Morphological and genetic divergence between Mediterranean and Caribbean populations of *Madracis pharensis* (Heller 1868) (Scleractinia, Pocilloporidae): too much for one species?

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Abstract

The colonial stony coral genus *Madracis* is cosmopolitan, lives in shallow and deep water habitats, and includes zooxanthellate, azooxanthellate and facultative symbiotic species. One of its species, *Madracis pharensis*, has been recorded from the Mediterranean and East Atlantic, where it forms small knobby and facultative zooxanthellate colonies (also named *M. pharensis f. pharensis*), and from the tropical Caribbean, where it also occurs in a massive and zooxanthellate form (named *M. pharensis f. luciphila* by some). These two forms have been previously found to host different *Symbiodinium* species. In this study, species boundaries and phylogenetic relationships between these two *Madracis pharensis* forms (from the Mediterranean Sea and the Caribbean), *M. senaria*, and the Indo-west Pacific *M. kirbyi* were analyzed through an integrated systematics approach, including corallite dimensions, micromorphology and two molecular markers (ITS and ATP8). Significant genetic and morphological differences were found between all the examined *Madracis* species, and between *M. pharensis* from the Mediterranean Sea and *M. pharensis f. luciphila* from the Caribbean in particular. Based on these results, the latter does not represent a

zooxanthellate ecomorph of the former but a different species. Its identity remains to be ascertained and its relationship with the Caribbean *M. decactis*, with which it bears morphologic resemblance, must be investigated in further studies. Overall, the presence of cryptic *Madracis* species in the Easter and Central Atlantic remains to be evaluated.

Keywords *Madracis senaria*, *Madracis kirbyi*, rDNA, ATP8, corallite diameter

Introduction

The genus *Madracis* Milne Edwards and Haime, 1849 is found throughout the tropics but also lives in temperate waters in shallow and deep habitats (Fricke & Hottinger 1983; Cairns 1984, 1991, 1999, 2000; Veron & Pichon 1976; Veron 2000; Santodomingo *et al.* 2007; Neves & Johnsson 2009). It includes colonial species forming encrusting, nodular, branching or massive coralla, which are either zooxanthellate, azooxanthellate or facultative symbionts thus occurring in zooxanthellate and azooxanthellate forms (Wells 1973a, b; Zibrowius 1980; Cairns *et al.* 1999, 2000; Frade *et al.* 2008a).

Madracis has been included in the Pocilloporidae by several authors (Wells 1956; Veron & Pichon 1976; Zibrowius 1980; Sheer & Pillai 1983; Cairns 1991, 1994, 1999, 2000; Veron 1986; Sheppard & Sheppard, 1991; Neves & Johnsson, 2009) until Veron (2000) moved it in the Astrocoeniidae Koby 1890 based on the presence of a "style-like columella and neatly arranged solid septa". Since, several studies based on different nuclear and mitochondrial makers confirmed the opinion of previous authors that *Madracis* is closely related to *Pocillopora* and allies rather than to the astrocoeniid *Stephanocoenia* Milne Edwards & Haime 1848 (Romano & Cairns 2000; Fukami *et al.* 2008; Kitahara *et al.* 2010; Stolarski *et al.* 2011; Huang 2012; Keshavmurthy *et al.* 2013; Arrigoni *et al.* 2017).

Madracis is characterized by a hexameral, octameral or decameral septal symmetry, and a styliform columella (Cairns & Kitahara 2012). Like in the other Pocilloporidae Gray, 1842, corallites are small (usually less than 2 mm in diameter) and typically present a limited number of characters that can be used to identify species and delineate species boundaries (Veron & Pichon 1976; Reyes Bonilla *et al.* 1995; Stefani *et al.* 2011; Schmidt-Roach *et al.* 2014). Veron & Pichon (1976) remarked that the genus *Madracis* "badly needs a revision on a world-wide scale, in the light of extensive and reliable ecological data". To date, no formal revision has been undertaken. However, much progress has been made in the current knowledge on the biology, ecology and systematics of the shallow water hermatypic *Madracis* species from the Caribbean (Diekmann *et al.* 2001; Vermeij & Bak 2002a, b; Vermeij *et al.* 2001, 2002, 2003a, b, c, 2004; Kaandorp *et al.* 2003, 2005; Vermeij & Sandin 2006; Frade *et al.* 2008a, b, c, 2010; Filatov *et al.* 2013) and Brazil (Muramatsu & Lang da Silveira 2008; Neves & Johnsson 2009; Capel *et al.* 2012). Outside of the Western Atlantic, the studies on deep and shallow water *Madracis* species deal mainly with their taxonomy and occurrence (Veron & Pichon 1976; Cairns 1991, 1994, 1999; Cairns & Zibrowius 1997; Veron 2000; Zibrowius *et al.* 2017), less frequently with aspects of their biology and ecology (Laborel & Vacelet 1958; Zibrowius 1980; Morri *et al.* 2000; Özalp & Alparslan 2015).

Madracis pharensis (Heller, 1868) was described from Hvarski Kanal, Croatia, in the Adriatic. The holotype appears to be lost (Zibrowius 1980) and in the species original description two drawings (Plate 1, Figs. 1-2) show a typically nodular colony and the expanded polyps, but no detail of the corallites. However, Zibrowius (1980) provided good illustrations of the species morphology based on his extensive reference collection now deposited at the National Museum of Natural History (MNHN) in Paris. *Madracis pharensis* forms small clumps or encrusting colonies (Figs. 1a, b) often found in caves and overhangs (Morri *et al.* 2000) and has two cycles of ten septa (S1 > S2) with well-formed paliform lobes (Zibrowius 1980). It is found at

various localities in the Mediterranean and in the Eastern Atlantic (Madeira, Canary, Cap Verde, and Azores islands) (Zibrowius 1980; Cairns 2000; Morri *et al.* 2000; Gerovasileiou *et al.* 2015; Özalp & Alparslan 2015). Laborel (1960) first reported this species from Brazil, and Goreau & Wells (1967) found a "cavernicolous" *M. pharensis* in Jamaica, thus extending the known species range to the Western Atlantic where it was later recorded also from various localities in the Caribbean and Bahamas, Gulf of Mexico, and Gulf of Campeche (Cairns 2000).

