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Phenotypic variation in the coral *Platygyra daedalea* in Kenya: morphometry and genetics

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ABSTRACT: High intraspecific variability and lack of adequate field descriptions or distinguishing skeletal features has made identification of the scleractinian coral *Platygyra daedalea* challenging. This species displays a number of distinct morphological types that co-occur on lagoonal reefs in Kenya and which often cannot be separated by field observations. To better understand how morphological and genetic variations are related, morphometric and molecular techniques were used to examine phenotypic variation in *P. daedalea*. A canonical discriminant analysis of measurements of 10 skeletal characters confirmed the existence of 2 morphotypes. No single diagnostic trait could be used to distinguish the 2 morphotypes, though a combination of 4 characters separated them. A mathematical equation is presented to separate colonies into the 2 morphotypes, where field identification is not possible. Genetic differentiation was studied using 5 microsatellite loci and sequence analysis of the internal transcriber spacer (ITS1 and ITS2) and 5.8S region of the nuclear ribosomal RNA gene. AMOVA of the microsatellite and ITS sequence data showed significant genetic differences between the 2 morphotypes. However, phylogenetic analysis of the ITS sequences showed no evidence of sequence divergence between morphotypes, which suggests that they share a gene pool, or that the genetic divergence is recent. We conclude that the occurrence of distinct morphotypes is a characteristic of *P. daedalea* and that there does appear to be a genetic basis for separating morphotypes. However, genetic differences in *P. daedalea* could only be detected when combined with morphometric data.

KEY WORDS: Coral reefs · Taxonomy · Morphotypes · Indian Ocean · *Platygyra daedalea*

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INTRODUCTION

Traditionally, the species unit has formed the basis for understanding ecology, evolutionary biology and biogeography. However, over recent decades increasing inclusion of novel molecular techniques in phylogeny has led to several cases where current classifications have been brought into question. Well-established species with clear differences in morphology and ecology may be almost indistinguishable at a genetic level. For example, the Old World buzzard group (birds of prey, genus *Buteo*) includes a plethora of species and subspecies based on variable characters such as plumage colour and body size, but almost no sequence variability was found in the pseudo-control region of mitochondrial DNA (Kruckenhauser et al. 2003). In contrast, there are cases where conservative morphological taxonomy has underestimated species diversity, as in the deep-sea mussel *Acharax* sp., which was thought to be a single species until molecular studies allowed separation into 2 distinct genetic clusters (Neulinger et al. 2006). In scleractinian corals, the high degree of intraspecific phenotypic variability makes taxonomic resolution of species in the field subjective (Veron 2000). Corals show morphological plasticity in response to habitat and environment variables (Foster 1979, Lang 1984). Field identification is further confounded by the potential for some species of coral to cross-fertilize (Willis et al. 1997) and form viable hybrids with distinct morphologies (Vollmer & Palumbi

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2002), or to form distinct morphological types ('morphotypes') within a species (Miller 1994).

Scleractinian coral taxonomy has been based on macroscopic and microscopic skeletal morphology (Vaughan & Wells 1943, Veron et al. 1977, Wallace 1999) on the premise that morphological discontinuities determine species divisions. While morphometric approaches have been successful in distinguishing many coral genera and species (e.g. Wallace 1974, Veron & Wallace 1984), difficulties have arisen in their applicability to taxa such as those of the Family Faviidae, where many of the skeletal characteristics overlap between different species from similar or different habitats (Wijsman-Best 1974, Veron et al. 1977, Veron 2000). Genetic studies in some cases have supported original classifications based on morphology (Ayre et al. 1991, Maté 2003), while in others they have led to the separation of intraspecific morphological groups into distinct species (Stobart & Benzie 1994, Stobart 2000). Hence, the integration of morphometric and molecular studies has not consistently demonstrated whether morphological species of corals should be separated or amalgamated.

High intraspecific variation occurs in *Platygyra* spp. on the Great Barrier Reef (GBR), Australia (Miller 1994). Seven morphotypes described by Miller (1994) did not correspond to any particular habitat type (Miller 1992, 1994). *Platygyra daedalea* displayed 4 distinct morphotypes on the GBR, and the skeletal characters of *P. daedalea* and *P. sinensis* differed consistently and significantly. Allozyme electrophoretic surveys of *P. daedalea, P. sinensis* and *P. pini* at 9 loci showed no relationship between genotype and morphological groupings at the species or intraspecies level, suggesting morphological species of *Platygyra* share a common gene pool (Miller 1992, Miller & Benzie 1997). In contrast, a study in Hong Kong on *P. pini* and 4 morphotypes of *P. sinensis* found genetic differences between the 2 species using ITS1, 5.8S and partial ITS2 rRNA sequence analysis, but not between morphotypes, with no diagnostic characters or clear groupings in morphological traits (Lam & Morton 2003). These conflicting results suggest that the use of more variable or rapidly evolving molecular markers may be more appropriate for distinguishing between similar species, and even more so when comparing 2 morphotypes of the same species. The disparities may also simply reflect the difficulties associated with field identification of these species.

Of the 8 species of *Platygyra* that have been recorded in Kenya, *P. daedalea* is dominant in lagoonal and fringing reefs, with a wide distribution along the coast (D. Obura unpubl. data). It is an important species in Kenya, particularly since it has shown a certain level of resistance to mass bleaching events, such as those in the western Indian Ocean during the 1997 to 1998 El-Niño Southern Oscillation (Obura 2001). Colonies within the Mombasa lagoon are massive, often hemispherical in form and show a variety of characters that are recognisable in the field. At the colony level, there is consistency in growth form, but at the corallite-level, colonies vary considerably in outward appearance, for example in septa shape, wall height, wall slope and the length and width of valleys (S. Mangubhai pers. obs.).

