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A modern description of *Crambionella stuhlmanni* (Scyphozoa: Rhizostomeae) from St Lucia Estuary, South Africa

SIMONE NEETHLING[1], ALAN CHANNING[1], LISA-ANN GERSHWIN[2] AND MARK J. GIBBONS[1]

[1]Department of Biodiversity and Conservation Biology, University of the Western Cape, Private Bag X17, Bellville 7535, South Africa, [2]Queen Victoria Museum and Art Gallery, Launceston, Tasmania 7250, Australia

A new record of Crambionella stuhlmanni *is reported from the east coast of South Africa. The material is described using quantitative morphological data, and mitochondrial (CO1) and nuclear (ITS-1) sequence data. The species can be diagnosed by a combination of morphological features including the presence of conical projections on velar lappets, the absence of orbicular appendages among mouthlets and the short length of the terminal club on the oral arm. Mitochondrial sequence data unambiguously delineate* C. stuhlmanni *as a separate species from* C. orsini*, and phylogenetic analyses support its placement within the monophyletic genus,* Crambionella.

Keywords: Rhizostomeae, taxonomy, systematics, morphological analyses, molecular analyses

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INTRODUCTION

South Africa is bathed by the warm Agulhas Current on the east coast and by the cool Benguela Current along the west coast: it is an environment that is characterized by the presence of a number of clear biogeographical provinces characterized by distinct communities of marine organisms (e.g. Emanuel *et al.*, 1992; Gibbons *et al.*, 2010a). As a consequence, it supports a remarkably high diversity of marine life (Gibbons *et al.*, 1999). While the diversity of organisms associated with the benthos is substantially greater than that in the pelagos, the region typically supports a greater proportion of global species in the latter than former environments (Gibbons *et al.*, 2010a). But, whilst 57% of the world's planktic urochordates or euphausiids have been reported from the region only ~7% of the world's scyphozoans have been formally logged (Gibbons *et al.*, 1999). Given both their conspicuousness (most Scyphozoa are of a relatively large size and many bloom on a seasonal basis) and the fact that the region has been explored by many of the Great Expeditions, this is somewhat surprising.

Globally, however, our understanding of scyphozoan diversity is relatively poor and a total of only ~200 species have been described to date (Mianzan & Cornelius, 1999). This number is probably artificially low, given their meroplanktic nature (Gibbons *et al.*, 2010b), and likely reflects the conserved nature of medusoid morphology (Hamner & Dawson, 2009). Many of the original descriptions of scyphozoans are archaic, being based on a few subjective and qualitative diagnostic characters (Bolton & Graham, 2004; Dawson, 2005a), so the foundation on which our knowledge has been based is weak at best. To add to this confusion, some species are now known to display considerable phenotypic plasticity (Dawson *et al.*, 2001; Dawson, 2005a), and crypsis is becoming more widely reported (Dawson & Jacobs, 2001; Schroth *et al.*, 2002; Holland *et al.*, 2004; Dawson, 2004, 2005b). Although morphological descriptions are still essential when documenting diversity, there is a desperate need to revise them using more objective and quantitative features, supplemented where possible with molecular data (Dawson & Jacobs, 2001; Schroth *et al.*, 2002; Dawson, 2003, 2004, 2005b, c; Holland *et al.*, 2004; McManus & Katz, 2009). Here we describe a species of *Crambionella* (Scyphozoa: Rhizostomeae) from the St Lucia Estuary using just such an approach, in part to set a modern descriptive standard for the wider taxon and in part to allow identification of the present material.

The genus *Crambionella* is an inscapulate daktyliophore (Scyphozoa: Rhizostomae), with an intra-circular network of anastomosing canals that communicates with the ring canal (not stomach), occasionally with adjacent radial canals (Stiasny, 1922; Kramp, 1961). Lappets are separated by a deep furrow and are free of any anastomosing canals (Stiasny, 1922, 1937; Rao, 1931). This genus possesses three winged oral arms with terminal clubs, pyramidal in shape, lacking any whip-like filaments (Rao, 1931; Kramp, 1961). Three species are presently known, and all are confined to the Indian Ocean. *Crambionella orsini* (Vanhöffen, 1888) was first described from the Red Sea and is known to bloom seasonally in the north-west Indian Ocean (Billet *et al.*, 2006; Daryanabard & Dawson, 2008). *Crambionella stuhlmanni* (Chun, 1896) was originally described from the mouth of the Quilimane River, East Africa and *C. annandalei* Rao, 1931 was first identified from material collected off of the coast of Chennai (formerly Madras), India. The three species can be separated primarily on the basis of the presence of conical projections on velar lappets, accessory orbicular

Corresponding author:
M.J. Gibbons
Email: mgibbons@uwc.ac.za

mouth appendages on the oral arms as well as the proportion of terminal club length to oral arm length, and their respectively isolated geographical ranges within the Indian Ocean (Vanhöffen, 1888; Chun, 1896; Mayer, 1910; Stiasny, 1922, 1937; Menon, 1930; Roa, 1931; Ranson, 1945; Nair, 1946; Kramp, 1956, 1961, 1970).

MATERIALS AND METHODS

Forty-eight specimens of *Crambionella* (Figure 1) were collected by dip-netting from the St Lucia Estuary (28°0′0″S 32°30′0″E) during December 2005, and were preserved immediately in 5% ambient seawater–formalin. The St Lucia Estuary forms part of the iSimangaliso Wetland Park (UNESCO, 2008) which is a World Heritage Site situated on the north-east coast of South Africa. This estuarine system is the largest in Africa occupying an area of approximately 325 km² (Fielding *et al.*, 1991), and is made up of three main lakes with an average depth of less than 1 m (Anonymous, 2008) that connect to the sea through a 22 km long channel (100–200 m wide) (Cyrus *et al.*, 2010). The wetland park lies between tropical and sub-tropical climatic zones, and is typified by warm moist summers and mild dry winters; mean annual temperatures routinely surpass 21°C (Anonymous, 2008). It is prone to seasonal flooding in summer and periods of drought leading to temporary mouth closures and associated fluctuations in salinity during winter (Fielding *et al.*, 1991; Cyrus *et al.*, 2010; Jerling *et al.*, 2010). By comparison with many estuarine systems in South Africa, that of St Lucia is considered to be fairly well understood (Pillay & Perissinotto, 2008).

Morphological data collection

After 22 months in preservation, thirty-six morphological features were measured under magnification from 44 specimens (Table 1; Figure 2) using Vernier callipers. After all bell measures were taken, the oral arms were removed and the radial canal system was injected with coloured latex to highlight its arrangement. Five un-dissected specimens were deposited at the Iziko South African Museum, Cape Town: accession number SAMCT H5110.