Wells (1973a) in a study of the Scleractinia from Jamaica, distinguished two forms of *M. pharensis*, one he defined lucifugous and ahermatypic with encrusting and nodular growth form, *M. pharensis* f. *pharensis* (Heller, 1868), and the other one luciphilous and hermatypic with laminar or encrusting growth form, *M. pharensis* f. *luciphila* Wells, 1973. Admittedly, the author did not look into detail at the corallite scale differences between the two forms, as he stated "attention is focused on the characters of the corallum". Some authors (Cairns 2000; Cairns *et al.* 2009) still separate *M. pharensis luciphila* and *M. pharensis pharensis* following Wells' (1973a) distinction between the hermatypic and the ahermatypic forms in the Caribbean. However, most authors have abandoned such distinction and referred to the zooxanthellate form (Figs. 1b, c) as *M. pharensis* in their works (Diekmann *et al.* 2001; Vermeij & Bak 2002b; Vermeij *et al.* 2004; Frade *et al.* 2008a, b, c, 2010; Neves & Johnsson 2009) although they did start separating different colour morphs having differences in their ecology and reproductive biology (Vermeij *et al.* 2004; Frade *et al.* 2008b, 2010).

Wells (1973a) stated that *M. pharensis* f. *luciphila* "may be a distinct species, but the morphology of the skeletal structures appears to be identical". Doubts have been also expressed by Zibrowius (1980) on the actual identity of the Caribbean and Brazilian specimens he could examine. Later, Fenner (1993) on the basis of his observations of living corals at different localities in the tropical Western Atlantic (Mexico, Martinique, Saint Lucia, the Cayman Islands, and Honduras) concluded that *M. pharensis* in this region is actually a form, or ecomorph, of the morphologically plastic zooxanthellate *Madracis decactis* (Lyman, 1859), a conclusion challenged by the overlapping light usage strategies of the two species (Vermeij & Bak 2002a). Diekmann *et al.* (2001) included two specimens of *M. pharensis* from the Mediterranean in their genetic analyses of Caribbean shallow water *Madracis*. In their phylogenetic reconstruction based on a nuclear ribosomal marker (nrDNA: ITS region), the specimens from the Mediterranean, chosen as an outgroup, were only distantly related to the Caribbean specimens which formed a paraphyletic species complex with *M. decactis*, *M. formosa* Wells, 1973, and *M. carmabi* Vermeij, Diekmann & Bak, 2003. Although later Frade *et al.* (2010) using a mitochondrial (mtDNA: NAD5) and two nuclear (nDNA: ATPSa and SRP54) intron markers confirmed that the species boundaries between all Caribbean species except *M. senaria* Wells, 1973 are semi-permeable, they did not include material from the Mediterranean. Furthermore, significant genetic differences were found between shallow and deep water populations of the examined *M. pharensis* from the Caribbean (with threshold at ca. 25 m depth), a pattern recently confirmed by another mitochondrial marker (mtDNA: ATP8, Bongaerts *et al.* 2015). Interestingly, this depth-related genetic divergence in *M. pharensis* strictly matches that of the dominant endosymbiont lineages harbored by this

species, with *Symbiodinium* type B7 associating with the shallow *M. pharensis* population and type B15 associating with the deep population (Frade *et al.* 2010). Such genetic partitioning of host and symbiont populations is not unique to *Madracis*, and highlights the potential role of depth-related processes in the evolution of brooding corals and their associated *Symbiodinium* (Frade *et al.* 2010, Bongaerts *et al.* 2013). To date, the genetic and morphological boundaries between *M. pharensis* f. *luciphila* and *M. pharensis* f. *pharensis* in the Caribbean, and between these two forms and the Mediterranean and West Atlantic *M. pharensis* remain unexplored.

In the Indo-Pacific, *M. kirbyi* Veron & Pichon, 1976 is the only known shallow water, zooxanthellate and reef dwelling *Madracis* species (Figs. 1g, h). First described from Australia, this species is found from the Red Sea to the central Pacific (Veron 2000). In their original description, Veron & Pichon (1976) remarked on the morphological similarities between this species and *M. pharensis* from the Mediterranean and *M. decactis*, yet they separated it from the congeners in reason of a much-reduced second cycle of septa. However, the degree of development of the second cycle of septa appears to be a rather variable character, questioned by some authors (e.g. Zlatarski & Estalella 1980). The species boundaries between *M. pharensis* and *M. kirbyi* remain unstudied, being the sequences of the latter species are still unavailable to date.

In this study, specimens of the hermatypic and zooxanthellate morph of *M. pharensis* (Figs. 1c, d) and *M. senaria* (Figs. 1e, f) from the Caribbean previously analyzed by Frade *et al.* (2010), *M. pharensis* from the Mediterranean (Figs. 1a, b), and *M. kirbyi* from the Indo-Pacific (Figs. 1g, h) are examined for the first time through an integrated approach combining two different markers (ITS and ATP8) and micro-morphological and morphometric data. Genetic and micromorphological differences, or lack thereof, between the typical *M. pharensis* from the Mediterranean and the Caribbean material are addressed. Moreover, species boundaries between *M. pharensis*, *M. senaria*, and *M. kirbyi* are investigated. A detailed description of the species' micromorphology is provided for the first time and its relevance from a systematic standpoint in the genus *Madracis* is discussed in an evolutionary context.

Methods

Sampling, examined collections and identification of the specimens

A total of 54 *Madracis* specimens were examined in this study (Table 1). Specimens were collected during SCUBA diving at various localities in the Mediterranean, on Curaçao (former Netherland Antilles) in the Southern Caribbean, and in the Indo-Pacific between 2 and 30 m depth (Fig. 2, Table 1). One specimen of *Madracis pharensis* was sampled from 30-60 m depth in Kotor Bay, Montenegro during the CROatian-Montenegrin Marine ecosystems (CROMA) cruise of RV Urania in February 2014.. After collection, corals were tagged and a fragment preserved in 95% ethanol for further molecular analyses. The corallum was then bleached in sodium hypochlorite, rinsed with freshwater, and air-dried for identification and morphological analyses. Corals were identified examining the type material and following Zibrowius (1980), Zlatarski & Estalella (1980) and Veron & Pichon (1976). The extensive collection of Mediterranean and Eastern Atlantic corals by Dr H. Zibrowius deposited at the MNHN was examined and specimens of *M. pharensis* were

observed at the stereo microscope. Fragments of five specimens were loaned to the first author for further analyses at the Scanning Electronic Microscope (SEM).

In order to avoid confusion and repetitions of the geographic origin of the examined specimens we henceforth refer to the non symbiotic Mediterranean and Eastern Atlantic material as *M. pharensis*, and to the zooxanthellate form from the Caribbean as *M. pharensis* f. *luciphila*. The azooxanthellate Caribbean form of *M. pharensis* (=*M. pharensis* f. *pharensis*), was not investigated in this study as material could not be retrieved.

