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Species delimitation in the coral genus *Goniopora* (Scleractinia, Poritidae) from the Saudi Arabian Red SeaTullia I. Terraneo¹, Francesca Benzoni^{2,3}, Roberto Arrigoni¹, Michael L. Berumen¹¹ Red Sea Research Center, Division of Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology, Thuwal 23955-6900, Saudi Arabia² Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, Milano 20126, Italy³ Institut de Recherche pour le Développement, UMR227 Coreus2, 101 Promenade Roger Laroque, BP A5, 98848 Noumea Cedex, New CaledoniaCorresponding author: Tullia I. Terraneo (tulliaisotta.terraneo@kaust.edu.sa)**Keywords:** GMYC, PTP, ABGD, haplowebs, DNA taxonomy, integrative taxonomy**Running Title:** Species delimitation in *Goniopora***Abstract**

Variable skeletal morphology, genotype induced plasticity, and homoplasy of skeletal structures have presented major challenges for scleractinian coral taxonomy and systematics since the 18th century. Although the recent integration of genetic and micromorphological data is helping to clarify the taxonomic confusion within the order, phylogenetic relationships and species delimitation within most coral genera are still far from settled. In the present study, the species boundaries in the scleractinian coral genus *Goniopora* were investigated using 199 colonies from the Saudi Arabian Red Sea and sequencing of four molecular markers: the mitochondrial intergenic spacer between CytB and NAD2, the nuclear ribosomal ITS region, and two single-copy nuclear genes (ATPs β and CalM). DNA sequence data were analyzed using a variety of methods and exploratory species-delimitation tools. The results were broadly congruent in identifying five distinct molecular lineages within the sequenced *Goniopora* samples: *G. somaliensis*/*G. savignyi*, *G. djiboutiensis*/*G. lobata*, *G. stokesi*, *G. albiconus*/*G. tenuidens*, and *G. minor*/*G. gracilis*. Although the traditional macromorphological characters used to identify these nine morphospecies were not able to discriminate the obtained molecular clades, informative micromorphological and

microstructural features (such as the micro-ornamentation and the arrangement of the columella) were recovered among the five lineages. Moreover, unique *in vivo* morphologies were associated with the genetic-delimited lineages, further supporting the molecular findings. This study represents the first attempt to identify species boundaries within *Goniopora* using a combined morpho-molecular approach. The obtained data establish a basis for future taxonomic revision of the genus, which should include colonies across its entire geographical distribution in the Indo-Pacific.

1. Introduction

Coral reefs occupy one-third of the world's tropical waters (Roberts et al., 2002) and represent one of the most biologically diverse ecosystems on Earth, (e.g., Bowen et al., 2013). Scleractinian corals are primary builders of coral reefs (Veron, 2000), and understanding the ecology, evolution, and biodiversity of corals is of fundamental importance for preserving these unique and threatened ecosystems (Carpenter et al., 2008). The knowledge of species boundaries within the order Scleractinia, as well as a clear understanding of their phylogenetic relationships and evolution, is critical for population genetics, physiological, and ecological studies. Nevertheless, we lack a comprehensive understanding of species boundaries within this group, which remains one of the most challenging taxonomic taxa for reliable species delimitation (Kitahara et al., in press). A major source of confusion is that the skeletal structures traditionally used to classify corals are extremely variable (Todd, 2008). Moreover, these taxa are influenced by convergent evolution and homoplasy, creating incongruence among different phylogenetic reconstructions (van Oppen et al., 2001; Fukami et al., 2004). Veron and Pichon (1976) first applied the “ecomorph” concept to corals, based on the fact that specific ecological conditions can influence intraspecific skeletal variations in corals. The environment can act on both phenotype and genotype, thus obscuring real evolutionary patterns. It has been experimentally demonstrated that several environmental factors can influence coral morphology, such as light, sedimentation, wave action, depth, and salinity (Randal, 1976, Miller, 1992, Bruno and Edmunds, 1997, Todd, 2008). High environmental heterogeneity in coral reefs creates several microhabitats that can have important impacts on colony growth form. In such a variable habitat, a high degree of environment-phenotype matching is expected (Lloyd, 1984). The amount of morphological plasticity varies widely among taxa as well as within the same taxa in different environmental conditions (Todd, 2008).

Increasingly accessible molecular tools have led to a rise in phylogenetic analyses in corals, often revealing hidden species diversity that was undetectable using traditional morphological analysis. In the last decade, numerous genetic identification algorithms have been proposed for species delimitation and estimation of evolutionary patterns among closely related species. Early approaches included DNA barcoding (Hebert et al., 2003) while more recent methods include analyses of a generalized mixed Yule coalescent (Pons et al., 2006), haplowebs (Flot et al., 2010), Automatic Barcoding Gap Detection (Puillandre et al., 2012), and Poisson-Tree-Processes (Zhang et al., 2013). The application of these techniques, generally known as DNA taxonomy (Tautz et al., 2003), has been proven to be a fast, objective, and rigorous assessment of biodiversity (Fontaneto et al., 2015). However, molecular analyses are ideally integrated with other analyses (e.g., morphology) to ensure a more accurate species delimitation (Schlick-Steiner et al., 2010). This is particularly true when dealing with organisms such as corals, where large population sizes and dispersal abilities increase the likelihood of incomplete lineage sorting and reproductive modes provide ample opportunity for hybridization (Knowlton, 2000). On one hand, the accuracy of genetic identification depends on the availability of fixed species-specific mutations within the analyzed DNA markers. It is therefore strongly encouraged to use multiple lines of genetic evidence in order to reduce the impact of limitations such as introgression, incomplete lineage sorting, retained ancestral polymorphisms, pseudogenes, or intragenomic variations (Degnan and Rosenberg, 2009). On the other hand, these molecular data should always be coupled with information from other fields, such as morphology, ecology, and reproduction, with the aim of a correct and broad formulation of species boundaries (de Queiroz, 2007).

The genus *Goniopora* de Blainville, 1830, common across the Indo-Pacific, is characterized by a genus-specific septal formula, *i.e.* a gonioporoid pattern (Bernard, 1903) (although this is not always discernable) and by having polyps with up to 24 tentacles usually extended in daytime (Veron and Pichon, 1982). Species identification to date has been conducted only using traditional macromorphology, such as colony growth form, corallite dimensions, and number and fusion pattern of septa (Veron, 2000). Unfortunately, these features are highly variable and plastic in *Goniopora*. In many other genera, molecular studies have demonstrated that these characters are evolutionary uninformative (Kitahara et al., in press). Bernard (1903) published the most comprehensive review of the genus in his “Catalogue of the Madreporarian Corals”. Despite more than five years of study, referring to both *Goniopora* and *Porites*, the author stated: “*I have no hesitation in asserting that in the present state of our knowledge coral species are indeterminable. The long list of names which were steadily growing as I proceeded to*

designate every apparent different form of Porites and Goniopora in the old way got completely out of hand". During 20th century more than 50 nominal species of *Goniopora* were described based on macromorphology (WoRMS, 2016). Meanwhile, Veron (2000) proposed that the *in situ* polyp morphology could be used as a key character for underwater species delimitation, leading to even further taxonomic confusion. Few molecular studies have been carried out in this group, limited primarily to assessing the phylogenetic position of *G. stokesi* (Kitano et al., 2013) and the evolutionary placement of the genus within the Poritidae (Kitano et al., 2014). Therefore we still lack a first attempt of species delimitation in *Goniopora*. According to the World Register of Marine Species, 32 extant species are ascribed to *Goniopora* to date (WoRMS 2016), while Veron (2000) recognizes only 24 of these.

