Marine Biology (2000) 137: 899–906

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Morphological and fluorescence analysis of the *Montastraea annularis* species complex in Florida

Received: 21 January 2000 / Accepted: 1 September 2000

Abstract The taxonomic status of the *Montastraea annularis* species complex is unclear. Much evidence has been accumulated to support the separation into 3 species, but the presence of intermediate morphotypes and the apparent lack of effective reproductive barriers in some areas are yet unexplained. Several authors have made a call for the introduction of new traits that can be used to resolve differences among closely related coral species. We collected skeletal and tissue samples from corals within the *M. annularis* species complex (15 each of *M. annularis*, *M. faveolata*, and *M. franksi*) and 10 morphological intermediates from several reefs in the Florida Keys. Multivariate analysis of corallite skeletal measurements supported the separation of the species complex into three taxa. We detected two main fluorescence emission peaks at 480 nm (turquoise) and 515 nm (green) that were not distributed equally among the three species. Every *M. annularis* colony had a major turquoise fluorescence peak. Some had a weak green secondary fluorescence peak. Colonies of *M. faveolata* and *M. franksi* had either the green or turquoise fluorescence peak, but at significantly different frequencies. The intermediate morphotypes proved to be highly heterogeneous with respect to both micromorphology and fluorescence, and their nature could not be fully explained. We were not able to separate the three species using fluorescence characters alone, however this new trait does increase our understanding of the taxonomic structure within the *M. annularis* species complex.

Communicated by N. H. Marcus, Tallahassee

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Introduction

The taxonomic relationships within several scleractinian genera have been under revision over the last two decades. Lang (1984) and Weil (1992) advocated the use of multiple character sets for investigating coral phylogeny and taxonomy. When congeners, previously separated solely by qualitative morphology, have been closely investigated using quantitative morphological, genetic, soft tissue, ecological, and behavioral characters, the new data have challenged accepted taxonomy [e.g. *Platygyra* (Miller and Babcock 1997; Miller and Benzie 1998), *Acropora* (Willis et al. 1997), and *Montastraea* (Knowlton et al. 1992; Weil and Knowlton 1994; Szmant et al. 1997)].

Until recently, the three species *Montastraea annularis* sensu stricto (Ellis and Solander 1786), *M. faveolata* (Ellis and Solander 1786), and *M. franksi* (Gregory 1895), were treated as a single taxon, *M. annularis* sensu lato. Evidence from electrophoretic, morphological, and behavioral characters has prompted the resurrection and re-description of the original three species (Weil and Knowlton 1994). However, unambiguous classification of individual colonies is complicated by the occurrence of intermediate morphotypes and by the lack of geographically widespread diagnostic features (Szmant et al. 1997). The boundary between species, as defined by gross colony morphology, is difficult to assess objectively. No study on the *M. annularis* species complex, to our knowledge, has explicitly included morphological intermediates.

Reported differences in the degree of separation of the three species throughout the Caribbean present an interesting situation. Van Veghel and Bak (1993), working in Curaçao, found significant differences in allozyme frequencies among the three species, but Nei's unbiased genetic differences (Nei 1978) were smaller

than those found in Panamá (Knowlton et al. 1992). Results of Van Veghel (1994) and Szmant et al. (1997), based on observations on the timing of spawning and on cross-fertilization experiments, suggest a high potential for hybridization. Knowlton et al. (1997) offer greater evidence for temporal and physiological pre-zygotic barriers for the Panamanian populations than do Szmant et al. for the Florida populations. Present genetic data do not help to resolve the situation. Sequencing of the ribosomal internal transcribed spacer (ITS) regions, the mitochondrial cytochrome *c* oxidase (COI) gene, and β-tubulin regions showed a high degree of homogeneity both between species and among geographic locations (Lopez and Knowlton 1997; Medina et al. 1999). However, the presence of at least one diagnostic amplified fragment-length polymorphism (AFLP) marker for *Montastraea faveolata* was found for the Panamanian region (Lopez and Knowlton 1997; Lopez et al. 1999). Other characters consistently separate *M. faveolata* from the other two species (Van Veghel and Bak 1993; Weil and Knowlton 1994). This is interesting, considering *M. annularis* and *M. franksi* are usually the easiest to separate from one another visually.

