



A review of zoanthid nematocyst types and their population structure

J. S. Ryland* & J. E. Lancaster

University of Wales Swansea, Swansea SA2 8PP, UK

(*Author for correspondence: E-mail: j.s.ryland@swan.ac.uk)

Key words: Cnidaria, Hexacorallia, *Palythoa*, *Protospalythoa*, cnidae, capsules, discriminant analysis

Abstract

Seven different types of nematocyst are detailed and illustrated from the Zoanthidea. We studied the size population structure of nematocyst capsules: how they are affected by preservation, and how and what should be measured. Populations of two types, large holotrichs and *p*-mastigophores, from the mesenterial filaments of *Protospalythoa heliodiscus* and *Pr. mutuki* have been compared using length, width, and (for *p*-mastigophores) shaft length. The analysis employed bivariate scattergrams and discriminant analysis. The two species are strongly differentiated by their holotrichs, less well by their *p*-mastigophores. The problem of bimodal size distributions is considered and resolved. It is recommended that nematocyst capsule population descriptors should always be included in species' descriptions, in addition to or instead of the customary minimum–maximum range.

Introduction

The presence of cnidae is one of the defining characteristics of the phylum Cnidaria. Within the Anthozoa, the types, characteristics, and capsule dimensions of one category of cnidae, the nematocysts, have been used in systematics for a century (Fautin, 1988), but the most appropriate data for species discrimination and higher level taxonomy remain open to debate. In this paper we first review the types of nematocyst recorded in Zoanthidea, updating the surveys of Schmidt (1972, 1974), and then explore the use of quantitative data, following the procedural recommendations recently formulated (Ryland et al., 2003). For recognition of nematocyst types, of which at least seven are known for Zoanthidea (Fig. 1), we follow Schmidt (1972, 1974), because of his clear diagrams (Fig. 1), but prefer a terminology based on that of Weill (1934). The first group of types comprises: large holotrichs (holotrichs I of Schmidt) or holotrichous isorhizas (i.e. with parallel sided tubule; Fig. 1D); small holotrichs

(holotrichs II) which, from Schmidt's depiction of a tapering tubule, appear to be holotrichous anisorhizas (Fig. 1B and C); and large atrichs – not included by Schmidt in the zoanthid assemblage although they had been recorded from the order by Cutress (1955) (Fig. 1A) and are present in the Actiniaria, from one division of which (Endomyaria) Schmidt (1974) derived the Zoanthidea. The second group comprises the various mastigophores: nematocysts with slender elongate capsules which, following England (1991) – who broadened Weill's (1934) original description to include mastigophores in which the shaft is wider, but only slightly wider, than the rest of the tubule – we identify as basitrichs (Fig. 1E and F; microbasic *b*-rhabdoids of Schmidt); *p*-mastigophores (*p*-rhabdoids of Schmidt), with a wide, sharply defined shaft (Fig. 1G); and microbasic *b*-mastigophores *sensu stricto* (special *b*-rhabdoids of Schmidt; Fig. 1H) which, in zoanthids, are more ovoid and less fusiform than basitrichs, and which – according to Schmidt (1974) – are restricted to the 'higher' Zoanthidea (a term of his own which

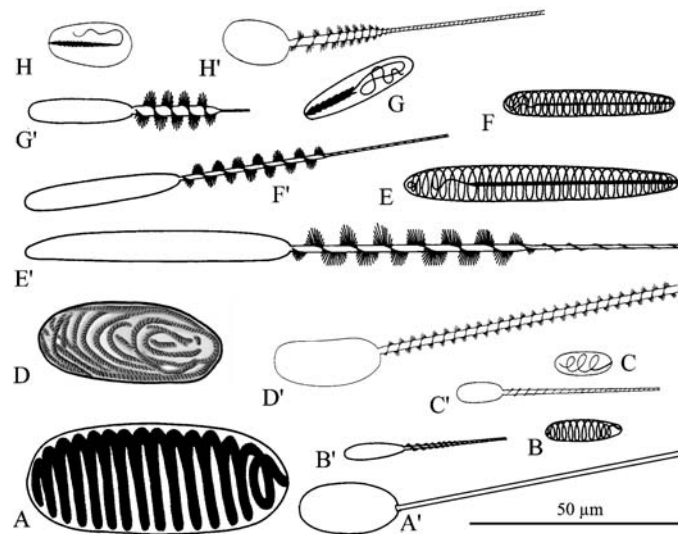


Figure 1. The types of nematocyst found in zoanthids. (A, A') Atrich, *Palythoa caesia*. (B, B') Small holotrich (holotrich II), *Palythoa tuberculosa*. (C, C') Small holotrich, *Parazoanthus axinellae*. (D, D') Large holotrich (holotrich I), *Palythoa caesia*. (E, E') Large basitrich, *Palythoa tuberculosa*. (F, F') Medium basitrich, *Palythoa tuberculosa* (a form with a short shaft occurs in *Parazoanthus axinellae*). (G, G') Microbasic *p*-mastigophore, *Palythoa tuberculosa*. (H, H') Microbasic *b*-mastigophores, *Parazoanthus axinellae* (H), *Zoanthus coppingeri* = *pacificus* (H'). Except A' and D, from Schmidt (1974).

we equate with all the Macrocnemina plus part (family Zoanthidae *sensu stricto*) of the Brachynemina (see Ryland et al., 2003, for discussion). Schmidt evidently perceived these 'special' *b*-mastigophores as different from those of anemones.

