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Variability of cnidae within a small clonal sea anemone (*Isactinia* sp.)

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Abstract

The cnidom and intraspecific variability of cnidae within the small sea anemone *Isactinia* sp. were verified. The specific cnidae within the cnidom of four discrete morphological structures (tentacle, actinopharynx, mesenterial filaments, and body column) within *Isactinia* sp. was investigated. Microbasic *b*-mastigophores, microbasic *p*-mastigophores, basitrichs, microbasic *p*-amastigophores, and spirocysts were found in this species. In addition, two morphologically distinct basitrich forms, distinguishable only in a discharged state, were also found, of which basitrichs with the distinctly shorter thread were found predominately only on the body column. The distribution and abundance of cnidae types differed significantly around the body in the sea anemones, as did the length of basitrichs and spirocysts among tissue types. Cnidae length also differed significantly among individuals. Correlations between cnidae length and sea anemone size were variable, whereby cnidae size was significantly negatively correlated to sea anemone size in seven cnidae–tissue combinations, positively correlated in one, and not correlated in two. Linear regression revealed that sea anemone size was able to explain 33%–68% of variation in size of *b*-mastigophores, *p*-amastigophores, and small basitrichs from within the mesenterial filaments. Correlations were negligible or not significant in remaining cnidae–tissue combinations, however. While providing key taxonomic cnidae information, this study also highlights the variability of cnidae that may occur within a species of *Isactinia*.

KEYWORDS
Actiniaria, cnidae, Cnidaria, ecology, intraspecific variation, taxonomy

1 | INTRODUCTION

Currently, there are 1170 recognized sea anemone species (WoRMS, 2023a). Except for pelagic and parasitic life stages (McDermott et al., 1982; Reitzel et al., 2009; Shick, 1991), sea anemones are predominately benthic and found in habitats ranging from intertidal tropical waters to the greatest depths of the polar seas (Daly et al., 2008; Fautin, 1989). This extensive distribution of sea anemones and their ability to survive in a wide spectrum of environments is often partly attributed to their suite of purpose-built microscopic armaments known as cnidae (Kass-Simon & Scappaticci, 2002).

Cnidae are the defining feature of the phylum Cnidaria (Fautin, 2009; Mariscal, 1984) and are collagenous organelles secreted within cnidocytes located in cnidarian ectodermal tissue of external body structures and the endoderm of some internal structures (Fautin, 2009). Cnidae are created through the complex process of cnidogenesis (Holstein, 1981; Skaer, 1973; Slautterback & Fawcett, 1959; Strömberg & Östman, 2017). Here, the foundations of the capsule are secreted from the Golgi apparatus within the cnidocyte (Holstein, 1981; Skaer, 1973; Slautterback & Fawcett, 1959; Strömberg & Östman, 2017). The thread proceeds to form externally to the capsule, before being withdrawn internally, after which the capsule closes and hardens (Skaer, 1973; Strömberg & Östman, 2017).

Cnidae encapsulate a densely coiled or pleated thread that, in many instances, may be laced with an array of barbs or spines (Mariscal, 1974; Mariscal, Conklin, & Bigger, 1977). Stimuli, whether they be physical, chemical, or neurological, or a combination thereof (Kass-Simon & Scappaticci, 2002), activate the cnidae, triggering a dramatic release of pressure that in turn ejects and everts the thread with immense force (Nüchter et al., 2006; Weber, 1989). The characteristics of these discharged threads enable the cnidae to be either glutinant (sticky), volvent (entangling), penetrative, or a combination of those abilities (Ewer & Fox, 1947; Fautin, 2009; Garese et al., 2023).

The specific ecological functions of cnidae have led to the diversification and specification in cnidae morphology (Kass-Simon & Scappaticci, 2002; Shick, 1991). Collectively, there are ~30 distinct types of cnidae, six of which are found within Anthozoa (Carlgren, 1945; Fautin, 1988, 2009; Kass-Simon & Scappaticci, 2002). Whereas some cnidae are equipped with large spines designed to penetrate predator or prey tissue (e.g., skin, exoskeleton, or fish scales), others have long threads with short spines that are perhaps better served for entangling prey (Ewer & Fox, 1947; Mariscal, 1974). Conversely, some cnidae may have no spines at all and are believed to be used as an adhesive aid (Mariscal, 1974, 1984; Mariscal, Conklin, & Bigger, 1977). In these ways, cnidae contribute to cnidarian survival by allowing for adhesion to substrates; the entanglement and penetration of tissue and toxin delivery in prey acquisition; or to defense from predators and spatial competitors (Fautin, 2009; Kass-Simon & Scappaticci, 2002; Mariscal, 1984).

Cnidae are broadly grouped into one of the three prominent categories: nematocysts, spirocysts, and ptychocysts (Mariscal, 1984). Nematocysts are the most diverse, with ~28 forms, whereas spirocysts and ptychocysts have only one form each (Mariscal, 1974; Mariscal, Conklin, & Bigger, 1977). In addition to being the most diverse category of cnidae, nematocysts are the only cnidae known to contain toxins (venom), which are injected directly into other organisms (Mariscal, 1974, 1984). The composition and concentration of biologically active components (proteins, peptides, and small molecules) that make up these venoms may vary among cnidae types (McClounan & Seymour, 2012), in tissue locality on a sea anemone's body, and among individuals (Ashwood et al., 2021; Mitchell et al., 2017, 2020). It is unknown, however, whether all nematocysts contain venom (Mariscal, 1974, 1984), and it has been suggested that spirocysts may lack venom (Mariscal et al., 1976), though this is yet to be confirmed.

In addition to the functional significance of the types of cnidae present, differences in the sizes of cnidae among different tissue types are particularly important in the taxonomic identification of actiniarians (Carlgren, 1940; Cutress, 1955; England, 1987; Fautin, 1988; Häussermann, 2004; Shick, 1991). Size is often useful in discern among families and genera, which may lack other distinguishing features (Fautin, 1988, 2009). The unique array of cnidae within discrete anatomical features of a sea anemone may also provide important clues into both the function of the cnidae (Rifkin & Endean, 1983; Shick, 1991) and the ecology of a species (Fautin, 1988).

The objective of this study was to identify and quantify the variety of cnidae within *Isactinia* sp. (Carlgren, 1900), a small tropical sea anemone. Specifically, this project aimed to verify the cnidom and biometrics of this species and further classify cnidae types, relative abundance, distribution, and cnidae size range within four anatomically discrete morphological structures: tentacle, actinopharynx, mesenterial filament, and body column. In addition to providing important taxonomic information for this species, we investigated the biometry of the cnidae, specifically to determine whether there were differences in size among cnidae types, and within cnidae types associated with different tissues around the body. Differences in cnidae size among individuals were quantified, and the relationship between cnidae size and sea anemone body size was investigated. In doing so, the information provided here contributes to the growing body of work on the complex factors that impact cnidae.

2 | METHODS

2.1 | Study organism

The taxonomic status of species in this genus is unclear and is currently being resolved by actiniarian taxonomists. Although it has been suggested, albeit anecdotally, that this species may be *Anemonia manjano* Carlgren, 1900, more recent work has identified specimens as *Isactinia* Carlgren, 1900, a genus that currently contains three recognized species (Fautin, 2016; WoRMS, 2023b). Due to morphological variation and different type localities for this species, the genus requires taxonomic revision. In the species of *Isactinia* we studied, individuals lack true marginal spherules (defined as marginal projections with cnidae distinct from those of the body column; Daly, 2003) and possess a diffuse endodermal sphincter (Ottaway, 1975). We deposited 10 representative voucher specimens and accompanying histology slides at the Museum of Tropical Queensland (MTQ) under the unique identifiers MTQ G80200 and MTQ G81176–G81178, respectively. As such, we refer to the sea anemones in this study as *Isactinia*-MTQ hereafter.

