# Cnidom in Ceriantharia, the exception or the rule?: new findings in the composition and micrometric variations of cnidocysts in sea anemones (#81353)



First submission

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# Cnidom in Ceriantharia, the exception or the rule?: new findings in the composition and micrometric variations of cnidocysts in sea anemones

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**Background.** Cnidaria is known for producing cnidocysts, a product of intracellular secretion found in all members of the phylum. Knowledge about cnidocysts has advanced since its discovery, mainly about its value in taxonomy. **Methods**. The present study aimed to understand the variability of the cnidom in the ceriantharians *Ceriantheomorphe brasiliensis* and *Ceriantus* sp.. For each individual, 30 intact capsules of each identified type of the following tissues were measured: marginal tentacles (4 from each individual), labial tentacles (4 from each individual), column, actinopharynx and mesenterial filaments. Each tissue was divided into three segments and the cnidome was analyzed. Statistics (mean, standard deviation, minimum and maximum) were made in all types of cnidae. The normality of the capsule length was tested by Shapiro-Wilk (α = 0.05), and due to the rejection of it, generalized linear mixed models (GLMM) were applied to test the cnidae size variations. **Results.** *Ceriantheomorphe brasiliensis* and *Cerianthus sp*. presented intra-specific variations in their cnidoms, both qualitatively and in cnidae sizes. However, the studied species also had qualitative intra-individual variations in their cnidoms between segments or sections of their structures. Some particular cnidocyst types, such as atrichs from the column of *C. brasiliensis* evidenced differences in their sizes between segments of the structure. In that case, the atrichs presented a gradient of size variations from the distal to the proximal segment of the column with the biggest size to the last one. **Conclusions**. This is the first study carried out on the variation of the composition and size of cnidocysts in Ceriantharia. Based on our results, we can conclude that the cnidom biometry presents intra-specific variation in the tube dwelling anemones *Ceriantheomorphe brasiliensis* and *Cerianthus* sp. which is coincident with the observed in other groups of sea anemones (*sensu lato*). Moreover, the species showed intra-individual

variations both in composition and size of cnidocysts. This characteristic was never reported with certainly before, not even in the more studied actiniaria sea anemones. This findings open a new vision about cnidae intra-individual variations that should be explored in others groups of sea anemones.

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# Abstract

**Background.** Cnidaria is known for producing cnidocysts, a product of intracellular secretion found in all members of the phylum. Knowledge about cnidocysts has advanced since its discovery, mainly about its value in taxonomy.

**Methods**. The present study aimed to understand the variability of the cnidom in the ceriantharians *Ceriantheomorphe brasiliensis* and *Cerianthus* sp.. For each individual, 30 intact capsules of each identified type of the following tissues were measured: marginal tentacles (4 from each individual), labial tentacles (4 from each individual), column, actinopharynx and mesenterial filaments. Each tissue was divided into three segments and the cnidome was analyzed. Statistics (mean, standard deviation, minimum and maximum) were made in all types of cnidae. The normality of the capsule length was tested by Shapiro-Wilk (α = 0.05), and due to the rejection of it, generalized linear mixed models (GLMM) were applied to test the cnidae size variations.

**Results.** *Ceriantheomorphe brasiliensis* and *Cerianthus* sp. presented intra-specific variations in their cnidoms, both qualitatively and in cnidae sizes. However, the studied

species also had qualitative intra-individual variations in their cnidoms between segments or sections of their structures. Some particular cnidocyst types, such as atrichs from the column of *C. brasiliensis* evidenced differences in their sizes between segments of the structure. In that case, the atrichs presented a gradient of size variations from the distal to the proximal segment of the column with the biggest size to the last one.

**Conclusions**. This is the first study carried out on the variation of the composition and size of cnidocysts in Ceriantharia. Based on our results, we can conclude that the cnidom biometry presents intra-specific variation in the tube dwelling anemones *Ceriantheomorphe brasiliensis* and *Cerianthus* sp. which is coincident with the observed in other groups of sea anemones (*sensu lato*). Moreover, the species showed intra-individual variations both in composition and size of cnidocysts. This characteristic was never reported with certainly before, not even in the more studied actiniaria sea anemones. These findings open a new vision about cnidae intra-individual variations that should be explored in others groups of sea anemones.

# Introduction

Cnidarians are known for producing cnidocysts, a product of intracellular secretion found in all members of the phylum, and are divided in three basic types: nematocysts, ptychocysts and spirocysts. These intracellular structures are responsible for directly assisting the capture of prey aggression and defense of the individual (Fautin, 2009), and in some cases tube construction (Mariscal, Conklin & Bigger, 1977; Stampar et al., 2015). Their diversity is observed in all cnidarians, since the cnidocysts have different shapes and sizes that are considered useful to characterize some genera or species (Fautin, 2009; Pica & Puce, 2017). The complete composition of cnidocysts of a species is called cnidom (Weill, 1934).

Knowledge about cnidocysts has advanced in different aspects since its discovery, among them its usefulness or not in taxonomy. As some types of cnidae are only found in specific groups, Stephenson (1929) states that is possible to differentiate species and/or genera of Actiniaria based on the characteristics of their cnidocysts. For Anthozoa, in general, the description of cnidocysts and their respective measurements were considered an ally in taxonomy by several authors (Carlgren 1940; Cutress 1955; Shick 1991) as long as Carlgren (1940) pointed out that no species description is complete unless it includes a description of the cnidom. However, a study conducted by Williams (1996) shows a variability of the cnidom in same species, questioning its usefulness for taxonomic purposes. It should be considered that in Actiniaria, for example, the size and type of cnidae may vary according to the environmental conditions in which the animal is submitted, as well as the size of the individual, in addition to the occurrence of distinct cnidae in some structures (Francis 2004; Acuña et al. 2007; Fautin 2009). Currently, studies on cnidom already cover statistical methods to test the intraespecific variations of the sizes of these structures, as presented by Garese et al. (2016). The intraespecific variation of cnidae sizes is the rule, at least, in actiniarian sea anemones (Garese, Carrizo & Acuña, 2016), and in consequence the taxonomic value of these data has little sustenance. However, quantitative analyses to distinguish closely related species or between morphotypes of the same species suggests that there is statistical significance between them in sizes of cnidae (González-Muñoz et al. 2017; Maggioni et al. 2021). On the other hand, other works have not found statistical support to distinguish specimens based on the differences between sizes of cnidae (González-Muñoz et al. 2018).