Regarding biogeography, the Red Sea seems to be a hotspot for *Goniopora* species diversity (Veron, 2000). Although the Red Sea is generally recognized as an important region of biodiversity and endemism (e.g., DiBattista et al., 2016), its biodiversity has remained largely understudied in recent years (Berumen et al., 2013). With regards to *Goniopora* in particular, the most recent morphological description of the genus from the Red Sea dates to Sheppard and Sheppard (1991), who identified eight main morphological species of *Goniopora*, namely *G. somaliensis* Vaughan, 1907, *G. djiboutiensis* Vaughan, 1907, *G. tenella* (Quelch, 1886), *G. stokesi* Milne Edwards and Haime, 1851, *G. columna* Dana, 1846, *G. tenuidens* (Quelch, 1886), *G. minor* Crossland, 1952, and *G. savignyi* Dana, 1846. Veron (2000) added Red Sea records of *G. lobata*, *G. burgosi*, *G. ciliatus*, *G. pearsoni*, *G. sultani*, and *G. planulata*, thus leading to a total of 14 *Goniopora* morphospecies recorded in the region.

Here, we employed a multi-locus approach combining sequence data of four DNA loci from both the mitochondrial and nuclear genome (the intergenic spacer between CytB and NAD2, the rDNA ITS region, the ATPs β gene, and the CalM gene) to delimit species of the genus *Goniopora* from the Saudi Arabian Red Sea. Genetic data were tested under different independent species delimitation strategies for the evaluation of putative molecular species. Finally, *in situ* morphology of polyps, skeletal macromorphology, and skeletal micromorphology were re-evaluated in light of genetic results with a perspective towards an integrative taxonomy (Padial et al., 2010).

2. Methods

2.1 Collection and morphospecies identification

A total of 199 *Goniopora* colonies were sampled in 27 sites along the Saudi Arabian coast of the Red Sea between October 2014 and March 2015 (Table S1, Fig. S1). Eleven coral colonies collected in Djibouti between

January and February 2010 during the Tara Oceans Expedition were also included in the analyses (Table S1, Fig. S1). Digital images of living colonies *in situ* were taken with a Canon Powershot G15 or a Canon Powershot G11 (Canon Inc., Tokyo, Japan) in an Ikelite underwater housing (Ikelite Underwater Systems, Indianapolis, USA). Specimens sampled in Saudi Arabia were preserved in 95% ethanol, while the samples from Djibouti were stored in CHAOS solution (not an acronym; 4 M guanidine thiocyanate, 0.1% N-lauroyl sarcosine sodium, 10 mM Tris pH 8, 0.1 M 2-mercaptoethanol). The rest of the corallum was bleached in a 2% sodium hypochlorite solution for 48 hours to remove soft tissues, rinsed with fresh water, and air dried for morphological analyses. Images of each corallum were taken with a Canon G15 or a Canon G11 digital camera.

Specimens were identified based on skeletal morphology after detailed observation of corallite features using a Leica M205 microscope (Leica Camera AG, Wetzlar, Germany) at King Abdullah University of Science and Technology (KAUST, Saudi Arabia) and a Leica M80 microscope (Leica Camera AG, Wetzlar, Germany) at University of Milano-Bicocca (UNIMIB, Italy). The number of septa, the presence of gonioporoid pattern and pali, the number of denticles, the depth of calice, and the dimension of columella were analyzed with reference to taxonomic original descriptions, type material, and bibliography illustrations of species recorded from the Red Sea (Sheppard and Sheppard, 1991). Details about morphospecies identification methods are provided in Data S1.

2.2 DNA extraction, amplification, and sequencing

Total DNA was extracted from samples stored in ethanol using DNAeasy® Tissue kit (Qiagen Inc., Hilden, Germany) whereas a phenol-chloroform-based method with a phenol extraction buffer (100 mM Tris-Cl pH 8, 10 mM EDTA, 0.1% SDS) was used to extract DNA from samples stored in CHAOS solution.

Four DNA markers (one mitochondrial region and three nuclear genes) were amplified by polymerase chain reaction (PCR). The mitochondrial fragment included a region spanning the 3' end of CytB and the 5' end NAD2 (hereafter IGR). The three nuclear genes were from a portion of the nuclear ribosomal DNA (including the 3' end of 18S, the entire ITS1, 5.8S, ITS2, and the 5' end of 28S, hereafter ITS region) and two single-copy genes, the ATPase β gene (ATPS β), and the calmodulin intron (CalM). All amplifications were conducted in a 15 μ l PCR volume composed of 0.2 μ M of each primer, 1X Multiplex PCR Master Mix (Qiagen Inc., Hilden, Germany), and < 0.1 ng DNA. The mitochondrial IGR was amplified using the primers CSF3 and CSR3 (Lin et al., 2011), the ITS region using the primers 1S and 2SS (Wei et al., 2006), the ATPS β gene using the primers ATPS β f2 (Forsman et al.,

2010) and ATPS β r2 (Concepcion et al., 2009), and the calmodulin intron using the primers CalMf and CalMr2 (Vollmer and Palumbi, 2002). All PCR products were purified by incubating with Illustra ExoStar (GE Healthcare, Buckinghamshire, UK) at 37°C for 60 min, followed by 85°C for 15 min and, subsequently, directly sequenced in forward and reverse directions using an ABI 3730xl DNA Analyzer (Applied Biosystems, Carlsbad, USA).

Forward and reverse sequences were assembled using CodonCodeAligner 3.7.0 (Codon Code Corporation, Dedham, USA). Chromatograms of the three nuclear genes showed intra-individual polymorphisms and double peaks, therefore these were statistically analyzed using PHASE (Stephens et al., 2001; available online at <http://stephenslab.uchicago.edu/software.html>). Sequences showing alleles of different length were discarded. For the other nuclear sequences with more than one heterozygous site, SeqPHASE (Flot, 2010; available online at <http://seqphase.mpg.de/seqphase/>) was used to generate PHASE input files from FASTA and convert PHASE output files back into FASTA (Flot et al., 2010). Final alignments of the four separate datasets were performed using E-INS-i option in MAFFT 7.130b (Kato and Standley, 2013) under default parameters and manually checked using BioEdit 7.2.5 (Hall 1999). (The alignment data are available on request from the corresponding author). The obtained alignments were run through GBLOCKS v0.91 b (Castresana 2000) to remove ambiguously aligned regions using the default “less stringent” settings. All new sequences generated in association with this study were deposited in EMBL (Table S1).

