Complete methods for quantifying morphological variation in *Mastigias*, and detailed stepwise-breakdown of results.

An accompaniment to Dawson, M. N, in press. Morphological variation and systematics in the scyphozoa: *Mastigias* (Rhizostomeae, Mastigiidae) - a golden unstandard? Hydrobiologia.

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: 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*, below) of the medusae were collected, usually within several hours and always within one day of collection, as described below (Fig. 1A-F).

A medusa was removed from the aquarium and placed in a small holding container. Colours, other than the browns attributable to zooxanthellae, in the (*f*1) bell, (*f*2) perradial and interradial canals, (*f*3) opaque flecks in the radial canals, (*f*4) oral arms, and (*f*5) terminal clubs, were recorded after comparison with a standardized colour chart. The (*f*6) relative abundance [including the distribution pattern] and (*f*7) colour of spots on the exumbrellar surface were noted and sketched. The medusa was then removed from the holding tank, placed in a mesh [1 mm^2] bag, drained of water for 20 s and (*f*8) weighed to the nearest 1g on an analytical balance. It was then placed, flat, exumbrella surface down with oral arms and terminal clubs gently teased out to radiate from the centre of the bell, on an horizontal transparent surface illuminated from below by a circular 40W fluorescent light. Using 0.1 mm precision calipers, the (*f*9) bell diameter [d_b] was re-measured, and the (*f*10) diameter of the ring canal [d_c] was measured. If mature, the medusa was sexed by the presence [female] or absence [male] of brood filaments on the oral arms and oral disk. Gonadal tissue also was biopsied and examined under a dissecting microscope to confirm the sex of the medusa. The (*f*11) length of the

unwinged portion, as well as the total length, of four oral arms was measured starting from the base of the arm at the oral disk (defined as the point at which the recumbent oral arm was creased). The (*f*12) length of the winged portion of the oral arms was then calculated by subtracting the length of the unwinged portion from the total length of the arm. The (*f*13) shape and (*f*14) number of terminal clubs was recorded. The (*f*15) lengths of four [or as many as possible if less than four] terminal clubs was measured. The oral arms were then amputated. The (*f*16) length, (*f*17) width, and (*f*18) depth of two oral pillars were measured. The (*f*19) width of two subgenital ostia was measured. The oral disk was then amputated and placed bell-facing surface down on the flat surface. The (*f*20) diameter of the oral disk (d_d) was measured across both perradial axes. The (*f*21a-d) thickness of the disk was measured at intervals of $d_d/6$ across one perradial axis using a mm-calibrated probe to determine disk-shape and thickness. The presence of (*f*22a,b) intermediate filaments [i.e. non-brood filaments] on the oral arms and on the oral disk were recorded. The (*f*23) number of non-rhopalar lappets was recorded in each of four, usually adjacent, octants. The shapes of (*f*24) the gastrovascular cavity and the gastric and gonadal tissue were sketched and classed as cruciform or not. Any (*f*25) colouration of the subgenital porticus was noted. The width of the gastrovascular cavity was measured along (*f*26) one interradial axis and (*f*27) one perradial axis. The subgenital porticus was then removed from the bell and (*f*28a-e) the bell depth was measured at intervals of $d_b/10$ across one interradial axis using a mm-calibrated probe to determine bell-shape and bell thickness. Finally, the radial canal system of the medusa was injected with food dye and photographed. The resulting picture was used to enumerate 12 features of the canal system, per quadrant (delimited by consecutive perradial canals). The number of originations of (*f*29) perradial canals, (*f*30) interradial canals, and (*f*31) adradial canals. The total number of anastomoses of (*f*32) perradial canals [i.e. perradial-perradial, perradial-adradial], (*f*33) interradial canals [i.e. interradial-interradial, interradial-adradial], and (*f*34) adradial canals [i.e. adradial-adradial]. Anastomoses of apparently ≥ 4 canals (*c*) might have been indistinguishable from ≥ 2 very close anastomoses each involving 3 canals and were therefore interpreted as comprising *c - 2* anastomoses separated by zero-length branches. The numbers of sinuses, i.e. branches that anastomosed at only one end, originating from the (*f*35) stomach pouch, (*f*36) perradial canal, (*f*37) interradial canal, (*f*38) adradial canals, and (*f*39) ring canal. The number of anastomoses that gave rise to (*f*40) two sinuses also was recorded, thus accounting for all branches in the radial canal system. The highly anastomosed canals marginal to the circular canal were too complex to enumerate reliably with the current methods and were therefore excluded from the analyses.

