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REPORT

Application of DNA barcoding in biodiversity studies of shallow-water octocorals: molecular proxies agree with morphological estimates of species richness in Palau

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Abstract The application of DNA barcoding to anthozoan cnidarians has been hindered by their slow rates of mitochondrial gene evolution and the failure to identify alternative molecular markers that distinguish species reliably. Among octocorals, however, multilocus barcodes can distinguish up to 70 % of morphospecies, thereby facilitating the identification of species that are ecologically important but still very poorly known taxonomically. We tested the ability of these imperfect DNA barcodes to estimate species richness in a biodiversity survey of the shallow-water octocoral fauna of Palau using multilocus (*COI*, *mtMutS*, 28S rDNA) sequences obtained from 305 specimens representing 38 genera of octocorals. Numbers and identities of species were estimated independently (1) by a taxonomic expert using morphological criteria and (2) by assigning sequences to molecular operational taxonomic units (MOTUs) using predefined genetic distance thresholds. Estimated numbers of MOTUs ranged from 73 to 128 depending on the barcode and distance threshold applied, bracketing the estimated number of 118 morphospecies. Concordance between morphospecies identifications and MOTUs ranged from 71 to 75 % and differed little among barcodes. For the speciose and ecologically dominant genus *Sinularia*, however, we were able to identify 95 % of specimens correctly simply by comparing *mtMutS* sequences and in situ photographs of colonies to an existing vouchered database. Because we lack a clear understanding of species boundaries in most of these taxa, numbers of morphospecies and MOTUs are both estimates of the true species diversity, and we cannot currently determine which is more accurate. Our results suggest, however, that the two methods provide comparable estimates of species richness for shallow-water Indo-Pacific octocorals. Use of molecular barcodes in biodiversity surveys will facilitate comparisons of species richness and composition among localities and over time, data that do not currently exist for any octocoral community.

Keywords Soft coral · Gorgonian · mtMutS · COI · 28S rDNA · *Sinularia*

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Introduction

Current estimates of the number of species inhabiting earth's marine environments span three orders of magnitude (Reaka-Kundla 1997; Bouchet 2006; Mora et al. 2011; Costello et al. 2012), a range that emphasizes how little we know of the organisms that populate even well-studied shallow-water ecosystems. Although our understanding of the diversity of coral reef-inhabiting fish and scleractinian corals is relatively complete, the numbers and identities of other invertebrate taxa living on reefs, including conspicuous and ecologically important macrofauna, remain poorly known (Reaka-Kundla 1997). Recent advances in biodiversity studies include the use of molecular proxies to estimate species diversity, an approach that is particularly

well established for studying microbial communities (Sogin et al. 2006; Amaral-Zettler et al. 2010). Among coral reef macrofauna, molecular approaches are also beginning to yield increased understanding of the biodiversity of difficult and understudied groups (Knowlton et al. 2010). For example, DNA barcoding of cryptic crustaceans collected from coral heads has revealed unexpectedly high levels of biodiversity including hundreds of previously undetected species (Plaisance et al. 2009, 2011). Molecular barcoding has also revealed unrecognized cryptic diversity in groups that have been thought to be well known taxonomically (e.g., Keshavmurthy et al. 2013). Such studies support the value of using molecular data to increase our knowledge of the biodiversity, geographical distributions and taxonomy of marine species.

Octocorallia is a prime example of a group of conspicuous and ecologically important macrofauna whose diversity and species boundaries remain very poorly known. This subclass of anthozoan cnidarians includes an estimated 3,400+ species of soft corals, gorgonians and sea pens found in marine habitats worldwide (Daly et al. 2007; Williams and Cairns 2013). Their highest diversity is in the deep sea (below 50 m depth; Cairns 2007), where they serve as critical structural species, generating complex three-dimensional habitats that support many other species (Krieger and Wing 2002; Stone 2006; Buhl-Mortensen et al. 2010). On shallow tropical coral reefs, octocorals are often dominant space-occupiers and competitors of scleractinian corals (Benayahu and Loya 1981; Tursch and Tursch 1982; Dinesen 1983; Fabricius 1997). These dynamics may be shifting as a result of coral bleaching, disease and other sources of mortality associated with ongoing climate change and anthropogenic impacts on reefs. In the Indo-Pacific, there is evidence that dominant space-occupiers such as the alcyoniid soft coral genera *Sinularia*, *Sarcophyton* and *Lobophytum* experience very high levels of mortality following bleaching (Fabricius 1999; Bruno et al. 2001; Loya et al. 2001) and that their recovery from such disturbances may be slow (Fabricius 1995; Cornish and DiDonato 2004). In contrast, other soft corals appear to recruit rapidly following disturbances and may opportunistically monopolize hard substrata, potentially inhibiting the recovery of scleractinians and other slow-growing species (Fabricius 1998; Fox et al. 2003; Stobart et al. 2005; Tilot et al. 2008). In the Caribbean, gorgonians may be relatively resistant to bleaching (Prada et al. 2010), and there is some evidence that their populations have been increasing as scleractinians have declined (Ruzicka et al. 2010). Few studies anywhere, however, have tracked long-term changes in octocoral populations, making it difficult to predict whether observed short-term responses to disturbance indicate permanent changes in community structure.