Individuals of *Isactinia*-MTQ are relatively small (Figure 1A), with oral discs ranging 5–50 mm in diameter. In captivity, specimens are observed to form dense aggregations on hard rocky or coral substrate (Figure 1B). These sea anemones are typically seen fully expanded

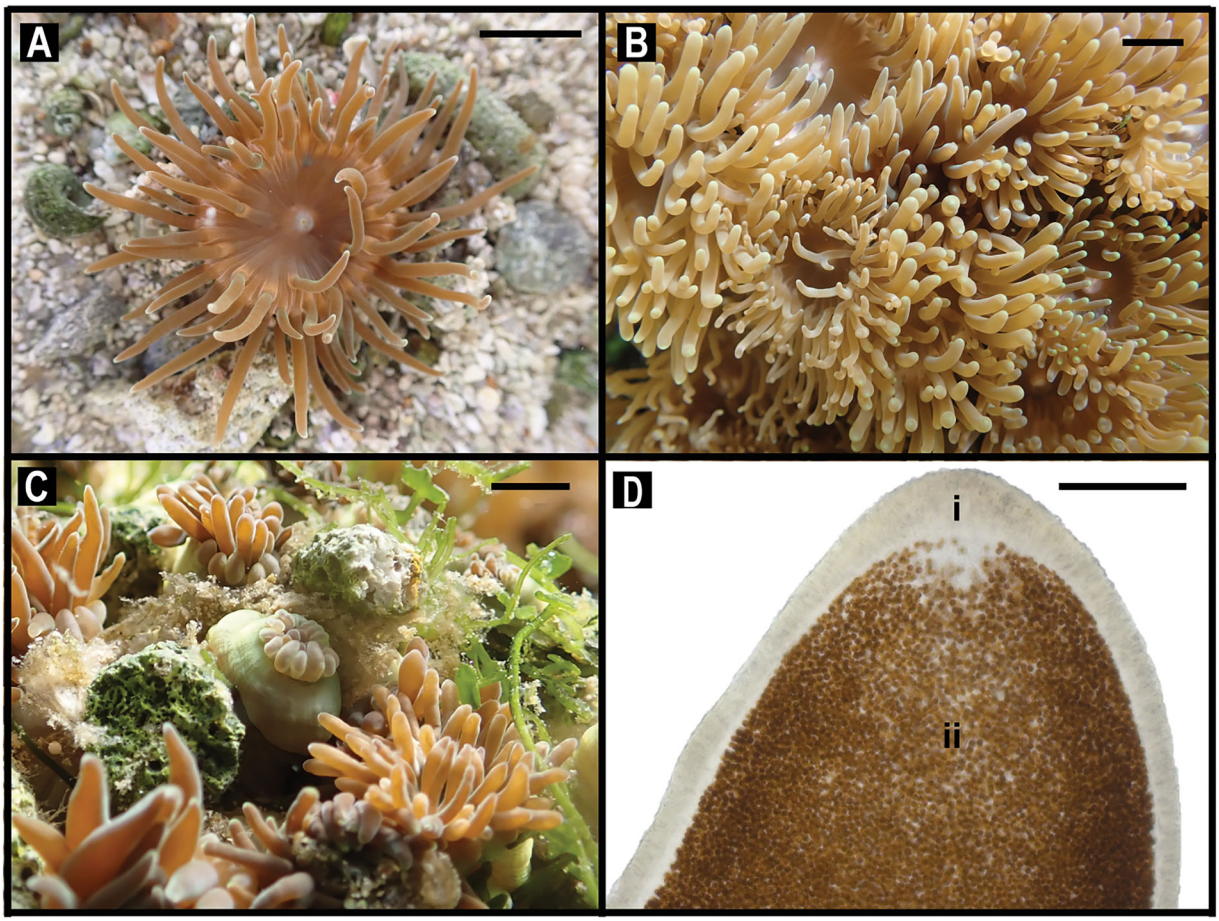

FIGURE 1 Representative examples of typical individuals of the sea anemone *Isactinia*-MTQ. (A) Expanded state. (B) Dense aggregation. (C) Contracted state. (D) Close-up of a tentacle. i, ectoderm; ii, endoderm with Symbiodiniaceae. Scale bars: A–C, ~5 mm; D, ~0.2 mm.

but provoked individuals retract (Figure 1C). In *Isactinia*-MTQ, individuals are photosymbiotic, with Symbiodiniaceae present within the endodermal tissues of the tentacle (Figure 1D). Asexual reproduction through longitudinal fission has also been observed in this species (K.L. Kaposi, unpubl. data).

Specimens were sampled from a large self-established population within the aquarium facility at James Cook University, Cairns, Australia. Sea anemones are readily found throughout a large 60,000-L recirculating aquaria system, comprising both indoor and outdoor tanks, with the greatest numbers appearing in large aggregations within the top 30 cm of the outdoor tanks (total depth 65 cm). The seawater within this aquaria system is typically maintained at 27 ± 1°C, salinity 35 ± 1‰, pH 8–8.5, NO_2 0 mg/L, NO_3 0 mg/L, and PO_4 0.5–1 mg/L (YSI Xylem temperature and conductivity probes and Serra Test Kits).

Sea anemones were gently removed from the substrate by agitating their pedal discs. Because this handling caused many of the sea anemones to retract, they were each then placed into 25-mL temporary holding containers and given 1 h to expand. Magnesium sulfate ($MgSO^4$) crystals were gradually added until all sea anemones appeared to be unresponsive to physical stimuli, after which point they were deemed to be anesthetized (Häussermann, 2004).

2.2 | Cnidom biometrics

2.2.1 | Cnidae identification

To determine the types of cnidae present within *Isactinia*-MTQ, samples of fresh tissue were taken from live anesthetized specimens. Sea anemones were dissected under a stereomicroscope by first cutting the polyp in half longitudinally through the mouth, which resides in the center of the oral disc. Four distinct anatomical tissue regions—tentacles, actinopharynx, mesenterial filaments, and body column—were sampled, as too were the pseudospherules, for purpose of taxonomic investigation (Figure 2). Due to variations in cnidae distribution and size within tissues (Ardelean & Fautin, 2004; Robson, 2004; Van-Praët, 1985; Watson & Mariscal, 1983), efforts were made to sample within the same regions consistently between specimens. For example, in the case of tentacles samples were taken from the tips, where cnidae are believed to be most densely packed. Minute tissue samples were wet mounted onto glass microscopy slides (Stephenson, 1929), and either freshwater, ethanol, or vinegar were used to induce cnidae to discharge (Häussermann & Försterra, 2001). Cnidae smears were prepared by applying gentle, even pressure to 22 × 22 mm coverslips covering sampled tissue on the histological slide.

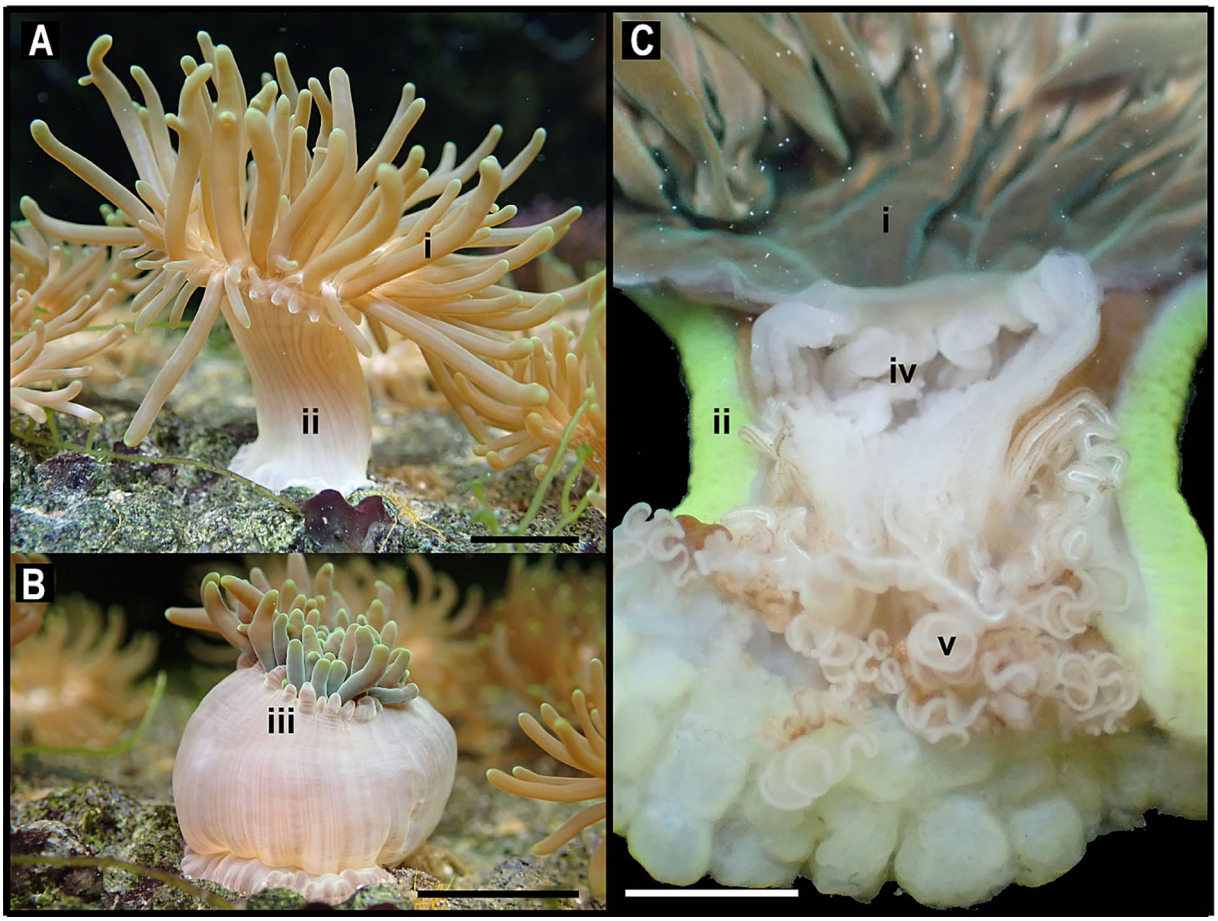

FIGURE 2 The anatomical features of *Isactinia*-MTQ examined in this study. i, tentacles; ii, body column; iii, pseudospherules; iv, actinopharynx; v, mesenterial filaments. Scale bars: A,B ~ 10 mm; C, ~0.2 mm.

