

# The cnidae of the acrospheres of the corallimorpharian *Corynactis carnea* (Studer, 1878) (Cnidaria, Corallimorpharia, Corallimorphidae): composition, abundance and biometry

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**ABSTRACT.** *Corynactis carnea* is a common corallimorpharian in the southwestern Atlantic Ocean, particularly in the Argentine Sea, and possesses spherical structures called acrospheres at the tips of its tentacles, characterized by particular cnidae. Twelve specimens were collected to identify and measure the types of cnidae present in the acrospheres, to estimate their abundance and to study the biometry of the different types. The cnidae of the acrospheres are spirocysts, holotrichs, two types of microbasic b-mastigophores and two types of microbasic p-mastigophores. Spirocysts were the most abundant type, followed by microbasic p-mastigophores and microbasic b-mastigophores; holotrichs were the least abundant. The size of only the spirocysts fitted well to a normal distribution; the other types fitted to a gamma distribution. A high variability in length was observed for each type of cnida. R statistical software was employed for statistical treatments. The cnidae of the acrospheres of *C. carnea* are compared with those of other species of the genus.

**KEY WORDS :** cnidocysts, biometry, acrospheres, Corallimorpharia, Argentina.

## INTRODUCTION

The Corallimorpharia form a relatively small, taxonomically-neglected group of skeletonless Anthozoa comprising no more than 40-50 species (DEN HARTOG et al., 1993). They are most closely related to the scleractinian corals and may even be considered as such. The taxonomy of the group is not well established and there is no consensus about the number of genera and families. In the most literal view, there are four families, one of which, Corallimorphidae, has three genera: *Corallimorphus* (Moseley, 1877), *Corynactis* (Allmann, 1846), and *Pseudocorynactis* (den Hartog, 1980). These are represented in both shallow coastal waters and the deep sea, and all three genera occur in the eastern Atlantic.

*Corynactis carnea* (Studer, 1878) is common in the southwestern Atlantic Ocean, mainly in the Argentine Sea, and its distribution was mentioned by many authors (CARLGREN, 1927; RIEMANN-ZÜRNECK, 1986; DEN HARTOG et al., 1993; GENZANO et al., 1996; ZAMPONI et al., 1998). This sea anemone forms pseudocolonies due to its asexual reproduction (DEN HARTOG, 1980; DEN HARTOG et al., 1993) and is characterized by a high colour variation. Spherical structures called acrospheres are found at the tips of its tentacles, and are diagnostic of the family Corallimorphidae (RIEMANN-ZÜRNECK & IKEN, 2003). Such structures have adhesive batteries extremely effective in the capture of zooplankton, mainly crustaceans (ROBSON, 1988). The acrospheres have cnidae (all types of cnidocysts present in a particular structure or species) composed of at least four different types of cnidocysts, while in tentacles only the spirocysts are present. The cnidocysts (nematocysts, spirocysts and ptychocysts) are subcellular structures characteristic of the phylum Cni-

daria. They vary in terms of their morphology and their functions, which include defence, aggression, feeding and larval settlement (FRANCIS, 2004). Cnidocysts also are important in the taxonomy of the group, and their description is necessary in any systematic work. The cnidae of *C. carnea* were incompletely documented by CARLGREN (1927), who did not distinguish the different types of cnida or describe the biometry of the capsules. ACUÑA et al. (2003; 2004 and 2007) provided important information on the size distribution of different types of cnidocysts, and also on modern statistical tools (generalized linear models) that allow statistical comparisons of non-normal size distributions, using more powerful proofs than traditional non-parametric tests. The goals of this paper are to study the composition, abundance and biometry of the cnidae of the acrospheres of the sea anemone *C. carnea* from the Argentine Sea.

## MATERIALS AND METHODS

Specimens of *C. carnea* were collected during a survey performed by the "Oca Balda" (10/9/88) (expedition 04-88). The individuals came from 38°11'S - 57°03'W, at a depth of 59m, temperature 10.3°C, salinity 33.7‰. Samples were fixed in 5% formaldehyde and subsequently in ethanol. In the laboratory, squashes of acrospheres from 12 individuals were made to identify and measure the length of cnidocysts, using a Zeiss Axiolab microscope with oil immersion at 1000x magnification. The cnidocysts were classified according to ENGLAND (1991). A microscope with a digital camera attached also was used for photography of the cnidae.