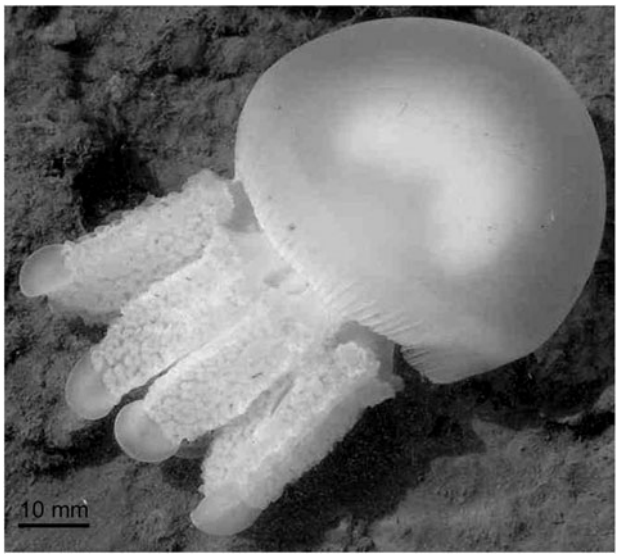

Fig. 1. A photograph of a live specimen from St Lucia Estuary (Ricky Taylor, Ezemvelo KZN Wildlife).

Comparisons were made between the measured variables of the St Lucia material and those of five good specimens of *Crambionella orsini* in the collections at the Natural History Museum, London (specimen numbers detailed below). Owing to the fact that this material had to be studied in a non-destructive way, not all measures could be repeated and discrepancies are indicated in Table 1.

Morphological data analyses

In order to determine the effect of individual size (external bell diameter, S1) on measured variables; Pearson's R correlations or Spearman's rank correlations (for those variables that failed tests of normality) were computed following $\log_{10}$ transformation of data (Zar, 1999). Test results were considered significant, following Bonferonni corrections for Type I errors (Quinn & Keough, 2002). Some morphological measures were expressed as proportions following Chun (1896), Mayer (1910), Menon (1930, 1936), Rao, (1931), Stiasny (1937) and Kramp (1961). These included: oral disc diameter (S 13) to external umbrella diameter (S1); length of the distal oral arm portion (S7) to length of the proximal oral arm portion (S6); length of terminal club (S11) to total oral arm length (S6 and S7); ostia width (S15) to inter-ostia width (S15) and umbrella height (S3) to external umbrella diameter (S1).

Given that many of the variables did change with individual size (see below), which complicates straightforward field comparisons, raw morphometric data were standardized by dividing them by external bell diameter (S1; except the aforementioned proportions), and $\log_{10}$ ratios were correlated with $\log_{10}$ size in an effort to eliminate size dependency. Comparisons between the standardized measurements of the St Lucia material and those of *C. orsini* were computed as above.

In order to test for morphological differences between individual logged ratios and between untransformed meristic measures, of the *Crambionella* specimens from the St Lucia Estuary and those of *C. orsini* two-tailed *t*-tests were computed, and the results inspected following Bonferroni corrections. All univariate statistical analyses were executed using STATISTICA v. 7.

In order to visualize and test for multivariate differences between the St Lucia material and that of *C. orsini* from the Natural History Museum, London, we have used a variety of the non-parametric routines within the PRIMER 6 & PERMANOVA+ software (Clarke & Gorley, 2006; Anderson *et al.*, 2008). A similarity matrix based on Euclidean distance was first constructed between the multivariate states (untransformed standardized measures) of all specimens. In order to maximize the number of individuals used, gaps were filled either by mean substitution (if there was no significant relationship of the considered feature with size) or from regression equations: invariant meristic features were excluded. Non-metric multi-dimensional scaling (NMDS) was used to visualize relationships in multivariate space (Clarke, 1993) and a one-way analysis of similarities (ANOSIM) test was used (*a priori*) to test the null hypothesis of no morphological dissimilarity between species (Clarke & Warwick, 2001). This latter routine computes an *R* statistic that measures the average distance between every specimen within a group and contrasts it to the average distance between every specimen from the other group. ANOSIM

Table 1. Morphological features (S #) of *Crambionella* specimens used in data analyses. Specimens were collected from St Lucia Estuary, on the north-east coast of South Africa, during December 2005 and preserved in 5% formalin in ambient seawater. Figure references are given where applicable. As *C. orsini* material examined at the Natural History Museum, London, had to be studied in a non-destructive way some measurements had to be excluded; measurements taken on *C. orsini* specimens are indicated (*).

Morphological feature number	Figure reference number	Morphological feature description (measured in mm)	Morphological feature number	Figure reference number	Morphological feature description (measured in mm)
S1*	–	External umbrella diameter to tip of lappets	S 19*	–	Width of oral pillars
S2*	–	External umbrella diameter to base of lappets	S 20	–	Internal umbrella diameter to tip of lappets
S3	Figure 2	Umbrella height	S 21	–	Internal umbrella diameter to base of lappets
S4	Figure 2	Umbrella thickness	S 22	Figure 2	Ring canal diameter
S5*	–	Width of oral arm base	S 23	Figure 2	Gonadal diameter along perradial axis
S6*	Figure 2	Length of the proximal (naked) portion of the oral arm	S 24	Figure 2	Gonadal diameter along adradial axis
S7*	Figure 2	Length of the distal portion (winged and terminal club) of the oral arm	S 25*	Figure 2	Number of velar lappets in octant
S8*	Figure 2	Depth of oral arm (including naked and ventral winged portion)	S 26	–	Number of conical projections on velar lappets
S9*	–	Depth of naked portion of oral arm	S 27*	–	Number of rhopalia
S10*	Figure 2	Depth of winged portion of oral arm	S 28	Figure 2	Number of rhopalial canals
S11*	Figure 2	Length of terminal clubs of oral arms	S 29	–	Point of termination for rhopalial canals
S12*	–	Width of terminal clubs of oral arms	S 30	Figure 2	Number of inter-rhopalial canals
S13*	Figure 2	Oral disc diameter	S 31	–	Point of termination for inter-rhopalial canals
S14*	Figure 2	Inter-ostia width	S 32	Figure 2	Number of anastomoses connecting with the ring canal
S15*	Figure 2	Width of ostia	S 33	Figure 2	Number of anastomoses connecting with adjacent inter- and rhopalial canals
S16*	–	Length of ostia	S 34	Figure 2	Number of anastomoses connections within the network
S17	Figure 2	Depth of oral pillars	S 35	Figure 2	Number of primary folds in each section of gonads
S18*	–	Length of oral pillars	S 36	Figure 2	Number of annular muscles

then performs a series (999) of permutation tests, wherein variables from each group (species) are randomly distributed between groups, and the *R* statistic is recalculated. If the original *R* statistic is more extreme than 95% of the permutations the null hypothesis is rejected at a level of $P < 0.05$.

