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Statistical relationships of cnidocyst sizes in the sea anemone *Oulactis muscosa* (Actiniaria: Actiniidae)

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ABSTRACT. This study analysis the multivariate statistical relationships among cnidocyst types and tissues in terms of cnidocyst capsule size in the sea anemone *O. muscosa*. In a total of 12 individuals we measured the length of 30 unfired capsules of each cnida type, taken randomly from each of the following tissues: tentacles, acrorhagi, column, actinopharynx and mesenterial filaments. In these tissues, we found spirocysts, basitrichs, microbasic b-mastigophores, microbasic p-mastigophores and holotrichs. Thus, a total of 5400 measurements were made. Looking for a common pattern of variation, a principal components analysis (PCA) was performed on the data matrix, among cnidocyst types for each tissue and between tissues for each cnidocyst type. The results showed that the lengths of most cnidocysts, whether from the same or different tissues, are not statistically related to each other, suggesting that different forces (cnidogenesis, replacement dynamics, etc.), could affect in various forms the development and therefore the length of each cnida, depending on the type or tissue of origin.

KEY WORDS: cnidae, PCA, *Oulactis muscosa,* intertidal, Argentina.

INTRODUCTION

Cnidocysts (nematocysts, spirocysts and ptychocysts) are formed from the secretions of specialized cells that reside mainly in the tentacles of cnidarians, such as sea anemones, cerianthids, jellyfish, corals and hydroids. These stinging organelles, for which Cnidaria is named, are the chief instruments by which the animals in this taxon obtain food, defend against predation, adhere to solid substrata during locomotion or settlement, or build protective tubes (Kass-Simon & Scappaticci, 2002). These authors proposed that the persistence of cnidarians in marine and freshwater niches since the Cambrian is due not least to the effectiveness with which their nematocysts-bearing tentacles act as lethal weapons of defense and predation.

Each cnidocyst is secreted by a cnidoblast, which matures into an epithelial cell, the cnidocyte. A mature cnidocyst consists of a three-layered wall that encloses a dense lumen containing an inverted tubule which, depending on the cyst type, features specialized structures such as shaft, barbs, spines, stylets, etc. In response to appropriate stimuli, a cnidocyst explosively evaginates its tubule. This enables the everting tubule to penetrate through the integument or skin of invertebrates, vertebrates and even humans. By this mechanism toxins and enzymes contained in the cysts can be introduced into the body of either the aggressor, or prey organism (Weber, 1989).

The cnidome refers to the assemblage of cnidae present in any cnidarian. A diagrammatic way of presenting information covering both the types of cnidae present and their sizes was developed by Hand (1955a; 1955b; 1956) and has become a standard procedure in the taxonomic description of sea anemones (Ryland et al., 2004). However, in this group of cnidarians their value has been debated (Fautin, 1988; Zamponi & Acuña, 1991; Acuña et al., 2003; 2004), mainly due to their high statistical variation. Several studies reported on this variation and other statistical parameters (Zamponi & Acuña, 1994; Acuna & Zamponi, 1997; Chintiroglou et al., 1997; Chintiroglou & Karalis, 2000; Williams, 2000;), while a few authors (Francis, 2004; Acuña et al., 2007) have related aspects of the cnidae to the body size of sea anemones. A complete review of the structural diversity, systematics and evolution of cnidae can be found in Fautin (2009).

The biometry of the sea anemone *Oulactis muscosa* (Drayton in Dana, 1846) was studied by Acuña et al. (2007). They showed that cnida length varies with specimen, tissue and cnida, and their results do not support a functional relationship between cnida length and anemone body weight. However, possible correlations between the lengths of different cnidocysts from all tissues of the sea anemone were not examined. Therefore, the aim of this study was to perform a principal components analysis (PCA) to explore the multivariate statistical relationships of cnidocyst capsule sizes among different types of cnidocysts and tissues in *O. muscosa*. To our knowledge, similar studies for other sea anemone species do not exist.

MATERIALS AND METHODS

Sampling

Individuals of *O. muscosa* were collected randomly from the rocky intertidal zone of Punta Cantera (38° 05' S, 57° 32' W), Mar del Plata, Argentina. Specimens were kept in aquaria with aerated sea water for up to two days, then anesthetized by the addition of crystals of magnesium chloride, and preserved in 5% formalin. A total of 12 individuals were used. For each specimen, we measured the length of 30 unfired capsules of each cnida type (we usually take N=30 since this is a statistically significant number), taken randomly, from each of the following tissues: tentacles, acrorhagi, column, actinopharynx and mesenterial filaments. In these tissues, we found spirocysts, basitrichs, microbasic b-mastigophores, microbasic p-mastigophores and holotrichs (see Acuña et al., 2007); terminology based on that of England (1991). Thus, a total of 5400 measurements were made using a Zeiss Axiolab Microscope with micrometric eyepiece at a magnification of 1000X (oil immersion). Where two or more discrete nematocyst sizes were present within a tissue, they were counted separately and suffixed I, II, etc., according to size. For tentacle tissue squashes, we used the tips, since the base is a cnidogenesis zone with cnidocysts in different stages of development and consequently with high variation in sizes. For more details see Acuña et al. (2007).

