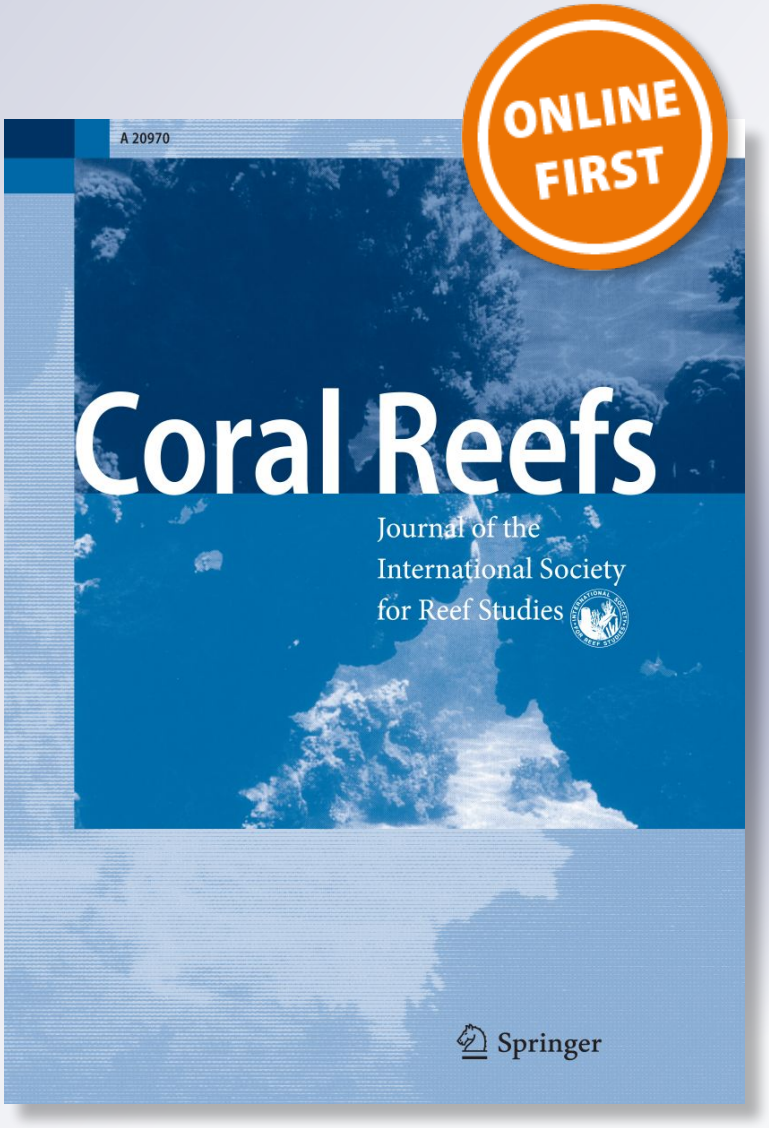
Corallite skeletal morphological variation in Hawaiian Porites lobata

Kaho H. Tisthammer & Robert H. Richmond

Coral Reefs
Journal of the International Society for Reef Studies

ISSN 0722-4028

Coral Reefs
DOI 10.1007/s00338-018-1670-5



Springer

Coral Reefs
https://doi.org/10.1007/s00338-018-1670-5

REPORT

Corallite skeletal morphological variation in Hawaiian *Porites lobata*

Kaho H. Tisthammer[1] · **Robert H. Richmond**[1]

Received: 16 May 2017 / Accepted: 19 February 2018

Abstract Due to their high morphological plasticity and complex evolutionary history, the species boundaries of many reef-building corals are poorly understood. The skeletal structures of corals have traditionally been used for species identification, but these structures can be highly variable, and currently we lack knowledge regarding the extent of morphological variation within species. *Porites* species are notorious for their taxonomic difficulties, both morphologically and genetically, and currently there are several unresolved species complexes in the Pacific. Despite its ubiquitous presence and broad use in coral research, *Porites lobata* belongs to one such unresolved species complex. To understand the degree of intraspecific variation in skeletal morphology, 120 corallites from the Hawaiian *P. lobata* were examined. A subset of samples from two genetically differentiated populations from contrasting high- and low-stress environments in Maunalua Bay, Hawaii, were then quantitatively analyzed using multivariate morphometrics. Our observations revealed high intraspecific variation in corallite morphology, as well as significant morphological differences between the two populations of *P. lobata*. Additionally, significant correlation was found between the morphological and genetic distances calculated from approximately 18,000 loci generated from restriction site-associated DNA sequencing. The unique morphological characters observed from the genetically differentiated population under environmental stress suggest that these characters may have adaptive values, but how such traits relate to fitness and how much plasticity they can exhibit remain to be determined by future studies. Relatively simple morphometric analyses used in our study can be useful in clarifying the existing ambiguity in skeletal architecture, thus contributing to resolving species issues in corals.

Keywords Porites · Morphometrics · Corallite · Corals · Coral morphology · Intraspecific variation

Introduction

Species are a fundamental unit of biological classification (de Queiroz 2007). However, species delimitation has been a controversial topic in evolutionary biology (e.g., de Queiroz 1998). Reef-building corals (order: Scleractinia) are one such taxon with taxonomic confusion, otherwise known as 'the species problem.' Coral taxonomy is traditionally based on morphology (Kitahara et al. 2016), but due to their high phenotypic plasticity, species delineation based on morphology has long been challenging in scleractinian corals (e.g., Bernard 1902; Hoffmeister 1926). Molecular phylogeny in the last several decades has greatly advanced our understanding of taxonomy and evolutionary relationships in many organisms (Yang and Rannala 2012; Kitahara et al. 2016). However, the species boundaries of certain reef-building corals remain poorly defined due to their complex evolutionary history (Knowlton 2000; Stat et al. 2012; Bosch and Miller 2016). Because colony-level morphology is extremely variable (Todd 2008), the skeletal

Topic Editor Dr. Line K. Bay

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00338-018-1670-5) contains supplementary material, which is available to authorized users.

✉ Kaho H. Tisthammer
kahot@hawaii.edu

[1] Kewalo Marine Laboratory, University of Hawaii at Manoa, 41 Ahui Street, Honolulu, HI 96813, USA

Published online: 26 February 2018

architecture of the corallite (the structure associated with individual polyps, Fig. 1), rather than the colony, has been used as a more reliable metric for coral taxonomic distinctions (Brakel 1977; Veron 2000). Yet, in many genera, this still does not solve the problem because corallites can be small, irregular and/or highly variable between and even within colonies. Morphological variation that exists among different geographic areas also adds confusion (Veron 2000). These morphological variations and plasticity of corals have caused widespread disparity between morphology-based taxonomy and molecular phylogeny (e.g., Fukami et al. 2004b; Forsman et al. 2009; Huang et al. 2009; Stat et al. 2012; Prada et al. 2014; Arrigoni et al. 2016).

Coral systematics, built on skeletal morphology, are continually being questioned and revised as new genetic data become available; for example, recent genetic studies have revealed (1) some morphospecies to be a single species (Eytan et al. 2009; Stefani et al. 2011; Pinzón et al. 2013), (2) an assumed single species with multiple colony forms to be separate species (Fukami et al. 2004a), and (3) multiple populations of an assumed single species to be cryptic species (Baums et al. 2005; Schmidt-Roach et al. 2012; Warner et al. 2015). Genetic delineation of reef-building corals has also been extremely challenging in some genera due to their slow rates of mitochondrial molecular evolution (Romano and Palumbi 1997; van Oppen et al. 1999), hybridization (e.g., Vollmer and Palumbi 2002; Hellberg et al. 2016), reticulate evolution (e.g., Veron 1995; Richards et al. 2013), and/or incomplete lineage sorting due to recent speciation (Miller and van Oppen 2003; Willis et al. 2006).

