CELL TYPES AS TAXONOMIC CHARACTERS IN *APLYSINA* (APLYSINIDAE, VERONGIDA)

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ABSTRACT

Some sponge groups are known to present a complex taxonomy, commonly associated with few morphological taxonomic characters. Consequently, techniques other than traditional morphology, such as isozymes, DNA fingerprinting and transmission electron microscopy, have been sought to enhance taxonomic diagnoses. Nevertheless, most require expensive equipment and time consuming procedures. In this work we used a simple technique, based on the characterization of definite cell types, to differentiate two species: *Aplysina caissara* and *Aplysina fulva*. Both show similar external and internal morphology, principally on smaller individuals, leading to erroneous identifications. Three shared cell types were used, namely spherulous cell I, spherulous cell II, and microgranular cell. Both species can be clearly identified based on characteristics of these cell types (general size and diversity of inclusions). The results show that the morphology of definite cell types, as observed on simple cytospins, can be an additional taxonomic character to the differentiation of cryptic species.

KEY WORDS

Aplysina, sponge cells, taxonomy, cytospins.

INTRODUCTION

Sponge species identification is based mainly on descriptions of the skeletal components and architecture, and external morphology. Some sponge taxa are known to present a complex taxonomy however, which is commonly associated with their possession of few readily accessible morphological taxonomic characters. In general, these taxa show a low variety of skeletal structures and/or extensive variations in external morphology characters within species. This problem is particularly significant in those groups with skeletons composed only of spongin fibers ("keratose" sponges) or even lacking distinct skeleton structures altogether (*e.g. Chondrosia*). Consequently, techniques other than traditional morphology have been sought to enhance taxonomic diagnoses. Analyses such as biochemical profiling (BERGQUIST *et al.,* 1990; KARUSO *et al.,* 1990), isozymes (BOURY-ESNAULT *et al.,*

1993; KLAUTAU *et al.,* 1994) and DNA/RNA sequencing (LÔBO-HAJDU *et al.,* 1999), have been used with promising results for sponges. In addition to these, descriptions of cell ultrastructure based on transmission electron microscopy images have been used as additional morphological characters (BOURY-ESNAULT *et al.,* 1994; MURICY *et al.,* 1996). Cellular structures and functions observed by light microscopy in stained tissue sections and cultivated sponge fragments were also used by SIMPSON (1968) with good results. Nevertheless, most of these techniques require expensive equipment or are time consuming procedures, and are prone in many sponges to interference resulting from the conspicuous presence of micro- as well as of macro-symbionts.

In this work we used a fast and simple technique, based on tissue dissociation and cell slides prepared with a cytocentrifuge. The isolated cells in these slides can be morphologically characterized and definite cell types selected and compared among different individuals.

MATERIALS AND METHODS

Species: Two species were used: *Aplysina caissara* Pinheiro and Hajdu, 2001 (Fig. 1A) and *Aplysina fulva* (Pallas, 1766) (Fig. 1B). Both show similar external and skeletal morphology, more striking on smaller specimens (Fig. 1B). Six individuals from each species were collected in the São Sebastião channel (23°44' S and 45°22' W, Southeastern Brazil). To verify the intraspecific cell morphology variability, two additional individuals of *A. fulva* were collected in different areas: Angra dos Reis (23º02' S and 44º19' W) and Arraial do Cabo (22º48' S and 41º55' W), respectively at a distances around 150 and 320 km from São Sebastião channel area. The identification of the species followed the usual methods described in PINHEIRO & HAJDU (2001), where detailed taxonomic descriptions of both species can be found.