The morphological features of both discharged and undischarged cnidae were examined using a combination of brightfield and phase-contrast microscopy on a Zeiss Axio Imager.M1 microscope with a magnification of 630× (oil immersion). To identify cnidae types, a combination of cnidae nomenclature by Carlgren (1949), England (1991), Mariscal (1974), and Östman (2000) was consulted. Collectively, these works are widely accepted sources used to classify actiniarian cnidae. We provide definitions and justifications of the final terminology we use in Section 3. When two distinct cnidae sizes were present within a tissue type, they were counted separately and identified with either the suffix -I or -II. In addition, some morphologically distinct cnidae types were further identified with either the prefix α- or β-.

2.2.2 | Cnidae distribution and relative abundance

To determine the percentage and relative abundance of each type of cnida within each of the tissues (tentacle, actinopharynx, mesenterial filaments, and body column), six anesthetized sea anemones (diameter of the oral discs ranging 0.7–1.2 cm) were individually fixed in an expanded state within a 100-mL bath of 4% formalin with seawater. After 2 min, fixed sea anemones were then transferred into individual containers with 15 mL of fresh 4% formalin in seawater. Following a minimum 7-day fixation period, sea anemones were dissected as described above, into four distinct anatomical tissue types: tentacles, actinopharynx, mesenterial filaments, and body column. Sea anemones were thoroughly rinsed in a 10-mM phosphate-buffered saline to mitigate the risks of formalin prior to dissections (O'Hara, 2018).

Methods similar to the samples taken from live specimens were used to wet mount small tissue samples from fixed sea anemones onto microscopy slides. Cnidae from within each of the respective tissue types were examined using brightfield microscopy at 630× magnification (oil immersion). The first 300 undischarged cnidae capsules, irrespective of cnidae classification and haphazardly encountered along a parallel-line search pattern within each tissue type of each specimen, were photographed. The number of tissue smears required to reach the target of 300 cnidae for each specimen and tissue type combination varied. Cnidae that were discharged, broken, malformed, or obscured by excessive tissue debris were not measured. Photomicrographs were taken using an AxioCam mounted directly onto the microscope, with the use of corresponding Zeiss Zen Software. The photomicrographs were then used to evaluate

the occurrence of each cnidae type, whereby each occurrence of each type of cnidae was counted.

A chi-square (χ^2) test of homogeneity was performed to determine whether the distribution and abundance of cnidae types (*b*-mastigophores, *p*-mastigophores, basitrichs-I, basitrichs-II, *p*-amastigophores, and spirocysts) differed among tissue types (tentacle, actinopharynx, mesenterial filament, and body column). The analysis was performed on the pooled totals from all specimens, of the absolute count of each respective cnidae type, within each of the anatomical regions. All observations were included to capture the presence of cnidae that may occur rarely (Fautin, 1988).

Although six sea anemones were prepared and sampled, data from the actinopharynx and mesenterial filaments could not be obtained from two of the specimens. As such, for these two tissue types, proceeding analyses on these were performed on data from four specimens. Six specimens were successfully sampled and used in tentacle and body column analyses.

Due to the inability to discern between α- and β-basitrich-I cnidae in undischarged forms, an additional qualitative survey was performed on a further six anesthetized sea anemones. Here, samples from each of the four distinct tissue types (tentacles, actinopharynx, mesenterial filaments, and body column) from each of the six live sea anemones were prepared on microscopy slides, and cnidae discharge was induced with freshwater. Using phase-contrast microscopy (200–400× magnification), the presence or absence of β-basitrich-I (evident by the presence of a short thread; Figure 4D) was recorded.

2.3 | Cnidae biometrics

2.3.1 | Cnidae size

When possible, the length and width measurements of undischarged capsules were taken from the first 100 of each cnidae type within each of the four distinct tissue types per sea anemone. Additionally, 10 cnidae from within the pseudospherules were also measured. Length and width were measured as direct lines from the posterior to anterior of the capsule and perpendicularly across at the widest point (Figure 3), excluding curvature. Measurements to the nearest 0.001 μm were taken using Zen-Lite 3.4 Software. From this, descriptive statistical parameters (range, mean, and standard deviation) were determined for the length and width of each cnidae type within corresponding tissue types, and subsequently presented in a traditional cnidae table.

To check the precision and accuracy of measurements used in this study, we followed methods of Strömberg and Östman (2017). To check for precision, a test with six replicate measurements (length and width) was made on three capsules of each cnidae type, and standard deviation calculated. Each replicate measurement was taken at independent sampling times. To check the accuracy of length and width measurements taken, the lead author and three other persons each measured 15–30 representative capsules of each cnidae type. The measurements obtained by each person were compared with univariate analysis of variance with least significant difference post hoc tests.

Data on cnidae width were included because this information is an important descriptive characteristic in actiniarian studies. Given this, scatterplots of capsule length against width were generated to provide a graphical representation of the cnidom signature (size and spread of all cnidae measured within each tissue type) of this species (Ryland et al., 2004).

2.3.2 | Variation in cnidae size among tissue types

For cnidae types (basitrich-I, basitrich-II, and spirocysts) found within more than two distinct tissue types, a Kruskal–Wallis test was performed to determine whether the length (dependent variable) of these cnidae types varied among the respective tissue types (independent variable) source. Specifically, we compared variation in the lengths of basitrichs-I, basitrichs-II, and spirocysts in tentacles, mesenterial filaments, and body column. Subsequent pairwise multiple comparison post hoc tests were performed to identify where differences lay. We used boxplots to show the minimum, median, and maximum quartile range of cnidae lengths, along with any outliers, to illustrate the differences in the lengths of cnidae among tissue types.

2.3.3 | Variation in cnidae size among individuals

To identify whether the length (dependent variable) of cnidae varied significantly among individuals (independent variable), a Kruskal–

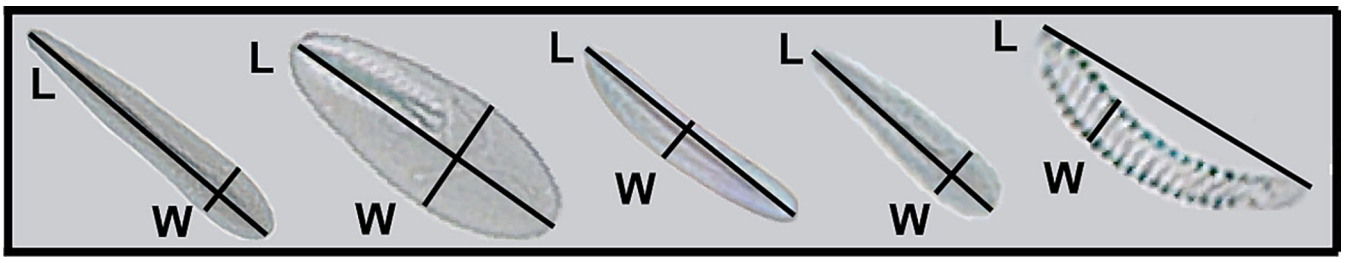

FIGURE 3 Examples of how length (L) and width (W) of cnidae were measured in specimens of *Isactinia*-MTQ. Measurements were direct lines from posterior to anterior of the capsule excluding curvature, and across at the widest point. From left to right, examples shown are a *b*-mastigophore, *p*-mastigophore, basitrich, *p*-amastigophore, and spirocyst. Cnidae are not to scale.

