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Morphological variation and systematics in the Scyphozoa: *Mastigias* (Rhizostomeae, Mastigiidae) – a golden unstandard?

Michael N Dawson
Department of Ecology and Evolutionary Biology, University of California, Los Angeles, USA; Coral Reef Research Foundation, Koror, Palau; Present address: Biological, Earth, and Environmental Sciences, University of New South Wales, Sydney, Australia (Tel.: +61-2-9385-3446; Fax: +61-2-9385-3450; E-mail: mndawson@unsw.edu.au)

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Abstract

Vagarious descriptions of species boundaries in jellyfishes have been attributed to inconsistent phenotypic variation between individuals, size-classes, populations, and species. However, the historical predominance of subjective and largely qualitative analyses of geographic variation has made it difficult to know where, if not in the analyses themselves, the real problems lie. Statistical analyses of morphological variation provide more objective and quantitative datasets. They also can be integrated with, for example, molecular genetics, geography, and paleoclimatology to provide an evolutionary perspective on biodiversity. Here, I illustrate some of the benefits of integrative statistical analyses of morphological variation in the golden jellyfish, *Mastigias* L. Agassiz, that inhabit lagoon and marine lake ecosystems in Palau, Micronesia. The morphology of *Mastigias* varies considerably between medusae, size-classes, populations, and environments and, although medusae generally showed location-specific morphologies, none of the variable features measured diagnosed all medusae from any location. DNA sequence data from cytochrome *c* oxidase subunit I and internal transcribed spacer one showed little variation and also did not reliably distinguish medusae from different locations. These results are consistent with post-glacial changes in sea-level and topography that suggest recent evolution of marine lake populations from an ancestral lagoonal form. Remarkably, many morphological features show greater variety in *Mastigias* in Palau than in all other members of the genus described from eastern Africa to the tropical South Pacific. Their morphological similarity, however, may mask considerable genetic divergence, as is the case for lagoonal forms in Palau and Papua New Guinea. There is, therefore, considerable heterogeneity in evolutionary process and morphological variation may be decoupled from variation in commonly used molecular markers. These results contribute to our understanding of inconsistencies in the taxonomy of scyphozoans and confirm that there is no widely applicable taxonomic standard for divining species. An evolutionary approach, however, provides a diverse set of tools for satisfactorily interpreting geographic variation for systematic purposes.

Introduction

Biodiversity in marine invertebrates has proven difficult to assess accurately using traditional morphological methods. Major problems include a dearth of characters in many taxa, 'rampant homoplasy' (i.e. widespread convergent or parallel evolution), ontogenetic variation, and phenotypic plasticity (e.g. Kramp, 1968; Gosliner & Ghiselin, 1984; Knowlton, 1993; Hamner, 1995; Féral, 2002; Finlay, 2002). These problems have been compounded by technical limitations on the observation, sampling, and culturing of marine organisms and our generally poor comprehension of the

marine environment (Hamner, 1985; Benzie, 2000; Vecchione et al., 2001; Kaeberlein et al., 2002). Moreover, a dearth of explicit, objective, and quantitative morphological analyses has made it difficult to know for sure where, if not in the analyses themselves, the real problems lie (Sneath & Sokal, 1973; Wiens, 2000; Dawson, 2003).

Molecular genetics has helped to clarify these issues. DNA sequencing, for example, has provided access to thousands rather than tens (or fewer) of characters, enabling the reconstruction of better resolved and more robust phylogenies (Hillis & Wiens, 2000). Molecular data have offered independent datasets and alternative hypotheses to phylogenetic trees potentially affected by adaptive morphological features or ontogenetic variation (e.g. Streelman et al., 2002). Molecular analyses also have indicated cases of phenotypic plasticity mistaken for species-level differences and, conversely, morphospecies mistaken for ecophenotypes (Miller et al., 2001; Finlay, 2002). The growing evidence for a multitude of cryptic marine species, even in some of the best studied macro-zooplankton and macro-benthos, is probably one of the most widely appreciated outcomes of molecular analyses of marine taxa (Knowlton, 1993, 2000; Féral, 2002). However, molecular evidence of cryptic species also has demonstrated that, *a posteriori*, many 'cryptic' species can be distinguished morphologically (Knowlton, 2000; e.g., Miller et al., 2001; Dawson, 2003). The implication is that earlier, often more qualitative and subjective, approaches to morphological taxonomy have, in many cases, been a significant source of systematic error.

Thus, there is a need for not only more widespread integration of molecular and morphological approaches, but also the application of improved morphometric methods – ones that are explicit, objective, and preferably quantitative (Poe & Wiens, 2000) – that will enable more reliable description and dissection of morphological variation (or the lack thereof). Elsewhere, I have integrated molecular data with statistical analyses of morphological data to differentiate cryptic species of the semaeostome moon jellyfish, *Aurelia* Péron and Lesueur (Dawson, 2003). Here, I use a similar approach to examine patterns of morphological variation in the rhizostome golden jellyfish, *Mastigias* L. Agassiz, that inhabit lagoon and marine lake ecosystems in Palau, Micronesia.

Materials and methods

Morphological data collection

Mastigias medusae (putatively *M. papua* L. Agassiz: see Uchida, 1947; P. F. S. Cornelius, pers. com. and in Massin & Tomascik, 1996; see also Tomascik et al., 1997, p. 779) were collected from six marine lakes and five lagoon locations between May 1996 and October 1998 (Table 1). Physical and biological attributes of the lake and lagoon environments are described elsewhere (e.g. Hamner, 1982; Hamner et al., 1982; Hamner & Hamner, 1998; Dawson et al., 2001; Dawson & Hamner, 2003). At each location, medusae with four gastric pouches were dipped from the water by hand and carefully transferred to a flat measuring tray. Medusae falling within three size categories, 50 ± 5 mm, 100 ± 5 mm, and 150 ± 5 mm bell diameter (between distal tips of opposed interradial rhopalia), were quickly transferred to buckets of native water and transported immediately to the Coral Reef Research Foundation, where they were placed in temporary aquaria containing ambient salinity water. Ten medusae per size class were taken from each location if possible, but small population sizes, patchy occurrence, and size variation limited collections to fewer individuals or size-classes in some populations. Two small medusae (10–20 mm) collected from OLO and reared to 100 mm in an aquarium (Dawson, 2000) were also taken for morphological analyses. Data describing 40 quantitative and qualitative meristic and morphometric features (denoted *f*#) of the medusae were collected, usually within several hours and always within 1 day of collection (Fig. 1a–f). *f*1–*f*7 were measured while medusae were in a temporary holding tank. They were then removed, drained, weighed (*f*8), and placed exumbrella surface down on a flat surface and identified as female or male. *f*9–*f*15 were measured on four oral arms, the oral arms amputated, then *f*16–*f*19 measured on two oral pillars or ostia. The oral disk was removed and *f*20–*f*28 measured; *f*20 across both perradial axes, *f*23 on

Table 1. The locations of *Mastigias* populations studied in Palau, and the times and sizes of samples

Location	Island	Period	Sample size (canals)		
			50 mm	100 mm	150 mm
Lake					
Big Jellyfish Lake (BJLK)	Koror[a]	May–December 1996	10 (9)	10	no
Goby Lake (GLK)	Koror[a]	May 1996–December 1997	10 (5)	10	10
Clear Lake (CLM)	Mecherchar	September–October 1998	10	10	10
Ongeim'l Tketau (OTM)	Mecherchar	March–April 1996	10	10 (9)	10
Intermediate lakes					
Ongael Lake (OLO)	Ongael	May 1997–October 1998	10 (6)	10	7
Tketau Lake (TLM)	Mecherchar	May 1997	3	ns	ns
Lagoon					
Big Jellyfish Cove (BJCK)	Koror[a]	May 1996	2 (0)	2 (0)	4 (2)
Risong Cove (RCA)	Auluptagel	May–June 1997	7 (7)	9 (5)	4 (3)
Ngerchaol Cove (NCN)	Ngerchaol	May 1996–June 1997	8 (6)	2	2
Omodes Lake (OLA)	Auluptagel	May 1996	ns	1 (0)	ns
Open lagoon (Lgn)	Koror[b]	May–June 1997	2	ns	ns

[a] Ngermeuangel; [b] M-dock and Malakal. BJLK also known as Uet era Ngermeuangel.
no, never observed in this lake; ns, not sampled as not present at time of collections.
For the purposes of this study, a lake is defined as a body of water that is entirely surrounded by land and impenetrable by free-diving through any large submerged conduit. The biota of lagoon locations, including Omodes Lake which is easily penetrable, includes corals and other typical reef fauna. Lake locations typically are more reminiscent of mangrove habitats. Tketau and Ongael lakes are intermediate.

four adjacent octants when possible, and all other features just once per medusa. The radial canal system of the medusa was injected with food dye and photographed and the resulting picture used to enumerate $f29$–$f40$. Anastomoses of apparently $\geqslant 4$ canals (c) were therefore interpreted as comprising $c-2$ anastomoses separated by zero-length branches. The highly anastomosed canals marginal to the circular canal were too complex to enumerate reliably and were excluded. A detailed protocol is available from *The Scyphozoan* website.

A subset of measurements, of features previously considered characteristic of species of *Mastigias* (e.g. Mayer, 1910, Kramp, 1961), were made on two formalin-fixed medusae from Tufi, Papua New Guinea. For comparison, the same measurements were made on two formalin-fixed medusae of similar size from RCA, Palau. These medusae were not included in the following morphological data analyses, but are compared subsequently in tabular form.