DNA extraction, amplification, and sequence analyses

Total genomic DNA was extracted from coral tissue using DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA, USA), following the manufacturer's protocol. Polymerase chain reaction (PCR) amplification was carried out for two molecular markers, a ~1400 bp portion of the mitochondrial DNA spanning a section of the 3' end of NAD5 gene, the entire trnW and the putative ATP8 genes, the entire intergenic spacer between ATP8 and COI genes, and a section of the 5' end of COI gene (hereafter ATP8) (Chen *et al.* 2008; Flot *et al.* 2008a) and a ~600 bp portion of the nuclear rRNA including the 3' end of the 18S rRNA, the entire ITS1, 5.8S, and ITS2, and the 5' end of the 28S rRNA (hereafter ITS region). These two molecular markers have proved to be powerful in the definition of species boundaries in the other pocilloporid genera *Pocillopora* (Flot *et al.* 2008a; Schmidt-Roach *et al.* 2012; Pinzon *et al.* 2013), *Seriatopora* (Flot *et al.* 2008b) and *Stylophora* (Flot *et al.* 2011; Stefani *et al.* 2011; Klueter & Andreakis 2013). The ATP8 locus was amplified for a total of 39 samples, the ITS region for 54. The ATP8 locus was amplified using the primers FNAD5.2deg and RCOI3 (Flot *et al.* 2008a) in a 25 µl PCR volume containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 µM for both of each primer, 0.1 mM of each dNTP, and 2 U of *Taq* polymerase. Thermal cycling conditions were 94° for 2 min; 35 cycles of 94° for 30 sec, 53° for 1 min, and 72° for 1 min; and 72° for 2 min. ITS region was amplified using the primers A18S (Takabayashi *et al.* 1998) and ITS4 (White *et al.* 1990), following the protocol proposed by Benzoni *et al.* (2011). All PCR products were purified with Illustra ExoStar (GE Healthcare, Buckinghamshire, United Kingdom) and directly sequenced in forward and reverse directions using an ABI 3130xl Genetic Analyzer (Applied Biosystems, Carlsbad, California). Sequences obtained in this study were deposited in EMBL, and accession numbers are listed in Table 1. Chromatograms were viewed, edited and assembled using CodonCode Aligner 5.0.2 (CodonCode Corporation, Dedham, MA, USA) and manually checked using BioEdit 7.2.5 (Hall 1999). Alignments of the two separated molecular loci were carried out using the E-INS-I option in MAFFT 7.164 (Katoh *et al.* 2002; Katoh & Standley 2013) under default parameters. Sequence alignments were converted into the Roehl file format using DnaSP 5.10.1 (Librado & Rozas 2009), removing invariable sites and not considering sites with gaps/missing. Relationships among haplotypes were reconstructed using NETWORK 4.612 (http://www.fluxus-engineering.com), based on the median-joining algorithm (Bandelt *et al.* 1999). Intra- and interspecific pair-wise distances (uncorrected *p*-distances) within and between the four examined *Madracis* species were calculated in MEGA 6 (Tamura *et al.* 2013).

Morphological analyses

Scleractinian coral samples were analyzed both at macro- and micromorphological level, using light microscopy (Leica M80 microscope) and SEM, respectively. In total, specimens 40 were studied and measured, 11 of *M. pharensis*, 10 of *M. pharensis* f. *luciphila*, 12 of *M. kirby*, and 7 of *M. senaria*. Corallum fragments size varied among species with nodular fragments of the colonies of *M. pharensis* being the smallest examined and reaching a maximum of 2 cm in length and width (Fig. 1). For imaging of corallite micromorphology at the scanning electron microscope (SEM), fragments of specimens were grinded, mounted on stubs using silver glue, sputter-coated with conductive gold film and examined using a Vega Tescan Scanning Electron Microscopy at University of Milano-Bicocca.

Coral skeletons for corallite measures were imaged through a Leica M80 microscope equipped with a Leica IC80HD camera at the same magnification to obtain images of 14 x 10.5 mm of the corallum surface. The Analyzing Digital Images tool was used to take measurements of the inter columella distance (ID) defined as the linear distance between the centre of the columella and two adjacent corallites, and of the calice diameter (CD) (Fig. 3). ID and CD were measured on five corallites for each specimen. Measures were taken between and within haphazardly selected corallites without any evidence of extracalicinal budding. In case of coralla with nodular growth forms, only corallites found on the sides and at least 5 mm from the top were considered. Variables were log-transformed, tested for normality (Shapiro-Wilk's W-test) and homogeneity of variance (Levene's test), then subjected to an analysis of variance. Turkey's test for unequal sample size (Spjotvoll & Stoline 1973) was used for *post hoc* comparisons of means. Alpha values were adjusted using the Bonferroni correction for multiple tests taking into account the average variable correlation (Simes 1986). For each character, the specimen mean from the five replicates was calculated.

Results

Molecular results

Sequence lengths of ATP8 ranged from 1155 (in *M. pharensis*) to 1275 bp (in *M. kirbyi*), and those of ITS region ranged from 599 to 603 bp. Relationships among haplotypes of the four examined *Madracis* species are shown in Figs. 4a, b for the separated mitochondrial and nuclear molecular loci, respectively.

A total of 38 ATP8 sequences for the four examined *Madracis* species were obtained and used for the haplotype network analysis. A total of nine haplotypes were identified from the ATP8 alignment: a single haplotype specific of *M. kirbyi*, a single haplotype specific of *M. pharensis luciphila*, three closely related haplotypes of *M. senaria* separated by up to four mutations, and four closely related haplotypes of *M. pharensis* separated by up to three mutations (Fig. 4a). No haplotypes are shared between the four lineages that are thus genetically isolated. *Madracis pharensis* f. *luciphila*, *M. senaria*, and *M. pharensis* varied among each other by 31-35 base changes, while *M. kirbyi* differed from the other three *Madracis* by at least 102 base changes. Intraspecific genetic distance (*p*-distance) ranged from 0 to 0.19, while pair-wise comparison of genetic distances between species were considerable higher. The average distance of *M.*