Establishing a clear and reliable taxonomic framework of reef-building corals is indispensable for recording and mapping patterns of biodiversity, understanding the ecological and evolutionary processes involved in speciation, predicting future changes, and determining appropriate conservation strategies (Veron 2013). Presently, a consensus has not been reached regarding the scale of genetic and morphological variations that characterize a species for certain reef-building coral taxa (Stat et al. 2012; Kitahara et al. 2016). We also lack knowledge on the degree of corallite morphological variability that exists within and between species, and how environment and genotype affect such plasticity (Todd et al. 2004a). The Endangered Species Act has listed 20 coral species as threatened in 2014 and three coral species as endangered in 2015. Evaluating extinction risk for coral species continues to be challenging, as taxonomic uncertainty hinders the determination of species ranges, population sizes, and management actions (Brainard et al. 2011).

The scleractinian genus *Porites* (Link, 1804) corals occur in tropical regions throughout the world, with the earliest fossil record from the Eocene (Veron 2000). Certain *Porites* species, such as *P. lobata*, have an especially extensive geographic distribution, throughout the Indo-Pacific Ocean from the Red Sea to the eastern Pacific. Despite its ubiquitous presence in the world, the genus *Porites* is among the most taxonomically challenging corals (Brakel 1977; Veron 2000, 2013). Over 50 *Porites* species are currently described (Veron 2000), but genetic studies on *Porites* are revealing unresolved species complexes, as well as cryptic species (Forsman et al. 2009, 2017). *Porites lobata* falls into the 'Clade I' species complex (Forsman et al. 2009), containing a mixture of endemic, rare, and cosmopolitan corals (*P. lobata*, *P. compressa* [endemic], *P. cylindrical*, *P. duerdeni*, *P. pukoensis* [rare], *P solida*, *P. annae*, and *P. lutea*) with various colony morphologies. Understanding accurate evolutionary relationships of the Clade I species has been

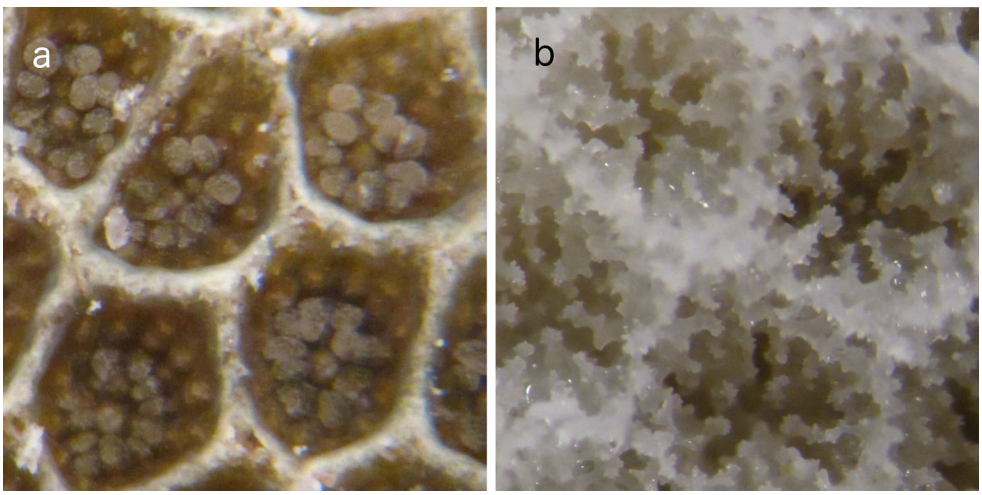


Fig. 1 *Porites lobata* corallites, live (**a**), and skeletal structure (**b**)

particularly difficult, since the genomic data with thousands of loci have not been able to resolve this species complex (Forsman et al. 2017). High plasticity and/or variability in *Porites*'s corallite morphology further add to the confusion. Although some *Porites* species appear to have distinct skeletal characters, which can aid in species identification, information is lacking regarding the extent of intra- and interspecific skeletal plasticity. This is partly because the skeletal characters are often summarized from a small set of samples without statistical analysis (Jameson 1995). To use corallite skeletal characters more efficiently in species identification, quantitative assessment of corallite structures is essential.

Corallite morphology between populations of *P. lobata* was investigated to capture the intraspecific skeletal variability using morphometrics. Our previous studies have identified clear genetic differentiation between *P. lobata* from the nearshore and offshore sites of Maunalua Bay, Oahu, Hawaii, which are less than 2 km apart (Tisthammer 2017). Due to large-scale urbanization, the corals in the nearshore areas of the bay are under chronic stress from sedimentation and terrestrial runoff containing pollutants (Richmond 2008). A reciprocal transplant experiment previously conducted in the bay revealed that the physiological and molecular responses to stress exposure significantly differed between these genetically differentiated nearshore and offshore *P. lobata* populations, leading us to conclude that selection has likely driven the nearshore corals to adapt to their high-stress environment (Tisthammer 2017). We used these populations as a study platform and tested whether and how the skeletal structures of the two populations differed. Additionally, over 100 *Porites* corallite samples were observed to capture the degree of variability. Lastly, correlation between the morphological and genetic distances was explored using the genomic data in a subset of *P. lobata* samples.

Materials and methods

Sample collection

Samples of *P. lobata* used in morphometric and genomic analyses were collected from the offshore site in Maunalua Bay, Oahu (21.26N, 157.71W), the nearshore site in Maunalua Bay, Oahu (21.27N, 157.71W), and Kewalo Basin, Oahu (21.29N, 157.86W) (Table 1). Samples were either flash frozen in liquid nitrogen and stored at − 80 °C, stored in 100% ethanol, or stored in DMSO buffer. For morphometric analysis, 12 nearshore, 12 offshore, and one Kewalo samples were used (Table 1). Additionally, a total of 120 samples collected from Maunalua Bay, Kewalo, and West Maui were used for general corallite observations (Fig. 2). Genomic data were available for five nearshore samples, two offshore samples, and one Kewalo sample from our previous study (Table 1, Forsman et al. 2017), which were used to calculate the genetic distance between the pairs of samples. The environmental conditions of the nearshore and offshore sites of Maunalua Bay are summarized in Table S1. All samples were collected under the State of Hawaii Special Activity Permit (SAP 2013-26, 2015-06).

General corallite observation

Corallite micro-skeletal characters of *P. lobata* were observed under a stereomicroscope, based on published morphological descriptions of the species (Veron and Pichon 1982; Weil 1992; Veron 2000; Ketchum and Reyes-Bonilla 2001; Forsman et al. 2015), and variability in skeletal characters was recorded. In Hawaii, there are two *Porites* species (*P. lobata* and *P. evermanni*) with similar mound/massive colony morphology, and the two species are hard to distinguish in the field. Therefore, all samples were confirmed genetically that they belonged to Clade I of the *Porites* species complex using existing genetic markers (ITS and/or Histone2 [H2]) with the method of Tisthammer (2017). With these genetic markers, *P. lobata* is identifiable only to the clade level (Forsman et al. 2009; Tisthammer 2017), but genetically, *P. lobata* and *P. evermanni* are distinguishable with all existing genetic markers.