In order to determine which of the variables contributed the most to dissimilarity between species, we used the similarity percentages (SIMPER) routine in PRIMER 6 (Clarke, 1993). SIMPER determines the average dissimilarity between all pairs of inter-group specimens (Clarke & Warwick, 2001). These averages are then disaggregated into the percentage that each variable contributes to overall dissimilarity amongst groups (Clarke & Warwick, 2001).

Finally, we used the canonical analysis of principal co-ordinates (CAP) routine in PRIMER 6 & PERMANOVA + , which is analogous to a discriminant functions analysis, in order to determine what percentage of St Lucia and *C. orsini* specimens were allocated to respective species groups. The CAP routine seeks a set of axes that best discriminates amongst *a priori* groups in multivariate space (Anderson *et al.*, 2008). Anderson *et al.* (2008) describe the processes executed within this routine. Numerous matrices are generated to produce a set of canonical axes. Conventionally in a canonical discriminant analysis a subset of principal co-ordinate (PCO) axes are chosen manually, based on the number of variables in the original data matrix. However, in the present study, as the number of standardized morphometric features approached the number of specimens, Anderson *et al.* (2008) suggest 'leave-one-out' diagnostics to determine the subset of PCO axes. The PCO axes determined are all orthonormal and are therefore independent of each other. Running parallel to this process is a matrix based on codes for groups identified by a factor associated with the Euclidean distance matrix, also orthonormalized. An additional matrix is then generated by relating the subset of PCO axes to an orthonormalized data matrix, yielding canonical eigenvalues and their associated eigenvectors which can be used to produce a CAP plot. These CAP axes, which are linear combinations of a subset

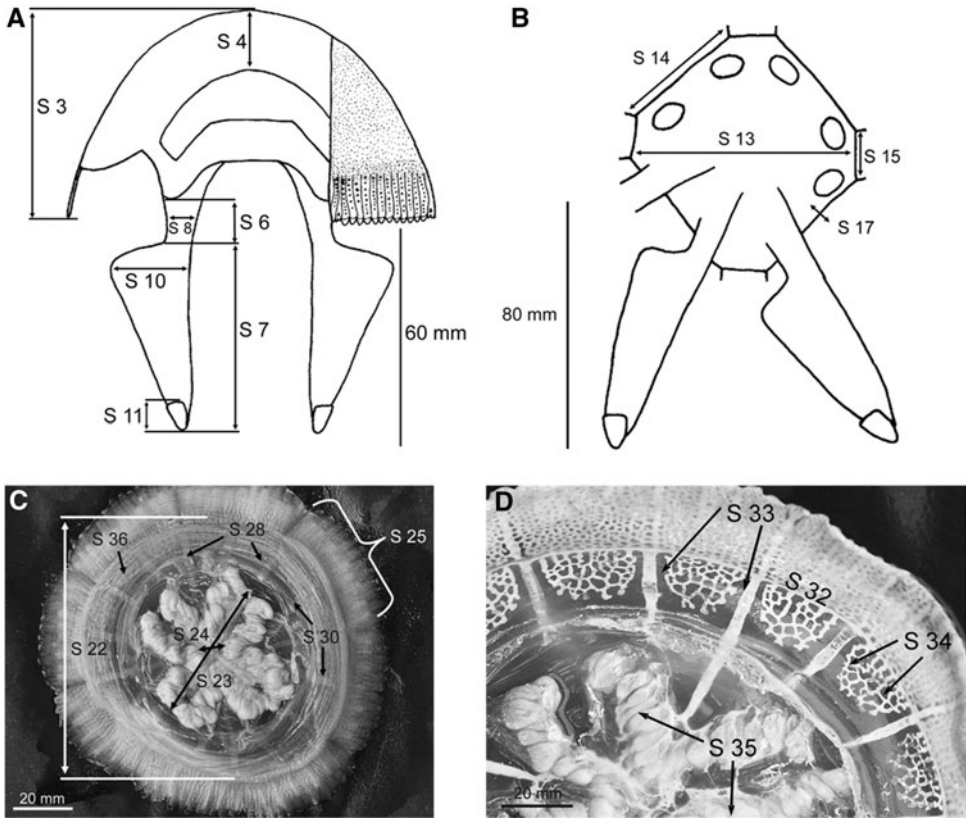

Fig. 2. Morphological features measured on *Crambionella* specimens collected from St Lucia Estuary, on the north-east coast of South Africa, during December 2005. (A) A schematic diagram of a longitudinal section along the perradial axis of a specimen (adapted from Dawson, 2005e); (B) a schematic diagram of the oral disc, from a subumbrella view (adapted from Dawson, 2005e). Only two of the eight oral arms are shown in A and B, and the key to all features (letters) measured should be sought from Table 1. Photographs showing the subumbrella view of a *Crambionella* medusa illustrating various morphological measurements (C) and the intra-circular and extra-circular anastomosing canal networks, after injecting coloured dye latex (D).

of orthonormal PCO axes, were used to determine if predefined groups were correctly classified. The CAP routine was also used to test the null hypothesis of no differences in the positions of centroids among groups in a multivariate space through a series of permutation tests (Anderson *et al.*, 2008). This routine makes no assumptions about the underlying distribution of variables rendering it suitable for non-parametric analyses (Anderson *et al.*, 2008).

DNA analysis

Three specimens of *Crambionella* were collected from the St Lucia Estuary at Charters Creek during September 2008, and immediately preserved in 99% ethanol. Material was stored at −20 °C prior to analysis in the laboratory.

DNA was extracted from ethanol-preserved oral arm tissues using a phenol–chloroform based method. Samples were placed in separate Eppendorf tubes. Extraction buffer (SDS 0.5%; 50 Mm Tris; 0.4 M EDTA; pH 8.0) in quantities of 0.5 ml were pipetted over each sample. Tissue samples were then macerated. Proteinase K (20 mg/ml) in quantities of 10 µl was then added. Samples were vortexed and incubated at 55°C for a minimum of three hours until the majority of protein was digested. Samples were then mixed with 500 µl phenol:chloroform:isoamyl alcohol (24:24:1), finger vortexed, then centrifuged at low speed (5000 × g) for 10 minutes. Supernatants were removed and placed in new Eppendorf tubes, mixed with 500 µl chloroform:isoamyl alcohol (24:1) and finger vortexed. Solutions were then centrifuged at low speed (5000 × g) for 10 minutes. Supernatants were removed and placed in new Eppendorf tubes. DNA was precipitated with 45 µl Na acetate and 650 µl of ice cold ethanol and left to incubate at −18°C overnight. Samples were then centrifuged at high speed (13000 × g) for 10 minutes and supernatants were discarded. Eppendorf tubes were inverted and left to air dry for a minimum of an hour. Each DNA sample was finally resuspended in 50 µl TE buffer.