To a small extent, higher anthozoan taxa may be characterized by their types of cnidae, as Hexacorallia are by spirocysts and Ceriantharia by ptychocysts. Carlgren (1940) believed that Ceriantharia were also characterized by lacking *p*-mastigophores, but they are present in arachnactis larvae (Schmidt, 1974). Schmidt considered that zoanthid holotrichs differed from those of all other orders in having 'spoon-shaped' spines, and that 'special' *b*-mastigophores were unique to 'higher' Zoanthidea. Certainly, we have not seen these *b*-mastigophores in the Sphenopidae (equivalent to Schmidt's 'lower' Zoanthidea) which are, however, the only zoanthids to have large basitrichs – of the type prevalent in Endomyaria – in the filaments.

In a comprehensive study of the cnidom of one zoanthid, *Acrozoanthus australiae* (Brachynemina Zoanthidae), Ryland et al. (2003) demonstrated the scope for error if species' nematocyst capsule sizes are expressed solely as a range between observed

upper and lower limits based on a sample of, say, $n = 20$. We investigated the practicalities of using standard statistical procedures to describe nematocyst samples representing populations (as by Hidaka, 1992; Pires & Pitombo, 1992; Chintiroglou & Simsiridou, 1997), rather as done for anemones by Williams (1996, 1998, 2000) but not always reaching the same conclusions. However, there are obvious limitations to the utility of conclusions based on a single species, and we here consider some data from species of Brachynemina Sphenopidae (Schmidt's, 1974 'lower' Zoanthidea).

Material and methods

For a few specimens, at the Australian Institute for Marine Science (AIMS), fresh nematocysts were obtained as aqueous squashes of fragments of tentacles and filaments. Discharge was encouraged with a drop of acetic acid and pressure on a cover slip, and the preparation viewed under Nomarski illumination. Photomicrographs were taken on colour film, using a $\times 40$ objective and $\times 3.3$ or $\times 5$ photo-ocular; the camera automatically imprinted a scale. Measurements were made from the film, as below.

Later, preserved zoanths fixed in seawater formalin (4% HCHO), Bouin's fluid, or 70% ethanol were used. After dab drying, since polyp size may be an important variable, volume was determined by displacement (all methods being described in more detail by Ryland et al. (2003)). Using a dissecting microscope, minute amounts of tissue were removed from filaments using extra-fine jewellers' forceps. The tissue was digested for 2–3 min in a few drops of 2% aqueous KMnO_4 on a microscope slide, excess fluid absorbed with a strip of filter paper, a drop of glycerol added, and the preparation macerated with the forceps (the method modified from England (1987)). The fragmented cells were spread by light, even pressure on the cover slip, 22×22 mm, which was then ringed with clear nail varnish to prevent evaporation.

Slides were systematically scanned for nematocyst capsules from the top left to the bottom right of the coverslip. All capsules lying wholly in the focal plane were measured; capsules oblique to the focal plane, being foreshortened, were ignored. When available, 100 capsule measurements were made of length, width and, for *p*-mastigophores, shaft length using bright-field or phase contrast illumination and $\times 40$ objectives. Except in the case of the freshly obtained holotrich/atrich sample, we have not used results when $n < 20$. A video camera attached via a $\times 6.7$ photo-ocular fed images to a dedicated image-analysis computer. Following calibration, measurements in μm were made on the screen image. The excellent but now obsolete DOS software MeasurementTV (Updegraff, 1990) was used at the time; we currently use Image-Pro Plus. Original basic statistics were performed in Quattro-Pro, later transferred to MS Excel[®] 2000 with XLstat add-in (Fahmy & Aubry, 2002) or BIOMstat 3.3 (Rohlf & Slice, 2002) for ANOVA.

Results

When freshly discharged nematocysts from the filaments of *Palythoa caesia* Dana were examined, it was observed that the large, oblong-oval (in plan view) capsules had discharged two different types of tubule. In one, the tubule was wide, $\sim 4.5 \mu\text{m}$, with large spines arranged in a triple helix.