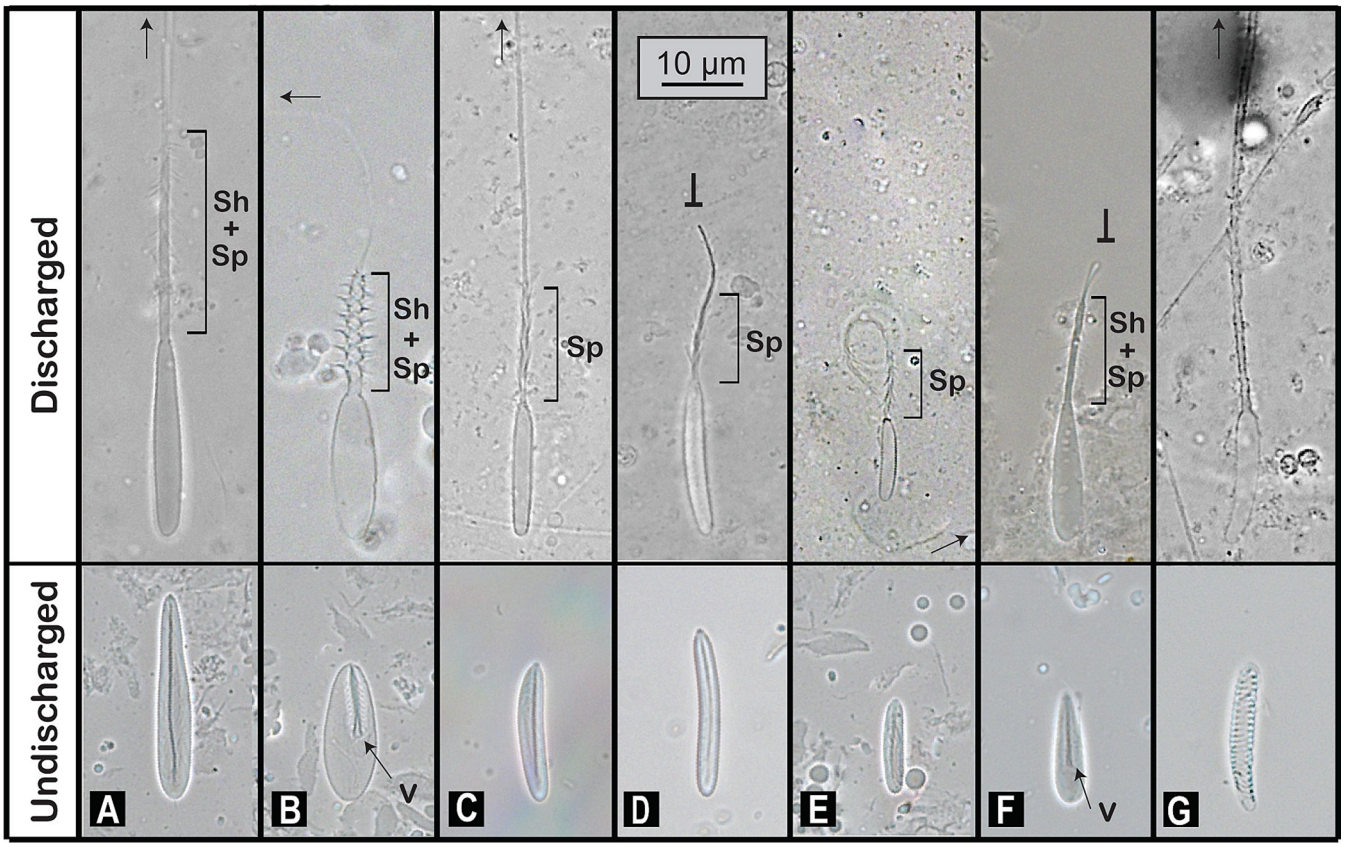

FIGURE 4 Representative cnidae of *Isactinia*-MTQ. (A–G) Different types of cnidae, with discharged cnidae in upper panels and undischarged in bottom panels: (A) microbasic *b*-mastigophore; (B) microbasic *p*-mastigophore; (C, D) basitrich-I; (E) basitrich-II; (F) *p*-amastigophore; (G) spirocyst. Basitrich-I (C, D) were further split into α- (C) and β- (D) forms because of distinct differences in discharged thread length. Note, because of difficulties distinguishing between undischarged α- and β-basitrich-I types, these have been grouped as "basitrich-I" in all analyses. Key distinguishing features that aided in identification are as follows: →, continuation of thread; ⊥, termination of thread; Sh, shaft; Sp, spines; v, v-notch. All images were taken at the same magnification. Scale bar = 10 µm.

Wallis test was performed on each possible combination of cnidae and tissue type. Cnidae–tissue combinations tested were *b*-mastigophores in mesenterial filaments; basitrich-I and -II in tentacles, actinopharynx, mesenterial filaments, and body column; *p*-mastigophores in the actinopharynx and mesenterial filaments; *p*-amastigophores in the mesenterial filaments; and spirocysts in the tentacle, mesenterial filaments, and body column. Subsequent pairwise multiple comparison post hoc tests were performed to locate where any differences lay.

2.3.4 | Correlation between cnidae length and sea anemone size

Finally, to examine whether cnidae size was correlated with sea anemone size, Pearson's correlation and a linear regression analysis were performed on the length of cnidae (for each possible combination of cnidae and tissue type) against the oral disc diameter of each sea anemone. All data were checked for normality using Shapiro–Wilk tests. Data were analyzed using IMB SPSS Statistics Software (version 28.0.1.0), and differences were considered statistically significant for $p < .05$.

3 | RESULTS

3.1 | Cnidom biometrics

3.1.1 | Cnidae identification

Based on distinct differences in the morphology of undischarged and discharged capsules and respective threads, a total of five cnidae types (*b*-mastigophore, *p*-mastigophore, basitrich, *p*-amastigophore, and spirocyst) were found in *Isactinia*-MTQ. Of these, basitrichs could further be divided into two size classes (basitrich-I and basitrich-II) and two subtypes (α-basitrich-I and β-basitrich-I), which brought the total number of cnidae within *Isactinia*-MTQ to seven (Figure 4). Cnidae were classified and characterized as follows.

Microbasic b*-mastigophore*

No v-notches were evident in the undischarged capsule. The discharged shaft, though not distinct, was less than three times the length of the capsule and gradually tapered into the thread. Spines were present (Figure 4A).

Microbasic p-*mastigophore*

The undischarged capsule had a distinct v-shaped notch at the end of the unfired shaft. Discharged cnidae had a distinct shaft <3× the length of the capsule. The length of the shaft was armed with prominent barbs and spines, and as such, no *Faltstück* (the area of the shaft without prominent spines) was present (Figure 4B).

Basitrichs

Although discharged cnidae lacked a shaft, well-developed spines were present at the base of the thread (Figure 4C–E). Because of a clear division in size, basitrichs were further divided into two size classes and were given the suffix "-I" for large types (Figure 4C,D) or "-II" for small types (Figure 4E). Additionally, close examination of the morphology of discharged basitrich threads revealed two distinct forms of basitrich-I, which were otherwise indistinguishable in an undischarged state: α-basitrich-I, with long threads (Figure 4C), and β-basitrich-I, with threads equal to or less than the length of the discharged capsule (Figure 4D). Because of difficulties in discerning between undischarged α-basitrich-I and β-basitrich-I capsules, these two types were treated and counted together as basitrich-I for all cnidae and measurement analysis of undischarged organelles.

Microbasic p-*amastigophore*

Undischarged cnidae had a distinct v-shaped notch at the end of the shaft, and if visible, the thread appeared faint. Threads beyond the fired shafts (which were <3× the length of the capsule) were often very thin or non-apparent (Figure 4F).

Spirocysts

The capsule had a thin wall, with a long coiled thread seen to be densely coiled within undischarged capsules (Figure 4G).

3.1.2 | Cnidae distribution and relative abundance

The distribution of cnidae types varied significantly throughout different tissues of the sea anemone bodies ($\chi^2 = 6134.374$, $df = 15$, $p < .001$; Figure 5, Table S1). Bastrichs were the most prevalent of all cnidae types and were encountered in all tissues sampled. In the tentacle cnidom, basitrich-I accounted for 68.2% of the present cnidae, whereas in the actinopharynx, they made up 98.7%. Basitrich-I were also the most abundant cnidae on the body column (94.6%). To this end, further investigations of discharged cnidae on the body column have shown β-basitrich-I to be the dominant of the two basitrich-I forms in this tissue type (Table S2). Additionally, except for a rare few seen in the actinopharynx and tentacles of one of six additional living sea anemones sampled during preliminary investigations, β-Basitrich-I were not seen in any other tissues (Table S2).

Of all tissue types, the mesenterial filaments had the greatest diversity of cnidae present, with all cnidae types encountered (Figure 5, Table S2). Here, *p*-mastigophores were the most abundant (36.8%), followed by *b*-mastigophores (26.7%) and *p*-amastigophores (14.6%). There were no *p*-amastigophores in any other tissue type; very few *p*-mastigophores were present in the actinopharynx (0.7% of the cnidom), and they were functionally absent (0.1%) in the body column; *b*-mastigophores were functionally absent from the actinopharynx and body column.

3.2 | Cnidae biometry

3.2.1 | Cnidae size

Across all cnidae types, precision tests determined that the standard deviation of length and width measurements ranged ±0.03–0.19 μm and ±0.05–0.15 μm, respectively. Accuracy tests revealed that, except for basitrich-I length ($H_{3,\ 72} = 9.109$, $p = .028$) and width ($H_{3,\ 72} = 8.012$, $p = .046$), *p*-mastigophore length ($H_{3,\ 72} = 15.903$, $p = .001$), and *p*-amastigophore width ($H_{3,\ 72} = 14.783$, $p = .002$) (Figure S1), there were no significant differences in the measurements among the people who did the measuring.