For estimation of cnidocyst abundance, four randomly-selected zones in each of the 12 squashes (one per individual) were chosen, and all identified capsules were counted. For statistical analyses, 30 capsules of each type from the acrospheres were measured. A total of 2160 measurements (30 capsules x 6 types of cnida x 12 individuals) were taken. Statistically-descriptive parameters, such as the types of size distribution and the cnidocyst sizes, were compared among individuals. Normality of size distribution was tested for each cnidocyst by means of a Shapiro-Wilks test ( $\alpha=0.05$ ). If normality was confirmed, an ANOVA was carried out in order to compare the cnidocyst sizes among specimens. In cases with non-normal distribution, a model with different distribution errors and other functional relationship between lengths and individuals for each cnidocyst type was evaluated. In this way, a Generalized Linear Model (GLM) with gamma errors was fitted, using the R program (EVERITT, 2005). The model form was:

$$g(\text{length}) = \beta_0 + \beta_1 \text{ individual} + \varepsilon$$

Then, in order to evaluate possible differences in the sizes of each cnida among individuals, a t-test was performed for the coefficients of the model ( $\beta_1$ ).

## RESULTS

### Cnidae composition

The cnidae of the acrospheres of *C. carnea* are composed of the following types: spirocysts with an elongated capsule and no spines distinguishable, spirally-coiled thread (Fig. 1a); holotrichs with a quite wide capsule and a thread with spines along whole length (Fig. 1b); microbasic b-mastigophore 1 with an elongated capsule and a thread with two distinct parts, a terminal tubule and a basal shaft with a length about 1/3 the length of the capsule, and a parallel-sided shape of the undischarged thread (Fig. 1c); microbasic b-mastigophore 2 with the same features as the previous type, except that the shaft has a length equivalent to 1/8 the length of the capsule (Fig. 1d); microbasic p-mastigophore 1 with an oval capsule and a thread with a terminal tubule and a basal shaft with a V-shape of the undischarged thread, which has a length equal to 1/2 the length of the capsule (Fig. 1e); and finally, microbasic p-mastigophore 2 with the same features as p-1, with the exception that the capsule has a more elongated shape and the length of the shaft is less than 1/4 the length of the capsule (Fig. 1f).

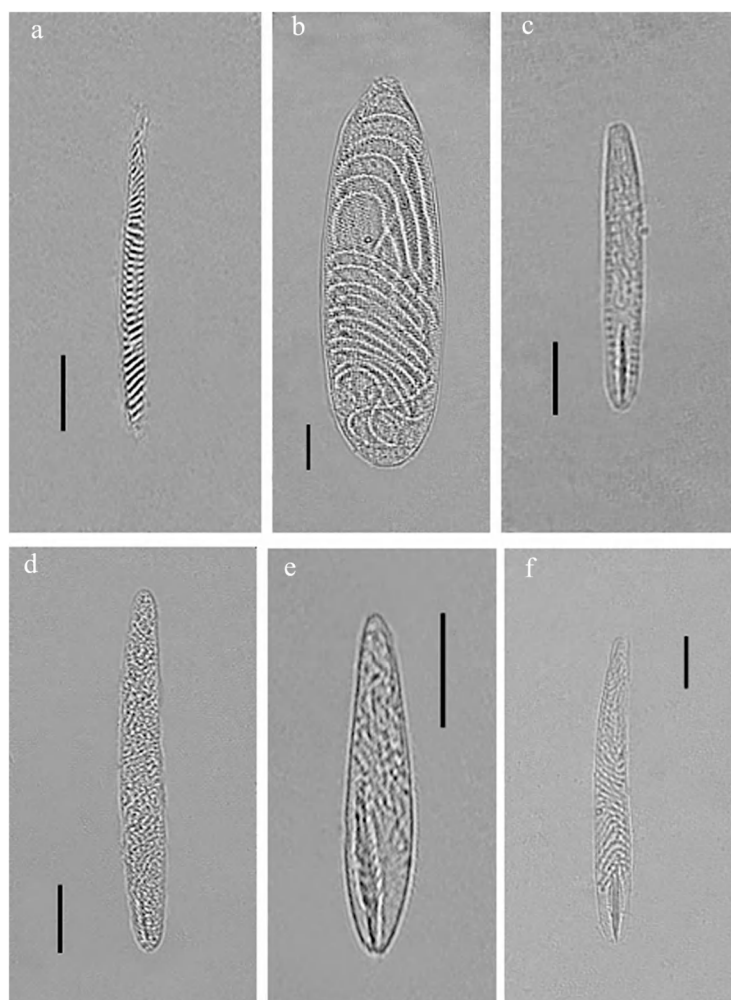


Fig. 1. – Cnidae of the acrospheres of *C. carnea*. (a) spirocyst, (b) holotrich, (c) microbasic b-mastigophore 1, (d) microbasic b-mastigophore 2, (e) microbasic p-mastigophore 1, (f) microbasic p-mastigophore 2. Bar: 10 $\mu$ m.