2.3 Haplotype networks and species delimitation analyses

In order to evaluate the relationships among different haplotypes, median-joining network analyses as implemented in Network 4.613 (Bandelt et al., 1999) were applied to each of the four separate datasets, i.e. IGR, ITS region, ATPS β , and CalM. Following Flot et al. (2010), nuclear haplonets were converted into haplowebs by drawing additional connections between the two haplotypes found co-occurring in heterozygous individuals using Network Publisher 2.0.0.1 (Fluxus Technology, Suffolk, UK) and Adobe Illustrator (Adobe Systems Inc., San Jose, USA), while sequences from the mitochondrial marker were simply sorted into haplogroups. To determine putative molecular species clusters in our dataset, we used three independent single locus species delimitation approaches: Automatic Barcoding Gap Discovery (ABGD) (Puillandre et al., 2012), Poisson-Tree processes (PTP) (Zhang et al., 2013), and generalized mixed Yule coalescent (GMYC) (Pons et al., 2006, Fujisawa and Barraclough, 2013). The ABDG was used to determine groups of candidate species on the basis of pairwise genetic distances. All the

alignments were imported in MEGA 6.0 (Tamura et al., 2013) to compute matrices of pairwise genetic distances using the Kimura 2-parameter (K2P) model (Table S2). These matrices were further used as input files on the ABGD webpage (<http://www.wabi.snv.jussieu.fr/public/abgd/abgdweb.html>) and a range of different settings were tested, showing concordant species delimitations results. Therefore, we set parameters as follows: $P_{\min} = 0.001$, $P_{\max} = 0.1$, Steps = 50, $X = 1.5$, and Nb bins = 20. The Poisson-Tree process is a tree-based species delimitation method that relies on coalescence theory and uses the branch lengths to estimate the average number of expected substitutions per site between two branching events. It requires a Maximum Likelihood (ML) gene tree as input, thus ML trees were obtained for each molecular marker using PhyML 3.0 with 500 bootstrap replicates to verify the robustness of the internal branches (Guindon and Gascuel, 2003). The best-fit substitution model for each locus was determined using jModeltest 2.1.7 (Darriba et al., 2012). The software suggested the following models as most suitable: AKY+I for IGR, ITS region, and ATPS β , and AKY+G for CalM. We ran PTP analyses for 400,000 MCMC generations with thinning value = 100 and burn-in = 0.25. We visually confirmed the convergence of the MCMC chain as recommended by Zhang et al. (2013). The GMYC approach combines models of stochastic lineage growth (Yule models) with coalescence theory (Pons et al., 2006, Fujisawa and Barraclough, 2013). It measures the degree of genetic clustering through detecting a threshold value at the transition of branching patterns that are characteristic of interspecific-level processes versus intraspecific-level processes of molecular evolution. The method starts with an inferred ultrametric gene tree and attempts to model the point on a time-calibrated phylogeny where this transition occurs. The ultrametric trees were obtained with BEAST 1.8.2 (Drummond et al., 2012) after removing identical haplotypes. A coalescent tree prior and the heterogeneity of the mutation rate across lineages were set under an uncorrelated lognormal relaxed clock. Analysis was run for 50 million generations, with a sampling frequency of 1000. After checking adequate mixing and convergence of all runs with Tracer 1.6 (Rambaut et al., 2014), the first 25% trees were discarded as burn-in and the maximum clade credibility tree was computed using TreeAnnotator 1.8.2 (Drummond et al., 2012). The resulting ultrametric tree was imported into R 3.1.3 (R Core Team, 2013) and GMYC analyses were run using the Splits (Ezard et al., 2009) and Ape (Paradis et al., 2004) libraries. Both the single threshold sGMYC (Pons et al., 2006) and multiple threshold mGMYC (Monaghan et al., 2009) methods were evaluated on each of the four separate datasets.

2.4 Morphological analyses

In situ polyp morphology and skeletal morphology were analyzed in light of the molecular results. For each voucher specimen, three images were taken with a Canon G15 camera at KAUST or with a Leica IC80HD camera at UNIMIB. The skeletal morphology of a subset of 166 specimens included in molecular analyses was analyzed using a Leica M205 FA stereo-microscope. Three 0.73x non-overlapping digital images were shot for each of the selected samples using a reference scale.

Skeletal features traditionally used for species identifications (Vaughan and Wells, 1943, Wells, 1956, Budd and Stolarski, 2009) were documented, namely the arrangement and shape of corallites, the number of septa and their organization (including the presence of the gonioporoid pattern of fusion), the development of pali and paliformes lobes, and the depth of the calice. *In-situ* characters, including polyp oral disc dimension, oral disc color, stalk length, tentacle length, and shape of tentacle tips were analyzed for each specimen. Micromorphology of skeletal structures was investigated using scanning electron microscopy (SEM). For SEM imaging, fragments of coral specimens were ground, mounted on stubs using silver glue, sputter coated using Au–Pd, and examined using Quanta 200 FEG SEM (FEI, Hillsboro, USA) at KAUST. Micromorphological characters evaluated for each specimen included: presence of the columella; the arrangement of columellar threads; the presence and development of spines and granules on septa, on septal face, on septa fusion points, and on corallite fusion points; and the number of denticles.

On the basis of the Leica M205 FA stereo-microscope digital images with a reference scale, three linear variables were measured on nine different corallites for each colony using ImageJ 1.49 (Rasband, 1997): the longest calice diameter (CaD), the longest columella diameter (CoD), and the distance between adjacent centroids (CD). For each character the specimen mean was calculated from nine replicates for each colony.

3. Results

3.1 Alignment statistics and haplotype determination

Table 1 provides a basic summary of sequence and alignment statistics, including the number of sequenced samples, the number of obtained sequences, the number of unique haplotypes, the total alignment length, the number of variable and parsimony informative sites, haplotype diversity, and nucleotide diversity. All collected *Goniopora* colonies were targeted at mitochondrial IGR locus, while sequences from ITS region, ATPs β , and CalM were obtained from 195, 175, and 184 samples, respectively. The alignment length varied considerably across the four

analyzed molecular markers, ranging from 254 bp (CalM) to 1238 bp (IGR), but the length of the sequences was similar within each locus. For example, within IGR alignment only a single deletion of 1 bp was observed in two IGR haplotypes of *G. somaliensis* and *G. savignyi* and three insertion sites totaling 11 bp occurred in all *G. gracilis* and *G. minor* sequences from the CalM alignment. The only notable exception concerned the ATPs β gene for which a long insertion of 514 bp was recovered in *G. stokesi* compared to all obtained sequences. Nevertheless, this long insertion was deleted by GBLOCKS and, therefore, excluded for further analyses. Overall, considering the number of variable and parsimony-informative sites, as well as the haplotype diversity, the four sequenced loci exhibited a sufficiently high degree of variability to allow for the evaluation and discussion of species boundaries within *Goniopora* (Table 1, Figs. 1-4).

Phase results were consistent across runs for the three nuclear loci. Indeed, phase calling for heterozygous nucleotide positions in the ITS region, ATPs β , and CalM was resolved at $P > 0.8$ in all samples.

Direct sequencing and subsequent phase analysis in a Bayesian framework revealed that all analyzed samples host either one or two dominant types of ITS region sequences, a situation previously reported in other coral genera (Flot et al., 2006, 2008a, 2010, 2011; Schmidt-Roach et al., 2012). Moreover, most ITS region types that co-occurred in some *Goniopora* colonies were found to be harbored alone in other specimens, suggesting that these variants might be allelic and each samples might be either homogeneous or heterogeneous for this molecular locus (Flot et al., 2006, 2011) as observed for single-copy nuclear genes such as ATPs β and CalM.

3.2 Mitochondrial haplonet and nuclear haplowebs

The mitochondrial haplonet (Fig. 1) and the three nuclear haplowebs (Figs. 2-4) were largely in agreement with each other even if some notable discrepancies were observed in the identification of the main *Goniopora* haplogroups. The median-joining haplotype network analysis inferred from the mitochondrial IGR resolved six well-differentiated haplogroups, corresponding to six different main lineages (clades I to VI) (Fig. 1). In particular, clusters I and VI consisted of both *G. somaliensis* and *G. savignyi*, cluster II was comprised of *G. djiboutiensis* and *G. lobata*, cluster III contained only *G. stokesi*, cluster IV consisted of *G. tenuidens* and *G. albiconus*, and cluster V was comprised of *G. minor* and *G. gracilis* (Fig. 2). All the three nuclear haplowebs resolved clades IV and V as separate and genetically isolated lineages in accordance with the IGR haplonet. On the other hand, the three haplowebs assigned clades I and VI into a single haplogroup (namely clade I) composed only of *G. somaliensis* and

G. savignyi (Figs. 2 -4). Lineages II and III were resolved as separated groups in both ITS region and ATPs β haplowebs (Figs. 2 and 3) but were merged into a single genetically isolated cluster based on the CalM haploweb (Fig. 4). The two *G. stokesi* specimens that constituted clade III were both heterozygous at the CalM locus and, while one allele was exclusive to *G. stokesi*, the other allele was the most common allele found in *G. djiboutiensis* and *G. lobata*. Notably, none of the heterozygous *Goniopora* colonies contained alleles from the two main clades in all three nuclear haplowebs (Figs. 2-4), suggesting that no allele recombination and sexual reproduction occurs across these lineages. This is evidence that these five main clades should likely be treated as separate species.