The presence of three distinct species in the *Montastraea annularis* complex would obviously complicate the interpretation of those studies that failed to distinguish between them (Knowlton et al. 1992; Van Veghel and Bak 1993). As Knowlton et al. (1992) pointed out, this oversight is of particular concern because *M. annularis* sensu lato has been the subject of many paleontological and ecological investigations. Weil and Knowlton (1994) and Medina et al. (1999) call for an investigation of additional characters to help resolve species differences.

Fluorescence, the absorption of short-wavelength light (excitation) with re-radiation at a longer wavelength (emission), occurs in many organisms. Since its initial description in the 1940s (Kawaguti 1944), fluorescence in corals has received limited attention in the literature, even though it is a common feature of the Scleractinia (Kawaguti 1944; Catalá-Stucki 1959; Mazel 1993). The details of its functional nature remain largely unknown (Mazel 1993, 1995). Recently, proteins similar to the green fluorescent protein (GFP) originally isolated from the jellyfish *Aequorea victoria* (Shimomura et al. 1962) have been found in non-scleractinian anthozoans (Matz et al. 1999). Laboratory analyses of the fluorescing substances found in several scleractinian species indicate that these compounds are proteins with similar molecular weight and chemical characteristics to GFP and the GFP-like proteins (Manica and Carter personal observations). This is strong evidence that the fluorescence in scleractinians is also caused by a GFP-like protein, yet the function of these putative proteins remains unknown.

Catalá-Stucki (1959) suggested that fluorescence might be a useful tool in coral systematics. To date, most studies have primarily been concerned with documenting the presence of fluorescence in a wide range of coral species (e.g. Logan et al. 1990; Mazel 1995), although Mazel (1993) did suggest that an investigation of closely related species would be interesting and specifically mentioned using fluorescence to potentially discriminate between members of the *Montastraea annularis* species complex.

In the present study, we describe the fluorescence patterns in the three members of the *Montastraea annularis* species complex and some specimens with intermediate morphologies. Combining this new character with corallite morphometrics, we investigate the degree of distinctiveness among the three species and the taxonomic affiliation of the intermediates in the Florida Keys.

Materials and methods

Sample collection

Samples of live *Montastraea* spp. were collected using SCUBA between November 1997 and September 1998 from six reef sites (two forereef at 5 to 10 m depth, two backreef at 2 to 5 m depth, and two turbid inshore reefs at 1.5 m depth) off Key Largo, Florida, USA (25°N; 80.4°W). We attempted to collect individuals from each species at each site, but this was not always possible. Small fragments ($\sim$15 cm^2) from 15 colonies of each of the 3 main morphotypes and 10 intermediate morphotypes (Table 1) were obtained using hammer and chisel. One of these intermediates (INT J) was competing for space with a typical *M. faveolata* colony. They were growing on the same boulder and were separated by a narrow groove of dead skeleton. This *M. faveolata* colony (designated INT K) was sampled in order to compare the two closely growing colonies. Colonies were identified to the species level

Table 1 *Montastraea* species groups. Physical descriptions of intermediate morphotypes and their location within the species groups generated by linear discriminant analysis (see Fig. 5)

Colony designation	Colony description	Location within species groups
INT A	*M. faveolata* – *M. franksi* intermediate	Outside *M. franksi* group
INT B	*M. faveolata* – *M. franksi* intermediate	Outside *M. franksi* group
INT C	*M. faveolata* – *M. franksi* intermediate	Outside *M. franksi* group
INT D	*M. faveolata* – *M. franksi* intermediate	Outside *M. franksi* group
INT E	*M. annularis*-like but without lobes	Within *M. annularis* group
INT F	*M. annularis*-like but without lobes	Within *M. annularis* group
INT G	*M. annularis*-like but oddly shaped	Within *M. annularis* group
INT H	*M. faveolata* with rounded knobs similar to small *M. annularis* columns	Within *M. annularis* group
INT I	*M. annularis* – *M. faveolata* intermediate	Between *M. annularis* and *M. faveolata* groups
INT J	*M. faveolata* with rounded knobs similar to small *M. annularis* columns	Between *M. annularis* and *M. faveolata* groups
INT K	*M. faveolata*	*M. faveolata*

according to gross morphology following Weil and Knowlton (1994). Most colony fragments were kept in seawater during transport to the laboratory (approx. 5 h), where they were maintained in individual rack compartments in a clean flow-through seawater aquarium. Several samples were collected and immediately frozen in liquid nitrogen. These were stored at −80 °C until analysis could be completed. Additional coral samples (20 *M. annularis*, 20 *M. faveolata*, and 10 *M. franksi*) were added to our fluorescence analysis as they became available. These were not used in the morphometric analysis.