Statistical analysis

A PCA was performed on the data matrix, among cnidocyst types for each tissue and among tissues for each cnidocyst type. Calculations were based on the correlation matrices, and the goal of this analysis was to express variance-covariance structures of the original variables (lengths of cnidocyst from different types and locations) through a few linear combinations or principal components. These components are determined by the eigenvectors of the correlation matrix and indicate the maximum variability directions that define the new factorial planes. Coordinates in the new system are called loadings and they are given by the correlations between each original variable and each principal component. Much of the total information contained in the data can be accounted for in the first principal components (see Johnson & Wichern, 1992 for a detailed description of PCA). The proportion of the total variance due to each component was also calculated. All calculations were done with the R statistical package (R, 2008).

RESULTS

Variation among cnidocysts within each type of tissue

In the tentacles there were only two types of cnidocysts (spirocysts and basitrichs), so for this location the analysis was just bivariate. Spirocyst and basitrich sizes were significantly correlated (ρ=0.78, p-value=0.003). In acrorhagi, the following types of cnida were present: holotichs (I and II), basitrichs and spirocysts. The bivariate association between each pair of cnidocysts, with their corresponding p-values is shown in the correlation matrix (Table 1); the highest correlation is between pairs holotrichs I – II. The associations between holotrichs I – basitrichs, holotrichs II – spirocysts and holotrichs II – basitrichs were weak, significant only at 10%. Fig. 1 illustrates the results of the multivariate PCA on this matrix. The first component (horizontal axis) is associated with holotrichs I and II, representing 54% of total variability or information. The second component (vertical axis) comprises the opposition between the latter two cnidocysts, while the third component (not shown in the figure) is associated to spirocysts with near 15% of total variability. Holotrichs, and basitrichs (I and II) are the cnida present in the column. Significant association at 5% was not observed (see Table 2), but the correlation between basitrich I and II was significant at 10%. As expected, given the correlation matrix, each component was strongly associated with one tissue, so the three-dimensional problem could not be reduced. The first component, represented by the horizontal axis in Fig. 2, comprised 56% of total variability and it represented the holotrichs. The second component (vertical axis) had 30% of variability, strongly comprising basitrichs II. Finally the third component explained 14% of the remaining variability and it was highly associated with basitrichs I and weakly with basitrichs II. In the actinopharynx, microbasic b-mastogophores, microbasic p-mastigophores and basitrichs were present. Significant correlations were not observed, similarly to the situation observed in the column (Table 3). Due to the low correlations, each component represented a moderate proportion of the variability. The first component represented microbasic b-mastigophores and explained near 50% of variability, the second was associated with microbasic p-mastigophores and the third with basitrichs (Fig. 3). The same types of cnida present in the actinopharynx were present in mesenterial filament, but they showed a different correlation matrix (compare Tables 3 and 4). Negative correlations between microbasic b-mastigophores and basitrichs, and microbasic p-mastigophores and basitrichs, were present, both significant at 5%. This means that large microbasic b-mastigophores and microbasic p-mastigophores were associated with small basitrichs. This means that large values of microbasic b-mastigophores are associated with large values of microbasic p-mastigophores. The three principal components represent each cnidocyst type, microbasic b-mastigophores, basitrichs and microbasic p-mastigophores, respectively. Given these correlations, 70% of the information of the three types of cnida can be represented by a single principal component (Fig. 4).

TABLE 1

Correlation matrix between cnidocysts from acrorhagi and p-values corresponding to the null hypotheses of no correlation.

	Basitrich	**Holotrich I**	**Holotrich II**	**Spirocyst**
Basitrich	1.000(0.000)			
Holotrich I	0.497(0.100)	1.000(0.000)		
Holotrich II	0.522(0.082)	0.612(0.034)	1.000(0.000)	
Spirocyst	0.160(0.620)	0.450(0.143)	0.524(0.080)	1.000(0.000)

TABLE 2

Correlation matrix between cnidocysts from column and p-values corresponding to the null hypotheses of no correlation.

	Basitrich I	**Basitrich II**	**Holotrich**
Basitrich I	1.000(0.000)		
Basitrich II	0.516(0.086)	1.000(0.000)	
Holotrich	0.287(0.365)	0.131(0.684)	1.000(0.000)

TABLE 3

Correlation matrix between cnidocysts from actinopharynx and p-values corresponding to the null hypotheses of no correlation.

