Identification of Cultured Xeniids (Octocorallia: Alcyonacea)

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An examination of xeniid octocorals was carried out on specimens collected from the coral culture aquariums of Oceans, Reefs, and Aquariums, Fort Pierce, Florida, USA. Gross morphological analysis was performed. Pinnule arrangements, size and shape of the colony, and sclerite shapes very closely matched the original description of *Cespitularia erecta*.

Keywords: Cnidaria; Coelenterata; Xeniidae; Cespitularia; soft corals

Introduction

The family Xeniidae has a broad geographical range from the Eastern coast of Africa, throughout the Indian Ocean to the Western Pacific Ocean. Extensive work has been published on the species diversity from the Red Sea (Benayahu 1990; Reinicke 1997a), Seychelles (Janes 2008), the Philippines (Roxas 1933), and as far north as Japan (Utinomi 1955). In contrast, there are only a few records from Indonesia (Schenk 1896; Ashworth 1899), Sri Lanka (Hickson 1931; De Zylva 1944), and the Maldives (Hickson 1903).

Within the family Xeniidae the genus *Cespitularia* contains seventeen nominal species. This genus is often confused with the xeniid genus *Efflatounaria* where living colonies can appear morphologically similar. There are few morphological differences between the two genera, the most notable of which are the polyps. Polyps from colonies of *Cespitularia* are only slightly contractile if at all, whereas polyps in living colonies of *Efflatounaria* are highly contractile when agitated. Colonies of *Efflatounaria* are typically considered more lobed compared to the branched stalks in *Cespitularia*. Some early SEM evidence suggests that the ultra-structure of *Cespitularia* sclerites differs from all other xeniid genera (M. Janes, unpublished data) but additional sampling needs to be collected. A systematic revision of the genera *Cespitularia* and *Efflatounaria* is currently underway (Y. Benayahu, personal communication).

There is a high degree of morphological plasticity in xeniid genera including Cespitularia and Efflatounaria that are kept in captivity. With this in mind it becomes very difficult to identify species of xeniids that have been grown in closed systems for prolonged periods of time. An effort to identify three captive grown specimens which visually appear very different from one another was carried out and the results are outlined below.

Materials and Methods

Live colonies attached to a single piece of substratum were collected. Specimens were fixed in 70% ethyl alcohol after collection. The material is deposited in the collections of AquaTouch, Phoenix, Arizona.

Initial morphological examination of the colony was performed under a dissecting microscope at 15-power. The polyps were examined for the number of pinnule rows and the number of pinnules in each row. Measurements to the nearest 0.01 mm were taken of colonies, tentacles, and pinnule sizes. Sclerites were isolated from the tissue and permanent mounts on microscopic slides were made with Durcupan AMC Fluka (Fabricius & Alderslade, 2001). The sclerites were measured to the nearest 0.005 mm on a compound microscope equipped with a Filar Micrometer. Observations were made at 100-power, which consisted of 10-power oculars combined with a 10-power objective.

There is a low degree of contrast between pinnules and tentacles when preserved polyps are viewed under the dissecting microscope. To improve the contrast and subsequently the accuracy of pinnule counts in each row of the tentacles a staining solution was used on fixed polyps. After staining the polyps were placed on a glass microscope slide, coated with Durcupan AMC mounting medium, a cover slip was added, and the microscope slide was dried on a slide warmer until the mounting medium hardened.

Systematic part

Class Anthozoa Ehrenberg, 1831
Subclass Octocorallia Haeckel, 1866
Order Alcyonacea Lamouroux, 1812
Family Xeniidae Ehrenberg, 1828
Genus *Cespitularia* Milne-Edwards & Haime, 1857

***Cespitularia erecta* Macfadyen, 1936**
(Figs. 1-4)

Cespitularia erecta Macfadyen, 1936: 26.

Material: AT 8312012A (also labeled as ORA Efflatounaria), AT8312012B (also labeled as ORA Blue Cespitularia), AT8312012C (also labeled as ORA Purple Cespitularia).