While there is a pressing need to better understand the ecological dynamics of octocoral populations and their responses to a changing environment, ecological studies are hampered by our poor understanding of octocoral taxonomy and the practical difficulties of discriminating and identifying species in most genera (Fabricius and Alderslade 2001). Identification of octocorals is time-consuming, requires a high level of taxonomic expertise, and there are few taxonomists actively working on the group (Cairns 2007). Large numbers of undescribed species and the need for taxonomic revisions of many genera mean it is often not possible to put species names on specimens with any confidence (Fabricius and De'ath 2008). As a result, most biodiversity surveys identify the majority of octocorals to genus only and do not attempt to quantify diversity at the species level (e.g., Stone 2006; Fabricius et al. 2007; Fabricius and De'ath 2008; Chanmethakul et al. 2010; Bridge et al. 2012). Others simply discriminate apparent morphospecies alphanumerically (e.g., van Oppen et al. 2005). Although the latter approach may allow species diversity to be estimated at a particular location, it does not enable comparisons of species composition across locations, as there is often no way to correlate the alphanumeric morphospecies identities used in one study or by one taxonomist with those of another. Establishment of a reference database of species-specific molecular barcodes linked to vouchered specimens would facilitate comparisons of faunal composition across locations and allow broad-scale patterns of distribution and long-term changes in octocoral biodiversity to be better assessed.

The development of molecular markers that reliably discriminate species has been a challenge in all groups of anthozoan cnidarians. Mitochondrial genes evolve 10–100X slower than nuclear genes in anthozoans (Hellberg 2006; Chen et al. 2009), as a result of which the "universal" animal *COI* barcode and other mitochondrial markers are frequently invariant within and among genera (Huang et al. 2008; Shearer and Coffroth 2008). In octocorals, an extended mitochondrial barcode of *COI* plus the octocoral-specific mitochondrial gene *mtMutS* has, however, been shown to discriminate approximately 70 % of the species that comprise the taxonomically well-known fauna of the northern Red Sea (McFadden et al. 2011). Although this barcode marker does not discriminate all species unequivocally, it is usually diagnostic at the genus level and typically allows species identifications to be narrowed to a small number of candidate sister taxa (McFadden et al. 2011).

Compared to the relatively well-studied and only moderately biodiverse Red Sea, the octocorals of the tropical western Pacific are both extremely diverse and poorly known taxonomically (Bayer 1981). The octocoral fauna of the Republic of Palau is, however, somewhat better known

Table 1 Species richness of octocorals in Palau estimated from the combined mt[a] + 28S molecular barcode and morphological criteria

Family	Genus	*N*	*H*	$K2p_{0.1\ \%}$	$K2p_{0.3\ \%}$	$K2p_{0.5\ \%}$	*S*	S_F
Acanthogorgiidae	*Acanthogorgia*	3	3	3	2	2	3	1
Alcyoniidae	*Cladiella*	1	1	1	1	1	1	4
Alcyoniidae	*Klyxum*	10	6	4	2	2	3	3
Alcyoniidae	*Lobophytum*	14	8	9	8	7	7	3
Alcyoniidae	*Paraminabea*	4	3	1	1	1	1	1
Alcyoniidae	*Sarcophyton*	51	25	15	12	10	9	>9
Alcyoniidae	*Sinularia*	52	34	27	20	16	26	38[c]
Briareidae	*Briareum*	5	3	2	1	1	2	2
Chrysogorgiidae	*Stephanogorgia*	2	1	1	1	1	1	1
Clavulariidae	*Carijoa*	1	1	1	1	1	1	1
Clavulariidae	*Clavularia*	5	2	1	1	1	1	1
Ellisellidae	*Dichotella*[b]	1	1	0	0	0	1	1
Ellisellidae	*Ellisella*	8	2	1	1	1	2	1
Ellisellidae	*Junceella*	3	2	3	2	1	2	3
Gorgoniidae	*Hicksonella*[b]	1	1	0	0	0	1	1
Gorgoniidae	*Pinnigorgia*	1	1	1	1	1	1	3
Gorgoniidae	*Rumphella*	6	2	1	1	1	1–2	1
Melithaeidae	*Melithaea*	11	7	6	6	6	4	5
Nephtheidae	*Dendronephthya*	11	9	5	5	3	6	6
Nephtheidae	*Lemnalia*	12	5	5	4	4	7	2
Nephtheidae	*Nephthea*	2	2	2	1	1	2	2
Nephtheidae	*Paralemnalia*	8	4	3	1	1	1	5
Nephtheidae	*Scleronephthya*	3	3	2	2	1	2	4
Nephtheidae	*Stereonephthya*	8	2	3	1	1	3	1
Nidaliidae	*Chironephthya*	15	9	6	4	4	4	3
Nidaliidae	*Nidalia*	2	1	1	1	1	1–2	1
Nidaliidae	*Siphonogorgia*	16	7	4	3	3	5	2
Parasphaerascleridae	*Parasphaerasclera*	2	2	2	1	1	2	4
Plexauridae	*Astrogorgia*	12	10	7	7	5	6	1
Plexauridae	*Bebryce*	4	3	2	1	1	1	1
Plexauridae	*Euplexaura*	4	1	1	1	1	3	2
Plexauridae	*Villogorgia*	1	1	1	1	1	1	1
Subergorgiidae	*Annella*	3	2	2	1	1	2	2
Subergorgiidae	*Subergorgia*	6	3	2	2	2	2	1
Tubiporidae	*Tubipora*	2	1	1	1	1	1	1
Xeniidae	*Asterospicularia*	2	1	1	1	1	1	1
Xeniidae	*Heteroxenia*	4	1	1	1	1	1	1
Xeniidae	*Sympodium*	1	1	1	1	1	1	0
Total		305	171	128	100	87	118	120