A total of 3015 cnidae were measured in this study. The raw cnidae length and width data are presented in Figure 6, in which different cnidae types appear to form broad clusters within each of the tissues. This is particularly evident between α- and β-basitrich-I types. A taxonomic summary regarding the length and width of cnidae from within each of the distinct tissue types is presented in Table 1. Irrespective of tissue type, the length × width of each cnidae type within *Isactinia*-MTQ ranged as follows: *b*-mastigophore, 19.82–31.27 × 3.14–6.57 μm; *p*-mastigophore, 12.7–20.58 × 3.74–6.91 μm; basitrich-I, 14.29–23.23 × 1.93–4.38 μm, basitrich-II, 8.17–13.36 × 1.63–2.79 μm; *p*-amastigophore, 10.79–23.97 × 2.88–4.84 μm; and spirocyst, 7.97–23.91 × 1.58–5.39 μm.

3.2.2 | Variation in cnidae size among tissues

The lengths of basitrich-I ($H_{3,\ 1659} = 1081.848$, $p < .001$), basitrich-II ($H_{3,\ 222} = 70.029$, $p < .001$), and spirocysts ($H_{2,\ 484} = 8.078$, $p = .018$) varied significantly among tissues (Figure 7). Basitrichs (both α- and β-basitrich types) in the body column were significantly larger than those in the actinopharynx, which were in turn larger than those found in the tentacle and mesenterial filaments (Figure 7). Among basitrich-II, capsule lengths were longest in the mesenterial filaments, followed by the tentacles, and the actinopharynx and body column, which were statistically similar (Figure 7). Similarly, spirocysts in the body column were also found to be significantly larger than those of the tentacles and mesenterial filaments (Figure 7).

3.2.3 | Variation in cnidae size among individuals

The length of some cnidae was also shown to vary significantly among individuals. The level of such variations differed among both cnidae and tissue types. For example, in the mesenterial filaments, significant differences in the length of cnidae among individuals was observed

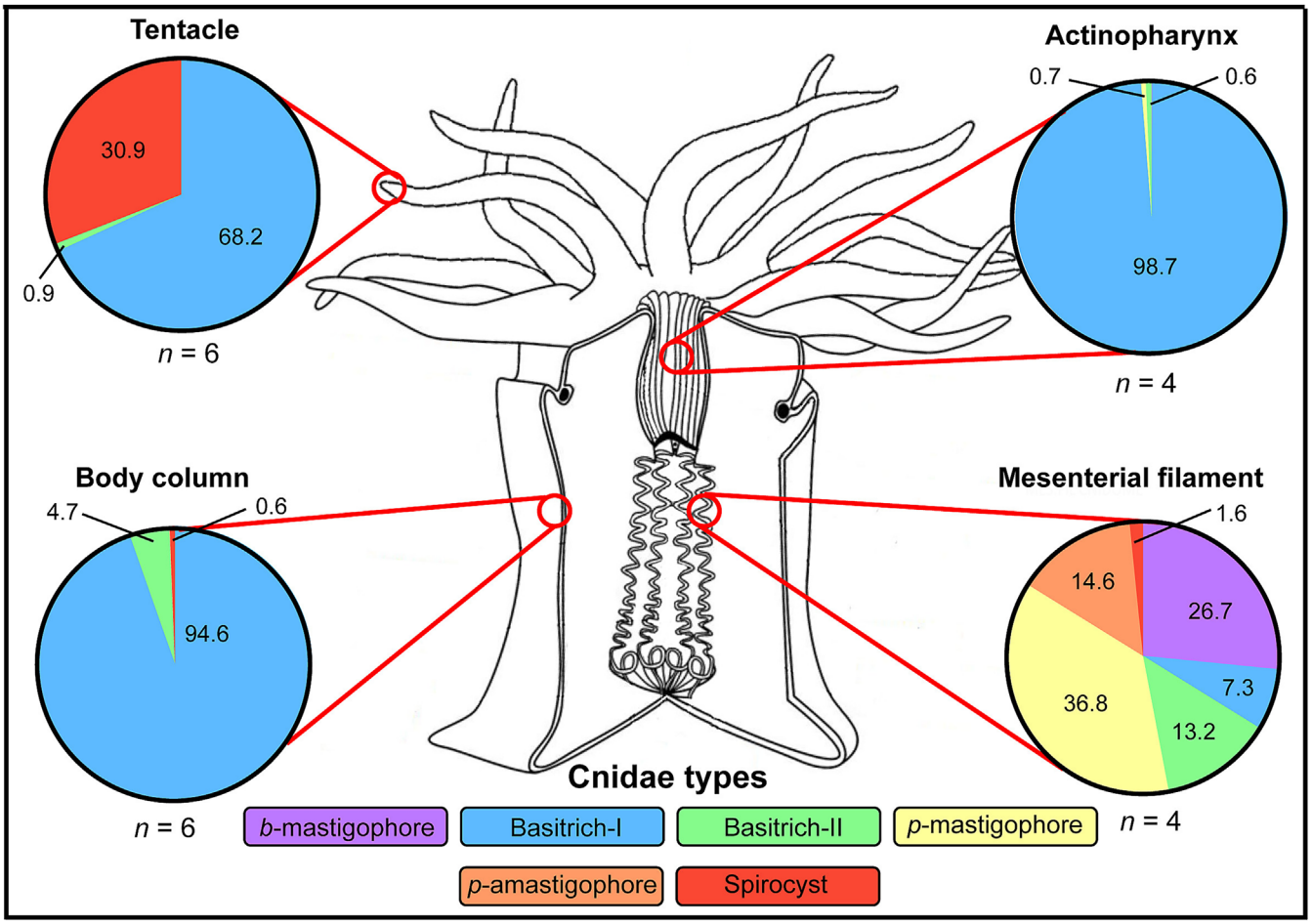

FIGURE 5 Distribution and relative abundance (%) of each cnidae type within different tissue types in specimens of *Isactinia*-MTQ. *N*, number of sea anemones sampled for each respective tissue type. Sea anemone artwork was modified from an image sourced from Bocharova and Kozevich (2011); originally credited to Ruppert et al. (2004).

for *b*-mastigophores ($H_{3,\ 230} = 169.135$, $p < .001$), basitrich-IIs ($H_{3,\ 130} = 46.515$, $p < .001$), *p*-mastigophores ($H_{3,\ 289} = 36.384$, $p < .001$), and *p*-amastigophores ($H_{3,\ 121} = 62.212$, $p < .001$), but not among basitrich-I and spirocysts.

Similarly, whereas the length of basitrich-II within the tentacle ($H_{1,\ 12} = 4.521$, $p = .027$), mesenterial filaments ($H_{3,\ 130} = 46.515$, $p < .001$), and body column ($H_{,\ 73} = 20.511$, $p = .001$) was significantly different among individuals, and no differences were seen among basitrich-II found in the actinopharynx. Similar unconformities in variations of cnidae length were also seen for basitrich-I, *p*-mastigophore, and spirocysts among tissue types.

3.2.4 | Correlation between cnidae length and sea anemone size

Of the 10 cnidae–tissue combinations that were tested, an increase in sea anemone size appeared to be significantly and inversely related to cnidae length in seven of these combinations (Table 2). Conversely, lengths of basitrich-I from within the body column were significantly positively correlated with sea anemone size, and lengths of cnidae in two other cnidae–tissue combinations were not correlated to sea anemone size. Among mesenterial filaments *b*-mastigophores, *p*-amastigophores, and basitrichs-I, sea anemone size was found to explain 67.8%, 52.6%, and 33.3% of the variation in cnidae length, respectively. In all other cnidae–tissue combinations, the diameter of the oral disc was weakly related (1.2% to 9.7%) to variation in cnidae length.