pharensis from *M. pharensis* f. *luciphila* (3.21 ± 0.51)was comparable to those between *M. senaria* and *M. pharensis* (3.04 ± 0.51), and between *M. senaria* and *M. pharensis* f. *luciphila* (3.49 ± 0.55) (Table 4). Alignment of the ITS region was conducted on a total of 63 sequences:31 sequences newly obtained in the present study and the remaining ones downloaded from GenBank (in particular, two sequences of *M. pharensis*, 18 of *M. pharensis* f. *luciphila*, and 12 of *M. senaria*). As detected also for the mitochondrial region, the network showed no shared haplotype among the four lineages, being, therefore, genetically separated. It revealed four main clusters of haplotypes that matched with the four examined *Madracis* lineages (Fig. 4b). Haplotypes of *M. kirbyi* were clearly distant from the other three lineages and separated by 11-19 mutation events. A minimum of eight base changes differentiated *Madracis pharensis* and *M. pharensis* f. *luciphila*, while at least seven mutations occurred between *M. senaria* and *M. pharensis* and a minimum of five mutations separated *M. senaria* and *M. pharensis* f. *luciphila*. The mean genetic distance between *M. pharensis* and *M. pharensis* f. *luciphila* (1.81 ± 0.4), was similar to the genetic distances between *M. senaria* and *M. pharensis* (1.73 ± 0.37) and between *M. senaria* and *M. pharensis* f. *luciphila* (1.71 ± 0.39) (Table 5).

Morphological results

The examined specimens of *M. pharensis* have round to polygonal corallites (Figs. 5a, e) with a cerioid or sub-plocoid arrangement (Fig. 5a). Average CD is 1.66 mm (± 0.22) (Table 2). Septal arrangement is decameral (S1 = 10) with S2 shorter than S1 (Figs. 5e, i). The top margin of the S1 is slightly exsert or flush, with a coenosteum ornamentation. Finely ornamented septal lobes are found at the inner end of the septa before fusing with the columella (Figs. 5e, i). A deep notch is found between the inner part of the corallite wall and the distal end of the septa (arrow in Fig. 5i). S1 sides with blunt spines all over the surface (Fig. 5m). The well developed styliform columella sits relatively deep in the fossa , with a wide base (Fig. 5e), pointed (Fig. 5i), and circular to oval in section (Figs. 5e, i). Columellae of adjacent corallites are on average 2.01 mm (± 0.27) apart. Coenosteum generally very reduced and composed by a series of juxtaposed granules corresponding to the tips of trabeculae (Figs.5i, m).

The skeletons of *M. pharensis* f. *luciphila* from Frade *et al.* (2010) included in our analyses have round corallites (Figs. 5b, f) with a plocoid arrangement (Figs. 5b). Average CD is 1.25 mm (± 0.12) (Table 2). Septal arrangement is decameral (S1 = 10) (Fig. 5f). S2 are shorter than S1 being in some specimens extremely reduced and visible only at the SEM as lobes alternating with S1 (Figs. 5f, j), while in others is more developed and visible (Fig. 3). The top margin of the S1 is exsert to the coenosteum, being also higher than the coenosteum ornamentation (arrow in Fig. 5j). Finely ornamented septal lobes are found at the inner end of the septa before they fuse with the columella (Figs. 3, 45f, j). S1 sides with blunt spines all over the surface (Fig. 5n). A styliform columella sits relatively deep in the fossa and is well developed, with a wide base (Fig. 5e), pointed (Fig. 4i), and circular in section (Figs. 5e, i). Columellae of adjacent corallites are on average 1.84 mm (± 0.28) apart. Coenosteum well developed, compact (Fig. 3) or presenting pits between the corallite wall and the coenosteum ornamentation (Figs. 5 b, f, n) which is composed of medium sized

granules (smaller than those observed in *M. pharensis*) (Figs. 5j, n) mostly arranged in lines forming a reticule separating adjacent corallites (Fig. 3).

The coralla of *M. senaria* from Frade *et al.* (2010), included in our analyses, have round corallites with a plocoid arrangement (Figs. 5c, g). Average CD is 1.45 mm (± 0.12) (Table 2). As described by Wells (1973b), S1 = 10, but septa are dimorphic. In fact, six protosepta (S1P, Figs. 5g, k) and four metasepta (S1M, Figs. 5g, k) can be distinguished, with S1M thinner and shorter than S1P. The top margin of the S1P is exsert with respect to the coenosteum (arrow in Fig. 5k) and higher than the S1M margin. Septal lobes at the inner end of the septa were not observed in the examined specimens (Fig. 5k). Septal side ornamentation present on S1P towards the outer end of the septa where they are thicker, reduced or absent on S1P (Fig. 5k). The styliform columella sits relatively deep in the fossa and is well developed and with a variably wide base, rounded at the top (Fig. 5k), and circular to oval in section (Figs. 5g, k). Columellae of adjacent corallites are on average 2.31 mm (± 0.36) apart. Coenosteum well developed, compact (Fig. 5c). Coenosteum ornamentation is composed of small sized granules (Fig. 5o) which can be well formed or very reduced.

The examined specimens of *M. kirbyi* have round to polygonal corallites with a cerioid or sub-plocoid arrangement depending of the specimen (Fig. 5d; Figs. 164, 165 in Veron & Pichon 1976). Average CD is 1.45 mm (± 0.18) (Table 2). Septal arrangement is decameral (S1 = 10) with reduced to extremely reduced S2 (Fig. 5h). The top margin of the S1 is slightly exsert or flush with the coenosteum ornamentation. Finely ornamented septal lobes are found at the inner end of the septa before they fuse with the columella (Fig. 5l). A notch is found between the inner part of the corallite wall and the distal end of the septa (arrow in Fig. 5l) resulting in a lobe similar to that observed in *M. pharensis* although in this species lobes are more pronounced. S1 sides with blunt spines all over the surface (Fig. 5l). The styliform columella sits relatively deep in the fossa and is well developed, with a wide base (Figs. 5h, l), rounded, and circular to oval in section (Figs. 5h, l). Columellae of adjacent corallites are on average 1.91 mm (± 0.32) apart. Coenosteum generally well formed, ornamentation composed by a series of large (Fig. 5p) or medium (Fig. 5h) sized granules forming bounding ridges shared by adjacent corallites (Fig. 5d) (Veron & Pichon 1976).

Statistically significant differences in CD were found between *M. pharensis*, which has the largest average CD among the examined species, *M. pharensis* f. *luciphila* (smallest CD) and *M. senaria*. Statistically significant differences in CD were also found between *M. pharensis* f. *luciphila* and *M. senaria* and *M. kirbyi* (Table 3). No statistically significant differences in calice dimensions were found between *M. pharensis* and *M. kirbyi*, and *M. senaria* (Table 3). Statistically significant differences in ID were found between *M. senaria*, which has the largest ID among the examined species, and *M. pharensis* (smallest ID), *M. pharensis* f. *luciphila*, and *M. kirbyi* (Table 3). No statistically significant differences ID were found between *M. pharensis* and *M. pharensis* f. *luciphila*, or *M. kirbyi*, nor between *M. pharensis* f. *luciphila* and *M. kirbyi* (Table 3).