Morphometric analyses

Multivariate morphometric analyses were conducted to reveal micro-skeletal differences in the corallites of *P. lobata* from the nearshore and offshore sites of Maunalua Bay. A set of 19 numerical and six descriptive characters were established in order to capture *Porites* corallite structural features, based on the previously published information as guidance (Brakel 1977; Veron and Pichon 1982; Jameson 1995; Ketchum and Reyes-Bonilla 2001; Forsman et al. 2015) (Table 2, Fig. 3). Collected coral skeletal samples were bleached in 15–50% sodium hypochlorite, rinsed with freshwater, and dried. Digital images of corallites were produced using a stereomicroscope, and 19 numerical characters were measured using ImageJ (Schneider et al. 2012) software. The six descriptive characters were measured under a stereomicroscope. Measurements from ten corallites per individual were taken randomly from each skeletal sample, avoiding recently formed small corallites by extra-tentacular budding. In order to assess whether ten corallite measurements per sample were enough to capture the within-sample variation, the average coefficient of variation of each numerical character was calculated using five and ten corallites, and

Table 1 *Porites* sample information used in morphometric and genomic analyses

	Sample ID	Species	Location	Analysis	Genbank or Sequence Read Archive Number
1	C4	*P. lobata*	O	M	KY502326
2	C6	*P. lobata*	O	M, G	SAMN06648852
3	C8	*P. lobata*	O	M	KY502329
4	C14	*P. lobata*	O	M	KY502332
5	C16	*P. lobata*	O	M, G	SAMN06648853
6	B2	*P. lobata*	O	M	KY502365
7	B3	*P. lobata*	O	M	KY502366
8	B4	*P. lobata*	O	M	pending
9	B7	*P. lobata*	O	M	KY502286
10	B9	*P. lobata*	O	M	KY502368
11	B10	*P. lobata*	O	M	KY502690
12	B11	*P. lobata*	O	M	KY502370
13	M2	*P. lobata*	N	M, G	SAMN06648857
14	M3	*P. lobata*	N	M	KY502336
15	M7	*P. lobata*	N	M, G	SAMN06648858
16	M9	*P. lobata*	N	M	pending
17	M12	*P. lobata*	N	M, G	SAMN06648859
18	N1	*P. lobata*	N	M, G	SAMN06648855
19	N3	*P. lobata*	N	M, G	SAMN06648856
20	N4	*P. lobata*	N	M	KY502357
21	N6	*P. lobata*	N	M	KY502358
22	N7	*P. lobata*	N	M	KY502359
23	N12	*P. lobata*	N	M	KY502362
24	N17	*P. lobata*	N	M	KY502364
25	K2	*P. lobata*	K	M, G	SAMN06648854

O offshore, Maunalua Bay; *N* nearshore, Maunalua Bay; *K* Kewalo Basin; *M* morphometrics; *G* genomics

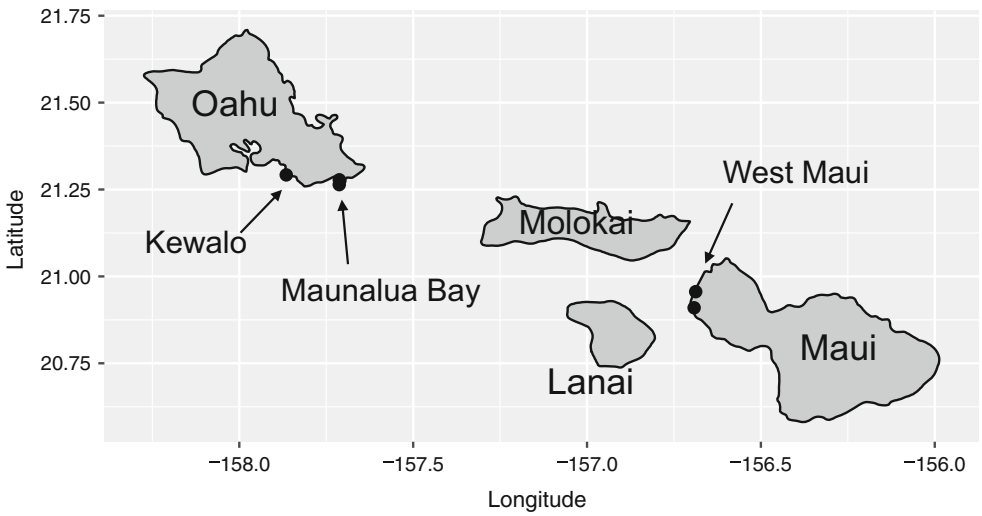


Fig. 2 Map of coral sampling locations

the values were compared. The average coefficients of variation did not differ between the two methods (Wilcoxon signed-rank test, $P = 1$), and therefore, ten measurements per sample were determined as adequate to capture the within-sample variation.

Principal coordinate analysis (PCO) was conducted to obtain an overall pattern of morphological variation using all 25 assessed characters in R version 3.3.1 (R Core Team 2016). Since highly correlated characters ($r > |0.95|$) may cause errors in certain multivariate analysis (Koutecký

Table 2 Corallite characters of Porites samples measured for morphometric analysis

	Characters	Type	Description
1	Corallite diameter (length)	N	Length parallel to dorsoventral axis
2	Corallite diameter (width)	N	Length perpendicular to dorsoventral axis
3	Corallite spacing	N	Average linear distance between centers of nearest and farthest neighboring corallites
4	Dorsal septum length	N	Linear distance from dorsal septum tip to inner theca margin
5	Ventral septum length	N	Linear distance from ventral septum tip to inner theca margin
6	Lateral septum length	N	Average of four linear distances from lateral septum tip to inner theca margin
7	Columella diameter	N	Average of maximum and minimum diameters of columella
8	Ventral palus diameter	N	Average diameters of ventral pali
9	Lateral palus diameter	N	Average of maximum and minimum diameters of lateral pali
10	Dorsal palus diameter	N	Diameter of dorsal palus
11	Fossa length	N	Distance measured across corallite center from middle ventral palus to dorsal palus
12	Fossa width	N	Average of distances measured across corallite center from a lateral palus to a diagonal lateral palus
13	Lateral septal spacing	N	Average of distances between lateral septa at thecal margin
14	Ventral septal spacing	N	Average of distances between ventral septa at thecal margin
15	Ventral palus spacing	N	Average of distances between ventral pali
16	Dorsal palus spacing	N	Average of distances between dorsal palus to a neighboring lateral plus
17	Lateral septum thickness	N	Average of cross-distances of lateral septa at midpoint
18	Dorsal septum thickness	N	Cross-distances of dorsal septum at midpoint
19	Ventral septum thickness	N	Average of cross-distances of lateral septa at midpoint
20	Number of pali	D	Number of pali per corallite
21	Lateral palus height	D	Degree of pronunciation of lateral pali (1 = not pronounced, 2 = slightly pronounced, 3 = moderately pronounced, 4 = highly pronounced)
22	Triplet form	D	1 = separated, 2 = fused, 3 = trident
23	Wall height	D	Pali to wall height: 1 = High walls, tips of pali are much lower than wall, 2 = Walls are slightly higher than pali, 3 = Pali come up to the wall height
24	Corallite shape	D	3 = round, 4 = diamond/square/rectangle, 5 = pentagon, 6 = hexagon
25	Columella shape	D	0 = not visible/none, 1 = rod shape, 2 = compressed

N numerical characters, *D* descriptive characters

2015), correlations were examined between characters, which showed no high correlation, and thus all numerical variables were used in the rest of the multivariate analyses. The 19 numerical character data were standardized to variables (scaling to [0,1] range), and the permutational multivariate analysis of variance (MANOVA) was performed to test the differences between the populations using the PERMANOVA + (Anderson et al. 2008) package of PRIMER version 6 (Clarke and Gorley 2006). Canonical discriminant analysis (CDA), which is a constraint ordination that maximizes differences between a priori defined groups, was used to test the discriminating