Fluorescence measurements

Tissue was removed from the skeleton of the live specimens within 8 d of collection by airbrushing with 5 ml of ice cold 0.1 *M* Tris buffer. The tissue was ground in a glass homogenizer for 5 min and then centrifuged at 2000 ×*g* for 20 min to pellet out zooxanthellae, cell debris, and most of the mucus. Samples were diluted further with 0.1 *M* Tris and divided into several 1.5 ml subsamples for the spectrofluorometric analysis described below.

For each specimen, we performed 16 emission scans at excitation wavelengths between 300 and 600 nm at 20 nm intervals using a Perkin-Elmer LS 3B spectrofluorometer (excitation slit width = 20 nm, emission slit width = 10 nm) connected to a personal computer. Emission wavelengths were sampled at 5 nm intervals. To avoid overlap between the excitation and emission light, we measured the fluorescence emission starting 35 nm above the excitation wavelength. Each emission scan was terminated at a wavelength less than twice the excitation wavelength to avoid the second-order effects inherent in the diffraction grating used. All readings were adjusted for the minimal background fluorescence of Tris buffer. Peaks of relative fluorescence intensity were scored as characters based on their excitation/emission couplet.

Several controls were used to verify the quality of the spectra obtained. Many fluorescent compounds are subject to photobleaching after prolonged exposure to intense light. From a preliminary investigation of *Montastraea annularis*, three major fluorescence couplets (excitation/emission, ex/em = 430/480 "turquoise", 530/580 "yellow", and 480/680 "red") were selected as calibration points to monitor subsample photobleaching. Calibration readings were taken as soon as a new subsample was prepared and after each scan. Subsamples were replaced when any of the calibration peaks decreased by more than 10%. Up to four subsamples were used to produce a full excitation–emission spectrum.

To control for laboratory-induced changes in fluorescence, additional fragments from eight of the colonies (2 *Montastraea faveolata*, 5 *M. annularis*, and 1 intermediate) were analyzed after 1 mo in our aquarium. These results were compared to those obtained for the same colonies analyzed within 8 d of collection.

Morphometric analysis

After tissue removal, skeletal fragments were cleaned by soaking overnight in a 10% household bleach solution, rinsed in tap water, and dried. We measured the same morphological features that Weil and Knowlton (1994) used in their re-description of the species (see Table 4), but we used half the number of calices (5 instead of 10). For *Montastraea faveolata*, *M. franksi* and the intermediate morphotypes, we measured 5 haphazardly chosen polyps per colony. Because of differences in corallite measurements along the length of a single column in *M. annularis* (Land et al. 1975; Van Veghel and Bak 1993; see also Riegl and Pillar 1997), 5 polyps from the side and 5 from the top of the column were selected. Images were captured on computer using a video camera mounted on a stereoscopic microscope. Measurements were made using Mocha (Jandel Scientific Co.).

Statistical analysis

Univariate analysis was used to test for significant differences for 13 micromorphometric measurements among the three species. Multivariate analysis on these 13 measurements was used to investigate the relationship among natural groups based on fluorescence and gross morphology. Principal-components analysis (PCA), which requires no assumption of grouping, served as an exploratory tool. Based on the natural grouping suggested by PCA, we used linear discriminant analysis to emphasize those components of variance that help to distinguish between groups. Cross-validation was used to compensate for the optimistic apparent error rate given by discriminant analysis. Cross-validation was obtained by omitting each sample one at a time, recalculating the discriminant functions using the remaining data, and then classifying the omitted observation. Statistical computations were performed using Minitab 12 (Minitab Inc.) and SPSS 8.0 for Windows (SPSS Inc., Chicago).