Cytochrome *c* oxidase subunit I (COI) was amplified using primers LCOjf (5′-ggtcaacaaatcataaagatattggaac-3′) and HCOcato (5′-ctccagcaggatcaaagaag-3′) (Dawson, 2005d) or HCO2198 (5′-taaacttcagggtgaccaaaaaatca-3′) (Folmer *et al.*, 1994). Internal transcribed spacer one (ITS1) was amplified using the primers jfITS1-5f (5′-ggtttcgtaggtgaacctgcggaa ggatc-3′) and jfITS1-3r (5′-cgcacgagccgagtgatccaccttagaag-3′) (Dawson & Jacobs, 2001). Sequences were amplified through polymerase chain reaction (PCR) and PCR conditions were different for each fragment analysed. PCR conditions (adapted from Daryanabard & Dawson, 2008) are summarized in Table 2. PCR products were purified and sequenced at the Central Analytical Facility, University of Stellenbosch. Electopherograms were checked visually, misreads corrected and poorly resolved terminal portions of sequences were discarded using Sequencher 4.9. Forward and reverse sequences were then aligned, using default settings, in Sequencher 4.9. Sequence identifications were verified by BLAST in GenBank.

Phylogenetic analyses were utilized to examine family level relationships using COI rhizostome sequences received from Professor M.N. Dawson, also available on GenBank. The

Table 2. Polymerase chain reaction (PCR) conditions used to amplify cytochrome *c* oxidase subunit I (COI) and internal transcribed spacer one (ITS1) from *Crambionella* specimens collected from St Lucia Estuary, on the north-east coast of South Africa, during December 2005 and preserved in absolute ethanol (adapted from Daryanabard & Dawson, 2008).

Number of cycles	PCR steps	COI	ITS1
One	Initial denaturation	8 minutes at 94 °C	8 minutes at 94 °C
	Annealing	2 minutes at 49 °C	2 minutes at 51.5 °C
	Extension	2 minutes at 72 °C	2 minutes at 72 °C
One	Denaturation	4 minutes at 94 °C	4 minutes at 94 °C
	Annealing	2 minutes at 50°C	2 minutes at 52.5°C
	Extension	2 minutes at 72 °C	2 minutes at 72 °C
33	Denaturation	45 seconds at 94 °C	45 seconds at 94 °C
	Annealing	45 seconds at 51 °C	45 at 53.5 °C
	Extension	1 minute at 72 ° C	1 minute at 72 ° C
One	Final extension	5 minutes at 72 °C	5 a minutes t 72 °C
One	Final hold	4 °C	4 °C

following sequences were utilized: *Cassiopea andromeda* (samples from Bermuda, AY319463 and AY319465); *C. frondosa* (sampled from Panama, AY319469 and AY319470); *C. ornata* (sampled from Palau, AY31945); *C. ornata* (sampled from Berau, AY319472); *Mastigias papua* (sampled from Palau, EU363340 and AY902982); *M. papua* (sampled from Berau, AY903048 and AY903049); *Phyllorhiza punctata* (sampled from Australia, EU363341 and EU363342; *Catostylus mosaicus* (sampled from Australia, AY737184 and AY737216); *Crambionella orsini* (sampled from Iran, EU363343 and EU363344); *Cephea cephea* (sampled from Palau, EU363345; *C. cephea* (sampled from Kwajalein, EU363346); *Acromitus flagellatus* (sampled from India, EU363347 and EU363348) and *Nemopilema nomurai* (AB243416). *Aurelia aurita* (Ulmaridae) has been used as an outgroup, as the order Semaeostomeae are now recognized to form the subclass Discomedusae with Rhizostomeae (Collins, 2002; Dawson, 2004; Marques & Collins, 2004; Collins *et al.*, 2006). Sequence data for *A. aurita* (sampled from Korea) were downloaded from GenBank (EF010537). Prior to further analyses, all sequence lengths were edited in Sequencher 4.9. A parsimony analysis was performed under direct optimization in the program POY 4.1.1 (Varón *et al.*, 2009) which simultaneously optimizes nucleotide homology and tree costs, thereby reducing the set of assumptions throughout the analysis. Bootstrap analyses (1500 pseudoreplicates) were performed to assess support of branch nodes. Mean pairwise sequence differences, using uncorrected '*P*', distances were calculated in PAUP* 10.4b.

RESULTS

SYSTEMATICS
Order RHIZOSTOMEAE Cuvier, 1799
Suborder DAKTYLIOPHORAE Stiasny, 1921
Superfamily INSCAPULATAE Stiasny, 1921
Family CATOSTYLIDAE Gegenbaur, 1857
Genus *Crambionella* Stiasny, 1921
Crambionella stuhlmanni (Chun, 1896)
(Figures 1–5; Tables 1–6)

SYNONYMY

Crambessa stuhlmanni Chun, 1896: 10, figure 1, pl. I, figure 1; Stiasny, 1922: 50, figure 3.

Catostylus stuhlmanni Mayer, 1910: 669; *Crambionella stuhlmanni* Stiasny, 1921: 129; Stiasny, 1937: 237; Ranson, 1945: 319; Kramp, 1961: 374.

COMPARATIVE MATERIAL EXAMINED

Five preserved specimens of *C. orsini* were examined from the Natural History Museum, London, all collected on the 'Murray' expedition in 1933: 1950.3.25.343 (Station 76; 29 November 1933; 2 m tow net, 2800 m wire out); 1950.3.25.346 (Station 71; 26 November 1933; otter net, 106 m); 1950.3.25.347 (Station 70; 25 November 1933; otter net, 199 m); 1950.3.25.356 (Anchorage Muscat; 22 November 1933; Hand net, surface); 1950.3.25.357 (Station 75; 28 November 1933; otter net, 210 m).

