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ARTICLE

Statistical analysis on the cnidome of genus *Hydra* using Generalized Linear Models

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Abstract. In the systematics of cnidarians, the different types of cnidocysts are considered an important taxonomic character. In *Hydra,* the four types of cnidocysts found in the ectoderm, concentrated in tentacles and their measurements, together with other morphological and reproductive characteristics, are very important for the taxonomy of the species. In this study, we explore in detail the biometric and statistical characteristics of the cnidome of three species of *Hydra* collected in three different environments for each climate season. A total of 17,378 capsules were measured. We used ANOVA test and Generalized Linear Model to analyze the distribution and differences reflected in each cnidome, considering the factors "individuals", "season", "lagoon" and "species". The results were clear: the cnidome keep specific information that, together with other taxonomic characteristics, allows us to discern between species of different groups. The same happens with cnidome of the same species but from different lagoons or climatic seasons: we observed a variation of parameters for each type of cnidocyst that could differentiate "ecological races", since these differences are not enough to declare different species.

Keywords. Argentina; Cnidocysts; Freshwater cnidarians; Shallow lakes.

INTRODUCTION

Cnidocysts are *the sine qua non* condition of the phylum Cnidaria; all cnidarians and only they produce them (Fautin, 2009). In the systematics of this group, the different types of cnidocysts have been considered an important taxonomic character and the cnidome have been defined as the inventory of all the cnidocysts or cnidae present in a species (Weill, 1934a). Cnidocysts are found in the ectoderm of hydrozoans and in *Hydra,* 95% of them are concentrated in the tentacles, growing in number from the base towards the tips (Bode & Flick, 1976). In this genus, we found four types: stenoteles, desmonemes, holotrichous isorhiza and atrichous isorhiza. The first two types are involved in feeding. Stenoteles pierce prey like a harpoon (even those with strong exoskeletons), injecting a certain amount of venom. Desmonemes evert their filament that strongly adheres to the surface of the prey (Burnett, 1973). The atrichous isorhiza are involved in the walking movements, responsible for adhering the tentacles and pedal disc in each "step" (Toppe, 1910; Ewer, 1947). Finally, holotrichous isorhiza are discharged in the presence of organisms that are not normally ingested by hydras and that do not cause a nutritional stimulus, such as *Paramecium.* This would indicate that the function of the holotrichous isorhiza could be one of the first defense reactions in nature (Burnett, 1973).

Cnidocysts are organized in bundles within the ectodermal epithelial cells (Slautterback, 1967). Ten to twenty cnidocysts are assembled in a single epithelial cell forming "batteries" (Gelei, 1927; Kanaev, 1969). Each battery is made up of at least one stenotele surrounded by several desmonemes and atrichous isorhiza (Ewer, 1947). Over time these structures have acquired different degrees of relevance. In Hydrozoa they have been examined in detail and many studies emphasize their usefulness in taxonomy (Itô & Inoue, 1962; Östman, 1979a, b, 1982, 1983, 1987, 1988; Bouillon, 1985; Watson, 1985; Cornelius & Östman, 1987). Deserti *et al.* (2010) and Deserti & Zamponi (2012) initiated a biometric and statistical approach in the study of the cnidome of three *Hydra* species and reported different morphotypes of the same cnidocyst, for the same species from different environments. However, there are still few studies that exploit their statistical qualities. In other groups, such as Anthozoa, certain studied investigate their nature. Acuña *et al.* (2004) and Garese *et al.* (2016) per-

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formed an exhaustive statistical study with a significant amount of data, applying GLM that become independent of the assumptions of normality, without losing information and power, as in non-parametric tests.

In this study, we investigated the biometric and statistical nature of the *Hydra* cnidome, expanding previous knowledge and adding a much larger amount of data, using the GLM as the main tool for the analysis and comparison of data. We used three *Hydra* species collected at three different sampling sites for each season of the year.

MATERIAL AND METHODS

Collection of polyps

Specimens of *Hydra vulgaris* Pallas, 1766, *H. vulgaris pedunculata* Deserti *et al.,* 2011 and *H. viridissima* Pallas, 1766 were collected in the following lakes: Nahuel Rucá (37°37′S, 057°26′W; 0.60 m deep; 245 ha), in Los Padres (37°56′S, 57°45′W; 1.24 m deep; 216 ha) and in La Brava (37°52′S, 57°58′W; 4.57 m; 400 ha), three shallows lakes located in the southeast region of the Pampa grasslands, Buenos Aires Province, Argentina.

Hydra vulgaris, of medium size, presented a brown coloration, separate sexes and long tentacles with a discontinuous growth pattern. The four types of cnidocysts with sizes within the ranges of the species were observed. *Hydra vulgaris pedunculata,* also brown and of medium size, presented the same general morphological and reproductive characteristics of *H. vulgaris,* but with a clearly distinguishable permanent peduncle. *Hydra viridissima,* small in size, presented a bright green coloration with short tentacles and the four types of cnidocytes present, the latter with sizes within the normal ranges cited for the species.

A total of 162 specimens were collected seasonally during two years: from autumn 2013 until summer 2014 and, from autumn 2014 until summer 2015. For the extraction of hydras, some substrates were collected: floating vegetation (different species depending on the season) and submersed macrophyte *Ceratophyllum demersum* L. The samples were packed and transported to the laboratory and conditioned in separated aquariums with water from the site and kept with aeration. The samples were maintained at 20° ± 3°C with a photoperiod of 12 hours of light and 12 hours of dark.

Cnidocyst sampling, description and typing

The width and length of 30 intact capsules of each type of nematocyst were measured by squash of entire and live polyps using a Zeiss Axiolab light microscope with a magnification of 1000X (immersion objective). The choice was random and with these sizes the data matrix was constructed for statistical analyses. In total 17,378 capsules were measured (average ~ 30 capsules × 4 types of cnidocysts × 162 specimens × 3 species). In some cases given the low abundance of some types, all of the cnidocysts found were considered.

For each species, the descriptive statistical parameters of each type of cnidocyst were detailed, such as the average length and width and the minimum and maximum of both measurements.

Relative abundance

To estimate the relative abundance, four different optical fields were randomly focused in the squash made for each polyp, counting in each field the different types of perfectly recognizable cnidocysts. The data were grouped and the average relative abundances for each species were calculated.

Statistical analysis

All statistical analysis were carried out using the R software (https://www.r-project.org). Only the length variable was used for each cnidocyst of each type (dependent or explained variable). This variable was grouped under three different factors:

1. "Individual" factor: the values of the length variable were compared between individuals of the same species, to detect a possible intraspecific variation.
2. "Season" factor: the values of the length variable were compared between seasons, of the same lagoon and species, to detect possible variations between the different climatic seasons.
3. "Lagoon" factor: the values of the length variable of the same species were grouped by lagoons, to detect possible intraspecific variations by environment.
4. "Species" factor: the values of the length variable were grouped entirely by species in order to detect interspecific variations.

The following data packages were used: (a) *H. vulgaris* and *H. vulgaris pedunculata*[1] from La Brava, (b) *H. vulgaris* and *H. vulgaris pedunculata* from Los Padres, (c) *H. vulgaris* and *H. vulgaris pedunculata* from Nahuel Rucá and (d) *H. viridissima*[1].

The normality of the capsule sizes was tested by a Shapiro-Wilks test ($\alpha = 0.05$) on the residuals of a Linear Model with normal distribution.

Comparisons of the sizes grouped under the four aforementioned factors were made using an ANOVA, in those cases where normality was accepted.