The inclusion of published ITS region sequences from Kitano et al. (2013, 2014) allowed the delimitation of three additional haplogroups in the ITS region haploweb (clades VII, VIII, and IX) (Fig. 2). These lineages were composed only by *Goniopora* colonies collected in the Pacific Ocean. Following the identifications proposed by Kitano et al. (2013, 2014), clade VII contained *G. cf djiboutiensis*, *G. pendulus*, and *G. cf cellulosa*, clade VIII contained *G. cf fruticosa*, *G. norfolkensis*, and *G. somaliensis*, while samples identified as *G. tenuidens* were the sole members of cluster IX. The other Pacific samples clustered together with their conspecific representatives collected in this study from the Saudi Red Sea in clade II, III, IV and V (Fig. 2). On the other hand, colonies from Japan identified as *G. somaliensis* by Kitano et al. (2013, 2014) belonged to clade VIII while our specimens of *G. somaliensis* were nested within clade I. A similar situation occurred also for *G. tenuidens* with Pacific samples in clade IX and our specimens in clade IV.

3.3 Molecular species delimitation

Single-locus species delimitation results from ABGD, PTP, and GMYC approaches are summarized in Table 2 and Figs. S2-S5. The partitions delimited by these three independent methods were mostly congruent within each of the four sequenced DNA loci and recovered a similar subdivision of sequences in putative lineages despite some exceptions that were recovered. As a general pattern, the numbers of putative entities suggested by ABGD and PTP were congruent with mitochondrial haplonet and nuclear haplowebs whereas sGMYC and mGMYC overestimated the number of putative species, fragmenting most molecular clades into several individual clusters (Table 2). There was therefore a high degree of congruence between species delimitation and haplonet / haploweb analyses, recovering mainly the following entities: *G. somaliensis* + *G. savignyi* group (clade I), *G.*

djiboutiensis + *G. lobata* group (clade II), *G. stokesi* group (clade III), *G. albiconus* + *G. tenuidens* group (clade IV), and finally *G. minor* + *G. gracilis* group (clade V).

In the ABGD analysis, the final results were identical using either Jukes-Cantor or Kimura distances. For IGR, the distribution of pairwise genetic distance using a $P_{\max}$ value = 0.1 displayed five to six lineages, depending on the gap width parameter. For gap width values ranging from 1 to 2% the ABGD analyses inferred six distinct groups, while higher threshold values resulted in five lineages, nesting all specimens identified as *G. somaliensis* and *G. savignyi* into a single group (Fig. S2). Based on the ITS region, using P values ranging from 0.001 and 0.1, nine entities were delimited for gap width values ranging from 4 to 5%. The delimited candidate lineages correspond to the network clusters (clade from I to IX) with the exception of cluster III (*G. stokesi*) that was split into two different groups. Considering ATPs β and CalM, the distribution of pairwise genetic distance using a $P_{\max}$ value = 0.1 (0.01 – 0.1 for CalM) displayed five (four for CalM) lineages for gap width values ranging from 7 to 10% (2 - 4% for CalM) in agreement with the identification of main haplogroups in nuclear haplowebs. Putative lineages delimited by PTP were identical to the ones proposed by ABGD with the exception of the ITS region results. Indeed, in this case, PTP identified four additional lineages, fragmenting clade VII in two groups and clade VIII in three groups (Fig. S3). Concerning the GMYC approach, a lineage-through-time plot based on the BEAST ultrametric tree revealed a sudden increase in branching rate towards the present, likely corresponding to the switch from interspecific to intraspecific branching events (Figs. S2-S5). The likelihood of the null model was lower than the maximum likelihood of both the sGMYC and mGMYC models in all analyses (Table S2) and, according to the latter, the transition from speciation to coalescence resulted in a partition with the numbers of putative species indicated in Table 2.

3.4 Morphological and morphometric analyses

In vivo polyp morphology of 199 *Goniopora* specimens was investigated in light of both morphospecies identifications and molecular results. In particular for each coral colony the stalk and tentacle length, the shape of tentacle tips, and the oral disc dimension and color were evaluated. A summary of polyp morphological analyses for each morphospecies is given in Table 3 and underwater photos of some representatives for each morphospecies and molecular clades are shown in Fig. 5. Although the morphology of the living colonies appeared meaningless in discriminating between morphologically-identified species, it was consistent within the molecular identified clades.

In fact, particular features of the polyps appeared to be diagnostic for each of the molecular clades, with the exception of samples nested within clade I. Within clade I, the two traditionally-defined morphospecies showed different dimensions of stalks and tentacles (always smaller in *G. somaliensis* than in *G. savignyi*).

A summary of macromorphological skeletal analyses is provided in Table 3 and pictures of skeleton colonies for some representatives for each morphospecies and molecular clades are shown in Fig. 6. In general the dimensions of the corallite and of the columellae, the number and arrangement of septa, the number of pali, and the depth of the calices were concordant with the traditional classification of the morphospecies within the genus. Nevertheless, no concordance with the molecularly-identified clades was recorded on the basis of the above-mentioned features.

A summary of SEM-based micromorphological skeletal analyses is shown in Table 3. SEM images of corallite and subcorallite details for some representatives for each morphospecies and molecular clades are shown in Fig. 7. Samples within the same molecular clade showed distinctive micromorphological and microstructural features. In particular, the micro-ornamentation and the arrangement of the columella were consistent within each molecular clade, with the exception of clade II wherein the columella arrangement in *G. djiboutiensis* exhibits a single process while *G. lobata* exhibits multiple processes. A detailed account of species identification and morphological species description is discussed in Data S1.

Three morphological variables (CaD, CoD, and CD) were scored for morphometric analyses from 166 specimens for a total of 1496 corallites. These three characters were largely overlapping among the nine morphospecies, except for *G. stokesi*, which was morphologically different from the other taxa and had the highest values of CaD, CoD, and CD among the analyzed *Goniopora* species (Fig. 8a). The outcome was the same if the three variables were assessed on the basis of the five molecular clades. Although clade III (composed only of *G. stokesi*) clearly showed the highest values of CaD, CoD, and CD, the three values were mostly overlapping among several clades (Fig. 8b).

4. Discussion

4.1 Molecular considerations

The four molecular markers used in the present work broadly resolved the same putative lineages within the analyzed samples of *Goniopora* (clades I to V), but these were not always in agreement with traditional morphospecies. Nevertheless, minor differences were detected across the DNA loci (Figs. 1-4). Each molecularly-defined lineage is comprised of at least two traditionally recognized morphospecies, with one exception (clade III is solely comprised of *G. stokesi* samples). Results from the ITS region suggest the presence of three additional molecular lineages within the *Goniopora* genus (clades VII to IX). These three clades are comprised only of samples from Japan (with the exception of one sample from Malaysia nested within clade VII).