DESCRIPTION

Umbrella between 62 and 181 mm in diameter (Table 3), finely granular, hemispherical or dome-shaped; margin cleft into narrow velar lappets, separated by deep furrows. Eight oral

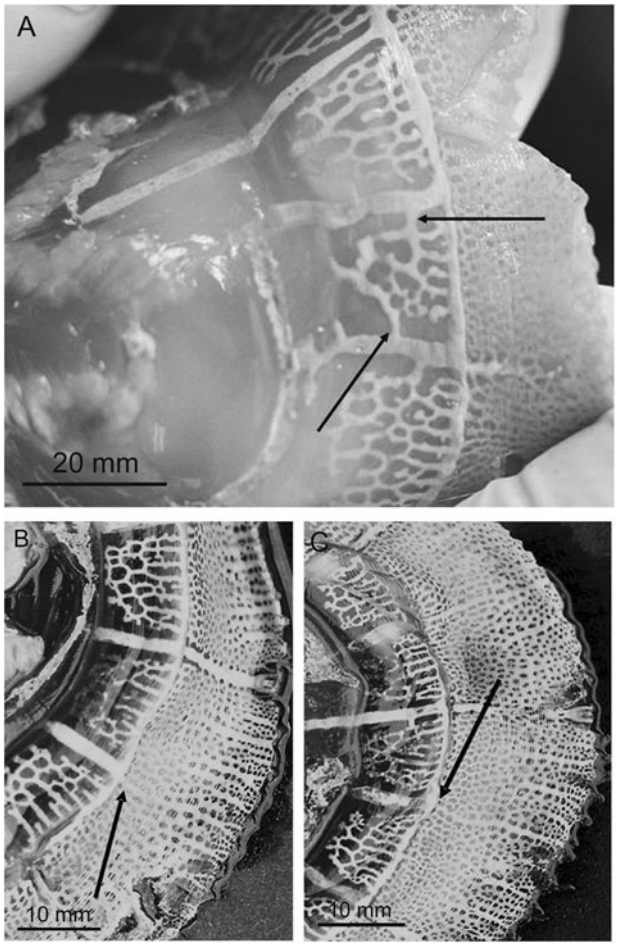

Fig. 3. Photographs showing the subumbrellar view of a *Crambionella* medusa collected in the St Lucia Estuary illustrating anastomosing canal network connections to both the rhopalial and inter-rhopalial canals, after injecting coloured dye latex (A) and inter-rhopalial canals that appear to extend beyond the ring canal (B, C). On closer inspection more than one canal originated from ring canal section and was thinner than canals that preceded the ring canal. Inter-rhopalial canals were therefore accepted to terminate at the ring canal.

Table 3. A summary of all *Crambionella* specimen measures from St Lucia Estuary and *C. osini* specimens examined from the Natural History Museum, London. Highlighted variables (bold typeface) are logged variables that were significantly correlated, using either Pearson's R ($P < 0.0014$, accepting Bonferonni correction) or Spearman's rank correlation ($P < 0.02$, accepting Bonferonni correction) test, with external bell diameter (S 1) in the case of the St Lucia specimens. Excluded measurements are S 1, S 2, S 29 and S 31 (indicated by †). Max, maximum; min, minimum.

Morphological feature	*Crambionella* (St Lucia)							*Crambionell orsini* (NHM)						
	Max	Min	Mean	SD	N	Median	Mode	Max	Min	Mean	SD	N	Median	Mode
S1†	181	62	119.53	29.75	38	121	135	165	114	147.6	19.53	5	152	152
S2†	158	54	96.79	24.70	38	98	102	144	100	132.8	18.46	5	139	139
S3	46.5	24.9	36.33	10.51	4	36.95	–	–	–	–	–	–	–	–
S4	29.4	4.7	13.90	6.17	44	11.8	11	–	–	–	–	–	–	–
S5	9.2	1	4.43	1.24	44	4.4	5	8	3.5	5.61	1.03	5	5.5	5
S6	19	1	9.35	3.13	44	10	11	14.5	5	9.27	2.78	5	8	8
S7	45.4	1.6	24.83	8.16	44	26.75	30	52	26	40.04	6.85	5	41	41
S8	19	3	10.66	3.09	44	11	11	17	8	12.07	2.26	5	12	12
S9	10	1.5	5.67	1.72	44	6	6	8.5	3.5	5.91	1.41	5	6	6
S10	29	3	15.19	4.26	44	15.6	15	26	12	18.85	3.68	5	18	17
S11	12.7	1	5.71	2.05	42	6	6	22	11	16.63	3.1	5	17	17
S12	11	1.4	5.79	1.78	42	6	5	15.5	1	11.06	2.84	5	11.75	13
S13	58.4	20.5	37.86	8.28	43	38.75	41	–	–	–	–	–	–	–
S14	26.4	7	15.62	4.40	44	15.4	17	26.5	17.5	22.81	2.99	4	24	25
S15	17	3.3	9.25	2.65	44	9	8	17.5	12	14.34	1.94	4	13.75	13
S16	11	2	4.47	1.24	44	4.1	4	6	3.5	4.79	0.72	3	6	5
S17	12	1	4.51	1.45	44	4.4	5	–	–	–	–	–	–	–
S18	25	6.4	16.52	3.71	44	16.9	19	19	7	11.65	2.85	5	11	8
S19	13	1	4.91	1.87	44	4.4	4	11	3	6.96	2.12	5	6.75	6
S20	122	40	86.66	20.68	38	24.9	78	–	–	–	–	–	–	–
S21	113.5	29.4	71.10	18.58	38	78.95	78	–	–	–	–	–	–	–
S22	90.5	28	63.59	15.68	43	63	50	–	–	–	–	–	–	–
S23	57	18	36.97	8.97	40	35.8	43	–	–	–	–	–	–	–
S24	12.6	1	5.61	1.99	40	5.2	6	–	–	–	–	–	–	–
S25	29	4	13.31	1.62	38	13	12	20	13	6.39	1.28	5	16	16
S26	19	1	10.18	3.36	34	10	12							
S27	10	6	7.91	0.75	34	8	8	8	8	8	0	5	8	8
S28	8	5	7.76	0.71	33	8	8	–	–	–	–	–	–	–
S29†	8	5	7.67	0.85	33	8	8	–	–	–	–	–	–	–
S30	8	5	7.72	0.77	32	8	8	–	–	–	–	–	–	–
S31†	8	8	8	0	32	8	8	–	–	–	–	–	–	–
S32	11	4	7.14	1.29	41	7	8	–	–	–	–	–	–	–
S33	4	0	0.22	0.37	41	0	0	–	–	–	–	–	–	–
S34	52	0	17.22	7.48	41	16	16	–	–	–	–	–	–	–
S35	33	6	18.15	4.17	39	18	19	–	–	–	–	–	–	–
S36	111	40	81.34	14.91	38	81.5	84	–	–	–	–	–	–	–
S3:S 1	0.37	0.28	0.32	0.03	4	0.32	–	–	–	–	–	–	–	–
S7:S 6	5.32	1.05	2.78	0.86	44	2.71	–	8.00	2.71	4.65	1.27	5	4.63	3
S13:S 1	0.41	0.24	0.32	0.04	37	0.32	–	0.44	0.24	0.33	0.09	5.00	0.28	–
S15:S 14	0.99	0.3	0.61	0.16	44	0.60	–	0.72	0.50	0.63	0.069	4	0.65	0.54
S11: oral arm length	0.243	0.1	0.17	0.04	42	0.17	–	0.44	0.24	0.34	0.053	5	0.65	0.31

arms, each divided into a naked proximal (ratio to bell diameter: 0.08; Table 4) and a three-winged distal (ratio to bell diameter: 0.21; Table 4) portion, latter almost three times longer than former (mean: 2.78, SD: 0.86; Table 3); distal portion with one adoral and two aboral rows of mouthlets and club-shaped appendages, adoral row originating proximal to and terminating distad of the two aboral rows; terminating in a naked pyramidal club, proportion of terminal club length to oral arm length low (mean: 0.17, SD: 0.04; Table 3). In life, exumbrella transparent-white; oral arms transparent-white, bearing light-brown mouthlets and appendages; terminal clubs transparent-white; gonads cream. In preservation, they are all transparent-cream.