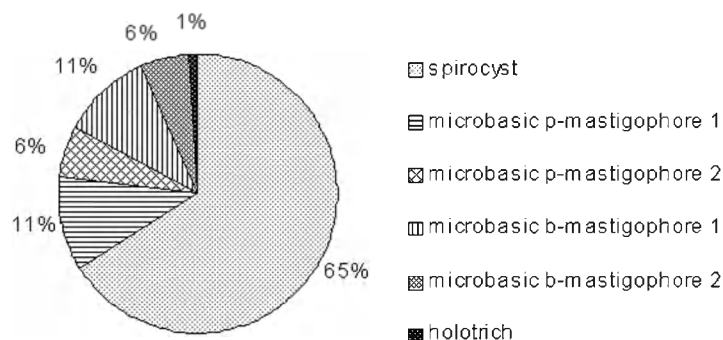


Fig. 2. – Abundances of the different cnidocyst types.

Size ranges of spirocysts were 22-80 x 2-4  $\mu\text{m}$  in length and width respectively, the holotrichs between 54-98 x 13-31  $\mu\text{m}$ , the microbasic b-mastigophores 1 between 31-70 x 4-6  $\mu\text{m}$ , the microbasic b-mastigophores 2 between 30-86 x 5-8  $\mu\text{m}$ , the microbasic p-mastigophores 1 between 20-55 x 5-10  $\mu\text{m}$  and the microbasic b-mastigophores 2 between 43-88 x 4-8  $\mu\text{m}$ .

#### Abundance of different cnidocysts

The pooled data of abundances for all types of cnidocysts in the 12 individuals are shown in Fig. 2. Clearly, the spirocysts are the most abundant cnida, followed by the microbasic p-mastigophores 1 and 2, and microbasic b-mastigophores 1 and 2, while the least abundant were the holotrichs, which were found in only five of the specimens.

#### Biometry

##### Statistically-descriptive parameters

The statistically-descriptive parameters for each cnida from the 12 analyzed individuals are shown in Tables 1-4. It can be observed that the holotrichs are the largest type (Table 2), while the microbasic p-mastigophores 1 are the smallest (Table 4).

TABLE 1

Descriptive statistical parameters of spirocysts

Indiv. N°	mean	std. dev.	CV	min.	max.
1	47.76	7.63	0.16	35	63
2	49.16	12.52	0.25	29	74
3	48.23	9.24	0.19	22	64
4	51.06	11.13	0.22	30	67
5	47.43	10.56	0.22	33	73
6	51.30	10.91	0.21	29	68
7	46.56	10.01	0.21	28	67
8	58.16	12.30	0.21	30	80
9	53.46	13.33	0.25	30	80
10	51.03	11.01	0.22	27	80
11	52.76	9.13	0.17	37	74
12	55.16	9.82	0.18	31	70

Mean, standard deviation (std. dev.), minimum (min.) and maximum (max.) in  $\mu\text{m}$ . CV (coefficient of variation).

TABLE 2

Descriptive statistical parameters of holotrich

Indiv. N°	mean	std. dev.	CV	min.	max.
1	74.50	7.55	0.10	55	89
2	75.50	7.62	0.10	61	87
3	75.76	6.21	0.08	65	88
4	76.26	7.74	0.10	57	91
5	84.63	6.85	0.08	72	98
6	72.60	8.98	0.12	54	89
7	82.26	6.37	0.08	65	95
8	79.40	7.00	0.09	66	90
9	86.80	7.64	0.09	67	98
10	76.60	5.51	0.07	61	85
11	79.03	5.62	0.07	68	92
12	78.76	7.93	0.10	62	94

Mean, standard deviation (std. dev.), minimum (min.) and maximum (max.) in  $\mu\text{m}$ . CV (coefficient of variation).