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 forty features in six stages (below). At each stage, as appropriate, it was first confirmed that bell diameters

were statistically 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. Depending on the extent of departures from normality (which often have few consequences) or homogeneity of variances (which can have severe consequences) data were excluded from further analyses (e.g. if they were invariable) or used without transformation (because initial exploration of several transformations did not solve all problems) in tests that either did not rely on normality and homoscedasticity or that were interpreted conservatively (Underwood, 1997: 194) to reduce the risk of Type I error.

(1) *Identification of sexually dimorphic features*. Forty-one female-male pairs of medusae, consisting of 25 pairs of 100 mm medusae from 6 locations (5 BJLK, 5 CLM, 5 GLK, 3 OLO, 5 OTM, 2 RCA) and 16 pairs of 150 mm medusae from 7 locations (5 CLM, 2 GLK, 3 OLO, 3 OTM, 1 RCA, 1 BJCK, 1 NCN), were compared using the paired t-test (for continuous, normally distributed data) and Wilcoxon signed ranks test (for categorical or continuous non-normal data).

(2) *Identification of variable features*. Data describing features that were not sexually dimorphic were grouped by location and compared within size-classes in a four-step process. First, boxplots describing all features were drawn to allow visual examination of diagnostic or distributional differences among populations. Second, the variances of continuous features were compared using Levene's test and, third, if variances were statistically similar the data sets were compared using ANOVA. Fourth, categorical features were examined for unique states or different frequencies of shared states. Features that were invariable according to these criteria were excluded from further analyses.

(3) *Investigation of correlations*. Comparisons involving only continuous features used Spearman's rank correlation. All other comparisons (continuous-categorical [i.e. ordinal or nominal] and categorical-categorical) were completed using contingency tables. Prior to contingency analyses, values for all continuous features were categorized into 3 even classes (using the "categorize variables" option in SPSS) in order to meet the assumption of χ^2 analyses that expected values are ≥ 5, although smaller expected values are acceptable when they are in a minority or when all are >2 (Steel & Torrie, 1980). Categorical (ordinal or nominal) values were grouped into two, three, or four classes, depending on how many states were defined when features were measured, as follows (some expected values were unavoidably low when 3 or 4 states had been defined). Bell colour (*f*1) was either absent or blue. Perradial and interradial canal colour (*f*2) was either absent or blue. Opaque flecks (*f*3) were absent, present, or common in the radial canals. Oral arm colour (*f*4) was absent, blue, white, or yellow, the last two typically being restricted to the 'fringe' of the oral arm (Figure 1). Terminal club colour (*f*5) was absent, blue, or yellow. Spots on the exumbrella (*f*6) were absent, present, common, or abundant. Spots, if not "absent", were coloured (*f*7) white, yellow, or greenish. Terminal club shape (*f*13) was categorized according to the minimum and maximum number of external angles in cross-section [circular to quadrangular, scored 0 to 4]. Intermediate filaments (*f*22) were absent or present on the oral arms and/or oral disk. The shape of the gastrovascular cavity (*f*24) was cruciform [normal] or crooked. Finally, the subgenital porticus (*f*25) was unpigmented or contained blue pigment. In the cases where character states were not mutually exclusive, for conceptual and computational convenience,

correlations among the states of the features were investigated (which approximate to correlations among features). Lack of correlation between features across all size-classes was interpreted as evidence of independence (Zelditch et al., 2000).

(4) *Elucidation of logical and partial-logical correlations*. Logical correlations were defined as those relationships that could be represented mathematically (or symbolically), at least in part, due to, for example, measurement of one feature using more than one method, covariance, or predication of one feature by another.

(5) *Recognition of meristic correlations*. By default, all non-logical correlations (i.e. correlations that were not representable mathematically or symbolically) were considered meristic correlations.

(6) *Character designation*. Based on the possible causes of partial logical and empirical correlations (e.g. development), "character" or "character-complex" status was proposed for each feature.

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 rescaled 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 five). MDS in SPSS used Euclidean distances (Sneath & Sokal, 1973: 249-250) and was considered complete when S-stress decreased by ≤ 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 Oxidase c subunit I (COI) was amplified using the primer pairs LCO1490 and AaCOIi-H [5'-ccatwgtcattccrggggcyctc] 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). All PCRs consisted of six steps of 94ºC for 8 mins, 49ºC for 2 mins, 72ºC for 3 mins, 94ºC for 4 mins, 50ºC for 2 mins, 72ºC for 2 mins, then 33 cycles of 94ºC/45s, 51ºC/45s, and 72ºC/60s, followed by

an extension step at 72ºC for 10 mins; the reaction was terminated by cooling to 4ºC. Amplified fragments were cloned using TOPO TA technology (Invitrogen), purified using Pharmacia's Flexiprep kit, labelled with BigDye and sequenced on ABI 377 automated sequencers. 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

