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ARTICLE

Morphological study of the sclerites of the species *Renilla muelleri* Kölliker, 1872 (Anthozoa, Octocorallia, Pennatulacea)

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Abstract. Some octocorals (Cnidaria, Anthozoa) can produce a sub-millimeter to a millimeter-range skeleton of calcium carbonate, known as sclerites. Sclerites have diverse morphological characters, such as size, and shape features that give them relevance as taxonomic characters at different levels. *Renilla* Lamarck, 1816, is a genus of the order Pennatulacea (Octocorallia), in which colonies are characterized by two distinct zones: a peduncle, which anchors them to the substrate, and a rachis that supports the polyps. In the case of *Renilla muelleri* Kölliker, 1872, prior research established the presence of similar sclerites in both the peduncle and the rachis. Nevertheless, the potential morphological variations of these sclerites among colonies and within different colony regions have yet to be assessed to determine the stability of these characteristics at the species level. This study aimed to describe and compare the external microscopic morphology and biometry of the sclerites of *Renilla muelleri,* enhancing their identification and assessing their consistency within the species. Sclerite composition was examined across the entire colony, and we analyzed length variation among colonies using generalized linear models (GLM) and within colony zones using generalized linear mixed models (GLMM). Additionally, the external microscopic morphology of all sclerites was examined through scanning electron microscopy (SEM). Based on size, two types of sclerites, namely large and small, were identified within the colonies. Both types exhibited significant size differences among colonies. Furthermore, the large sclerites displayed notable variations between zones, with those in the rachis being the largest and holding the highest rank within the colonies. In terms of external microscopic morphology, the sclerites exhibited considerable variability, making it challenging to establish clear groupings based on these characteristics. Based on the obtained results, it can be concluded that both the biometry and external microscopic morphology of sclerites do not exhibit consistency as characteristics in *Renilla muelleri* highlighting the ambiguity in defining *Renilla* species.

Keywords. Dimorphic colonies; Octocorals; Taxonomy; SEM.

INTRODUCTION

The size, and external microscopic morphology of sclerites are pivotal features in the taxonomy of organisms within the subclass Octocorallia (Cnidaria: Anthozoa), as they serve as diagnostic characteristics (Sethmann *et al.,* 2007). Nonetheless, the identification and classification of different sclerite types pose a challenging task due to the considerable variation in size and shape observed both within and between species (Lewis & von Wallis, 1991; Vargas *et al.,* 2010; Mastrototaro *et al.,* 2015; Gutiérrez-Rodríguez *et al.,* 2009; West *et al.,* 1993; Everton *et al.,* 2015). Some authors showed that SEM analyses can provide information on the morphological differentiation between them and can be considered diagnostic at the species level (Sánchez, 2007). Aharonovich & Benayahu (2011), Benayahu *et al.* (2018), and Halász *et al.* (2019) attributed this morphological difference to the modes of growth and aggregation of calcite crystals.

The history of the genus *Renilla* Lamarck, 1816 (Pennatulacea) has been marked by challenges in species description. It previously included 13 species, of which only 6 are currently considered valid. Incomplete descriptions and the difficulty in identifying reliable taxonomic

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characteristics have contributed to these taxonomic issues, exemplified by cases such as *Renilla chilensis* Philippi, 1892 (considered a *nomen dubium*) (Pérez & Zamponi, 1999). Prior literature has suggested that the sclerites of the genus *Renilla* consist of three-flanged rods and exhibit similar lengths in both the peduncle and rachis (Pérez & Ocampo, 2001). *Renilla musaica* Zamponi & Pérez, 1995 represent the unique exception showing variations in the lengths of sclerites between rachis and peduncle. *Renilla muelleri* Kölliker, 1872, is among the most well-documented species within the genus and is widely distributed along the Argentine coast (Zamponi & Pérez, 1995). Previous research, as conducted by Clavico *et al.* (2007), has specifically examined variations in sclerite sizes in *R. muelleri* while also established links between these variations and the depth at which samples were collected. While sclerites have been recognized as diagnostic features between species, it is essential to consider the possibility of morphological variation within a given species. Based on quantitative data, the significance of these features and their potential utility for future taxonomic purposes remains to be comprehensively evaluated. This study aimed to describe and compare the microscopic external morphology and biometry of *Renilla muelleri* sclerites in order to facilitate the identification and comparison of these structures within the species. Additionally, the study aimed to assess the consistency of sclerites as a taxonomic character at the species level.

MATERIAL AND METHODS

Material studied

The studied specimens were identified as *Renilla muelleri* following the morphological characteristics presented by Zamponi & Pérez (1995). Colonies have a horseshoe or kidney-shaped form. They exhibit a relatively short peduncle. Autozooids feature five calycinal teeth. In the dorsal section, the rachis is completely covered with zooids, while the ventral part of the rachis and peduncle display a deep violet coloration (Fig. 1). According to the original description (Zamponi & Pérez, 1995), *R. muelleri* presents peduncle sclerites of the same length as those of the rachis. This characteristic will be discussed below, as the study of sclerite lengths is the focus of this investigation. On the other hand, the original description of *R. muelleri* identifies the cnidocysts: microbasic amastigophores, holotrichous isorhizas, atrichous anisorhizas, and macrobasic mastigophores (Zamponi & Pérez, 1995). However, in the specimens analyzed here, only one type of cnidocyst could be identified, probably atrichous isorhiza, although without certainty.

Study site

We examined ten colonies of *Renilla muelleri* Kölliker, 1872, collected in the Atlantic Ocean. Four of these col-