Tissue dissociation: Methods are those described in CUSTÓDIO *et al.* (1998). The sponges were collected, cleaned, cut in small fragments (2 - 3 mm) and incubated with CMFSW+EDTA (calcium-magnesium free seawater - NaCl 460 mM, Na_2SO_4 7 mM, KCl 10 mM, Hepes 10 mM, EDTA 2.5 mM) for 30 min in a rotary shaker. The supernatant was discarded and the fragments resuspended in fresh CMFSW+EDTA solution. After 45 min the supernatant was collected and filtered through a double 40 μm nylon mesh. The cells were then pelleted by centrifugation (500 x g for 10 min) and washed once with CMFSW (without EDTA).

Cells: The slides of isolated cells were made with a cytocentrifuge (FANEM). This equipment is of common use in cell culture laboratories and uses slide carriers with sample compartments leading to an orifice that is placed against a microscope slide (FRESHNEY, 2000). After the run, the cells are flat on the slide and its morphological features can easily be observed.

To prepare the slides the final pellet from dissociation was resuspended in CMFSW and adjusted to 5 x 10^5 cells/ml and cytospinned (100 μl per spot - 100 x g for 5 min). The slides were fixed in formaldehyde sublimate for 45 minutes, stained either with Mallory Trichrome or Ramon-Cajal, (MARTOJA & MARTOJA, 1967) and mounted with Entellan (Merck). The cell characterization was based on overall shape and cytoplasmic/nuclear features, and the type identification was based on morphological similarities with the cell types described in SIMPSON (1984). To evaluate the cell type concentration, 200 cells of each species were counted in random fields and each type expressed as percent of total.

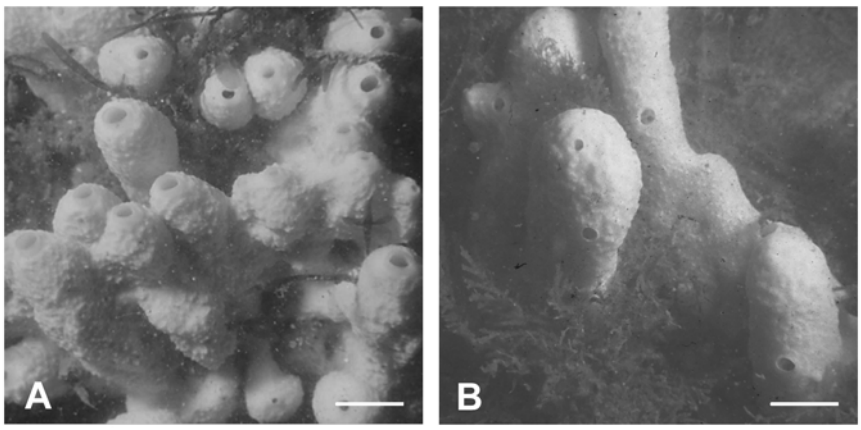

Fig. 1. Underwater photography. *Aplysina caissara* **(A)** and *Aplysina fulva* **(B)**, both from the São Sebastião channel area. Scale bars 2 cm.

RESULTS

There were no significant morphological differences among the *Aplysina fulva* cells from individuals collected in São Sebastião, Angra dos Reis and Arraial do Cabo. The counts show that the three most frequent cell types, identified as spherulous cells I, spherulous cells II and microgranular cells, are shared by both species. In *Aplysina caissara* spherulous cells I comprise 55 % of the total cell population in the slides, spherulous cells II 15 %, and microgranular cells 20 %. All other cell types in this species comprise only 10 % of the total cell population. In *A. fulva* spherulous cells I comprise 49 %, spherulous cells II 31 %, and microgranular cells only 8 %. The other types are also 12 % of the total. These three main types were then selected and compared. The characteristics of each type are described below:

Aplysina caissara

Spherulous cells I (Fig. 2A) - Cytoplasm filled with unstained marked vacuoles of varied sizes. All vacuoles show a well defined rod-like structure,
which divides the interior in two halves. Average cell diameter of 10.5 μm and nucleus with 2.4 μm, without nucleolus.

Spherulous cells II (Fig. 2C) - Small cell with cytoplasm loaded with dense irregular granules showing dark brownish color. Average diameter of 8,4 μm and nucleus with 1.9 μm, without nucleolus.