Morphological data analyses

Characters and character-complexes were identified from the 40 features in six stages. (1) Sexually dimorphic characters, identified by paired t-test or Wilcoxon signed ranks test of twenty five 100 mm and sixteen 150 mm medusae, if any, were excluded. (2) Invariable features, i.e. continuous features with statistically indistinguishable variance and mean or categorical features with no differences in the frequency of states, were excluded. (3) Correlations were identified among continuous features by Spearmans rank correlation and by contingency analyses for all other comparisons and classed as either (4) logical or partial logical (*sensu* Sneath & Sokal, 1973) or, by exclusion, (5) meristic. (6) Features that were not correlated in all size-classes were interpreted as independent characters (Zelditch et al., 2000), other features were grouped in character complexes. At each stage, as appropriate, it was first confirmed that bell diameters were statistically

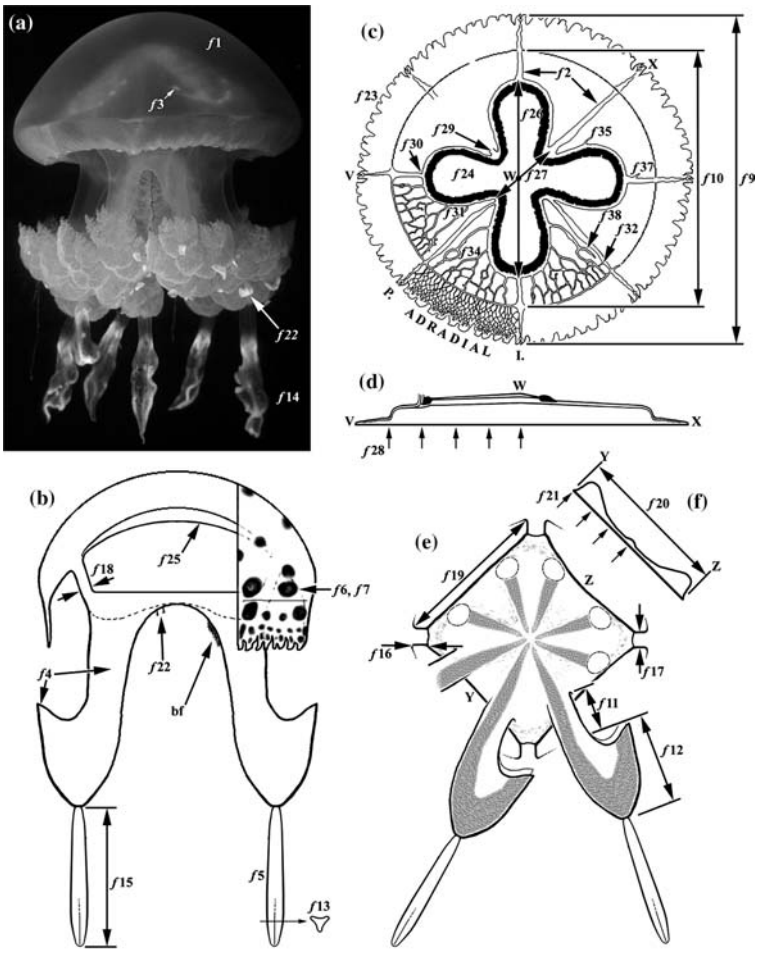

Figure 1. The morphology of *Mastigias*. (a) Photograph of an approximately 15 cm [bell diameter] medusa from Goby Lake. (b) Sketch of a longitudinal section along the interradial axis of the medusa [i.e. through the plane of the paper after rotation of the medusa in A through approximately 30° to the left]. (c) Sketch of the subumbrellar surface of the bell, and (d) cross-section through the bell along line VWX. (e) Schematic of the oral disc, in oral aspect, showing two of the eight oral arms, and (f) cross-section through the oral disc along line YZ or its orthogonal. Features discussed in the text include the following. Bell colour (*f*1). Perradial and interradial canal colour (*f*2). The presence of pigmented flecks at the roots or along the length of radial canals (*f*3). Oral arm colour (*f*4; lower arrow points to the 'fringe'). Terminal club colour (*f*5). Abundance (*f*6) and colour (*f*7) of spots on the exumbrellar surface. Bell diameter (*f*9). Ring canal diameter (*f*10). Length of the simple, unwinged, portion of the oral arm (*f*11). Length of the winged portion of the oral arm (*f*12). Shape (*f*13), number (*f*14), and length (*f*15) of the terminal clubs. Length (*f*16), width (*f*17), and depth (*f*18) of the oral pillars. Width of the subgenital ostia (*f*19). Diameter (*f*20) and depth (*f*21) of the oral disc (Uchida, 1926; a.k.a. 'arm disk' of Mayer [1910] and Kramp [1961]). Presence of intermediate filaments on the oral arm and oral disc (*f*22). Number of velar lappets [i.e. all lappets bar the two at each rhopalium] (*f*23). Shape of the gastrovascular cavity (*f*24). Colour of the subgenital porticus (*f*25). The interradial (I. *f*26) and perradial (P. *f*27) diameters of the gastrovascular cavity. Bell thickness (*f*28). The number of perradial (*f*29), interradial (*f*30), and adradial (*f*31) canal origins at the gastrovascular cavity. The number of perradial (*f*32) and adradial (*f*34) anastomoses in the radial canals that are circumscribed by the ring canal. The number of sinuses originating at the gastrovascular cavity (*f*35), and interradial (*f*37) and adradial (*f*38) canals. Radial canals are named consistent with the phylogenetic hypothesis of Uchida (1926, p. 87). Five features are not indicated in the figure: the mass of the whole medusa (*f*8), the number of interradial anastomoses (*f*33), the number of sinuses originating at perradial (*f*36) and ring canals (*f*39), and the number of anastomoses leading to two sinuses (*f*40). bf, brood filaments which are found only on mature female medusae.

similar between all samples (using analysis of variance [ANOVA] or the *t*-test) and then that datasets were normally distributed with homogeneous variances (using Lilliefors' and Levene's tests, respectively), which was most often the case. Datasets that did not meet these basic parametric statistical assumptions were excluded from further analyses, used in tests not reliant on such assumptions, or were interpreted conservatively (Underwood, 1997, p. 194) to reduce the risk of Type I error.

Multivariate analyses

Two-dimensional plots representing morphological similarity in continuous characters between medusae, within size-classes, were calculated by multi-dimensional scaling (MDS) of re-scaled, weighted, continuous features. Values were re-scaled between 0 and 1 by dividing each observed value by the maximum value observed for that feature in the relevant size-class. Features were then down-weighted by a factor equivalent to the number of significant correlations they showed within character-complexes. Finally, characters represented by multiple measurements in the dataset (e.g., bell depth was measured in five different positions) were downweighted further according to the number of measurements (e.g., *f* 28a–e were downweighted by a factor of 5). MDS in SPSS used Euclidean distances (Sneath & Sokal, 1973, pp. 249–250) and was considered complete when S-stress decreased by $\leqslant 0.001$ during successive iterations.

Two-dimensional plots representing morphological similarity in categorical characters between medusae, within size-classes, were calculated by categorical principal components analysis (CATPCA) of weighted categorical features in SPSS. Within features, similarity was assessed on a nominal scale using the categories created at data collection. Missing values were excluded from analyses. The object principal was optimized to provide maximum resolution of distances between medusae. Values were downweighted by a factor equivalent to the number of significant correlations they showed within character complexes and the number of repeated measurements of the character in the dataset.

Molecular genetics

Gastric and gonadal tissues were biopsied from 19 medusae in Palau, including representatives of all populations, and two medusae in Tufi, Papua New Guinea. DNA was extracted from these tissues following the protocol of Dawson & Jacobs (2001). Cytochrome *c* oxidase subunit I (COI) was amplified using the primer pairs LCO1490 and AaCOIi-H [5′-ccatwgtcattccrggggc yctc] and AaCOIi-L and HCO2198 (Folmer et al., 1994; Dawson & Jacobs, 2001). Internal Transcribed Spacer One (ITS1) was amplified using primers jfITS1-5f and jfITS1-3r (Dawson & Jacobs, 2001). PCR, cloning, and sequencing followed the protocol outlined in Dawson (in press). Electropherograms were checked visually, misreads corrected, and poorly resolved terminal portions of sequence discarded. The remaining sequences were aligned in ClustalX (Jeanmougin et al., 1998) and mean pairwise sequence differences calculated in PAUP* 4.0b10 (Swofford, 2002) and Arlequin 2.0 (Schneider et al., 2000) for Macintosh.

Results

Morphological data analyses

All *Mastigias* < 65 mm were immature. Bell diameters within larger size classes did not differ between sexes (100 mm: $t_{24} = -1.014$, $p = 0.308$; 150 mm: $t_{15} = 0.493$, $p = 0.629$). Excepting the occurrence of brood filaments, which was used to differentiate female and male medusae *a priori*, there was no evidence of significant sexual dimorphism ($p \geqslant 0.027$) in any continuous or categorical feature in either size-class after Bonferroni correction for multiple tests (a total of 47 paired *t*-tests and 59 Wilcoxon signed ranks tests were completed).