Detailed skeletal or molecular studies on corals are time-consuming, costly and not practical for routine ecological studies. Most researchers rely heavily on field guides, so it is important to define the variety of physical features a species such as *Platygyra daedalea* displays in different localities to investigate whether specific morphotypes do exist, their potential ecological role, and to determine whether there is a genetic basis for separating them. Where intermediate forms exist, quantitative methods are needed that are less subjective than univariate approaches and can distinguish colonies into specific morphotypes (Miller 1994). The purpose of our study was to (1) identify intraspecific morphological variation in *P. daedalea,* expressed in both the field observable traits and skeletal morphology, and to determine its relationship with genotypic traits, (2) develop a methodology for distinguishing different forms of *P. daedalea* into specific morphotypes, and (3) assess the level of molecular distinction between morphotypes using microsatellite markers and sequence variation at 2 ITS regions of the rRNA gene. By examining relationships between field and skeletal morphologies and genetic traits, we can start to understand intraspecific variation and taxonomic boundaries in *P. daedalea* and hence reduce the possibility of taxonomic error (Miller 1992, 1994).

MATERIALS AND METHODS

Field identification. Adult colonies of *Platygyra daedalea* were identified in the field in accordance with Veron et al. (1977) and Veron (2000) at 3 patch reefs in the Mombasa Marine National Park and Reserve, Kenya between 2003 and 2005 (Fig. 1). Kijembe and Nyali Reefs are separated by about 250 m, and Coral Gardens is situated approximately 7 km north of Nyali Reef. All 3 sites were in the lagoon at 0.5 to 1.5 m mean low water depth and experienced similar temperature and tidal regimes (S. Mangubhai unpubl. data). Patch reefs within the Mombasa lagoon are fairly uniform, and the benthic cover and coral community structure and composition were similar at the 3 sites (S. Mangubhai, unpubl. data). Thirty-nine corals were selected at Coral Gardens (3°59′26″S,

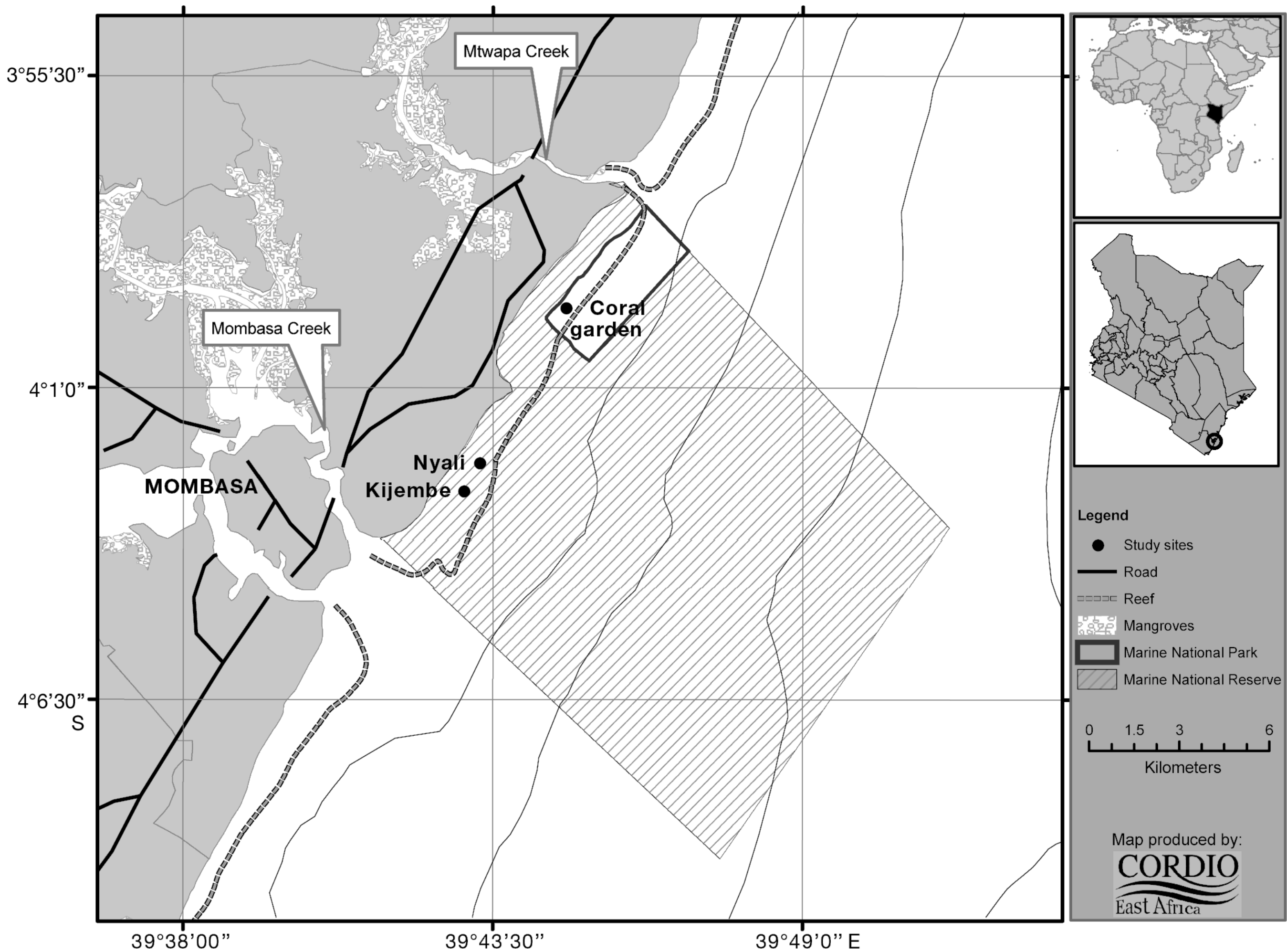

Fig. 1. *Platygyra daedalea.* Study sites in the Mombasa Marine National Park and Reserve in Kenya

39° 45′ 1″ E), 48 at Nyali Reef (4° 3′ 44″ S, 39° 42′ 44″ E) and 46 at Kijembe Reef (4° 3′ 37″ S, 39° 42′ 44″ E) with the aim of including the full range of phenotypic traits or 'morphological varieties' observed in the lagoon.