Microgranular cells (Fig. 2E) - Cytoplasm irregularly stained in dark green with Mallory, presenting tiny and scattered vacuoles with bright appearance. Average cell diameter of 11.3 μm and nucleus with 1.9 μm, without nucleolus.

Aplysina fulva

Spherulous cells I (Fig. 2B) - Cytoplasm filled with vacuoles of varied sizes showing light green staining with Mallory. As in *A. caissara*, rod-like structures divide

the interior in two halves, but both the vacuole limits and internal rod structures are less conspicuous. Average cell diameter of 15.4 μm and nucleus with 2.5 μm, without nucleolus.

Spherulous cells II (Fig. 2D) - Large cell with cytoplasm loaded with irregular granules stained with lighter brownish color than *A. caissara*. Average diameter of 11.7 μm and nucleus with 2.1 μm, without nucleolus.

Microgranular cells (Fig. 2F) - Granular cytoplasm with tiny and scattered vacuoles with bright appearance. The cell shows a regular bright green color on Mallory staining. Average cell diameter of 13.3 μm and nucleus with 2.5 μm, without nucleolus.

DISCUSSION AND CONCLUSION

The morphological similarity between *Aplysina caissara* and smaller individuals of *Aplysina fulva* has caused some misidentifications. MOTHES DE MORAES (1987) and LERNER (1996) classified their specimens of the yet undescribed *A. caissara* in the known range of morphological plasticity exhibited by *A. fulva* along the Brazilian coast. PINHEIRO & HAJDU (2001) argued that both species can be distinguished, by *A. caissara*'s obligatory possession of relatively large apical oscule in opposition to *A. fulva*'s different arrangement, with irregularly distributed oscules. Far from being unique, these misidentifications are common in sponge taxonomy, happening mainly on those groups with few readily accessible taxonomic characters and a large range of morphological variability, as in these *Aplysina* species. Both share similar colors, habits and skeletal architecture that can not be easily differentiated without direct comparison. However, in the cytospins both species can be clearly discriminated.

Despite their morphological differences, *A. fulva* individuals show a considerable similarity among their cell populations. This observation corroborates the idea that this species really possesses a large morphologic plasticity, in contrast to the hypothesis of *A. fulva* comprising a species complex instead. In this case, this variability can be related to factors such as different habitats, water currents, light exposition and symbionts in each locality and/or distribution boundary (PINHEIRO & HAJDU, 2001).

The general differences among the cell populations of *A. caissara* and *A. fulva* are striking, and only a superficial observation of the cytospins in low magnification is enough to differentiate both species. The most notable distinction is cell sizes, always smaller in *A. caissara* than in *A. fulva*. Other differences are observed in cell contents between structurally similar cell types, as revealed by staining. Noteworthy is the fact that the general features of other cell types, such as archeocytes and pinacocytes, seem to be more similar among the species than the observed features for the category of "cell with inclusions". It is tempting to hypothesize that cells playing fundamental functional or physiological roles (such as pinacocytes) may be less plastic than those with putative storage functions.

Sponge cell categorization is nevertheless complex, and there is still no definite terminology. As pointed out previously (SIMPSON, 1984), there is a necessity to know their developmental physiology and functional role in order to accurately define cell types. However, the approach of this study was to use cytological characteristics not to determine their physiological or structural role, but as simple