Undischarged, the tubule was loosely and obliquely coiled, in the manner of a vertical coil of rope that has been pulled sideways from the top, with the spines producing a banded appearance under Nomarski illumination (Fig. 1D). This type corresponded to Schmidt's (1974) holotrich I, described from *Pa. tuberculosa*, although he illustrates the spines in a single helix (Fig. 1D'). In the second type (despite the larger capsule, see below) the tubule was thinner, $\sim 3.7 \mu\text{m}$, and appeared totally devoid of spines, even when using a $\times 100$ oil immersion objective; prior to discharge the tubule was coiled in the capsule like a spring (Fig. 1A and A') – very different from the holotrichs. It corresponded with the atrich described by Schmidt (1974) in actinians but not recorded by him in *Pa. tuberculosa* or any other zoanthid, although he had evidently overlooked that Cutress (1955) had mentioned them from unspecified zoanths as well as actinians. Our specimens matched Cutress' description perfectly. The holotrich and atrich capsules were also readily separated by size and shape: holotrichs ($n = 4$) length 61.18 ($\text{SD} \pm 2.59$) μm , width 26.85 (± 1.66) μm , ratio 2.28 (± 0.05); atrichs ($n = 10$) length 82.80 (± 2.93) μm , width 34.02 (± 4.55) μm , ratio 2.47 (± 0.29). In a much larger, preserved sample ($n = 100$) of holotrichs the dimensions were 62.29 (± 2.78) \times 28.60 (± 2.08) μm ; no atrichs were present. We retain the term atrich, since no spines are visible under the light microscope, though aware that Westfall (1965) – using transmission electron microscopy – could identify small spines around the tubule in the atrichs of *Metridium*.

Photographs were also taken of discharged and undischarged basitrichs and *p*-mastigophores of *Protopalpythoa mutuki* Haddon & Shackleton from the rocky shore at Kissing Point, Townsville, for later comparison with preserved material. Using measurements from the film we compared discharged and undischarged basitrichs by ANOVA, the mean lengths being not significantly different: means (\pm SD) undischarged 57.69 (± 3.68), discharged 56.70 (± 3.68), $p \approx 0.2$, but width had slightly increased: undischarged 6.69 (± 1.00), discharged 7.12 (± 0.89), $p = 0.045$. Three methods of fixation were compared: sea water Bouin's fluid for 48 h or more followed by washing and storage in 70% ethanol, direct immersion in 70% ethanol, and 4% formaldehyde in sea water

(though later samples were washed and transferred to 70% ethanol). The dimensions for basitrichs, holotrichs, and *p*-mastigophores are in Table 1. While the long, slender basitrichs (Fig. 1E and F) may perhaps be adequately characterized by length, *p*-mastigophores are quite oval in plan view and holotrichs are oblong-oval (Fig. 1G and D respectively). We have, therefore, measured both length and width. ANOVA of preserved basitrich lengths showed significant differences ($p = 2.67 \times 10^{-9}$), with a similar (though less pronounced, $p = 0.003$ – 0.0001) result for length and width together using XLstat for discriminant analysis. Moreover, when measurements of fresh undischarged and exploded basitrichs were included, the differences were even more marked ($p < 0.0001$ in all tests). For clarity in the diagram, all the preserved samples have been combined (Fig. 2). On holotrich lengths the corresponding ANOVA was significant ($p = 6.54 \times 10^{-6}$) but discriminant analysis of lengths and widths gave mixed results ($p < 0.0001$ – 0.12 according to test). There was a different result for *p*-mastigophores with ANOVA indicating no differences in length ($p = 0.168$) but discriminant analysis gave values of $p = 0.002$ – 0.095 . Since we used separate whole polyps, the results do not necessarily indicate that method of fixation produces different degrees of shrinkage, and the three methods have not produced a consistent pattern for the three nematocyst types (Table 1); whether or not fixation method is important requires verification using a partitioned polyp fixed in three ways. The shrinkage caused by any method of fixation is, however, very marked in contrast to the fresh material, exploded or not (Table 1).

The remaining results, all based on preserved material, investigate differences between two sympatric species of *Protopalythoa* from the Great Barrier Reef and Fiji archipelago: *Pr. mutuki* and *Pr. heliodiscus* Ryland & Lancaster (2003). Full details of the types of nematocyst occurring in these two species have been tabulated elsewhere (Ryland & Lancaster, 2003: Tables 2 and 3). Since basitrichs are absent from *Pr. heliodiscus*, our present comparisons are restricted to holotrichs and *p*-mastigophores, which are abundant in the filaments of both species. Holotrich samples of *Pr. mutuki* came from 12 polyps from five sites and of

Pr. heliodiscus from 11 polyps from seven sites; *p*-mastigophores of *Pr. mutuki* came from seven polyps from five sites and of *Pr. heliodiscus* from 11 polyps from seven sites (Table 1). Additionally, the clearly visible, sharply demarcated shaft in the capsule of *p*-mastigophores (Fig. 1G) can readily be measured, providing a third variable (Table 1).