ANOVA indicated heterogenous size distributions among samples in the 50 mm size-class ($F_{9,62} = 2.485$, $p = 0.017$; but post-hoc Tukey test $p > 0.100$ for all pairwise comparisons) attributable to the three largest OLO medusae (55, 55, 54 mm) in the class. Analyses of a dataset

excluding these medusae gave the same pattern of significant and non-significant results as analyses of the original 50 mm dataset so the analyses of the original entire dataset are reported. The size distributions of medusae in samples of 100 mm ($F_{8,55} = 1.662$, $p = 0.129$) and of 150 mm ($F_{6,40} = 1.330$, $p = 0.267$) were statistically similar.

Of the 39 quantitative and qualitative features analysed, 37 were variable in at least one size-class (according to the stated criteria) and 31 were variable in all of the size-classes. In the 50 mm size-class, 18 of 28 continuous features (24 of 35 features and components thereof) showed significant variation among populations in either their variance or their mean, seven features were non-significantly variable among populations, and five features were constant. In the 100 mm size-class, 25 of 28 features (31 of 35 features or components) varied significantly among populations, two features varied non-significantly, and two features were constant. In the 150 mm size-class, 25 of 28 features (32 of 35 features or components) varied significantly, one varied non-significantly, and two were constant (Fig. 2; Appendix i). All 11 categorical features were variable in all size-classes bar subgenital porticus colour ($f25$) in 150 mm medusae (Appendix ii). Two features, $f29$ and $f30$, were invariable in all size-classes and were excluded from all subsequent analyses.

In the 50 mm size class, 204 of 861 (23.7%) pairwise comparisons among variable features resulted in significant correlations after Bonferroni correction for multiple tests. The correlation coefficient, r, ranged from 0.41 to 0.97. In the 100 mm size class, 146 of 1176 (12.4%) comparisons were significantly correlated and $0.40 \leqslant r \leqslant 0.88$. In the 150 mm size class, 101 of 1225 (8.2%) of comparisons were significant and $0.48 \leqslant r \leqslant 0.90$. Thirty of these comparisons, involving 16 features, were significant in all three size-classes. Schematic representation of these correlations revealed three distinct networks of interactions (Appendix iii). Further consideration of likely logical, partial logical, and (by default) meristic correlations identified, in addition to eight independent characters, seven different character complexes related to mass, largesse, colour, radial canal complexity, the frequency of sinuses, form of the gastrovascular cavity, and the form of the terminal clubs (Appendix iv).

Multivariate analyses

Multi-Dimensional Scaling (MDS) of rescaled, weighted, characters and character-complexes summarized the morphological differences among *Mastigias* (Fig. 3) that were evident in the box-plots of individual features (Fig. 2) clarifying, graphically, the variation both within and between populations in all size-classes. MDS-plots showed considerable overlap between populations in the smallest, 50 mm, size-class but greater segregation in the larger (100 and 150 mm) size-classes. This pattern of greater morphological difference between populations in larger medusae was also obvious when considering lagoon populations, as a group, versus lake populations (Fig. 3).

CATPCA plots of weighted data summarised graphically the morphological differences among *Mastigias* evident in categorical data (Fig. 4; see Appendix ii). Differences between populations and between lagoon and lake morphologies were evident in all size-classes. The loadings of features on the principal components varied between size-classes but, in general, features related to blue colouration exerted the strongest influence on separation (along dimension 2). In the 50 mm size-class, the maximum and minimum loadings along dimension 1 were 9% ($f6$) and 3% ($f5y$) of the total loading, respectively. In contrast dimension 2, along which the maximum and minimum loadings were 30% ($f25$) and $< 0.1\%$ ($f2$) of the total loading, respectively, was strongly influenced by just three components ($f25$, $f3$ [24%], $f1$ [10%]; remainder $\leqslant 6\%$). In the 100 mm size-class, the maximum and minimum loadings along dimension 1 were 10% ($f6$) and 2% ($f25$) of the total, respectively, and along dimension 2 they were 41% ($f25$) and $< 0.1\%$ ($f24$); dimension 2 was again strongly influenced by just three components ($f25$, $f1$ [29%], $f2$ [20%]; remainder $\leqslant 9\%$). In the 150 mm size-class, the maximum and minimum loadings were 11% ($f6$) and 1% ($f13max$) along dimension 1, and 21% ($f1$) and 0.7% ($f7y$) along dimension 2; dimension 2 was moderately influenced by four components ($f1$, $f3$ [11%], $f4y$ [10%], $f5y$ [10%]; remainder $\leqslant 6\%$).

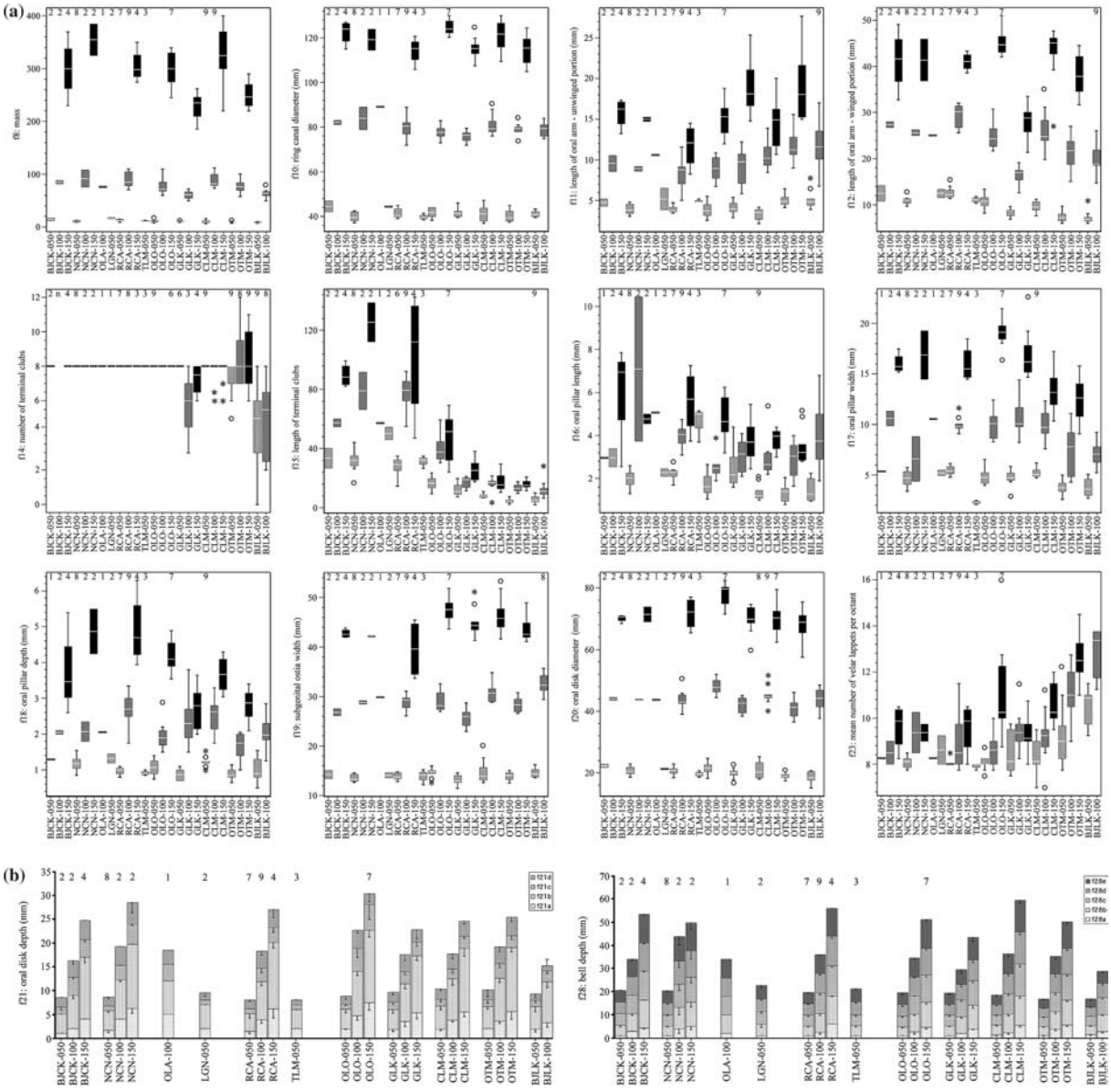

Figure 2. Differences among size-classes and populations of *Mastigias* in the 26 variable continuous macromorphological features. Populations and size-classes are indicated along the abscissa, characters and character-states along the ordinal axis. The boxplots show the median (line), upper and lower quartiles (box), and the largest and smallest values (whiskers) excluding outliers (o; 1.5 to 3 box-lengths from the box-end) and extreme values (*; > 3 box-lengths from the box-end). Boxes are shaded according to size-class (bell diameter; black, 150 mm; dark grey, 100 mm; light grey, 50 mm) except in charts for *f*21 and *f*28 in which colours represent the different positions at which repeat measures were taken.