Only 4 characters, viz. wall height, septa slope, septa shape and the prominence of the wall relative to the valley were useful in distinguishing corals into groups recognisable in the field, and on this basis 2 distinct morphotypes were identified *a priori*: (1) prominent valley (PDV), i.e. colonies with septa of mixed sizes and spacing, with short to medium walls that slope, and valleys that are more prominent (and appear wider) than the walls (Fig. 2a,b); while colour is not a useful distinguishing trait, in many instances this morphotype has brown walls and green valleys; (2) straight-walled (PDSW) i.e. colonies have septa of equal size and even spacing, with tall and straight walls, such that the walls are more prominent (and partially hide) the valleys; growth form is more consistent in this morphotype, is mostly hemispherical, and in most instances wall and valley colours are similar (beige/cream), with a distinctive ridge forming at the top of the wall (Fig. 2c,d).

Many corals had field characteristics that were a mixture of PDV and PDSW morphotypes, and these were classified as 'intermediate forms' and subdivided into 3 smaller groups: E with uneven septa, wall either short or medium in height, walls and valleys equally prominent; F with even septa, walls straight and medium height, wall equal or more prominent than valley; and H including the remaining corals with a mix of many characters that did not allow assignment into larger groupings. Of the 133 colonies sampled, 27 were identified in the field and by photographs as PDV, 18 as PDSW, and 88 as intermediate forms.

Skeletal characters. Samples for morphometric analysis of the skeletons were approximately 10 × 10 cm. They were removed from colonies using a hammer and chisel. We avoided injured areas and the growing edge of the colonies, which can have atypical skeletal char-

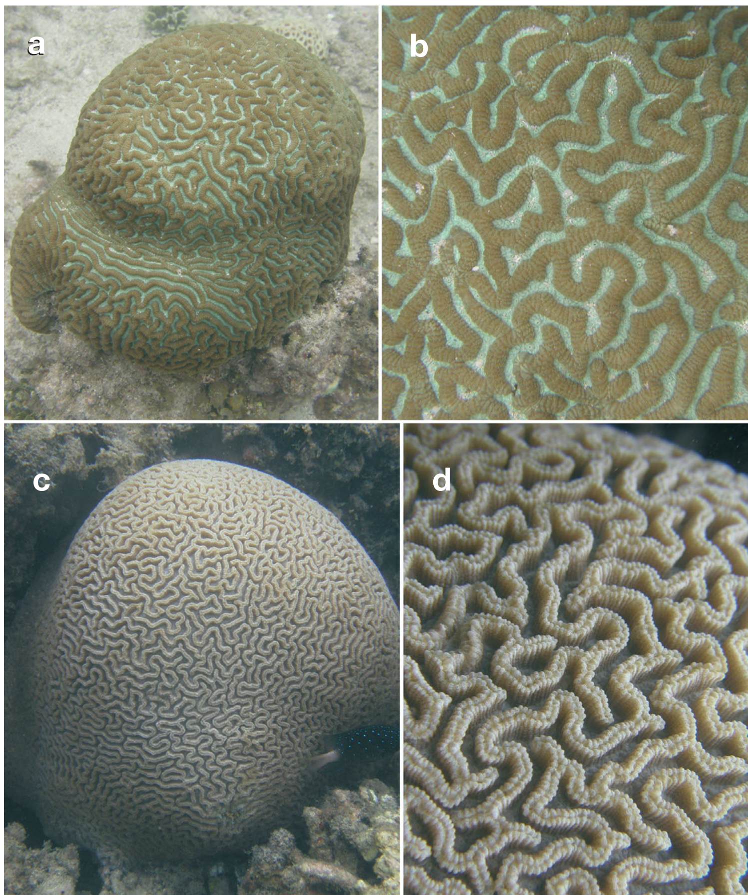

Fig. 2. *Platygyra daedalea*. Morphotypes found in Kenyan lagoonal reefs. (a,b) Prominent valley (PDV, max. colony diameter 49 cm); (c,d) straight-walled form (PDSW, max. colony diameter 75 cm)

acters (Miller 1994). Nine skeletal characters defined by Miller (1994) were selected for this study, viz. valley length (VL), valley width (VW), valley depth (VD), columella width (CW), septa thickness (ST), theca thickness (TT), exertness of septa (ES), polyp area (PA), and number of septa per cm length (SCM). A tenth character, wall width (WW), defined as the length of individual septa that formed the width of a wall, was also added (Fig. 3). Numerical values were averaged across a minimum of 5 (and where time permitted, 10) measurements taken randomly on the skeletal piece under a stereomicroscope with a calibrated eyepiece micrometer. Valley length and polyp area were measured from photographs of the colony (with a scale in the image frame) and in the software program ImageJ 1.32j (available at http://rsb.info.nih.gov/ij/download.html).

All analyses were performed on the mean values of the skeletal characters measured on each colony. Only colonies that were clearly identified in the field as PDV (n = 27) or PDSW (n = 18) were included in morphometric analyses to distinguish these morphotypes. We used t-tests to examine differences between PDV and PDSW for each of the 10 characters, and all p values <0.05 were considered significant. A canonical discriminant analysis (CDA) was performed on the 10 skeletal characters to determine whether they separated the 2 morphotypes, and if so, which best discriminated between the 2. All characters were normally distributed except for PA, which had slightly skewed data ($p < 0.01$, Kolmogorov-Smirnov test). Given that CDA is robust to deviations from normality, PA was included the analysis. The CDA generated a function to produce discriminant scores, which was then used to predict how closely related the remaining intermediate forms were to PDV or PDSW. All analyses were done in SPSS 11.0 for Windows.