Although there is considerable knowledge in Anthozoa, in general there is no information about the variations of cnidom in Ceriantharia. The description of the cnidom for species of this subclass has been made by few authors, when is compared to anemones.  As an example: *Arachnanthus australiae* (Carlgren, 1937), *Pachycerianthus curacaoensis* (den Hartog, 1977), *Isarachnanthus nocturnus* (den Hartog, 1977) and *Botruanthus mexicanus* (Stampar, González-Muñoz & Morandini, 2016), but there are not studies that highlight its variability and micrometrics in details. Although limited, the study of the Ceriantharia cnidom helps as one of the main resources of identification due to the highly difficult of collecting these animals (Spier, Stampar & Prantoni, 2012). In consequence, the present study aimed to test the variability of the cnidom in *Ceriantheomorphe brasiliensis* (Mello-Leitão, 1919) and *Cerianthus sp.*, as study cases in Ceriantharia, including a novel intra-structure variation approach.

# Materials & Methods

## 2.1 Studied species

The cnidom of ten specimens of *Ceriantheomorphe brasiliensis* and seven specimens of *Cerianthus* sp. were analyzed (Table S1). All specimens were collected manually by the SCUBA method and preserved in 4% formaldehyde.

## 2.2 General cnidom analysis

For each specimen, 30 intact capsules of each cnida type identified (when was possible) were measured from the following structures in three segments (base, middle, tip) for each one: marginal tentacle (4 tentacles from each specimen), labial tentacle (4 tentacles from each specimen), column, actinopharynx and mesenterial filaments. In the case of *Cerianthus* sp. the total number of specimens studied for the different structures was variable due to their conservation status. The nomenclature was based on Mariscal (1974). A total of 25317 measurements were performed. All measurements were made using a 1000x objective in the Motic Images Plus 2.0 program. The cnidom was described and their sizes compared between individuals in each structure without discriminate between segments in this case. Statistics descriptive parameters (mean, standard deviation, minimum and maximum) were calculated in all types of cnidae present. The normality of the capsule length was tested by the Shapiro-Wilk test ($\alpha = 0.05$) on the residuals of a linear model with normal distribution. In cases where normality was accepted, the ANOVA test was used to test differences between individuals. In data sets in which normality was not accepted, a generalized linear model (GLM) was fitted with gamma distribution for errors and inverse as link function (following Garese, Carrizo & Acuña, 2016).

The model used was:

$$g\,(\textit{cnida length}) = \beta_0 + \beta_1\,(\textit{individual}) + \varepsilon$$

Then a T-test ($\alpha = 0.05$) for the $\beta_1$ parameters was produced to evaluate differences in the cnidae sizes between individuals.

## 2.3 Intra-structure cnidom composition

For this purpose each structure was separated an analyzed into three different segments: *base* (proximal), *middle*, *tip* (distal). The number of individuals that presented each type of cnidocysts on each segment was recorded, and a percentage of occurrence for all cnida type was calculated for each region in the different structures. Then, those percentages were used to produce radar charts using the R package ggplot2 (Wickham, 2016).

A statistical comparison of cnidae sizes between segments was just carried out in cases where the cnida type was present in the three segments of a structure of all specimens studied or at least in 90% of them. A linear mixed model (LMM) or a Generalized Linear Mixed Model (GLMM) was fitted testing the normality of the residuals of it. The general model form was:

$$\text{cnida length} \sim \beta_0 + \beta_1\,\text{segment} + (1 \mid \text{Individual}) + \varepsilon.$$

Where “*segment*” variable was considered as fixed effect and “*individual”* variable as random effect due to several measures were taken in each individual. In cases where normality was rejected a GLMM with Gamma distribution for errors and identity link function was fitted (following Garese, Carrizo & Acuña, 2016).Then, confident intervals of cnidae sizes for each segment was calculated from the LMM or GLMM, and compared.

Also Kernel density plots (Sheather, 2004) were produced to explore graphically the variations of cnidae sizes between segments. These graphics were mainly useful in those types that had an adequate representativeness in the three segments in several individuals but which it was not possible to apply the statistical approach according to the criteria adopted (presence in 90% of individuals/segment). The density plots were obtained for those cnidocysts that were present in the three segments in more than 70% of the individual sampled.
All statistical analyses were performed with the R program (R Core Team, 2020). The models were produced with the R package 'lme4' (Bates et al., 2015). All graphics were made using ggplot2 R package (Wickham, 2016).

# Results

## 2.1 *Ceriantheomorphe brasiliensis*

### 2.1.1 General cnidom analysis

The cnidom of *Ceriantheomorphe brasiliensis* presents a total of nine types of cnidae (Fig. 1), three more than reported by Spier, Stampar & Prantoni (2012). The cnidae found are reported in Table 1. From all data-sets obtained (all different cnidae types in the structures sampled; 20 data-sets in total, 3 no analyzed due to a low "n") just 4 fitted to a normal distribution (Table 1). These data-sets corresponded to microbasic b-mastigophore I (b I) and III (b III) from actinopharynx, microbasic b-mastigophore V (b V) from column and atrich from marginal tentacles. The ANOVA produced to test the variation between individuals of the cnidae sizes indicated significant differences in all cnidocysts analyzed [Actinopharynx: b I (F = 39.07; $P < 2e\text{-}16$), b II (F = 61.6; $P < 2e\text{-}16$); Column: b_5 (F = 109.8; $P < 2e\text{-}16$); Marginal tentacle: atrich (F = 135.3; $P < 2e\text{-}16$)].
For the remaining16 data-sets, that were not fitted to a normal distribution, GLMs were applied to test differences between individuals in cnidae sizes. All types of cnidocysts showed significant differences between individuals (Table S2).
All the cnidocysts analyzed, independently of type and structure where they came from, showed differences in their sizes between individuals.

### 2.1.2 Intra-structure qualitative variations

About qualitative composition of cnidom in *C. brasiliensis,* the different segments (base, middle, tip) of the structures showed some patterns of variations.
The actinopharynx included *atrichs* and *microbasic b-mastigophores I* and *III* in its cnidom. The atrichs were observed in the "base" of practically all specimens

conforming the cnidom of the segment along with the less frequent *b-mastigophores I* and *III*. Around 50% of individuals presented *atrichs*, *b I* and *b III* in the "upper" (*tip*) region of actinopharynx. While the middle section presented *atrichs* and *b III* in the 50% of specimens, whereas *b I* were rare being present in less than 30% of the individuals for the segment (Fig 2A, Table S3).

The column showed a consistent presence of *atrichs* along the three segments. Also, appeared *pticocyst* mainly in the middle of the column, in the 50% of the specimens and in a little percentage in the base of the specimens, while were absent in the upper section of the structure. The *microbasic b-mastigophore I* was present exclusively at the tip of the column being part of the cnidom where the *atrichs* were dominant. *Holotrichs*, *b-mastigophores V* and *VI* presented a very low frequency of ocurrence in the three segments, thus they could be defined as rare (Fig. 2A, TableS3).