Molecular genetics

COI

A region 330 nucleotides long was amplified from 19 medusae from Palau (1 from BJCK, NCN, RCA, and OLA; 2 from BJLK, CLM, GLK, lagoon, OTM, and TLM; 3 from OLO) and two from Tufi, Papua New Guinea. Maximum and minimum uncorrected pairwise sequence differences between Palau medusae were 2.1 and 0%, respectively

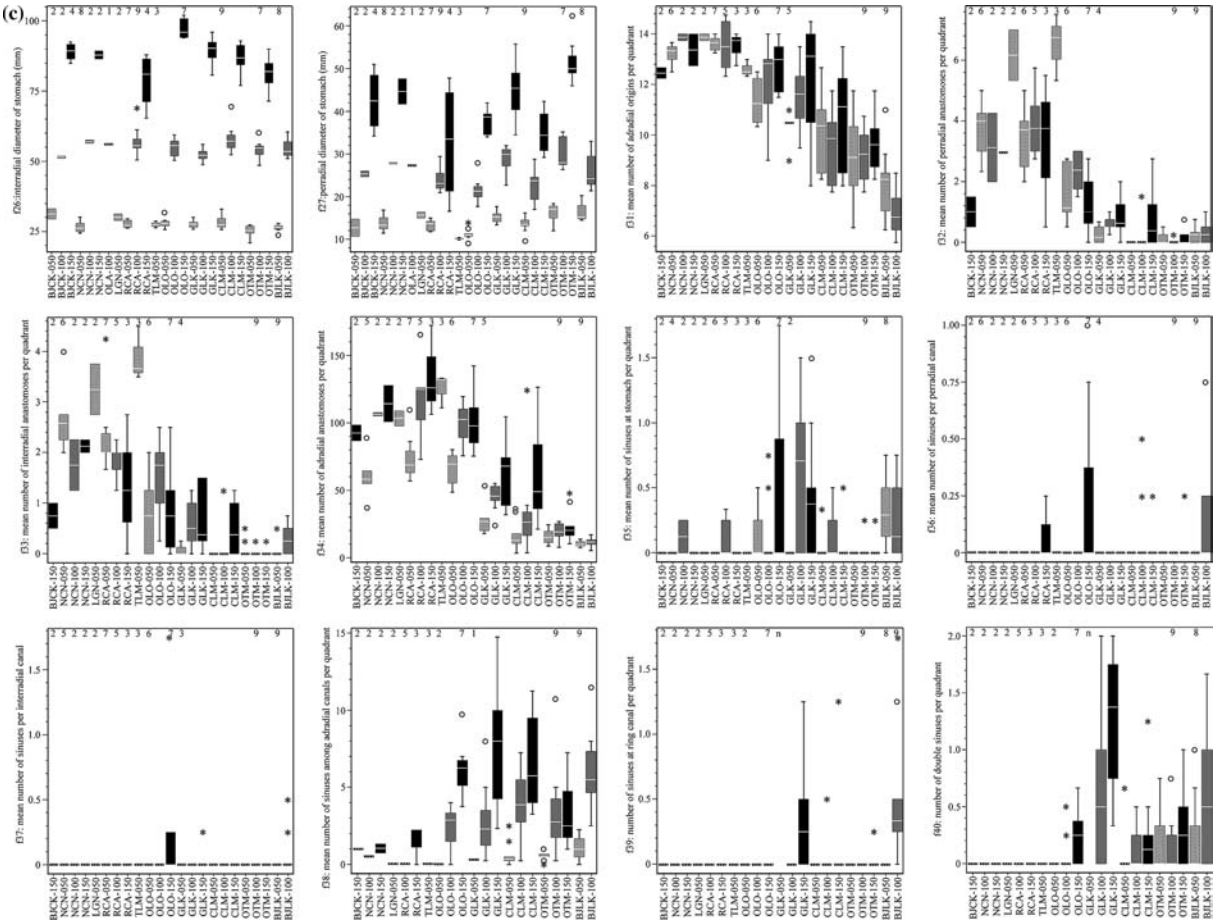

Figure 2. (Continued).

(median and mean = 0.6%). Mean sequence difference, in Palau, between lagoon locations was 0.26% (sd, 0.19%), between lake locations 0.77% (sd, 0.52%), and between lagoon and lake locations 0.52% (sd, 0.42%). All Palau medusae were more distantly related to Tufi *Mastigias*. Mean uncorrected pairwise sequence difference between Palau and Tufi medusae was 7.6% (median 8.0%, minimum 6.3%, maximum 9%).

ITS1

A region of between 438 and 479 nucleotides length (aligned length 491 positions) was amplified from 9 medusae (1 from TLM, OLO, BJCK, RCA, CLM, GLK, and OTM; 2 from BJLK). Maximum and minimum pairwise sequence differences were 14% and 0% respectively (median = 10.0%, mean = 8.5%) based on 350 characters for which there was < 5% missing data. Excluding gapped positions, of which 69% (49/71) occurred in or immediately adjacent to potentially hypervariable regions (i.e. perfect or imperfect repeat units), maximum and minimum pairwise sequence differences were 4% and 0%, respectively (median = 0.9%, mean = 1.5%). Mean sequence difference, excluding hypervariable regions, between lagoon locations was 3.39% (sd, 0.72%), between lake locations 0.58% (sd, 0.18%), and between lagoon and lake locations 1.74% (sd, 1.22%).

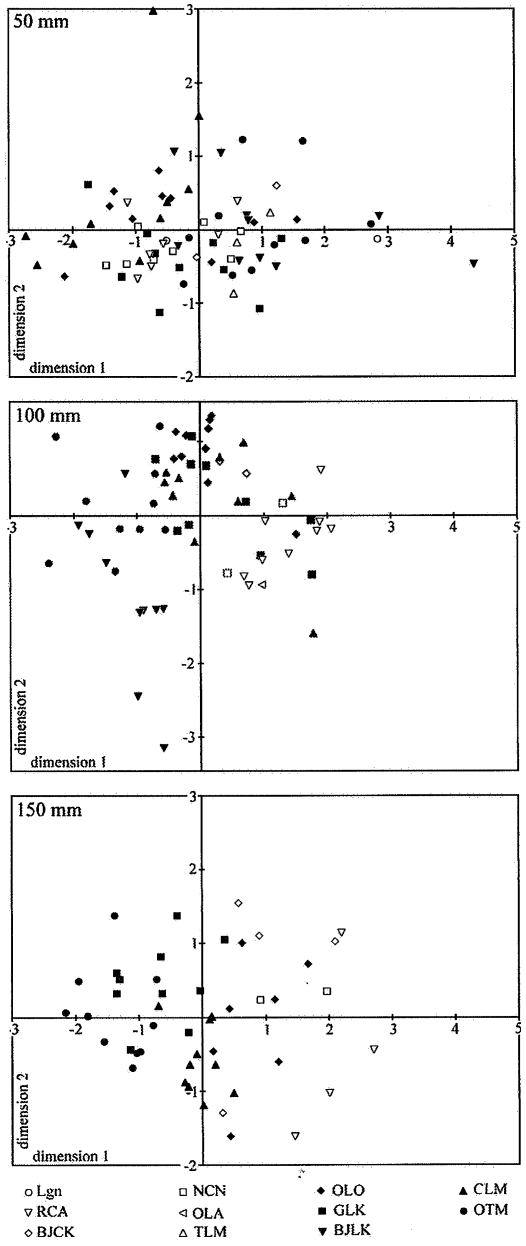

Figure 3. Morphological similarity of *Mastigias* medusae represented in two dimensions by multi-dimensional scaling (MDS) of quantitative characters and complexes within size-classes. 50 mm, stress = 0.110 and RSQ = 0.958; 100 mm, stress = 0.180, RSQ = 0.85; 150 mm, stress = 0.175, RSQ = 0.852.

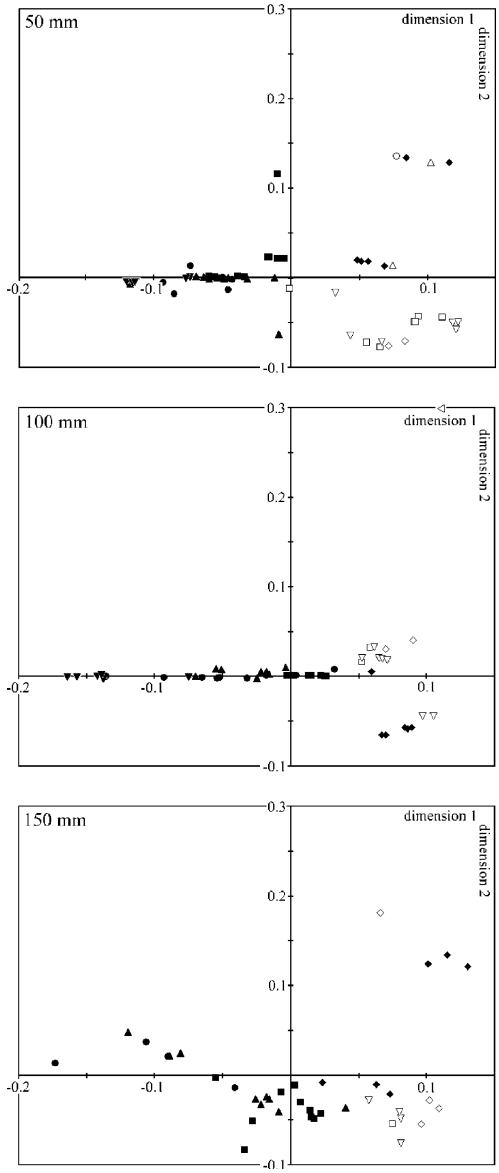

Figure 4. Morphological similarity of *Mastigias* medusae represented in two dimensions by categorical principal components analysis (CATPCA) of qualitative characters and complexes. 50 mm size-class: dimension 1 explains 20.1% of the total variation, Cronbach's alpha (α) = 0.992; dimension 2, 7.9%, $\alpha = 0.971$. 100 mm size-class: dimension 1, 18.0%, $\alpha = 0.991$; dimension 2, 6.7%, $\alpha = 0.967$. 150 mm size-class: dimension 1, 15.1%, $\alpha = 0.986$; dimension 2, 7.6%, $\alpha = 0.967$. Symbols as in Figure 3.