Results

Fluorescence

Analysis of the spectrofluorometric data revealed several common features in the excitation–emission (ex/em) matrices (Fig. 1). Two red peaks under visible light excitation (excitation/emission = 480/670, and 440/670) represent chlorophyll (Hardy et al. 1992; Mazel 1993). In the control experiments, two yellow peaks with wide emission bands (one peak around 530/580, and a weaker peak at 380/575 to 585: Fig. 1c) could be induced in the laboratory. Six out of eight control colonies (3 *Montastraea annularis*, 2 *M. faveolata*, and 1 intermediate) initially did not show any yellow. Five of these colonies (all 3 *M. annularis*, 1 *M. faveolata*, and the intermediate) displayed strong yellow fluorescence after 1 mo. Because of their lack of power in resolving species differences, the chlorophyll and yellow peaks will not be considered further.

The remaining characters, a turquoise (420 to 440/480 to 485), and a green (460 to 480/505 to 515) peak (Fig. 1a, b), were variable in their presence and were significantly associated with different species (chi-square = 38.5; $df = 2$; $p < 0.001$; Table 2). Several specimens of *Montastraea annularis* also had a very weak green shoulder close to the major turquoise peak (Fig. 1d). Since this feature was likely to be missed in weakly fluorescing colonies, it was not used in the analysis. The *M. faveolata* specimen sharing a boulder with an intermediate (INT K) had a green peak, whereas the intermediate colony (INT J) was turquoise. Eight of the remaining intermediates were turquoise and one (INT I) was green. Under natural light, those individuals with a green fluorescence peak were noticeably green in coloration, while individuals with turquoise fluorescence appeared brown.

Morphometrics

A comparison of the sides and tops of *Montastraea annularis* colonies revealed significant differences for most of the measured variables (MANOVA Wilk's $F = 3.25$; $df = 7, 22$; $p = 0.016$; Table 3). The sides were