For the rejection cases, GLM with gamma distribution for the errors were applied. The model was:

$$\mathbf{g\ (length) = ß_0 + ß_1\ factor + \varepsilon}$$

In addition, for the GLM, t tests were applied to compare the coefficients of the model (ß1). The fit of these

1 The species *H. viridissima* was excluded from analyzes under the season and lagoon factor, given the low number of individuals in each group to avoid the possibility that the results obtained could not be reliable. However, it is included for comparisons of lengths between individuals and between species. Under the same criteria, *H. vulgaris pedunculata* from La Brava was excluded under the season factor (since it appears only during the winter of 2013).

models was graphically evaluated using Q-Q Plots of the residuals vs. the theoretical quantiles of the model and scatter diagrams of the deviation of the residuals vs. the fitted values of the model.

RESULTS

Cnidocyst sampling, description and typing

The cnidome of the species *H. viridissima, H. vulgaris* and *H. vulgaris pedunculata* are respectively presented in Figs. 1-3. The different holotrichous isorhiza morphotypes, found in each species are shown in Fig. 4. Additional details of the measurements (sizes of each cnidocyst of each species for each lagoon) are presented in the tables of Appendix A as supplementary material.

Relative abundances

In the three species, the most abundant type was the desmoneme, followed by the stenotele, atrichous isorhiza and finally, holotrichous isorhiza (Fig. 5).

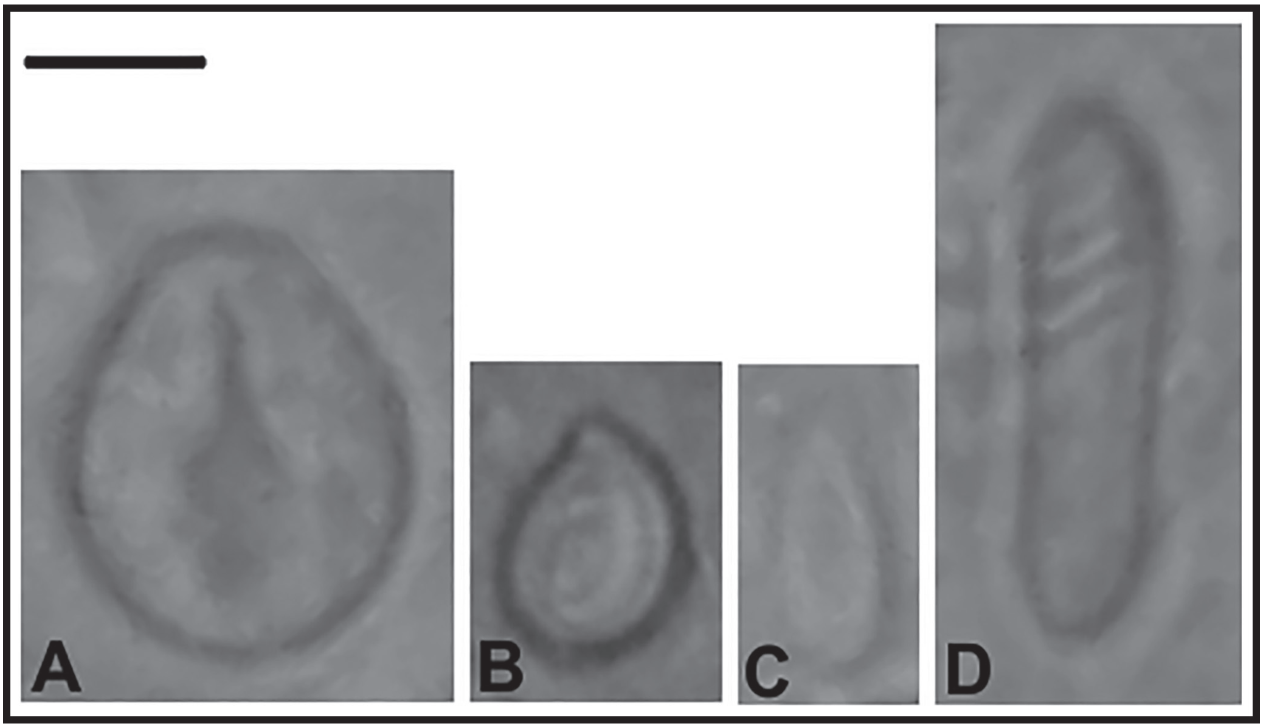

Figure 1. Cnidome of *Hydra viridissima.* (A) stenotele, (B) desmoneme, (C) atrichous isorhiza and (D) holotrichous isorhiza. Scale bar: 3 µm.

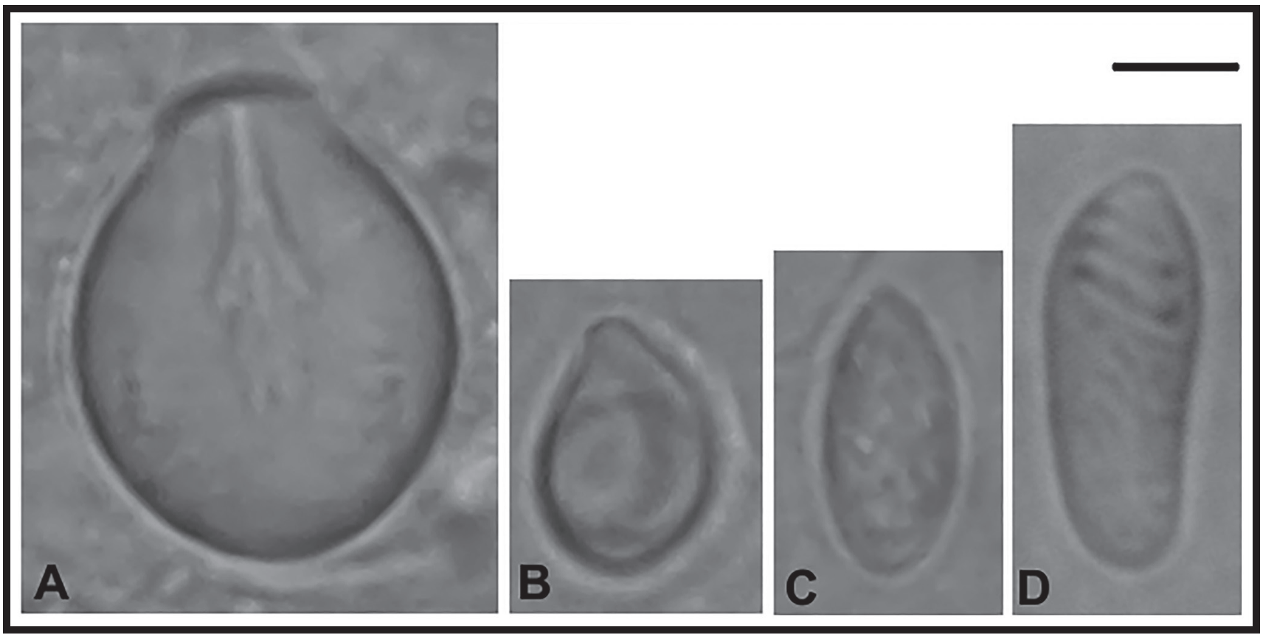

Figure 2. Cnidome of *Hydra vulgaris.* (A) stenotele, (B) desmoneme, (C) atrichous isorhiza and (D) holotrichous isorhiza. Scale bar: 2.85 µm.