	Microb. b-mastigophore	**Basitrich**	**Microb. p-mastigophore**
Microb. p-mastigophore	1.000(0.000)		
Basitrich	0.488(0.107)	1.000(0.000)	
Microb. p-mastigophore	0.440(0.152)	0.003(0.993)	1.000(0.000)

TABLE 4

Correlation matrix between cnidocysts from mesenterial filament and p-values corresponding to the null hypotheses of no correlation.

	Microb. b-mastigophore	**Basitrich**	**Microb. p-mastigophore**
Microb. b-mastigophore	1.000(0.000)		
Basitrich	-0.700(0.011)	1.000(0.000)	
Microb. p-mastigophore	0.676(0.016)	-0.586(0.045)	1.000(0.000)

TABLE 5

Correlation matrix between basitrichs from different tissues and p-values corresponding to the null hypotheses of no correlation.

	Tentacle	**Acrorhagi**	**Column**	**Actinopharynx**	**Mes. filament**
Tentacle	1.000(0.000)				
Acrorhagi	0.798(0.002)	1.000(0.000)			
Column	0.636(0.026)	0.278(0.381)	1.000(0.000)		
Actinopharynx	0.409(0.187)	0.387(0.214)	-0.161(0.616)	1.000(0.000)	
Mes. filament	0.715(0.009)	0.398(0.199)	0.538(0.071)	0.442(0.151)	1.000(0.000)

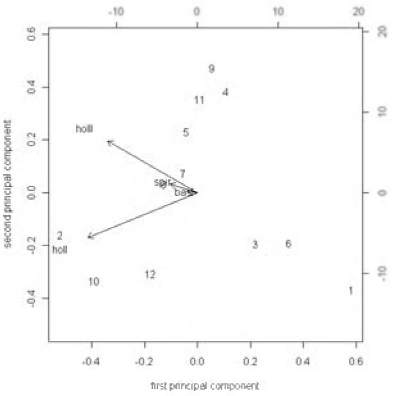

Fig. 1. – Scatterplot of the two first principal components for acrorhagi. Arrows represent the mean values for each cnidocyst. holI: holotrich I, holII: holotrich II, basI: basitrich I, spiro: spirocyst.

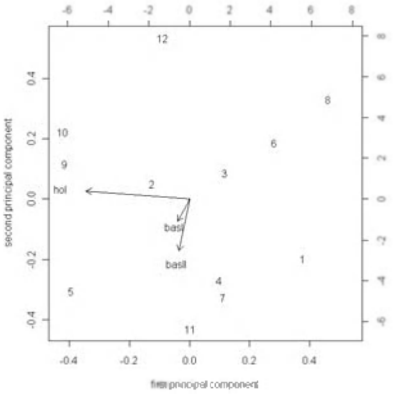

Fig. 2. – Scatterplot of the two first principal components for column. Arrows represent the mean values for each cnidocyst. hol: holotrich, basI: basitrich I, basII: basitrich II.

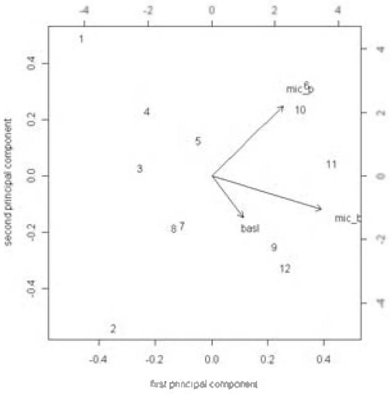

Fig. 3. – Scatterplot of the two first principal components for actionpharynx. Arrows represent the mean values for each cnidocyst. mic_p: microbasic p-mastigophore, basI: basitrich, mic_b: microbasic b-mastigophore.

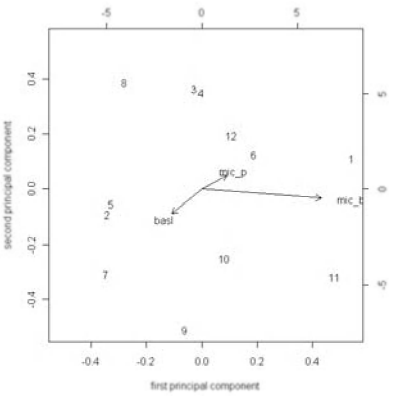

Fig. 4. – Scatterplot of the two first principal components for mesenterial filament. Arrows represent the mean values for each cnidocyst. mic_p: microbasic p-mastigophore, mic_b: microbasic b-mastigophore. basI: basitrich.