With regards to the mitochondrial DNA, the amplified IGR between CytB and NAD2 showed sufficient levels of sequence variation to provide high resolution within the genus *Goniopora*. Mitochondrial haplotype analyses resolved six main molecular lineages (clades I - VI) congruent with the lineages identified by the three nuclear markers, with the exception of clade VI (which clustered together with clade I based on the nuclear haplotypes). The average genetic distance between the six molecular groups was $1.6 \pm 0.4\%$, ranging from $2.5 \pm 0.5\%$ between clades II and V to $0.6 \pm 0.2\%$ between clades III and IV (Table S3). These results represent a further demonstration that, even though the mitochondrial DNA of scleractinian corals is characterized by slow evolution rates and a resultant low number of nucleotide variations among species (Shearer et al., 2002; Hellberg, 2006; Huang et al., 2008), some intergenic non-coding regions can be highly informative in resolving species boundaries. Indeed, the utility of these IGRs as phylogenetic variable markers has been already proven in different scleractinian families and genera (reviewed by Kitahara et al., in press). Kitano et al. (2013) used the mitochondrial region between the 5' of NAD5 and the 3' of COI, comprising the entire trnW and ATP8 and a total of three IGRs, to infer the phylogenetic position of the species *G. stokesi*. Unfortunately, this region turned out to be uninformative, explainable by the fact that, despite the 1640 bp length of the sequenced molecular locus, only a total of 197 bp belonged to the three variable IGRs (Lin et al., 2011; Kitano et al., 2013). On the contrary, the IGR selected in the present study is the longest occurring in the mitochondrial genome of *G. columna*, the only mitogenome available for the genus to date (GenBank Accession Number NC015643; Lin et al., 2011). This region consists of 585 bp, of which 90 were found to be variable sites (Table 1).

The three utilized nuclear markers, *i.e.* ITS region, ATPs β , and CalM, broadly led to the same results between each other and the mitochondrial IGR (Figs. 2-4). Previous works successfully used rDNA for low-level taxonomic comparisons, especially thanks to the presence of the two high variable internal transcribed spacer

regions, ITS1 and ITS2, which have fewer functional constraints than the ribosomal genes 18S, 5.8S, and 28S (Chen et al., 2004; Wei et al., 2006). Although rDNA represents a multigene family, this region is subjected to concerted evolution (Arnheim et al., 1980), a mechanism that homogenizes different repeated ITS units through unequal crossing-over and gene conversion (Dover 1982). In many cases, the rate of concerted evolution is enough to homogenize the variation among unit repeats within species, but interspecific divergence can be high (Hillis and Dixon 1991). The utility of rDNA to infer phylogenetic relationships in corals has been long debated due to high levels of intraindividual and intraspecific variation in *Acropora* Oken, 1815 caused by the presence of pseudogenes, hybridization, incomplete lineage sorting, and intracolony genetic variability (van Oppen et al., 2002, Marquez et al., 2003, Vollmer and Palumbi 2004, Schweinsberg et al., 2015). Nevertheless, detailed studies demonstrated that these features are unique to the *Acropora* genus (Chen et al., 2004, Wei et al., 2006), whose rDNA shows a peculiar secondary structure (Chen et al., 2004) and the shortest ITS sequence among any eukaryotes measured to date (Odorico and Miller, 1997). Conversely, the ITS region has been successfully used for all other coral taxa for which it was tested (Kithara et al., *in press*), therefore highlighting that the rate of homogenization among rDNA tandem repeats is variable in different lineages of corals. The ITS region was recently targeted by Kitano et al. (2013, 2014) to assess species level relationships in the genus *Goniopora*. Although the authors failed to resolve some *Goniopora* lineages, these unresolved phylogenetic patterns may probably be related to misinterpretations of the variability and plasticity of the skeletal structures rather than the unsuitability of the chosen marker. For example, the authors stated that colonies identified as *G. cf djiboutiensis*, *G. pendulus*, and *G. cellulosa* may all represent morphological variations of *G. pendulus*. We find that samples from all three of these morphospecies belong to clade VII based on our ITS regions haploweb (Fig. 2). The ATPs β gene resolved the same haplogroups suggested by the ITS region (Figs. 2-3) and both nuclear loci were highly variable in terms of mutations (Table 1). The mean genetic distance between the five main clades was $10.5 \pm 3.5\%$ using ATPs β and $5.7 \pm 1\%$ with the ITS region (Table S3). CalM also showed high levels of variation (mean genetic distance between clades was $6.5 \pm 2.4\%$) (Table S3). Nevertheless, CalM detected only four groups, clustering clades II and III together (Fig. 4). Different hypotheses can be proposed to explain this discrepancy. Calmodulin is one of the calcium binding proteins that are highly conserved in their protein and gene structure (Yuasa et al., 1999) and, therefore, may exhibit only low levels of gene sequence variation, thus obscuring real evolutionary relationships. Alternatively, considering that *G. stokesi* colonies harbored two alleles of CalM gene (one of which was exclusive to *G. stokesi* and the other was the most common allele

shared also by *G. djiboutiensis* and *G. lobata* (Fig. 4)), it is likely that the shared allele might represent a retention of a shared ancestral allele, resulting in an unresolved phylogeny (Degnan and Rosenberg, 2009). Another possible explanation might be incomplete lineage sorting due to a deceleration in the evolutionary rate of CalM gene within the lineage. This phenomenon is known to cause discrepancies in comparative phylogeny (Maddison and Knowles, 2006).

Molecular results were corroborated by three species delimitation algorithms, *i.e.* ABGD, PTP, and GMYC, which broadly endorsed the presence of five putative species of *Goniopora* from the Saudi Arabian Red Sea and Djibouti (Table 2), and were congruent with mitochondrial haplonet and nuclear haplowebs (Figs. 1-4). The slightly different partitions provided by the various molecular approaches to species delimitation is expected since they rely on different criteria and data inputs (Fontaneto et al. 2015) and, moreover, it has been demonstrated that their performance depends on several factors, such as the variability of sequenced molecular locus, the number of analyzed species, the population size, and the speciation rate (Tang et al., 2014; Dellicour and Flot, 2015). For example, Dellicour and Flot (2015) showed that single locus species delimitation approaches are heavily challenged by species-poor datasets, resulting in artifactual species boundaries definitions and overestimates of the number of actual putative lineages. In particular, ABGD and GMYC are not suitable when dealing with all-conspecific samples (Dellicour and Flot, 2015). On the other hand, haplowebs outperform sGMYC and mGMYC when dealing with one to six-species datasets because the latter two approaches usually require several internode intervals to fit the intraspecific branching rates, leading to an excess in the estimates of the total number of entities (Talavera et al., 2013, Dellicour and Flot, 2015). In any case, it must be always taken into account that haploweb reliability depends on dense sampling, consisting of several individuals per species. With regards to population size, both ABGD and GMYC perform better when dealing with small populations and thus are often applied to haploid datasets, *i.e.*, mitochondrial sequence alignments and nuclear ribosomal markers, (*e.g.*, ITS1 and ITS2) due to their concerted mode of evolution (Navajas and Boursot, 2003). Moreover, the accuracy of GMYC-based methods sharply decreases when dealing with an increasing speciation rate (Zhang et al., 2013, Dellicour and Flot, 2015). Similarly, haplowebs perform poorly when population sizes, speciation rates, and mutation rates are large. Moreover, although both GMYC and PTP are tree-based methods, the former is more sensitive to differences among gene trees introduced by smoothing steps. Further, unresolved nodes have greater effects on GMYC estimates than on PTP estimates (Tang et al., 2014). Even if the development of these molecular tools was primarily targeted to disentangle

species diversity of meiofauna and other poorly studied taxa (Fontaneto, 2014; Fontaneto et al., 2015), our study suggests that these combined approaches are powerful in providing first-pass species partition hypotheses, even for hard corals, a group in which the great variability of skeletal features has challenged taxonomists for centuries.