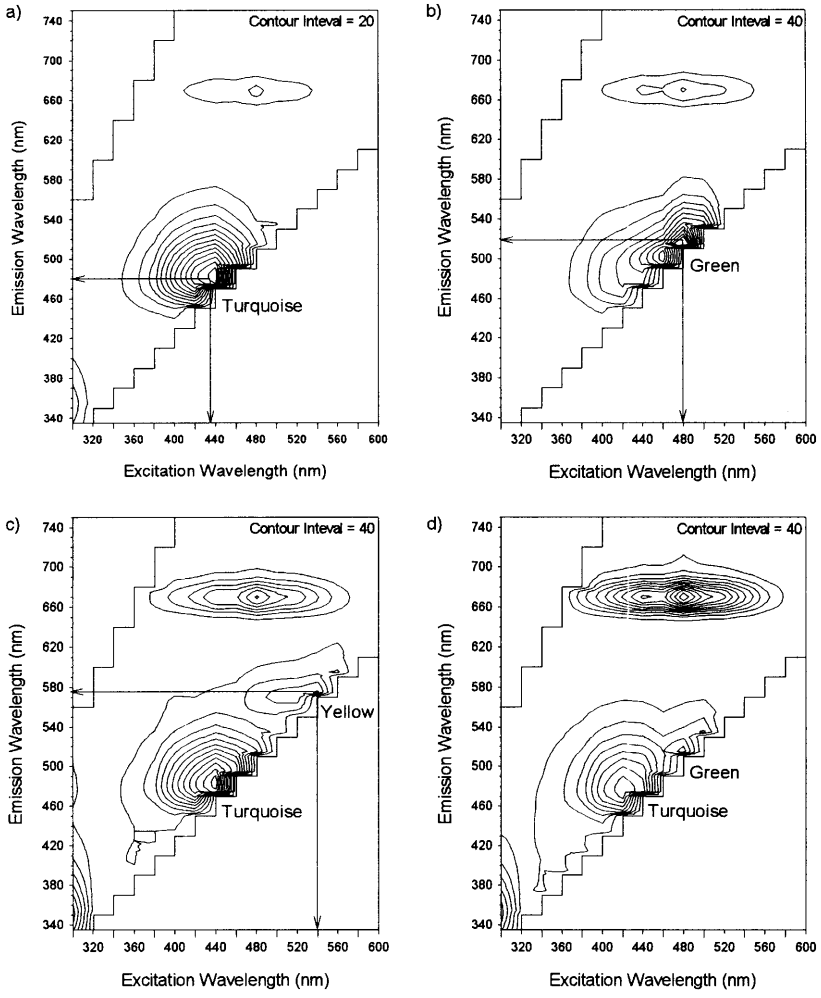

Fig. 1 *Montastraea annularis* species complex. Excitation–emission (ex/em) Scans displaying four common fluorescence patterns found in the complex: **a** *M. annularis* turquoise fluorescence (435/480); **b** *M. faveolata*, green fluorescence (480/520); **c** *M. annularis*, turquoise and yellow fluorescence (530/575); **d** *M. annularis*, turquoise fluorescence with small green shoulder. Ovoid peak in top middle of each graph is caused by chlorophyll (480/670 and 440/670); upper left corner, where emission wavelength is approximately twice excitation wavelength is blank because of presence of second-order optical effects in this area; lower right corner, where excitation wavelength is greater than emission wavelength, was not measured

Table 2 *Montastraea* spp. Occurrence of green and turquoise fluorescence in the three species. Individual comparisons among each species pair were all significant (chi-square: $p < 0.05$ using a sequential Bonferroni correction; Rice 1989)

Fluorescence color	*M. annularis*	*M. faveolata*	*M. franksi*
Green	0	25	11
Turquoise	35	10	14

chosen for interspecific comparisons, since the tops are highly unusual areas of fast polyp division, and their adoption would be circular in directly correlating gross and fine morphology.

For the 45 specimens used in the morphometric analysis (i.e. the intermediate morphotypes were not used), univariate analysis revealed significant differences among the species for most measured variables (Table 4). Fluorescence as a nested factor was significant only in the ANOVA on columella diameter ($F = 5.97$; $df = 2, 40$; $p = 0.005$), but the variable was not significantly different among species ($F = 1.60$; $df = 2, 40$; $p > 0.2$).

Preliminary inspection of the variables revealed that measurements from opposite septa were highly correlated (Pearson coefficient >0.85, $p < 0.01$) and redundant (tolerance $= 0.005$), so the opposite septal variables were dropped. The same applied to maximum and

Table 3 *Montastraea annularis*. (Paired *t*-tests of 8 variables in sides and tops of colonies (means ± SE). Significance levels (using sequential Bonferroni correction; Rice 1989): $**p < 0.01$; $*p < 0.05$

Area	Calix diam	Columella diam	Primary septal length	Primary septal width	Tertiary septal length	Min. inter-calical distance	Max. inter-calical distance	No. of septa[a]
Sides	2.41 ± 0.04	1.55 ± 0.05	0.48 ± 0.02	0.31 ± 0.01	0.21 ± 0.01	1.13 ± 0.06	2.36 ± 0.12	23.71 ± 0.20
Tops	2.20 ± 0.05	1.43 ± 0.05	0.42 ± 0.01	0.29 ± 0.00	0.25 ± 0.01	0.89 ± 0.03	1.95 ± 0.08	23.60 ± 0.15
Sign. Level	**	**	*	NS	**	*	*	NS

[a] Sign-rank test for paired comparisons

Table 4 *Montastraea annularis* species complex. Univariate ANOVAs of the eight variables with species as main factor and fluorescence as nested factor (means ± SE). (*MAN* = *M. annularis*; *MFA* = *M. faveolata*; *MFR* = *M. franksi*; *GR* = green; TURQ = turquoise) ANOVA significance levels (Sum of squares adjusted for multiple comparisons): ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$

	Calix diam	Columella diam	Primary septal length	Primary septal length	Tertiary septal length	Min. inter-calical distance	Max. inter-calical distance	No. of septa[a]
M. annularis	2.41 ± 0.05	1.55 ± 0.05	0.48 ± 0.02	0.31 ± 0.01	0.25 ± 0.01	1.13 ± 0.06	2.36 ± 0.12	23.71 ± 0.20
M. faveolata	2.80 ± 0.06	1.70 ± 0.06	0.59 ± 0.02	0.32 ± 0.01	0.28 ± 0.01	1.02 ± 0.06	2.28 ± 0.09	25.36 ± 0.44
M. franksi	2.73 ± 0.07	1.65 ± 0.05	0.58 ± 0.02	0.36 ± 0.01	0.31 ± 0.02	1.21 ± 0.06	2.80 ± 0.08	24.72 ± 0.37
ANOVA by species	***	NS	***	**	*	NS	***	*
Tukey's	MFA, MFR > MAN		MFA, MFR > MAN	MFR > MFA, MAN	MFR > MAN		MFR > MAN, MFA	MFA > MAN, MFR
ANOVA by fluorescence	NS	**	NS	NS	NS	NS	NS	NS
Tukey's		GR > TURQ						