N = number of specimens sequenced for at least 2 of 3 loci; H = number of unique 3-locus genotypes; $K2p_{0.1}$ = number of MOTUs differing by average Kimura 2-parameter genetic distance >0.1 %; $K2p_{0.3}$ = number of MOTUs differing by >0.3 %; $K2p_{0.5}$ = number of MOTUs differing by >0.5 %; S = estimated number of morphospecies, this study; S_F = number of morphospecies estimated by Fabricius et al. (2007)

[a] mt = *mtMutS* + *igr1* + *COI* (McFadden et al. 2011)

[b] Genera not distinguished as MOTUs

[c] Estimate from van Ofwegen (2008)

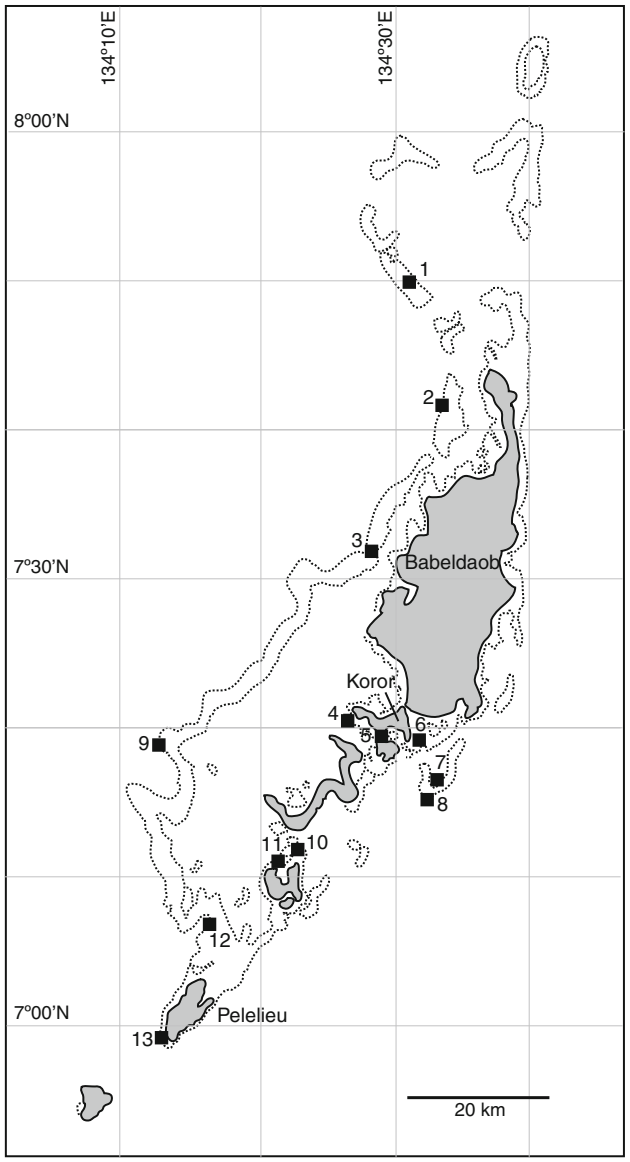

Fig. 1 Map of the main islands (*shaded areas*) and surrounding shallow-reef habitat (*dotted outlines*) of the Republic of Palau. Octocorals were collected at 13 sites (*numbered squares*) spanning a N–S distance of approximately 115 km. *1* NW barrier reef, 7°50.087′N, 134°31.132′E; *2* NW barrier reef, 7°41.888′N, 134°33.833′E; *3* West Channel, 7°32.496′N, 134°28.347′E; *4* Pinchers, 7°20.402′N, 134°25.682′E; *5* Ngerikuul Gap, 7°19.254′N, 134°29.751′E; *6* KB Channel, 7°18.592′N, 134°31.485′E; *7* Short Drop-Off, inner wall, 7°16.470′N, 134°31.523′E; *8* Short Drop-Off, outer slope, 7°15.533′N, 134°31.436′E; *9* Siaes Tunnel, 7°18.686′N, 134°13.596′E; *10* Wonder Channel, 7°10.869′N, 134°21.612′E; *11* Neco Channel, 7°12.316′N, 134°22.646′E; *12* Turtle Cove, 7°05.078′N, 134°15.730′E; *13* Pelelieu wall, 6°58.612′N, 134°13.330′E

than that of most other regions of the western Pacific and has been summarized in several recent papers (Fabricius et al. 2007; van Ofwegen 2008). Excluding those species that are only known from deep water (>60 m), ~144 morphospecies of octocorals belonging to 63 genera have been recorded. Only half of those species have been assigned to known taxa, however, and the others have simply been identified to genus (Fabricius et al. 2007). In the absence of species names or other unique identifiers (e.g., molecular barcodes) for half of the octocoral fauna, it is currently not possible to compare the species composition of Palau to that of any other location. Here, we test further the ability of molecular barcodes to distinguish morphospecies of octocorals for the purposes of (1) estimating the species richness of a region and (2) identifying species by comparison to an established database of barcode sequences and in situ photographs.