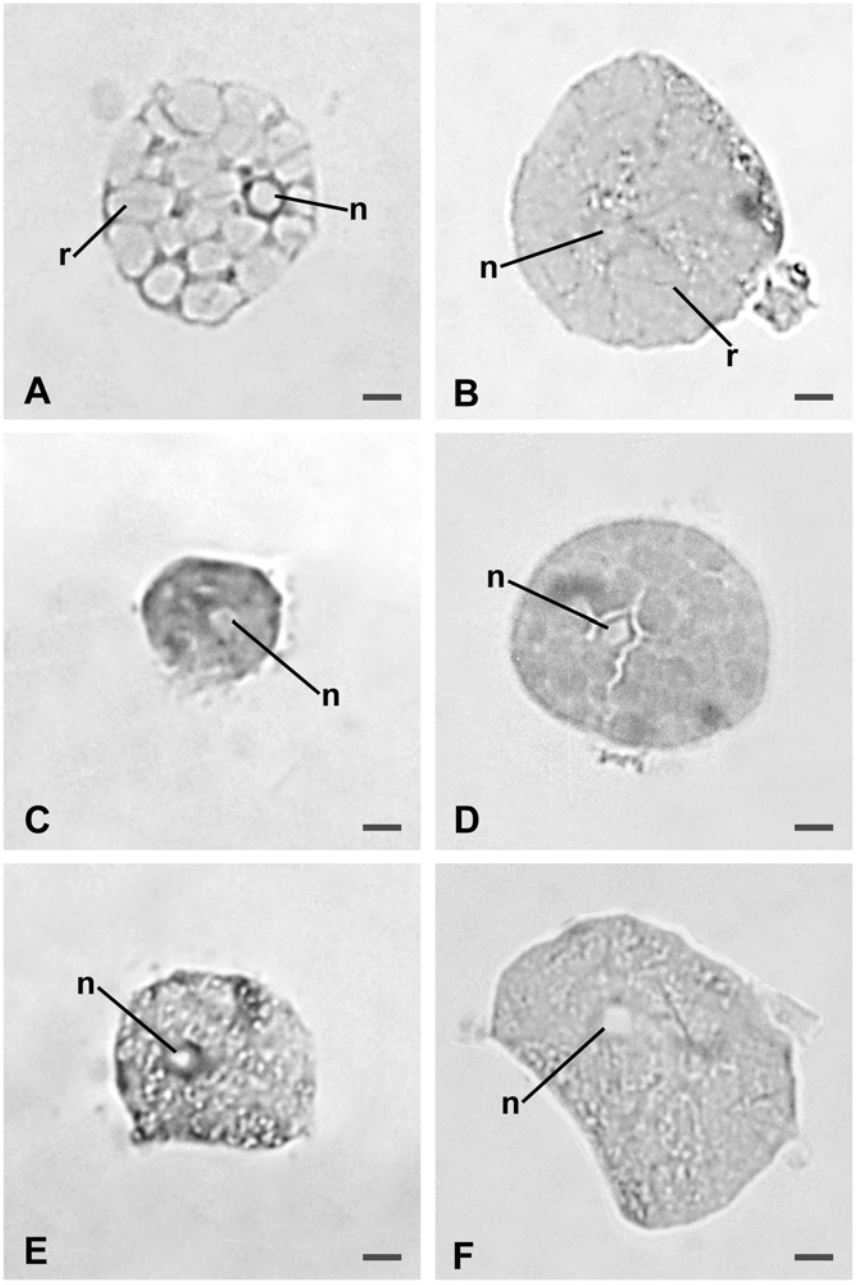

Fig. 2. Cell types. Spherulous cells I of *Aplysina caissara* **(A)** and *Aplysina fulva* **(B)**. Spherulous cells II of *A. caissara* **(C)** and *A. fulva* **(D)**. Microgranular cells of *A. caissara* **(E)** and *A. fulva* **(F)**. r - intravacuolar rod-like structure; n – nucleus. Scale bars 2 μm.

structures to be compared. As a parallel, microscleres are a basic taxonomic character in several groups, although their structural function in the skeletal framework is rather unknown and not necessarily the same in different taxa.

This work demonstrates that sponge species can be clearly distinguished by comparing cell types using a simple and fast method. Our results show that cellular morphology as observed on cytospins could be an additional character to those traditionally used for taxonomic differentiation of cryptic species.

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