The advent of next-generation sequencing tools has revolutionized our understanding of evolutionary processes and provided practical applications into phylogenomics and species delimitation (Davey et al., 2011). Therefore, it is important to highlight that the four used molecular markers still represent only a small portion of the entire genome of *Goniopora*; we cannot exclude the presence of further genetic divergence in other regions of the nuclear genome that would better explain the different morphologies of the skeletons. However, this currently seems unlikely considering the general concordance observed among several independent mitochondrial and nuclear loci.

4.2 Morpho-molecular evidence as a tool to evaluate species delimitation

Using mitochondrial and nuclear sequences, we determined that *Goniopora* in the Saudi Arabian Red Sea is represented by five genetically isolated entities. This is in contrast to the nine traditional species identified based on skeletal macromorphology. Independent DNA loci from both the mitochondrial and nuclear genome suggested similar scenarios and congruent individual clustering patterns, suggesting that reproductive isolation among lineages is likely to have occurred between these five groups. Nuclear haplowebs revealed that the five lineages are actually multi-locus fields for recombination (Carson, 1957; Doyle, 1995; Flot et al., 2010) characterized by mutual allelic exclusivity and their own allele pool, and therefore should likely be considered as distinct species (de Querioz, 2007). An alternate hypothesis is that these clades represent a species complex exhibiting incipient speciation and incomplete lineage sorting causes the failure in the delimitation of boundaries between morphospecies. However, we feel this hypothesis is extremely unlikely primarily because of the agreement in the various methods we employed. Further, diverging populations are expected to reach mutual allelic exclusivity faster than reciprocal monophyly; allelic haploweb analysis is thus a powerful and sensitive criterion to study the process of speciation and delimit species (Flot et al., 2010). A simpler and more probable hypothesis to explain the presence of two distinct morphs in each of the genetic lineages is that the levels of phenotypic plasticity of the species have been previously underappreciated by traditional taxonomy, leading to overestimates of the real biodiversity within the genus. This is common scenario in corals. For example, it has been shown that the traditional coral species *S. danae* Milne Edwards and Haime, 1850, *S. kuehlmanni* Sheer and Pillai, 1983, and *S. subseriata* (Ehrenberg, 1834) from the Gulf

of Aden likely represent ecomorphs of *S. pistillata* Esper 1797 (Stefani et al. 2011). Similarly, recent genetic analyses based on six molecular loci showed that the Red Sea endemic species *S. mamillata* Sheer and Pillai, 1982 and *S. wellsi* Sheer, 1964 cluster together in a single molecular clade with the widespread *S. pistillata*. This calls into question the assumption that the region represents a hotspot for *Stylophora* diversity (Arrigoni et al. *in press*). Our phylogenetic reconstructions based on the three nuclear loci (ITS region, ATPs β , and CalM) evidence the presence of widespread allele sharing among morphospecies within each molecular clade, suggesting possible reticulate patterns and hybridization between ecomorphs. Gene exchange and introgressive hybridization have been extensively documented in the *Acropora* species complexes *A. cytherea* (Dana, 1846) and *A. hyacinthus* (Dana, 1846). These *Acropora* species have shown to form a syngameon across the Indo-Pacific (Lander and Palumbi, 2012). Nevertheless, further analyses to test for admixture in our specimens are needed before arriving at any general, definitive conclusions.

Results from macromorphological, micromorphological, and morphometric analyses were re-evaluated in light of our molecular results, suggesting a complex scenario for each of the identified molecular clades. Skeletal characters traditionally used to identify *Goniopora* species allowed the delimitation of nine morphospecies within the analyzed colonies (Table 3 and Fig. 6). Nevertheless, none of these traditional species were supported by genetics, morphometrics, and micromorphology except *G. stokesi* (Tables 2 and 3). This species was detected as a distinct lineage (clade III) composed of specimens from both the Red Sea and the Pacific Ocean (Figs. 1-4) and, including skeletal morphology, it was consistently separated from the other clades (Table 3 and Fig. 7).

Although macromorphology of colony and traditional skeletal features failed to resolve the evolutionary lineages of *Goniopora* revealed by molecular data, sub-corallite-scale analyses were congruent with genetics. For example, despite *G. somaliensis* and *G. savignyi* (clade I) exhibiting different calice and columella dimensions and differing numbers of septa (Fig. 6), they showed a conserved micro-ornamentation of the skeletal structures (Fig. 7). A similar situation occurred in clade IV (*G. albiconus* and *G. tenuidens*) (Figs. 1-4). With regards to clade V, although we found several colonies that clearly exhibited the typical traditional macromorphological characters of either *G. minor* or *G. gracilis*, some of the analyzed specimens presented both corallites with an internal crown formed by fusions between pali (typical of *G. minor*) and corallites with separated pali (typical of *G. gracilis*). These finding suggested that the two traditional morphospecies might represent the two extremes of a single plastic species. Conveniently, SEM analyses showed shared micro-ornamentation among all the specimens clustered into

clade V (Table 3, Fig. 7). The main discordance among genetic, morphological, and micromorphological data was related to clade II (composed of *G. djiboutiensis* and *G. lobata*) (Table 3). With the exception of a common type of micro-ornamentation, the two species differed in micromorphological characters (e.g., the columella arrangement and in the number of denticles present on the upper side of the primary septa) (Fig. 7). Some samples identified as *G. djiboutiensis* by Kitano et al. (2013) nested within clade II according to the ITS region haploweb (Fig. 2) but showed deeper calices compared to the other *G. djiboutiensis* samples, as well as a reduced number of pali and smaller columella. Similarly, some colonies identified as *G. lobata* by Kitano et al. (2013) presented pronounced pali and wider columella. These results suggest the presence of intermediate morphologies between the two traditional morphospecies

4.3 *In situ* polyp morphology as a diagnostic character for underwater identification of *Goniopora* molecular clades

Unlike the majority of scleractinian corals, the genus *Goniopora* has fleshy polyps with 24 tentacles usually extended in daytime. The polyps and their tentacles vary considerable in shape and color and it has been proposed that *in situ* morphology of the polyps can be used as a diagnostic character to assess species boundaries within the genus (Veron and Pichon, 1982; Veron, 1986; Nishihira and Veron, 1995; Veron, 2000). On this basis, we assessed the morphology of the living polyps in the context of a) each traditionally identified morphospecies and b) the molecularly-defined lineages.