Sexual dimorphism – All *Mastigias* < 65 mm were immature. Bell diameters within size classes did not differ between sexes (100 mm: $t_{24} = -1.014$, $p = 0.308$; 150 mm: $t_{15} = 0.493$, $p = 0.629$). There was no evidence of sexual dimorphism in any continuous feature in either size-class. The smallest *p*-value observed was 0.027, which was non-significant for $\alpha = 0.05$ after Bonferroni correction for 2 or more tests (a total of 47 paired t-tests and 21 Wilcoxon signed ranks tests were completed). Similarly, excepting the occurrence of brood filaments which was used to differentiate female and male medusae, there was no evidence of sexual dimorphism in any categorical feature of *Mastigias*; the smallest *p*-value observed was 0.041 in a total of 38 Wilcoxon signed ranks tests (19 per size class).

Variability – 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). Stepwise removal of populations then individuals from the analysis indicated that large medusae in the OLO sample were the principle source of variation and that exclusion of just the three largest OLO medusae (55 mm, 55 mm, 54 mm) produced an otherwise intact dataset in which size distributions were statistically similar among populations ($F_{9,58} = 2.018$, $p = 0.053$). Analyses of this modified 50 mm dataset gave the same pattern of significant and non-significant results as analyses of the original 50 mm dataset, although *p*-values for some comparisons differed slightly. The analyses of the original entire dataset are reported below. There were no statistically significant differences in the size distributions of medusae among populations within the 100 mm ($F_{8,55} = 1.662$, $p = 0.129$) nor, despite significantly heterogenous variances ($p = 0.038$), among populations within the 150 mm size classes ($F_{6,40} = 1.330$, $p = 0.267$).

Of the 39 quantitative and qualitative features analyzed, 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 (32 of 35 features or components) varied significantly, one varied non-significantly,

and two were constant (Fig. 2; Table 2). All eleven categorical features were variable in all size-classes bar subgenital porticus colour (*f*25) in 150 mm medusae (Table 3). Two features, *f*29 and *f*30, were invariable in all size-classes and were excluded from all subsequent analyses.

Correlations – 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 $0.40 \leq r \leq 0.88$. In the 150 mm size class, 101 of 1225 (8.2%) of comparisons were significant and $0.48 \leq r \leq 0.90$. 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 (Fig. 3). The smallest network paired together two of the three states observed for spot colour (*f*7). A larger network involved mass (*f*8), the length of the winged portion of the oral arms (*f*12), three measurements of bell depth (*f*28), and the diameter of the ring canal (*f*10), of which all but the last were linked by multiple correlations. The largest network linked three measures of terminal club morphology (*f*15, *f*13min, *f*13max), four measures of radial canal structure (*f*31, *f*32, *f*33, *f*34), and five measures of colour (*f*2, *f*3, *f*4, *f*5, *f*6).

Logical correlations – Mass (*f*8) was partially logically correlated with the lengths of unwinged and winged portions of the oral arm (*f*11, *f*12), the shape, number, and length of the terminal clubs (*f*13, *f*14, *f*15), dimensions of the oral pillars (*f*16, *f*17, *f*18), the diameter and depth of the oral disc (*f*20, *f*21), and the bell depth (*f*28) via the relationship mass = Σ(dimensions * density). The contribution of each feature to the mass, however, varied (e.g. compare the dimensions of the oral pillars and the terminal clubs) and the features were not necessarily correlated with each other (but see bell depth below), and overall mass was not a predictor of the dimensions of any particular part. Thus, only three features (ring canal diameter, length of the winged portion of the oral arms, and bell depth) were significantly correlated with mass in all three size classes and only six (oral disc diameter, the width and depth of oral pillars, the shape, length, and number of terminal clubs) were significantly correlated with mass in any size class.

The colours of different parts of the medusa were logically correlated if colour was an attribute of the individual rather than of its parts (i.e. $fC \supseteq fc$, where c was the colour of a part of the individual which was of colour C). The occurrence of (blue) pigmentation was correlated in two of four features (*f*2 and *f*5, but not *f*1 nor *f*4) and with the occurrence of exumbrellar spots (*f*6) in all size classes, but these were not correlated with the occurrence of other pigmentation, except in a subset of size classes.

Measurements describing each aspect of the structure of adradial, interradial, and perradial canals (i.e. origins, anastomoses, and sinuses) also were logically correlated if the frequency of anastomoses (or origins, or sinuses) was an attribute of the individual's radial canal system (R) rather than of adradial, interradial, or perradial parts (r) of the canal system (i.e. $fR \supseteq fr$). Empirically, the frequencies of anastomosing in adradial, interradial, and perradial canals were significantly correlated, and the frequency of adradial anastomoses was significantly correlated with the frequency of adradial origins, in all size classes. Comparisons among other features of the canal system, such among multiple measures of the frequency of sinuses, were non-significant in at least one size-class due to low frequencies and low variation (Fig. 2).