TABLE 3

Descriptive statistical parameters of microbasic b-mastigophores

Type	Indiv. N°	mean	std. dev.	CV	min.	max.
1	1	42.83	3.98	0.09	38	54
	2	41.30	3.36	0.08	33	47
	3	40.00	4.41	0.11	32	49
	4	39.96	3.89	0.10	32	46
	5	42.00	4.82	0.11	35	59
	6	40.26	4.26	0.11	31	46
	7	45.90	6.74	0.15	38	70
	8	43.33	4.17	0.10	36	50
	9	40.96	3.09	0.08	35	46
	10	40.83	3.54	0.09	32	46
	11	39.10	3.77	0.10	31	45
	12	41.00	2.57	0.06	35	46
2	1	48.00	5.27	0.11	42	61
	2	51.33	8.14	0.16	40	65
	3	51.93	12.84	0.25	37	86
	4	43.30	5.05	0.12	31	53
	5	43.70	6.69	0.15	30	53
	6	44.40	6.30	0.14	35	64
	7	59.76	10.08	0.17	40	76
	8	53.10	5.56	0.10	44	66
	9	61.80	12.01	0.19	43	83
	10	51.73	7.95	0.15	41	74
	11	45.73	4.94	0.11	40	59
	12	51.50	8.73	0.17	31	72

Mean, standard deviation (std. dev.), minimum (min.) and maximum (max.) in  $\mu\text{m}$ . CV (coefficient of variation).

TABLE 4

Descriptive statistical parameters of microbasic p-mastigophores

Type	Indiv. N°	mean	std. dev.	CV	min.	max.
1	1	33.23	1.83	0.06	30	38
	2	31.36	4.68	0.15	21	41
	3	36.36	5.35	0.15	27	46
	4	31.43	4.93	0.16	20	43
	5	40.60	7.81	0.19	27	55
	6	30.83	3.29	0.11	23	36
	7	34.43	5.68	0.16	25	45
	8	35.40	6.28	0.18	26	48
	9	31.86	2.73	0.09	26	38
	10	31.83	3.47	0.11	26	39
	11	31.80	4.52	0.14	23	41
	12	29.56	3.69	0.12	21	35
2	1	69.30	7.42	0.11	56	88
	2	59.73	6.57	0.11	43	70
	3	67.26	7.85	0.12	55	82
	4	69.76	7.10	0.10	53	79
	5	61.76	6.12	0.10	50	73
	6	59.23	6.71	0.11	45	70
	7	75.56	5.98	0.08	57	84
	8	69.13	5.39	0.08	58	78
	9	72.50	6.58	0.09	60	85
	10	59.86	5.17	0.09	51	70
	11	60.56	6.18	0.10	48	70
	12	65.36	6.29	0.10	52	75

Mean, standard deviation (std. dev.), minimum (min.) and maximum (max.) in  $\mu\text{m}$ . CV (coefficient of variation).

### Normality test

According to the analyses of residuals, normality of size distribution was accepted for the spirocysts ( $p=0.653$ ). However, for the holotrichs ( $p=0.003$ ), microbasic b-mastigophores 1 ( $p<0.001$ ), microbasic b-mastigophores 2 ( $p=0.001$ ), microbasic p-mastigophores 1 ( $p=0.024$ ) and microbasic p-mastigophores 2 ( $p=0.017$ ), a normal distribution of the capsule sizes was rejected. Fig. 3 shows the Q-Q plots of residuals vs. normal expected values of each cnida.

### ANOVA

An ANOVA test revealed that the sizes of spirocysts varied significantly among the 12 individuals ( $F=3.11$ ,  $p<0.0005$ ). These differences are illustrated with box-plots (Fig. 4).