The metamesenteries showed a uniform cnidom with *microbasic b-mastigophores I* in the three sections in the 50 % of individuals, meanwhile also appeared *microbasic b-mastigophores IV* at the tip in around 30% of specimens (Fig. 2A, TableS3).

In labial tentacles both the *microbasic b-mastigophores I* y *III* appear as uniform and very frequent between the individuals in the three segments (Fig. 2A, TableS3). The *microbasic b-mastigophores II* were generally present in the middle and tip of the labial tentacles of all specimens, whereas in the base were present in the 50% of individuals. The pattern of variation of the *atrichs* was the most particular in labial tentacles. This cnida type was present in high percentage of the specimens in the base of the structure, meanwhile was observed in less than 30% of the individuals in the segments middle and tip. On the other hand, the *microbasic b-mastigophores V* appeared very rarely in the three segments (Fig. 2A, TableS3).

The marginal tentacles showed a similar pattern than the labial tentacles, with several *b-mastigophores* distributed in the three segments and a particular pattern for the *atrichs.* However, unlike labial tentacles *atrichs* were present exclusively in the base of marginal tentacles, in the 70% of the individuals, and they were absent in middle and tip sections. Also, the *microbasic b-mastigophores* where randomly present in near 50% of the specimen both the middle and tip of the structure. In the base, just *microbasic b-mastigophores V* and *VI* were observed in the 50% of the specimens, meanwhile the presence of the rest of *b-mastigophores* was rare (Fig. 2A, TableS3).

### 2.1.3 Intra-structure cnidae size variations

As was mentioned in section 2.1.2, uniquely the atrichs of the column of *Ceriantheomorphe brasiliensis* were observed in all specimens at the three-segment sampled. Hence, a LM and LMM were fitted for those data sets due to the adjusted to a normal distribution (*P* = 0.2378, α = 0.05). The LMM resulted the best model (Table S4) and its form was:  Atrich length ~ segment+ (1 |Individual). The variable "Individual" resulted significant comparing the LMM versus the null LM (Atrich length ~ segment); its standard deviation and those of the residuals of the model are shown in Table S5. The mean estimates by the LMM shown that the sizes of atrichs from the base are slightly larger than those from middle segment, however they were quite larger if compares with those from the distal zone (Table S6). The confident intervals of the LMM clearly evidenced a gradient in the length of atrichs from proximal to distal segments of the column of *C. brasiliensis* being those from the distal zone the smallest. Also, the CI from the distal segment presented the particularity that its higher size values were similar to the smallest sizes from the middle segment. Moreover, the CI of the atrichs from the distal zone was absolutely not overlapped with the one from the basal zone. Between the CIs from middle and basal zone there were a little overlapping around the larger and smaller sizes, respectively (Table S6).

The comparisons between segments were also carried out for the microbasic b-mastigophore I and III from the labial tentacles of *C. brasiliensis.* They were found practically in all individuals in the three segments with the exception of one of ten specimens at “tip” segment, (see Table S3). For both data sets the normality of residuals of a linear model was tested and rejected (microbasic b-mastigophore I: W = 0.99827, $P$ = 0.001; microbasic b-mastigophore III: W = 0.98645, $P$ < 0.001). In consequence generalized linear models were fitted. For both types the GLMM was the best model (Table S4) taking the form as follow: microbasic b-mastigophore length ~ segment + (1 | Individual). The incorporation of the variable “individual” as random effect resulted significant, its standard deviation and those of the residuals of the GLMM are shown in Table S5.

The CIs for the GLMM showed a similar pattern in both microbasic p-mastigophores I and III. A clear superposition of the size distribution of the cnidocysts between the three segments of the labial tentacles for both cnida types was observed (Table S6).

The differences of sizes between segment was also explored by mean of density plots (Fig. 3A), including those types with high representativeness but where not possible fit the models due to the absent in several specimens in some segments. This was just the case of microbasic b-mastigophores I from marginal tentacles, beyond the analyzed atrichs of column and microbasic b-mastigophores I and III of labial tentacles. For the atrichs of column the density plots reflected the statistic differences observed in the models, where the distribution of sizes in the segments exhibit a gradient from smallest to largest sizes from distal (tip) to base segment (Fig. 3A). Meanwhile, for the microbasic b-mastigophores both for those of the labial tentacles and that of the marginal tentacles the graphs showed a clear superposition of the distribution of size between segments as observed in the fitted models (Fig. 3A).

## 2.2 *Cerianthus* sp.

### 2.2.1 General cnidom analysis

The cnidom of *Cerianthus* sp. presents eight types of cnidae (Fig. 4). Also spirocysts were found in the tentacles although were not included in the analyses. The cnidae found are reported in Table 2.
ANOVA tests showed significant differences in all cnidocysts analyzed whose length adjusted to normal distribution [Marginal tentacles: atrich (F = 19.58; *P* = 3.9e-08); b VI: (F = 17.15; *P* = 4.2e-05) Actinopharynx: b I (F= 62.13; *P* < 2e-16); Column: pticocyst (F = 210.8; *P* < 2e-16), atrichs I (F = 20.48; *P* < 2e-16), atrichs II (F = 2.75; *P* = 0.029)].
GLMs were applied to evaluate differences between individual for resting datasets of cnidocysts: atrichs from actinopharynx and labial tentacles; microbásic b-mastigophore I from mesenteries, labial and marginal tentacles; and microbásic b-mastigophore II and III from both tentacles. In all cases significant differences were observed between individuals (Table S7).

### 2.2.2 Intra-structure qualitative variations

Concerning to the qualitative composition of cnidom in the different segments of the specimens of *Cerianthus* sp. some variations were observed mainly in the marginal and labial tentacles. While in actinopharynx, column and metasenteries the pattern was quite uniform between segments (Fig. 2B).
The cnidom of the actinopharynx of *Cerianthus* sp*.* was formed by *atrich*s and *microbasic b-mastigophores I* and *III*. The *atrich* was the main cnida type in the structure being observed in all specimens at all sections of it. Also, the *microbasic b-mastigophore I* appeared in a high percentage of individuals (near 70%) at the basal segment (base) of the pharynx, while in the half of specimens in the middle and tip sections. The *microbasic b-mastigophore III* was rare and observed in very few individuals at all regions. A similar pattern of composition of the cnidom was observed between the segments (Fig. 2B, Table S8).
In column, the cnidom is compound mainly by two types of atrichs, and pticocysts. A similar pattern is observed between segments, where all cnidae types appear in near the 50% of individuals at the three segments (Fig. 2B, Table S8). The *microbasic b-mastigophore I* was also part of the cnidom but appearing in a very low percentage of individuals at the upper segment (*tip*) while it was absent in the base and middle segments. The *atrichs* showed a quite uniform distribution along all the structure, being both types founded in around 50% of specimens in the three segments, with the exception of the *atrichs I in the middle segment* of the individuals where were less frequent. Around 50% of individuals evidenced *pticocysts* at the middle of the column, and in a little lower percentage at the tip (distal) and ~~moreover~~ at base (in around 30%

of individuals). Very low percentage of individuals presented *microbasic b-mastigophores I*, in those cases mainly at middle and tip of the column, although they must be considered rare (Fig. 2B, Table S8).