The interradial and perradial diameters of the gastrovascular cavity should predict to some extent (i.e. be logically correlated with) the shape of the gastrovascular cavity. However, these measures were not significantly correlated in any size class indicating that the measures of diameter alone were insufficient to predict whether the GVC was or was not cruciform.

Measurements of bell depth (*f*28) and oral disc depth (*f*21) at adjacent positions were logically autocorrelated. Significant correlations were found among three measures of bell depth in all size classes and among all-bar-one measures of bell depth in at least one size class; significant correlations occurred only twice among any measure of oral disc depth. States also were correlated within two other features: spot colour (*f*7) and terminal club shape (*f*13).

Meristic correlations – Features related to size were empirically correlated indicating a mechanism such as allometric growth leading to "largesse" (*sensu* Dawson, 2003; Fig. 3). Meristic correlations also were found between the length of the winged portion of the oral arm and bell depth (*f*12-*f*28), aspects of terminal club structure (*f*13-*f*15), terminal club structure and canal structure (*f*13/*f*15-*f*31/*f*32/*f*33/*f*34), terminal club length and several aspects of pigmentation (*f*15-*f*2/*f*3/*f*4/*f*5/*f*6), the number of adradial origins and adradial anastomoses (*f*31-*f*34), the number of adradial origins and spot abundance (*f*31-*f*6), the abundance of spots and pigmentation in the terminal clubs (*f*6-*f*5), and among aspects of pigmentation (*f*2-*f*5-*f*6).

Designation of characters and complexes – The logical and meristic correlations detailed above provide preliminary evidence for eight characters and seven 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 (Table 4).

Multivariate Analyses

Sixteen continuous features that were not significantly empirically correlated with any other feature—*f*11, *f*14, *f*16, *f*17, *f*18, *f*19, *f*20, *f*23, *f*26, *f*27, *f*35, *f*36, *f*37, *f*38, *f*39, *f*40—received unit weight. Other continuous features were down-weighted by dividing the rescaled observed value by the number of significant correlations within complexes and the number of repeat measures (Table 4), as follows (feature/[number-of-correlations-within-complex*number-of-repeat-measures]): *f*8/[4*1], *f*10/[4*1], *f*12/[4*1], *f*15/[2*1], *f*21/[1*4], *f*28/[4*5], *f*31/[4*1], *f*32/[4*1], *f*33/[4*1], *f*34/[4*1]. Multi-dimensional Scaling (MDS) of the rescaled, weighted, characters and character-complexes summarised the morphological differences among *Mastigias* (Fig. 4) 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. Notably, MDS-plots showed considerable overlap between populations in the smallest, 50 mm, size-class but greater segregation in the larger (100 mm 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. 4). Down-weighting all sinus characters by a factor of six—to explore the effect of designating a single "sinus" character complex in which *f*35 to *f*40 constitute six repeat measures—did not change the results shown in Fig. 4 (e.g. Fig. 7).

The independent categorical features *f*1, *f*3, *f*24, and *f*25 received unit weight while the remaining seven categorical features were downweighted as follows

(feature/[number-of-correlations-within-complex*number-of-repeat-measures]): *f*2/[3*1], *f*4/[1*3], *f*5/[6*2], *f*6/[3*1], *f*7/[1*3], *f*13/[4*2] (repeat measures for *f*13 were minimum and maximum shapes), *f*22/[1*2]. CATPCA plots of the weighted data summarised graphically the morphological differences among *Mastigias* evident in categorical data (Fig. 5; see Table 3). 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% (*f*6) and 3% (*f*5y) of the total loading, respectively. In contrast dimension 2, along which the maximum and minimum loadings were 30% (*f*25) and <0.1% (*f*2) of the total loading, respectively, was strongly influenced by just three components (*f*25, *f*3 [24%], *f*1 [10%]; remainder ≤6%). In the 100 mm size-class, the maximum and minimum loadings along dimension 1 were 10% (*f*6) and 2% (*f*25) of the total, respectively, and along dimension 2 they were 41% (*f*25) and <0.1% (*f*24); dimension 2 was again strongly influenced by just three components (*f*25, *f*1 [29%], *f*2 [20%]; remainder ≤9%). In the 150 mm size-class, the maximum and minimum loadings were 11% (*f*6) and 1% (*f*13max) along dimension 1, and 21% (*f*1) and 0.7% (*f*7y) along dimension 2; dimension 2 was moderately influenced by four components (*f*1, *f*3 [11%], *f*4y [10%], *f*5y [10%]; remainder ≤6%).

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 (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 PNG 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%).