Genetics. A total of 69 colonies of *Platygyra daedalea* were selected in May 2005 for genetic analyses. At the time of collection, morphometric analyses of the skeletons had not been completed and colonies were identified in the field as 25 PDV, 5 PDSW and 39 intermediate forms. Following morphometric analyses, genetic samples were correctly labelled and analysed as follows: 59 PDV (Coral Gardens n = 21, Nyali Reef n = 20, Kijembe Reef n = 18) and 10 PDSW (Coral Gardens n = 4, Nyali Reef

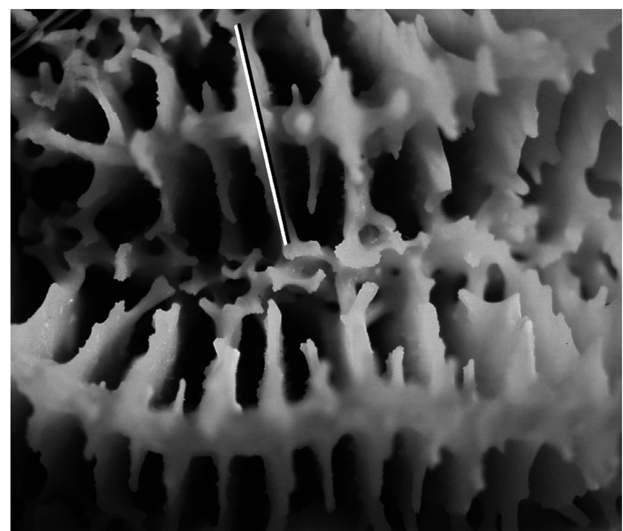

Fig. 3. *Platygyra daedalea*. Skeleton: line (scale: 4 mm) indicates how wall width was measured. See Fig. 1 in Miller (1994) for diagrammatic representation of remaining 9 skeletal characters used in this study

n = 6). Small pieces, approximately 1 cm^2, were removed from the 69 colonies using a hammer and chisel and placed in separate labelled Ziploc® bags. The samples were subsequently transferred into 10 ml centrifuge tubes filled with 70% ethanol and kept at ambient temperature until transported back to the laboratory.

DNA was extracted using the Qiagen® DNEasy kit according to the protocol for rodent tails. Fragments were placed directly into lysis buffer and kept in a water bath at 50°C overnight. We amplified a fragment of nuclear ribosomal DNA (rRNA) that has been used in a number of genetic studies of scleractinian corals (Takabayashi et al. 1998, Lam & Morton 2003) using the coral-specific forward primer A18S (Takabayashi et al. 1998) (GenBank accession number X53498) and the universal reverse primer ITS4. The Polymerase Chain Reaction (PCR) was carried out in a 20 µl reaction using 100 ng DNA, 0.75 U AmpliTaq, 0.5 µl of each primer (conc. 10 ng μl^{-1}) and a concentration of 0.5 mM of each dNTP, and 0.15 mM of $MgCl_2$. PCR products were purified and sequenced by Macrogen Inc., using the same primers that had been used for the PCR reaction.

Given that microsatellite loci are more polymorphic and evolve more rapidly than other DNAs such as rDNA (Baker 2000), they may show a higher degree of divergence between morphotypes or potentially unique alleles, provided there is some reproductive barrier in place. For this study we used 5 fluorescently labelled microsatellite primers developed for *Platygyra* species (Miller & Howard 2004). The PCR was carried out in a 10 µl reaction using 25 ng of DNA, 0.5 U AmpliTaq, 0.25 mM of each dNTP, and 0.5 mM of each primer. Cycling protocol was initiated with 5 min at 94°C followed by 30 cycles of 94°C for 30 s, 54 to 60°C (locus Pd29 at 54°C, Pd31 at 56°C, Pd48 at 60°C, Pd61 at 58°C, Pd62 at 57°C) for 30 s, and 72°C for 1 min, and finalised with an extra extension period at 72°C for 10 min. PCR products, as well as positive and negative controls, were genotyped on an ABI 3700 capillary sequencer (Applied Biosystems) using a Gene Scan-500 Rox size standard provided by ABI, and visualised using GeneMapper v. 3.7 (Applied Biosystems). One microsatellite locus, Pd29, was found to be monomorphic and was subsequently excluded from analysis.

The ITS rRNA sequences were aligned manually using BioEdit v. 7.0 (Hall 1999) and exported to MEGA v. 3.1 (Kumar et al. 2004), which was used to reconstruct a phylogeny of the individuals based on the Maximum Parsimony optimality criterion with heuristic search. To align our genetic samples with a previous study of *Platygyra* species from Hong Kong, we included sequences of each morphotype of *P. sinensis* (GenBank accession numbers AF481885, AF481888, AF481895) and *P. pini* from the same subfamily (GenBank accession number AF481902) (Lam & Morton 2003). *Montastrea franksi*, which belongs to the same family but different subfamily (GenBank accession number AB065349) was used as an outgroup. Branch support levels were estimated using 1000 bootstraps. Sequence divergence was estimated using Kimura's 2-parameter model (Kimura 1980).

To examine genetic differences between the 2 morphotypes both within each site and after pooling the sites, and to assess the difference between the study sites with morphotypes pooled, an Analysis of Molecular Variance (AMOVA) was done using the programme Arlequin (Laval & Schneider 2005) with both ITS and microsatellite data. The genetic distance method used by the AMOVA was pair-wise population subdivision (F_{ST}), which was estimated according to Cockerham & Weir (1984). The significance values were calculated using 20000 non-parametric permutations. This procedure is appropriate when dealing with unbalanced sample sizes, as it does not assume normality or equality of variances among samples.