Bivariate data are conveniently displayed in scattergrams (Figs 3 and 4) but not readily analysed by ANOVA or other univariate methods. Moreover, because two related variables are involved, regressions conform to Model II (Sokal & Rohlf, 1995) and cannot be compared by ANCOVA, although comparisons between correlation coefficients are possible (Sokal & Rohlf, 1995). This situation is discussed in detail, with respect to *Acrozoanthus australiae*, elsewhere (Ryland et al., 2003). Confidence ellipses (normally the 95%) for each bivariate mean (see Sokal & Rohlf, 1995) were calculated in BIOMstat and added to the Excel graph (Fig. 3); these ellipses are analogous to the confidence limits of a single mean (Fig. 4). The separation between the species based on filament holotrichs is particularly clear (Fig. 3), that for *p*-mastigophores less so, largely on account of an anomalous *Pr. mutuki* site (7 in Fig. 4). With two or three variables, discriminant analysis was used. As would be predicted from the plot, a comparison of sites shows significant differences between them. Although the pooled data show a strong difference between the two species ($p < 0.0001$, reinforcing the validity of their separation based on other characters), the amount of overlap would make it difficult to identify any particular specimen on this criterion alone. Of 1606 individual capsule measurement sets, ~16% of *Pr. heliodiscus* capsules would be confounded with *Pr. mutuki*, and ~26% of *Pr. mutuki* would be confounded with *Pr. heliodiscus*. While shaft length (as percentage of capsule length – arcsine transformed in the data analysis but not in the graph) – rather than capsule width is used for the *y*-variate in Fig. 4, because it appeared to be proportionally longer in *Pr. heliodiscus*, the scattergram shows that this is true only for two sites (3 and 8 in Fig. 4).

The statistical analysis of the capsule measurements showed further complications. Just as we had found in *Acrozoanthus australiae* (Ryland et al., 2003), occasional populations of *Pr. mutuki*

Table 1. Measurement data for nematocyst capsules from filaments of two species of *Protopalpithoa*, arranged by type

Nem. type	Sp #	Polyp vol. (ml)	n	Treatment	Mean length ± SD (µm)	Mean width ± SD (µm)	Shaft L ± SD (µm)	Locality and identification	Loc #
Bas	1	NR	100	Form	48.09 ± 2.49	5.77 ± 0.45	–	Kissing Pt 101/529	5
Bas	1	NR	100	Fresh, expl	56.70 ± 3.23	7.12 ± 0.89	–	Kissing Pt, Townsville	5
Bas	1	NR	23	Fresh, unex	57.69 ± 3.68	6.69 ± 1.00	–	Kissing Pt, Townsville	5
Bas	1	NR	100	Ethanol	49.29 ± 2.49	6.19 ± 0.45	–	Kissing Pt 101/529	5
Bas	1	NR	100	Bouins	46.51 ± 3.83	5.37 ± 0.49	–	Kissing Pt 101/529	5
Hol	1	0.20	100	Form	50.01 ± 2.05	27.11 ± 1.71	–	Caloundra, Qld 106/9613	7
Hol	1	0.10	100	Ethanol	54.39 ± 4.96	22.27 ± 1.71	–	Heron I., GBR 140/9669	6
Hol	1	NR	100	Bouin	50.35 ± 3.68	22.20 ± 2.48	–	Kissing Pt 101/529	5
Hol	1	0.09	100	Bouin	55.98 ± 4.15	28.90 ± 2.63	–	Kissing Pt 101/9642	5
Hol	1	NR	100	Ethanol	51.72 ± 2.63	22.42 ± 2.04	–	Kissing Pt 101/529	5
Hol	1	NR	100	Form	49.51 ± 3.09	22.37 ± 2.12	–	Kissing Pt 101/529	5
Hol	1	0.06	25	Form	47.54 ± 4.70	22.01 ± 1.90	–	Korotogo, Fiji 4/965	11
Hol	1	0.75	100	Form	49.08 ± 2.68	19.16 ± 1.33	–	Korotogo, Fiji 4/961	11
Hol	1	0.11	100	Bouin	55.89 ± 5.65	23.41 ± 3.15	–	Torres Strait 527/9630	1
Hol	1	NR	100	Bouin	53.42 ± 3.89	24.36 ± 2.32	–	Torres Strait 527/202	1
Hol	1	0.10	100	Bouin	49.61 ± 2.78	19.88 ± 1.40	–	Torres Strait 528/9638	1
Hol	1	NR	100	Bouin	47.37 ± 3.57	20.24 ± 1.88	–	Torres Strait 528/104	1
Hol	2	0.11	100	Form	42.63 ± 5.04	16.66 ± 2.13	–	Fitzroy I., GBR 111/9653	3
Hol	2	0.08	26	Form	40.01 ± 5.80	15.45 ± 1.99	–	Fitzroy I., GBR 111/9657	3
Hol	2	0.12	30	Form	41.42 ± 4.62	16.44 ± 2.34	–	Low Is, GBR 210/9651	2
Hol	2	0.25	100	Ethanol	42.58 ± 4.71	20.28 ± 2.69	–	Makuluva Pass 66/9619	9
Hol	2	0.32	100	Ethanol	40.31 ± 3.84	19.35 ± 1.91	–	Makuluva Pass, Fiji 66/9616	9
Hol	2	0.11	90	Ethanol	41.55 ± 4.08	19.67 ± 1.84	–	Makuluva Pass 66/9617	9
Hol	2	0.13	78	Ethanol	40.34 ± 4.13	18.71 ± 2.63	–	Orpheus I., GBR 125/9633	4
Hol	2	0.25	100	Form	40.91 ± 4.92	19.91 ± 2.39	–	Toberua Pass, Fiji 3/966	10
Hol	2	0.15	62	Form	39.25 ± 4.43	19.34 ± 2.39	–	Toberua Pass, Fiji 3/9610	10
Hol	2	0.10	50	Ethanol	34.90 ± 5.98	16.65 ± 2.31	–	Torres Strait BB 1824	1
Hol	2	0.07	100	Form	37.89 ± 4.04	16.26 ± 1.63	–	Yarawa reef, Fiji 43/96101	8
Hol	2	0.08	90	Ethanol	34.65 ± 5.01	15.63 ± 2.29	–	Yarawa reef 44/9701	8
p-Mas	1	0.10	100	Ethanol	22.54 ± 1.95	5.09 ± 0.54	10.28 ± 1.04	Heron I., GBR 140/9669	6
p-Mas	1	NR	100	Ethanol	21.56 ± 1.54	4.91 ± 0.42	NR	Kissing Pt 101/529	5
p-Mas	1	0.09	52	Bouin	22.92 ± 1.56	5.49 ± 0.47	11.34 ± 0.93	Kissing Pt 101/9642	5
p-Mas	1	NR	21	Fresh, unex	26.17 ± 1.80	6.46 ± 0.74	NR	Kissing Pt, Townsville	5
p-Mas	1	NR	100	Form	21.89 ± 1.39	4.78 ± 0.29	10.72 ± 0.97	Kissing Pt 101/529	5