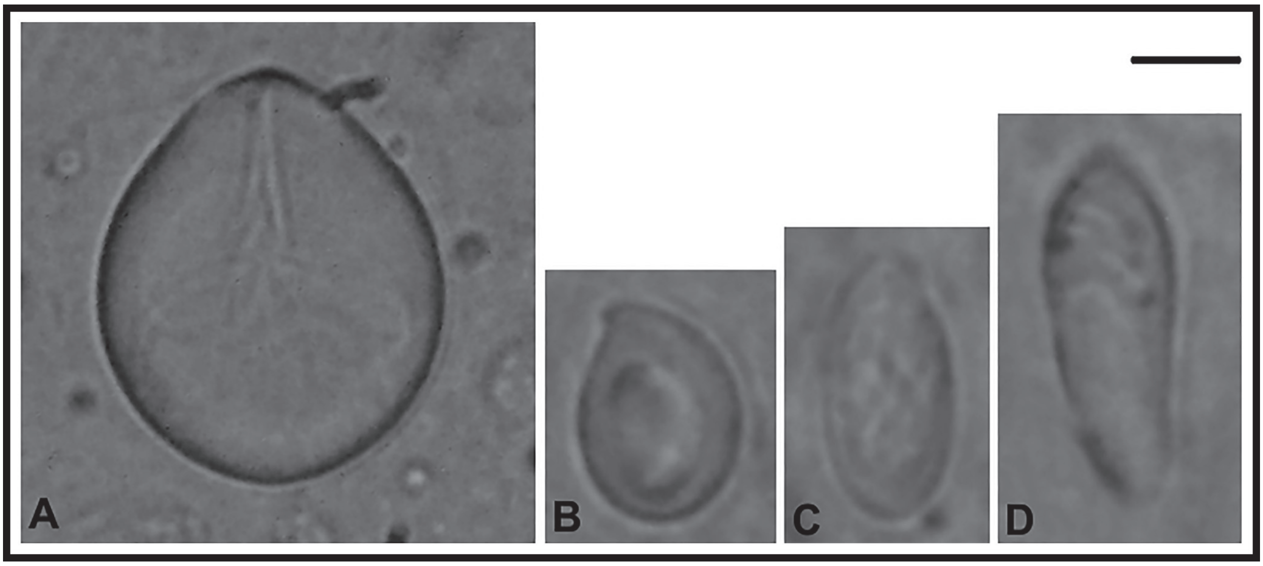

Figure 3. Cnidome of *Hydra vulgaris pedunculata.* (A) stenotele, (B) desmoneme, (C) atrichous isorhiza and (D) holotrichous isorhiza. Scale bar: 2.7 µm.

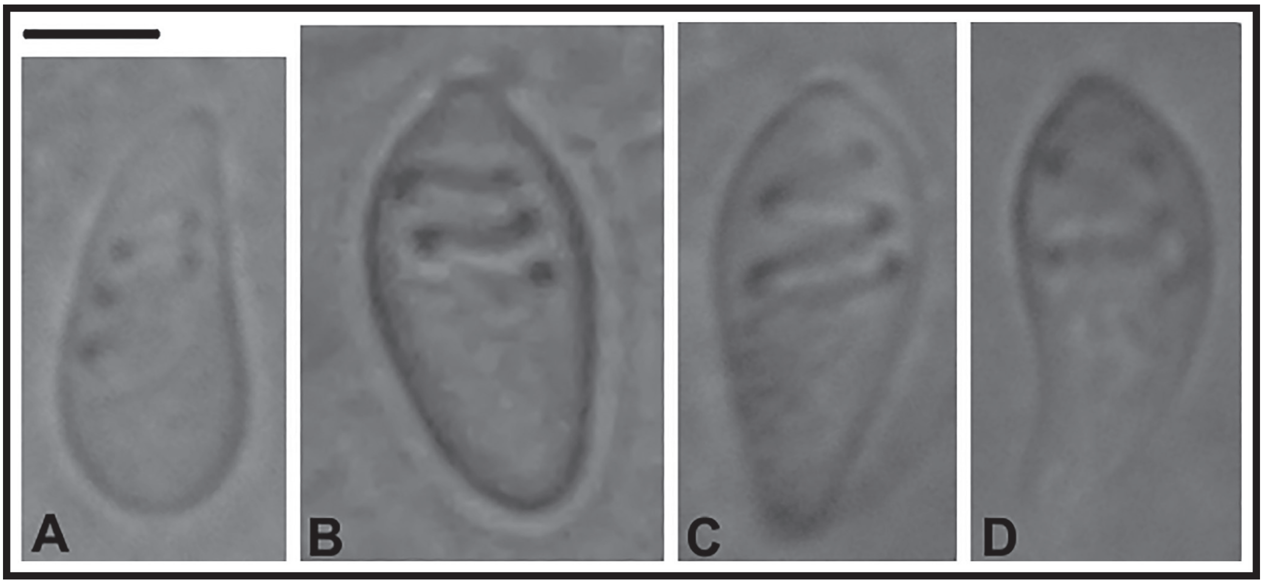

Figure 4. Different morphotypes of holotrichous isorhiza. (A) *Hydra viridissima,* (B) *Hydra vulgaris pedunculata,* C and (D) *Hydra vulgaris.* Scale bar: 2.45 µm.

Statistical analysis

Normality: for the "length" variable of the four types of cnidocysts in each species, normality ($\alpha = 0.05$) was rejected (Table 1).

The analysis of normality ($\alpha = 0.05$) of the residuals of a Linear Model with normal distribution, yielded different results. On the one hand, for *H. viridissima* normality was rejected in all cases (p-values) (atrichous isorhiza = 0.0004, desmoneme = 1.2×10^{-6}, holotrichous isorhiza = 0.0002, stenotele = 0.001). For *H. vulgaris pedunculata,* however, the length of the desmoneme showed a normal distribution (p-value = 0.06) while for the remaining three, it was rejected (atrichous isorhiza = 0.004, holotrichous isorhiza = 2.2×10^{-16}, stenotele = 0.006). Finally, for *H. vulgaris,* normality was also rejected in all cases, the p-values being the following: atrichous isorhiza = 2×10^{-10}, desmoneme = 2.3×10^{-16}, holotrichous isorhiza = 1.4×10^{-6}, stenotele 2.4×10^{-15}.

Table 1. Normality test: p = values corresponding to "length" variable of the four types of cnidocysts in each species ($\alpha = 0.05$).

	atrichous isorhiza	desmoneme	holotrichous isorhiza	stenotele
H. viridissima	6.83×10^{-10}	4.8×10^{-9}	0.0002	1.7×10^{-7}
H. vulgaris	2×10^{-8}	2×10^{-16}	3.4×10^{-16}	2.2×10^{-10}
H. vulgaris pedunculata	2.2×10^{-16}	2.3×10^{-16}	2.1×10^{-14}	5.5×10^{-13}

1. "Individuals" factor: The existence of significant differences between individuals was evaluated using a t test for the coefficients of each model. For the species *H. viridissima,* 25% of the individuals presented differences in the sizes of their isorhizas and desmonemes and 100% of the individuals presented differences in the sizes of their stenoteles. For the subspecies *H. vulgaris pedunculata,* 47.6% of the individuals presented differences in the sizes of their stenoteles, 71.4%