The canal system with a continuous ring canal, and four perradial and four inter-radial canals extending to umbrella margin and eight adradial canals terminating at the ring canal. Intra-circular network of anastomosing canals originating from ring canal (~7 connections with the ring canal; Table 3), no communication with the gastric cavity except occasionally through the perradial and inter-radial canals (0.22 connections with adjacent radial canals; Table 3 & Figure 3), less dense (~17 connection points within intra-circular network; Table 3) than that of the extra-circular network, which does not extend into lappets.

Eight rhopalia (range: 6–10; Table 3), situated in pits with radiating furrows, flanked laterally by ocular lappets that are smaller than, and slightly dorsal to, velar lappets. Twelve velar lappets per octant (range 4–29; Table 3), each with a row of small conical projections (mode: 12, range: 1–19; Table 3) mid-dorsally. There are approximately 84 (range 40–111; Table 3) annular muscles on subumbrellar surface. Four crescent shaped ostia lead from the gonadal and gastro-vascular cavity; ostial and inter-ostial widths approximately equal (mean: 0.61, SD: 0.16; Table 3). Gonads at the time of sampling were either immature and thin or mature and plump. Maturity in specimens was reached when external bell diameter reached ~100 mm. Of the 48 medusae examined 26 had developed gonads.

VARIATION

The majority of the meristic measurements taken were found to be size dependant (Table 3), although some were not. These features are highlighted in Table 3, as they can be useful in species-level comparisons. Adradial canals sometimes appear to extend beyond the ring canal but on closer inspection extensions are thinner, and seem to be more subdivided than those of the rhopalial canals (Figure 3).

REMARKS

A comparison of the key morphological and meristic features that can be used to distinguish the three recognized species of *Crambionella*, together with the appropriate data from this study are shown in Table 5. From this it can be seen that the number of velar lappets in each octant of the present material was similar to that of *C. stuhlmanni* (Chun, 1896). The presence of conical projections on the dorsal median line of each lappet was also consistent with observations for *C. stuhlmanni*, and this feature can be used to distinguish the material from *C. orsini* (Menon, 1930, 1936; Stiasny, 1937) but not from *C. annandalei* (Rao, 1931; Stiasny, 1937). However, the high ratio of terminal club length to the oral arm length as well as the ratio between distal winged portion and naked proximal portion of the oral arm separate *C. annandalei* (Menon, 1930; Rao, 1931) from the present material. Both *C. annandalei* and *C. orsini* possess accessory orbicular mouth appendages (Rao, 1931; Menon, 1936; Stiasny, 1937; Kramp, 1961), which both the present material and *C. stuhlmanni* lack (Stiasny, 1922; Kramp, 1961).

Although meristic differences (Table 3) between the present material and *C. orsini* are pronounced enough to allow ready separation, and generally agree with the literature (see Table 5) (Vanhöffen, 1888; Chun, 1896; Mayer, 1910; Stiasny, 1922, 1923, 1937; Menon, 1930, 1936; Rao, 1931; Ranson, 1945; Nair, 1946; Kramp, 1956, 1961, 1970), there are also clear differences in some of the standardized morphometric data (Table 4).

Table 4. Summary of all ratios (morphological features divided by external bell diameter) of *Crambionella* specimens from St Lucia Estuary and *C. orsini* specimens examined from the Natural History Museum (NHM), London. Highlighted (bold typeface, left-hand side column) are those logged ratios that were significantly correlated with external bell diameter ($P < 0.05$, accepting Bonferonni correction) in the case of the St Lucia material. Excluded standardized measurements included in correlation analyses S2 and S3 as well as all proportions and meristic data except S35 and S36 (indicated by †). Highlighted *P*-values (right-hand side column) are the standardized variables that differed significantly ($P < 0.05$, accepting Bonferonni correction) between *C. orsini* and the specimens of *Crambionella* from the St Lucia Estuary, as determined using two-tailed *t*-tests.

Morphological feature	*Crambionella* (St Lucia)			*Crambionella orsini* (NHM)			*P*
	Mean	SD	N	Mean	SD	N	
S3†	0.03	0.09	4	–	–	–	–
S4	0.10	0.05	38	–	–	–	–
S5	0.04	0.01	42	0.04	0.01	5	0.55
S6	0.08	0.02	42	0.06	0.01	5	0.31
S7	0.21	0.04	42	0.27	0.01	5	0.01
S8	0.09	0.03	42	0.08	0.01	5	0.51
S9	0.05	0.01	42	0.04	0.01	5	0.17
S10	0.13	0.02	42	0.13	0.02	5	0.52
S11	0.05	0.02	40	0.11	0.01	5	**<0.0025**
S12	0.05	0.01	40	0.07	0.01	5	**<0.0025**
S13	0.28	0.11	37	0.33	0.09	5	0.33
S14	0.13	0.02	42	0.12	0.07	5	0.009
S15	0.08	0.02	42	0.08	0.05	5	0.04
S16	0.04	0.01	42	0.02	0.02	5	0.23
S17	0.04	0.01	42	–	–	–	–
S18	0.12	0.01	42	0.08	0.01	5	**<0.0025**
S19	0.04	0.01	42	0.05	0.01	5	0.03
S20	0.54	0.19	38	–	–	–	-
S21	0.66	0.23	38	–	–	–	–
S22	0.49	0.17	38	–	–	–	–
S23	0.26	0.13	34	–	–	–	–
S24	0.04	0.03	34	–	–	–	–
S25†	0.11	0.05	38	0.11	0.02	5	0.38
S26†	0.07	0.04	34	–	–	–	–
S27†	0.06	0.03	37	0.06	0.01	5	0.27
S28†	0.06	0.03	37	–	–	–	–
S29†	0.06	0.03	34	–	–	–	–
S30†	0.05	0.03	36	–	–	–	–
S31†	0.07	0.02	42	–	–	–	–
S32†	0.06	0.02	38	–	–	–	–
S33†	0.00	0.00	16	–	–	–	–
S34†	0.13	0.07	38	–	–	–	–
S35	0.13	0.08	33	–	–	–	–
S36	0.65	0.28	38	–	–	–	–
S3:S 1†	–	–	–	–	–	–	–
S7:S 6†	–	–	–	–	–	–	**<0.0025**
S13:S 1†	–	–	–	–	–	–	0.90
S15:S 14†	–	–	–	–	–	–	0.77
S11: oral arm length†	–	–	–	–	–	–	**<0.0025**