Materials and methods

Collections

A total of 391 specimens representing 38 genera of octocorals (Table 1) were collected during 8 d of field work in the Republic of Palau from 19–26 May 2010. Dives were made at 13 sites in shallow-reef environments (to 30 m depth) spanning a 115 km transect from the barrier reefs NW of Babeldaob (7°50.087′N) to the southern end of Pelelieu (6°58.612′N) (Fig. 1). Two genera of soft corals, *Sinularia* and *Sarcophyton*, were sampled extensively for ongoing chemical ecology projects ($n = 161$ specimens). In addition, we collected representatives of all other species of octocorals that we encountered at each site; collections were made haphazardly without attempting to quantify relative abundance of species or to search areas systematically for new species. Specimen vouchers were preserved in 70 % EtOH or frozen at −20 °C for chemical analyses; small subsamples of tissue to be used for DNA analysis were stored in 95 % EtOH. Voucher material for all species has been deposited at Naturalis Biodiversity Center [formerly Rijksmuseum van Natuurlijke Historie, Leiden (RMNH)]; specimens of *Heteroxenia*, *Klyxum* and *Cladiella* have also been deposited at the Zoological Museum, University of Tel Aviv (ZMTAU) and California Academy of Sciences (CAS).

Taxonomic identifications

All specimens were initially identified to genus using established morphological criteria (Fabricius and Alderslade 2001). Independently and without knowledge of the molecular results, a taxonomic expert (LPvO) estimated numbers of morphospecies collected per genus based on assessment of traditionally used morphological characters. Tissue samples from different anatomical regions of a colony were dissolved in bleach to extract sclerites that

were examined using light microscopy. For the majority of genera, morphospecies were merely discriminated as sp. A, sp. B, etc., and no attempt was made to identify specimens to known species.

DNA sequencing and analysis

Extraction of DNA from ethanol-preserved tissue samples, amplification by PCR, and sequencing of the *mtMutS* (*msh1*) and *COI* genes followed the protocols published in McFadden et al. (2011). In addition, we sequenced an approximately 765-nt fragment of the *28S* nuclear ribosomal gene using primers 28S-Far,with either 28S-Rar or 28S-Rab (McFadden and van Ofwegen 2013), and the same PCR protocol used for *COI*. Sequences were aligned using the L-INS-i method in MAFFT v. 6 (Katoh and Toh 2008), and pairwise genetic distances (Kimura 2-parameter) among specimens were calculated using the DNADist program in PHYLIP v. 3.69 (Felsenstein 2005). MOTHUR v. 1.29 (Schloss et al. 2009) was used to cluster sequences into MOTUs (molecular operational taxonomic units; Floyd et al. 2002) based on average neighbor distance thresholds of 0.1, 0.3 and 0.5 % (see below). MOTUs were defined separately for four different barcode combinations: (1) *mtMutS* alone; (2) 28S rDNA alone; (3) the mitochondrial (mt) barcode of *mtMutS* + *igr1* + *COI* (McFadden et al. 2011); and (4) a combined multilocus barcode of mt + 28S rDNA. For each barcode and distance threshold, we calculated species richness accuracy (SA) as the number of MOTUs detected divided by the estimated number of morphospecies sequenced. The concordance of species identifications was estimated as the percentage of specimens for which molecular and morphospecies classifications were in agreement. For example, if a MOTU included three specimens of morphospecies A and one specimen of morphospecies B, it would be counted as three concordant identifications and one non-concordant identification for a total concordance of 0.75. If a fourth individual of morphospecies A belonged to a different MOTU, it would also be counted as a non-concordant identification, for an overall concordance of 0.60 among the five specimens.

Determination of average neighbor thresholds to define MOTUs

Because taxonomic boundaries and intraspecific variation have been so little studied in octocorals, when two different morphospecies share identical barcode markers, we cannot often discriminate between two possible interpretations: (1) the molecular marker failed to distinguish species or (2) our interpretation of species boundaries based on morphology is incorrect. For this reason, we have restricted testing of the discriminatory abilities of barcode markers to a test group of *Alcyonium*, a genus within which species boundaries have been assessed previously using allozymes and reproductive differences (McFadden 1999; McFadden et al. 2001). A subset of the same specimens that were used in a previous study to estimate average intra- and interspecific genetic distances for *mtMutS* and the mt barcode (*mtMutS* + *igr1* + *COI*; McFadden et al. 2011) were sequenced for 28S rDNA. Pairwise genetic distances (Kimura 2-parameter) within and among species were calculated, and means and ranges of values were compared among the four barcode markers used here (*mtMutS,* 28S, mt and combined mt + 28S). In addition, mean and maximum coalescent depths were calculated for each barcode, where coalescent depth is the maximum pairwise distance observed among individuals of the same species (e.g., Meyer and Paulay 2005). MOTHUR was used to cluster specimens into MOTUs to determine the average neighbor distance at which concordance of species identification was highest (i.e., MOTUs most closely reflected actual species).