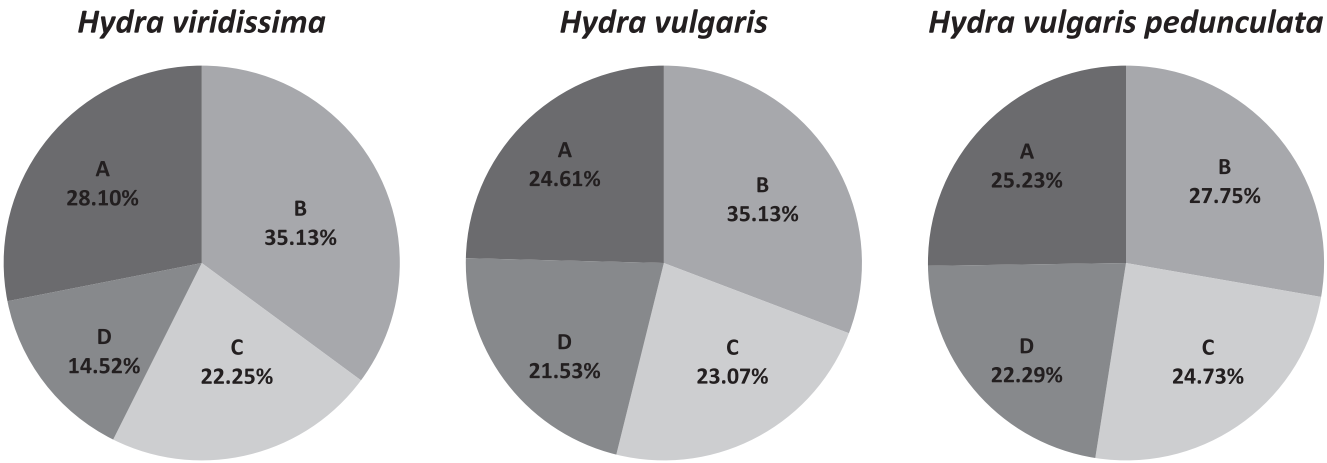

Figure 5. Relative abundances of each type of cnidocyst for each species. (A) stenotele, (B) desmoneme, (C) atrichous isorhiza and (D) holotrichous isorhiza.

for the atrichous isorhiza, 81% for the desmonemes and 95.2% for the holotrichous isorhizas. Finally, for *H. vulgaris,* 11.8% of the individuals presented differences in the sizes of their desmonemes, 35.5% for the stenoteles, 75.5% for the holotrichous isorhiza and 76.3% for the atrichous isorhiza.

2. "Season" factor: The *H. vulgaris* individuals collected in La Brava were found in winter 2013 and autumn 2014. Comparing both seasons, desmonemes were the only cnidocysts that presented differences, yielding a p-value < 0.01. In the case of the same species collected in Los Padres, the atrichous and holotrichous isorhiza and stenotele species showed differences between the spring 2014 and summer 2015 seasons, with p-values < 0.01, < 0.05 and < 0.001, respectively. The subspecies *H. vulgaris pedunculata,* also found in Los Padres, was collected in spring 2014 and summers 2014 and 2015. Atrichous and holotrichous isorhiza showed differences between the spring 2014-summer 2014 seasons (p < 0.01 and < 0.001, respectively) and the desmonemes showed differences in all the stations (with p-values < 0.001). For the species found in Nahuel Rucá, *H. vulgaris pedunculata* was found in three seasons, spring 2013 and 2014 and summer 2014. The atrichous isorhiza and desmonemes showed differences in all seasons (p-values < 0.001 in all cases) and the stenoteles, only between the spring 2013-spring 2014 pair (p < 0.001). Finally, also in Nahuel Rucá, the *H. vulgaris* species was collected in the eight sampled stations. Table 2 summarizes those stations that presented differences in the sizes of their cnidocysts.
3. "Lagoon" factor: For *H. vulgaris pedunculata* all types of cnidocysts showed significant differences between the three lagoons. The p-values of the t-test for the coefficients (ß1) of the models in all cases were < 0.001, except for the desmoneme that only registered variation between La Brava and Los Padres (p-value = 0.29).

For *H. vulgaris,* all cnidocysts showed differences between the three lagoons. In all cases, the p-values obtained by the t test for the coefficients (ß1) of the different models were < 0.001.

Table 2. Differences in the sizes of the four types of cnidocysts of *Hydra vulgaris* from Nahuel Rucá. Winter season 2014 does not appear because it is the one used to make the comparisons.

seasons	atrichous isorhiza	desmoneme	holotrichous isorhiza	stenotele
winter 13				
autumn 13	***	**	**	***
autumn 14	***		***	
spring 13			***	
spring 14	***		*	
summer 14	***		***	
summer 15				**

Signification levels: "***" 0.001, "**" 0.01, "*" 0.05

Table 3. Variations between species. P-values of the t-test for the coefficients (ß1) of the GLM of each type of cnidocyst. The species *Hydra vulgaris pedunculata* is not shown as it is the one taken for comparisons.

species	atrichous isorhiza	desmoneme	holotrichous isorhiza	stenotele
Hydra viridissima	***	***	***	***
Hydra vulgaris			***	

Signification levels: "***" 0.001

Figures 6 and 7 show, for the species *H. vulgaris* and *H. vulgaris pedunculata,* respectively, the adjustment of each model for each type of cnidocyst, by means of the Q-Q Plots graphs of the residuals vs. the theoretical quantiles of the model and scatter diagrams of the deviation of the residuals vs. the fitted values of the model.

4. "Species" factor: Table 3 lists the p-values of the t test for the coefficients (ß1) of each model for the comparison of each pair of species. Figure 8 shows the fit of the models, for each type of cnidocyst, using the Q-Q Plots graphs of the residuals vs. the theoretical quantiles of the model and scatter diagrams of the deviation of the residuals vs. the fitted values of the model. The confidence intervals for each model are: *H. vulgaris pedunculata* vs. *H. viridissima:* atrichous isorhiza [0.08-0.09], desmoneme [0.08-0.01], holotrichous isorhiza [0.003-0.008] and stenotele [0.06-0.07]. For *H. vulgaris pedunculata* vs. *H. vulgaris:* holotrichous isorhiza [(-0.003)-0.002].