As PDV was found to be phenotypically more variable, we investigated whether this variability was reflected at the molecular level by assessing average molecular diversity over loci for each morphotype (using Arlequin), in accordance with Tajima (1983, 1993). Hardy-Weinberg equilibrium tests were performed to gauge population structures, and assignment tests were conducted on the log-likelihood of individual genotypes being classified correctly as PDV or PDSW, as predicted by the CDA. The significance values for the Hardy-Weinberg tests and F_{ST} values were calculated using permutation tests (Laval & Schneider 2005).

The programme Structure v. 2.0 (Pritchard et al. 2000) was used to examine possible subpopulations by assigning individuals to clusters according to their 4 –loci microsatellite genotypes. The underlying hypothesis was that the sampled individuals should cluster into the 2 morphotypes, or alternatively into the 3 sample sites. A series of analyses at a range of K-values (1 – 4) was done to ascertain the most probable number of clusters. A burn-in of 25000, and a 250000 run length were used.

RESULTS

Morphometrics

A comparison of mean values and their ranges for PDV and PDSW for each of the 10 skeletal characters found that there was no single diagnostic character suitable for separating the morphotypes (Table 1). There were significant differences between the 2

Table 1. *Platygyra daedalea.* Mean values of 10 skeletal characters for all colonies and for morphotypes PDV and PDSW. p values generated by *F*-statistics apply to comparisons of morphotypes PDV and PDSW. All measurements in mm, except polyp area in cm^2

Character	Code	*P. daedalea*	PDV	PDSW	p
No. of septa cm^{-1} length	SCM	12.9 (8.8 – 15.7)	13.4 (11.0 – 14.9)	11.2 (8.8 – 13.4)	<0.001
Wall width	WW	4.0 (2.9 – 5.1)	3.7 (3.2 – 4.3)	4.4 (3.8 – 5.1)	<0.001
Polyp area	PA	11.4 (6.4 – 28.7)	9.7 (6.6 – 12.3)	15.4 (10.4 – 28.7)	<0.001
Valley depth	VD	4.3 (2.7 – 7.2)	4.0 (3.0 – 6.4)	5.2 (3.4 – 7.2)	<0.001
Valley width	VW	5.6 (3.9 – 7.2)	5.4 (4.1 – 7.1)	6.2 (5.5 – 7.2)	<0.001
Valley length	VL	5.9 (1.4 – 12.1)	5.5 (1.6 – 11.6)	7.3 (2.2 – 11.8)	0.018
Corallum width	CW	1.1 (0.8 – 1.5)	1.1 (0.8 – 1.4)	1.1 (0.9 – 1.4)	0.055
Septa thickness	ST	0.2 (0.1 – 0.4)	0.2 (0.1 – 0.2)	0.2 (0.1 – 0.4)	0.097
Theca thickness	TT	0.9 (0.5 – 1.7)	0.9 (0.6 – 1.3)	0.1 (0.5 – 1.2)	0.134
Exertness of septa	ES	1.0 (0.3 – 2.4)	1.0 (0.4 – 2.0)	0.8 (0.3 – 1.5)	0.162
Number of colonies		133	27	18	

morphotypes for 6 of 10 characters (PA, SCM, VD, VW, WW and VL, Table 1).

A stepwise CDA identified the 4 skeletal variables SCM, WW, PA and ST as the best set of predictors of membership in the 2 groupings PDV and PDSW. One discriminant function was produced with an eigenvalue of 1.806, which accounted for 100% of the variance among the 4 variables. Discriminant scores for the function were highly correlated with the groupings (r^2 = 0.64). Standardised coefficients showed all 4 characters contributed to the discriminant function in roughly equal proportions, with SCM being inversely related to the other 3 characters (Table 2).

A pooled within-groups covariance matrix classification procedure for the 2 morphotypes was done, where membership in groups was based on prior probabilities that took into account different group sizes. The classification procedure showed that 26 (96.3%) colonies of PDV and 15 colonies of PDSW (83.3%) were correctly identified (Table 3). Overall, 41 of the 45 of colonies (91.1%) were correctly identified. Cross-validation (where each colony in the analysis is classified by the functions derived from all colonies other than that colony) was also performed to test whether the original classification procedure provided overly-optimistic estimates of group membership. The test showed there was little difference between the original and cross-validation classifications (i.e. only one coral changed its grouping), and therefore the original classification scheme was robust and the number of variables used in the model was correct (Table 3).

Table 2. *Platygyra daedalea*. The unstandardised and standardised discriminant function coefficients generated by the CDA for morphotypes PDV and PDSW colonies. See Table 1 for character codes

Characters	Unstandardised	Standardised
WW	1.678	0.578
ST	10.090	0.424
PA	0.122	0.423
SCM	–0.319	–0.361
Constant	–5.903	

Table 3. *Platygyra daedalea*. The percentages of corals correctly and incorrectly classified into the 2 morphotypes PDV and PDSW by the canonical discriminant analysis. Numbers of corals in parentheses

Classification	Morphotype	Correct	Incorrect	Overall success (%)
Original	PDV	96.3 (26)	3.7 (1)	91.1 (45)
	PDSW	83.3 (15)	6.7 (3)	
Cross-validated	PDV	96.3 (26)	3.7 (1)	88.9 (45)
	PDSW	77.8 (14)	22.2 (4)	

A discriminant function coefficient matrix was produced from the CDA solution on the 4 diagnostic variables (Table 2), and the unstandardised coefficient values were used to calculate the discriminant score (DS) for each intermediate form colony using the equation below:

$$DS = -5.903 + (WW \times 1.678) + (ST \times 10.09) + (PA \times 0.122) - (SCM \times 0.319)$$

Unstandardised coefficients were selected to enable application of the function to new data using raw variables. A bi-plot of the discriminant scores for PDV, PDSW and intermediate forms was produced (Fig. 4). The 4 incorrectly grouped colonies (identified by classification procedure) were responsible for observable overlap between morphotypes. Once colonies were reassigned to the group designated by the classification procedure, it was clear that all negative discriminant scores corresponded to PDV, and all positive scores corresponded to PDSW, and there was no large gap between the 2 morphotypes' scores (Fig. 4).