Table 5. A character matrix highlighting morphological features that differ among the three *Crambionella* species. (Vanhöffen, 1888; Chun, 1896; Mayer, 1910; Stiasny, 1922, 1923, 1937; Menon, 1930, 1936; Roa, 1931; Ranson, 1945; Nair, 1946; Kramp, 1956, 1961, 1970) and the *Crambionella* material under investigation. Recorded geographical ranges are also given for all species.

Feature	*C. orsini*	*C. annandalei*	*C. stuhlmanni*	*Crambionella stuhlmanni*—present study
Umbrella diameter	55–210 mm	80–200 mm	80–200 mm	62–181 mm (Table 3)
Proportion of umbrella height to umbrella diameter	0.3	0.3	0.3–0.5	Mean: 0.32 ±0.03 (Table 3)
Number of velar lappets in each octant	16	14	12	Mode: 12; range: 4–29 (Table 3)
Conical projections on velar lappets	Absent	Present	Present	Present
Number of conical projections	–	14–16	15–18	Mode: 12; range: 1–19 (Table 3)
Proportion of oral disc to external umbrella diameter	0.5–0.6	≤0.5	0.5	Mean: 0.32 ±0.04 (Table 3)
Accessory orbicular mouth appendages on distal winged portion	Present	Present	Absent	Absent
Proportion of distal winged portion to naked proximal portion	Three to four times as long	More than six times as long	Two to three times as long	Mean: 2.78 ±0.86 (Table 3)
Proportion of terminal club length to oral arm length	0.125	0.5	0.33	Mean: 0.17 ±0.04 (Table 3)
Proportion of ostia to inter-ostia width	1/3–1/2 as wide as inter-ostial columns	1/2 as wide as inter-ostial columns	1/4–1/3 as wide as inter-ostial columns	Mean: 0.61 ±0.16 (Table 3)
Inter-rhopalial canals termination	Ring canal	Ring canal	Ring canal	Ring canal
Number of intra-circular anastomosing canals connected to ring canal	Rare	Rare	Rare	Rare (Table 3)
Intra-circular anastomosing canal connections to inter-rhopalial or rhopalial canal	Inter-rhopalial canals	–	Rhopalial canals	Connections to both inter- and rhopalial canals (Figure 3)
Geographical range	South-west and south-east coast of India, Krusadai Islands, Persian Gulf to Red Sea and Kenya to Seychelles Islands	Bay of Bengal and Andaman Islands	Along the coasts of Mozambique and Madagascar	St Lucia Estuary, South Africa

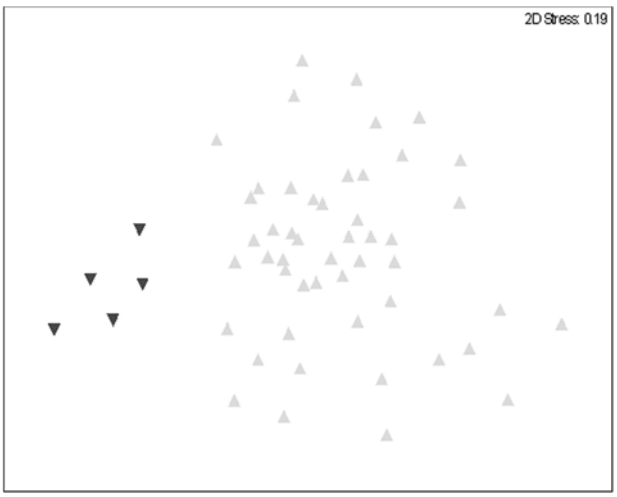

Fig. 4. Non-metric multi-dimensional scaling (NMDS) of standardized morphometric data illustrating the morphological dissimilarity between *Crambionella* medusa collected in the St Lucia Estuary (grey) and *C. orsini* (black) examined at the Natural History Museum, London. Stress value is indicated.

The results of the NMDS analysis (Figure 4) show that the two species are well separated, and even though the stress value is relatively high, the CAP was able to successfully categorize all specimens into the correct group (the permutation test results were significant: $P < 0.001$). For the canonical procedure a subset of three PCO axes were used based on the 'leave-one-out' diagnostics, which accounted for 66.74 % of the total variation in the species data. The first squared canonical correlation (δ_1^2) was high: 0.56. Similar results were also obtained from the ANOSIM, where the Global *R* value of 0.67 was highly significant ($P < 0.001$). The variables contributing to the dissimilarities between species are highlighted in Table 6, foremost of which are the various characteristics of the oral arm.

For cytochrome *c* oxidase subunit I (COI) a maximum length of 660 nucleotides was amplified from three *Crambionella* specimens sampled in the St Lucia Estuary (GenBank accession numbers HM348770–HM348772) and compared to two *C. orsini* specimens (sequences downloaded from GenBank, accession numbers: EU363341 and EU363342). DNA sequence data from COI showed an average of 11.84% pairwise sequence difference between the material examined in this study and *C. orsini*. Dawson & Jacobs (2001) suggest that differences of

Table 6. Standardized morphometric data that contributed most to the dissimilarity between *Crambionella* material collected from the St Lucia Estuary and *C. orsini* specimens examined at the Natural History Museum, London, as determined by SIMPER analysis.

Morphological feature	Contribution %	Cumulative %
Length of the terminal club	24.10	24.10
Terminal club length: total oral arm length	17.89	41.99
Length of the distal portion of the oral arm: length of proximal portion of the oral arm	12.77	54.76
Width of terminal club	6.78	61.54
Length of oral pillar	5.81	67.35
Length of distal portion of oral arm	4.13	71.48
Length of proximal portion of oral arm	4.11	75.60
Width of ostia	3.20	78.80
Oral disc diameter	2.81	81.61
Length of ostia	2.76	84.37
Width of oral pillar	2.53	86.91
Ostia width: inter-ostia width	2.49	89.40
Depth of naked portion of oral arm	2.01	91.41

10–20% between COI sequences set the standard for species level divergence. Phylogenetic analyses computed using COI sequence data demonstrate a monophyletic *Crambionella* clade (Figure 5). The consensus tree was supported by generally high bootstrap values, except at the branch that illustrated Catostylidae to be paraphyletic to the other rhizostome families represented. This is in contrast to previous molecular phylogenetic analyses executed on rhizostomes using COI (Daryanabard & Dawson, 2008) and future work is needed to verify the findings in the present study. For internal transcribed spacer one (ITS1) a maximum length of 335 nucleotides was amplified from two *Crambionella* specimens sampled in the St Lucia Estuary (GenBank accession numbers HM348773 and HM348774); no comparative data for *C. orsini* were available.