[a] ANOVA performed on ranked data

minimum estimates of calix and columella diameter, which were subsequently averaged. The 12 initial measurements were reduced to 8 variables: calix and columella average diameters, primary septal length and width, tertiary septal length, minimum and maximum inter-calical distance, and number of septa. The number of septa variable failed a Kolmogorov–smirnov normality test ($p < 0.01$) and was excluded from any multivariate analysis.

Principal components were computed from the 45 identified samples (the 9 intermediates were not included). Following Kaiser's criterion, the first three components were taken to be sufficient to explain most of the variance, as the eigenvalues of further components fell below 1. The combination of the first and second components revealed clustering by the three separate species, despite a high degree of overlap (Fig. 2). A similar trend was observed for all other combinations of the first three components. In a plot of the second and third component, when fluorescence was superimposed on the species classification, 4 out of 5 green *Montastraea franksi* were found in close proximity to the green *M. faveolata* (Fig. 3). Also, 2 of the 3 turquoise *M. faveolata* fell away from their species cluster. No effect of sampling location, date or depth was found.

Linear discriminant analysis was used to further separate the species. All seven variables were included to obtain maximum separation. The three species groups

Fig. 2 *Montastraea annularis* species complex. First and second principal components from 45 specimens positively identified to species level. First component was mostly influenced by calix diameter, primary septal length and maximum intercalical spacing; second component was mostly influenced by minimum and, to a lesser extent, maximum intercalical distance [*Dotted ellipses* Gaussian bivariate ellipses for each species ($p = 0.683$); *continuous ellipses* confidence intervals on centroids ($p = 0.95$); ○ *M. annularis* (*MAN*); □ *M. faveolata* (*MFA*); △ *M. franksi* (*MFR*)] Symbol shading is for distinction only

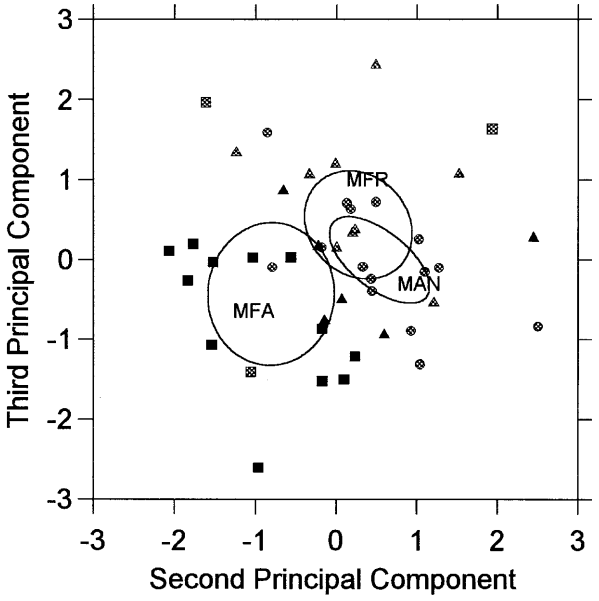

Fig. 3 *Montastraea annularis* species complex. Second and third principal components based on 45 specimens positively identified to species level. Second component mostly represented minimum and, to lesser extent, maximum intercalical distance; third component was mostly influenced by columella diameter [*Continuous ellipses* confidence intervals on centroids for each species (ELM; $p = 0.95$); ○ *M. annularis*; □ *M. faveolata*; △ *M. franksi*; *hatched* turquoise fluorescence; *black* green fluorescence]