Discriminant scores calculated for each intermediate form showed 78.4% of colonies to be PDV and 21.6% to be PDSW (Table 4). Almost all form E

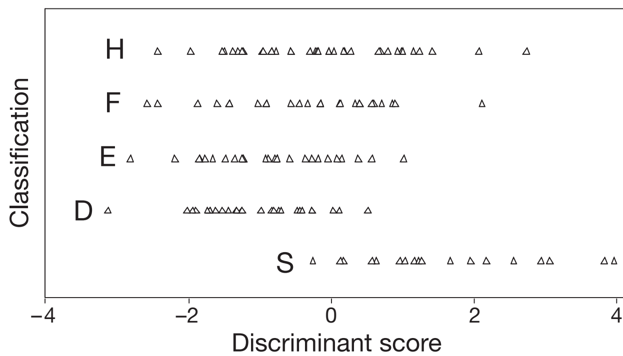

Fig. 4. *Platygyra daedalea*. Groupings by discriminant scores. S: PDSW; D: PDV; E, F, H: intermediate forms

colonies belonged to PDV (92.3%) and only 2 colonies were misidentified in the field. By contrast, colonies belonging to forms F and H were more difficult to classify into the 2 morphotypes with accuracy, based on field identifications alone (Table 4). The inclusion of form E within PDV means that the field description for PDV should read 'colonies have septa of mixed sizes and spacing, with short to medium walls that slope, and valleys that are more prominent (and appear wider) **or are equal to** the walls'. These results show PDV to be the dominant morphotype in Kenyan lagoonal reefs, with 73.7% of all corals in this study belonging to this group.

ITS sequencing

The rRNA fragment was successfully sequenced to include the ITS1, 5.8S and the complete ITS2 region in 52 individuals, of which 10 were classified as PDSW and 42 as PDV by the CDA. Sequences of the remaining 17 individuals (16 PDV and 1 PDSW) were poor or incomplete and were excluded from further analysis. No specific morphotype or sample site was dominant among the excluded genetic samples.

The sequence aligned 639 positions of which 72 were variable and 29 were potentially parsimony informative. Cloning was not performed as we found no evidence of sequence heterogeneity at any of the parsimony informative sites that were used to infer the phylogenetic tree. The complete sequence contained less than 2% ambiguous sites. The ITS1 region had an average length of 209 bases and varied between 207 and 210. One colony from Kijembe Reef had an additional insertion of 18 bases. The 5.8S gene comprised 168 positions and the ITS2 region varied between 210 and 218 positions. A total of 31 haplotypes were found. There were 9 haplotypes among 10 individuals of PDSW, and 22 haplotypes among 40 individuals of PDV, with no identical haplotypes shared between the 2 morphotypes. Parsimony tests were constructed for the complete rDNA sequence. The average nucleotide substitution per site, measured as Kimura's 2-parameter mean distance, was 0.018 ± 0.003 SD between PDV and PDSW, and 0.020 ± 0.005 between Kenyan samples and the *Platygyra sinensis* sequences derived from GenBank. No inferred parsimonious tree grouped PDV and PDSW separately, nor did it separate them from sequences of *P. sinensis* (Fig. 5).

Table 4. *Platygyra daedalea*. Percentages of intermediate forms (E, F, H) classified into 2 morphotypes. Numbers of corals indicated in parentheses

Group	PDV	PDSW
E	92.3 (24)	7.7 (2)
F	76.9 (20)	23.1 (6)
H	69.4 (25)	30.6 (11)
Overall	78.4 (69)	21.6 (19)

Contrary to the phylogenetic analysis, AMOVAs on the sequence data revealed a significant genetic difference between PDV and PDSW, where 22% of the genetic variation was explained by the difference between the 2 morphotypes when treating all sampling sites as one population (F_{ST} = 0.20, p = 0.017). There was no genetic structure between populations at the different sites (p = 0.604).

Microsatellites

Microsatellite data revealed a slight difference in genetic diversity measured by expected heterozygosity (Hs) over loci between the 2 morphotypes (PDV 0.26 ± 0.184 SD, PDSW 0.37 ± 0.24 SD). Two of 5 loci had significant excesses of heterozygotes in PDV, whereas all loci were in Hardy-Weinberg equilibrium in PDSW. In concordance with the ITS sequence analysis, AMOVAs showed significant subdivisions both between morphotypes within sites (F_{ST} = 0.055, p = 0.016) and between morphotypes when pooling sampling sites (F_{ST} = 0.028, p = 0.040). When doing the AMOVA on each locus separately, it becomes apparent that this difference is caused by a significant differentiation at one locus, Pd 48 (F_{ST} = 0.335, p < 0.0001). None of the other loci showed signs of differentiation. No significant difference was found between sampling sites when pooling morphotypes (F_{ST} = −0.041, p = 0.60) or between sites within each morphotype (PDSW F_{ST} = −0.15, p = 0.89; PDV F_{ST} = 0.001, p = 0.30).