Discussion

Genetic and morphological boundaries between the examined species

Significant genetic and morphological differences were found among the examined *Madracis* species in general, and between *M. pharensis* and *M. pharensis* f. *luciphila* in particular. All the examined species presented a decameral arrangement of the septa (S1 = 10; S2 < S1 or extremely reduced) with the case of *M. senaria* presenting dimorphism of S1 (S1P = 6; S1M = 4) (Fig. 5). Overall, the development of S2 varied in all the examined species, being always extremely reduced in *M. senaria* but variable in the other species. According to Cairns (2000) and to Neves & Johnsson (2009), *M. pharensis* specimens from southwestern Atlantic have 12 septa, 6 S1 and 6 S2. Although we agree with these authors that "septal number may be an unreliable character if considered exclusively" (Neves & Johnsson 2009), none of the specimens we examined had 12 septa and we are aware of no description or illustration of *M. pharensis* with such arrangement. Moreover, the figures of *M. pharensis* in Cairns (2000: Figs. 36–41) show a typical decameral arrangement of the septa. In fact, the author kindly acknowledged a *lapsus calami* in the text of his publication (S.D. Cairns pers. comm.).

Our results show that the examined Mediterranean specimens of *M. pharensis* and the Caribbean *M. pharensis* f. *luciphila* are genetically distinct (Tables 4-5). Moreover, although the average inter corallite distance is not significantly different between the two supposed morphs of the same species, corallites in *M. pharensis* are statistically significantly larger than in *M. pharensis* f. *luciphila* (Fig. 6; Table 3). These remarkable differences, highlighted in this study for the first time, suggest that *M. pharensis* f. *luciphila* is not simply a different morph of *M. pharensis*, but that it is a distinct species and that the two can be distinguished based on their morphology, genetics and ecology. Therefore, the doubts expressed by previous authors (Wells 1973a; Zibrowius 1980) on the actual identity of the Caribbean and Brazilian specimens of *M. pharensis* are confirmed by our findings. However, two points remain to be clarified before any formal taxonomic action can be undertaken. Firstly, it is needed to verify if, as suggested by Fenner (1993), the zooxanthellate *M. pharensis* f. *luciphila* which has been called *M. pharensis* in the Caribbean and in Brazil is actually a form, or ecomorph, of the morphologically plastic and zooxanthellate *Madracis decactis*. In fact, Frade *et al.* (2010) showed that the genetic boundaries between these species are semi-permeable. Secondly, the genetic and morphologic boundaries between the azooxanthellate and sciaphilous *M. pharensis* from the Caribbean and the forms examined in this study need to be investigated. The difference in size between the zooxanthellate (larger) and azooxanthellate (smaller) Caribbean forms were already remarked by Cairns (2000) who also noted that the former has more widely spaced corallites than the latter.

There is recognized ecophenotypic plasticity at both colony and corallite level in *Madracis* (Todd 2008). However, Filatov *et al.* (2013) argued that morphological characteristics at the colony level in addition to corallite morphology, genetic and ecological information, are in particular useful to delineate different coral species in the genus. They focused on Caribbean species characterized by a branching growth form and did not include either form of *M. pharensis*. Although we did not include measures of the corallum shape in our morphological analyses, all the coralla of the examined *M. pharensis* specimens were from knob-shaped and/or encrusting colonies, while all the examined specimens of *M. pharensis* f. *luciphila* were from typically encrusting or massive coralla.

Our results showed that *M. pharensis* and *M. kirbyi* are genetically distinct. However, no statistically significant difference in corallite diameter or inter columellar distance was found between these two species. In the original description of *M. kirbyi*, Veron & Pichon (1976) remarked that this species has a much-reduced second cycle of septa compared with that of the Mediterranean specimens of *M. pharensis*, but that the two species seem related. The degree of development of the second cycle of septa considered by some authors as an informative character seems actually rather variable intraspecifically as also remarked by Zlatarski & Estalella (1980) in their description of *M. decactis*. Morphological analyses at the SEM showed, however, differences in columellar morphology between the two species. In *M. pharensis* the columella is wider at the base (Fig. 5e) than in *M. kirbyi* (Fig. 5h) and its tip is pointed (Fig. 5i), while in the latter it is rounded (Fig. 5l). Moreover, in several of the examined *M. kirbyi* specimens the septa reaching the columella form a pattern of radiating ridges on its surface (Fig. 5h). Schmidt-Roach *et al.* (2014) suggested that in the genus *Pocillopora* the shape of the columella could be a reliable character to tell species apart despite a remarkable reduction and morphologic variability of the septa. Our results indicate that the columella could be also an informative morphological character in in *Madracis*, suggesting that this feature should be taken into account in future studies addressing species level differences in other pocilloporids as well. *Madracis senaria* is morphologically and genetically a distinct species being the only one among the Caribbean species examined by Frade *et al.* (2010) for which clear genetic boundaries were evidenced. Our results confirm this finding, showing that this species is also genetically distinct from *M. pharensis* and *M. kirbyi*. Despite significant genetic differences between *M. kirbyi* and *M. senaria*, and although corallites are more distant in the latter than in the former, these two species have similar corallite diameter. The peculiar dimorphism of S1 (S1P = 6; S1M = 4) (Fig. 5) in *Madracis senaria*, however, allows to readily tell these two species apart from all the other described species in the genus.

Difference in hosted endosymbiont species between M. pharensis *and* M. pharensis *f.* luciphila

In addition to the genetic and morphological differences discussed above, *M. pharensis* and *M. pharensis* f. *luciphila* differ also in some ecological traits. In the temperate Mediterranean and Eastern Atlantic, zooxanthellate colonies of the facultative symbiotic species (Cairns *et al.* 1999) host the cold-loving *Symbiodinium pysgmophilum* (LaJeunesse *et al.* 2012) harbored by subtropical and temperate corals, previously named type B2. However, all specimens from the Caribbean examined by Frade *et al.* (2008a, b, c) hosted *Symbiodinium* B7 type, while the colonies below 25 m hosted the B15 type instead. This genotype dominates the deeper symbiont populations associated with *M. pharensis* f. *luciphila* all the way down to the host's lower limit of distribution of >90 m depth (Bongaerts *et al.* 2015). Such observed niche partitioning relates to the physiological capabilities of the symbionts involved (Frade *et al.* 2008b) and matches the evolutionary split between shallow and deep *M. pharensis* f. *luciphila* lineages, suggesting depth-related environmental gradients as driving factors to disruptive selection and the evolution of corals (Frade *et al.* 2010, Bongaerts *et al.* 2013).