4 | DISCUSSION

The small clonal sea anemone *Isactinia*-MTQ was found to have five types of cnidae: *b*-mastigophores, *p*-mastigophores, basitrichs, *p*-amastigophores, and spirocysts (Figure 4). The biometry of these cnidae is highly variable, although this variation may be associated with differences in measurements made by different people. Significant differences in the length of undischarged capsules were seen among cnidae types, among tissue types (Figure 7), and among individuals, and were typically negatively correlated with sea anemone size (Table 2). Whereas the overall differences in size among cnidae types may be

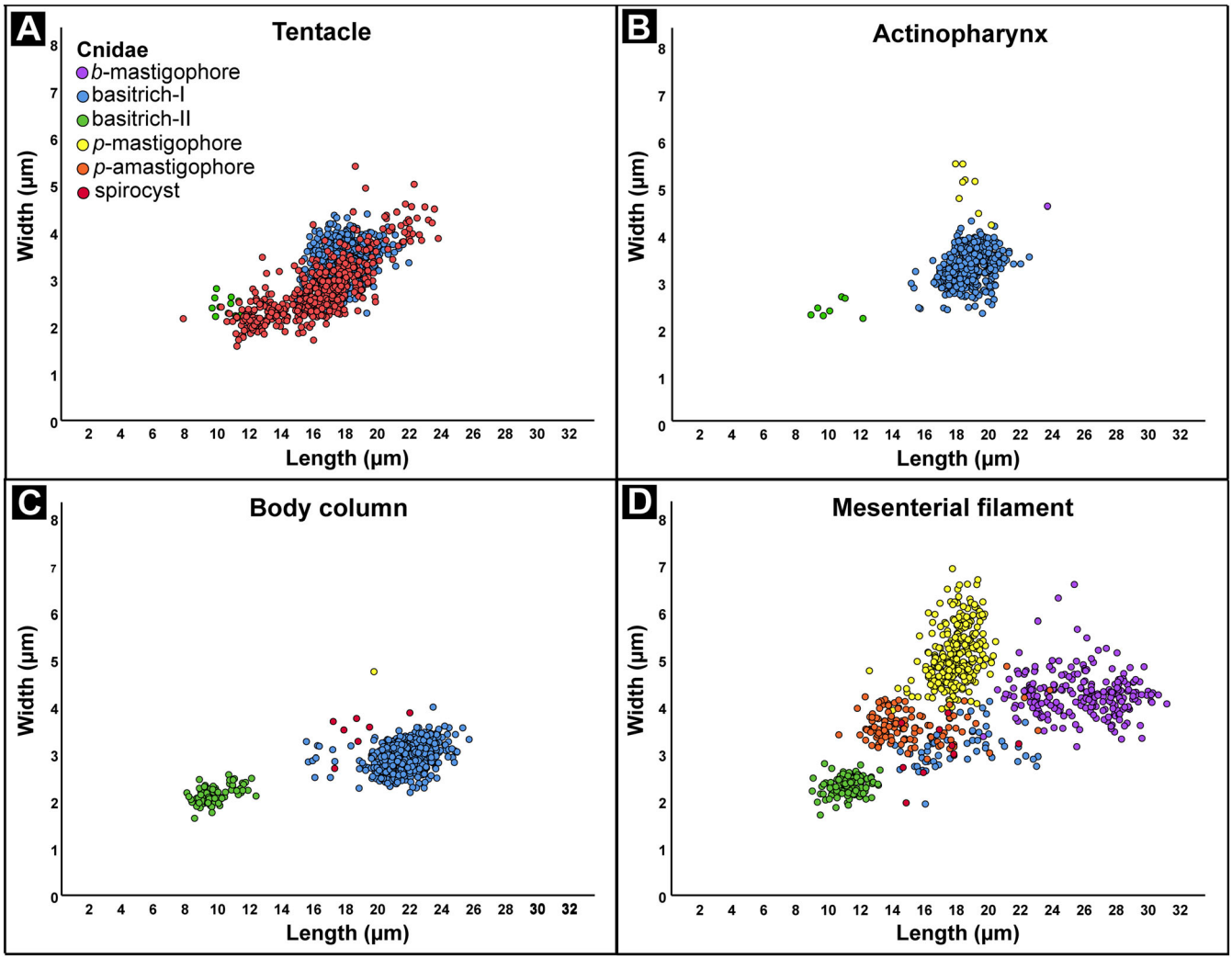

FIGURE 6 Cnidom biometrics of *Isactinia*-MTQ. Each panel shows the length of undischarged cnidae capsules plotted against the width of all cnidae types from different tissues. (A) Tentacles. (B) Actinopharynx. (C) Body column. (D) Mesenterial filaments. Purple, *b*-mastigophore; blue, basitrich-I; green, basitrich-II; yellow, *p*-mastigophore; orange, *p*-amastigophore; red, spirocyst.

attributed to the specific function of cnidae (Francis, 2004), the difference among individuals may be the result of a range of contributing endogenous and exogenous factors such as the morphology of the respective tissue (Francis, 2004; Garese et al., 2023), size of the individual (Chintiroglou et al., 1997; Chintiroglou & Simsiridou, 1997; Dunn, 1981; Francis, 2004; Karalis & Chintiroglou, 1997; Kramer & Francis, 2004; Stephenson, 1929), specific population (Acuña & Zamponi, 1997), environmental conditions (Cerrano et al., 1997; Chintiroglou, 1996; Karalis & Chintiroglou, 1997; O'Hara, 2018; Zamponi & Acuña, 1991 [as cited in Acuña et al., 2003; Fautin, 2009]), and predatory pressure (Francis, 1976; Gochfeld, 2004; Jennings, 2014). Collectively, the results presented here add to a growing body of work demonstrating the complexity of actiniarian cnidom biometrics.

Basitrichs were found to include two size classes; the larger of which, basitrich-I, was found to include two morphologically different types discernable only in a discharged state. The importance of investigating both the undischarged and discharged states in parallel was highlighted by finding that the larger basitrichs were seen to be further differentiated into those that possessed a long thread and another type that had a vastly shorter thread.

The cnidae found within *Isactinia*-MTQ conform with the cnidom descriptions of previous studies. Basitrichs, *p*-mastigophores, and spirocysts are common among Actiniaria (Fautin, 2009; Kass-Simon & Scappaticci, 2002; Mariscal, 1974), with most species seen to possess them within their cnidom (Carlgren, 1945, 1949; Fautin, 1988, 2009). Species within the Actinioidea superfamily have commonly been shown to possess *b*-mastigophores and *p*-mastigophores (Carlgren, 1945, 1949; Rodríguez et al., 2014). Additionally, the cnidom of species within Actiniidae have also been found to contain spirocysts, basitrichs, and *p*-mastigophores (Carlgren, 1945).

The presence of two distinct size classes of cnidae, particularly basitrich types, is not uncommon among sea anemones (Carlgren, 1945; Daly, 2017; Fautin, 2009; Mitchell, 2010). Furthermore, unlike cnidae in hydrozoans, anthozoan cnidae do not migrate

TABLE 1 Lengths and widths of undischarged cnidae from different tissue types of *Isactinia*-MTQ.

Tissue type	Cnida type	Figure 4 panel	Proportion of individuals with cnida type	Number of cnidae	Length (μm)			Width (μm)		
					Range (outlier)	Mean	SD	Range (outlier)	Mean	SD
Tentacle	Basitrich-I	C, D	6/6	600	15.11–22.08	17.86	1.43	2.27–4.38	3.37	0.36
	Basitrich-II	E	3/6	12	9.77–11.56	10.68	0.65	2.2–2.79	2.41	0.2
	Spirocyst	G	6/6	467	10.34–23.91 (7.97)	16.34	2.75	1.58–4.59 (5.39)	2.76	0.63
Actinopharynx	*b*-mastigophore	A	1/4	1	23.8	–	–	4.55	–	–
	p-mastigophore	B	3/4	8	18.05–20.29	18.88	0.75	4.15–5.45	4.93	0.47
	Basitrich-I	C, D	4/4	400	15.29–22.66	19.01	1.1	2.28–4.24	3.28	0.37
	Basitrich-II	E	3/4	7	9.01–12.26	10.39	1.13	2.17–2.63	2.37	0.18
Mesenterial filament	*b*-mastigophore	A	4/4	230	19.82–31.27	26	2.49	3.14–5.45 (6.57)	4.25	0.45
	p-mastigophore	B	4/4	289	14.16–20.58 (12.7)	18.04	1.1	3.74–6.91	5.08	0.6
	Basitrich-I	C, D	4/4	59	14.29–23.23	18.17	2.4	2.61–4.1 (1.93)	3.15	0.4
	Basitrich-II	E	4/4	130	9.13–13.36	11.37	0.87	1.67–2.79	2.32	0.18
	p-amastigophore	F	4/4	121	10.79–19.89 (23.97)	14.91	2.32	2.88–4.34 (4.84)	3.58	0.33
	Spirocyst	G	3/4	10	14.7–22.03	17.11	2.19	1.953–3.85	3.06	0.56
Body column	*p*-mastigophore	B	1/6	1	19.86	–	–	4.74	–	–
	Basitrich-I	C, D	6/6	600	15.64–25.82	21.84	1.34	2.18–3.99	2.94	0.26
	Basitrich-II	E	6/6	73	8.17–12.49	9.95	1.04	1.63–2.56	2.13	0.18
	Spirocyst	G	2/6	7	17.32–18.79	17.87	0.67	2.69–3.74	3.4	0.49
Pseudospherules	Basitrich-I	C, D	6/6	60	19.18–25.719	22.84	1.46	2.41–3.8	3.11	0.31