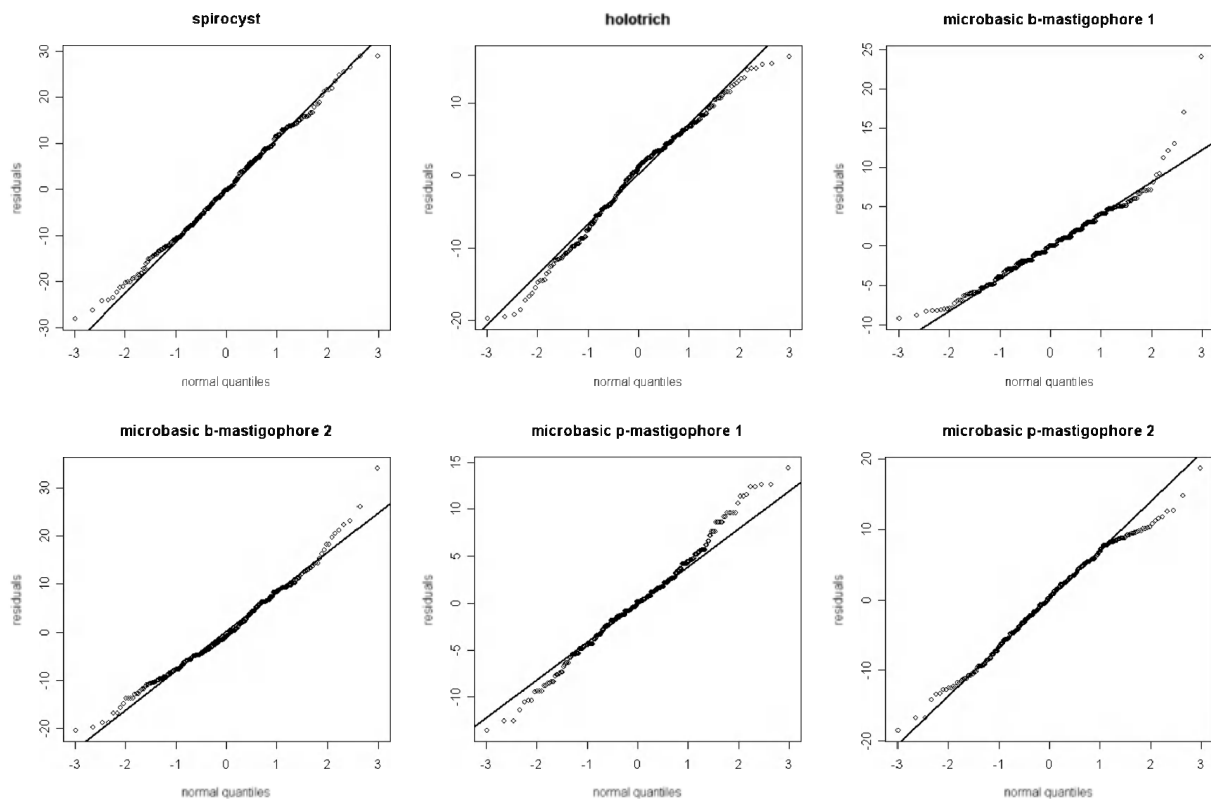


Fig. 3. – Q-Q plots of residuals vs. normal distribution.