Continued on p. 184

Table 1. (Continued)

Nem. type	Sp #	Polyp vol. (ml)	<i>n</i>	Treatment	Mean length ± SD (µm)	Mean width ± SD (µm)	Shaft L ± SD (µm)	Locality and identification	Loc #
<i>p</i> -Mas	1	NR	100	Bouin	21.52 ± 1.60	4.71 ± 0.44	NR	Kissing Pt 101/529	5
<i>p</i> -Mas	1	0.10	49	Form	19.66 ± 1.45	4.80 ± 0.49	9.51 ± 1.01	Torres Strait 528/9638	1
<i>p</i> -Mas	1	0.11	52	Bouin	22.40 ± 1.58	4.73 ± 0.34	9.85 ± 0.97	Torres Strait 527/9630	1
<i>p</i> -Mas	1	NR	100	Bouin	18.67 ± 1.51	5.01 ± 0.46	NR	Torres Strait 528/104	1
<i>p</i> -Mas	1	NR	100	Bouin	21.73 ± 2.01	4.81 ± 0.46	NR	Torres Strait 527/202	1
<i>p</i> -Mas	2	0.11	53	Form	27.97 ± 2.45	5.24 ± 0.69	14.81 ± 1.45	Fitzroy I., GBR 111/9653	3
<i>p</i> -Mas	2	0.08	51	Form	27.81 ± 2.12	5.17 ± 0.60	15.60 ± 1.40	Fitzroy I., GBR 111/9657	3
<i>p</i> -Mas	2	0.11	100	Ethanol	27.81 ± 1.85	5.10 ± 0.45	12.47 ± 1.12	Makuluva Pass 66/9617	9
<i>p</i> -Mas	2	0.32	100	Ethanol	27.07 ± 1.54	4.76 ± 0.44	12.56 ± 1.05	Makuluva Pass 66/9616	9
<i>p</i> -Mas	2	0.25	100	Ethanol	26.81 ± 1.98	4.91 ± 0.48	12.06 ± 1.67	Makuluva Pass 66/9619	9
<i>p</i> -Mas	2	0.13	100	Ethanol	25.01 ± 2.36	4.74 ± 0.47	11.94 ± 1.41	Orpheus I., GBR 125/9633	4
<i>p</i> -Mas	2	0.15	100	Form	23.90 ± 1.67	5.15 ± 0.64	11.83 ± 1.29	Toberua Pass, Fiji 3/9610	10
<i>p</i> -Mas	2	0.25	100	Form	23.65 ± 2.10	4.83 ± 0.68	10.44 ± 1.45	Toberua Pass, Fiji 3/966	10
<i>p</i> -Mas	2	0.10	50	Ethanol	25.00 ± 2.10	5.17 ± 0.49	11.30 ± 1.25	Torres Strait BB1824	1
<i>p</i> -Mas	2	0.07	100	Form	22.51 ± 1.10	4.70 ± 0.47	13.02 ± 1.06	Yarawa reef 43/96101	8
<i>p</i> -Mas	2	0.08	100	Ethanol	27.41 ± 1.65	6.21 ± 0.71	12.96 ± 0.83	Yarawa reef 44/9701	8
<i>p</i> -Mas1	1	0.75	100	Form	20.68 ± 1.45	3.83 ± 0.45	8.91 ± 0.72	Korotogo, Fiji 4/961-1	11
<i>p</i> -Mas2	1	0.75	100	Form	42.29 ± 4.42	6.49 ± 1.16	17.68 ± 1.97	Korotogo, Fiji 4/961-2	11
<i>p</i> -Mas2	1	0.26	100	Form	40.56 ± 3.69	6.84 ± 0.69	17.22 ± 1.98	Korotogo, Fiji 4/963	11
<i>p</i> -Mas1	1	0.20	100	Form	28.22 ± 3.63	6.24 ± 1.05	13.95 ± 1.57	Caloundra, Qld 106/9613	11