Identification of *Sinularia* species

mtMutS sequences for *Sinularia* were aligned to an existing reference database of sequences for 80 species of that genus, including 36 of 38 species recorded previously from Palau (McFadden et al. 2009). A specimen was considered to be identified to species if its *mtMutS* haplotype exactly matched that of a species in the reference set, provided the reference sequence was unique. In cases where a haplotype was similar to but not a direct match to any species in the reference alignment, or, conversely, was shared with two or more species in the database (i.e., the reference sequence was not unique), species identifications were narrowed further by (1) restricting comparisons to reference sequences of species known to have been recorded previously in Palau (van Ofwegen 2008) and (2) comparing in situ photographs of colony morphology to reference photographs available in a curated online repository (http://science.naturalis.nl/research/people/cv/ofwegen/sinularia-images). All species identifications were subsequently confirmed or corrected based on microscopic examination of sclerites by a taxonomic expert.

Results

Average neighbor distance thresholds for species discrimination

28S rDNA sequences (~765 bp) were obtained for 5–10 individuals of each of 10 species of *Alcyonium* for which

Table 2 Genetic distance values (Kimura 2-parameter) within and between 10 North Atlantic and Mediterranean species of *Alcyonium* assessed using different molecular barcodes

Gene region (s)	nt	Mean coalescent depth (±S.D.)	Max. coalescent depth Observed	Mean interspecific distance (±S.D.)	Min. interspecific distance	SA (K2p)	SA (PCA)
mtMutS	735	0.0018 (±0.0024)	0.0070	0.0367 (±0.0246)	0	$0.5_{(0.5\ \%)}$	0.5
mt[a]	1,648	0.0012 (±0.0012)	0.0035	0.0262 (±0.0170)	0	$0.5_{(0.3\ \%)}$	0.6
28S rDNA	765	0.0015 (±0.0025)	0.0080	0.0338 (±0.0185)	0	$0.6_{(0.7\ \%)}$	0.8
mt + 28S	2,413	0.0015 (±0.0016)	0.0049	0.0279 (±0.0173)	0	$0.6_{(0.3\ \%)}$	0.9

nt = total length (nucleotides) of aligned sequence; coalescent depth = maximum intraspecific distance within a species; $SA_{(K2p)}$ = species richness accuracy (#MOTU/#species) at K2p threshold that minimized false positives; SA(PCA) = species richness accuracy (#MOTU/#species) based on assigning MOTUs using pure characteristic attributes

[a] (*mtMutS* + *igr1* +*COI*)

pairwise genetic distances and pure characteristic attributes (sensu Rach et al. 2008) had been assessed previously for *mtMutS* and the mt barcode (McFadden et al. 2011). Pairwise genetic distances (Kimura 2-parameter) among 28S sequences ranged from 0 to 0.8 % among individuals of the same species, and 0–6.4 % among congeneric species. Mean coalescent depth (0.15 %) and mean interspecific genetic distance (3.38 %) were both slightly greater for 28S than for the mt barcode (values of 0.12 and 2.62 % respectively, Table 2). When 28S was combined with mt for a multilocus barcode of 2,413 bp, the mean coalescent depth was 0.15 %, with a maximum observed coalescent depth of 0.49 % and a mean interspecific distance of 2.79 %. Two species pairs (*A. glomeratum*-*A. palmatum*, *A. digitatum*-*A.* sp. A) shared identical 28S genotypes (interspecific distance = 0); when 28S was combined with mt, however, only one species pair (*A. glomeratum*-*A. palmatum*) shared identical genotypes and could not be distinguished on the basis of pure characteristic attributes. Single-nucleotide polymorphisms in the 28S sequences also identified several individuals within the *A. coralloides* clade that are likely of hybrid origin, consistent with previous evidence for reticulate evolution within this clade (McFadden and Hutchinson 2004).

Species of *Alcyonium* were considered to have been discriminated correctly (concordance = 1) when all sequences from a species were assigned to the same MOTU and that MOTU included sequences from only one species. The average neighbor distance threshold that maximized the number of correct species identifications and minimized the number of false positives ranged from 0.3 % for the mt and combined mt + 28S barcodes to 0.5 and 0.7 % for *mtMutS* and 28S, respectively. At thresholds below 0.3 %, no additional species were identified correctly, but false positives (i.e., intraspecific variants classified as different MOTUs) were common. The maximum number of species discriminated correctly using average genetic distance thresholds was 6 of 10 using both 28S and the combined mt + 28S barcodes; 5 species were identified correctly using either *mtMutS* or mt. For all barcodes except *mtMutS*, the number of species that could be discriminated correctly was greater when MOTUs were defined using pure characteristic attributes rather than genetic distance thresholds (Table 2).