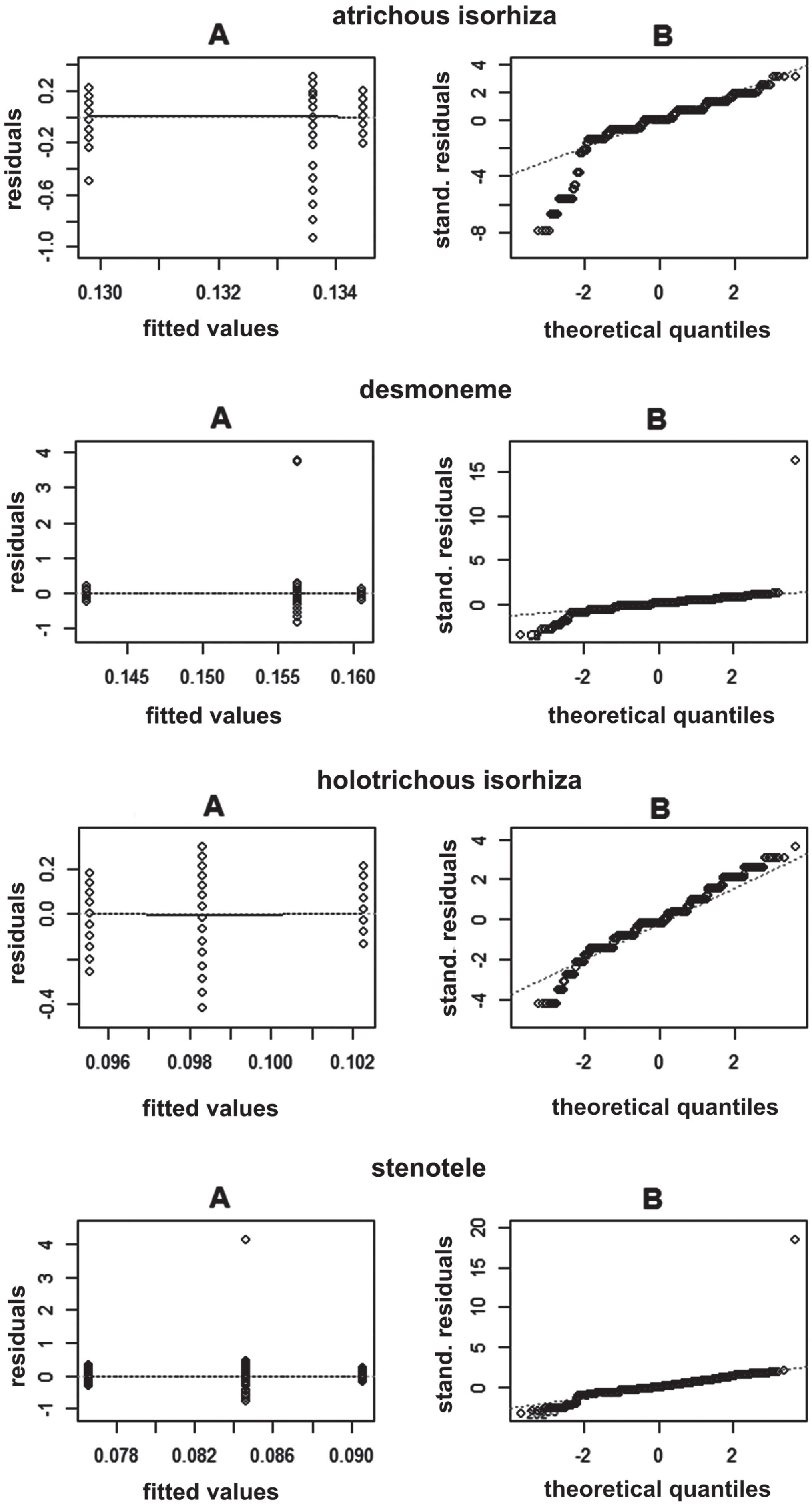

Figure 6. GLM adjustment charts for *Hydra vulgaris.* (A) scatter plot, (B) Q-Q Plots.

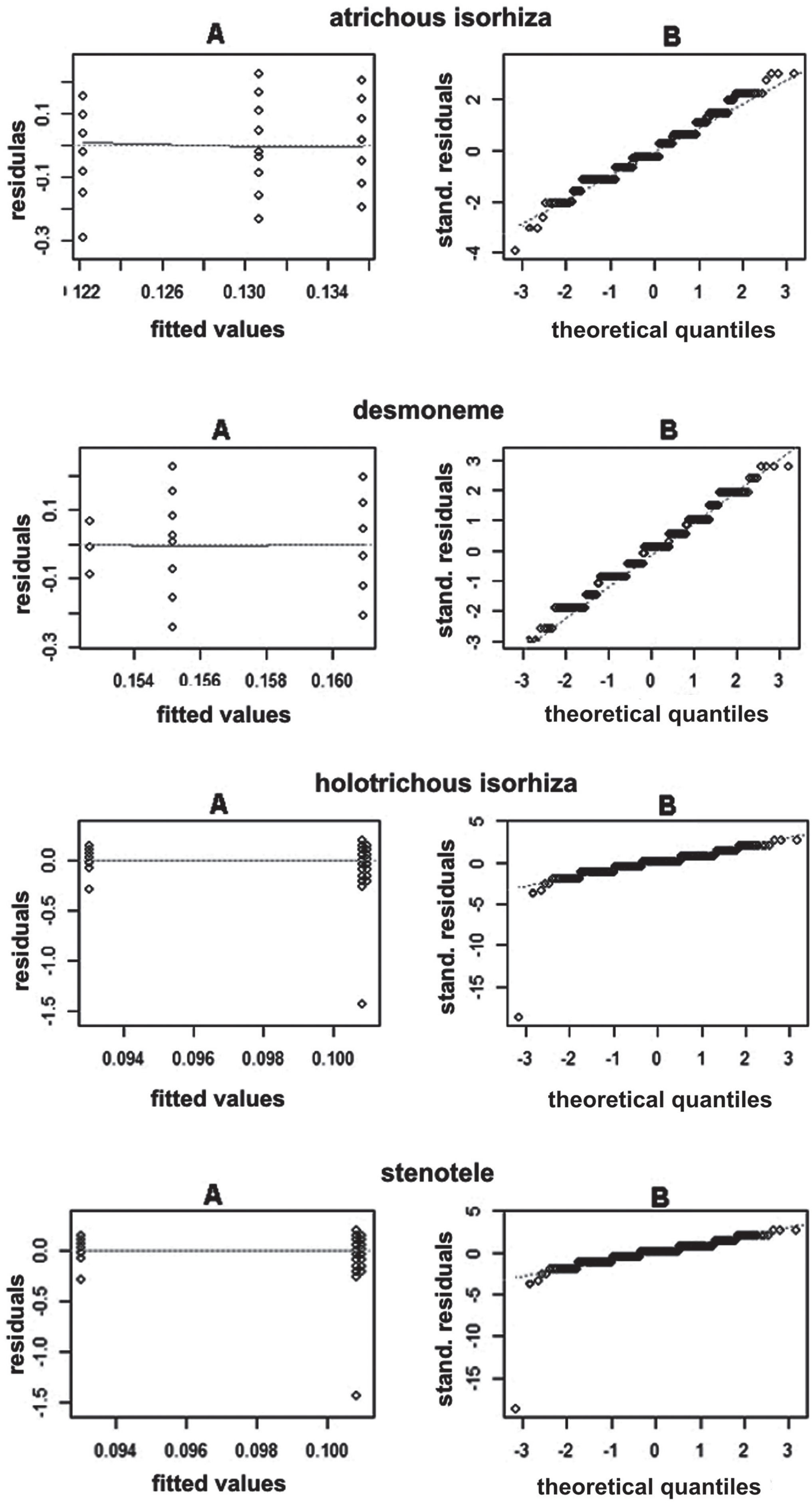

Figure 7. GLM adjustment charts for *Hydra vulgaris pedunculata.* (A) scatter plot, (B) Q-Q Plots.