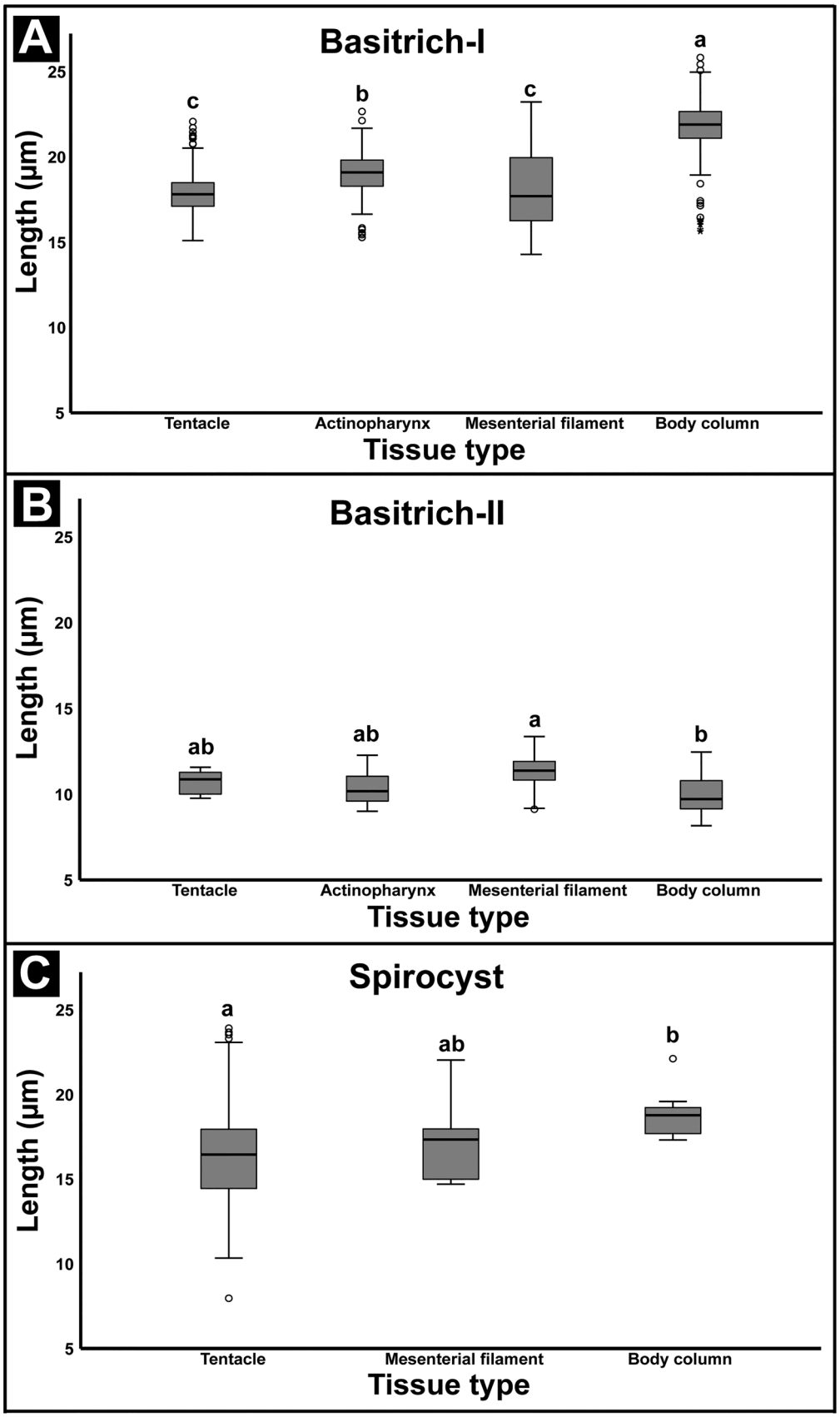

FIGURE 7 Lengths of cnidae in *Isactinia*-MTQ across different tissue types. (A) Basitrichs-I. (B) Basitrichs-II. (C) Spirocysts. The minimum, median, and maximum quartile range of cnidae lengths, along with any outliers, are illustrated. Letters denote significant differences among tissue types as shown by pairwise post hoc comparisons. Error bars are 95% confidence intervals.

TABLE 2 Pearson's correlation and linear regression of length of each cnida within different tissue types as a function of sea anemone size (measured as the diameter of the oral disc) in *Isactinia*-MTQ. –, no data.

	Tissue type													
	Tentacle				Actinopharynx				Mesenterial filament				Body column			
Cnida type	Slope	Intercept	R^2	p	Slope	Intercept	R^2	p	Slope	Intercept	R^2	p	Slope	Intercept	R^2	p
b-mastigophore	–	–	–	–	–	–	–	–	−12.73	38.25	0.678	<.001	–	–	–	–
p-mastigophore	–	–	–	–	–	–	–	–	−2.27	20.28	0.1	<.001	–	–	–	–
Basitrich-I	−2.09	19.81	0.097	<.001	−1.68	20.69	0.059	<.001	−1.92	20.17	0.012	.405	1.71	20.24	0.047	<.001
Basitrich-II	–	–	–	–	–	–	–	–	−3.52	14.59	0.333	<.001	−1.16	10.92	0.012	.362
p-amastigophore	–	–	–	–	–	–	–	–	−12.34	28.28	0.526	<.001	–	–	–	–
Spirocyst	−1.8	18.04	0.012	.019	–	–	–	–	–	–	–	–	–	–	–	–

around the body between tissues; rather, they develop underneath mature cnidae (Strömberg & Östman, 2017). As such, the small basitrichs sampled here are not likely to be immature cnidae and instead may represent a true smaller basitrich type, which may serve a unique purpose. Further research is required, however, to discern the benefits or reasons for possessing both large and small basitrichs concurrently.

The specific cnidom of each discrete morphological region of the body in individuals of *Isactinia*-MTQs varied significantly (Figure 5). Some cnidae types (basitrichs and spirocysts) were found between different tissue types, although their abundance was not equal between tissues. Conversely, *b*-mastigophores, *p*-mastigophores, and *p*-amastigophores were largely restricted to one location (mesenterial filaments) within the body.

Cnidae are single use (Tardent, 1995) and are theorized to potentially be energetically costly to produce (Bode & David, 1978; Fautin, 2009; Francis, 2004; Sachkova et al., 2020), particularly in small species (Robson, 1988). As such, the differences in abundance and distribution of cnidae among tissue types are likely to be the result of evolutionary pressures favoring cnidae that achieve maximum benefit in line with the function and purpose of the respective tissue in which they are found.

One of the key roles of sea anemone tentacles is to capture prey items such as zooplankton (e.g., copepods, isopods, and amphipods) and fish from the water (Van-Praët, 1985). The combination of basitrichs (both large and small types) and spirocysts present within this tissue are likely to facilitate this. Spirocysts are known for their adhesive properties (Mariscal, 1974, 1984; Mariscal, McLean, & Hand, 1977), enabling the tentacles to quickly adhere to and hold onto prey and providing the penetrative basitrichs an opportunity to inject the prey with the venom payload (Strömberg & Östman, 2017; Van-Praët, 1985). Whereas spirocysts accounted for less than one-third of cnidae within the tentacles of *Isactinia*-MTQ, in many species, spirocysts are the most common form of cnidae (Fautin, 2009; Mariscal et al., 1976; Schmidt, 1982; Shick, 1991; Watson & Mariscal, 1983). The reasons for the variations in composition of cnidae within the tentacles of different sea anemone species remain unknown. Some cnidae are theorized to be polyfunctional (Daly, 2017; Fautin, 2009; Prudkovsky, 2014; Shick, 1991), so perhaps for species such as *Isactinia*-MTQ, in which basitrichs dominate, the greater proportion of basitrichs within the tentacles may serve a dual purpose in both penetrating and entangling prey, thus negating the requirement to have many spirocysts. Research into the ecology of this species is required before full conclusions may be drawn.

Once food has been captured, sea anemones use their tentacles to pass it towards the mouth, where it enters the actinopharynx (Lampitt & Paterson, 1987; Lindstedt, 1971; Nicol, 1959). In *Isactinia*-MTQ, the cnidom of the actinopharynx was almost exclusively made up of large basitrichs, complimented by very low numbers of small basitrichs and *p*-mastigophores. This is consistent with other species in which basitrichs and sometimes mastigophores are found within the actinopharynx (Acuña et al., 2007; Chintiroglou et al., 1997;

Garese et al., 2019; González-Muñoz et al., 2018; Mitchell, 2010). Cnidae within the actinopharynx are believed to further assist in the immobilization and killing of prey (Van-Praët, 1985) before it is passed further down the gastrovascular cavity towards the mesenterial filaments.

Sea anemone mesenterial filaments may be used to kill and facilitate the digestion of prey (Fautin, 2009; McMurrich, 1899; Nevalainen et al., 2004; Nicol, 1959; Schlesinger et al., 2009). In addition to the enzyme-producing glands (Shick, 1991), it is widely reported that mesenterial filaments are also heavily laced with penetrative nematocysts (Carlgren, 1940, 1945; Mitchell, 2010; Östman et al., 2010). Several studies have shown that these nematocysts often exhibit cytolytic (cellular digestion) and neurotoxic (disrupts nerves) properties, supporting the theory that these filaments are important in digestion (Fautin, 2009; Nevalainen et al., 2004). Given that *b*-mastigophores, *p*-mastigophores, and *p*-amastigophores were all but exclusively found here, it is probable that these nematocysts are particularly used for this purpose. Furthermore, unlike many scleractinian and corallimorpharian species, most sea anemones do not typically extrude their mesenterial filaments during antagonistic encounters (Robson, 2004). Some sea anemones have been reported to use their mesenterial filaments during prey acquisition (M.L. Mitchell, unpubl. data), however, which supports the theory that the mastigophores and amastigophores found in *Isactinia*-MTQ serve a digestive purpose.