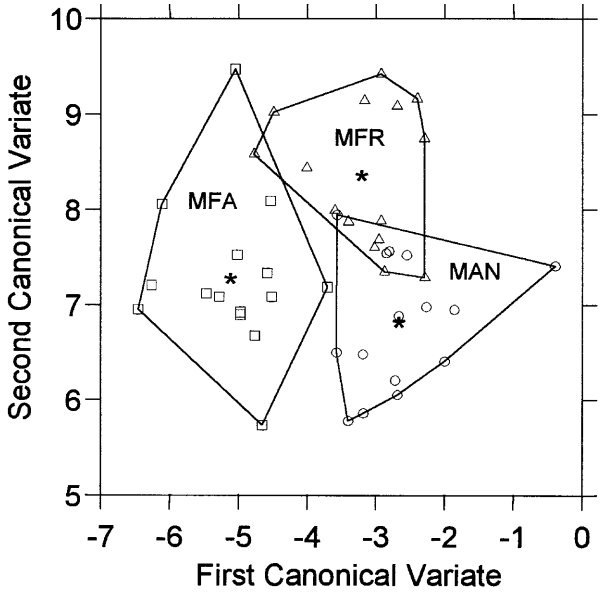

Fig. 4 *Montastraea annularis* species complex. First and second canonical variates based on 45 specimens positively identified to species level. First canonical variate mostly represented columella diameter and primary septal length; second canonical variate was mostly influenced by maximum intercalical distance and primary septal width and, to lesser extent, primary septal length (*Polygons* represent largest convex envelope including all specimens belonging to one species; ○ *M. annularis*; □ *M. faveolata*; △ *M. franksi*)

showed clear separation, with limited overlap between *Montastraea annularis* and *M. franksi* (Fig. 4). The estimated accuracy of 87% dropped to 73% after cross-validation. Using the first two discriminant functions, it was possible to investigate the nature of the two specimens forming a single boulder as well as of the remaining intermediates (Fig. 5). The *M. faveolata* (INT K) specimen fell clearly in its species cluster, whereas the intermediate (INT J) collected from the same boulder was placed at the very edge of the *M. faveolata* envelope. As expected, the remaining intermediates appeared to be a heterogeneous group. Five specimens fell between the species clusters, but four were found close or outside the external edges of the *M. franksi* cluster.

MANOVA with species as the main fixed factor and fluorescence as a nested factor revealed a significant effect by the former ($F = 10.11$; $df = 2, 40$; $p < 0.001$) but not the latter ($F = 1.76$; $df = 2, 40$; $p > 0.10$).

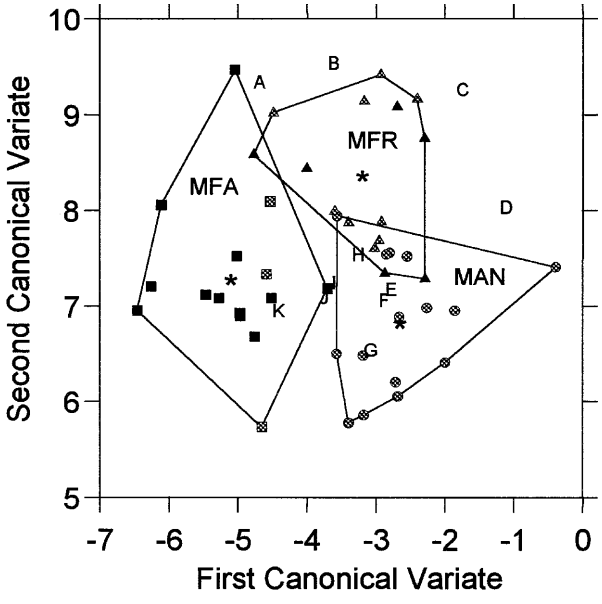

Fig. 5 *Montastraea annularis* species complex. First and second canonical variates based on 45 specimens positively classified to species level. Scores for intermediate morphotypes were computed using discriminate functions for the three species [*Polygons* represent largest convex envelope including all specimens belonging to one species; ○ *M. annularis*; □ *M. faveolata*; △ *M. franksi*; *A–K* intermediates (Table 1); *hatched* turquoise fluorescence; *black* green fluorescence]

Discussion

Our data provided evidence for significant differences in both micromorphological and fluorescence characters among the three *Montastraea* species. The separation of the species complex has already received support from central Caribbean areas (Van Veghel and Bak 1993; Weil and Knowlton 1994), but our study is the first analysis of the *Montastraea* populations in the northern Caribbean. The lack of fully diagnostic features and the problematic classification of the intermediate morphotypes greatly complicate the picture.