The occurrence of M. pharensis *outside the Mediterranean and the Eastern Atlantic*

In addition to the aforementioned records of *M. pharensis* in the Western Atlantic, an azooxanthellate form of *Madracis* named *M.* sp. cf *M. pharensis* has been recorded in the Indo-Pacific in the Philippines and Fiji (Cairns & Zibrowius 1997), the Galapagos (Wells 1983, Cairns 1991, Hickman 2008), and in the Guf of California, Mexico (Reyes Bonilla *et al.* 1995; Cairns & Zibrowius 1997)(;;;;. Not much is known about this form which has never been analyzed genetically. Reyes Bonilla *et al.* (1995) suggested that skeletal differences among *M. pharensis* and *Madracis* sp. cf *M. pharensis* "are important and consistent enough that they may represent two different species" mainly based on the extreme reduction of the secondary septa in the Pacific form (e.g. Hickman 2008:24 SEM figure). Our results confirm the suspicions expressed by some authors (see Wells 1973a; Zibrowius 1980; Fenner 1993) that the Caribbean zooxanthellate and hermatypic form previously named *M. pharensis* (Diekmann *et al.* 2001; Vermeij & Bak 2002b; Vermeij *et al.* 2004; Frade *et al.* 2008a, b, c, 2010) and *M. pharensis* f. *luciphila* (Wells 1973a; Cairns 2000; Cairns *et al.* 2009) is not the same species as *M. pharensis* from the Mediterranean (Heller 1868; Laborel & Vacelet 1958; Zibrowius 1980; Gerovasileiou *et al.* 2015). However, the azooxanthellate Caribbean form *M. pharensis* f. *pharensis* was not investigated in this study. Nevertheless, it cannot be ignored that this sciaphilous azooxanthellate form with smaller encrusting to nodular coralla (Cairns 2000) is morphologically and genetically the same as *M. pharensis* from the Mediterranean and the Azores. Thus, further analyses including *M. pharensis* f. *pharensis* are needed to clarify the status of this form and, most of all, the actual geographic distribution of the typical *M. pharensis*. Moreover, the species boundaries between *M. pharensis* f. *luciphila* and the other Caribbean species examined by Diekmann *et al.* (2001) and Frade *et al.* (2010), namely *M. decactis*, *M. formosa*, *M. mirabilis* (= *Madracis auretenra* Locke, Weil & Coates, 2007) and *M. carmabi*, need to be analyzed using ATP8 as a marker together with detailed micromorphological tools and will be addressed in a paper in preparation.

Conclusions

The results presented in this paper indicate that *M. pharensis* from the Mediterranean Sea and the form c*M. pharensis* f. *luciphila* found in the Caribbean are two genetically and morphologically different species. Moreover, *Madracis kirbyi*, examined for the first time in this study through an integrated systematics approach, and *M. senaria* are valid species genetically and morphologically distinct from the formers. However, whether *M. pharensis* f. *luciphila* is an undescribed species or a morph of *M. decactis* remains to be ascertained. Also, the possibility that *M. pharensis* f. *luciphila* is a complex of species with host depth partitioning and genetic segregation of its symbionts can not be excluded. Moreover, the identity of the azooxanthellate Caribbean form of *M. pharensis* remains unresolved. These issues need to be addressed on a broad morphological and genetic comparative study for the whole genus *Madracis* including material from a wide depth range.

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Figure legends

Fig. 1 *In situ* images of *Madracis pharensis* (a-b), *M. pharensis* f. *luciphila* (c-d), *M. senaria* (e-f), and *M. kirbyi* (g-h) showing corallum growth form and polyps; **a** azooxanthellate colony in a cave at 15m (UNIMIB MD856, Sicily, Italy); **b** zooxanthellate colony with expanded polyps in an under-hang at 20m (UNIMIB MD846, Liguria, Italy); **c** a colony of the green morph (PF2006.936, Curaçao, Dutch Caribbean); **d** a colony of the brown morph (PF2006.699, Curaçao, Dutch Caribbean); **e**, **f** sub-massive colony; **g** encrusting colony at 15m (UNIMIB TOMY299, Bouéni Bay, Mayotte); **h** knobby colony at 35 m (IRD HS2868 Prony Bay, New Caledonia).

Fig. 2 Maps showing the localities where *Madracis* specimens examined in this study were sampled in the Caribbean, Southern Red Sea and Indo-Pacific (a) and in the West Atlantic, Mediterranean Sea and Northern Red Sea. Numbers in the white-filled circles indicate the sampling localities: 1, Curaçao, Dutch Caribbean; 2, Santa Maria Island, Azores; 3, La Ciotat, France; 4, Port-Cros, France; 5, Bergeggi, Italy; 6, Paraggi, Italy; 7, Zembra Island, Tunisia; 8, Taormina, Italy; 9, Kotor, Montenegro; 10, Therasia Island, Greece; 11, Yambu, Saudi Arabia; 12, Farasan Banks, Saudi Arabia; 13, Socotra Island, Yemen; 14, Mayotte Island; 15, Madang, Papua New Guinea; 16, south of the Grande Terre, New Caledonia. The color filled circles symbols indicate which species or form was sampled at each locality (brown = *M. senaria*; green = *M. pharensis* f. *luciphila*; orange = *M. pharensis* f. *luciphila*; turquoise = *M. kirbyi).* Further information on sampling sites in Table 1.

Fig. 3 Linear variables considered for morphological analysis of the examined *Madracis* species: calice diameter (CD) and inter-columellar distance (ID) (specimen PF2006.883, *M. pharensis luciphila*).

Fig. 4 Median-joining network of **a** ATP8 and **b** ITS region haplotypes of *M. pharensis* (orange), *M. pharensis* f. *luciphila* (green), *Madracis senaria* (grey), *M. kirbyi* (blue). The circle diameter is proportional to the frequency of specimens sharing the same haplotype. The length of the branches is proportional to the number of mutation events that separates the haplotypes.