An assignment test performed using the programme Arlequin on the log-likelihood of individual multiloci genotypes, correctly assigned 87% of the colonies to

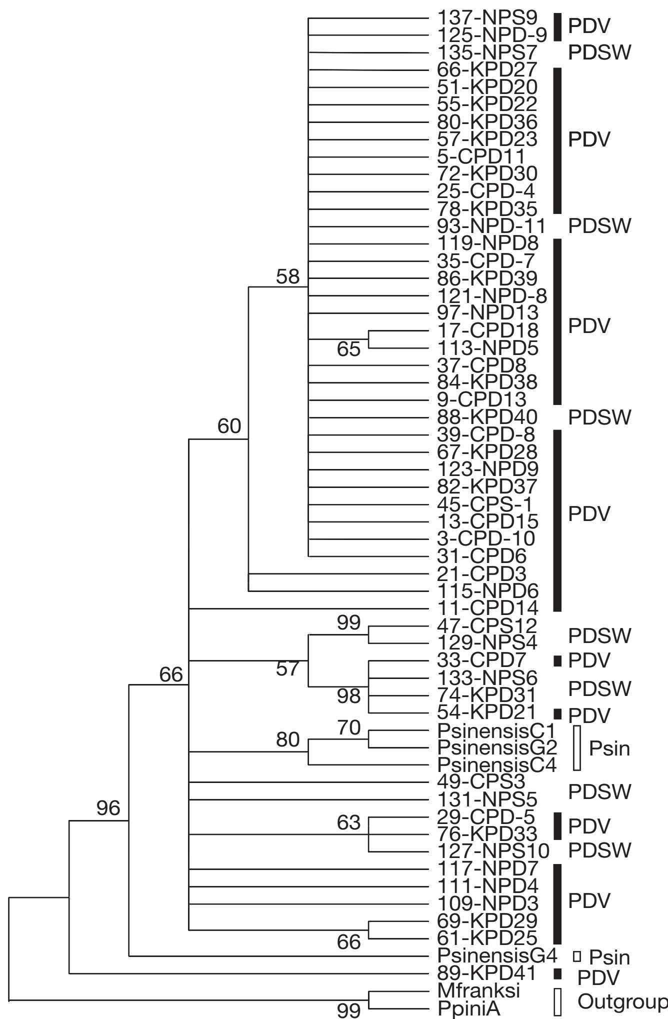

Fig. 5. One of 297 equally parsimonious trees of the complete gene ITS–1 + 5.8S + ITS–2 constructed using maximum parsimony with 1000 replicates and 1000 bootstraps. Initial tree inferred by random addition. Individual samples are named according to sample site and field identification (CPD/S – Coral Gardens PDV/PDSW; NPD/S – Nyali PDV/PDSW; KPD/S – Kijembe PDV/PDSV). Psinensis: *Platygyra sinensis*; Mfranksi: *Montastrea franksi*; Ppini: *P. pini*

the same morphotype designated by the morphometric analysis (94% of PDV and 63% of PDSW). Nine individuals were assigned differently to morphotype by the genetic assignment test versus the morphometric analyses. Six of these individuals were originally classified in the field as intermediate forms.

When using only microsatellite genetic data and no prior information on morphotypes, the Bayesian population cluster analysis failed to assign any individuals to specific clusters, and the maximum likelihood values were almost identical for models of K = 1 to K = 4 (where K = the inferred number of subpopulations in the data set). The programme Structure was also unable to assign the 39 colonies identified in the field as intermediate forms to either of the 2 morphotypes when using the microsatellite genotypic information of colonies classified in the field as PDC or PDSW. However, after the genetic samples were relabelled following morphometric analysis, we found that the model for K = 2 had the highest maximum likelihood and the programme successfully assigned 98% of PDV and 97% of PDSW to correct morphotype.

DISCUSSION

Intraspecific phenotypic variability in scleractinian corals occurs in response to environmental variables (Wijsman-Best 1974, Foster 1980) and genetic differences (Willis & Ayre 1985), or a combination of both (Foster 1979, Miller 1992). In Kenya, 2 morphotypes of *Platygyra daedalea* were identified in a single lagoonal habitat type, and these observations were confirmed through morphometric studies. PDV was the dominant morphotype in the lagoon, comprising 73.7% of the colonies sampled. Skeletal variation was continuous between the 2 morphotypes, and while there was no single diagnostic trait that could be used to distinguish between PDV and PDSW, there were significant differences in 6 of the 10 skeletal characters. These findings contrast with Miller (1994) who found that significant differences could only be detected between morphological species on the GBR when 9 skeletal characters were considered simultaneously.

With the exception of polyp area and valley length, the mean skeletal measurements and their ranges were consistent with those reported for *Platygyra daedalea* on the GBR (Miller 1994). The narrower range in values in Kenyan colonies most likely reflects the selection of one habitat type for this study, compared to 4 in the GBR study. The overlapping ranges in mean measurements between the different GBR morphotypes means it is not possible to determine whether PDV and PDSW correspond to any of the morphotypes described by Miller (1994) without running a CDA with the data from both regions. Valley length has been used to differentiate between species of *Platygyra* (Veron et al. 1977, Veron 2000), but was not found to be a useful trait for describing Kenyan colonies, which have valley lengths comparable to *P. ryukyuensis* and *P. pini* on the GBR (Miller 1994).

The field descriptions for PDV and PDSW, and the CDA-generated mathematical equation presented in this paper can be used by other researchers in Kenya separately, or in combination, to assign *Platygyra daedalea* colonies to one of the 2 morphotypes. Measurements of 4 skeletal characters were adequate to distinguish between morphotypes. The CDA classification

procedure is currently under-utilised by taxonomists, and this study has shown its value in separating taxonomic groups that have overlapping skeletal characters, such as corals belonging to the Family Faviidae.