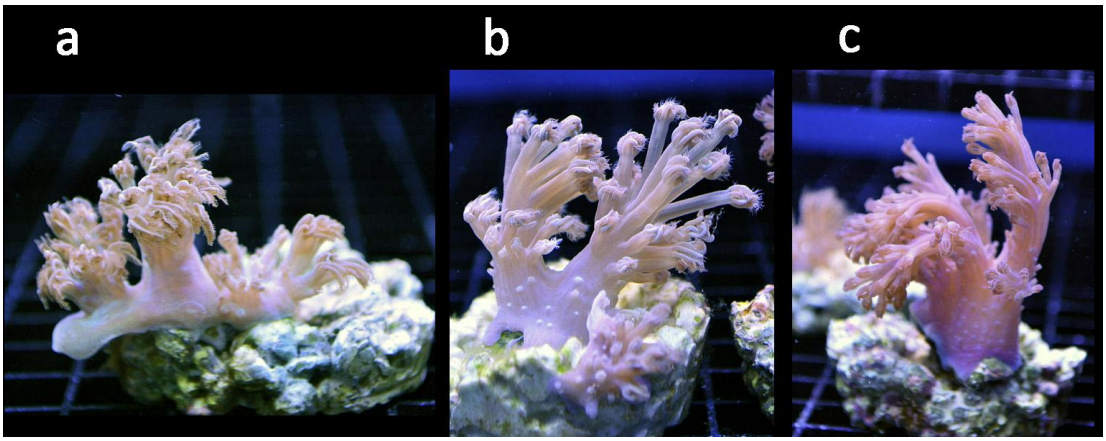

Figure 1. Images of live colonies: a, AT 8312012A; b, AT 8312012B; c, AT 8312012C.

Description. Cultured colonies are comprised of short, branched stalks attached coral rock. Individual colonies are from 4.0 to 6.0 cm tall in life (Fig. 1).

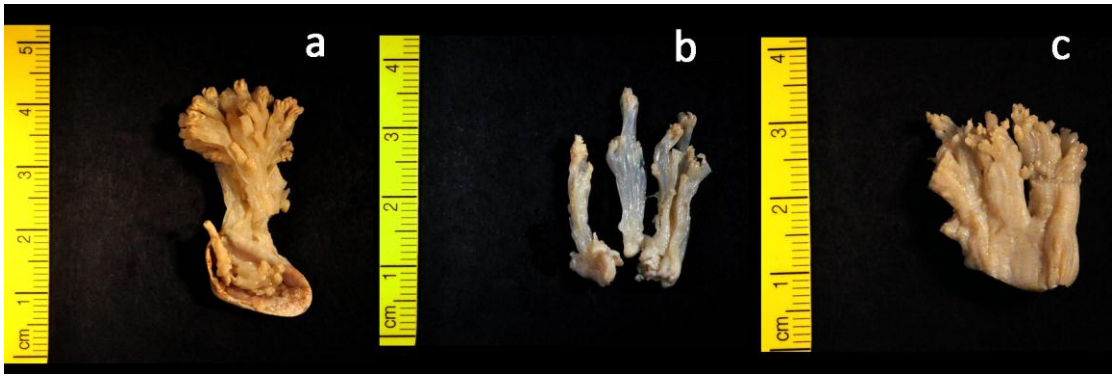

Figure 2. Preserved colonies: a, AT 8312012A; b, AT 8312012B; c, AT 8312012C.

Preserved colonies are notably shrunken (Fig.2), with primary stalks measuring 2.5 to 4.0 cm tall and up to 0.5 cm wide. Colonies form from primary stalks with shorter secondary branches developing above the basal attachment. Polyps are elongated measuring up to 4.0 mm long by 0.8 mm wide at the base in preserved specimens. The stalks exhibit longitudinal grooves and notable shrinkage after fixation. The tentacles are slightly conical in shape with a blunt tip; they are flattened laterally and up to 2.0 mm long and 0.5 mm wide (Fig. 3). There is a bare median present along the oral side of the tentacles that extends nearly the entire length. Along each side of the tentacle there is one row of pinnules. There are from 17 to 20 pinnules in a row. The pinnules are tapered with a rounded tip at the distal end. They measure up to 0.8 mm long by 0.05 mm wide at the base.

Figure 3. Polyps mounted from preserved colonies: a, AT 8312012A; b, AT 8312012B; c, AT 8312012C.