In vivo polyp morphology was congruent to genetic data and consistent within clades II, III, IV, and V (Table 3 and Fig. 5), suggesting that polyp morphology can be used as a helpful tool for underwater discrimination of these five molecular lineages. Indeed, all colonies of both *G. djiboutiensis* and *G. lobata* nested within clade II had long stalks and long pointed or bulbous tentacles, with medium sized white oral discs, sometimes characterized by distinctive pink or blue mouths (Table 3). These features were also consistent with *G. sultani* and *G. ciliatus* (Veron, 2000: p. 355, fig. 4 and pp. 372-373, figs. 1-5, respectively) but no colonies of the latter two species have been analyzed in this work so we hesitate to make further conclusions regarding these morphospecies. Samples of *G. stokesi* clustering within clade III had a distinctive morphology in the field (Fig. 5). They presented polyps with very long stalks and tentacles, a distinctive brown coloration, and white oral discs of medium dimensions. However, these morphological polyp features were shared also by *G. pendulus* (Veron, 2000: p. 350, figs. 1-2; Kitano et al., 2013: p. 8, fig. 4a). All specimens of *G. pendulus* analyzed by Kitano et al. (2013) were included in our ITS region

haploweb and they were grouped in clade VII (Fig. 2). In this case, *in vivo* polyp morphology cannot be used to discriminate between lineages III and VII. Concerning clade IV, all the samples identified as *G. albiconus* and *G. tenuidens* showed a common *in vivo* morphology (Fig. 5) that was clearly distinct from the other analyzed specimens and molecular clades due to the presence of white large oral cones in the polyps (Table 3). This feature was unique for all the specimens nested within this molecular clade but, according to our results, no *in vivo* traits can be used to discriminate between the two morphospecies nested in clade IV. The inclusion in the analyses of two samples (BA002, DJ074) collected from Gulf of Aden and Djibouti and identified by Kitano et al. (2013) as *G. albiconus* on the base of *in vivo* polyp morphology provided further evidence that the morphology of the polyps of *G. albiconus* and *G. tenuidens* is identical. Our evidence with respect to these two morphospecies leads us to tentatively conclude that these represent one lineage. Finally, all the colonies belonging to molecular clade V shared a distinctive and identical polyp morphology, characterized by small polyps with no visible stalks, small white oral cones, and short pointed tentacles (Table 3). Apart from the general dimensions of the polyps shared with *G. somaliensis* (which can be easily discriminated by the presence of short stalks), no further feature was common among colonies nested within clade V and any of the other identified *in vivo* morphologies (Fig. 5).

The only discordance between genetics and polyp morphology occurred in clade I, a lineage composed of *G. somaliensis* and *G. savignyi* morphospecies (Fig. 5). All *G. somaliensis* colonies exhibited small polyps with short stalks and short pointed tentacles, a general pale brown color, and a white oral disc. However, polyps of *G. savignyi* were very similar to the ones of *G. somaliensis* but differed greatly in dimensions, always bigger and with stalks up to two-fold longer than *G. somaliensis* (Table 3). Therefore, *in vivo* polyp characters of colonies ascribed to clade I were unique with comparison to the other four lineages but, despite a general similarity between *G. somaliensis* and *G. savignyi*, their polyps showed distinctive dimensions (Fig. 5).

To conclude, although polyp morphology is not useful in discriminating among morphospecies, it may be a diagnostic character to differentiate among the five molecular lineages of *Goniopora* that we found in the Saudi Arabian Red Sea. Integrating these results with micromorphological and molecular findings, we suggest that the most likely scenario is that the traditional nine macromorphology-based species of *Goniopora* are not valid biological units, at least for the Saudi Arabian Red Sea. Instead, we propose that this geographic region is populated by five main evolutionary lineages, genetically isolated from each other and characterized by distinct corallite micromorphology and *in vivo* polyp morphology. We qualify this proposal with acknowledgement that this is based

only on samples from the Saudi Arabian Red Sea and Djibouti, so analysis of further specimens from different localities in the Red Sea and Indo-Pacific should be added before drawing general conclusions on the genus.

Conclusions

The present study represents a stepping stone in understanding the evolutionary relationships within the genus *Goniopora*. Although widespread in the Indo-Pacific (Veron, 2000), the genus remained understudied except for two recent works by Kitano et al. (2013, 2014) that investigated the phylogenetic relationships of several species with a particular focus on specimens from Japan. This is the first work using molecular approaches to the biodiversity of the genus within an underexplored and species-rich region such as the Red Sea, where a wide range of marine organisms are increasingly being investigated using combined morpho-molecular data (e.g., Haverkort-Yeh et al., 2013; DiBattista et al., 2013, 2015; Terraneo et al., 2014; Huang et al., 2014; Arrigoni et al., 2015; van der Meij et al., 2015; Spaet and Berumen, 2015).

The strong genetic evidence of the present study highlights the potential shortcomings of traditional taxonomic efforts to delimit species boundaries of scleractinian corals based exclusively on skeletal macromorphology. Unfortunately, scleractinian morphology exhibits extensive phenotypic plasticity, high variability, and/or evolutionary convergence that often obscure natural patterns of evolution (e.g., Gittenberger and Hoeksema, 2006; Fukami et al., 2004; Todd, 2008; Flot et al., 2011; Budd et al., 2012). The integration of molecular tools with morphological analyses has proven to be powerful in clarifying misleading and complex phylogenies, with recent examples in the genera *Pocillopora* (Schmidt Roach et al., 2014), *Psammocora* (Stefani et al., 2008; Benzoni et al., 2010), and *Desmophyllum* (Addamo et al., 2016). In the present study, a multi-locus genetic approach provides the basis for a reevaluation of evolutionary-informative morphological features to assess species boundaries in *Goniopora*. The results demonstrate the utility of molecular species delimitation methods such as ABGD, PTP, GMYC, and haplowebs in taxonomically complex groups of organisms (Pons et al., 2006; Puillandre et al., 2012; Fontaneto, 2014, 2015; Tang et al., 2014). We advocate for additional studies incorporating genetic and morphological characters of *Goniopora* from different geographic areas in the Indo-Pacific to clarify species boundaries within this genus and to enable final taxonomic clarity.

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Figures and Tables

Table 1 Sequence statistics for each of the four molecular loci. Number of sequenced samples (N), number of obtained sequences (N_{seq}), number of unique haplotypes (H), total alignment length as number of sites (bp), number of parsimony informative sites (N_{PS}), haplotype diversity (H_D), and nucleotide diversity (N_D).

Table 2 Summary of species delimitation methods results (ABGD, PTP, sGMYC, and mGMYC) across each of the four molecular loci. Number of lineages identified by species delimitation method (n), molecular clade successfully delimited (+), molecular clade lumped with other lineages (L), molecular clade further subdivided into two or more lineages (S).

Table 3 Summary of characters of polyp morphology, skeleton macromorphology, and skeleton micromorphology analyzed in this study to delimitate morphospecies of *Goniopora*.

Fig. 1 Haplonet of mitochondrial IGR. Colored circles represent haplotypes and their sizes are proportional to the frequencies of coral colonies sharing the same haplotype. Numbers on the branches indicate the number of mutated positions in the alignment that differentiate each haplotype. Cohesive clusters of haplotypes are enclosed in dashed black circles.

Fig. 2 Haploweb of nuclear ribosomal ITS region. Colored circles represent haplotypes and their sizes are proportional to the frequencies of coral colonies sharing the same haplotype. Colored lines connect haplotypes of heterozygous individuals while the colors refer to morphospecies assignments. Branch length is proportional to the number of mutations that differentiate each haplotype. Cohesive clusters of haplotypes are enclosed in dashed black circles.

Fig. 3 Haploweb of nuclear ATPs β . Colored circles represent haplotypes and their sizes are proportional to the frequencies of coral colonies sharing the same haplotype. Colored lines connect haplotypes of heterozygous individuals while the colors refer to morphospecies assignments. Numbers on the branches indicate the number of mutated positions in the alignment that differentiate each haplotype. Cohesive clusters of haplotypes are enclosed in dashed black circles.

Fig. 4 Haploweb of nuclear CalM. Colored circles represent haplotypes and their sizes are proportional to the frequencies of coral colonies sharing the same haplotype. Colored lines connect haplotypes of heterozygous individuals while the colors refer to morphospecies assignments. Numbers on the branches indicate mutated positions in the alignment that differentiate each haplotype. Cohesive clusters of haplotypes are enclosed in dashed black circles.