In labial tentacles (Fig. 2B, Table S8), the *microbasic b-mastigophores I* and *III* were present in the three segments in high percentage (around 70-80%) of individuals. The others *b* types (*II* and *V*) were found in few individuals (around 25% or less) in middle and tip sections, while were absent at the base. The atrichs marked a clear pattern of variation between segments of the labial tentacles. This type of cnidocyst was observed in almost all individuals in the base of the labial tentacles, meanwhile it was found in scarce number of specimens at middle and tip sections (Fig. 2B, Table S8).

Besides, the marginal tentacles showed several types of microbasic b-mastigophores in around 50% of individual in middle and tip regions. At base, just *b V* and *b VI* were observed also in around 50% of individuals. The pattern of distribution of the different types of b-mastigophores was quite variable between segments. The atrichs were present in the majority of specimens exclusively at the base segment, and absent at the middle and tip sections of the marginal tentacles. As was the case in labial tentacles, the marginal tentacles evidenced as clear variation in the distribution of atrichs between segments (Fig. 2B, Table S8).

In the metamesenteries the *microbasic b-mastigophore I* was the unique cnidocyst found and it was observed in totally specimens at all segments of the structure (Table S8, graph not included).

### 2.2.3 Intra-structure cnidae size variations

For *Cerianthus* sp. there were just two types of cnidocysts that were present in the three segments of all specimens analyzed. These were the cases of atrichs from actinopharynx and microbasic b-mastigophores from metamesenteries in six and three specimens studied, respectively (Table S8). Both data sets of cnidae sizes did not fit to normal distribution [atrichs (actinopharynx): W = 0.98527, $P < 0.001$; microbasic b-mastigophores I (metamesenteries): W = 0.91328, $P$ = 2.727e-11], then GLMs were fitted and compared versus a GLMM to obtain the best model. For atrichs the GLMM, including the variable “individual” as random effect, resulted the best model (Table S9), and standard deviation of that variable is showed in Table S10. Meanwhile, for the microbasic b-mastigophores the best model was the GLM being not significant the variable “individual” as random effect (Table S9).

The CIs of the model for the atrichs from actinopharynx showed a partial superposition of the sizes of cnidocysts between the three segments (Table S11). The middle segment presented the lowest values (24.9 µm) meanwhile the CI for proximal (base) segment was almost completely overlapped with it. The distal segment evidenced the highest values (38.2 µm) and was also superpositioned with both previous segments at exception of the highest values (Table S11).

The exploration of differences between segments of cnidae sizes using density plots included to the microbasic b-mastigophores I from labial tentacles besides the two types analyzed above by mean of statistic models. The density plots for atrichs from actinopharynx showed wide curves and not evident range of distribution, probably explained by the low *n* (3), although an overlapping of sizes between segments was observed (Fig. 3B). Both microbasic b-mastigophores I, from metamesenteries and labial tentacles, evidenced clear ranges of distribution of sizes and overlapping between segments (Fig. 3B). All graphs were consistent with the results of the fitted models.

# Discussion

The present paper has as novelty a new methodology for sampling cnidocysts in the individuals exploring different segments or regions into each structure. The classical approach to study the cnidom in sea anemones implies to sample cnidocysts in all the structures present in the species, taking portions of tissues from a particular zone of them (Williams, 1996). For instance, the middle region of the column, the tip of tentacles, or the middle zone of actinopharynx, etc. That methodology supposed certain uniformity of the presence of determinate cnidae types along a structure. However, the results of this work, in base to a new methodology implemented, revealed that supposed uniformity intra-structure of the cnidom composition is not true at least in the studied ceriatharian sea anemones. There were several cnida types that presented not uniform distribution between segments of structures of the sea anemones. This is evident pointing that just the atrichs from the column of 23 total cnida types sampled in *Ceriantheomorphe brasiliensis* were present in the three segments in all the specimens analyzed. This qualitative variation could be explaining the new cnidae types found here in relation to the reported by Spier, Stampar & Prantoni (2012), probably due to the wider sampling used. Moreover, also the atrichs from actinopharynx of *Cerianthus* sp. were present in the three zones sampled (base, middle and tip) in all specimens explored but, in this case, the "n" was just equal three. For the rest of cnidocysts of that species, the conformation of the cnidom presented variability between segments even with the relative low number of specimens sampled. The clearest patterns of variability were observed in the labial and marginal tentacles similarly in both species; where the atrichs were present almost exclusively at the base of tentacles but not in the middle and tip zones. A possible explanation to that pattern for the atrichs could be related to different functions of the distinct regions of the tentacles. Then, according to our findings the classical approach to establish the cnidom composition of a sea anemone species is questioned due to a qualitative variation of the cnidom into a same structure.

Cnidom is usefull in taxonomic studies, although its reliability for this purpose is questioned, especially for the cnidae sizes data in sea anemones (Fautin, 1988; Williams, 1996, 1998, 2000; Acuña et al., 2003, 2004; Acuña, Excoffon & Ricci, 2007; Acuña, Ricci & Excoffon, 2011). The statistic approach of that kind of data have been widely approached. Some authors have reported the normal distribution of cnidae sizes (Williams, 1996, 2000; Ardelean & Fautin, 2004). However, other authors have found that biometry data of cnidocysts may not fit normal distribution (Acuña et al. 2003; 2004; Acuña, Excoffon & Ricci, 2007; Garese, Carrizo & Acuña, 2016). Based on the results of this study, both possibilities were observed in ceriantharia sea anemones: Generalized Linear Model and ANOVA were applied due to the results of normality which is coincident with the observed in actiniaria and corallimorpharia sea anemones by Garese et al. (2016).