Although on balance the material most closely resembles *C. stuhlmanni*, which is in agreement with its geographical distribution (Table 5), there was one feature at odds with previous descriptions. In the present specimens the intra-circular anastomosing canal network sometimes connected to both the rhopalial and inter-rhopalial canals (Figure 3), whilst in the original descriptions the anastomosing canals were only connected to rhopalial canals (Stiasny, 1922). It is unlikely that these discrepancies reflect erroneous observations on the part of Stiasny; but rather it is probable that previous descriptions overlooked this rare feature due to small sample sizes examined. Scyphozoans often display considerable intra-specific morphological variation between geographically isolated or separated populations (Brewer, 1991; Bolton & Graham, 2004; Dawson, 2005a). Morphological variation is often as a result of phenotypic plasticity, a response to variable environmental conditions (Dawson *et al.*, 2001; Bolton & Graham, 2004). Dawson (2005b) highlights the importance of thorough geographical sampling, in combination with adequate sample sizes (as

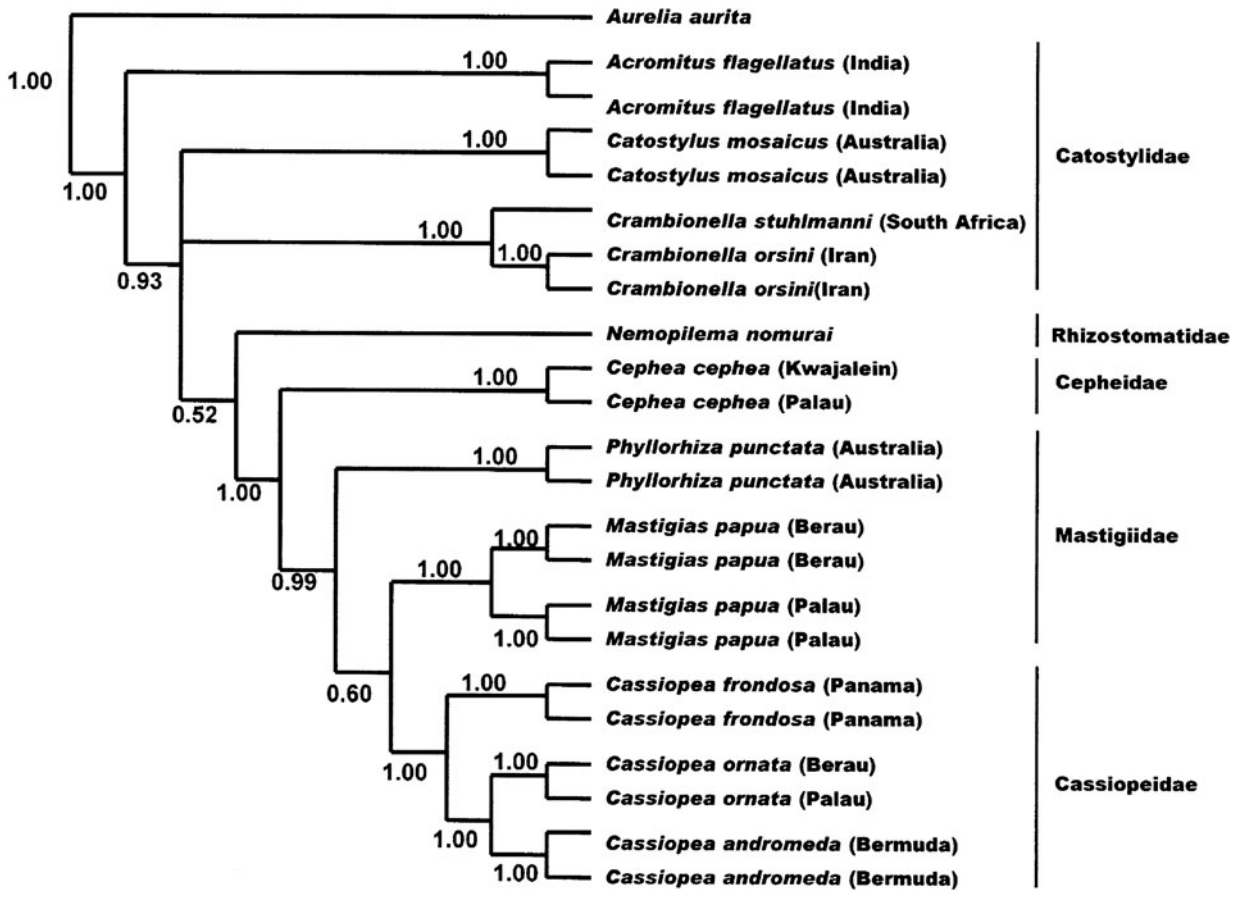

Fig. 5. A consensus tree of Rhizostomeae (original sequence data received from Professor M.N. Dawson), and outgroup, based on 474 nucleotides from cytochrome *c* oxidase subunit I (COI). Analysed by direct optimization in POY. Bootstrap values are indicated.

observed in this study), to get a more accurate representation of morphological variation.

Molecular analyses are increasingly being used in scyphozoan systematics (Dawson & Jacobs, 2001; Schroth *et al.*, 2002; Dawson, 2003, 2004, 2005a, b, c, d, e; Holland *et al.*, 2004) and the decision about whether to use molecular or morphological analyses when describing species is subject to much debate (Dawson, 2005f). Molecular data increase the number of objective characters used, which enhances the likelihood of distinguishing taxa and permits phylogenetic reconstruction, free of impractical or inappropriate morphological features (Dawson, 2004). However, in some studies molecular analyses have failed to differentiate groups that showed significant morphological, behavioural and physiological differences (Dawson, 2005a). An approach which combines all data available is therefore required in scyphozoan systematics (Dawson, 2003, 2005f). Although this study did not utilize ecological or behavioural data, integrating molecular and morphological data is an important stepping stone for future work on this species.

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Correspondence should be addressed to:
M.J. Gibbons
Department of Biodiversity and Conservation Biology
University of the Western Cape
Private Bag X17
Bellville 7535
South Africa
email: mgibbons@uwc.ac.za