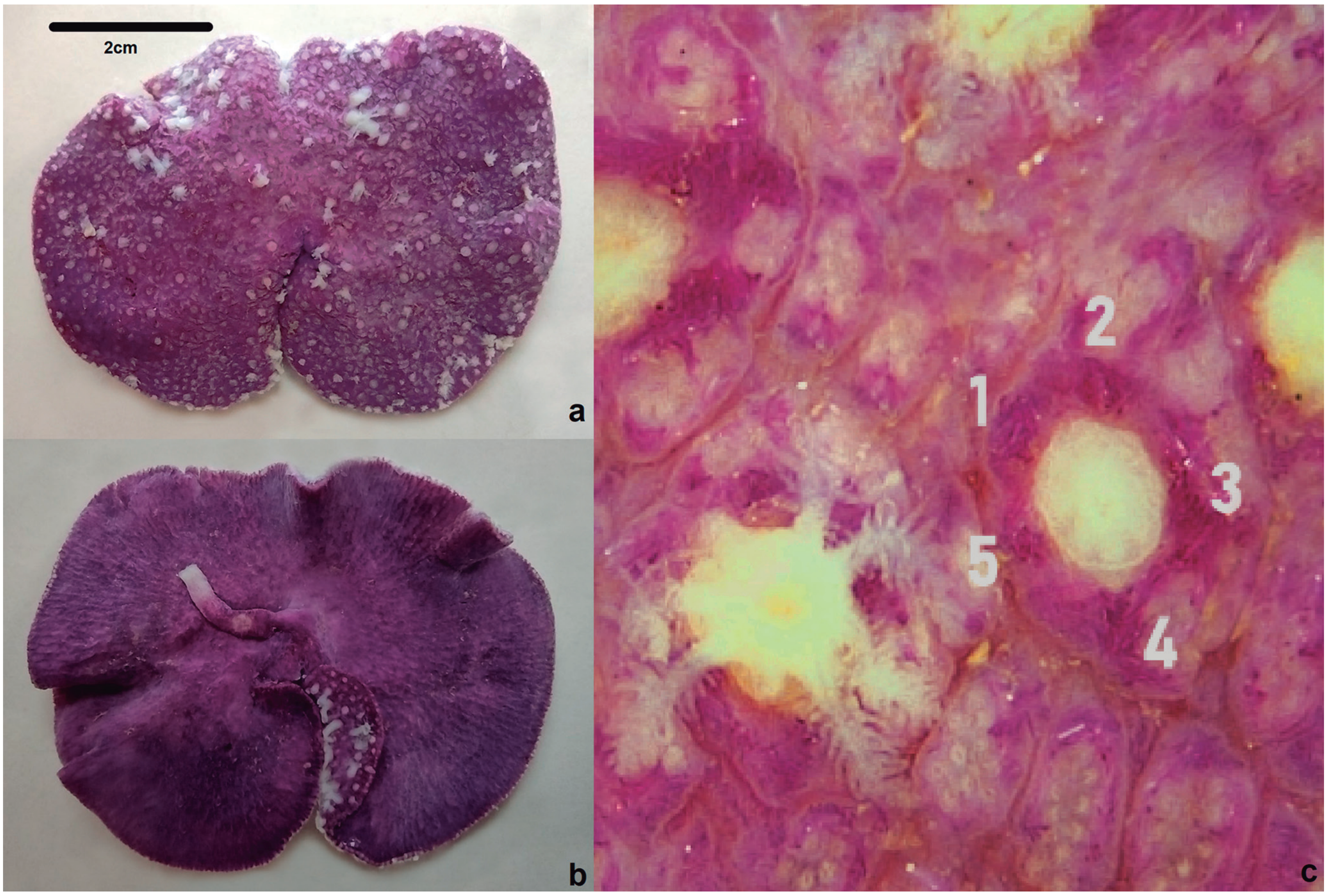

Figure 1. *Renilla muelleri.* (a) Dorsal view; (b) Ventral view; (c) Autozooids with calycinal teeth numbered 1 to 5.

Figure 2. Study area and sampling stations. The triangle corresponds to the place of the Undine ('U') campaign and the circle to the place of the Holmberg ('H') campaign.

onies (hereafter referred to as 'U') were obtained in 24/July/1926, on an expedition aboard the ship "Undine," operated by Gardella-Cap. Alexandersson Company (35°08′S, 52°35′W, at a depth of 50 meters). The remaining six colonies (hereafter referred to as 'H') were collected during the H09/92 B.I.P. "Dr. Eduardo L. Holmberg" INIDEP expedition (37°08′S, 56°41′W, at a depth of 16 meters) in 1992 (Fig. 2).

Protocol of sclerite preparation

To obtain sclerite samples, 5 mm^3 sections of tissue were carefully extracted with a scalpel from both the rachis and peduncle. Subsequently, these samples were subjected to treatment with sodium hypochlorite to facilitate sclerite dissociation. Finally, ethanol and distilled water were used to clean the sample.

Sclerite composition and biometry

As the data originated from different collection sites, we kept the separation by distinguishing between the "Undine" (referred to as "U") and "Holmberg" (referred to as "H") campaigns for our analysis. We identified the distinct types of sclerites according to their size, and their location (peduncle and rachis), and these were subsequently measured and photographed under an optical microscope at 40X magnification. To aid in these tasks, we employed the Leica Application Suite EZ software (version 3.4.0, Leica Microsystems, Wetzlar, Germany).

The normality of sclerite length was evaluated by means of a Shapiro-Wilk test ($\alpha = 0.05$), applied to the residuals obtained from a linear model assumed to have a normal distribution. If normality was not supported, a Generalized Linear Model (GLM) with a gamma distribution for error terms was employed as an alternative approach. The adequacy of these models was assessed through graphical methods, including Quantile-Quantile (Q-Q) plots, depicting the relationship between the residuals and the theoretical quantiles of the model, as well as scatter plots illustrating the deviation of residuals about the model's predicted values.

Analysis of sclerite type length variation among colonies

As our data originated from two collection sites, we conducted an assessment of their influence on sclerite length variations among colonies. To account for potential dependencies, a Generalized Linear Mixed Model (GLMM) was fitted, with the variable 'Campaign' included as a random effect. The model was formulated as follows:

$$y_i\,(\text{Length}) = \beta_i \times (\text{Colony}) + z_i \mu_i\,(\text{Campaign}) + \varepsilon_i$$

To evaluate the statistical significance of the random effect associated with the variable 'Campaign,' we performed a comparison between a Generalized Linear Model (GLM) and the GLMM using ANOVA ($\alpha = 0.05$). The Akaike Information Criterion (AIC) was employed to determine the model that best fit the data. Subsequently, differences between the β_1 coefficients of the colonies were assessed using a t-test ($\alpha = 0.05$) to ascertain disparities in sclerite length among the various colonies for each sclerite type, separately for both peduncle and rachis.

Analysis of sclerite length variation between peduncle and rachis

Given that the dataset comprised measurements from 10 colonies across two campaigns conducted at various depths, similar to the previous analysis, the variables "Campaign" and "Colony" were treated as random effects during the significance assessment in the analysis. The variable 'Zone' was included as fixed effect, assessing rachis and peduncle data. Consequently, the formulated model was as follows:

$$y_i\,(\text{Length}) = \beta_i \times (\text{Zone}) + z_i \mu_i\,(\text{Colony}) + z_i \mu_i\,(\text{Campaign}) + \varepsilon_i$$

To select the optimal model, the significance of each random variable was assessed by iteratively removing them one at a time, employing Akaike's Information Criterion. Subsequently, the best model was compared against a null model using ANOVA ($\alpha = 0.05$).

From the Generalized Linear Mixed Model (GLMM), the variance explained by the variables 'Colony' and 'Campaign' was examined. Subsequently, a t-test (α = 0.05) was performed on the coefficients β_i of the GLMM to compare sclerite-type lengths between the peduncle and rachis zones.

Data analysis

All data visualization, modeling, and statistical analyses were conducted using the R programming language (R Core Team, 2020) and the following packages: ggplot2 (Wickham, 2016), lme4 (Douglas *et al.,* 2015), and Rcmdr (Fox & Bouchet-Valat, 2020).

Size of the colony

The estimation of colony size was conducted by measuring the area of the dorsal face of the rachis on scaled photographs through the utilization of the ImageJ software. To assess the potential correlation between colony size and sclerite size, a Spearman correlation test was employed, with a significance level set at α = 0.05.

External microscopic morphology analysis of sclerites

The sclerites derived from both the rachis and peduncle were prepared following the protocol outlined in Section "Protocol of Sclerite Preparations". Following preparation, the samples were affixed to a scanning electron microscope (JEOL JSM-646LV) slide surface using bifacial tape and subsequently coated with a gold-palladium layer for observation. Photomicrographs of the sclerites from the rachis and peduncle were captured to analyze the ultrastructural features of their external morphology.

RESULTS

Composition and Biometry of Sclerites

The composition and biometry of the sclerites exhibited similarity across both campaigns. Two discernible types of sclerites were identified in both the peduncle and rachis based on their size, labeled as "large" and "small." The size distinction between the two sclerite types was defined by a length threshold of 200 ± 3 µm according to the distribution of sizes in a kernel density plot (Fig. 3-5, Table 1). A total of 2,400 sclerites were measured (60 per type and zone of the colony).

Analysis of sclerite length variations among colonies

The variable "Campaign" was determined to be non-significant in the analysis and was subsequently excluded. The GLM model was selected for all cases (Table 2). According to the t-test for β_i of the GLM, significant differences in sclerite length between colonies were observed for both types of sclerites in both the rachis and peduncle (Tables 3-6).