Two *p*-mastigophore samples, one for each sub-population, were taken from one of the Korotogo (4/961). Note 1: the Caloundra sample is the full sample, before separation into components (Fig. 5). Nem. type (nematocyst types): Bas, basitrich; Hol, holotrich; *p*-Mas, *p*-mastigophore. Species are 1, *Pr. mutuki*, 2, *Pr. heliodiscus*; NR, not recorded; *n* = sample size; form, seawater formalin, often later stored in ethanol; expl, exploded; unex, unexploded. Locality numbers (loc #) refer to Figures 3 and 4. In the Korotogo sample there are two populations of *p*-mastigophores: *p*-Mas1, *p*-Mas2.

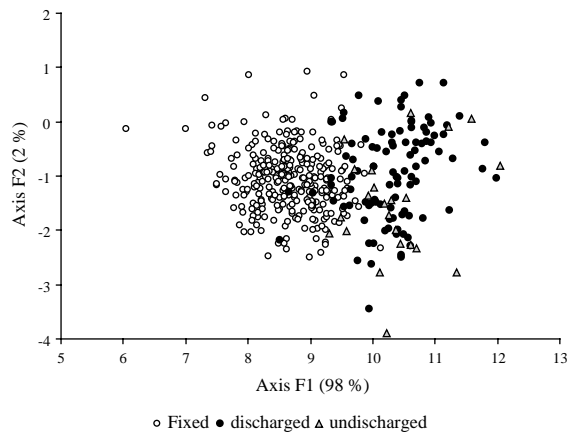


Figure 2. Discriminant analysis of capsule measurements (length, width) of filament basitrichs from *Protopolythoa mutuki* from the rocky intertidal of Kissing Point, Townsville. The comparison is between fixed material (Bouins fluid, 70% ethanol, and 4% sea water formaldehyde, $n = 100$ each, with the samples merged), and un-fixed discharged ($n = 100$) and undischarged ($n = 23$) nematocysts (see text).

(but not of *Pr. heliodiscus*) contained two separate populations of *p*-mastigophores in their filaments. In one sample (Korotogo reef, Fiji; 11 in Fig. 4) the frequency distributions of capsule length were totally separated: p_1 ($n = 96$) mean 20.68 (SD ± 1.45), range 16.58–24.02 μm ; p_2 ($n = 100$) mean 42.95 (± 3.07), range 35.96–50.61 μm . In a second population (Caloundra, coast of southern Queensland; 7 in Fig. 4), the length-frequency ranges overlap resulting in a bimodal distribution

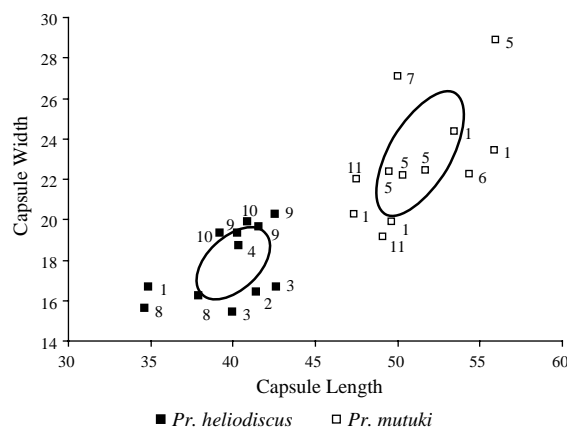


Figure 3. Scattergram, filament holotrich capsule sample means of width versus length in two *Protopolythoa* species, with ellipses indicating 95% confidence limits of the mean of means. Labels are localities (see Table 1 for key).

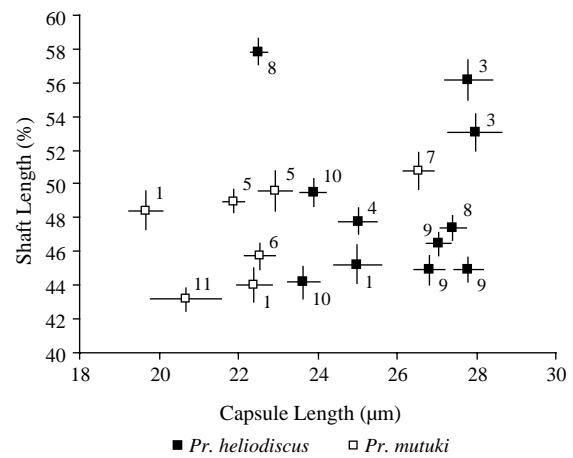


Figure 4. Scattergram, filament *p*-mastigophore capsule shaft length (as percentage of capsule length) versus capsule length sample means in two *Protopolythoa* species. Labels are localities (see Table 1 for key). Error bars are 95% confidence limits.