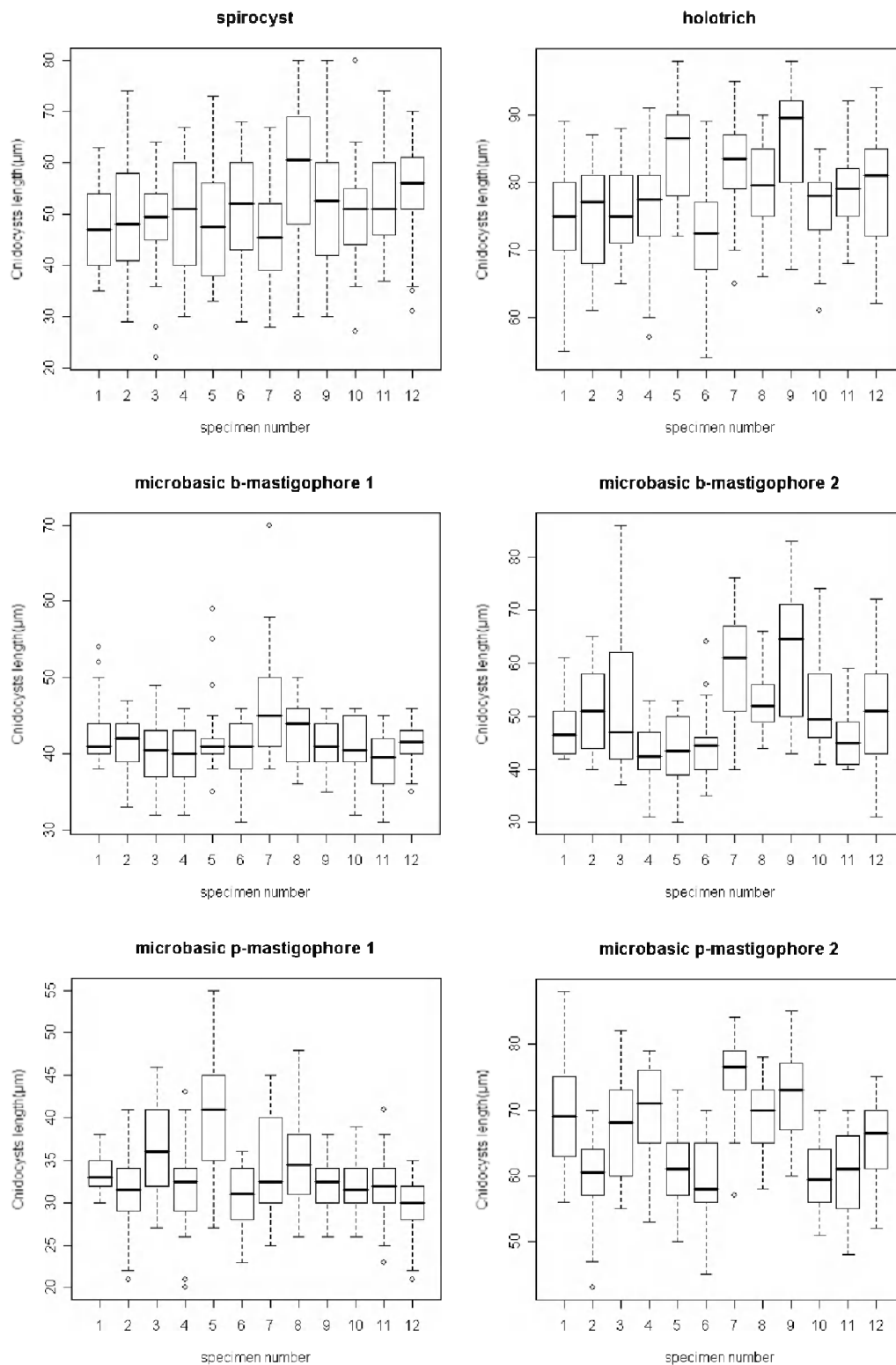


Fig. 4. – Box-plots of cnidocysts lengths. Outliers: o

### Generalized Linear Models (GLM)

Sizes of holotrichs, microbasic b- mastigophores 1 and 2, and microbasic p- mastigophores 1 and 2 fitted very well to a GLM with gamma errors (Fig. 5). This was con-

firmed by the homogeneous coefficients of variation (Tables 2-4), despite the variation of the mean (ACUÑA et al., 2007). The sizes of the above cnidocysts also varied significantly between individuals (Table 5 and Fig. 4).