Table 1. Descriptive statistics of *Renilla muelleri* sclerite length biometry discriminated by campaigns. Range (minimum-maximum), mean ± standard deviation (SD), N: total number of measurements.

Zone	Type	Campaign	Range: min-max. (µm) (mean ± SD)	N
Rachis	Small	Holmberg	103 – 197 (156.44 ± 23.97)	600
		Undine	102 – 197 (159.87 ± 26.07)	
	Large	Holmberg	200 – 516 (310.64 ± 70.27)	600
		Undine	205 – 524 (358.48 ± 83.90)	
Peduncle	Small	Holmberg	103 – 200 (159.28 ± 24.69)	600
		Undine	103 – 197 (155.43 ± 24.70)	
	Large	Holmberg	200 – 516 (290.24 ± 60.94)	600
		Undine	205 – 524 (337.61 ± 73.24)	

Table 2. ANOVA of the models for length differences between individuals. * Denotes the selected model. AIC: Akaike's Information Criterion, with the lowest value indicating the best-fitted model.

Zone	Type	Model	AIC	p-value
Rachis	Small	Length~ Colony*	5557	
		Length~ Colony + (1 | Campaign)	5561	1
	Large	Length~ Colony*	6752.8	
		Length~ Colony + (1 | Campaign)	6756.8	1
Peduncle	Small	Length~ Colony*	5520.2	
		Length~ Colony + (1 | Campaign)	5524.2	1
	Large	Length~ Colony*	6224.2	
		Length~ Colony + (1 | Campaign)	6228.2	1

Analysis of sclerite length variations between peduncle and rachis

Normality tests for sclerite length data led to rejection in all datasets [rachis: small ($p < 0.001$), large ($p = 0.004905$), peduncle: small ($p < 0.001$), large ($p < 0.001$)]. Consequently, a generalized linear model was fitted, demonstrating a good fit (Appendix: Figs. 1-4). For small sclerites, the optimal model incorporated the variable "Colony" as a random effect. In the case of large sclerites, the model included both "Colony" and "Campaign" as random effects (Table 7). This suggests that the "Colony" variable had a significant effect on both large and small sclerites, while the "Campaign" variable was only significant for large sclerites.

The small sclerites exhibited no significant differences ($p = 0.845$) in length between those from the peduncle and rachis. Conversely, the large sclerites displayed significant differences in length ($p < 0.001$), and based on the estimated model values, those from the rachis (estimated = 338.53 µm) were longer than those from the peduncle (estimated = 298.18 µm). The variables "Colony" (standard deviation = 64.81) and "Campaign" (standard deviation = 73.55) accounted for a substantial portion of the model's variance, while the variance explained by the

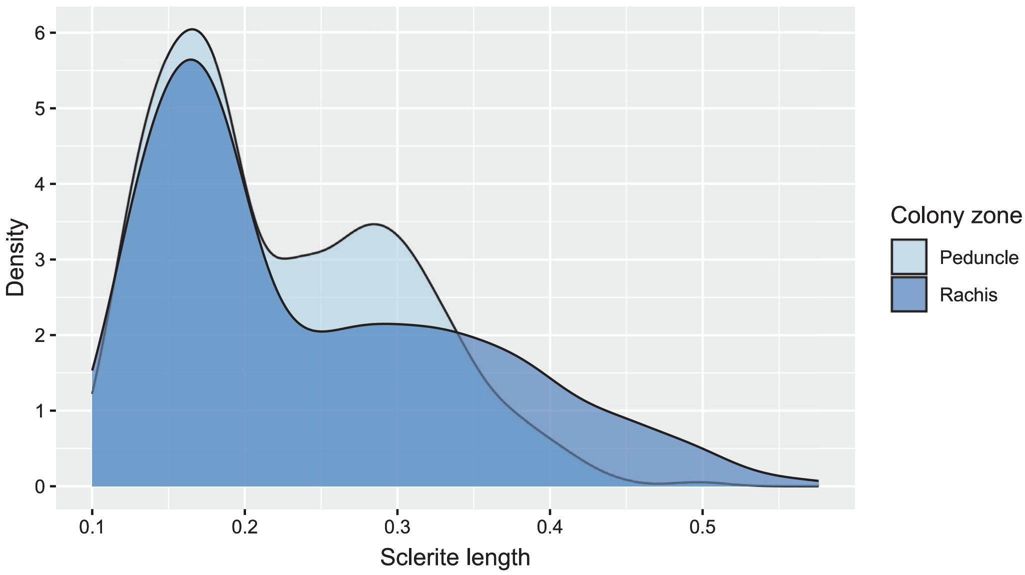

Figure 3. Kernel density plot of the length (µm) of all sclerite types according to the zone of *Renilla muelleri* colonies.

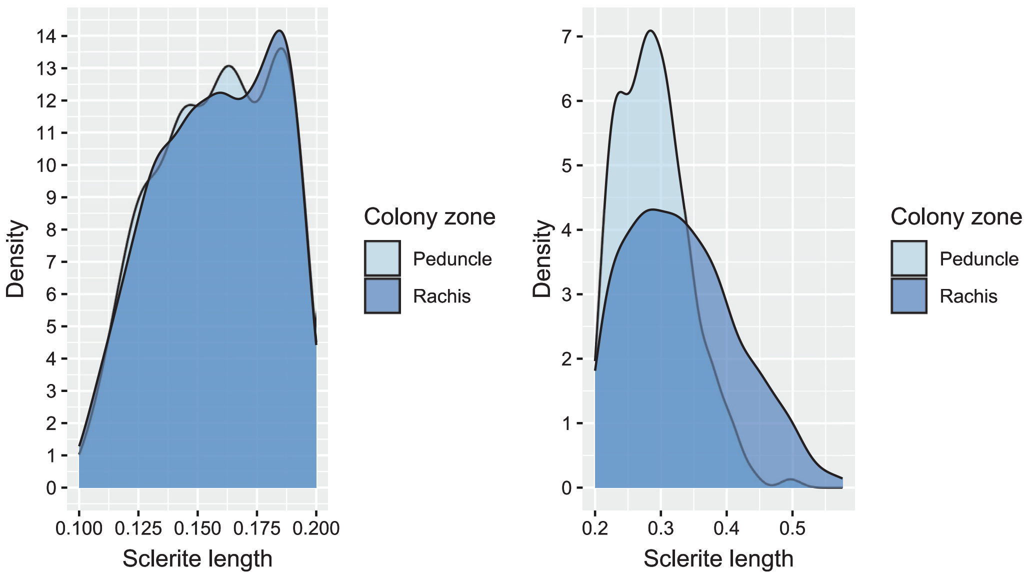

Figure 4. Kernel density plot of the length (µm) of (a) small and (b) large sclerites according to the zone of the *Renilla muelleri* colony.