(Fig. 5). In the diagram we have separated the two components by the standard graphical method (Harding, 1949; Cassie, 1954), reconstituting two separate population distributions (on the assumption of normality) using the proportions of the normal distribution curve found in standard statistical tables (e.g. Zar, 1996). We describe the

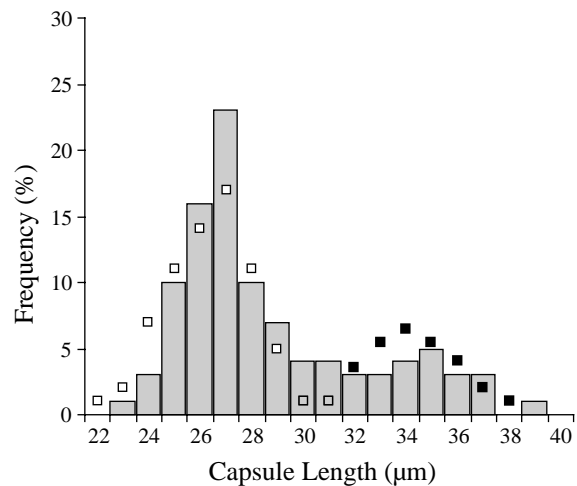


Figure 5. Analysis of bimodal distribution (filament *p*-mastigophores) of *Protopolythoa mutuki* from Caloundra, southern Queensland (grey bars), with the calculated distribution plots of the two sub-populations, p_1 \square and p_2 \blacksquare , assuming normal distributions (see text). The 30–31 and 31–32 μm bins contain not only the same number of original measurements but equal numbers of each sub-population. Arbitrary division has therefore been made at 31 μm (see Fig. 6).

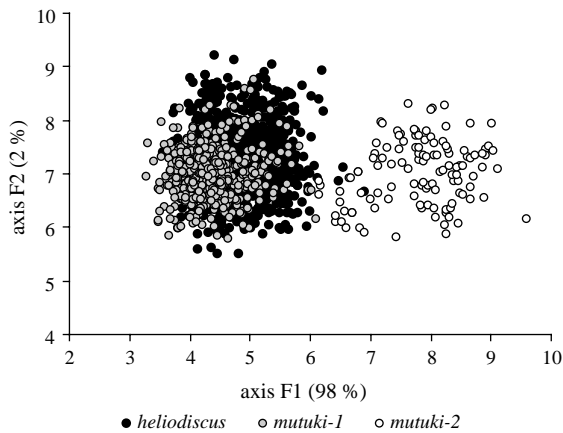


Figure 6. Discriminant analysis of filament *p*-mastigophore capsule measurements (length, width) from two species of *Protopalpythoa*, using both p_1 and p_2 sub-populations found in *Pr. mutuki* (see Fig. 5 and text).

method fully elsewhere (Ryland et al., 2003). The method makes no subjective assumptions and shows, in this case, that there is a slight overlap at the tails of the distribution between 30 and 32 μm . Since the two relevant 1 μm bins happened to contain the same number of measurements, and the predicted occurrence of the two types was approximately the same, an arbitrary separation was made at 31 μm . As seen in Figure 4, the point for sample 7 (based solely on the smaller, p_1 , population) is still anomalously high; but the analysis establishes that this is *not* the result of sample contamination. The overall result of discriminant analysis, including all *p*-mastigophores (Fig. 6), shows a very strong separation of the p_2 size-group and would almost certainly have indicated if there had been contamination of any other p_1 samples.

Our analyses showed that several of the capsule length–frequency distributions departed from normality. One possible cause of this, as just indicated, would be the inclusion of a few measurements from a distinct, but smaller or larger, population. In other cases the cause of the departure remains unexplained. The overall problem of non-normality is not further considered here, having been thoroughly discussed elsewhere (Ryland et al., 2003).

Discussion

The greatest problem affecting our knowledge of nematocyst types within zoanthid higher taxa is

the lack of ultra-structural studies, and hence ability to be able to differentiate types precisely; also, so few species have yet been studied in detail that generalization is unwise. Apart from the identity of ‘atrichs’, all zoanthid *b*-mastigophores and holotrichs II require SEM investigation. Treating nematocysts as populations depends on the availability of an image-analyser, since sample sizes are ideally at least 50 (Ryland et al., 2003). Digital images are more readily obtainable now but not all imaging software (as the University of Texas’ shareware ImageTool) easily permits more than one measurement per object: we consider it desirable to measure as many capsule variables as practicable. Much information can be conveyed in bivariate scattergrams (Figs 3 and 4). The samples can be analysed by multivariate methods, such as discriminant analysis (Figs 2 and 6). The examples given in this paper demonstrate the utility of this approach in revealing differences between species, although inter-colony variation must also be properly evaluated. Discriminant analysis can help to resolve the identity of any particular collection with reference to known species and to decide whether two morphotypes constitute separate species even when capsule size ranges overlap (Fig. 6). The measurements from more than one nematocyst type can be combined in any analysis, increasing the resolving power. The erratic occurrence of differently sized sub-populations, particularly of *p*-mastigophores, is curious, observed in such unrelated species as *Acrozoanthus australiae* (Ryland et al., 2003) and *Protopalpythoa mutuki*. Normality must be checked, and bimodal distributions separated into component parts.