Phylogenetic analysis of the rRNA ITS sequences revealed no consistent grouping pattern for the 2 morphotypes of *Platygyra daedalea* in Kenya (Fig. 5). However, both microsatellite and sequence data indicate that there is a small, but significant genetic difference between the 2 morphotypes, though not between study sites, indicating that morphotypes are more genetically separated than sampling sites within the Mombasa Marine National Park and Reserve. In addition, ITS sequence data indicate that these 52 samples of *P. daedalea* are genetically indistinguishable from published sequences from 4 genetic clusters of *P. sinensis* from Hong Kong (Lam & Morton 2003), raising the issue of the taxonomic status of the *P. daedalea/ sinensis* species complex, or the validity of using ITS sequences to separate species.

An earlier study of the phylogenetic relationships between species of *Platygyra* used allozymes as genetic markers and found morphological, but no clear genetic differences between species (Miller & Benzie 1997). As it is often assumed that allozymes are non-neutral markers, and therefore potentially under the influence of stabilising selection, they have relatively low mutation rates (Hartl & Clark 1997). Also, most mutations that occur in allozyme markers are silent, and as such will not cause a difference in motility on an electrophoretic gel, and hence will be undetected. This will decrease the signals of divergence and increase the chances of not detecting a genotypic difference that may exist.

In contrast, ITS sequencing found genetic distinctions, but no clear morphological clustering was found between *Platygyra sinensis* and *P. pini* in Hong Kong (Lam & Morton 2003). We found morphological differences, but no clear genetic differences using both ITS sequences and microsatellites within the Kenyan corals. The lack of morphological clustering in the Hong Kong study may be attributable to their method of choice for morphometric analysis, which differed from the CDA used by Miller (1994) and our study. Based on these 3 analyses, it appears that the ITS region is sufficiently divergent to be useful at the species level, but may not be variable enough to detect genetic differences between morphotypes with a current or recently shared gene pool.

The ITS sequences presented here are highly diverse, with a large proportion of unique haplotypes, consistent with other studies of corals (van Oppen et al. 2002, Marquez et al. 2003, Fukami et al. 2004). The rate of evolution of nuclear rRNA is believed to allow for differences between species to accumulate through genetic drift, while maintaining similarities between populations within species (Hillis & Dixon 1991). The use of ITS sequences for phylogenetic and population genetic studies of corals has been questioned based on evidence that corals display a faster rate of speciation than the concerted evolution of the rDNA, resulting in shared ancient rDNA lineages which would obscure processes such as introgressive hybridisation (Vollmer & Palumbi 2004). However, in the present study the combined results from the analysis of microsatellites and rDNA sequences support the existence of 2 morphotypes of *Platygyra daedalea* that have evolved and existed in sympatry. While it is not possible to distinguish between these morphotypes using molecular markers alone, in combination with morphological data a significant genetic division is apparent. We used only 4 variable microsatellite loci. Additional variable markers would probably improve the chances of discriminating between the 2 morphotypes based on genetics alone. Individual AMOVA of each microsatellite locus shows that one locus, Pd48, is solely responsible for the inferred differentiation. Hence, it may be speculated that this locus is linked to a gene that is under differential selection in the 2 morphotypes.

It is not clear how morphological boundaries are maintained in *Platygyra* species or morphotypes, with earlier studies showing there are no apparent gamete-level barriers to fertilisation between species on the GBR (Miller & Babcock 1997). A recent study in Kenya suggests the timing and duration of gametogenic cycles for PDV and PDSW are similar, with both morphotypes capable of biannual spawning at similar times in August/September and February/March each year (Mangubhai & Harrison 2007).

In contrast to PDV, PDSW was more genetically diverse (Hs), was in Hardy-Weinberg equilibrium at all microsatellite loci and displayed a far greater proportion of unique haplotypes in the ITS region. PDSW was also more difficult to correctly assign to a morphotype using microsatellite markers. Although it is possible that the prevalence of unique haplotypes is a result of the small PDSW sample size, it may be speculated that these 2 morphotypes share a recent ancestral past and that PDSW is the more ancestral type. Despite being less genetically diverse, PDV is more prevalent and phenotypically diverse, and if it has a selective advantage over PDSW in the sampled environment, it may have evolved assortative mating mechanisms within its morphotype to allow for maintenance of morphological boundaries. However, due to the limited sample size and overall low polymorphism of the microsatellite loci included in the study (Table 5), the genetic data should be interpreted with a degree of caution.

Platygyra morphotypes have now been identified in 3 different locations in the Indo-Pacific Region (Miller

Table 5. *Platygyra daedalea*. Allele frequencies for each morphotype (PDV, PDSW)

Locus	Allele	PDV	PDSW
Pd31	145	0.595	0.591
	155	0.388	0.409
	160	0.017	0.000
Pd48	206	0.000	0.045
	265	0.017	0.227
	273	0.983	0.727
Pd61	198	0.000	0.091
	202	0.121	0.090
	209	0.836	0.818
	218	0.043	0.000
Pd62	190	0.009	0.045
	202	0.500	0.545
	206	0.491	0.409

1992, 1994, Lam & Morton 2003) and the western Indian Ocean (this study). Morphotype differentiation appears to be characteristic of this genus, though it is not known if morphotypes exist in all *Platygyra* species. We are currently unable to explain why PDV is more prevalent than PDSW in the lagoon and to say whether this prevalence extends to deeper waters of the fringing reef and further along the East African coast. Given the prevalence of morphotypes in this genus, the next logical step would be to determine whether there are ecological differences between morphotypes in *P. daedalea*. For example, is one morphotype more resilient to bleaching than the other, and does the prevalence of PDV in Kenya reflect differences in responses to bleaching? Therefore, further studies are required to understand the role of different morphotypes, and whether there are costs, benefits and limits to phenotypic plasticity that have ecological and evolutionary consequences.

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