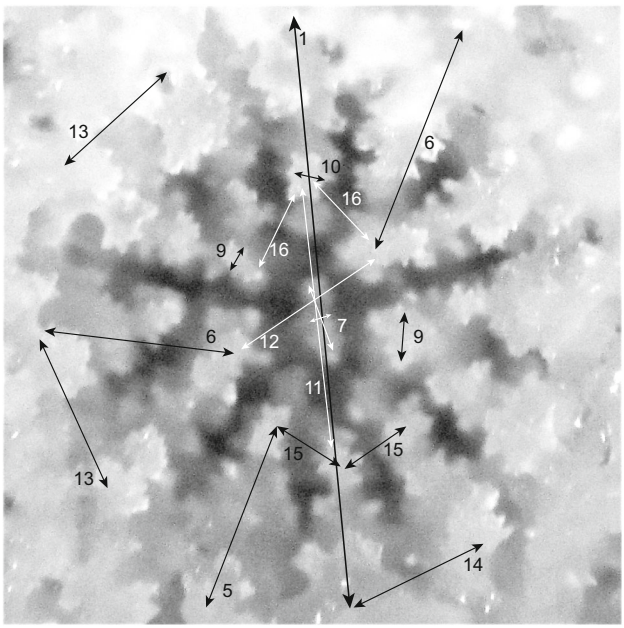


Fig. 3 Diagram of examples of measurement locations of *P. lobata* corallite listed in Table 2

power of morphometric characters and to find characters with a significant conditional effect using MorphoTools (Koutecký 2014) in R. Canonical discriminant analysis does not produce a scatter biplot for two groups, and therefore, an additional sample from the Kewalo Basin was added for analytical purposes (better visualization) for conducting CDA. Forward selection of characters with nonparametric Monte Carlo permutation tests (5000 permutations) was used to identify the characters with significant conditional effects. Classificatory discriminant analysis with cross-validation was conducted to obtain the posterior probabilities of classification into each group, as implemented in MorphoTools.

Morphological and genetic distance comparison

A distance matrix of corallite measurements for pairs of samples was created using the vegan package in R based on Euclidian and Manhattan distances to compare the results using the Euclidian and non-Euclidian distances. The single nucleotide polymorphism (SNP) loci used in this study were part of the dataset from Forsman et al. (2017). The methods for generating the SNP loci are described in detail in Forsman et al. (2017), and the data are publicly accessible (Table 1 of Forsman et al. 2017). Briefly, SNP loci were identified by aligning the reads generated by ezRAD (Toonen et al. 2013) to the *P. lobata* transcriptomic reference sequences (available at http://comparative.reefgenomics.org/). VCFtools (Danecek et al. 2011) was used to extract and filter the SNPs of the eight *P. lobata* samples. This process yielded 17,956 loci with a mean depth of 37.6 (± 21.7), which were used to estimate the genetic distances in the *pofadinr* package (Joly et al. 2015) in R. In *pofadinr*, the *genpofad* method was chosen to estimate genetic distance, since *genpofad* was more accurate and precise than other options in the package (Joly et al. 2015). The estimated morphological and genetic distance matrices were tested for correlation using the Mantel test using the *ape* (Paradis et al. 2004) and *ade4* (Dray et al. 2007) packages in R, as well as in PRIMER v6 using RELATE function.

Results

General corallite observation

Observation of *P. lobata* corallites revealed a great amount of structural variation from the published keys (Veron and Pichon 1982; Veron 2000). Hawaiian *P. lobata* samples displayed variable numbers of pali, ranging from five to eight. The variability in the number of pali was also observed within a sample. Most corallites were moderately excavated with relatively undeveloped pali, forming a concave V- to U-shape, which represents 'typical' *P. lobata* corallite architecture (Fig. 4a). However, there were samples with flat corallites, in which the tips of pali and septal denticles aligned with the wall, resembling the corallites of *P. evermanni* (Fig. 4b). These samples were genetically confirmed as *P. lobata* using ITS and/or H2 (i.e., belonging to the *Porites* Clade I complex, Forsman et al. 2009) and thus were not misidentified. The ventral triplet had free margins in the majority of observed samples, yet samples with a trident or a fused triplet were observed more frequently (~ 20% of samples) than expected (Fig. 4c, d). Some samples had highly developed pali, especially on the lateral pairs of septa (Fig. 4e). The diameter of pali was often greater for such tall, developed pali. A difference in pali development was among the most distinct characters observed between the nearshore and offshore *P. lobata* samples from Maunalua Bay; highly developed lateral pairs of pali were frequently observed in the nearshore samples, while none of the offshore samples showed such a feature. A columella was present in most samples examined. The shape of the columella ranged from rod shaped to compressed flat shaped (Fig. 4f), and the nearshore samples had more rod-shaped columella (83%) than the offshore samples (68%) (Chi-squared test, $\chi^2 = 5.298$, $df = 1$, $P = 0.0214$). Approximately 35% of the examined samples showed intra-colonial variation in the columella shape.