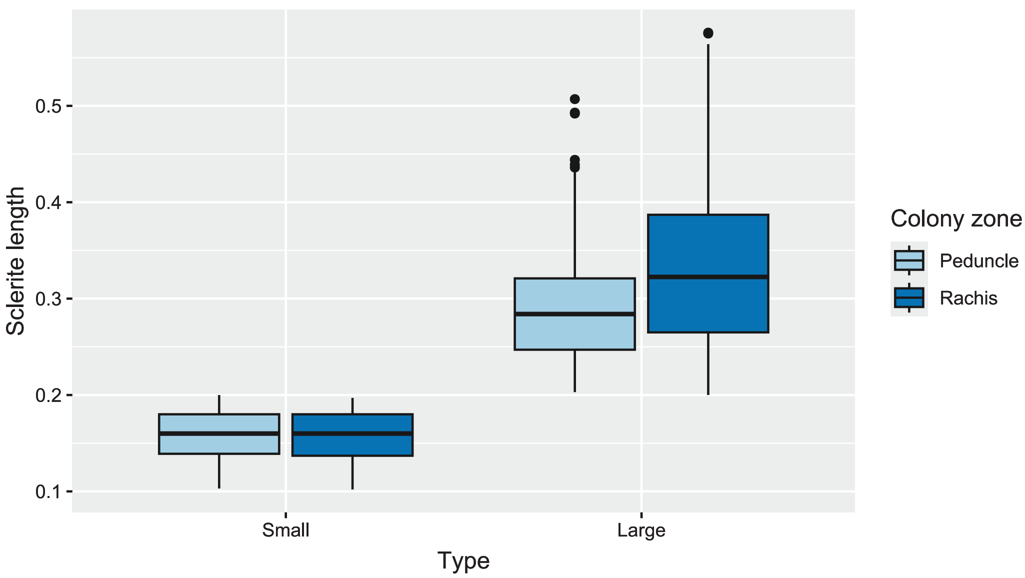

Figure 5. Stratified boxplot for the length (µm) of each type of sclerite according to the zone of *Renilla muelleri* colonies.

Table 3. *Renilla muelleri.* Rachis, sclerite small. Estimated values and confidence intervals derived from GLM, along with the p-value of the t-test for β_1 of the model. * Indicates significant differences for α = 0.05. The p-value for individual 1H is not displayed, as it was employed for comparisons. "H" denotes colonies from the Holmberg campaign, and "U" corresponds to colonies from the Undine campaign.

Colony	Estimated calculated (µm)	2,50%	97,50%	p-value
1H	150,35	156,36	144,64	—
2H	153,07	169,09	139,69	0,52428
3H	166,03	184,60	150,73	0,000459 *
4H	164,64	167,43	138,49	0,756488
5H	158,95	176,10	144,72	0,048403 *
6H	158,57	175,64	144,39	0,058959
1U	169,73	189,07	153,87	0,0000198 *
2U	151,33	167,03	138,21	0,816653
3U	153,17	169,21	139,79	0,509271
4U	165,23	183,64	150,05	0,000852 *

Table 4. *Renilla muelleri.* Rachis, sclerite large. Estimated values and confidence intervals derived from GLM, along with the p-value of the t-test for β_1 of the model. * Indicates significant differences for α = 0.05. The p-value for individual 1H is not displayed, as it was employed for comparisons. "H" denotes colonies from the Holmberg campaign, and "U" corresponds to colonies from the Undine campaign.

Colony	Estimated calculated (µm)	2,50%	97,50%	p-value
1H	272,70	288,45	258,07	—
2H	339,41	402,71	292,83	9,89E-08 *
3H	336,03	398,13	290,23	3,47E-07 *
4H	293,38	341,39	256,77	0,0694 *
5H	301,15	351,56	262,93	0,0139
6H	321,20	378,15	278,70	5,70e-05 *
1U	357,17	427,09	306,46	8,09e-11 *
2U	368,54	444,17	314,38	2,26E-12 *
3U	345,22	410,64	297,31	1,05E-08 *
4U	364,34	437,04	311,91	3,82E-12 *

residuals was low (standard deviation = 0.04) (Table 8). The confidence intervals for the rachis sclerites were broader compared to those of the peduncle sclerites.

Correlation between sclerite size and colony size

The colonies collected in the "Undine" campaign were smaller in size than the colonies from the "Holmberg" campaign (Table 9). In the rachis, the large sclerite length showed a barely negative correlation ($p < 0.001$; rho = –0.28) with colony size; meanwhile, no significance was found for

Table 5. *Renilla muelleri.* Peduncle, sclerite small. Estimated values and confidence intervals derived from GLM, along with the p-value of the t-test for β_1 of the model. * Indicates significant differences for α = 0.05. The p-value for individual 1H is not displayed, as it was employed for comparisons. "H" denotes colonies from the Holmberg campaign, and "U" corresponds to colonies from the Undine campaign.

Colony	Estimated calculated (µm)	2,50%	97,50%	p-value
1H	155,00	161,02	149,27	—
2H	163,83	180,98	149,53	0,04322 *
3H	165,25	182,66	150,74	0,01961 *
4H	157,68	173,69	144,26	0,5304
5H	169,05	187,20	153,98	0,00161 *
6H	161,95	178,74	147,92	0,10931
1U	156,12	171,84	142,91	0,79298
2U	141,93	155,22	130,62	0,00137 *
3U	145,77	159,69	133,96	0,02515 *
4U	160,13	176,58	146,37	0,23394

Table 6. *Renilla muelleri.* Peduncle, sclerite large. Estimated values and confidence intervals derived from GLM, along with the p-value of the t-test for β_1 of the model. * Indicates significant differences for α = 0.05. The p-value for individual 1H is not displayed, as it was employed for comparisons. "H" denotes colonies from the Holmberg campaign, and "U" corresponds to colonies from the Undine campaign.

Colony	Estimated calculated (µm)	2,50%	97,50%	p-value
1H	267,58	252,88	257,49	—
2H	276,77	275,54	252,48	0,22734
3H	253,43	252,98	232,41	0,05225
4H	273,28	272,18	249,50	0,45055
5H	254,67	254,17	233,48	0,07701
6H	293,31	291,55	266,51	0,00114 *
1U	321,22	318,36	290,02	1,98E-10 *
2U	310,20	307,79	280,78	2,11E-07 *
3U	328,37	325,14	296,05	1,16e-12 *
4U	309,20	306,77	280,00	3,45E-07 *

Table 7. ANOVA of the models for differences in Length between zones. * Denotes the selected model. ** Indicates significant differences for α = 0.05. AIC: Akaike's Information Criterion, with the lowest value corresponding to the best-fitted model.

Type	Model	AIC	p-value
Small	**Length~ Zone**	11148	
	Length~ Zone + (1 | Colony) *	11095	1.452e-13 **
	Length~ Zone + (1 | Colony) + (1 | Campaign)	11097	1.369e-12 **
Large	**Length~ Zone**	13270	
	Length~ Zone + (1 | Colony)	13096	< 2.2e-16 **
	Length~ Zone + (1 | Colony) + (1 | Campaign) *	13093	< 2.2e-16 **

Table 8. Estimate and confidence intervals calculated from the GLMM model. P-value of a t-test of the β_i of the peduncle vs. rachis model. * Denotes significant differences for α = 0.05. N: total number of measurements. CI: confidence intervals.