Discussion

The morphology of *Mastigias* varied between medusae, size-classes, populations, and environments. However, none of the 37 variable features measured diagnosed all medusae from any location in Palau; only 15 constituted independent characters or character complexes. Previously,

inter-individual, ontogenetic, and inter-population variation have been considered problematic for the taxonomy of other scyphozoans (e.g., Mayer, 1910; Kramp, 1968); phenotypic plasticity, rampant homoplasy, and a dearth of characters have confounded species identification in other marine invertebrates (e.g., Gosliner & Ghiselin, 1984; Knowlton, 1993). As discussed below, these problems also impinge upon morphological studies of *Mastigias* and again complicate taxonomic efforts. An evolutionary perspective, however, facilitates the interpretation of variation for systematic purposes.

Patterns of morphological variation in Mastigias *in Palau*

Variation within populations: i. Among individuals
Most features were variable within most size-classes and most populations (Appendices i, ii; Fig. 2). All medusae possessed an unique combination of traits but were most similar to other individuals within the same size-class and population (Figs 2–4). Thus, inter-individual variation was distributed non-randomly and, although inter-individual variation may complicate some taxonomic efforts, in this case, it was insufficient to overwhelm higher-level effects.

Variation within populations: ii. Among size-classes
Although the growth of individuals was not followed through time, it is clear that the appearances of *Mastigias* medusae change ontogenetically (Appendices i, ii; Fig. 2). Only two features were invariable among all size-classes and populations: the number of perradial and interradial origins (*f*29, *f*30). Five other features were invariable across all size-classes in at least one population: the number of terminal clubs (*f*14) and four measures of sinus abundance (*f*36, *f*37, *f*39, *f*40). All other features were variable in at least one size-class in all populations. In features that showed strong trends in variation between populations, such as the length of terminal clubs (*f*15) and the mean number of adradial origins per quadrant (*f*31), the same trend generally occurred in all size-classes (Fig. 2). Thus, like inter-individual variation, ontogenetic variation did not necessarily mask higher-level patterns of variation. To the contrary, as is generally accepted, knowledge of ontogenetic variation can elucidate developmental and evolutionary trends (e.g., Futuyma, 1998, pp. 651–676; Mabee, 2000; Zelditch et al., 2000). In this case, for example, variation within features increases and the number of correlations among features decreases with increasing size of medusae (Appendix i; Fig. 2) which is consistent with morphological variation by terminal change (either addition or subtraction; Futuyma, 1998, p. 653; Mabee, 2000). Greater similarity among earlier ontogenetic stages (Figs 3, 4), therefore, is not as problematic as it is potentially informative. Ontogenetic variation presents additional phylogenetic information (e.g. Uchida, 1926) and the ontogenetic criterion may be used to polarize at least some higher-level, for example inter-population or inter-species, patterns of evolution (Mabee, 1996; Meier, 1997).

Variation among populations and habitats
Mastigias medusae were most similar to other medusae from the same location, although the disparity between populations was less in smaller medusae (Figs 2–4). A number of features showed no obvious geographic pattern (e.g. mass [*f*8], ring canal diameter [*f*10], oral disk diameter [*f*20], perradial diameter of the stomach [*f*27]) whereas others were strongly geographically structured (e.g., length of terminal clubs [*f*15], mean number of adradial origins per quadrant [*f*31]; Fig. 2). Overall, a morphological trend emerged apparently related to environment – from medusae inhabiting the lagoon, through intermediate marine lakes (e.g., OLO), to shallow (GLK) and then deeper meromictic marine lakes (e.g. OTM, BJLK; Figs 2–4) – suggesting causal and perhaps functional explanations for morphological variation. For example, terminal clubs, which are easily detached (Uchida 1926), may be an anti-predatory device (P. Cornelius, L. Martin, pers. com.) and are most obvious in the lagoon where visual predators (e.g. turtles) occur and vestigial in meromictic lakes where there are apparently no visual predators of consequence. The form of the terminal clubs, and other aspects of morphology, might also be related to hydrodynamics because lagoon medusae, which are more likely to be advected

away from the breeding population, swim faster than lake medusae but the difference cannot be explained simply by differences in pulse-rates (Dawson & Hamner, 2003). Morphological variation therefore may also manifest in behavioural variation (e.g., Podos, 1997, 2001). Lagoon and intermediate marine lake *Mastigias*, for instance, have blue, possibly photoprotective (Arai, 1997; but see Dove et al., 1995), pigmentation and generally occupy surface waters even during the most intense sunlight, whereas *Mastigias* in meromictic marine lakes almost always lack the pigmentation and swim deeper when sunlight is brighter (Dawson & Hamner, 2003). Velar lappets are almost invariably angled acutely clockwise of the bell diameter (Fig. 1) and thus shed water clockwise off the bell forcing the medusa to rotate anti-clockwise (when looking at the subumbrella). This rotational behaviour illuminates the photosymbiotic zooxanthellae of *Mastigias* evenly all around the bell (Hamner et al., 1982). It is important however, to reserve judgement on the adaptive benefit of these features, which include plesiomorphies and apomorphies, until appropriate experimental evidence is available (e.g., Gould & Lewontin, 1979; Dawson & Hamner, 2003).

Phenotypic plasticity

While phenotypic plasticity may be an evolutionarily important source of variation, aspects thereof, such as ecophenotypic variation, may compromise systematic analyses of morphological data. It is important, therefore, to assess the possible effects of phenotypic plasticity on patterns of geographic variation. Ecophenotypic variation has been documented in *Mastigias* in Palau. For example, *Mastigias* in OTM looked different before and after the 1997–1998 ENSO event that induced dramatic changes in physical lake structure and the density of medusae (Dawson et al., 2001). The differences, which, unfortunately, generally were not quantified, included changes in the frequency of intermediate filaments, the shape of the terminal clubs, and an increase in the maximum size attained by medusae

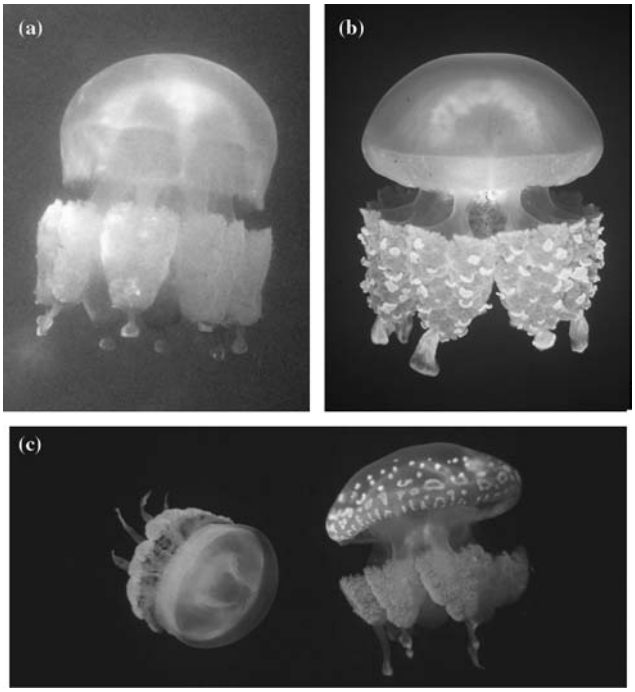

Figure 5. Possible extent of phenotypic plasticity. Large mature *Mastigias* medusae in OTM (a) during high population size, $\sim 10^6$–10^7 medusae, before the 1997–1998 ENSO event and (b) during low population size, $\sim 10^4$ medusae, after the 1997–1998 ENSO event [2000; picture courtesy of P. Colin, Coral Reef Research Foundation]. *Mastigias* medusae in OTM during high population size, $\sim 10^7$ medusae, in 2002 look more like medusae before than after the ENSO event. (c) Co-occurring phenotypes in BJLK, 2001.