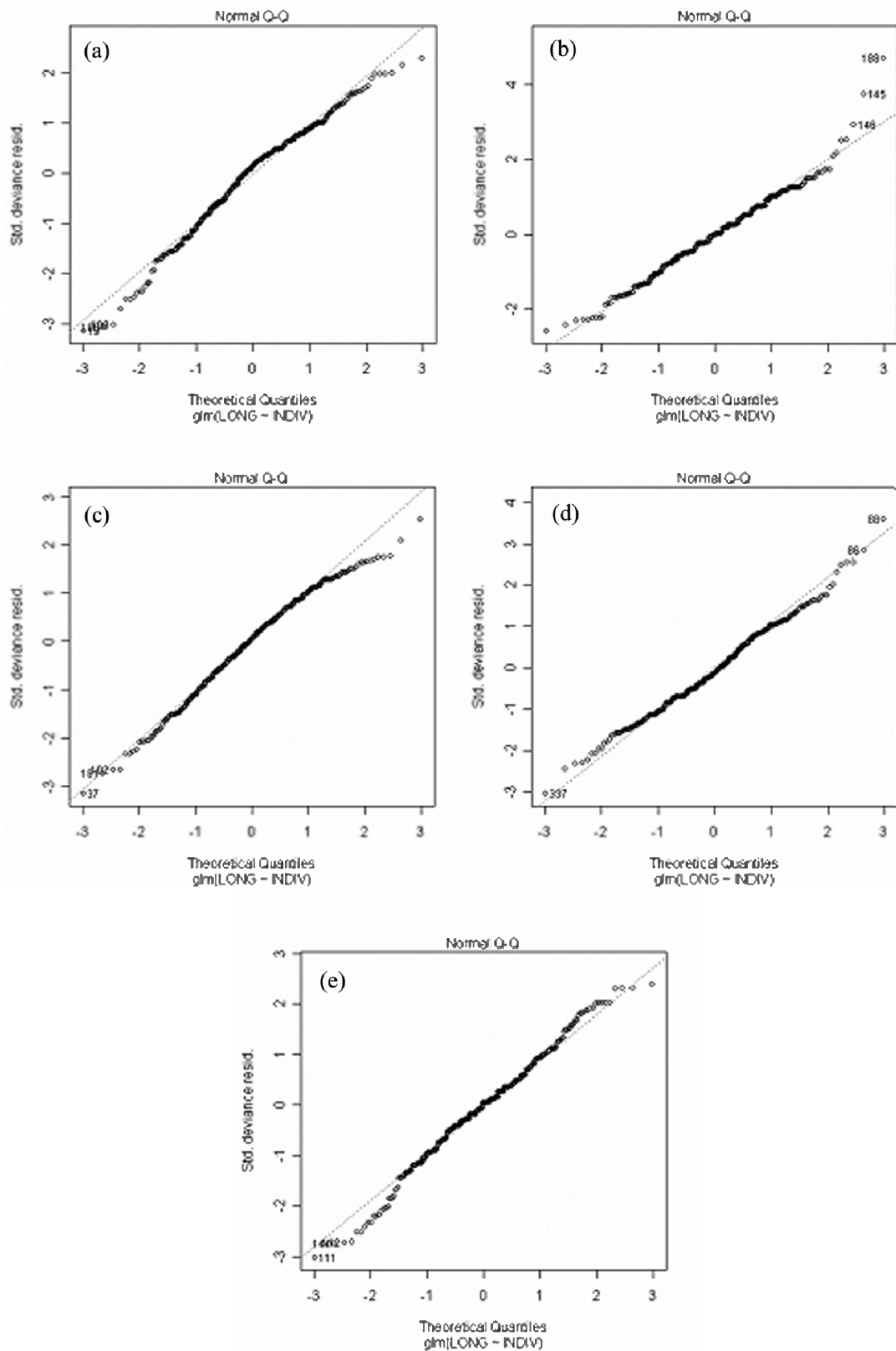


Fig. 5. – Q-Q plots of residuals vs. theoretical quantiles of GLM. (a) holotrich, (b) microbasic b-mastigophore 1, (c) microbasic b-mastigophore 2, (d) microbasic p-mastigophore 1, (e) microbasic p-mastigophore 2.

TABLE 5

P values of t test for the coefficients of the model ( $\beta_1$ ). The individual N° 1 is not shown because it was utilized to carry out the comparisons

	holotrich	microbasic b-mastigophore 1	microbasic b-mastigophore 2	microbasic p-mastigophore 1	microbasic p-mastigophore 2
2	0.575	0.157	0.100	0.110	< 0.0001 ***
3	0.478	0.008 **	0.054	0.013 *	0.246
4	0.324	0.007 **	0.012 *	0.124	0.793
5	< 0.0001 ***	0.445	0.022 *	< 0.0001 ***	< 0.0001 ***
6	0.277	0.017 *	0.056	0.040 *	< 0.0001 ***
7	< 0.0001 ***	0.007 **	< 0.0001 ***	0.326	0.0008 ***
8	0.008 **	0.652	0.014 *	0.081	0.925
9	< 0.0001 ***	0.084	< 0.0001 ***	0.245	0.079
10	0.242	0.064	0.066	0.234	< 0.0001 ***
11	0.013 *	< 0.0001 ***	0.235	0.222	< 0.0001 ***
12	0.020 *	0.090	0.084	0.001 **	0.023 *

significant at levels '\*\*\*\*' 0.001 '\*\*\*' 0.01 '\*\*' 0.05 respectively.