Sclerites. Within the tissue of stalks and polyps the sclerites are rather numerous. They are most abundant in the tentacles and pinnules of the polyps. There are relatively fewer sclerites in the base and branches of the colonies. The polyp sclerites range from 0.017 to 0.021 mm in diameter and in the lower portions of the lobes located close to the area of basal attachment the sclerites are up to 0.023 mm in diameter (Fig. 4). All of the sclerites have a dark textured surface under a compound light microscope. They are oval to disc-shaped. Most have irregularly formed edges

with indentations or scored fractures across parts of the surface. Scanning electron microscopy of the sclerites was not performed.

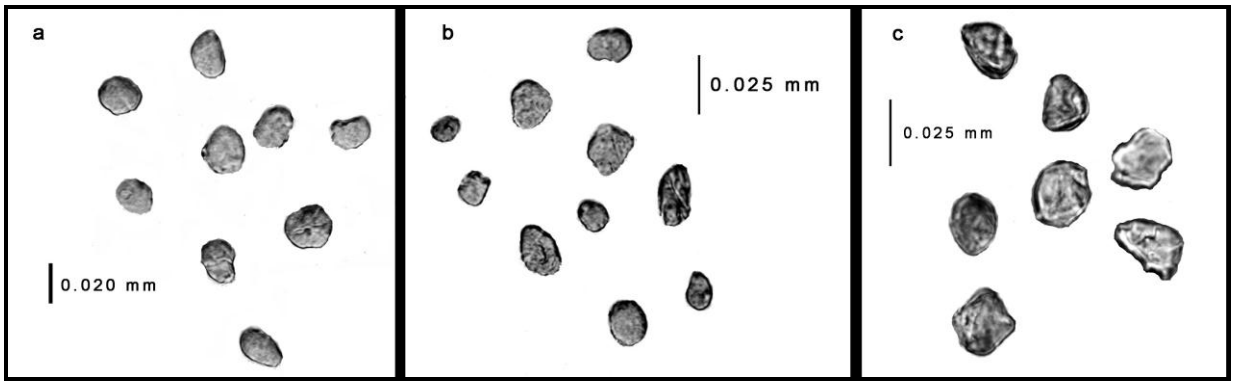

Figure 4. Light micrographs of *Cespitularia erecta* sclerites isolated from colony stalks and polyps: a, AT8312012C; b, AT8312012B; c, AT 8312012A.

Molecular results. Not available.
Color. Living colonies range from light tan with brown polyps (Fig. 1a), light pink with darker polyps (Fig. 1b) to uniform purple in color (Fig. 1c). In a preserved state the colonies are light tan (Figs. 1a, 1c) or cream colored (Fig. 1b).
Variability. The most significant variations among the three samples appear to be the color of living specimens and their sizes. The tan color of AT 8312012A was notably different from the pinkish color of AT8312012B and the purple color that was observed in AT8312012C. In life AT 8312012A was also the smallest, measuring 4.0 cm in height. Both AT8312012B and AT8312012C were up to 6.0 cm tall when fully expanded. In all three samples the row and number of pinnules, tentacle size, sclerite size, and sclerite shape were nearly identical.
Distribution. Originally described from the Great Barrier Reef, Australia (Macfadyen 1936).
Remarks. Many features of these three specimens agree well with the original description given by Mrs. Macfadyen (1936). Notable is the colony size which is described as 3.5 cm in the largest preserved colony compared to 4.0 cm in the largest preserved colony examined in this collection. The type material also has short branches as was observed in the cultured material (Fig. 1). She describes polyps as having tentacles up to 2.6 mm long, one row of pinnules on either side of the tentacle with a bare median on the oral side of the tentacles. There are 18-20 pinnules per row which have a finger-like shape with a pointed tip in the type description. This matches well with the sampled material which had tentacles that were 2.0 mm long (a difference likely due to fixation), a single row pinnules on either side of the tentacles, 17-20 pinnules in a row, and the same conical shape to the pinnules. The pinnules in the type material were only 0.4 mm long where as these preserved specimens had a pinnule length up to 0.8 mm long, a difference that could also be attributed to the preservation method. The color of the living type specimen remains unknown however the preserved color was described as “cream white”, this is very similar to the preserved state of the cultured specimens (Fig. 2). The sclerites of the type specimen were observed to have a maximum diameter of 0.045 mm. This is larger than the 0.023 maximum diameter observed in the cultured material.