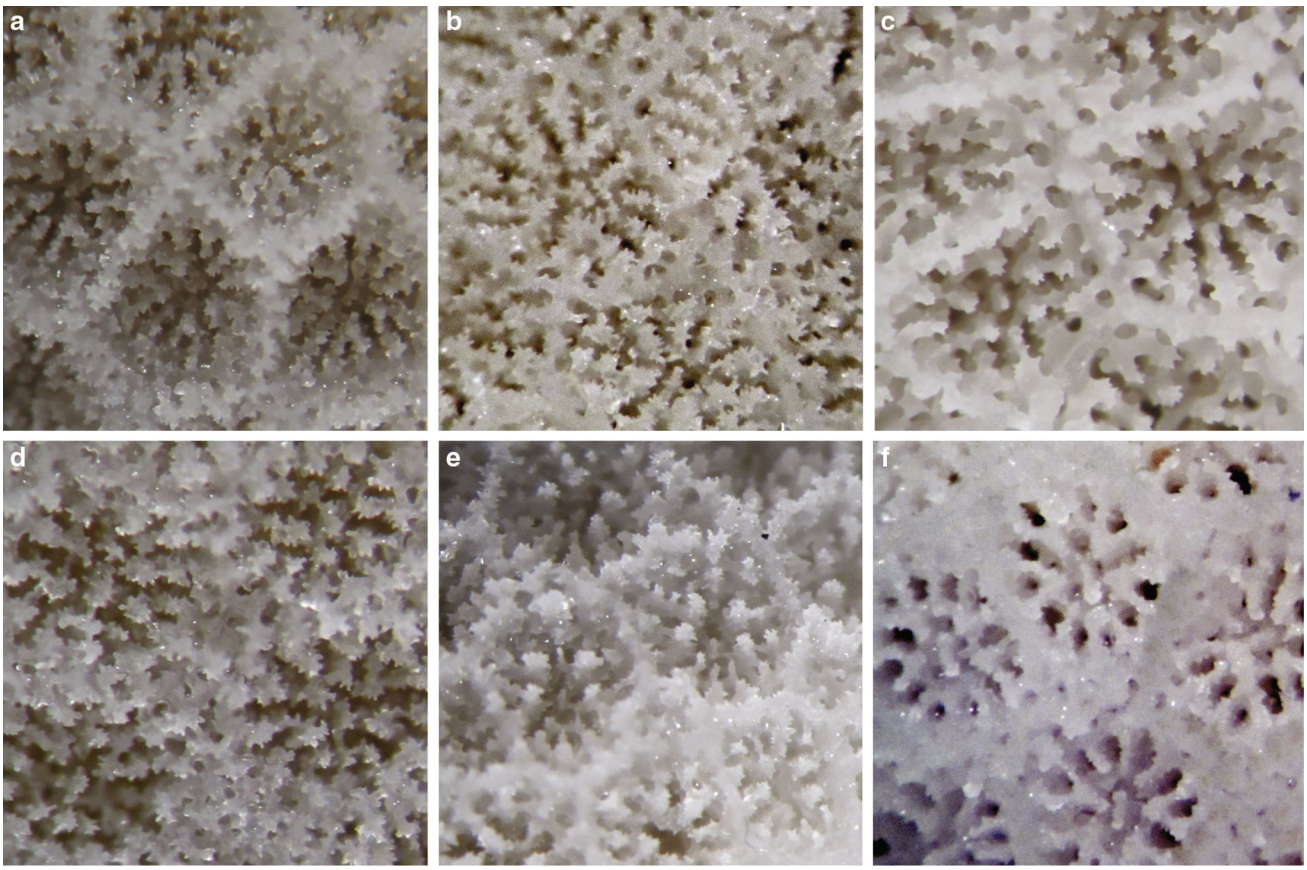


Fig. 4 Images of *P. lobata* corallites; **a** 'typical' corallite structure (represents the offshore site of Maunalua Bay), **b** flat corallite variation (shallow calice), **c** reduced number of pali (5–6), **d** ventral triplet forming a trident, **e** tall, pronounced lateral pali (represent the nearshore site of Maunalua Bay), and **f** compressed columella

Morphometric analysis

A total of 25 samples were used to quantitatively assess the differences in corallite skeletal morphology between *P. lobata* from the nearshore and offshore sites in Maunalua Bay. One Kewalo sample was included to better visualize the canonical scores. Principal component analysis resulted in the first axis explaining 25.3% of the variance and the second axis explaining an additional 18.9% between the three locations (Fig. S1). The results of PERMANOVA revealed significant morphological differences between colonies from the three locations (pseudo-F [pF] = 18.70, $P = 0.001$, perm = 1000), as well as between the nearshore and offshore sites (p$F = 8.669$, $P = 0.001$, perm = 1000).

Canonical discriminant analysis revealed that more than half of the characters (12 out of 19) contributed significantly to define the morphological distinctiveness between locations, rather than just a few characters influencing the differences (Table S2). The characters which contributed the most ($P \leqq 0.005$) were corallite spacing, ventral septum length, columella diameter, ventral palus diameter, lateral palus diameter, fossa width, ventral palus spacing, and lateral septa thickness. The forward selection procedure identified seven characters with a significant conditional effect, which were, in the order of significance; lateral palus diameter, ventral palus spacing, corallite spacing, ventral palus diameter, columella diameter, fossa length, dorsal palus spacing. (Table S3). All of these characters had significant marginal effects (i.e., when a character is tested alone in the model), except for dorsal palus spacing. In CDA, the first axis explained 22.6% of the variance, and the second axis explained an additional 17.1%. The Kewalo sample had almost no overlap of the canonical scores with other samples, while a portion of the canonical scores of the nearshore and offshore samples overlapped (Fig. 5a). Kewalo and Maunalua Bay samples separated primarily along the second discriminant axis, while the nearshore and offshore corals of Maunalua Bay separated along the first axis (Fig. 5a). The pattern of canonical scores observed using 19 characters was well preserved even when CDA was run with the seven characters identified as significant conditioning effects in the forward selection, in which the first and second axes still

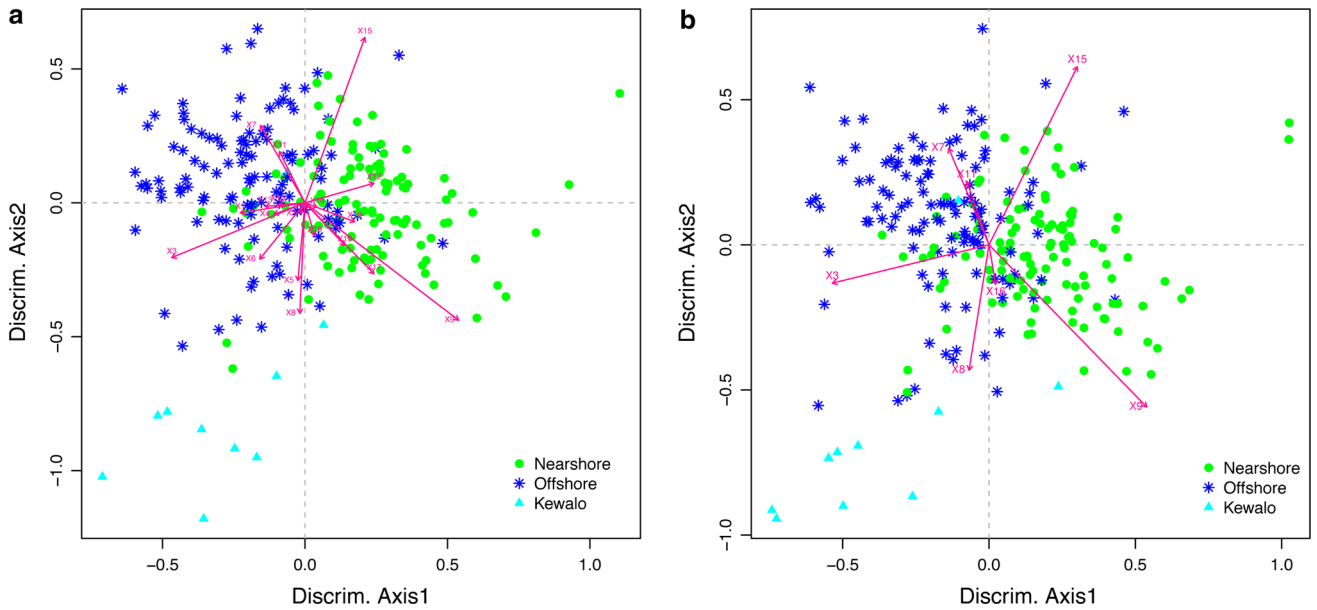


Fig. 5 Canonical discriminant analysis using 19 morphological characters of 25 *P. lobata* corallites (**a**), and using the seven characters identified in the forward selection process (**b**). The first and second discriminant axes are displayed, which explained 22.6% and 17.1% in (**a**), and 20.2 and 15.7% in (**b**)

explained 20.2 and 15.7% of the variance, respectively (Fig. 5b). Canonical discriminant analysis was also run using the averages of the individual samples, based on the seven significant characters. The results also retained the pattern of canonical scores well (Fig. S2) with the first and second axes explaining 39.3 and 32.8%, respectively. The average values of these seven characters revealed that corallite spacing, columella diameter, and fossa length were larger in the offshore samples, while ventral palus diameter, lateral palus diameter, ventral palus spacing, and dorsal palus spacing were larger in the nearshore samples.

The classificatory discriminant analysis with cross-validation resulted in correct classification approximately 85% of the time for all the samples. (Table 3, Fig. 6). For each group, the samples were correctly identified 82.5% of the time in the nearshore corals, 86.7% in the offshore corals, and 90% in the Kewalo sample.