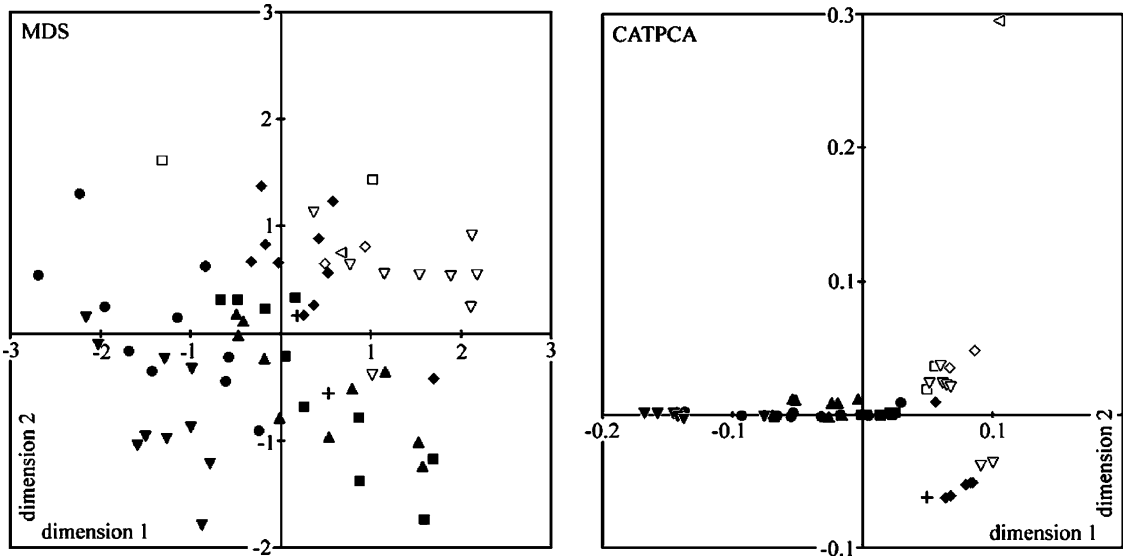

Figure 6. Limited phenotypic plasticity shown by *Mastigias* reared in aquaria. Multi-dimensional scaling of quantitative data and categorical principal components analysis (CATPCA) of qualitative data showing similarity of *Mastigias* medusae from Ongael Lake that grew to 100 mm in the lake (black diamonds) that were reared to 100 mm in an aquarium (crosses; $n = 2$; both with the same coordinates in CATPCA). Analyses were the same as those used to generate Figures 3 and 4 bar down-weighting of sinus characters by a factor of six to indicate the potential effect on MDS of designation of a single 'sinus' complex as opposed to six independent sinus characters. MDS: stress = 0.137, RSQ = 0.911. CATPCA: dimension 1, 18.0%, $\alpha = 0.991$; dimension 2, 6.9%, $\alpha = 0.967$. Symbols as in Figure 2, except "+" aquarium reared OLO medusae.

(Fig. 5a–b). The changes have been reversed since lake structure and population size reverted to their pre-ENSO states (pers. obs.). For example, maximum size decreased by over 30% (from 25 to 17 cm) between June 2000 and June 2002 (M. Dawson, L. Martin, & L. Bell, unpubl. data). Unpigmented and spotted forms of *Mastigias* in BJLK which otherwise look similar (Fig. 5c) also may indicate phenotypic plasticity, in this case perhaps stochastic developmental plasticity (e.g., Elowitz et al., 2002). However, a genetic component cannot be excluded at this time because *Mastigias* medusae retain location-specific morphologies, including spots, when reared in aquaria (Fig. 6; Dawson, 2000). Thus, although there is evidence of phenotypic plasticity in *Mastigias*, ecophenotypic variation is unlikely to overwhelm the population-level differences that have been described herein.

Patterns and implications of molecular variation in Mastigias *in Palau*

In contrast to the many morphological differences seen among *Mastigias* from different locations in Palau, there were few molecular differences in COI or ITS1 (excluding hypervariable regions). Based on COI data, genetic distances among habitats paralleled morphological differences being, on average, greater among medusae from different lakes (0.77 ± 0.52%) than among medusae from different lagoonal locations (0.26 ± 0.19%). Genetic distances among habitats in ITS1, though, showed the opposite pattern, being greater among lagoon medusae (3.39 ± 0.72%) than lake medusae (0.58 ± 0.18%). The relatively small genetic distances between medusae and the discordance between patterns of variation in COI and ITS1 are consistent with recent establishment of populations in marine lakes and very close, putatively conspecific, relationships of lagoon and lake medusae (e.g. Avise, 2000, p. 63). Considering medusae from all locations in Palau, mean genetic distances in COI (2.1% maximum) and ITS1 (4.2% maximum) were considerably less than distances from *Mastigias* in Papua New Guinea (COI: 6–9%) and differences between congeneric species of moon jellyfish *Aurelia* (COI:

Table 2. Summary of some macro-morphological characters used by Mayer (1910) and Kramp (1961) to differentiate species or varieties of *Mastigias*, and comparison with the results of this study describing lagoon medusae, putatively *M. papua* (Uchida, 1947), and marine lake *Mastigias*

Mastigias species or variety	Filaments	Velar lappets (octant^{-1})	Mouth-arm length	Terminal club length	Terminal club shape	Adradial origins (octant^{-1})	Perradial anastomoses	Subgenital ostia width	Colour (Mayer 1910)	Geographic range
M. albipunctatus Stiasny 1920	One long and several short on arm-disk	6–14	$\approx r$	Variable	Variable	12 to 14	None to few	–	–	Indian Ocean, Malay Archipelago, Australia
M. andersoni Stiasny 1926	One central and four peripheral on arm-disk only	6	$\approx r$	Long	Clubbed	12 to 15, or 18	None	–	–	Australia
M. gracilis (Vanhöffen 1888)	Particularly long on arm-disk; also on oral arms	5–10 (K); 5–10 (M)	$< r$; w = 3u to 4u	Short; r/6	Rounded knob	6 to 7	–	2 pw	–	Red Sea
M. ocellatus (Modeer 1791)	Few on arm-disk; also on oral arms	$\approx$ 12	$\leqslant r$; $w <$ u	$= r$	Triangular	15 to 20	None	–	Bell: rd-bn Spots:w-bn	Indian Ocean, to Philippines, Hong Kong
M. pantherinus Haeckel 1880	–	16	$\approx 2r$; $w \geqslant$ 2u	4*r* to 6*r*	–	$>$ 10	–	–	Bell: bn Spots:w-bk	Samoa
M. papua (Lesson 1830)	Numerous on oral arms	8	r; w = 2u/3	2r; sometimes less, or absent	Triangular	$<$10 (K); 7–9 (M)	Usually $>$ 0	$\approx$ 2 pw	Bell: be, bn g-ol. Spots: w, bn, y	Indian Ocean, Malay Archipelago, Fiji, Palau, Japan
M. sibogæ Mass 1903	Numerous on oral arms; also on terminal clubs	9	r	$\sim 2r$	Triangular	7–10	–	3 pw	Bell: y? Spots: or?	Malay Archipelago
M. sidereus Chun 1896	–	6 (K); 8 (M)	2 r; $u < w$	Long; r	Clubbed	$\sim$ 7	Always	2 pw	Bell: be, bn g-ol. Spots: w, bn, y	E. Africa, W. Indian Ocean
lagoon (100-150 mm) [min, med, max]	On oral arms and arm-disk	7.8, 9, 11.5	0.7, 0.7, 0.8 r; w = 1.9, 3.0, 6.2 u	0.6, 1.4, 1.8 r	Triangular	6.1, 6.8, 7.4	0.5, 3.0, 5.8	1.9, 2.8, 6.5 pw	Bell: be Spots: w, y, g	Palau
marine lake (100-150 mm) [min, med, max]	None; on oral arms only; on oral arms and arm-disk	7, 10, 16	0.4, 0.7, 0.9 r; w = 0.8, 2.2, 4.3 u	0.1, 0.3, 1.2 r	Circular, flattened, triangular, quadrangular	2.9, 5.2, 7.3	0, 0.4, 3.0	1.7, 3.2, 7.0 pw	Bell: be, nc Spots: w, y, ns	Palau

M. roseus (Reynaud) excluded as Mayer (1910) and Kramp (1961) considered it a dubious species.
Mastigias sp. Rao excluded as no data presented by Kramp (1961).
Minimum, median, and maximum values summarise the states of quantitative features in this study.
r, bell radius; w, length of the winged portion of the oral arm; u, length of the unwinged portion of the oral arm; pw, oral pillar width.
Colours abbreviated as follows: w, white; y, yellow; be, blue; bk, black; bn, brown; g, green; rd, red; rs, rose; ol, olive; or, orange; v, violet; nc, no colour; ns, no spots.
– no information in Mayer (1910) nor Kramp (1961). Where information differed the source is indicated as K (Kramp, 1961) or M (Mayer, 1910).

Table 3. Comparison of morphological features in genetically distinct *Mastigias* from Papua New Guinea ($n = 2$) and Palau ($n = 2$)

Mastigias species or variety	Bell diameter (mm)	Velar lappets (octant^{-1})	Mouth-arm length	Terminal club length	Terminal club shape	Subgenital ostia width[a]	Colour[b]	Geographic location
Mastigias sp. (fixed)	43, 65	~8[c]	0.6 to 0.7	1.0 to 1.1	Triangular	2.4 to 2.6 pw	Purple[b]	Papua New Guinea
Mastigias papua (fixed)	49, 50	~7 or 8[c]	0.6	0.6 to 0.9	Triangular	2.8 pw	Purple[b]	Palau
Mastigias papua (live; min to max)	47 to 55	8 to 8.5	0.6 to 0.7	0.5 to 1.5	Triangular	2.2 to 2.8 pw	blue	Palau

The comparison is made between preserved medusae from Tufi (Papua New Guinea) and Risong Cove (Palau). For context, mean measurements on live medusae from Risong Cove are presented as an indication of the effects of preservation.
[a] As a multiple of the width of oral pillars (pw).
[b] Purple pigment was obvious in one preserved specimen from each location and is interpreted, by comparison with live specimens from Palau, as representing blue pigmentation in life.
[c] Approximate counts because lappets were difficult to enumerate in small preserved specimens.

13–24%; ITS1: 10–40% [excluding microsatellites]; Dawson & Jacobs, 2001), upside-down jellyfish *Cassiopea* (COI: 11–23%; Holland et al. in press), and many other marine organisms (e.g. Chen et al., 1996; Meyran et al., 1997; Odorico & Miller, 1997; Peek et al., 1997; Bucklin et al., 1998).