Zone	Type	Estimated (µm)	CI		Standard Deviation			p-value	N
			2,50%	97,50%	Individual	Campaign	Residuals		
Rachis	**Small**	158,04	149,80	166,29	14,53	—	0,02	0,845	—
	Large	338,53	305,71	371,31	64,81	73,55	0,04	< 2e-16 *	600
Peduncle	**Small**	157,77	152,30	163,24	14,53	—	0,02	—	—
	Large	298,18	271,98	324,38	64,81	73,55	0,04	—	600

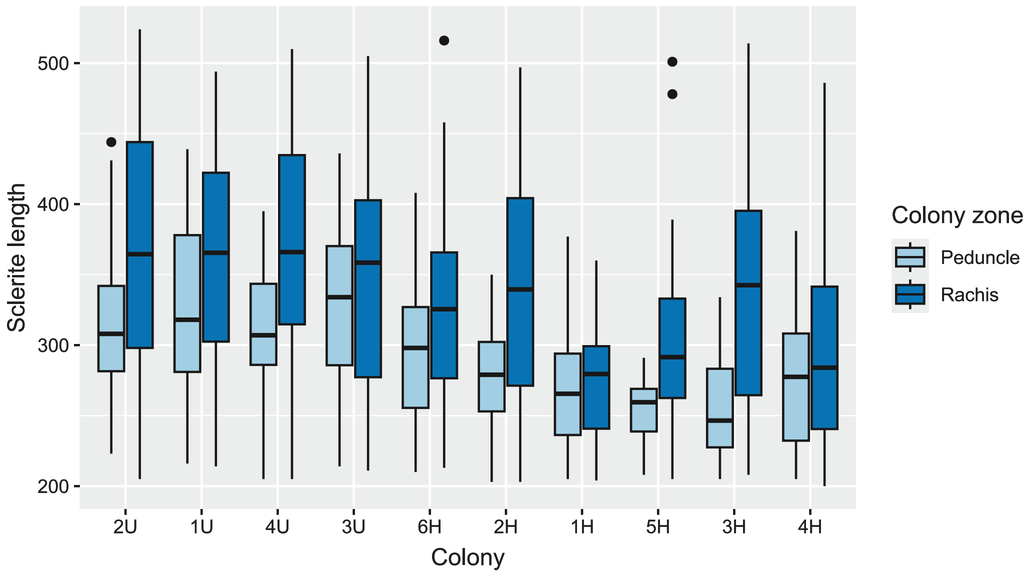

Figure 6. Stratified boxplot for large sclerites of *Renilla muelleri* by colony zone. Colonies are arranged on the x-axis in increasing order of size. "H" denotes colonies from the Holmberg campaign, and "U" corresponds to colonies from the Undine campaign

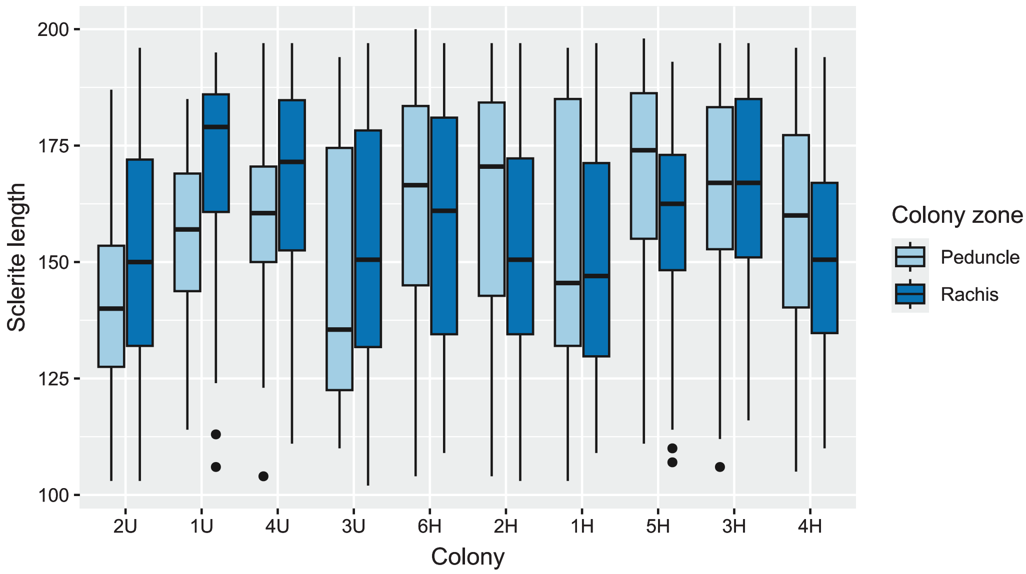

Figure 7. Stratified boxplot for small sclerites of *Renilla muelleri* by colony zone.. Colonies are arranged on the x-axis in increasing order of size. "H" denotes colonies from the Holmberg campaign, and "U" corresponds to colonies from the Undine campaign

Table 9. Size of *Renilla muelleri* colonies estimated from the rachis area (cm^2) of the colonies. "H" denotes colonies from the Holmberg campaign, and "U" corresponds to colonies from the Undine campaign.

Colony	1H	2H	3H	4H	5H	6H	1U	2U	3U	4U
Rachis area(cm^2)	16,11	14,07	19,51	24,23	18,93	11,13	4,72	4,61	7,87	6,07

the small ones ($p = 0.09$). In the peduncle, the lengths of large sclerites had a negative correlation ($p = < 0.001$; $rho = -0.42$), and for small sclerites, the correlation was slightly positive ($p < 0.001$; $rho = 0.20$) (Figs. 6 and 7).

External microscopic morphology

In terms of the microscopic external morphology of sclerites, both the peduncle and rachis exhibited spindles. SEM observations revealed considerable variability in the external morphology of each sclerite type. Within the specimens, sclerites displayed predominantly smooth and regular margins, while others exhibited mostly irregular margins. All sclerites featured grooves along the longitudinal axis, some linear and/or spiral, complete from one end of the axis to the other and/or incomplete, present only in a variable portion of the sclerite. These morphological characteristics were consistent across both sizes and in both areas of the colony in both campaigns. The external microscopic morphology variability encountered precluded discrimination or grouping based on these characteristics (Fig. 8).

DISCUSSION AND CONCLUSION

The analysis of sclerite composition in the octocoral *Renilla muelleri,* considering external microscopic morphology, revealed the presence of spindles in both the peduncle and rachis. Two size categories were identified: small and large. This information represents a novel contribution to the understanding of this species and enhances the original description by Zamponi & Pérez (1995).

The results highlight intraspecific variation in the length of both types of sclerite among colonies, a pattern consistent with findings from other studies on alcyonarian colonies (West *et al.,* 1993; Gutiérrez-Rodríguez *et al.,* 2009; Everton *et al.,* 2015). Clavico *et al.* (2007) similarly observed size variation among colonies of *Renilla muelleri,* attributing it to phenotypic variability associated with colony depth. These observations reflect uncertainty regarding the size of sclerites in defining *Renilla* species. The descriptions of *R. muelleri* and *R. musaica* are very similar, and the terminology used to describe the sclerites is somewhat imprecise. For instance, phrases like "sclerites short and wide in the rachis" and "long and thin in the peduncle" used in the description of the species (Zamponi & Pérez, 1995), lack clarification on what constitutes short or long. Indeed, just two differences stand out between the descriptions of *R. muelleri* and *R. musaica.* Firstly, there is the issue of cnidocyst identification. We could not definitively identify any of the cnidocyst types mentioned in the descriptions of either species. Only one structure resembling a cnidocyst could be probably identified as an atrich isorhiza. This type of cnidocyst is mentioned in the original description of *R. muelleri.* Secondly, *R. musaica* is defined by exhibiting differences in sclerite length between the rachis and peduncle; while *R. muelleri* shows no differences. Our work reveals differences between the "large" sclerites from the rachis and peduncle in *R. muelleri,* with those from the rachis being longer. Zamponi & Pérez (1995) reported only one type of sclerite based on size in both species without distinguishing between "small" and "large". Figure 9 compiles and shows graphically the size of sclerites from *R. muelleri* obtained in this work and those from *R. muelleri* and *R. musaica* extracted from Zamponi & Pérez (1995). The values indicated by these authors for the sizes of the sclerites of both *R. muelleri* and *R. musaica* overlapped with the sizes found in this study for the "large" sclerites (Fig. 9). Additionally, it is observed that the "small" sclerites correspond to those found in the peduncle of a colony of *R. musaica* from Zamponi & Pérez (1995) (Fig. 9). These observations, added to the variability observed in sclerites of *Renilla* species (Clavico *et al.,* 2007), raise doubts regarding the validity of *R. musaica.* It could potentially be considered a junior synonym of *R. muelleri,* although a detailed analysis of type material is required to clarify this matter.