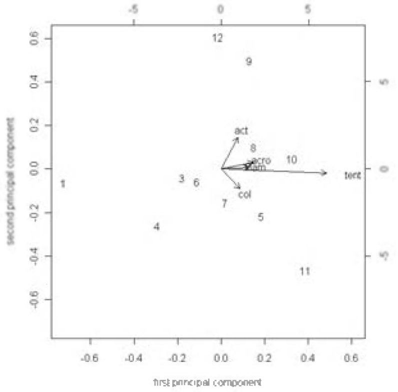

Fig. 5. – Scatterplot of the two first principal components for basitrichs. Arrows represent the mean values for each tissue. acro: acrorhagi, tent: tentacle, col: column, filam: mesenterial filament, act: actinopahrynx.

Variation within each type of cnidocyst from different tissues

Basitrichs were present in all sampled tissues. The capsule lengths measured in tentacles were significantly associated with acrorhagi, column and mesenterial filament and this last tissue was also associated with column (Table 5). Actinopharynx was not significantly correlated with any other cnida. The PCA showed a first component explaining 82.5% of total variability, strongly associated with tentacles (Fig. 5, horizontal axis). This was due to the fact that tentacle lengths have a very high variance (Acuña et al., 2007) and so it determines the first component. Spirocysts were present in tentacles and acrorhagi; the correlation between the two localities was $\rho=0.622$ and its p-value=0.031, meaning that big spirocysts appeared simultaneously in tentacles and acrorhagi and the same for small spirocysts. The cnidocyst holotrich was present in acrorhagi and column. The calculated correlation between these tissues was moderately significant: $\rho=0.571$, p-value=0.052. Microbasic b-mastigophores were recorded in actinopharynx and mesenterial filament; their corresponding lengths were not correlated ($\rho=0.142$, p-value=0.659). Microbasic p-mastigophores were also in actinopharynx and mesenterial filament; their correlation was a little higher, but still not significant ($\rho=0.486$, p-value=0.109).

DISCUSSION

Sea anemones are equipped with two major types of cnidocysts (nematocysts and spirocysts), with functions in food capture, defence, aggression and larval settlement. The description of the size and distribution of cnidocysts is commonplace in taxonomic studies of these marine invertebrates, but its value is discussed here. In a previous paper we examined all 15 types and sizes of cnida distributed in all different tissue from specimens of different sizes of the sea anemone *O. muscosa* (Acuña et al., 2007). Complementary to this, the goal of this paper was to study the multivariate statistical relationships of the sizes, between types in each tissue and between tissues for each type of cnidocyst.

An important correlation was observed between spirocysts and basitrichs from tentacles, despite being different types, while the holotrichs I and II from acrorhagi showed a moderate correlation. This could be explained by a similar cnidogenesis and replacement dynamics. In the other tissues no significant correlations were observed between the different cnidocysts. The strong correlations observed between lengths of basitrichs measured from tentacles and acrorhagi are possibly due to ontogeny of the last structure, since Bigger (1982) concluded that they are homologs of tentacles. However, Daly (2003) mentioned that an acrorhagus must not be considered a highly modified tentacle, as it is topologically and anatomically distinct. Also the basitrichs from tentacles are significantly associated with those from column and mesenterial filament, but not with those from actinopharynx. It is important to mention that the actinopharynx is a tissue with a complex pattern of nematocyst size-distribution (Ardelean & Fautin, 2004).

These results showed that the lengths of most cnidocysts, both from the same or different tissues, are not statistically related; suggesting that different forces (cnidogenesis, replacement dynamics, etc.); could be affecting in various forms the development and therefore the length of each cnida, depending on the type or tissue of origin. Doumenc et al. (1989) concluded that for the genus *Telmatactis*, nematocyst biometry is associated to a significant degree with the "relative age" of the anemone. According with Chintiroglou (1996) in his study on *Edwardsia claparedii,* it appears that certain nematocyst dimensions of sea anemones can be affected by various physiological conditions. Also Karalis & Chintiroglou (1997) suggested that each sea anemone species uses a different mode of cnidome construction. More studies involving other species and types of cnida could shed light on this issue. The cnidome and the distribution and dimensions of the cnidocysts are now considered essential to any taxonomic description or redescription of a cnidarian species of most taxa (Fautin, 1988; Östman, 2000). However the extremely complex biometry of these subcellular organelles reduces their value as taxonomical tools at least in sea anemones. Usefulness of cnidome size investigations in Medusozoa (hydra, hydroids, jellyfishes, etc.) and Anthozoa (including sea anemones, cerianthids, zoanthids, corals, etc.) vary considerably. In Anthozoa, given the continuous growth of cnidocytes (perhaps explaining the large size variability), the taxonomical value of this character is greatly reduced and it requires time-consuming investigation, whereas in Medusozoa cnidocyst size may be a key to discrimination between sister species or geographically isolated populations (Östman et al, 1987). Further understanding of which morphological aspects are informative is needed for their systematic and phylogenetic value to be understood and their potential as reflections of evolution to be realized (Fautin, 2009).

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