When compared to other species of *Cespitularia C. erecta* differs from *C. multipinnata* (Quoy & Gaimard 1833) which has 3-4 rows of pinnules; *C. coerulea* (May 1898), *C. hypotentaculata* (Roxas 1933), *C. quadriserta* (Roxas 1933), *C.*

taeniata (May 1899), and *C. wisharti* (Hickson 1931) all lack sclerites; *C. exigua* (Verseveldt 1970), *C. infirmata* (Verseveldt 1977), *C. robusta* (Tixier-Durivault 1966), *C. schlicteri* (Janes 2008), *C. simplex* (Thomson & Dean 1931), *C. subviridis* (Quoy & Gaimard 1833), and *C. turgida* (Verseveldt 1971) (determined to be a species of *Efflatounaria* Benayahu 1993) have fewer pinnules per row; *C. densa* (Tixier-Durivault 1966) has both fewer pinnules and two rows on each side of the tentacle; and *C. stolonifera* (Gohar 1938) which has 22-27 pinnules in a row and smaller sclerites. Based on the differences described here and the similarities listed above the cultured specimens are most likely to be *C. erecta*.

Discussion

The three potentially different cultured xeniid specimens examined in this project were selected based on their color differences and from available information that they originated from three different locations: AT 8312012A, Philippines; AT 8312012B, Indonesia; and AT 8312012C Kenya. As such they were suspected to be different species. The results of this study shows that based on morphological characters all three specimens appear to be the same species, *Cespitularia erecta*.

Color differences in xeniids are not a good indicator of speciation. Specifically because the sclerites are colorless in reflected and transmitted light and any pigments within the tissue are soluble in most fixatives and ethanol (Alderslade 1995: 111). As well as due to varied concentrations of zooxanthellae within the tissues colonies will take on different degrees of brownish color depending on their exposure to light (Reinicke 1997b). In a closed culture system populations of both *Xenia* and *Heteroxenia* species were recorded to show a change in the amount of brown pigmentation in colony stalks and polyps over periods exceeding one year (M. Janes unpublished data).

To date there have been no published studies indicating how significant the morphological plasticity of Xeniidae octocorals are when grown under captive conditions for prolonged periods (or in the wild). However, anecdotal observations of changes in gross morphology such as colony size, polyp size, color, and basal attachment surface area have been noted by aquarium hobbyists. Alderslade (2000: 245) mentions that rapid changes in octocoral sclerite shapes and densities can occur when kept in aquaria. He also sites an observation by Julian Sprung where pinnule number and arrangement can change in specimens raised in aquariums. With the potential to undergo significant morphological changes under captive conditions it is unknown if the similarities in sclerite size and shape, polyp size, pinnule row, and pinnule number observed in the three cultured specimens is a result of prolonged exposure to identical parameters of light, water flow, temperature, and water chemistry or if all three specimens are in fact identical species.

There are two components in the process of describing species of xeniids that could produce more accurate results. The first is DNA analysis which was not performed in this study due to budget limitations. Using currently available genetic markers for mitochondrial DNA has shown to significantly improve the accuracy of species identification when combined with traditional morphology in some octocorals (McFadden et al. 2011) and initial work on DNA analysis with the inclusion of nuclear DNA also showed similar results in a collection of xeniids (Janes et al. in process). If molecular tools had been applied to the three cultured samples and showed them to be three different species then all we would be able to determine is

that these three samples are genetically different species but have no morphological variation, a result of captive conditions. This would also suggest their identification to the species level would be impossible. The second advantage which would help improve the accuracy of identification would be to have fixed some of the wild sourced or parental material prior to its introduction into aquaria. Conducting a taxonomic examination of this material would provide baseline identification to the species level and allow for comparative analysis of future offspring after they have "grown out" under captive conditions over prolonged periods of time. Unfortunately, in this case none of the initial material was fixed. Applying both DNA analysis and traditional taxonomy to the identification of wild sourced material prior to being introduced into an aquarium would improve the accuracy of identifying samples of cultured *Cespitularia* to the species level.

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