Multivariate analysis based on micromorphometrics supported the separation of the three species. However, as previously detected in Curaçao and Panamá (Van Veghel and Bak 1993; Weil and Knowlton 1994), there was a degree of overlap within every trait analyzed, and no measurement provided diagnostic power. Remarkably, our centroids differed greatly from the values published for Panamá (Weil and Knowlton 1994). Van Veghel and Bak (1993) have already reported biogeographic variation between Curaçao and Panamá. Observer bias is a major concern in all morphometric studies, and it could certainly explain quantitative differences between our measurements and those of other investigators. However, any bias should apply to all species and is not expected to affect the relationship among them. Our study revealed several patterns that were incompatible with the data from Panamá. For example, *Montastraea faveolata* has the smallest average number of septa in Panamá, but the largest in Florida. Environmental and genetic differences among the three species provide more convincing explanations for the discrepancies found throughout the distribution range. Budd (1993) reported a correlation between environment and micromorphology in *M. faveolata*, and it is not unreasonable to suspect that the other two morphotypes will also be affected by abiotic factors. Allozyme frequencies also differ between Curaçao and Panamá, confirming the presence of genetic differences among regions. The heterogeneity of all the traits throughout the distribution range further strengthens the significance of morphological distinctiveness being retained in all the investigated areas.

The study of fluorescence also provided evidence for the separation of the three species. We sampled specimens of each species from the same areas, and no link could be found between color and localities. Furthermore, the two specimens forming a single boulder (INT J and K) fluoresced differently, strongly arguing against environmental control on the color of fluorescence. In the laboratory, both the turquoise and green colors were stable in their expression, but the yellow proved to be inducible. Incidentally, the yellow fluorescence trait might be an interesting target for the investigation of stress indicators or environmental parameters such as temperature or light intensity. With the exception of columella diameter, micromorphology did not suggest strong differences between fluorescent variants of the same species. Observations of colormorphs are informative in this regard, since coloration is influenced by fluorescence. Differences in aggressive interactions and allozyme frequencies have been reported between colormorphs, but the evidence is mostly anecdotal (Dustan 1975; Logan 1985; Weil and Knowlton 1994) and lacks any consistent trend (Weil and Knowlton 1994). Despite the lack of diagnostic features, fluorescent traits offer a further dimension to our understanding of these species.

One of the major problems in dealing with the *Montastraea annularis* species complex is the presence of highly variable intermediate morphotypes as defined by gross morphology (Szmant et al. 1997). The process of assigning colonies to an intermediate class is in itself highly arbitrary. The intermediates in our data set could not be easily categorized. Several specimens had an intermediate micromorphology that could fit Wagner's intermediacy criterion (1969, 1983) for hybrids. Cross-fertilization studies in Florida (Szmant et al. 1997) have shown the potential for hybridization between the three species in this area. However, some of the intermediates appeared to belong well outside the common range of measurements of the putative parent species. The possibility of extreme morphological variability of one or more species can thus not be ruled out. Until fully diagnostic characters for all three species are found, the nature of the intermediate morphotypes is in doubt.

In conclusion, our study provided further evidence for the separation of species within the *Montastraea annularis* species complex and represents the first quantitative morphological analysis of Florida populations. Our knowledge of the species complex, however, is still very unsatisfactory. The presence of highly heterogeneous morphological intermediates, coupled with the lack of diagnostic characters for all three species, greatly complicates the picture. Researchers in the fields of ecology and paleontology are urged to keep a clear record of which growth forms are encountered in any survey or experiment, as further work on these species might reveal unexpected issues. Fluorescence proved a useful tool in investigating the *M. annularis* species complex, even though it failed to provide fully diagnostic features. New traits, especially genetic markers, are needed to fully understand the taxonomic relationships among the three *Montastraea* species.

Acknowledgements We thank J. Janco and P. Darche for valuable field support, P. Gibbs and P. Whitney for technical advice, A. Szmant, P. Walsh and M. Schmale for the use of their laboratories, A. Szmant, M. Miller, E. Weil, and C. Mazel for critical reviews of our manuscript, and D. Brown of Perkin-Elmer for help in writing the spectrofluorometer control program. Corals were collected under Permits FKNMS-09-97, FKNMS-051-98, FKNMS-056-98, and FKNMS-057-98 issued by the Florida Keys National Marine Sanctuary and Permits 97S-364 and 98S-364 issued by the Florida Department of Environmental Protection. Both authors contributed equally to this paper.

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