The colonies from the "Holmberg" campaign were collected at a shallower depth than the colonies from the smaller "Undine" campaign and were also larger. The findings indicated that, for both the rachis and the peduncle, the reduction in colony size is associated with a slight increase in the size of the large sclerites, while such a trend is not observed for the small ones.

On the other hand, it was observed that the large sclerites exhibit variations in their sizes within colonies and between campaigns, with those from the rachis being larger than those from the peduncle. Clavico *et al.* (2007) associated the difference in sclerite length with variations in the depth where the colonies develop, indicating larger sclerites at greater depths. While the pri-

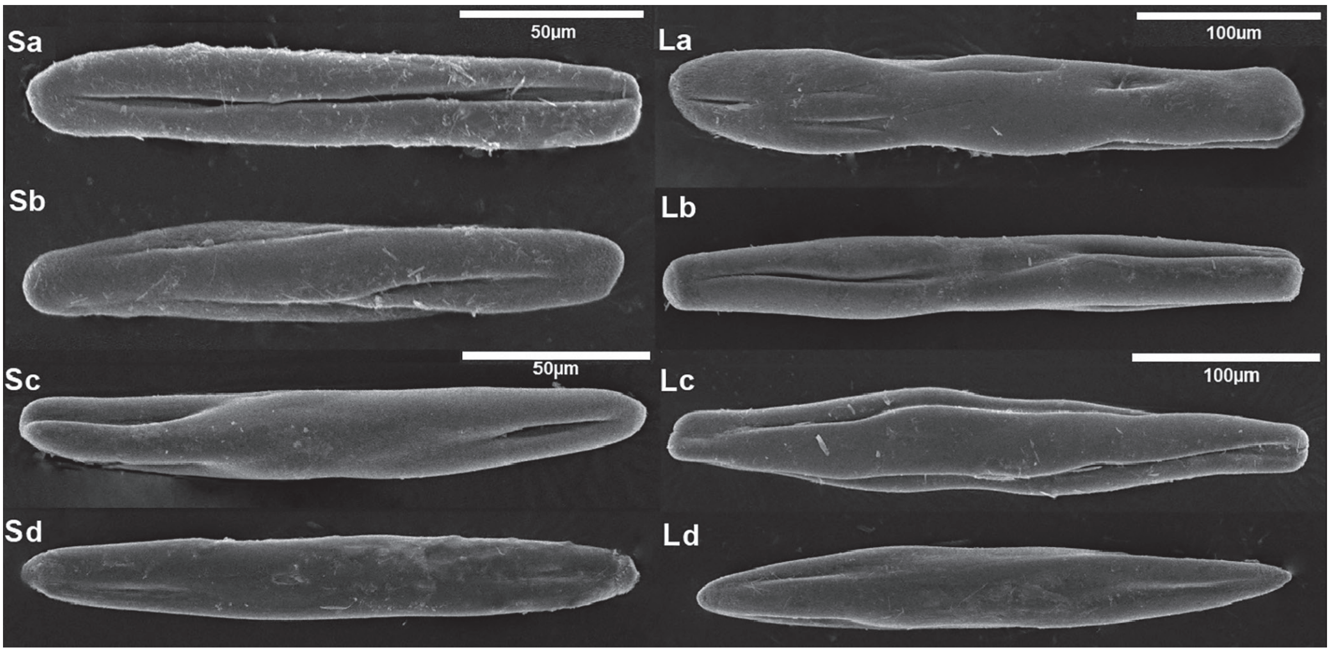

Figure 8. (a) and (b): axis with rounded ends of the same width as the central region. Sa: small sclerite with regular edges and complete linear longitudinal grooves. Sb: small sclerite with irregular edges and incomplete spiral grooves. La: large sclerite with irregular edges and incomplete linear longitudinal grooves, Lb: large sclerite with regular edges and complete longitudinal spiral grooves. (c) and (d): Axis with acute ends, the central region is the widest, Sa: small sclerite with irregular edges and incomplete longitudinal spiral grooves. Sb: small sclerite with regular edges and incomplete linear longitudinal grooves. La: large sclerite with irregular edges, and complete longitudinal spiral grooves. Lb: large sclerite with regular edges and incomplete linear longitudinal grooves.

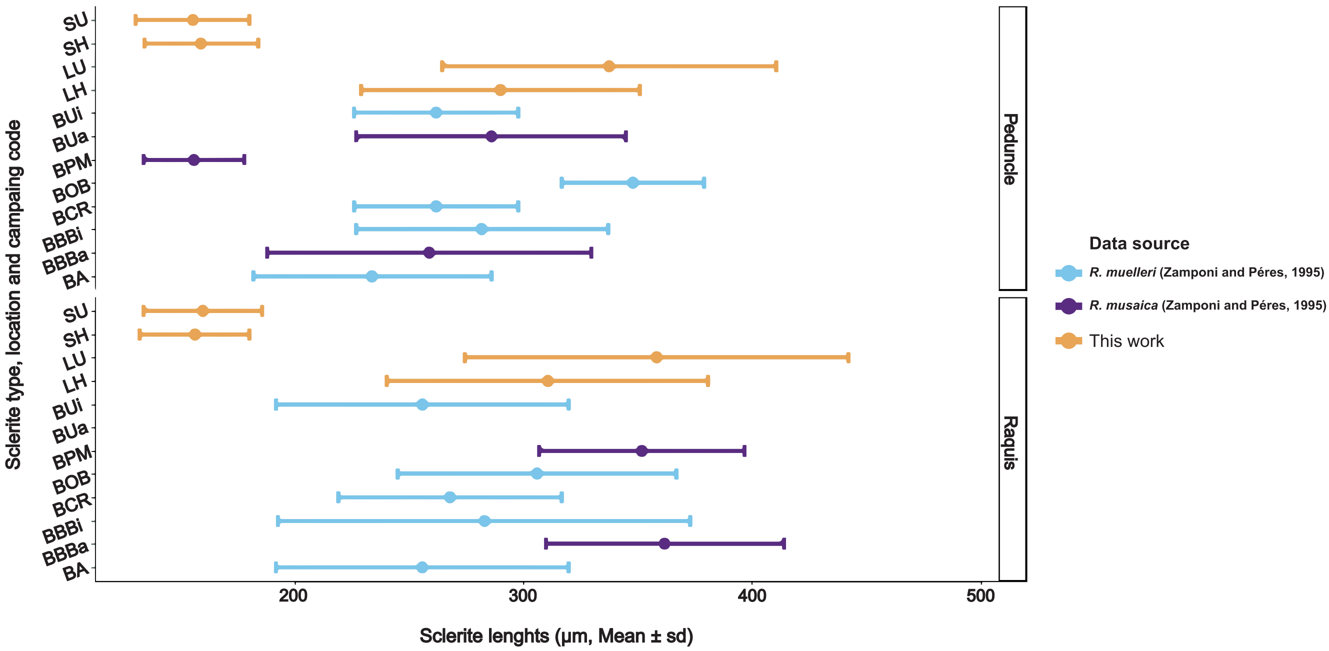

Figure 9. Size of sclerites from *R. muelleri* obtained in this work and those from *R. muelleri* and *R. musaica* extracted from Zamponi & Pérez (1995). From this work SU: Small Undine, SH: Small Holmberg, LU: Large Undine, LH: Large Holmberg; From Zamponi & Pérez (1995) BUi: *R. muelleri* Vessel Undine, BUa: *R. musaica* Vessel Undine, BPM: *R. musaica* Vessel Presidente Mitre, BOB: *R. muelleri* Vessel Oca Balda, BCR: *R. muelleri* Vessel Comodoro Rivadavia, BBBi: *R. muelleri* Vessel Bahía Blanca, BBBa: *R. musaica* Vessel Bahía Blanca, BA: *R. muelleri* Vessel Angélica.

mary objective of this study was not to assess the potential cause of the variation, our results align with those of Clavico *et al.* (2007) only in the context of the large sclerites, where smaller colonies collected at greater depths displayed a slight increase in size. As a hypothesis, the larger size sclerites could potentially provide structural reinforcement to the colonies, serving either to withstand environmental pressure or to compensate for the fragility associated with smaller sizes, or perhaps both. However, this needs further investigation for validation. Variations in sclerite size have also been related to gradients of variation in predation pressure and the mechanical force of waves and currents that vary with depth (West, 1997).