Even through different statistical approaches, the sizes of the cnidocysts varied between individuals both *Cerianteomorphe brasiliensis* and *Ceriantheus* sp. in agreement with the results observed in Actiniaria sea anemones (Williams, 1996, 2000; Acuña et al., 2003, 2004; Francis 2004; Acuña, Excoffon & Ricci, 2007; Acuña & Garese 2009; Acuña, Ricci & Excoffon, 2011) and mentioned as rule by Garese, Carrizo & Acuña (2016).

About the size variations between segments just some cnida types were well represented in the specimens studied to analyze them statistically. The above mentioned atrichs from column, and microbasic b-mastigophores I and III from labial tentacles of *C. brasiliensis* and the atrichs from actinopharynx and microbasic b-mastigophores I from metamesenteries of *Ceriantheus* sp. were statistically studied. Of all of them, uniquely the atrichs from column of *C. brasiliensis* evidenced differences in sizes between the base, middle and tip segments of the structure. That cnida type showed a gradient of size variation from base to distal segments of higher to lower sizes respectively. A similar pattern was found by Ardelean & Fautin, 2004 for the microbasic b-mastigophores from the column of one specimen of the sea anemone *Actinodendron arboreum* (Quoy & Gaimard, 1833). Robson (1988) suggests that the variation of cnidae sizes may be a result of cnidogenesis (stages of development of the cnidae), and the high variability in the sizes and types of cnidocysts between individuals of the same species can be explained by the interaction between the demand and replacement of the product of intracellular secretion. Then, a possible explanation of the gradient observed intra-structure in the column of *Ceriantheomorphe brasiliensis* could be attribute to the burrowing form of life of the ceriantharian sea anemones. That makes the distal zone of the column more exposed and the use and replace of the cnidom could be more frequent in the zone provoking to find more cnidocyst not completely developed with smaller sizes.

The atrich was the most abundant type of cnidae in both species, more than the ptichocyst (exclusive cnidae of Ceriantharia). Since the formation of the tube in Ceriantharia can be done in different ways according to species, the ptichocyst may be in a specific development stage according to the strategy used by the animal (Mariscal, Conklin & Bigger, 1977; Stampar et al., 2012).

# Conclusions

This is the first study carried out on the variation of the composition and size of cnidocysts in Ceriantharia with considerable sample number. Based on the results, we can conclude that the size of cnidocysts in ceriantharians sea anemones vary intra-specificaly as in other groups (Acuña et al., 2003, 2004; Francis 2004; Acuña, Excoffon & Ricci, 2007; Acuña & Garese 2009; Acuña, Ricci & Excoffon, 2011; Garese, Carrizo & Acuña, 2016). The data obtained in this study reinforce the observation of authors such as Schimdt (1972) and Fautin (2009) that the variation of the cnidom between individuals of the same species is sometimes higher than different species, but also prove that could exist both qualitative variations of the cnidom and cnidae sizes variations intra-structure of an individual of a species. The new findings presented open a new question for further investigations about if these variations be an exception in ceriantharian or could be found in other sea anemones such as the actiniaria ones which could call in question all the previous descriptions of the cnidom of sea anemones species.

# Acknowledgements

We thank to Lic. Nicolás Vazquez for his suggestion in graphic presentations and Fabrizio Scarabino for providing specimens of *Cerianthus* sp. from Uruguay.

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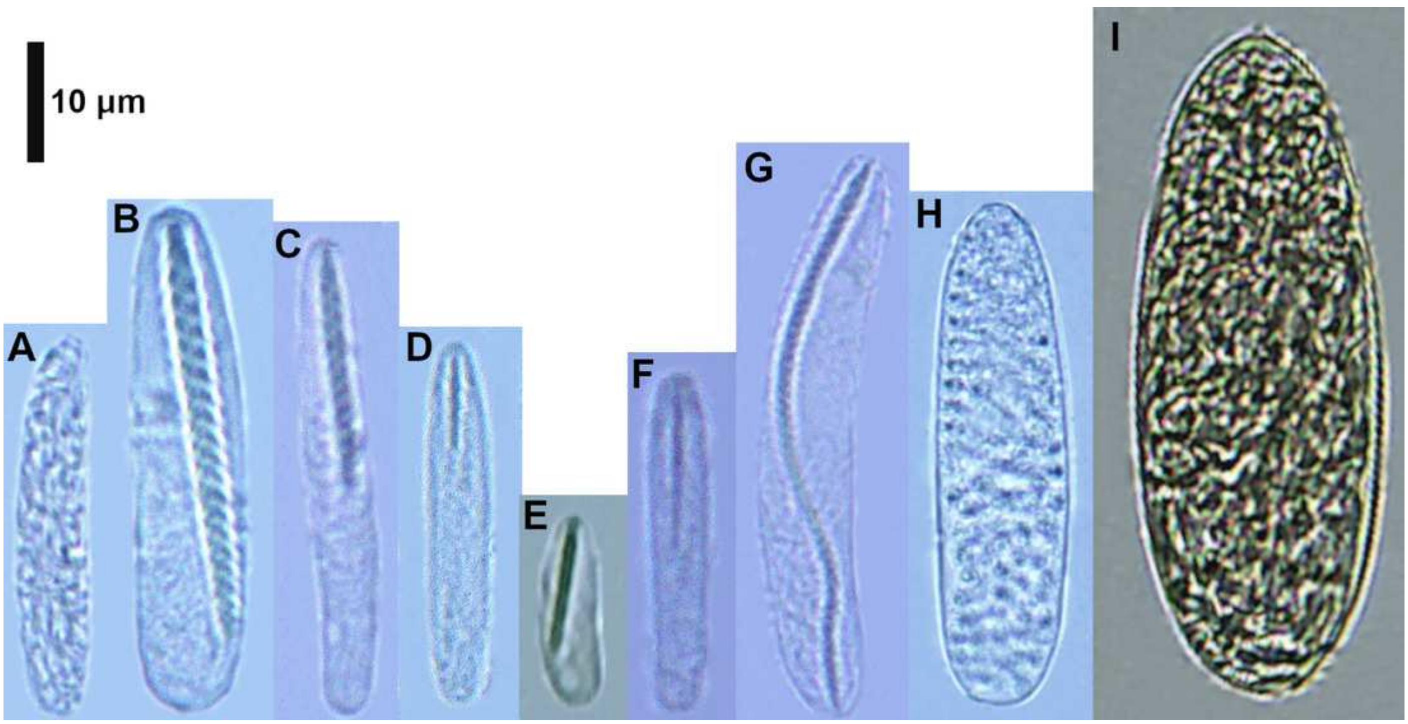
# Figure 1