Based on these results, we selected 0.1 and 0.5 % as minimum and maximum average neighbor distance thresholds to use for detecting MOTUs among octocoral specimens from Palau. These values approximate the mean (0.15 %) and maximum (0.49 %) coalescent depths observed among *Alcyonium* species for the mt + 28S barcode (Table 2). Although no barcode gap was observed in *Alcyonium* (i.e., intraspecific and interspecific distances overlapped one another) only 20 % of interspecific comparisons had genetic distance values <0.5 and 10 % had values <0.1 %. We selected 0.3 % as an additional, intermediate threshold value to use for species discrimination; among *Alcyonium* species, an average distance threshold of 0.3 % minimized false positive assignment of MOTUs while simultaneously maximizing correct species identifications, thereby yielding the highest concordance.

Palau biodiversity estimates

We obtained *mtMutS* sequences (834 nt) for a total of 339 specimens, *igr1* + *COI* (977 nt) for 319 and 28S (864 nt) for 273 (of the 109 specimens of *Sarcophyton* collected, only 61 and 34 were sequenced for *igr1* + *COI* and 28S, respectively) (Table 3). All sequences have been deposited in GenBank (Electronic Supplemental Material, ESM S1). Complete mt + 28S barcodes were obtained for 231 specimens, and two-locus barcodes were obtained for an additional 89. Among all specimens that were sequenced, a total of 171 unique mt + 28S barcode sequences were detected; specimens for which only 1 or 2 loci were sequenced were considered to be unique only if they had an allele that was not detected in any specimens for which complete barcodes were obtained (Table 1). The largest numbers of unique barcode sequences were found for

Table 3 Summary of MOTUs estimated using different barcode markers and detection thresholds

	Molecular barcode			
	mtMutS	28S	mt[a]	mt[a] + 28S
No. of specimens sequenced (N)	339[b]	273	284	231
No. of morphospecies sequenced (S)	110	113	118	118
Distance threshold = 0.1 %				
No. MOTUs	96	121	**117**	128
Species richness accuracy (SA)	0.873	1.071	**0.992**	1.085
Concordance	0.749	0.715	0.729	0.737
Distance threshold = 0.3 %				
No. MOTUs	85	101	87	101
Species richness accuracy (SA)	0.773	0.894	0.737	0.847
Concordance	**0.753**	0.726	0.721	0.740
Distance threshold = 0.5 %				
No. MOTUs	73	95	68	87
Species richness accuracy (SA)	0.664	0.841	0.576	0.737
Concordance	0.711	0.726	0.643	0.719

S = morphospecies richness; species richness accuracy (SA) = MOTUs/S. Concordance = fraction of specimens for which MOTU and morphospecies assignments are in agreement (see text). Boldface: highest accuracy

[a] mt = *mtMutS* + *igr1* + *COI*

[b] 44 of 105 *Sarcophyton* specimens sequenced for *mtMutS* only

Sinularia ($n = 34$), *Sarcophyton* ($n = 25$) and *Astrogorgia* ($n = 10$), while in 13 genera, only a single barcode sequence was detected. Identical barcode sequences were shared between genera in three cases: *Rumphella* sp. A with *Hicksonella* sp. A, *Dichotella gemmacea* with *Junceella juncea*, and *Paralemnalia digitiformis* with *Lemnalia* sp. C.

The total number of morphospecies collected was estimated to be $S = 118$, based on a taxonomic expert's assessment of the morphological characters traditionally used for octocoral taxonomy (Table 1, Suppl. S1, S2). Estimates of the number of MOTUs ranged from a low of 73 using *mtMutS* only (0.5 % threshold) to 128 using the combined mt + 28S barcode (0.1 % threshold) (Table 3). For most genera, the range of MOTUs estimated using different threshold values was equal to or bracketed the number of morphospecies (Table 1). Numbers of MOTUs slightly exceeded morphospecies estimates for *Sarcophyton* ($S = 9$, MOTU = 10–15) and *Melithaea* ($S = 4$, MOTU = 6), but underestimated numbers of morphospecies in *Lemnalia* ($S = 7$, MOTU = 4–5), *Dendronephthya* ($S = 6$, MOTU = 3–5), and *Siphonogorgia* ($S = 5$, MOTU = 3–4). There were also several genera for which up to three morphospecies were distinguished, but only a single MOTU was found, e.g., *Nephthea* and *Euplexaura*.

For all four barcodes, species richness accuracy (SA) was greatest (i.e., closest to 1.0) at an average genetic distance threshold of 0.1 % and declined when higher thresholds were used to define MOTUs (Table 3). In contrast, concordance of identifications was highest at a distance threshold of 0.3 % for all barcodes except mt. The difference in concordance between the 0.1 and 0.3 % thresholds was, however, less than 1 % for all barcodes; for all barcodes except 28S, concordance dropped by 2–8 % when a threshold of 0.5 % was used (Table 3).

Sinularia species identification

mtMutS sequences were obtained for 49 of 52 *Sinularia* specimens (ESM S2); *igr1* + *COI* and 28S sequences were obtained for 52 and 47 specimens, respectively. The greatest number of unique sequences was detected for *mtMutS* ($n = 29$), followed by 28S ($n = 24$), and *igr1* + *COI* ($n = 23$). Thirty-four unique barcode sequences were found among the 49 specimens for which two loci were sequenced, and 34 of the 45 specimens for which all three loci were sequenced had unique mt + 28S barcodes (Table 1).