Table 3 Results of cross-validated classificatory discriminant analysis of 25 *P. lobata* corallites using the seven morphological characters identified as discriminant variables in the forward selection process

	N	O	K	% correct
Nearshore	99	20	1	82.5
Offshore	10	104	6	86.7
Kewalo	1	0	9	90
Total			250	84.8

N nearshore, *O* offshore, *K* Kewalo

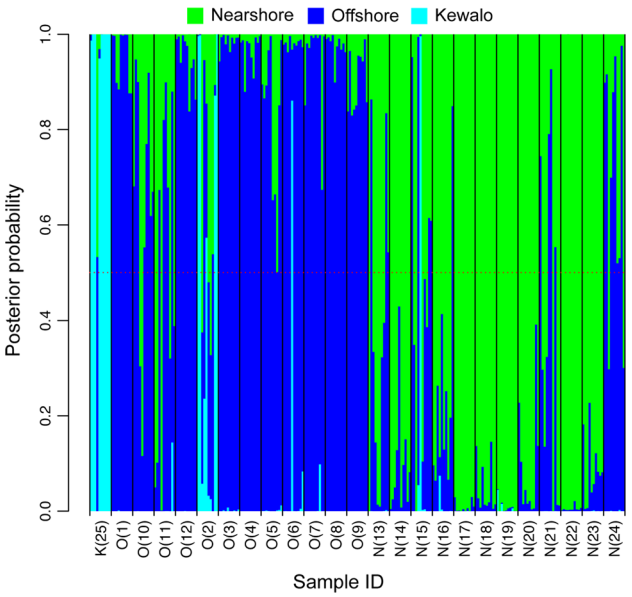


Fig. 6 Classificatory discriminant analysis of 25 *P. lobata* corallites. Each bar represents each observation. Table 3 shows the corresponding numerical results of classificatory discriminant analysis (average probabilities for each group of samples)

Morphological and genomic distance comparison

The morphological distance obtained from the 19 numerical characters and the genetic distance estimated from the 17,956 SNPs showed a significant correlation between the nearshore and offshore *P. lobata* samples from Maunalua Bay (*Relate* in PRIMER: $\rho = 0.641$–0.667,

$P = 0.029$–0.03, Mantel test: $r = 0.559$–0.632, $P = 0.030$–0.046, $n = 8$) (Fig. 7).

Discussion

This study provided new insights into the range of variation in corallite morphology of *P. lobata*, as well as potential genetic basis on their morphological variation. The results highlight a need to establish clearer diagnostic morphological characters for *Porites* taxa. If only a handful of taxonomic experts can identify the species, this will not aid future research progress. Methods that require sophisticated technology, such as scanning electron microscopy or 3D reflex microscopy, are also not practical, as most researchers will not have access to such instruments or resources to conduct expensive analysis. As in Forsman et al. (2015), our study showed that using a simple stereomicroscope and imaging software for morphometric analysis can be an efficient tool to capture character differences in *Porites* corallites.

Corallite observation

Assuming all Clade I samples assessed in our study were *P. lobata*, a high level of variation in corallite morphology of *P. lobata* was found, including some of the key diagnostic characters, such as the number of pali. Ketchum and Reyes-Bonilla (2001) noted detailed variations in *P. lobata* corallite architecture, such as columella being compressed or rod shaped, and the developmental state of pali in their report on corals from Revillagigedo Islands, Mexico, and

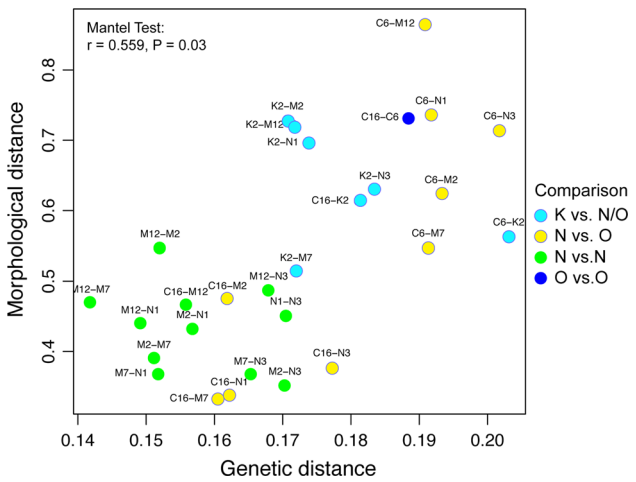


Fig. 7 Relationship between *P. lobata* corallite morphology and genetics. The morphological distance matrix was obtained using the Euclidian method based on 19 characters, and the genetic distance matrix was calculated using the *genpofad* method based on 17,956 SNP loci of coral host

classified the morphological variation of *P.lobata* into three forms. The authors also categorized the variants of massive *Porites* samples that did not match with any existing corallite descriptions as '*Porites* sp.1,' which included samples having 5–8 pali with a fused to free ventral triplet. In our study, all of these forms were observed, which suggests some or all of their samples of *Porites* sp.1 could well be the variation of *P. lobata*. However, since no genetic analysis was conducted on their samples, we can only speculate at this point. The Eastern Tropical Pacific and the Hawaiian Islands represent a marginal habitat for reef corals, geographically apart from the central Pacific by thousands of kilometers (Baums et al. 2012; Hellberg et al. 2016). These isolated regions, with their low species diversity, offer an excellent opportunity to efficiently study *Porites* skeletal structures, since the uncertainty of species identification can be reduced from the equation.

Morphometric analysis

Since there were no changes in the average coefficients of variation between using five and ten measurements per sample, our previous assumption (that using ten measurements per sample was sufficient to capture the within-sample variation) was valid. This is not to be confused with intra-colonial variation, since the majority of our observations were made from one skeletal fragment per colony, with each fragment ranging in size from 1 to 16 cm^2. An ideal way to assess corallite morphology is to take skeletal samples from various parts of a colony to capture intra-colonial variation, since the top and the bottom of a colony may show different skeletal characters. However, this is often not possible due to limited resources and/or restrictive collection permits. The samples used in this study were collected from an area of a colony facing upward to minimize the effect of intra-colonial variation. In terms of capturing the 'within-sample' variation, our analysis suggested that taking even five measurements per sample may be enough. This result will increase the efficiency of future morphometric studies, as taking multiple measurements from a small corallite is the most time-consuming part of the analysis.

The morphometric analysis of *P. lobata* corallites showed strong grouping based on geographic locations, which also corresponded to genetic distance. The characters with a significant conditional effect, identified by the stepwise forward selection in CDA, were congruent with the characters that significantly contributed to defining the groups. These seven characters also retained the separation of canonical scores among the sites (Fig. 5b), suggesting that the number of characters to be measured can also be substantially reduced in future morphometric studies of *P. lobata*. Two of the seven key characters were associated

with ventral triplet (ventral palus spacing and ventral palus diameter). Ventral triplet is one of the diagnostic characters for identifying *Porites* species and is reported as 'usually have free margins' in *P. lobata* (Veron and Pichon 1982; Veron 2000). Our study revealed that the skeletal formation of the ventral triplet could vary substantially for *P. lobata*, with up to 20% of colonies having some of their corallites with a fused triplet or a trident, indicating that ventral triplet may not be a reliable diagnostic character in species identification. The characters associated with lateral pali/septa were also important features in defining the groups in *P. lobata*. This was not surprising, since the most noticeable differences in *P. lobata* corallites (between the nearshore and offshore sites) were the prominent development of lateral pali.

Our results stress the importance of using the quantitative approach, as qualitative observations will unlikely to capture the subtle differences in morphology. For example, 'corallite spacing' was another of the seven key characters, which is a difficult feature to compare without a quantitative assessment. Weil (1992) reported corallite density (the number of corallites per area) as the most important distinguishing character in his discriminant analysis of *Porites* corallites. However, Jameson (1995) stated that corallite density was not a reliable character, since budding corallites would influence the number. Our results showed that corallite spacing, which is related to corallite density, is one of the key characters in defining the groups. (Jameson (1995) did measure corallite spacing in his study, but the corallite spacing was not one of the five characters identified as important in his discriminant analysis.) Certain environmental stressors, such as sedimentation, are also known to influence corallite density (Mwachireya et al. 2015). We, therefore, recommend corallite spacing as a useful diagnostic character.