Renilla muelleri exhibits simple sclerites visible with the naked eye under a light microscope, yet significant external morphological variability becomes apparent under SEM. Regarding ultrastructural characteristics, the observation of diverse external morphologies was possible, displaying a wide range of variation for each zone of the colony. This variability hindered discrimination and grouping based on these characteristics under optical microscopy. Consequently, the ultrastructure of external morphology, according to our results, does not appear to be a consistent character at a specific taxonomic level. However, it is worth noting that other authors, such as Aharonovich & Benayahu (2011), found the utility of ultrastructure as a character for distinguishing species. This is observed at a much higher level of detail, focusing on the depositional form of calcium carbonate crystals.

The present study offers a new and comprehensive morphological description of *Renilla muelleri* sclerites, accompanied by an analysis of their size variations among colonies and within different zones of colonies. Furthermore, it presents evidence suggesting that both the external microscopic morphology and biometry of the sclerites exhibit variability between colonies and within colonies, challenging their reliability as taxonomic characters at a specific level. To further validate these observations, future studies employing the same methodology on other congeneric species are recommended, allowing for comparative analyses. Finally, future studies on *Renilla muelleri* should aim to explore explanations for the patterns of variation in sclerite sizes and their relationships with colony size, as well as environmental variables such as water column pressure, currents, and predation.

AUTHORS' CONTRIBUTIONS: FMG, AG: Methodology, Software, Data curation, Formal analysis, FMG: Writing – original draft, Visualization, Investigation; AG: Supervision; FMG, FHA, AG: Conceptualization, Writing – review & editing. All the authors actively participated in the discussion of the results, they reviewed and approved the final version of the paper.
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APPENDIX

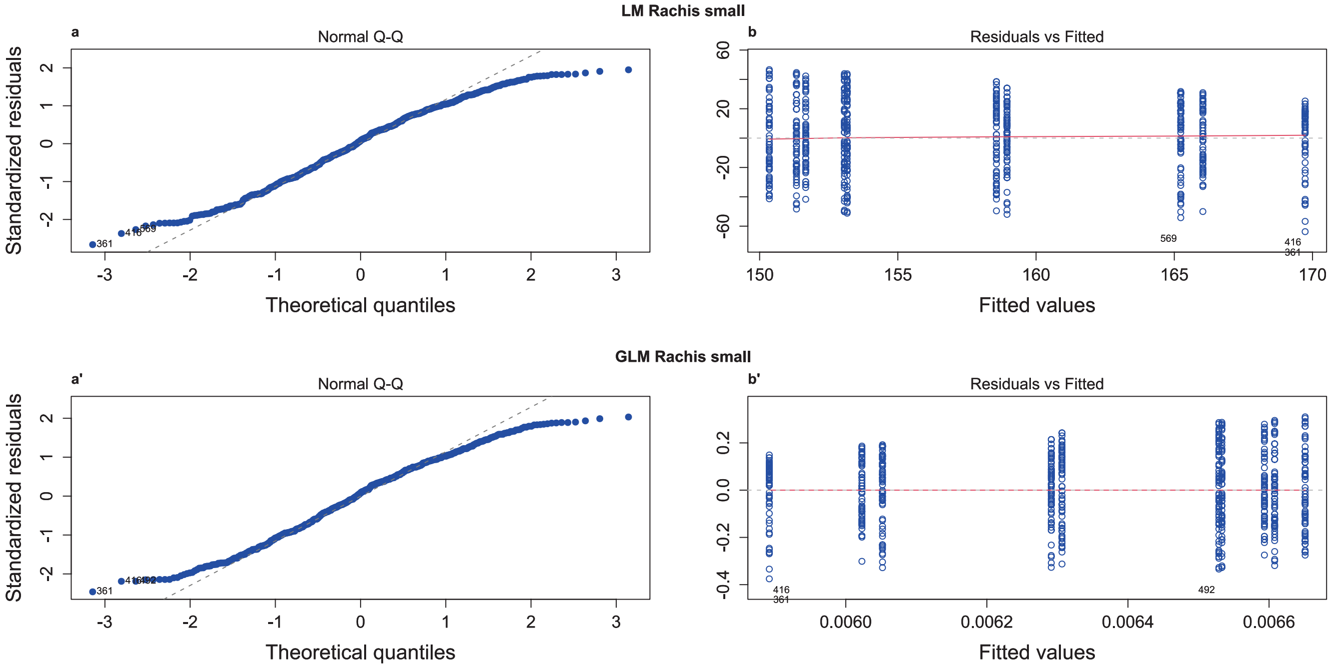

Figure 1. LM (normal) and GLM (gamma) fit plots. Q-Q plots for *Renilla muelleri* rachis small sclerites from LM (a) and GLM (a'); Standardized Residuals vs. Fitted Values from (b) and GLM (b').