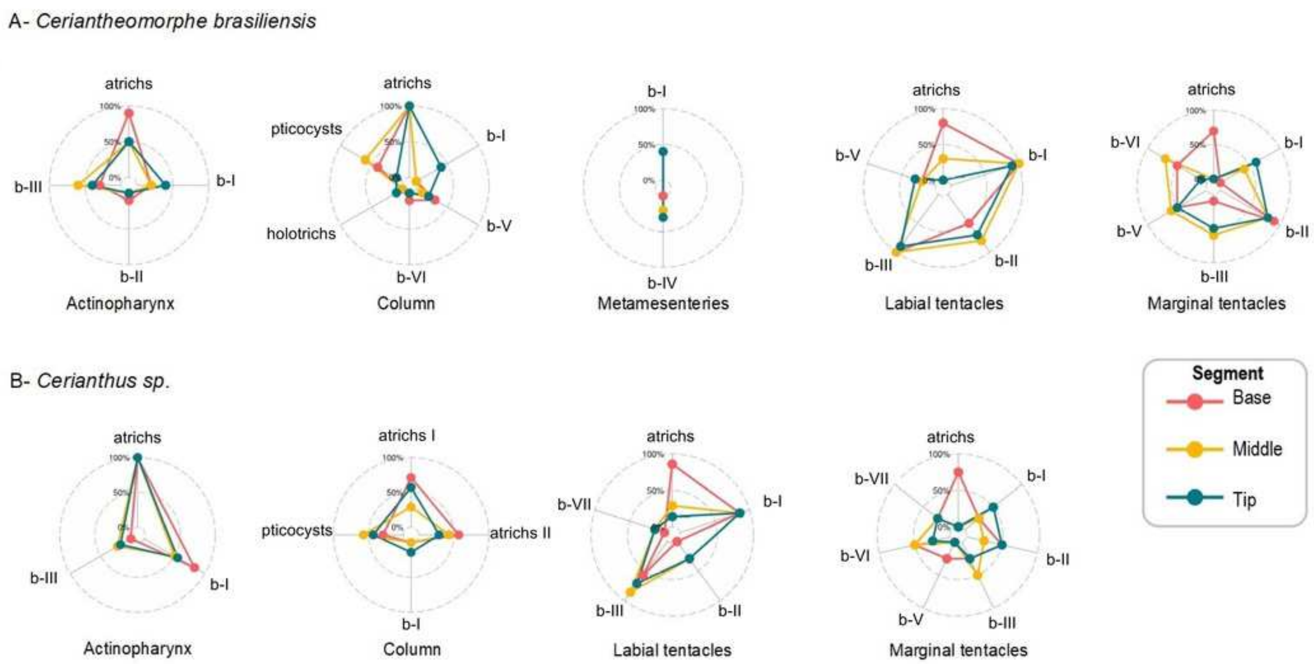
Cnidocysts of *Ceriantheomorphe brasiliensis.*

(A) Atrich. (B) Microbasic b-mastigophore I. (C) Microbasic b-mastigophore II. (D) Microbasic b-mastigophore III. (E) Microbasic b-mastigophore IV. (F) Microbasic b-mastigophore V. (G) Microbasic b-mastigophore VI. (H) holotrich. (I) Pticocyst.



# Figure 2

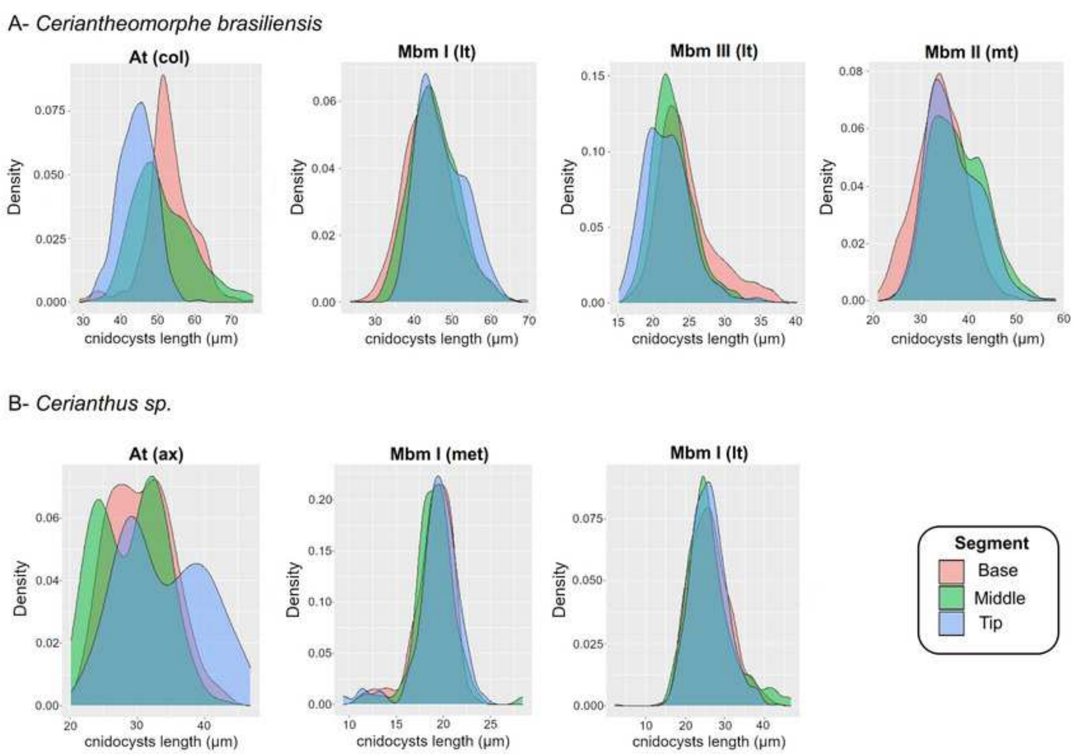
Intra-structure qualitative variations of the cnidoms of *Ceriantheomorphe brasiliensis* (A) and *Ceriantheus* sp. (B)



# Figure 3

Density plot of cnidae sizes in the different segments of the structures of *Ceriantheomorphe brasiliensis* (A) and *Ceriānthus* sp. (B).