Although not unheard of (Garese et al., 2016, 2019), actiniarian mesenterial filaments are believed to rarely contain spirocysts (Van-Praët, 1985). Although it is possible that the spirocysts reported here in the mesenterial filaments may be an artifact of exogenous contamination between samples, Fautin (1988) has cautioned that the presence of unexpected cnida may indeed signify a rare variety within the tissue. Given the degree of precaution taken in this study to avoid contamination, the presence of spirocysts within the mesenterial filaments of *Isactinia*-MTQ cannot be ruled out. Nevertheless, although this has yet to be confirmed, it is thought that spirocysts do not contain venom (Mariscal, 1974). Given that spirocysts are hypothesized to predominately serve an adhesive role (Mariscal, 1974, 1984; Mariscal, McLean, & Hand, 1977), and thus are unlikely to have a penetrative function, it is unlikely that they would aid in the killing and direct digestion of prey. The presence of spirocysts within the mesenterial filaments of *Isactinia*-MTQ may contribute to securing the prey items close to the digestive tissues (Goldberg, 2002).

The ectodermal tissue of the body column in individuals of *Isactinia*-MTQ was found to be dominated by basitrichs and a small proportion of spirocysts. Unlike many other species of sea anemone, *Isactinia*-MTQ lack specific aggressive and defensive structures such as catch tentacles, acrorhagi, and acontia. Instead, the primary defensive strategy in this species appears to be the ability to contract when threatened. Because individuals of *Isactinia*-MTQ are small, they may be particularly vulnerable to predation by sea spiders, starfish, gastropods (nudibranchs and sea snails), polychaetes, and fish, which are known to commonly feed on sea anemones (Ottaway, 1977; Shick, 1991). Interestingly, the ectodermal tissue of the body column was the only morphologically discrete tissue type in which the short-thread basitrichs (β-basitrich-I) were located, and indeed, these basitrichs made up most of the cnidom of the body column. The reasons for the presence of two different types of basitrichs within this area of the body warrant further investigation.

Whereas documentation of both the length and width of undischarged actiniarian cnidae is taxonomically important (Carlgren, 1940; Cutress, 1955; England, 1987; Fautin, 1988; Häussermann, 2004; Shick, 1991), studies that statistically compare cnidae sizes typically do so on length data alone (Acuña et al., 2003, 2004, 2007, 2011; Allcock et al., 1998; Chintiroglou et al., 1997; Chintiroglou & Simsiridou, 1997; Francis, 2004; Karalis & Chintiroglou, 1997; Kramer & Francis, 2004; Watts et al., 2000). Alternative variables include surface area (Chintiroglou et al., 1997; Chintiroglou & Simsiridou, 1997), volume (Chintiroglou et al., 1997; Chintiroglou & Simsiridou, 1997), and length–width ratio (Chintiroglou et al., 1997; Karalis & Chintiroglou, 1997). Similar to the results of the precision tests in this study, measurements of cnidae length have been previously shown to exhibit a lower coefficient of error than measurements of cnidae width (Ryland et al., 2004). Given that only cnidae length was selected as the proxy for cnidae size in this study and that all reported measurements were obtained by one person, the accuracy of measurements obtained in this study was deemed acceptable for analysis.

The length of undischarged basitrichs and spirocysts varied among the tissue types in which they were located. Similar findings have been reported in the scleractinian coral *Desmophyllum pertusum* (formerly *Lophelia pertusa*), in which *b*- and *p*-mastigophores in the tentacles and in mesenterial-like filaments resembling those of actiniarian acontia, respectively, were shown to be dramatically larger than those in other tissues (Strömberg & Östman, 2017). Similarly, Francis (2004) found differences in length among spirocysts in the tentacles and acrorhagi in *Anthopleura elegantissima* (Actiniaria). Such differences in cnidae size among anatomical regions may be yet another example of the subtle specialization of cnidae (Francis, 2004), or there could be differences in rates of use or replacement among tissue types (Acuña et al., 2007, 2011). Alternatively, differences in cnidae size may simply be the result of the physical characteristics of the surrounding ectodermal tissue (Francis, 2004). Thickness and cell size may apply external pressure on developing cnidae, thereby restricting their ultimate size (Francis, 2004).

Intraspecific variation in cnidae size among individuals has been reported previously in sea anemones (Acuña et al., 2003, 2007; Allcock et al., 1998; Chintiroglou et al., 1997; Chintiroglou & Simsiridou, 1997; Dunn, 1981; Francis, 2004; Garese et al., 2016; Karalis & Chintiroglou, 1997; Kramer & Francis, 2004; Stephenson, 1929; Watts et al., 2000; Williams, 1998; Zamponi & Acuña, 1991 [in Acuña et al., 2003; Fautin, 2009]) and seems to be typical of most sea anemones. Although differences in cnidae size may be attributed to genetic differences (Allcock et al., 1998), there is evidence that cnidae size may be correlated with differences in sea anemone size or weight (Chintiroglou et al., 1997; Chintiroglou & Simsiridou, 1997; Dunn, 1981; Francis, 2004; Karalis & Chintiroglou, 1997; Kramer & Francis, 2004; Stephenson, 1929).

Several studies have reported scaling of cnidae size among individuals, whereby larger individuals are typically seen to have larger cnidae (Chintiroglou et al., 1997; Chintiroglou & Simsiridou, 1997; Dunn, 1981; Francis, 2004; Karalis & Chintiroglou, 1997; Kramer & Francis, 2004). This is contrary to what we found in *Isactinia*-MTQ, in which the size of most cnidae (distinguished among respective tissue types) was negatively related to the size of the individual (measured as the diameter of the oral disc). It remains unclear why the size of only some cnidae, and not others, is correlated with body size, and why cnidae size appeared to decrease with increasing sea anemone size. Not all species display a relationship between cnidae length and body size, however (Acuña et al., 2007; Williams, 1998). More research, with increased replication and a broader range of sizes, may be required to understand this phenomenon within both *Isactinia*-MTQ and other species.

Although the causes of intraspecific variability in the sizes of cnidae within *Isactinia*-MTQ remain unclear, other studies found temporal and spatial variation in the size of cnidae. For example, location, latitude, and depth (Karalis & Chintiroglou, 1997; Zamponi & Acuña, 1991 [in Acuña et al., 2003; Fautin, 2009]) are associated with variation in cnidae size among individuals. Similarly, the presence of physical threats (Francis, 1976; Gochfeld, 2004; Jennings, 2014), changes to energy availability (food and photosynthetic energy; Gundlach & Watson, 2018, 2019; Hiebert & Bingham, 2012; Hoepner et al., 2019; Koch, 2014), and environmental conditions such as temperature (O'Hara, 2018) and water movement (Cerrano et al., 1997) are associated with a plastic change in the size and composition of cnidae in cnidarians.

5 | CONCLUSION

In addition to providing a detailed taxonomic description of the cnidom within a small, clonal tropical sea anemone, this study sought to quantify the intraspecific variability of cnidae both within and among individuals. The length of cnidae within this species was found to be highly variable and unpredictable, highlighting the complexity of size variation within individual anemones. Further to this, variation in measurements of cnidae by different people demonstrates that for statistical analyses, it's importance for a single person to do the measurements. This study provides valuable baseline information on the distribution and density of different cnidae types associated with different biological functions and highlights subtle intraspecies differences that should also be considered when working on the cnidae of this species. It should be noted, however, that the results here are for these specific individuals within this captive population that were sampled. Further research is required to determine whether wild conspecifics are equally variable, both in terms of cnidae types and sizes, but also in how these results compare with other species. The latter would be valuable in making accurate ecological inferences. Regardless, it is clear that there are numerous challenges when documenting the cnidae characteristics of actiniarians.

AUTHOR CONTRIBUTIONS

Concept: Katrina L. Kaposi and Jamie E. Seymour. *Methodology*: Katrina L. Kaposi, Jamie E. Seymour, and Michela L. Mitchell. *Taxonomy*: Michela L. Mitchell. *Formal analysis and investigation*: Katrina L. Kaposi, Michela L. Mitchell, Robert L. Courtney, and Jamie E. Seymour. *Writing—original draft*: Katrina L. Kaposi. *Writing—review and editing*: Katrina L. Kaposi, Robert L. Courtney, Jamie E. Seymour, and Michela L. Mitchell. *Resources*: Seymour. *Supervision*: Jamie E. Seymour and Robert L. Courtney.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interests.

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