Fig. 5 SEM images of the corallites arrangement (a-d), top view (e-h) and side view (i-l) of a calice, and detail of the coenosteum ornamentation in *Madracis pharensis* (a, e, i, m), *M. pharensis* f. *luciphila* (b, f, j, n), *M. senaria* (c, g, k, o), and *M. kirbyi* (d, h, l, p); **a**, **e** MNHN IK 2010-784 (Therasia, Greece); **b**, **f**, **j**, **n** PF2006.884 (Curaçao, Dutch Caribbean); **c**, **g**, **k**, **o** PF2006.902 (Curaçao, Dutch Caribbean); **d**, **h** IRD HS2868 (Prony Bay, New Caledonia); **i** MNHN IK 2010-786 (La Ciotat, France); **l** IRD HS672 (Banc Gail, New Caledonia); **m** UNIMIB MD844 (Liguria, Italy); **p** UNIMIB SO168 (Socotra Island, Yemen); arabic numerals on the septa in e-h indicate the cycle number; in *M. senaria* 1P and 1M indicate protosepta and

metasepta (*sensu* Wells 1973b), respectively; arrows indicate the top lobe of a septum; arrowheads indicate the coenosteum ornamentation.

Fig. 6 Average (± S.E.) calice diameter (CD) and inter-columellar distance (ID) for the *Madracis* species examined in this study.

Tables

Table 1

List of the examined *Madracis* samples, including collection code, sampling locality (the number in parentheses refers to the locality shown in Fig. 1), latitude and longitude of the sampling site, expedition and collection year (Exp.), collector (Coll.) andEMBL accession numbers for the examined markers (ITS region and ATP8), and presence of morphological data (Morph.). Specimens deposited at Institut de Recherche pour le Développement, Noumea, New Caledonia (IRD); King Abdullah University of Science and Technology, Thuwal, Kingdom of Saudi Arabia (KAUST); National Museum of Natural History, Paris, France (MNHN); University of Milano-Bicocca, Milano, Italy (UNIMIB). - = sampling did not occur in the frame of any scientific expedition onboard a research vessel. Further details on the research vessels and DOI on the expeditions, when available, can be found in the acknowledgements.

Species	**Code**	**Locality**	**Latitude**	**Longitude**	**Exp.**	**Coll.**	**ITS region**	**ATP8**	**Morph.**
M. pharensis	UNIMIB MD844	Paraggi, Liguria, Italy (6)	44° 18.673'N	9° 12.772'E	- 2011	A. Caragnano	LN875783	LN875815	Yes
M. pharensis	UNIMIB MD845	Paraggi, Liguria,Italy (6)	44° 18.673'N	9° 12.772'E	- 2011	F. Benzoni	LN875784	LN875816	-
M. pharensis	UNIMIB MD846	Paraggi, Liguria,Italy (6)	44° 18.673'N	9° 12.772'E	- 2011	A. Caragnano	LN875785	LN875817	Yes
M. pharensis	UNIMIB MD853	Bergeggi, Liguria, Italy (5)	44° 14.047'N	8° 26.630'E	- 2012	S. Montano	LN875786	LN875818	-
M. pharensis	UNIMIB MD856	Taormina, Sicily, Italy (8)	37° 50.694'N	15° 17.943'E	- 2012	F. Benzoni	LN875787	LN875819	Yes
M. pharensis	MD02	Taormina, Sicily, Italy (8)	37° 50.694'N	15° 17.943'E	- 2012	F. Benzoni	-	-	Yes
M. pharensis	MD03	Taormina, Sicily, Italy (8)	37° 50.694'N	15° 17.943'E	- 2012	F. Benzoni	-	-	Yes
M. pharensis	MD01	Kotor, Montenegro (9)	42° 28.833'N	18° 43.267'E	CROMA 2014	M. Taviani	LN875788	LN875820	Yes
M. pharensis	MNHN IK2010.783	Pointe du Tuf, Port-Cros, France (4)	43° 0.153'N	6° 24.815'E	- 1970	H. Zibrowius	-	-	Yes

M. pharensis	MNHN IK2010.784	Cap Tripiti, Therasia Island, Greece (10)	36° 24.542'N	25° 21.028'E	MEDOR 1967	J.G. Harmelin	-	-	Yes
M. pharensis	MNHN IK2010.785	Sao Lourenco Bay, Santa Maria Island, Azores (2)	36° 59.291'N	25° 02.592'W	Bioaçores 1971	H. Zibrowius	-	-	Yes
M. pharensis	MNHN IK2010.786	Grotte des Trois Pépés, La Ciotat, France (3)	43° 9.763'N	5° 36.042'E	- 1991	H. Zibrowius	-	-	Yes
M. pharensis	MNHN IK2010.787	Zembra Island, Tunisia (7)	37° 7.191'N	10° 47.247'E	- 1969	H. Zibrowius	-	-	Yes
M. pharensis f. *luciphila*	PF2005.243	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	-	-	Yes
M. pharensis f. *luciphila*	PF2005.399	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	LN875789	LN875821	Yes
M. pharensis f. *luciphila*	PF2005.397	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	LN875790	LN875822	Yes
M. pharensis f. *luciphila*	PF2005.555	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	-	-	Yes
M. pharensis f. *luciphila*	PF2006.693	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	LN875791	LN875823	-
M. pharensis f. *luciphila*	PF2006.694	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	LN875792	LN875824	-
M. pharensis f. *luciphila*	PF2006.699	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	LN875793	LN875825	-
M. pharensis f. *luciphila*	PF2006.705	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	LN875794	LN875826	Yes
M. pharensis f. *luciphila*	PF2006.711	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	LN875795	LN875827	-
M. pharensis f. *luciphila*	PF2006.712	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	LN875796	LN875828	Yes
M. pharensis f. *luciphila*	PF2006.783	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	LN875797	LN875829	Yes

M. pharensis f. *luciphila*	PF2006.883	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	LN875798	LN875830	Yes
M. pharensis f. *luciphila*	PF2006.884	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	LN875799	LN875831	-
M. pharensis f. *luciphila*	PF2006.914	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	-	-	Yes
M. pharensis f. *luciphila*	PF2006.936	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	-	-	Yes
M. senaria	PF2005.303	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	-	LN875832	Yes
M. senaria	PF2005.510	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	-	LN875833	-
M. senaria	PF2005.543	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	-	LN875834	Yes
M. senaria	PF2005.571	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	-	LN875835	Yes
M. senaria	PF2005.572	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	-	LN875836	Yes
M. senaria	PF2006.670	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	-	-	Yes
M. senaria	PF2006.901	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	-	LN875837	Yes
M. senaria	PF2006.902	Curaçao, Dutch Caribbean (1)			-	P.R. Frade	-	LN875838	Yes
M. kirbyi	KAUST SA037	Dolphen Lagoon, Farasan Banks, Saudi Arabia (12)	19° 00.320'N	40° 08.889'E	KAUST FARASAN BIODIVERSITY 2013	F. Benzoni	LN875800	LN875839	-
M. kirbyi	KAUST SA074	Ablo, Farasan Banks, Saudi Arabia (12)	18° 39.571'N	40° 49.618'E	KAUST FARASAN BIODIVERSITY 2013	F. Benzoni	LN875801	LN875840	-
M. kirbyi	KAUST SA1181A	Yambu, Saudi Arabia (11)	24° 26.561'N	37° 14.860'E	KAUST AQABA BIODIVERSITY 2013	G. Paulay	LN875802	LN875841	-