Genetic correlation to corallite morphology

The significant correlation found between the morphological and genetic distances (Fig. 7) suggests that corallite architecture is potentially determined genetically in *P. lobata*. This is congruent with previous studies, which concluded that much of the observed corallite/morphological variation in *Porites* species was genetically based (Brakel 1977; Weil 1992; Forsman et al. 2015; Dimond et al. 2017). Recent studies also recognize that microskeletal characters of scleractinian corals are more genetically controlled and less affected by environment than previously thought (Janiszewska et al. 2011, 2013; Kitahara et al. 2016). However, because of the contrasting environments of the nearshore and offshore sites, and since phenotypes can be both genetically and environmentally controlled, the observed morphological differences could be a phenotypic response to environmental differences.

As pointed out in Todd (2008), phenotypic plasticity and intraspecific variation are not the same, though they are often used interchangeably, causing confusion and misunderstanding. Our study measured the intraspecific variation in corallite morphology; whether, or how much, these corals would exhibit plasticity in a different environment is unknown. We attempted to assess plasticity in corallite morphology in the reciprocal transplant experiment conducted between the nearshore and offshore sites. However, no measurable changes were observed (results not shown), likely due to the 30-d experimental period being too short to capture such changes. Therefore, further studies will be needed to understand the exact nature of the relationship between the genetic and morphological distances and how much phenotypic plasticity these corals exhibit in corallite morphology due to environmental factors.

So, does the corallite structure have adaptive values? We can speculate from previous studies that the unique corallite structure of the nearshore genotype is likely not by coincidence. For example, light and water movement are known to induce changes in corallite morphology, and sedimentation also likely plays a role (Todd 2008). The coral fragments transplanted to the shallow water site (with greater light intensity and higher total suspended sediment concentration) showed increased calice size, skeletal topology, and fragment rugosity in *Favia speciosa* (Todd et al. 2004a, b). The authors concluded that these induced changes likely had an adaptive value, since the increase in calice size correlates with the sediment shedding capacity in many coral species (Stafford-Smith and Ormond 1992). The shape of calice also appeared to affect the sedimentation shedding ability (Riegl 1995). The Maunalua Bay nearshore site has considerably lower light intensity (Tisthammer 2017) and higher total suspended sediment concentration (Richmond 2009; Storlazzi et al. 2010) than the offshore site (Table S1). Therefore, it is possible that the corallite morphology of the nearshore corals is beneficial for surviving in such an environment. The nearshore corals overall had shallower corallites and much more pronounced pali than the offshore corals. These traits may help prevent the accumulation of sediments and/or facilitate the removal of sediments, which may reduce the energy required to shed sediments, and/or help polyps extend under limited light.

In summary, our results show high intraspecific variation in corallite morphology of *P. lobata*, with significant differences in morphological characters between the geographic locations. Our previous studies have shown molecular and physiological response differences between the nearshore and offshore *P. lobata* from Maunalua Bay, which are genetically differentiated, suggesting that the

nearshore corals are genetically adapted to their environment (Tisthammer 2017). The distinct morphological characters seen in the nearshore *P. lobata*, therefore, also suggest that the observed corallite characters may have an adaptive value. The high level of variation in skeletal morphology observed in *P. lobata* in our study spells out the need for further understanding the extent of skeletal variability and plasticity in *Porites* species, as well as establishing a new classification system that integrates morphological data and genetic information.

Acknowledgements The authors thank Z. Forsman for the study directions he provided us in early part of this research. Coral samples were collected under Department of Land and Natural Resources permit SAP 2013-26 and 2015-06. Following people assisted in collecting coral samples: M. Stiber, V. Sindorf, F. Seneca, J. Murphy, J. Martinez, A. Lyman, K. Richmond, V. Hölzer, N. Spies, and A. Irvine.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA + for PRIMER: guide to software and statistical methods. PRIMER-E Ltd

Arrigoni R, Berumen ML, Chen CA et al. (2016) Species delimitation in the reef coral genera *Echinophyllia* and *Oxypora* (Scleractinia, Lobophylliidae) with a description of two new species. *Molecular Phylogenetics and Evolution*, **105**, 146–159.

Baums IB, Boulay JN, polato NR, Hellberg ME (2012) No gene flow across the Eastern Pacific Barrier in the reef-building coral Porites lobata. *Molecular Ecology*, **21**, 5418–5433.

Baums IB, Miller MW, Hellberg ME (2005) Regionally isolated populations of an imperiled Caribbean coral, *Acropora palmata*. *Molecular Ecology*, **14**, 1377–1390.

Bernard HM (1902) The species problem in corals. *Nature*, **65**, 560.

Bosch TCG, Miller DJ (2016) Corals. In: *The Holobiont Imperative: Perspectives from Early Emerging Animals*, pp. 99–111. Springer, Vienna.

Brainard RE, Birkeland C, Eakin CM, McElhany P, Miller MW, Patterson M, and Piniak GA (2011) Status review report of 82 candidate coral species petitioned under the U.S. Endangered Species Act. U.S. Dep. Commerce, NOAA Tech. Memo., NOAA-TM-NMFS-PIFSC-27, 530 p. + 1 Appendix

Brakel WH (1977) Corallite variation in Porites and the species problem in corals. *Proceedings of Third International Coral Reef Symposium*, 457–462

Clarke KR, Gorley RN (2006) PRIMER v6: User Manual/Tutorial. PRIMER-E, Plymouth, 192 pp.

de Queiroz K (1998) The general lineage concept of species, species criteria, and the process of speciation: a conceptual unification and terminological recommendations. In: *Endless forms: species and speciation* (eds Howard DJ, Berlocher SH), pp. 57–75. Oxford University Press on Demand

de Queiroz K (2007) Species Concepts and Species Delimitation. *Systematic Biology*, **56**, 879–886.

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, McVean G, Durbin R (2011) The variant call format and VCF tools. *Bioinformatics* **27**, 2156–8.

Dimond JL, Gamblewood SK, Roberts SB (2017) Genetic and epigenetic insight into morphospecies in a reef coral. *Molecular Ecology,* 1–12, https://doi.org/10.1111/mec.14252.

Dray S, Dufour AB, Chessel D (2007) The ade4 package-II: Two-table and K-table methods. *R news*, **7**, 47–52.

Eaton DAR (2014) PyRAD: Assembly of de novo RADseq loci for phylogenetic analyses. *Bioinformatics* **30**:1844–1849.

Eytan RI, Hayes M, Arbour-Reily P, Miller M, Hellberg ME (2009) Nuclear sequences reveal mid-range isolation of an imperilled deep-water coral population. *Molecular Ecology*, **18**, 2375–2389.

Forsman ZH, Barshis DJ, Hunter CL, Toonen RJ (2009) Shape-shifting corals: molecular markers show morphology is evolutionarily plastic in Porites. *BMC Evolutionary Biology*, **9**, 45.

Forsman ZH, Wellington GM, Fox GE, Toonen RJ (2015) Clues to unraveling the coral species problem: distinguishing species from geographic variation in Poritesacross the Pacific with molecular markers and microskeletal traits. *PeerJ*, **3**, e751.

Forsman ZH, Knapp ISS, Tisthammer KH, Eaton DAR, Belcaid M, Toonen RJ (2017) Coral hybridization or phenotypic variation? Genomic data reveal gene flow between *Porites lobata* and *P. Compressa. Molecular Phylogenetics & Evolution* http://dx.doi.org/10.1016/j.ympev.2017.03.023.

Fukami H, Budd AF, Levitan DR et al. (2004a) Geographic differences in species boundaries among members of the *Montastraea annularis* complex based on molecular and morphological markers. *Evolution*, **58**, 324–337.

Fukami H, Budd AF, Paulay G, Solé-Cava A, Chen CA (2004b) Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals. *Nature*, **427**, 832–835.