Fig. 5 *In vivo* colony morphology and polyp details of *Goniopora*. Clade I: (a-b) *G. somaliensis* SA1989; (c-d) *G. savignyi* SA1431; Clade II: (e-f) *G. djiboutiensis* SA1541; (g-h) *G. lobata* SA1395; Clade III: (i-l) *G. stokesi* SA1572-BU039; Clade IV: (m-n) *G. albiconus* SA1631; (o-p) *G. tenuidens* SA1627; Clade V: (q-r) *G. minor* SA1597; (s-t) *G. gracilis* SA1804.

Fig. 6 Skeletal macromorphology of *Goniopora* colonies and corallite details. Clade I: (a-b) *G. somaliensis* SA1691; (c-d) *G. savignyi* SA1431; Clade II: (e-f) *G. djiboutiensis* SA1740; (g-h) *G. lobata* SA1396; Clade IV: (i-j) *G. albiconus* SA1631; (k-l) *G. tenuidens* SA1627; Clade V: (m-n) *G. minor* SA1597; (o-p) *G. gracilis* SA1804; Clade III: (q-r) *G. stokesi* BU039.

Fig. 7 Skeletal micromorphology of *Goniopora*. Clade I: (a-b) *G. somaliensis* SA1691; (c-d) *G. savignyi* SA1431; Clade II: (e-f) *G. djiboutiensis* SA1740; (g-h) *G. lobata* SA1396; Clade IV: (i-j) *G. albiconus* SA1631; (k-l) *G. tenuidens* SA1627; Clade V: (m-n) *G. minor* SA1597; (o-p) *G. gracilis* SA1804; Clade III: (q-r) *G. stokesi* BU039.

Fig. 8 Box plots of morphometric variables examined for (a) each morphospecies of *Goniopora*, and (b) for each molecular-defined clade of *Goniopora*; CaD: long diameter of calice; CoD: long diameter of columella; CD: distance between centroids. Colors indicate (a) morphospecies and (b) genetic lineages labeled at the top and at the bottom.

a.

b.

Table 1. Sequence statistics for each of the four molecular loci.

	N	N _{seq}	H	bp	N _v	N _{ps}	H _D	N _D
IGR	200	200	14	1238	90	50	0.795	0.012
ITS region	195	501	155	986	251	148	0.957	0.035
ATPsβ	175	267	24	695	41	41	0.817	0.064
CalM	184	299	39	254	60	55	0.902	0.053

Number of sequenced samples (N), number of obtained sequences (N_{seq}), number of unique haplotypes (H), total alignment length as number of sites (bp), number of variable sites (N_v), number of parsimony informative sites (N_{ps}), haplotype diversity (H_D), and nucleotide diversity (N_D).

Table 2. Summary of species delimitation methods results (ABGD, PTP, sGMYC, and mGMYC) across each of the four molecular loci.

	IGR				ITS region				ATPsβ				CalM			
	ABGD	PTP	sGMYC	mGMYC	ABGD	PTP	sGMYC	mGMYC	ABGD	PTP	sGMYC	mGMYC	ABGD	PTP	sGMYC	mGMYC
n	6	6	7	7	8	12	14	14	5	5	5	6	4	4	6	7
clade I	+	+	S	S	+	+	+	+	+	+	+	+	+	+	S	+
clade II	+	+	+	+	+	+	S	S	+	+	+	+	+	+	S	S
clade III	+	+	+	+	+	S	S	S	+	+	+	+	L	L	L	L
clade IV	+	+	+	+	+	+	+	+	+	+	+	S	+	+	+	+
clade V	+	+	+	+	+	+	S	S	+	+	+	+	+	+	+	+
clade VI	+	+	+	+	L	L	L	L	L	L	L	L	L	L	L	L
clade VII	n.a.	n.a.	n.a.	n.a.	+	S	+	+	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
clade VIII	n.a.	n.a.	n.a.	n.a.	+	S	S	S	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Number of lineages identified by species delimitation method (n), molecular clade successfully delimited (+), molecular clade lumped with other lineages (L), molecular clade further subdivided into two or more lineages (S).

Table 3. Summary of characters of polyp, skeleton macromorphology, and skeleton micromorphology analyzed in this study to delimitate morphospecies of *Goniopora*.

		CLADE I		CLADE II		CLADE III	CLADE IV		CLADE V	
		<i>G. somaliensis</i>	<i>G. savignyi</i>	<i>G. djiboutiensis</i>	<i>G. lobata</i>	<i>G. stokesi</i>	<i>G. albiconus</i>	<i>G. tenuidens</i>	<i>G. minor</i>	<i>G. gracilis</i>
POLYPS	ORAL DISC DIMENSIONS	< 3 mm	3 - 5 mm	3 - 5 mm	3 - 5 mm	3 - 5 mm	> 5 mm	> 5 mm	< 3 mm	< 3 mm
	ORAL DISC COLOUR	white	white	white, white and pink/blue	white, white and pink/blue	white	white	white	white	white
	STALK LENGTH	0.1 - 5 cm	0.1 - 10 cm	> 10 cm	> 10 cm	> 10 cm	0.1 - 10 cm	0.1 - 10 cm	< 1 cm	< 1 cm
	TENTACLE LENGTH	< 3 mm	3 - 5 mm	3 - 5 mm	3 - 5 mm	> 5 mm	3 - 5 mm	3 - 5 mm	< 3 mm	< 3 mm
MACROMORPHOLOGY	TENTACLE TIPS	pointed	pointed	pointed, bulbous	pointed, bulbous	pointed	pointed	pointed	pointed	pointed
	CALICE DIAMETER	1.7 - 2.7 mm	2.7 - 3.2 mm	3.5 - 5.1 mm	3.1 - 5 mm	4.1 - 6.1 mm	2.9 - 4.7 mm	2.5 - 3.4 mm	1.8 - 2.9 mm	2.1 - 3.4 mm
	COLUMELLA DIAMETER	1.2 - 1.4 mm	1.5 - 1.8 mm	2 - 2.7 mm	1.5 - 2.6 mm	2.1 - 3.6 mm	1.5 - 2.4 mm	1.3 - 2 mm	1 - 1.9 mm	1.5 - 1.9 mm
	NUMBER OF SEPTA	20 - 24	24	20 - 26	16 - 24	20 - 24	24	16 - 20	12	14 - 20
	GONIOPOROID PATTERN	yes	yes	yes	yes	yes	yes	no	no	no
	NUMBER OF PALI	5	5	5	no	no	5	5	fused	5
	DEPTH OF CALICE	shallow	shallow	shallow	deep	deep	medium	medium	medium	medium
	TIPS OF	spines	spines	single	single	paddles	single	single	paddles	paddles
MICROMORPHOLOGY										

HOLOGY	COLUME LLAR THREADS COLUME LLA	single proces s	es singl e proc ess	spines multipl e process	spine s single proce ss	les multi ple proc ess	spine s single proce ss	spines single proce ss	les multi ple proc ess	les multi ple proc ess
	NUMBER OF DENTICLE S	1, 2	1,2	2, 3	1, 2	1	1, 2	1, 2	1	1

Highlights

Highlights

- We sequenced IGR, ITS region, ATPs β , CalM for the genus *Goniopora* from the Red Sea
- We used haploweb, ABGD, PTP and, GMYC to evaluate species delimitation in *Goniopora*
- The four markers and molecular species delimitation methods were broadly congruent
- Five molecular lineages were resolved in contrast to nine traditional morphospecies
- Corallite micromorphology and *in vivo* polyp aspect were concordant with genetics