The actual number of *Sinularia* species collected as determined by morphological criteria was $S = 26$. The mt and combined mt + 28S barcodes yielded the highest species richness accuracy; the number of MOTUs estimated using the mt barcode (0.1 % threshold) was 25 (SA = 0.961) and using mt + 28S (0.1 % threshold) it was 27 (SA = 1.039). Concordance was, however, higher with mt (76.9 %) than with mt + 28S (73.1 %). Consistent with the results for all octocoral species, *mtMutS* alone (0.1 % threshold) yielded the highest concordance (77.6 %) for *Sinularia*, but a lower species richness accuracy (21 MOTUs; SA = 0.808) than either multilocus barcode.

Forty-two of 52 *Sinularia* specimens (81 %) were identified correctly to species based on matching *mtMutS* haplotypes to species in the reference database, and, in cases where molecular identifications were ambiguous (i.e., no direct match or direct matches to >1 species), by comparisons of colony morphology as judged from in situ

photographs (ESM S2). Five specimens with combinations of *mtMutS* haplotypes and colony morphologies that did not match any species in our reference set could also not be identified based on sclerite morphology and may represent undescribed species. Three additional specimens matched sequences in the reference set but were identified incorrectly because the reference taxon had been misidentified. Two specimens were simply identified incorrectly: one specimen of *S. tumulosa* was misidentified as *S. babeldaobensis* and a specimen of *S. humilis* was left unidentified. Both of these specimens had unique *mtMutS* haplotypes and belonged to clades within which a number of species share identical or very similar haplotypes. If we consider the five specimens that could not be matched genetically and also could not be identified morphologically to represent correct identifications of undescribed species, our success rate at identifying *Sinularia* species using a combination of *mtMutS* haplotypes and in situ colony morphology was 50/52 (96 %). Among the morphospecies we identified correctly using the *mtMutS* barcode were three that had not previously been recorded from Palau (*S. humesi*, *S. polydactyla*, *S. verseveldti*).

Discussion

Our estimates of total species richness using MOTUs defined by average genetic distances were very similar to the estimates made by a taxonomist based on assessment of morphological characters. The number of species detected by both methods also agreed well with previous estimates of octocoral species richness in Palau (Fabricius et al. 2007; Table 1). This result suggests that total numbers of species present among a sample of octocorals can be estimated reasonably well using molecular proxies (MOTUs), without the need to discriminate species morphologically. The molecular methods employed require no taxonomic expertise, and their use could greatly accelerate comparisons of biodiversity over space and time, information that we currently lack for octocoral populations in most parts of the world. Although the haphazard sampling method we employed was not intended to estimate either the total species richness of octocorals in Palau or relative abundance-based measures of species diversity (e.g., Plaisance et al. 2011), our results suggest that molecular methods of species discrimination would facilitate such studies.

Although both molecular and morphological methods yielded comparable estimates of the total number of species present in our sample, identification of specimens to species level remains a much more complex problem in octocorals. The concordance between molecular and morphospecies identifications was 70–75 %, similar to the identification success rate that was estimated in a previous survey of octocoral biodiversity in the Red Sea (McFadden et al. 2011). Our understanding of species boundaries in Octocorallia, however, is still very poor for the majority of genera recorded in the Indo-Pacific (Fabricius and Alderslade 2001). Many species remain undescribed, and taxonomic revisions of most genera will be required before species names can reliably be assigned to specimens. For this reason, it is in most cases impossible to know whether an observed lack of concordance between a MOTU and morphospecies assignment is due to a failure of the molecular barcode to discriminate valid species or to flawed taxonomic interpretations of morphological variation. Few studies of intraspecific morphological variation or environmental plasticity exist for octocorals (West et al. 1993; West 1997; Skoufas 2006), and only a handful have examined both genetic and morphological variation (Kim et al. 2004; Prada et al. 2008; Gutiérrez-Rodríguez et al. 2009; Bilewitch et al. 2010). In some cases, genetic differentiation has been concordant with observed morphological variation, suggesting that distinct ecotypes represent different species (Prada et al. 2008). In other studies, observed morphological differences among populations have not correlated with genetic differences, implying that colony growth forms or sclerites may vary within species (Gutiérrez-Rodríguez et al. 2009; Bilewitch et al. 2010). Determining whether morphological variants have been inappropriately lumped as a single species (e.g., Prada et al. 2008) or inappropriately split into separate species (e.g., Bilewitch et al. 2010) requires time-consuming case-by-case studies. Approaches such as the one we have taken here, however, offer a first step toward identifying taxa that exhibit a lack of concordance between morphological species concepts and MOTUs.

