). The present examination of the dry gold-coated sclerites of *O. biseriata*, *O. faraunensis* and *O. impulsatilla* has provided novel microstructural details that can be considered as diagnostic for the genus. Future examination of sclerites of other *Ovabunda* species will enable a better evaluation of the taxonomic importance of high-resolution images in relation to other xeniid genera.

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