Substantial shrinkage of capsules during fixation, such as we have demonstrated, would be expected but the effects of different fixation and preservation protocols – which we have been unable to resolve here – require further investigation. We have not considered in this paper any correlation of nematocyst size with polyp size, a very real consideration in zoanthids, but draw attention to the inconsistent patterns found in *Acrozoanthus australiae* (Ryland et al., 2003): polyp size must always be recorded. So far as species descriptions are concerned, size ranges (i.e. minimum to maximum) should be replaced, or at least supplemented, by calculated statistical parameters.

References

- Carlgren, O., 1940. A contribution to the knowledge of the structure and distribution of the cnidae in the Anthozoa. *Kungliga Fysiografiska Sällskapets i Lund Förhandlingar* 51: 1–62.
- Cassie, R. M., 1954. Some uses of probability paper in the analysis of size frequency distributions. *Australian Journal of Marine and Freshwater Research* 5: 513–522.
- Chintiroglou, C. & M. Simsiridou, 1997. Biometric investigations on the cnidae of the sea anemone *Actinia equina mediterranea* form I Schmidt, 1971. Proceedings of the Sixth International Conference on Coelenterate Biology, Nationaal Natuurhistorisch Museum, Leiden: 101–108.
- Cutress, C. E., 1955. An interpretation of the structure and distribution of cnidae in Anthozoa. *Systematic Zoology* 4: 120–137.
- England, K. W., 1987. Certain Actiniaria (Cnidaria, Anthozoa) from the Red Sea and tropical Indo-Pacific Ocean. *Bulletin of the British Museum (Natural History), Zoology* 53: 205–292.
- England, K. W., 1991. Nematocysts of sea anemones (Actiniaria, Ceriantharia and Corallimorpharia: Cnidaria): nomenclature. *Hydrobiologia* 216/217: 691–697.
- Fahmy, T. & A. Aubry, 2002. XLstat. Addinsoft, Paris.
- Fautin, D. G., 1988. Importance of nematocysts to actinian taxonomy. In Hessinger, D. A. & H. M. Lenhoff (eds), *The Biology of Nematocysts*. Academic Press, London: 487–500.
- Harding, J. P., 1949. The use of probability paper for the graphical analysis of polymodal frequency distributions. *Journal of the Marine Biological Association of the United Kingdom* 28: 141–153.
- Hidaka, M., 1992. Use of nematocyst morphology for taxonomy of some related species of scleractinian corals. *Galaxea* 11: 21–28.
- Pires, D. O. & F. B. Pitombo, 1992. Cnidae of the Brazilian Mussidae (Cnidaria: Scleractinia) and their value in taxonomy. *Bulletin of Marine Science* 51: 231–244.
- Rohlf, F. J. & D. E. Slice, 2002. *BIOMstat: Statistical Software for Biologists*. Applied Biostatistics, Port Jefferson, NY.
- Ryland, J. S. & J. E. Lancaster, 2003. Revision of methods for separating species of *Protopalythoa* (Hexacorallia: Zoanthidea) in the tropical West Pacific. *Invertebrate Systematics* 17: 407–428.
- Ryland, J. S., M. M. Brasseur & J. E. Lancaster, 2003. Use of cnidae in taxonomy: implications from a study of the cnidome of *Acrozoanthus australiae* (Hexacorallia: Zoanthidea). *Journal of Natural History* 38: 1193–1223.
- Schmidt, H., 1972. Die Nesselkapseln der Anthozoen und ihre Bedeutung für die phylogenetische Systematik. *Helgoländer Wissenschaftliche Meeresuntersuchungen* 23: 422–458.
- Schmidt, H., 1974. On evolution in the Anthozoa. Proceedings of the Second International Symposium on Coral Reefs 1: 533–560.
- Sokal, R. R. & F. J. Rohlf, 1995. *Biometry*. W. H. Freeman, San Francisco.
- Updegraff, G., 1990. *MeasurementTV*. DataCrunch, San Clemente.
- Weill, R., 1934. Contribution à l'étude des cnidaires et de leurs nematocystes. *Travaux de la Station Zoologique de Wimereux* 10–11: 1–702.
- Westfall, J. A., 1965. Nematocysts of the sea anemone *Metridium*. *American Zoologist* 5: 377–393.
- Williams, R. B., 1996. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria): statistical parameters and taxonomic relevance. *Scientia Marina* 60: 339–351.
- Williams, R. B., 1998. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria), II: further studies of differences amongst sample means and their taxonomic relevance. *Scientia Marina* 62: 361–372.
- Williams, R. B., 2000. Measurements of cnidae from sea anemones (Cnidaria: Actiniaria), III: ranges and other measures of statistical dispersion, their interrelations and taxonomic relevance. *Scientia Marina* 64: 49–68.
- Zar, J. H., 1996. *Biostatistical Analysis*. Prentice Hall, Upper Saddle River, NJ.