Comparison with morphospecies of Mastigias

Approximately eight morphospecies of *Mastigias* have been described (Mayer, 1910; Kramp, 1965). Mayer (1910) noted four morphological differences that he considered most useful for identifying species and varieties of *Mastigias*: the number and shape of the velar lappets, the length of the mouth arms relative to bell radius, the length of the terminal clubs relative to bell radius, and colour. Kramp (1961) also listed these characters but did not explicitly give them greater weight than other distinctive characters. Presumably on the basis of such characters, Uchida (1947) identified *M. papua* in Palau which, implicit in his lack of comment, did not differ remarkably from conspecifics in Japan (Uchida, 1926) although there clearly is variation within and between populations of *M. papua* (Table 2). It seems highly unlikely that Uchida studied lake medusae. However, lake medusae exhibit a range of character values that span almost all states recorded in, and considered discriminatory for, existing morphospecies of *Mastigias* (Table 2). The identification of Palau medusae as *M. papua* and the radical morphology of lake medusae despite their close genetic relationship to lagoon medusae raises questions regarding the status of the previously described morphospecies. For example, if *Mastigias* in Palau are used as a benchmark, the eight described morphospecies of *Mastigias* could be genetically closely related and simply morphological variants of a single wide-spread species. In contrast, if existing morphospecies are used as a benchmark, there may be up to six species of *Mastigias* in Palau alone. However, the reality is clearly a complex mix of these and other scenarios because *Mastigias* in Papua New Guinea are morphologically similar to *M. papua* in Palau (Table 3) but distinguished by as much as 9% sequence difference in COI.

An evolutionary perspective

These apparently conflicting patterns of morphological and molecular variation can be reconciled by taking an evolutionary perspective that incorporates heterogeneity in processes. Distinguishing species from this perspective, however, will require additional data describing their ecology, morphology, genetics, and distribution (e.g. Temple-

ton, 1989; Knowlton, 2000; see also Crandall et al., 2000). For example, within Palau, an approximate continuum of forms of *Mastigias* inhabit an approximate continuum of environments ranging from semi-enclosed holomictic coves characterized by coral reef metazoans, through holomictic or occasionally mixed 'intermediate' marine lakes with few reef fishes, sponges, and algae, to isolated meromictic marine lakes characterized by mangroves and associated taxa (Hamner & Hamner 1998; W. Hamner, M. Dawson, L. Martin, L. Bell, P. Colin, unpublished data). Patterns of sea-level change, local topography, molecular data (e.g., COI), and the ontogenetic criterion, are consistent with the evolution of lake medusae from an ancestral lagoon population during the late-Pleistocene and Holocene (Hamner & Hamner, 1998). Ecological and morphological similarities – such as vertical migration, reduced colouration, shortened terminal clubs, and a simpler radial canal system – among lake medusae suggest they may be variants along a cline within a single derived clade. However, geography suggests that most, and perhaps all, lake populations are isolated from each other as well as from the lagoon, an interpretation that is also consistent with differences in molecular (e.g., ITS1) and morphological data (this study), as well as differences in behavior (Dawson & Hamner, 2003). In this case, each lake population likely diverged independently and in parallel from a common lagoonal ancestor within the last 18,000 years, and differences between lake populations are attributable to variation in, for example, environment, time since divergence, founder effects, genetic drift, and intensity of selection (e.g., Hamner & Hamner, 1998; Dawson et al., 2001; Dawson & Hamner, 2003). In this case, their similar evolutionary trajectories are evidence of repeated evolution and considerable homoplasy.

The implications for the existing taxonomy of *Mastigias*, and of other jellyfishes in general, are significant. Relatively large morphological differences may accrue rapidly despite little genetic divergence in commonly assayed markers (e.g. Figs 2–4). Conversely, relatively little morphological difference may mask deep genetic divergence (e.g., Table 3; see also Dawson & Jacobs, 2001; Schroth et al., 2002). Morphological and molecular differences might also both be small, or both be large. Thus, it is impossible to know *a priori* what is the systematic significance of morphological variation in *Mastigias* (Table 2) and it is unlikely that any morphological standard can be applied to identify species.

Conclusion

Vagaries in the species-level taxonomy of some scyphozoans have been attributed to subjective analyses of phenotypic variation between individuals, size-classes, and populations (e.g., Mayer, 1910; Kramp, 1961, 1965, 1968; Gershwin, 2001) and may have been exacerbated by phenotypic plasticity and a dearth of characters. Modern analytical methods, integrating univariate and multivariate statistical analyses of quantitative and qualitative morphological data have gone some way to resolving these issues, but also indicate a dearth of diagnostic characters (e.g., Dawson, 2003; this study). Indeed, in many medusae, there may be insufficient independent and non-homoplasious macro-morphological characters to ever generate a robust phylogeny for species within many genera or even for higher taxa (e.g., Gershwin & Collins, 2002; Dawson, 2003) although geographic variation may be commonplace. Statistical analyses of morphology can enrich evolutionary discourse by increasing the range of characters suitable for study and explicitly considering variation, which is both a pervasive phenomenon and an integral component of evolution by natural selection (e.g., Darwin, 1859; Futuyma, 1998, p. 231). More detailed description of morphological variation should improve systematic studies and facilitate improved understanding of the patterns of and processes influencing evolution in the Scyphozoa.

The integration of morphological with molecular and other data provides additional benefits. For example, molecular methods provide an independent metric by which to gauge the success of morphological approaches and have provided strong evidence for numerous cryptic species (Knowlton, 1993, 2000; Dawson & Jacobs, 2001). However, other approaches come with problems of their own (e.g., Hillis & Wiens, 2000), as demonstrated here. For example, COI and ITS1, which are widely used markers for marine invertebrates, indicated different relationships between and did

not reliably differentiate among morphologically, behaviourally, and physiologically divergent golden jellyfish, *Mastigias*, in Palau (McCloskey et al., 1994; Dawson & Hamner, 2003; this study). Genetic differences were considerably less than those typically considered indicative of species-level relationships in other cnidarians (e.g., Chen et al., 1996; Odorico & Miller, 1997; Dawson & Jacobs, 2001) although morphological variation exceeded differences previously considered sufficient to delineate species (Table 2). These data, past confusion, and recent studies (e.g., Dawson, 2003) indicate that there is no gold standard for designating species in the Scyphozoa.

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Appendix i. Variability in continuous features measured in *Mastigias* in Palau. Levene's statistic is reported for all features in all size-classes while *F*-statistics are presented only for the features that had homogeneous variances among populations (after sequential Bonferroni correction [Rice, 1989] for multiple Levene's tests within size-classes; $\alpha = 0.05$). Features whose means differed significantly among populations (after sequential Bonferroni correction for multiple ANOVAs within size-classes; $\alpha = 0.05$) are indicated by asterisks.

f[a]	Feature	50 mm[b]			100 mm[c]			150 mm[d]		
		Levene	*F*	*p*	Levene	*F*	*p*	Levene	*F*	*p*
8	Mass	1.408	7.032	*	1.979	5.932	*	1.137	7.438	*
10	ring canal diameter	1.606	1.458		1.468	3.015	*	0.803	3.358	*
11	OA unwinged length	1.310	4.632	*	1.538	2.741	*	1.551	4.575	*
12	OA winged length	0.946	17.796	*	1.435	13.110	*	0.988	12.416	*
14 [6]	Terminal club number	7.431			8.857			8.144		
15	Terminal club length	2.849			4.210			12.634		
16	Oral pillar length	2.099	13.870	*	2.961	3.562	*	3.701	4.478	*
17	Oral pillar width	1.306	8.703	*	2.153	5.831	*	0.918	9.805	*
18	Oral pillar depth	2.256	3.833	*	1.115	4.041	*	1.630	11.141	*
19	Subgenital ostia width	2.348	1.568		0.901	9.421	*	3.186	3.965	*
20	Oral disc diameter	1.897	2.822		1.065	3.810	*	0.851	3.449	*
21a [2]	Oral disc depth (edge)	6.041			4.480			1.349	3.253	*
21b [1]	Oral disc depth (2/3)	3.606			0.934	2.562		2.109	5.099	*
21c [3]	Oral disc depth (1/3)	9.452			2.942	5.737	*	10.660		
21d [1]	Oral disc depth (center)	2.162	1.487		3.365	6.539	*	4.259		
23	Velar lappet number	4.197			1.135	15.275	*	2.534	7.521	*
26	GVC interradial diameter	1.132	3.998	*	1.058	2.349		1.859	8.345	*
27	GVC perradial diameter	1.397	9.259	*	2.194	5.767	*	4.563		
28a [5]	Bell thickness (4/5)	33.410			6.830			5.398		
28b	Bell thickness (3/5)	2.431	2.132		3.518			1.942	6.731	*
28c [1]	Bell thickness (2/5)	4.958			5.371			1.751	10.202	*
28d	Bell thickness (1/5)	5.099			4.262			3.570	13.066	*
28e	Bell thickness (centre)	2.510	7.113	*	5.442			2.997	8.950	*
29 [8]	Perradial origins	invariant			invariant			invariant		
30 [8]	Interradial origins	invariant			invariant			invariant		
31	Adradial origins	3.612			1.480	28.216	*	3.945	4.450	*
32 [1]	Perradial anastomoses	7.307			9.712			5.881		
33 [1]	Interradial anastomoses	4.162			3.179	23.457	*	6.021		
34	Adradial anastomoses	2.202	57.827	*	2.658	37.585	*	2.392	13.071	*
35 [5]	GVC sinuses	6.237			5.449			6.800		
36 [8]	Perradial sinuses	invariant			7.140			13.054		
37 [8]	Interradial sinuses	invariant			8.606			4.335		
38	Adradial sinuses	2.480	1.885		2.060	5.474	*	2.310	5.989	*
39 [6]	Ring canal sinuses	invariant			9.521			2.877	1.466	
40 [3]	Double sinuses	3.105	0.596		6.970			3.665	9.497	*

[a] superscript numbers report the number of populations ($\geqslant 1$) in which any feature was invariant in the 50 mm size-class of all populations of *Mastigias* for which data were available (maximum 8 due to too few observations in BJCK and open lagoon populations).