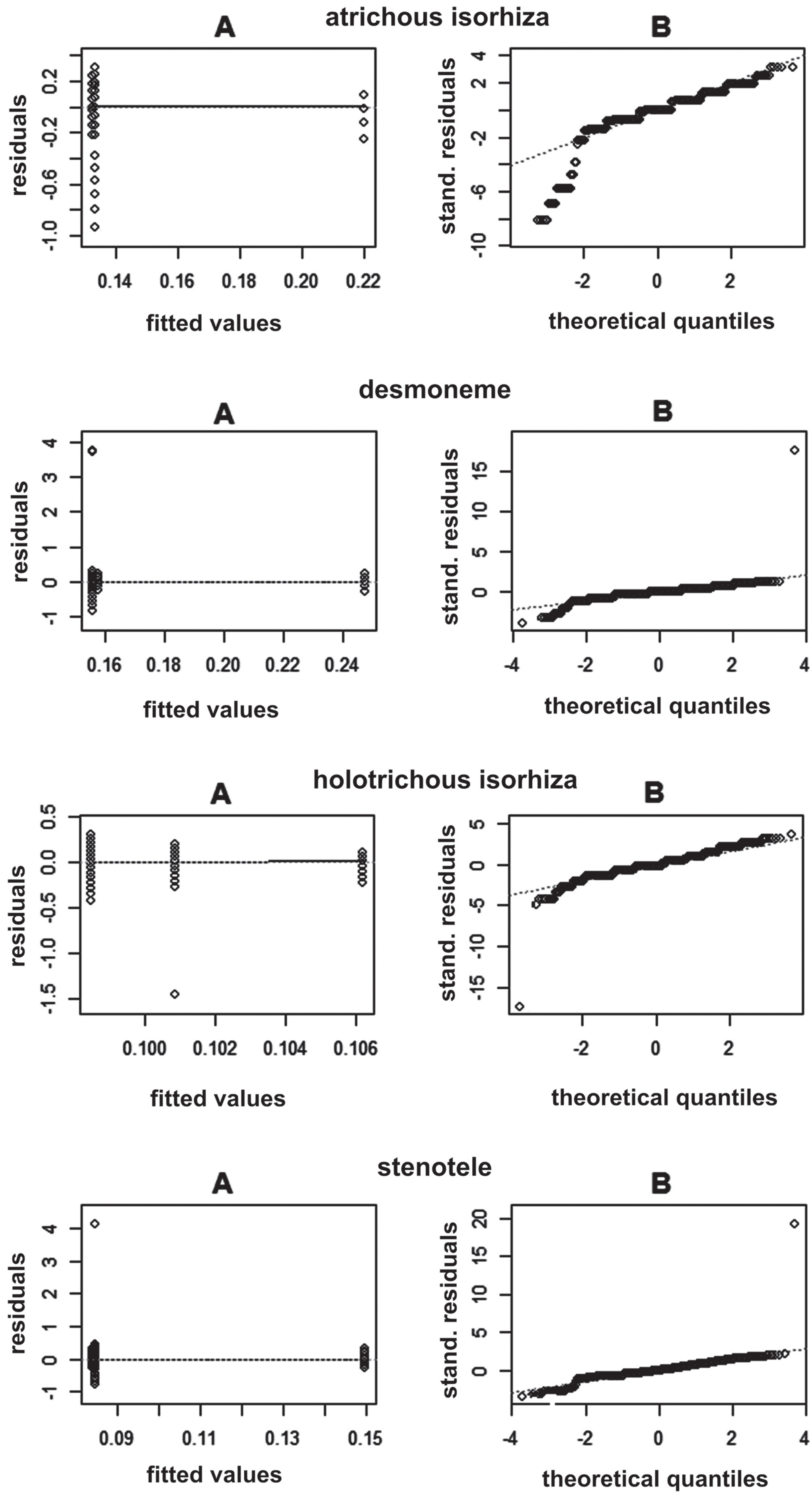

Figure 8. GLM adjustment graphs used for comparison between species. (A) scatter plot, (B) Q-Q Plots.

DISCUSSION AND CONCLUSION

The taxonomic value of the cnidome has already been much discussed (Weill, 1934a, b; Itô & Inoue, 1962; Kubota, 1976; Östman, 2000). Nowadays it is mandatory the use of the statistical distribution of the data in taxonomic issues. For *H. viridissima, H. vulgaris* and *H. vulgaris pedunculata,* at least two different morphotypes of holotrichous isorhiza have been recorded. However, despite varying in their shape, different from the classic shape of paramecium or shoe sole, their average measurements do not vary between the studied species. Especially the *length* value, which is the usual measurement used for statistical analysis. Deserti *et al.* (2010) reported two different morphotypes for *H. vulgaris* from Los Padres and Nahuel Rucá. In Nahuel Rucá they described morphotype I, in a classic form, and II in the form of a seed. The latter morphotype is similar to that recorded for *H. vulgaris pedunculata* from Los Padres and *H. vulgaris* from Nahuel Rucá. Other authors also described more than one holotrichous isorhiza capsules (Cordero, 1941a, b; Dioni, 1968; Hemmrich, *et al.,* 2007; Wang *et al.,* 2009). In these studies the shapes and sizes were described and included within the cnidome of the species. In the case of the cnidome reported by Dioni (1968) for the green hydra *H. plagiodesmica,* the forms are clearly different from those detailed here for *H. viridissima.* Although there is no theory that explains the appearance of different morphotypes, it is likely that these differences respond to variations during cnidogenesis, in response to physiological changes. This could be explained if we consider that the capsules, once formed, do not present a certain degree of plasticity that could respond to pressures within the tissues or even during their migration through the body. However, for the same species collected in Nahuel Rucá by Deserti *et al.* (2010), only two morphotypes were found, while in this study, an additional third morphotype is added. Taking into account that the structure of holotrichous isorhiza is in many cases of importance for the taxonomic status of a species (for example, the differentiation between *H. oligactis* from *H. pseudoligactis*), it is imperative to pay special attention and care in the typification of its different forms. Despite the fact that many times the average sizes remain constant beyond their morphology, the perfect distinction between one and the other is necessary.

Regarding the relative abundances, the same pattern was observed in all species. Although the percentages yielded different values, the abundance was, in decreasing order, desmonemes, stenoteles, atrichous and holotrichous isorhiza. These abundances agree with that recorded by Deserti *et al.* (2010) and Deserti & Zamponi (2011) for *H. vulgaris* from Los Padres and Nahuel Rucá and Deserti *et al.* (2011) for *H. vulgaris pedunculata* from Los Padres. Other authors report different abundances; for example Bode & Flick (1976) and Zumstein (1973) for the North American *H. attenuata* (= *H. vulgaris*) place the atrichous isorhiza in second place and the stenoteles in third. Although these differences may be real, based on physiological responses to different environments, they could also respond, in part, to the methodology used for counting the cnidocysts. While we used here the relative abundance based on visual counts, the aforementioned authors perform solutions in sodium duodecyl sulfate, physically separating one capsule from another and counting all the cnidocysts present in a polyp. This technique would maximize the visualization of atrichous isorhiza, which turns out to be the most difficult type to individualize, since the structure of their capsule quickly melts with the surrounding medium.

The analysis of the distribution of the data yielded several results. On the one hand, although the most frequent is the absence of normality. Many studies have shown that the normality of the data can vary. The statistical tests frequently assumed a normal distribution of the lengths of the capsules, a concept that has currently been refuted by several authors (Acuña *et al.,* 2003, 2004, 2007; Acuña & Garese, 2009; Garese, 2013), at least for several species of sea anemones. The progress in the studies at the level of the data distribution, in principle, was overturned in the use of non-parametric tests, for the case of rejected normality. These tests, in certain situations, waste information and are less powerful, characteristics that are much more minimized in the GLMs developed by Nelder & Wedderburn (1972).

In particular, GLMs with gamma errors tend to fit very well to data from biological studies and are more appropriate for analyzing skewed data with constant coefficient of variation, so that it is not necessary to resort to transformations that force us to work within unnatural scales. Furthermore, in these cases, the equality of variances is not a requirement and the link between the dependent variable and the linear combination of independent variables (in the classic case it is the identity), can take different forms (Hastie & Tibshirani, 1990). In other cnidarians, the use of these GLMs was very appropriate for quantitative comparisons (Allcock *et al.,* 1998; Watts *et al.,* 2000; Ardelean & Fautin, 2004; Acuña *et al.,* 2004; Garese, 2013). Either by parametric tests, when normality was accepted or by GLM, when it was rejected, the sizes of the cnidocysts vary between individuals of the same species, in the three species studied. Of the totality of the cases analyzed, the holotrichous isorhiza were the ones that presented the higher variation, with a value of 76.8%. They are followed by atrichous isorhiza, with 74.4%, stenoteles in third place, with 38.7% and finally, desmonemes, with a 21.2% of variation in all cases. If we observe carefully, this order in the percentage of variation is exactly opposite to the order of the relative abundances. This indicates that the holotrichous isorhiza despite being the least abundant type, is the most variable. This is another characteristic that reinforces the importance that its analysis requires.

When we analyze the difference in sizes between individuals of the same species, collected in the same lagoon, we note that variations are recorded between some climatic seasons. In *H. vulgaris,* again the holotrichous isorhiza were the ones that presented the higher variations between seasons, however in *H. vulgaris pedunculata,* the first place was for the desmonemes. When analyzing these results as a whole, the holotrichous occupy the second place in terms of variation with a percentage of 54% below the atrichous isorhiza with 61%.