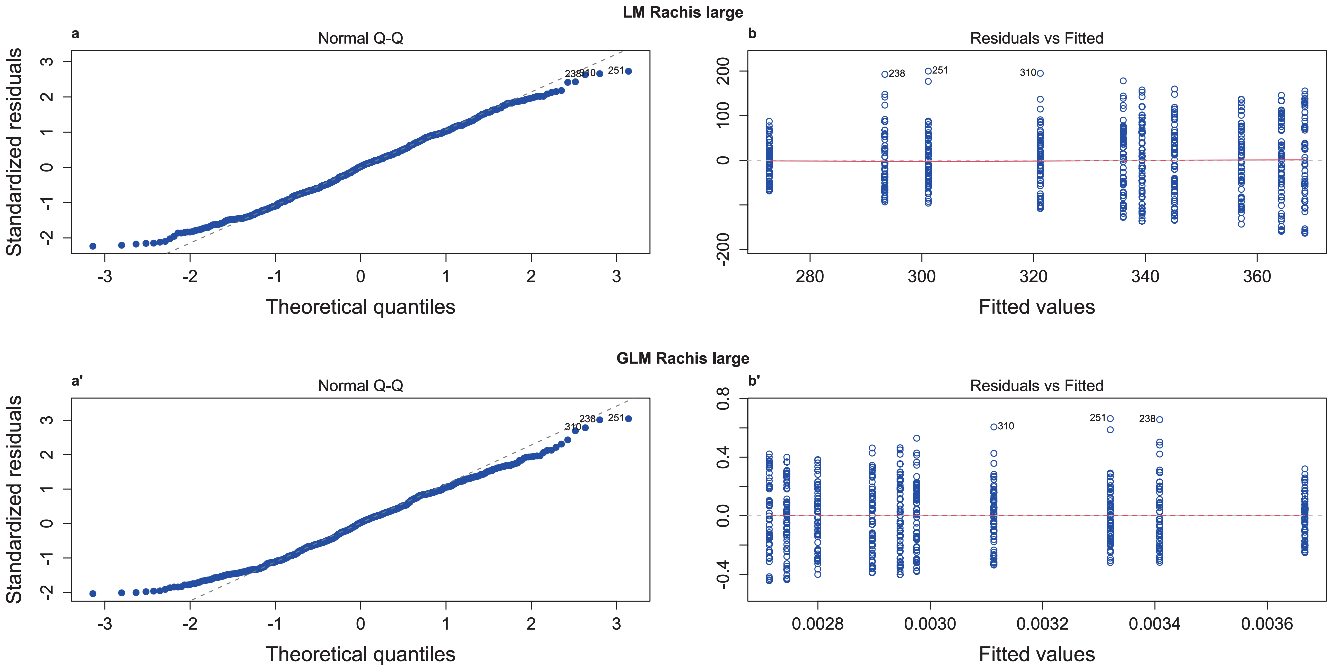

Figure 2. LM (normal) and GLM (gamma) fit plots. Q-Q plots for *Renilla muelleri* rachis large sclerites from LM (a) and GLM (a'); Standardized Residuals vs. Fitted Values from LM (b) and GLM (b').

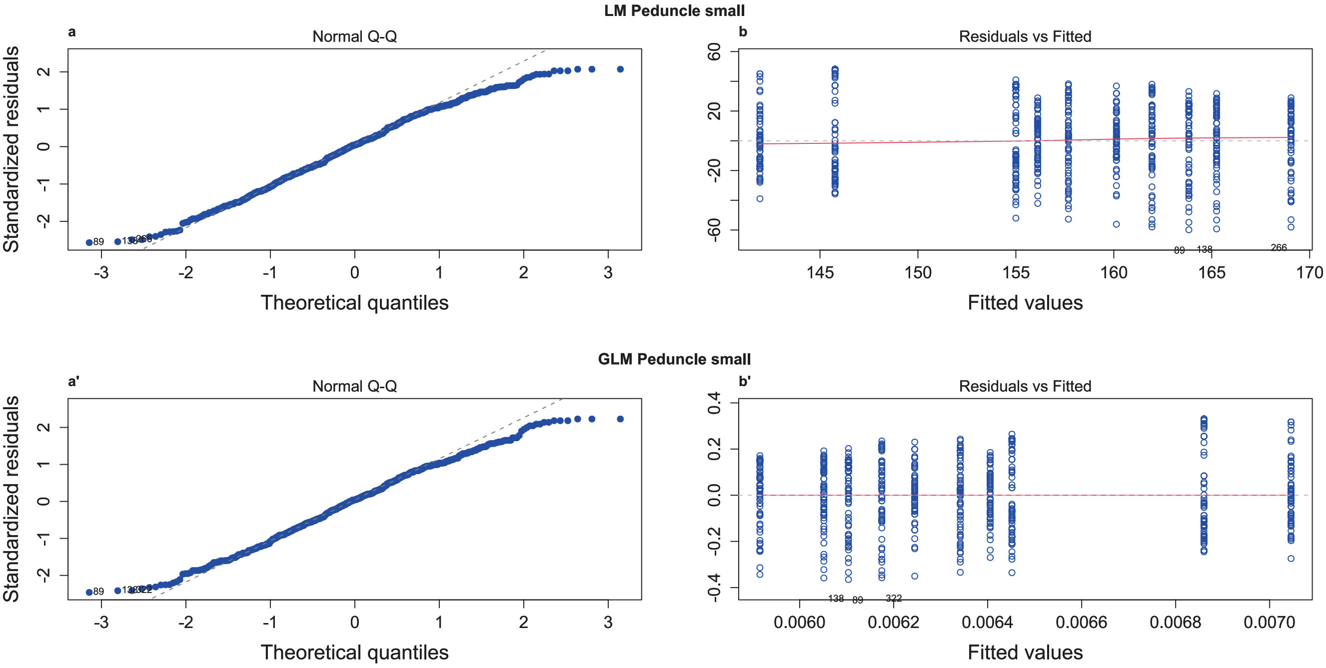

Figure 3. LM (normal) and GLM (gamma) fit plots. Q-Q plots for *Renilla muelleri* peduncle small sclerites from LM (a) and GLM (a'); standardized residuals vs. fitted values from LM (b) and GLM (b').

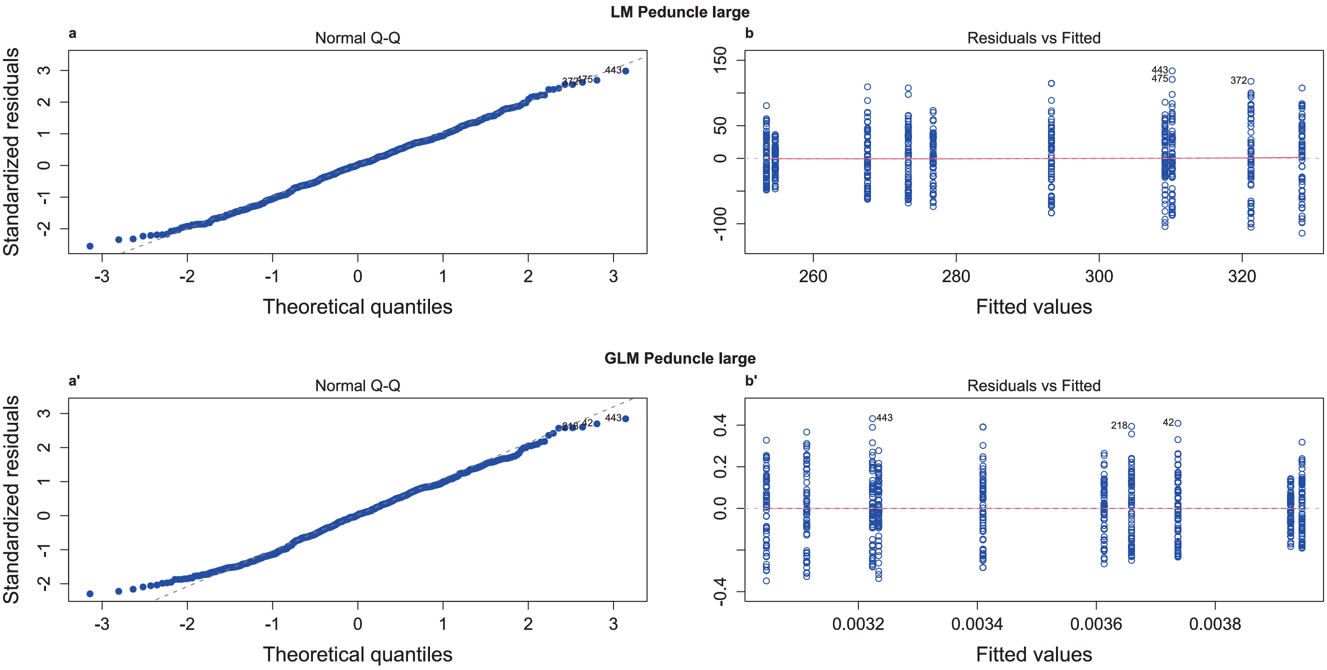

Figure 4. LM (normal) and GLM (gamma) fit plots. Q-Q plots for *Renilla muelleri* large peduncle sclerites of LM (a) and GLM (a'); standardized residuals vs. fitted values of LM (b) and GLM (b').

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