[b] Degrees of freedom 9,62 except *f*14 (9, 52), *f*15-18 *f*20 (9,60), *f*8 *f*10 *f*23 (9,61), *f*31 (8,49), *f*32 (8,47), *f*33-*f*34 (8,48), *f*35 (8,42), *f*40 (5,29).

[c] Degrees of freedom 8,55 except *f*8 *f*11 *f*20 *f*21d *f*28a-e (8,54), *f*14 (7,42), *f*19 (8,53), *f*26-27 (8,52), *f*31-40 (6,49).

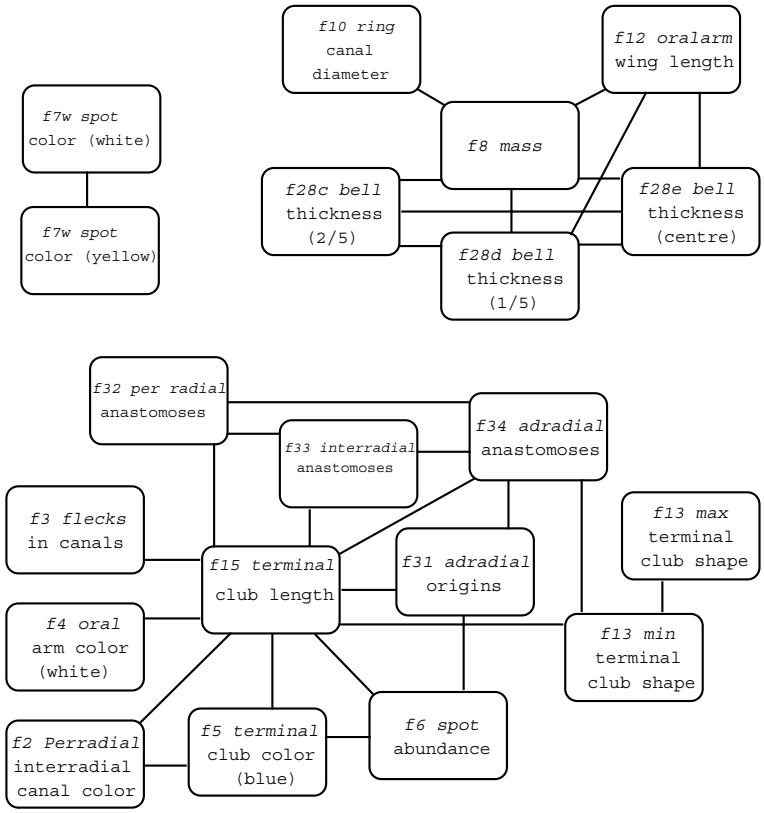
[d] Degrees of freedom 6,40 except *f*14 (6,31), *f*20 *f*31-40 (6,37).

Appendix ii. Variation in categorical features of *Mastigias* in Palau

f	Feature	50 mm size class Population[a]										100 mm size class									150 mm size class						
		0	1	2	3	5	6	7	8	9	10	0	1	3	4	6	7	8	9	10	0	1	3	6	7	8	9
1	Bell colour	0	b 0	b	b 0	b	b	0 b	0	0	0	0	0	0 b	0	b 0	0	0	0	0	0 b	0	0	b 0	0	0	0
2	Perradial and interradial canal color	b	b 0	b	b	b	b	0	0	0	0	b	0b	b	b	0	0	0	0	0	b	b	b	0	0	0	0
3	Flecks in canals	1	1 0	0	1 2	0	0	0	0 1	0 2	0	2	12	2	2	1 2	2 1	0 12	0 1 2	0	2 0	1	2	2 1	2 1 0	02 1	0 1
4	Oral arm color	bw	y w b	0	b w y	b	w b y	0 w	0	0	0	b wy	by	w 0 y	w	w 0	0 w	0	0 w	0	bw y	y	0 y	1 0	0 y w	0	0
5	Terminal club color	b	by	b	b y	b	b	y 0 b	0	0	0	b	by	b y	b	b	y 0	0	0	0	b	by	b y	0 b	y 0	0	0
6	Abundance of spots	23	3 2	1	3	3	3 2	1	1 0	1 2	0	3	23	3 2	3	3 2	1 2	0 1 23	0 1 2	0	3 2	3	23	3	1 2	0 1	0
7	Spot color	w y	w y	w	w y	w	w	y	w y	w	n/a	g	w y	w y	w	w	y	w y	w	n/a	y w g	w	w	w	y	w	n/a
13	Terminal club shape	3	3	3	3	3	3 0	3 2 10	3 0 2	2 3 0 1	2 0 3	3	3	3 2	3	3	3 2 1	3 2 1 0	3 2 0	2 0 3	3	3	3 2	3 2	32	4 3 02	3 2 1
22	Oral arm filaments	ad	a d	ad	a d	ad	a	0 a	0 a d	0	0	ad	ad	ad	ad	a d	a	a d	a d 0	a 0	a d	ad	a d	a d	a d	a d	a d
24	GVC shape	c	c	c	c	c	c	c	c	c k	k c	c	c	c	c	c	c	c	c k	k c	c	c	c	c	c k	c	c k
25	Subgenital porticus colour	0	0	b	0	b 0	0 b	0 b	0	0	0	0	0	0	b	0	0	0	0	0	0	0	0	0	0	0	0

[a] Key to populations: 0 BJCK, 1 NCN, 2 open lagoon, 3 RCA, 4 OLA, 5 Tketau, 6 OLO, 7 GLK, 8 CLM, 9 OTM, 10 BJLK. Key to character states: b, blue; g, greenish; w, white; y, yellow. 0, absent; 1 present; 2, common; 3, abundant. a, on oral arms; d on oral disk. c, cruciform; k, crooked. n/a, not applicable to this dataset (but see Fig. 5). The distribution of character states is shown by feature, size-class, and location. The relative frequencies of states within samples is indicated by their order, with the most frequent top, ties on the same line, and least frequent bottom.

Appendix iii. Networks of 30 significant correlations among 16 macromorphological features, or states thereof, that occurred within all size classes of *Mastigias*.



Appendix iv. Summary of macro-morphological features studied in *Mastigias*, showing those that were variable in 50, 100, or 150 mm medusae, and hypothesized character complexes. Complexes in which significant correlations occurred between features within all size classes appear in bold.

Character		Variable	Complex		
f	Description	(50, 100, or 150)	Logical		Meristic
1	Bell colour	All			
2	Per-, inter-radial canal colour	All			**Colour**
3	Flecks in canals	All			
4	Oral arm colour	All			
5	Terminal club colour	All			**Colour**
6	Abundance of spots	All			**Colour**
7	Spot colour	All			
8	Mass	All	**Mass**		**Largesse**[a]
10	Ring canal diameter	All	**Mass**		
11	OA unwinged length	All	Mass		
12	OA winged length	All	**Mass**		**Largesse**
13	Terminal club shape	All	Mass		**Club form**
14	Terminal club number	All	Mass		
15	Terminal club length	All	Mass		**Club form**
16	Oral pillar length	All	Mass		
17	Oral pillar width	All	Mass		
18	Oral pillar depth	All	Mass		
19	Subgenital ostia width	100, 150			
20	Oral disc diameter	100, 150	Mass		
21	Oral disc depth[b]	All	Mass		
22	Oral arm filaments	All			
23	Velar lappet number	All			
24	GVC shape	All	gvc form		
25	Subgenital porticus colour	50, 100			
26	GVC interradial diameter	50, 150	gvc form		
27	GVC perradial diameter	All	gvc form		
28	Bell thickness[b]	All	**Mass**		**Largesse**
29	Perradial origins at gvc	Invariant			
30	Interradial origins at gvc	Invariant			
31	Adradial origins at gvc	All		**Canal complexity**	
32	Perradial anastomoses	All		**Canal complexity**	
33	Interradial anastomoses	All		**Canal complexity**	
34	Adradial anastomoses	All		**Canal complexity**	
35	GVC sinuses	All		Sinus	
36	Perradial sinuses	100, 150		Sinus	
37	Interradial sinuses	100, 150		Sinus	
38	Adradial sinuses	100, 150		Sinus	
39	Ring canal sinuses	100, 150		Sinus	
40	Double sinuses	100, 150		Sinus	

[a] Largesse is used in the context of its original, now obsolete, meaning of 'liberality, bountifulness …' wherein 'liberal' includes the meaning 'of outline, parts of the body: ample, large' and 'bountiful' is defined as 'of things: characterized by bounty, abundantly yielding; also, ample, abundant, plenteous.' (Oxford English Dictionary online http://dictionary.oed.com/).
[b] Considering all four measurements of oral disk depth and all five measurements of bell thickness.

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