The analysis by species at the lagoon level showed that the sizes of all the cnidocysts showed variations, with the exception of the *H. vulgaris pedunculata* desmoneme, which only presented differences between La Brava and Los Padres. This result may be representing changes at the physiological level related to the different limnological variables that characterize each lagoon. We could assume, that the cnidome of a species has certain flexibility when it comes to environments. This situation is similar to that recorded by Acuña & Zamponi (1997) in sea anemones belonging to different intertidal zones. The authors observed that some of their nematocysts present significant variations in their sizes according to the area they inhabit, describing these species from different areas as "ecological races", since these differences are not enough to declare different species.

The last of the comparisons, and perhaps the most relevant and most applicable, is the one that allows us to discern between different species. Although we already know that a characteristic alone does not determine one species or another, it is important to know if the cnidome has sufficient statistical power to be used as a taxonomic feature. Since the cnidome is a fundamental requirement when describing a cnidarian species, these data can become an important taxonomic argument and not simply be a numerical description.

In our analysis, the three species were different from the point of view of their cnidocysts. When we compare *H. viridissima* with the remaining species, we see that all its cnidocysts show variations. However, when we compare *H. vulgaris* with *H. vulgaris pedunculata,* they only differ at the level of their holotrichous isorhiza.

These results reinforce the fact that *H. viridissima* belongs to *viridissima* group, while *H. vulgaris* and *H. vulgaris pedunculata* belong to *vulgaris* group. When we analyze different groups we note that the variations intensify, while in those of the same group they decrease. For *H. vulgaris* and *H. vulgaris pedunculata* we are analyzing species and subspecies, so a much smaller variation in their cnidocysts makes even more sense. It is not surprising that, once again, holotrichous isorhiza are the exception in the latter case. The results show that the subspecies retains, for most of its cnidocysts, the biometric identity of the species that it is derived from. Deserti & Zamponi (2011) carry out a similar study, using a smaller amount of data and different statistical methods. However, the final results were the same: species from different groups showed differences in their cnidocysts, while those belonging to the same group did not. It is likely that in the analysis presented here, the increase in the number of measurements has allowed us to detect the difference of the holotrichous isorhiza for *H. vulgaris* and *H. vulgaris pedunculata.*

It seems clear that cnidomes of different species have well-defined identities that can be used to distinguish, at least, between groups. The measurement of cnidocysts, although often not very seductive due to the difficulty represented by the small sizes and the structural complexity in the Hydrozoa (David *et al.,* 2008), seems to have its reward; gives us a powerful statistical result to be used in a taxonomy. However, knowledge of cnidocysts at the level of data distribution and intraspecific variations is of vital importance. Such knowledge will prevent us from believing that certain "ecological races" are, in fact, different species. As we can see, there is a large amount of information contained within the cnidome of a species. As the exploration of its statistical characteristics progresses in greater depth, we can choose more suitable methodologies for its analysis that yield more robust results. Many times, the amount of data used in a study is not what gives robustness to the results, but rather the application of a correct methodology based on the intrinsic characteristics of the measurements. As we advance in this knowledge, the use of the cnidome in taxonomic issues will be more reliable.

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APPENDIX A

Table 1. Measurements of the four types of nematocysts of *Hydra vulgaris* found in LB in winter 2013 and autumn 2014. St dev: standard deviation, w: width, l: length.

		stenotele (µm)		desmoneme (µm)		atrichous isorhiza (µm)		holotrichous isorhiza (µm)	
		w	l	w	l	w	l	w	l
winter 2013	mean	10.23	13	5.08	7.11	3.95	7.66	4.65	10.42
	median	10.50	13	5	7	4	7.5	4.5	10.5
	st dev	1.21	1.45	0.60	0.64	0.40	0.75	0.51	0.75
	min	7.5	9.5	3.5	5.5	3	4.5	3.5	8
	max	13.5	17	6.5	8.5	5	9	6	12
autumn 2014	mean	10.41	13.13	5.05	6.91	4	7.75	4.54	10.52
	median	10.5	13	5	7	4	8	4.5	10.50
	st dev	1.34	1.59	0.64	0.65	0.41	0.79	0.49	0.76
	min	7.5	9.5	3.5	5.5	3	4.5	3.5	8
	max	15	17.5	6.5	8.5	5	9.5	5.5	12.5
totals	mean	10.31	13.05	5.06	7.02	3.97	7.70	4.61	10.46
	median	10.5	13	5	7	4	8	4.5	10.5
	st dev	1.27	1.51	0.61	0.65	0.40	0.77	0.50	0.76
	min	7.5	9.5	3.5	5.5	3	4.5	3.5	8
	max	15	17.5	6.5	8.5	5	9.5	6	12.5

Table 2. Measurements of the four types of nematocysts of *Hydra vulgaris pedunculata* found in LB in winter 2013. St dev: standard deviation, w: width, l: length.

		stenotele (µm)		desmoneme (µm)		atrichous isorhiza (µm)		holotrichous isorhiza (µm)	
		w	l	w	l	w	l	w	l
winter 2013	mean	11	13.75	4.88	6.55	4.18	8.18	4.33	10.75
	median	10.75	13.75	5	6.5	4	8	4.5	11
	st dev	1.66	1.91	0.38	0.40	0.30	0.80	0.37	0.79
	min	7.5	10	4	6	4	6	3.5	8
	max	15	17.5	5.5	7	5	9	5	12.5

Table 3. Measurements of the four types of nematocysts of *Hydra viridissima* found in LP in autumn 2013. St dev: standard deviation, w: width, l: length.

		stenotele µm)		desmoneme (µm)		atrichous isorhiza (µm)		holotrichous isorhiza (µm)	
		w	l	w	l	w	l	w	l
autumn 2013	mean	5.53	7.03	2.73	4.21	2.27	4.72	2.5	9.47
	median	5.5	7	3	4	2	5	2.5	9.5
	st dev	0.46	0.67	0.38	0.40	0.34	0.34	0.54	0.67
	min	5	6	2	3.5	2	4	2	8
	max	7	9	3.5	5	3	5	5	10.5

Table 4. Measurements of the four types of nematocysts of *Hydra vulgaris pedunculata* found in LP in spring and summer 2014. St dev: standard deviation, w: width, l: length.

		stenotele (µm)		desmoneme (µm)		atrichous isorhiza (µm)		holotrichous isorhiza (µm)	
		w	l	w	l	w	l	w	l
summer 2014	mean	9.35	11.92	4.43	6.38	3.62	7.31	4.14	10.04
	median	9	11.5	4.5	6.5	3.5	7.5	4	10
	st dev	1.46	1.64	0.36	0.43	0.36	0.45	0.41	0.71
	min	7	9.5	4	5.5	3	6	3	8.5
	max	13	16.5	5.5	7.5	4.5	8.5	5	11.5
spring 2014	mean	9.34	11.53	4.29	6.02	4.08	7.44	4.32	9.66
	median	9.5	11.5	4.25	6	4	7.5	4.5	9.5
	st dev	1.33	1.30	0.36	0.44	0.29	0.51	0.39	0.65
	min	7	9	3.5	5	3.5	6.5	3.5	8
	max	12.5	14.5	5	7	5	9	5.5	11
Totals	mean	9.39	11.76	4.37	6.21	3.81	7.37	4.22	9.9
	median	9	11.5	4.5	6	4	7.5	4	10
	st dev	1.39	1.48	0.37	0.47	0.41	0.48	0.42	0.70
	min	7	9	3.5	5	3	6	3	8
	max	13	16.5	5.5	7.5	5	9	5.5	11.5