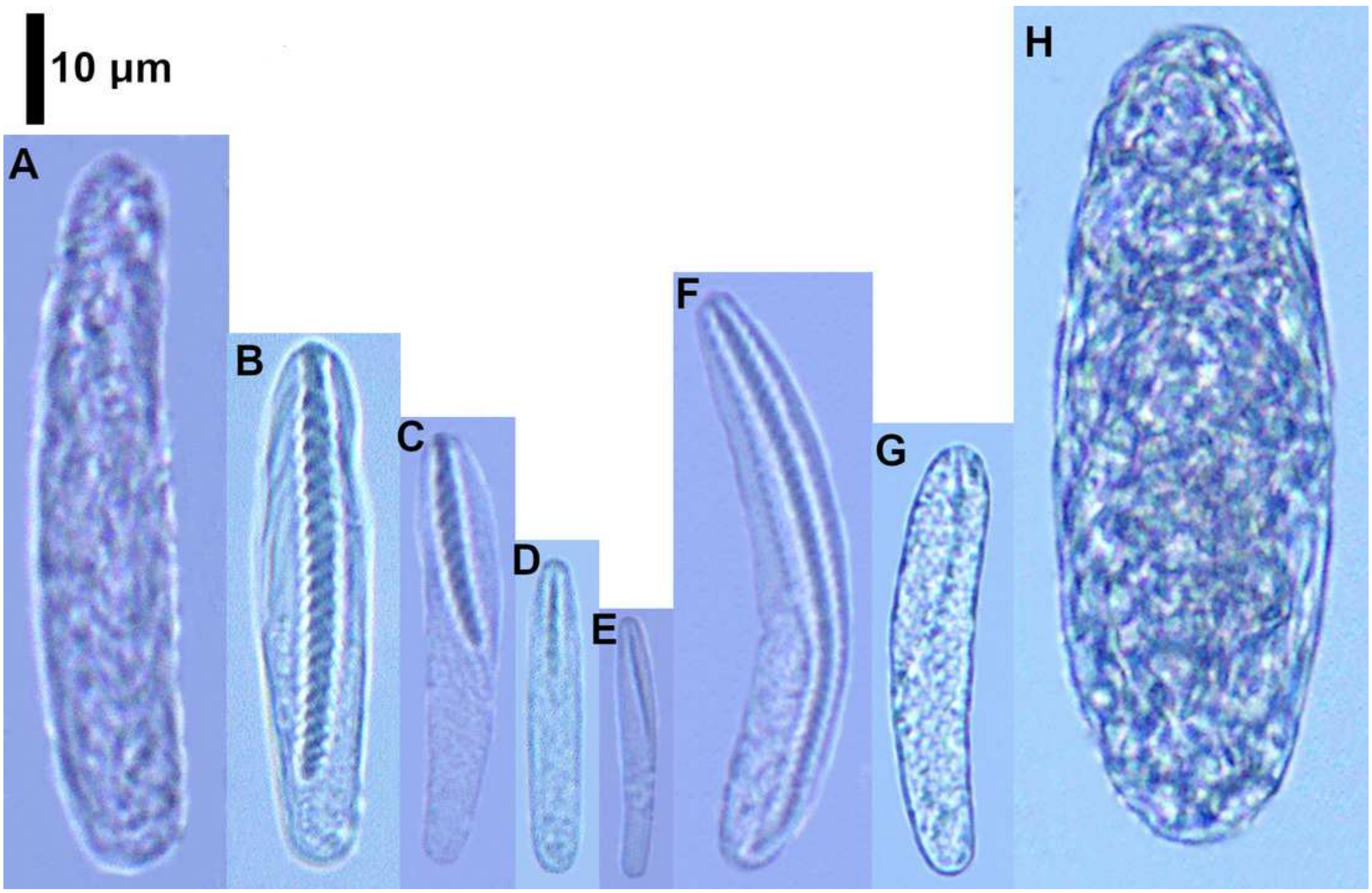
At = atrich, Mbm = microbasic b-mastigophore, (col) = column, (lt) = labial tentacles, (mt) = marginal tentacles, (ax) = actinopharynx, (met) = metamesenteries.



# Figure 4

Cnidocysts of *Cerianthus* sp.

(A) Atrich. (B) Microbasic b-mastigophore I. (C) Microbasic b-mastigophore II. (D) Microbasic b-mastigophore III. (E) Microbasic b-mastigophore V. (F) Microbasic b-mastigophore VI. (G) Microbasic b-mastigophore VII. (H) Pticocyst.



## Table 1 (on next page)

Cnidom composition of *Ceriantheomorphe brasiliensis.*

**Table 1:**

**Cnidom composition of *Ceriantheomorphe brasiliensis.***

n = number of specimens that present the cnida/total number of specimens. N = total number of sized cnidocysts. Underlined *P*-values significant at α=0.05; # Shapiro Test was not applied due to low N.

| **Tissue / Cnida type** | **Range [min-max]**<br>**Length (mean ± SD) x Width (mean ± SD) (µm)** | ***n*** | ***N*** | ***P*-value** |
|---|---|---|---|---|
| **Actinopharynx** | | | | |
| atrich | 27.09-56.28 (38.74 ± 5.80) x 3.96-11.79 (6.98 ± 1.46) | 4/10 | 570 | 7.42E-04 |
| microbasic b-mastigophore I | 31.97-67.98 (47.99 ± 6.94) x 3.12-14.52 (8.45 ± 2.64) | 6/10 | 240 | 0.054 |
| microbasic b-mastigophore II | 37.53-47.29 (41.93 ± 2.80) x 4.45-7.16 (6.18 ± 0.66) | 1/10 | 30 | # |
| microbasic b-mastigophore III | 19.38-45.92 (30.41 ± 5.29) x 1.86-5.59 (3.67 ± 0.75) | 9/10 | 389 | 0.854 |
| **Column** | | | | |
| atrich | 28.86-75.74 (49.62 ± 7.45) x 4.97-22.44 (12.26 ± 2.63) | 10/10 | 895 | 4.54E-04 |
| microbasic b-mastigophore I | 26.36-46.89 (34.02 ± 4.03) x 4.13-10.07 (6.76 ± 1.05) | 4/10 | 120 | 0.000454 |
| microbasic b-mastigophore V | 23.52-36.02 (29.04 ± 2.98) x 2.29-5.69 (3.76 ± 0.65) | 3/10 | 179 | 0.815 |
| microbasic b-mastigophore VI | 46.55-58.46 (53.15 ± 2.71) x 4.37-8.02 (5.82 ± 0.86) | 1/10 | 30 | # |
| holotrich | 33.06-55.04 (46.63 ± 4.81) x 6.23-16.34 (10.33 ± 1.91) | 1/10 | 30 | # |
| ptychocyst | 53.30-92.93 (71.97 ± 8.02) x 16.08-44.12 (28.16 ± 4.36) | 8/10 | 330 | 6.91E-03 |
| **Metamesenteries** | | | | |
| microbasic b-mastigophore I | 32.02-73.54 (57.12 ± 7.02) x 6.07-18.54 (12.45 ± 2.35) | 4/10 | 360 | 1.91E-12 |
| microbasic b-mastigophore IV | 13.55-30.21 (19.67 ± 3.44) x 3.04-8.28 (4.77 ± 1.12) | 4/10 | 150 | 0.001212 |
| **Labial Tentacles** | | | | |
| atrich | 25.54-49.99 (37.33 ± 4.10) x 3.57-11.41 (6.71 ± 1.28) | 8/10 | 868 | 3.73E-02 |
| microbasic b-mastigophore I | 23.38-69.61 (45.36 ± 6.44) x 4.32-14.76 (8.53 ± 1.66) | 10/10 | 3180 | 0.004 |
| microbasic b-mastigophore II | 19.20-51.93 (32.74 ± 4.26) x 2.69-8.31 (4.88 ± 0.84) | 8/10 | 1620 | 2.38E-10 |
| microbasic b-mastigophore III | 15.18-46.49 (23.47 ± 3.99) x 1.46-6.80 (3.02 ± 0.60) | 10/10 | 2369 | 5.86E-16 |
| microbasic b-mastigophore V | 14.82-28.39 (21.50 ± 2.76) x 1.72-5.08 (3.18 ± 0.61) | 4/10 | 360 | 0.004 |
| **Marginal Tentacles** | | | | |
| atrich | 25.54-62.55 (41.76 ± 7.40) x 4.37-18.33 (7.64 ± 2.50) | 7/10 | 570 | 0.16 |
| microbasic b-mastigophore I | 50.32-98.38 (71.03 ± 8.47) x 1.09-18.86 (12.12 ± 2.32) | 7/10 | 600 | 0.001 |
| microbasic b-mastigophore II | 20.94-58.23 (36.12 ± 5.66) x 2.87-9.50 (5.42 ± 1.03) | 9/10 | 2248 | 6.80E-06 |
| microbasic b-mastigophore III | 15.67-48.93 (26.49 ± 6.15) x 1.76-6.50 (3.54 ± 0.87) | 10/10 | 780 | < 2.2e-16 |
| microbasic b-mastigophore V | 15.08-35.75 (23.68 ± 4.31) x 1.49-5.54 (3 ± 0.58) | 7/10 | 1138 | 2.51E-09 |
| microbasic b-mastigophore VI | 29.56-74.05 (53.17 ± 9.78) x 1.71-9.12 (5.45 ± 1.36) | 7/10 | 687 | 0.0006 |