Hellberg ME, Prada C, Tan MH, Forsman ZH, Baums IB (2016) Getting a grip at the edge: recolonization and introgression in eastern Pacific *Porites* corals. *Journal of Biogeography*

Hoffmeister JE (1926) The Species Problem in Corals. *American Journal of Science*, **12**, 151–156.

Huang DW, Sherman BT, Lempicki RA (2009) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Research*, **37**, 1–13.

Jameson SC (1995) Morphometric analysis of the Poritidae (Anthozoa: Scleractinia) off Belize. In:, pp. 1591–1596

Janiszewska K, Jaroszewicz J, Stolarski J (2013) Skeletal ontogeny in basal scleractinian micrabaciid corals. Journal of Morphology 274:243–257

Janiszewska K, Stolarski J, Benzerara K, Meibom A, Mazur M, Kitahara MV, Cairns SD (2011) A unique skeletal microstructure of the deep-sea micrabaciid scleractinian corals. Journal of Morphology 272:191–203

Joly S, Bryant DJ, Lockhart PJ (2015) *Flexible methods for estimating genetic distances from nucleotide data*. Methods in Ecology and Evolution

Ketchum JT, Reyes-Bonilla H (2001) Taxonomía y distribución de los corales hermatípicos (Scleractinia) del Archipiélago de Revillagigedo, México. *Revista de Biología Tropical*, **49**, 803–848.

Kitahara MV, Fukami H, Benzoni F, Huang D (2016) The New Systematics of Scleractinia: Integrating Molecular and Morphological Evidence. In: Goffredo S., Dobinsky Z. (eds) The Cnidaria. Past, Present and Future.In Past Present and Future Springer International Publishing, Cham, pp 41–59

Knowlton N (2000) Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia*, **420**, 73–90.

Koutecký P (2014) MorphoTools: a set of R functions for morphometric analysis. *Plant Systematics and Evolution*, **301**, 1115–1121.

Miller DJ, van Oppen MJ (2003) A "fair go"* for coral hybridization. *Molecular Ecology*, **12**, 805–807.

Mwachireya SA, McClanahan TR, Hartwick BE, et al (2015) Growth and corallite characteristics of Kenyan scleractinian corals under the influence of sediment discharge. *International Journal of Biodoversoty and Conservation,* **7**:364–377.

Paradis E, Claude J, Strimmer K (2004) APE: Analyses of Phylogenetics and Evolution in R language. *Bioinformatics*, **20**, 289–290.

Pinzón JH, Sampayo E, Cox E et al. (2013) Blind to morphology: genetics identifies several widespread ecologically common species and few endemics among Indo-Pacific cauliflower corals (*Pocillopora*, Scleractinia). *Journal of Biogeography*, **40**, 1595–1608.

Prada C, DeBiasse MB, Neigel JE et al. (2014) Genetic species delineation among branching Caribbean Porites corals. *Coral Reefs*, **33**, 1019–1030.

R Core Team. 2016. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org

Richards ZT, Miller DJ, Wallace CC (2013) Molecular phylogenetics of geographically restricted Acropora species: Implications for threatened species conservation. *Molecular Phylogenetics and Evolution*, **69**, 837–851.

Richmond RH (2008) Watersheds impacts on coral reefs in Maunalua Bay, Oahu, Hawaii. HCRI Project Final Report FY2007

Richmond RH (2009) Watersheds impacts on coral reefs in Maunalua Bay, Oahu, Hawaii. HCRI Project Final Report FY2008

Riegl B (1995) Effects of sand deposition on scleractinian and alcyonacean corals. *Marine Biology*, **121**, 517–526.

Romano SL, Palumbi SR (1997) Molecular evolution of a portion of the mitochondrial 16S ribosomal gene region in scleractinian corals. *Journal of Molecular Evolution*, **45**, 397–411.

Schmidt-Roach S, Lundgren P, Miller KJ, Gerlach G, Noreen AME, Andreakis N (2012) Assessing hidden species diversity in the coral *Pocillopora damicornis* from Eastern Australia. *Coral Reefs*, **32**, 161–172.

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, **9**, 671–675.

Stafford-Smith MG, Ormond R (1992) Sediment-rejection mechanisms of 42 species of Australian scleractinian corals. *Australian Journal of Marine and Freshwater Research*, **43**: 583–705.

Stat M, Baker AC, Bourne DG et al. (2012) Molecular Delineation of Species in the Coral Holobiont. In: *Advances in Marine Biology*, pp. 1–65. Elsevier Ltd

Stefani F, Benzoni F, Yang SY et al. (2011) Comparison of morphological and genetic analyses reveals cryptic divergence and morphological plasticity in *Stylophora* (Cnidaria, Scleractinia). *Coral Reefs*, **30**, 1033–1049.

Storlazzi CD, Presto KM, Logan JB, Field ME (2010) Coastal Circulation and Sediment Dynamics in Maunalua Bay, Oahu, Hawaii. USGS Open-File Report 2010-1217

Tisthammer, KH (2017) Understanding the adaptive ability of corals to rapidly changing environments. Ph.D. Dissertation. University of Hawaii at Manoa

Todd PA (2008) Morphological plasticity in scleractinian corals. *Biological Reviews*, **83**, 315–337.

Todd PA, Ladle RJ, Lewin-Koh N (2004a) Genotype × environment interactions in transplanted clones of the massive corals Favia speciosa and Diploastrea heliopora. *Marine ecology Progress Series*, **271**, 167–182.

Todd PA, Sidle RC, Lewin-Koh NJI (2004b) An aquarium experiment for identifying the physical factors inducing morphological change in two massive scleractinian corals. *Journal of experimental marine biology and ecology*, **299**, 97–113.

Toonen RJ, Puritz JB, Forsman ZH, Whitney JL, Fernandez-Silva I, Andrews KR, Bird CE (2013) ezRAD: a simplified method for genomic genotyping in non-model organisms. *PeerJ*, **1**, e203.

van Oppen MJ, Willis BL, Miller DJ (1999) Atypically low rate of cytochrome b evolution in the scleractinian coral genus *Acropora*. *Proceedings of the Royal Society B: Biological Sciences*, **266**, 179–183.

Veron JEN (1995) *Corals in Space and Time:The Biogeography and Evolution of the Scleractinia*. Cornell University Press, Ithaca, NY.

Veron JEN (2000) *Corals of the World* (MG Stafford-Smith, Ed,). Australian Institute of Marine Science, Townsville, Australia

Veron JEN (2013) Overview of the taxonomy of zooxanthellate Scleractinia. *Zoological Journal of the Linnean Society*, **169**, 485–508.

Veron JEN, Pichon M (1982) *Scleractinia of Eastern Australia Part IV Family Poritidae*. Australian Institute of Marine Science and Australian National University Press, Miami, FL.

Vollmer SV, Palumbi SR (2002) Hybridization and the evolution of reef coral diversity. *Science*, **296**, 2023–2025.

Warner PA, van Oppen MJH, Willis BL (2015) Unexpected cryptic species diversity in the widespread coral Seriatopora hystrix masks spatial-genetic patterns of connectivity. *Molecular Ecology*, **24**, 2993–3008.

Weil E (1992) Genetic and Morphological Variation in Caribbean and Eastern Pacific Porites (Anthozoa, Scleractinia). *Proc. of the Seventh International Coral Reef Symposium*, **2**, 643–656

Willis BL, van Oppen M, Miller DJ, Vollmer SV (2006) The role of hybridization in the evolution of reef corals. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 489–517.

Yang Z, Rannala B (2012) Molecular phylogenetics: principles and practice. *Nature Reviews Genetics*, **13**, 303–314.