Table 5. Measurements of the four types of nematocysts of *Hydra vulgaris* found in LP in spring 2014 and summer 2015. St dev: standard deviation, w: width, l: length.

		stenotele (µm)		desmoneme (µm)		atrichous isorhiza (µm)		holotrichous isorhiza (µm)	
		w	l	w	l	w	l	w	l
spring 2014	mean	9.13	10.18	4.38	6.20	3.95	7.49	4.24	9.84
	median	9	11	4.5	6	4	7.5	4	10
	st dev	0.91	0.88	0.38	0.39	0.36	0.46	0.45	0.56
	min	7	9	3.5	5.5	3	6.5	3.5	8.5
	max	11.5	13.5	5.5	7	5	9	5.5	12
summer 2015	mean	8.51	10.84	4.33	6.26	3.80	7.35	4.06	9.70
	median	8.5	10.5	4.5	6.5	4	7.5	4	10
	st dev	0.75	0.79	0.44	0.38	0.39	0.49	0.34	0.54
	min	7	9	3	5	3	6	3	8.5
	max	11.5	14	5.5	7	5	8.5	5	11.5
Totals	mean	8.8	11.04	4.37	6.23	3.89	7.44	4.16	9.77
	median	9	11	4.5	6	4	7.5	4	10
	st dev	0.90	0.87	0.41	0.39	0.39	0.48	0.41	0.56
	min	7	9	3	5	3	6	3	8.5
	max	11.5	14	5.5	7	5	9	5.5	12

Table 6. Measurements of the four types of nematocysts of *Hydra viridissima* found in NR in autumn 2013. St dev: standard deviation, w = width, l = length.

		stenotele (µm)		desmoneme (µm)		atrichous isorhiza (µm)		holotrichous isorhiza (µm)	
		w	l	w	l	w	l	w	l
autumn 2013	mean	4.92	6.45	2.68	3.92	2.37	4.43	3.27	9.11
	median	5	6.5	3	4	2.5	4.5	3	9
	st dev	0.48	0.50	0.43	0.45	0.41	0.43	0.44	0.69
	min	4	5	2	3	2	3.5	3	7.5
	max	6	7.5	3.5	5	3.5	5	4	10

Table 7. Measurements of the four types of nematocysts of *Hydra vulgaris* found in NR in autumn and spring of 2013, summer, autumn, winter and spring of 2014 and summer of 2015. St dev: standard deviation, w: width and l: length.

		stenotele (µm)		desmoneme (µm)		atrichous isorhiza (µm)		holotrichous isorhiza (µm)	
		w	l	w	l	w	l	w	l
autumn 2013	mean	7.95	10.38	4.01	5.82	3.2	6.82	4.29	10.26
	median	8	10.5	4	6	3.5	7	4.5	10.5
	st dev	2.28	2.92	0.80	1.23	0.73	1.62	0.68	1.5
	min	4	5	2	2.5	1.5	2.5	2.5	6.5
	max	13	16	5.5	7.5	4.5	9.5	6	13
spring 2013	mean	9.59	12.20	4.64	6.72	3.68	7.70	4.42	10.77
	median	9.5	12	4.5	7	3.5	7.5	4.5	10.5
	st dev	1.75	1.97	0.58	0.63	0.45	0.65	0.47	0.95
	min	6.5	9	3	5	3	6.5	3	8
	max	14.5	18	7	8.5	5	10	6	13.5
summer 2014	mean	8.98	11.45	4.18	6.28	3.29	6.96	4.02	10.06
	median	9	11.5	4	6.5	3.5	7	4	10
	st dev	1.08	1.15	0.51	0.48	0.30	0.45	0.57	0.66
	min	7	9.5	3	5.5	3	6	3	9
	max	11	13.5	5	7.5	4	8	5	11.5
autumn 2014	mean	9.16	11.99	4.41	6.51	3.56	7.25	4.58	10.87
	median	9	12	4.5	6.5	3.5	7	4.5	11
	st dev	1.60	1.74	0.48	0.51	0.44	0.64	0.48	0.76
	min	6	9	3.5	5	2.5	6	3.5	9
	max	13.5	16	5.5	7.5	4.5	9.5	6	13
winter 2014	mean	10.24	12.52	4.24	6.29	3.71	7.64	4.18	10.01
	median	10	12.5	4	6.5	4	7.5	4	10
	st dev	1.58	1.86	0.44	0.55	0.42	0.50	0.43	0.58
	min	7.5	9	3	5	3	6	3	8
	max	15	17.5	5.5	7.5	5	9	5.5	12
spring 2014	mean	9.61	11.75	4.19	6.17	3.49	7.42	4.05	9.81
	median	9.5	11.5	4	6	3.5	7.5	4	10
	st dev	1.42	1.51	0.40	0.52	0.40	0.51	0.49	0.59
	min	6.5	8.5	3	5	3	3.9	3	8
	max	14	16.5	5.5	7.5	5	9	5.5	11.5
summer 2015	mean	9.35	11.49	4.40	6.35	3.73	7.68	4.12	9.87
	median	9	11.5	4.5	6.5	4	7.5	4	10
	st dev	1.19	1.23	0.42	0.48	0.45	0.57	0.39	0.62
	min	7	9	3	5	2.5	5	3	7.5
	max	14	17	6	8	5	10	6	12
Totals	mean	9.42	11.74	4.35	6.35	3.61	7.49	4.21	10.13
	median	9	11.5	4.5	6.5	3.5	7.5	4	10
	st dev	1.60	1.79	0.53	0.66	0.49	0.79	0.50	0.88
	min	4	5	2	2.5	1.5	2.5	2.5	6.5
	max	15	18	7	8.5	5	10	6	13.5

Table 8. Measurements of the four types of nematocysts of *Hydra vulgaris pedunculata* found in NR in spring 2013 and 2014 and summer 2015. St dev: standard deviation, w: width and l: length.

		stenotele (µm)		desmoneme (µm)		atrichous isorhiza (µm)		holotrichous isorhiza (µm)	
		w	l	w	l	w	l	w	l
spring 2013	mean	10.35	13	4.93	7.21	4.13	8.1	4.09	10.22
	median	10.25	13.25	5	7.5	4	8	4	10
	st dev	1.30	1.27	0.40	0.52	0.34	0.69	0.20	0.71
	min	8	10	4	6	3.5	7	4	9
	max	13	15	6	8	5	9	4.5	11.5
spring 2014	mean	9.44	11.59	4.24	6.19	3.51	7.44	4.11	9.78
	median	9	11.5	4	6	3.5	7.5	4	10
	st dev	1.48	1.60	0.43	0.46	0.41	0.52	0.45	0.67
	min	7	9	3	5	2.5	6	3	7.5
	max	13	15.5	5	7.5	5	9	5	12
summer 2015	mean	10.24	12.36	4.61	6.60	3.77	7.83	4.20	10.06
	median	10	12	4.5	6.5	4	8	4	10
	st dev	1.68	1.77	0.39	0.46	0.46	0.57	0.39	0.88
	min	7.5	9.5	3.5	5.5	3	6.5	3.5	1.5
	max	14	16.5	5.5	7.5	5	9.5	5	12
Totals	mean	9.85	12.02	4.45	6.44	3.67	7.65	4.15	9.92
	median	9.5	12	4.5	6.5	3.5	7.5	4	10
	st dev	1.61	1.72	0.47	0.55	0.47	0.61	0.42	0.79
	min	7	9	3	5	2.5	6	3	4.5
	max	14	16.5	6	8	5	9.5	5	12

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