## Table 2(on next page)

Cnidom composition of *Cerianthus sp.*

**Table 2:**

**Cnidom composition of *Cerianthus sp.***

n=number of specimens that present the cnida/total specimens; N= total number of cnidocyst. Underlined *P*-values significant at α=0.05; # Shapiro Test was not applied due to low N.

| Tissue / Cnida type | Range [min-max]<br>Length (mean ± SD) x Width (mean ± SD) [µm] | *n* | *N* | *P*-value |
|---|---|---|---|---|
| **Actinopharynx** | | | | |
| atrich | 20.17-46.82 (31.09 ± 5.67) x 2.56-9.82 (5.65 ± 1.32) | 6/6 | 514 | 2.55e-08 |
| microbasic b-mastigophore I | 21.41-54.93 (34.68 ± 5.46) x 3-10.24 (6.01 ± 1.21) | 6/6 | 270 | 0.2058 |
| microbasic b-mastigophore III | 16.52-36.90 (25.54 ± 5.16) x 1.61-4.55 (2.83 ± 0.65) | 1/6 | 52 | # |
| **Column** | | | | |
| atrich II | 26.46-40.80 (33.72 ± 3.15) x 5.27-16.36 (9.33 ± 1.77) | 6/7 | 257 | 0.65 |
| atrich II | 40.68-62.77 (50.56 ± 4.24) x 6.02-20.90 (2.86 ± 3.74) | 5/7 | 198 | 0.1 |
| microbasic b-mastigophore I | 23.26-38.91 (30.35 ± 2.78) x 4.23-9.21 (6.02 ± 0.98) | 1/7 | 30 | # |
| ptychocyst | 26.33-81.38 (55.43 ± 10.98) x 11.50-34.18 (21.17 ± 3.61) | 5/7 | 250 | 0.197 |
| **Metamesenteries** | | | | |
| microbasic b-mastigophore I | 9.43-28.42 (19.12 ± 2.27) x 2.59-6.81 (4.36 ± 0.64) | 3/3 | 266 | 3.53e-10 |
| **Labial Tentacles** | | | | |
| atrich | 15.66-46.41 (25.29 ± 3.71) x 2.67-6.88 (4.41 ± 0.63) | 6/7 | 585 | 2.20E-16 |
| microbasic b-mastigophore I | 21.21-46.61 (26.07 ± 5.14) x 3.89-9.77 (4.80 ± 1.08) | 6/7 | 1816 | 2.20E-16 |
| microbasic b-mastigophore II | 16.26-41.42 (24.56 ± 6.34) x 1.92-5.81 (3.32 ± 0.76) | 3/7 | 127 | 9.00E-03 |
| microbasic b-mastigophore III | 10.11-34.44 (17 ± 3.80) x 1.10-4.90 (2.08 ± 0.48) | 6/7 | 982 | 2.20E-16 |
| microbasic b-mastigophore VII | 16.20-32.87 (22.17 ± 3.44) x 2.03-6.38 (3.47 ± 0.97) | 1/7 | 188 | # |
| **Marginal Tentacles** | | | | |
| atrich | 19.26-31.94 (26.14 ± 2.39) x 3.54-7.54 (5 ± 0.83) | 3/4 | 129 | 0.581 |
| microbasic b-mastigophore I | 23.37-47.16 (32.68 ± 4.92) x 3.87-8.26 (5.83 ± 0.96) | 2/4 | 298 | 0.002 |
| microbasic b-mastigophore II | 12.74-32.26 (22.67 ± 3.70) x 1.79-6.05 (3.57 ± 0.64) | 3/4 | 647 | 0.014 |
| microbasic b-mastigophore III | 13.33-26.09 (19.17 ± 2.43) x 1.43-2.94 (2.18 ± 0.24) | 2/4 | 294 | 0.004 |
| microbasic b-mastigophore V | 12.77-17.87 (15.14 ± 1.41) x 1.60-2.46 (2.04 ± 0.21) | 1/4 | 20 | # |
| microbasic b-mastigophore VI | 23.83-39.06 (32.90 ± 5.52) x 2.58-6.04 (4.33 ± 0.70) | 2/4 | 376 | 0.085 |
| microbasic b-mastigophore VII | 17.39-26.72 (22.80 ± 1.72) x 2.49-5.17 (3.66 ± 0.38) | 1/4 | 365 | <0.001 |