## DISCUSSION

Fourteen species within the genus *Corynactis* are considered valid according to FAUTIN (2006). The morphological differentiation of these species is very complicated, due to the difficulty of distinguishing the morphological characters usually used in sea anemone taxonomy (CARLGREN, 1927). Besides, the descriptions of several species are incomplete, mainly in terms of the composition of their cnidae. In the case of *C. carnea*, CARLGREN (1927) only mentioned some types without providing data related to their biometry. In this paper, we examined in detail the cnidae of the acrospheres of many individuals of this corallimorpharian species, and we clearly differentiated the following cnidocysts: spirocysts, microbasic b-mastigophores 1 and 2, microbasic p-mastigophores 1 and 2, and holotrichs. Within the 14 valid species mentioned by Fautin in her database, there are detailed descriptions of the cnidae of only three species: *C. viridis* (Allman, 1846) (DEN HARTOG et al., 1993), *C. californica* (Calgren, 1936) (HAND, 1955) and *C. denhartogi* (Ocaña, 2003). The cnidae of *C. carnea* are very similar to those of *C. viridis* from the Northern Hemisphere. Although DEN HARTOG et al. (1993) used a different nomenclature, this similarity in cnidae also occurs when applying the nomenclature used in this paper (ENGLAND, 1991). In the corallimorpharian *C. californica*, the cnidae composition within the acrospheres also is very similar, but only a single type of microbasic b-mastigophore is recognized. HAND (1955) does not differentiate the cnidae of the acrospheres and tentacles. In the same way, OCAÑA (2003) detailed the cnidae of the acrospheres and tentacles of *C. denhartogi* combined, recognising spirocysts, two holotrichs, two microbasic b-mastigophores and two microbasic p-mastigophores. It is interesting to mention that in other corallimorpharians such as *Rhodactis rhodostoma* (Ehrenberg, 1934), the marginal tentacles contain similar types of nematocysts (types 1 and 2 holotrichs, microbasic p-mastigophore, types 1 and 2 microbasic b-mastigophore) but not spirocysts (LANGMEAD & CHADWICK-FURMAN, 1999).

The abundances of the cnidocysts of *C. carnea* and *C. viridis* follow a similar pattern: the spirocysts are clearly

the most abundant, while the other types occur at a low frequency. However, a difference was observed concerning the holotrichs, which in *C. carnea* is the least abundant type, but in *C. viridis* is the second most abundant type. It is interesting to note that in scleractinian corals [closely related to the order Corallimorpharia and sharing the same categories of cnidocysts (DEN HARTOG, 1980; DEN HARTOG et al., 1993; PIRES, 1997)], there are modified tentacles (sweeper tentacles), whose tips are referred to as acrospheres and that contain a different proportion among the different cnidocyst types in comparison with *C. carnea*. For example, the tips of the tentacles of *Montastrea cavernosa* (Linnaeus, 1766) have 63% holotrichs (DEN HARTOG, 1977), while in *Galaxea fascicularis* (Linnaeus, 1758) the microbasic b-mastigophores make up 50% and the spirocysts 40% of the cnidae (HIDAKA & MIYAZAKI, 1984; HIDAKA & YAMAZATO, 1984).

Some authors have found a normal distribution of the capsule lengths of cnidocysts (WILLIAMS, 2000; ARDELEAN & FAUTIN, 2004; FRANCIS, 2004), but others have not, at least for acontiarian sea anemones, the actinarian *Oulactis muscosa* (Drayton in Dana, 1846) and zoanthids (ACUÑA et al., 2003; 2007 and references therein). THOMASON & BROWN (1986) showed clearly that generalizations cannot be made concerning the statistical distribution of the cnidae in scleractinian corals. In *C. carnea*, normality of size distributions was rejected for five of the six types of cnidocysts. However, a normal model fitted very well to the spirocysts, this being coincident with the results obtained by FRANCIS (2004) for the sea anemone *Anthopleura elegantissima* (Brandt, 1835). According to our results and those of other authors, we conclude that a normal distribution is uncommon, and the statistical distribution of cnidae size must be tested before any biometric analysis.

Sizes of the cnidocysts of *C. carnea* varied between individuals. Studies carried out in the acontiarian sea anemones *Haliplanella lineata* (Verrill, 1869), *Tricnidactis errans* (Pires, 1988), *Anthothoe chilensis* (Lesson, 1830) and the actinarians *O. muscosa* and *A. elegantissima* (ACUÑA et al., 2003; 2007; FRANCIS, 2004), also showed intraspecific variation in capsule sizes. This variability reduces the taxonomic value of cnidocysts, and

could be explained by the supply and demand of cnidocysts for the sea anemones (ROBSON, 1988).

According with the results obtained here, the cnidae of the acrospheres of *C. carnea* are similar to those of other species of the genus. This species is part of the *C. viridis*-complex, along with *C. viridis*, *C. annulata* (Verrill, 1866); *C. chilensis* (Carlgren, 1941) and *C. delawarei* (Widersten, 1976), because of their distribution (Atlantic Ocean and Pacific of South America) and morphological similarity (DEN HARTOG et al., 1993). Because of this similarity and high intraspecific variability (colour and cnidocyst sizes), it is clearly necessary to revise the genus *Corynactis* and this species complex, perhaps using molecular characters that are useful especially when the morphological ones are obscure or difficult to distinguish.

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