M. kirbyi	KAUST SA1181B	Yambu, Saudi Arabia (11)	24° 26.561'N	37° 14.860'E	KAUST AQABA BIODIVERSITY 2013	G. Paulay	LN875803	LN875842	-
M. kirbyi	IRD HS unreg	Prony Bay, Grande Terre, New Caledonia (16)	22° 19.927'S	166° 49.806'E	HYDROPRONY 2011	C. Payri	-	-	Yes
M. kirbyi	IRD HS2672	Ilot Casy, Grande Terre, New Caledonia (16)	22° 21.208'S	166° 50.304'E	- 2009	G. Lasne	-	-	Yes
M. kirbyi	IRD HS2868	Prony Bay, Grande Terre, New Caledonia (16)	22° 21.230'S	166° 49.300'E	- 2011	F. Benzoni	LN875804	LN875843	Yes
M. kirbyi	IRD HS2977	Pouembout, Grande Terre, New Caledonia (16)	21° 16.847'S	164° 47.081'E	CORALCAL 4 2012	F. Benzoni	-	-	Yes
M. kirbyi	UNIMIB TOMY012	Ile Blanche, Mayotte Island (14)	12° 42.891'S	45° 10.455'E	TARA OCEANS 2010	F. Benzoni	LN875805	LN875844	Yes
M. kirbyi	UNIMIB TOMY088	Bouény Bay, Mayotte Island (14)	12° 54.698'S	45° 07.871'E	TARA OCEANS 2010	F. Benzoni	LN875806	LN875845	Yes
M. kirbyi	UNIMIB TOMY298	Bouény Bay, Mayotte Island (14)	12° 54.698'S	45° 07.871'E	TARA OCEANS 2010	F. Benzoni	LN875807	LN875846	Yes
M. kirbyi	UNIMIB TOMY299	Bouény Bay, Mayotte Island (14)	12° 54.698'S	45° 07.871'E	TARA OCEANS 2010	F. Benzoni	LN875808	LN875847	Yes
M. kirbyi	UNIMIB PFB024	Madang, Papua New Guinea (15)	5° 11.347'S	145° 49.637'E	NIUGINI 2012	F. Benzoni	LN875809	LN875848	Yes
M. kirbyi	UNIMIB PFB242	Wonad Island, Madang, Papua New Guinea (15)	5° 11.624'S	145° 49.521'E	NIUGINI 2012	F. Benzoni	LN875810	LN875849	-
M. kirbyi	UNIMIB PFB291	Madang, Papua New Guinea (15)	5° 05.020’S	145° 49.358'E	NIUGINI 2012	F. Benzoni	LN875811	LN875850	Yes
M. kirbyi	UNIMIB PFB363	Madang, Papua New Guinea (15)	5° 06.716'S	145° 49.354'E	NIUGINI 2012	F. Benzoni	LN875812	LN875851	Yes

M. kirbyi	UNIMIB SO080	Shalintaitin, Socotra Island, Yemen (13)	12° 40.156'S	54° 02.850'E	YEMEN BIODIVERSITY 2010	F. Benzoni	LN875813	LN875852	Yes
M. kirbyi	UNIMIB SO091	Alamah, Socotra Island, Yemen (13)	12° 36.861'S	53° 49.137'E	YEMEN BIODIVERSITY 2010	F. Benzoni	LN875814	LN875853	-

Table 2 Average (± standard deviation) calice diameter (CD) and inter-columellar distance (ID) for the *Madracis* species examined in this study.

Species	**n**	**CD**	**ID**
M. pharensis	9	1.66 (± 0.22)	2.01 (± 0.27)
M. pharensis f. *luciphila*	10	1.25 (± 0.28)	1.85 (± 0.12)
M. senaria	7	1.45 (± 0.36)	2.31 (± 0.12)
M. kirbyi	12	1.46 (± 0.32)	1.91 (± 0.18)

Table 3 One-way ANOVA results for differences between the examined *Madracis* species (Mp = *M. pharensis*; Mpl = *M. pharensis* f. *luciphila*; Ms = *M. senaria*; Mk = *M. kirbyi*). *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001; n.s., not significant.

	Mp - Mpl		**Mp - Ms**		**Mp - Mk**		**Mpl - Ms**		**Mpl - Mk**		**Ms - Mk**	
	F	p	F	p	F	p	F	p	F	p	F	p
CD	27.46	****	5.01	*	5.98	n.s.	12.06	**	10.19	**	0.00	n.s.
ID	1.87	n.s.	4.03	*	0.60	n.s.	9.01	**	0.28	n.s.	6.19	*

Table 4 Pair-wise comparison of genetic distance values (*p*-distance) within (bold) and between the examined *Madracis* species based on mitochondrial ATP8. Values are means (± standard deviation).

	M. pharensis	***M. pharensis* f. *luciphila***	***M. senaria***	***M. kirbyi***
M. pharensis	**0.0013 (± 0.0008)**			
M. pharensis* f. *luciphila	0.0321 (± 0.0051)	**0 (± 0)**		
M. senaria	0.0304 (± 0.0051)	0.0349 (± 0.0055)	**0.0019 (± 0.0009)**	
M. kirbyi	0.0869 (± 0.0087)	0.0968 (± 0.0086)	0.0887 (± 0.0086)	**0 (± 0)**

Table 5 Pair-wise comparison of genetic distance values (*p*-distance) within (bold) and between the examined *Madracis* species based on the ITS region of the nuclear rDNA. Values are means (± standard deviation).

	M. pharensis	***M. pharensis* f. *luciphila***	***M. senaria***	***M. kirbyi***
M. pharensis	**0.0117 (± 0.0027)**			
M. pharensis* f. *luciphila	0.0181 (± 0.0040)	**0.0065 (± 0.0018)**		
M. senaria	0.0173 (± 0.0037)	0.0171 (± 0.0039)	**0.0072 (± 0.0018)**	
M. kirbyi	0.0292 (± 0.0059)	0.0273 (± 0.0055)	0.0333 (± 0.0052)	**0.0118 (± 0.0029)**