For some of the genera we collected in Palau, the observed lack of concordance between molecular and morphospecies identifications can almost certainly be attributed to our lack of understanding of the taxonomic significance of morphological variation rather than to deficiencies in the molecular barcode used. Among the genera we sampled, the agreement between MOTU and morphospecies assignments was particularly poor for *Sarcophyton*, with only 45.7 % of specimens exhibiting concordance (*mtMutS*, 0.3 % threshold). Previous molecular phylogenetic work on this genus has demonstrated, however, that the commonly encountered species *Sarcophyton glaucum* represents a cryptic species complex of six or more phylogenetically distinct lineages that cannot reliably be distinguished morphologically (McFadden et al. 2006). Applying the most conservative barcode and genetic distance threshold we used for species identification (mt, 0.5 % threshold; Table 3), the 19 specimens in our collection that were identified morphologically as *S. glaucum* belonged to 5 different MOTUs. In this case, the number of

MOTUs is undoubtedly a more realistic estimate of species richness than the morphospecies identification. Likewise, we collected two distinct growth forms of *Sinularia brassica* that were once classified as different species, but have subsequently been synonymized based on the similarity of their sclerites (Benayahu et al. 1998). At distance thresholds <0.5 % all barcodes divided these two morphotypes among two different MOTUs, each concordant with a different colony growth form. This result suggests that the original taxonomic interpretation of the different growth forms as two different species is likely to be correct.

In the two examples cited above, the number of MOTUs exceeded the estimated number of morphospecies, suggesting the existence of morphologically cryptic species. In other genera we collected, however, the estimated number of morphospecies exceeded the number of MOTUs observed. For example, three morphospecies of *Euplexaura* were distinguished, but all specimens of that genus had identical barcode sequences. In this poorly known genus, we are currently unable to interpret the discrepancies between morphospecies classification and MOTUs. Are the barcode sequences employed in our study insufficiently variable to detect interspecific differences in *Euplexaura* and other genera, or is our interpretation of morphological variation incorrect? As a first step to answer such questions, we require larger sample sizes of barcoded specimens collected across a range of ecological situations and geographic locations in order to determine whether different morphotypes are (1) discrete or continuous, and (2) associated with any environmental parameters (e.g., depth, water movement, light). In short, integrated taxonomic approaches are needed to further test species boundaries in groups in which morphological differentiation is not accompanied by genetic differences in the barcode markers. Reciprocal transplantation experiments can also shed light on the genetic versus environmental control of morphology when ecotypic morphological variation is observed (e.g., Prada et al. 2008).

Barcoding and taxonomic revision

Although the primary purpose of this study was to estimate species richness rather than to explore phylogenetic relationships among taxa, sequence data obtained for the former purpose may also generate insights into the latter, motivating and informing subsequent taxonomic revisions. The results of our barcoding efforts in Palau highlight several groups within which observed phylogenetic relationships among taxa conflict with morphological definitions of genera. In three cases, morphospecies considered to belong to different genera nonetheless shared identical multilocus barcode sequences. Ellisellid specimens identified as *Dichotella gemmacea* and *Junceella juncea* were genetically identical. The similarity between these two species has been noted previously (Grasshoff 1999); they share identical sclerite forms, and the genera are distinguished by colony growth form alone (dichotomously branched vs. unbranched). Likewise, specimens of the gorgoniid genera *Rumphella* and *Hicksonella* shared identical barcodes. The primary morphological distinction between these two genera is the presence in *Hicksonella* of small numbers of long rod-like sclerites in the coenenchyme, a variable character of questionable value for generic distinction (Alderslade 1986). Although the majority of morphospecies belonging to the nephtheid genera *Lemnalia* and *Paralemnalia* were well separated, a specimen identified as *Paralemnalia digitiformis* was genetically identical to three specimens of *Lemnalia*. The generic affiliation of *P. digitiformis* has also been questioned previously and its similarity to certain species of *Lemnalia* noted (Fabricius and Alderslade 2001). Finally, although no morphospecies of *Chironephthya* shared an identical barcode sequence with a morphospecies of *Siphonogorgia*, these two nidaliid genera exhibited a paraphyletic relationship, with no evidence of a clear phylogenetic distinction between them (Fig. 2). Moreover, at average distance thresholds >0.1 %, one MOTU included two specimens identified as *Siphonogorgia* cf. *godeffroyi* plus two specimens of *Chironephthya*. These two genera, distinguished primarily by differences in the structure and prominence of the calyces, have been widely confused in the literature (Fabricius and Alderslade 2001), and are clearly in need of revision. In each of the four cases illustrated above, the molecular data obtained in the course of this biodiversity survey provide insights into the phylogenetic relationships among genera and a springboard for future revisionary work.

Molecular barcodes for octocorals

Slow rates of mitochondrial gene evolution (Shearer et al. 2002; Huang et al. 2008) have hindered the search for molecular barcodes that reliably discriminate species of octocorals and other anthozoan cnidarians. Testing of candidate barcodes for octocorals has been further confounded by the uncertainty of our definitions of species boundaries in most groups, as discussed above. Previous tests comparing the mitochondrial protein-coding genes *COI* and *mtMutS* as barcodes concluded that species discrimination within the genus *Alcyonium*—one of the few octocoral genera in which species boundaries have been assessed using the criterion of reproductive isolation—was highest using a combined mt barcode of *mtMutS* + *igr1* + *COI* (McFadden et al. 2011). In the present study, use of 28S rDNA or the three-locus combination of 28S + mt yielded slightly higher species richness accuracy among the *Alcyonium* species than either mt or *mtMutS* alone (Table 2). The expense and time required to barcode specimens increases with the number of

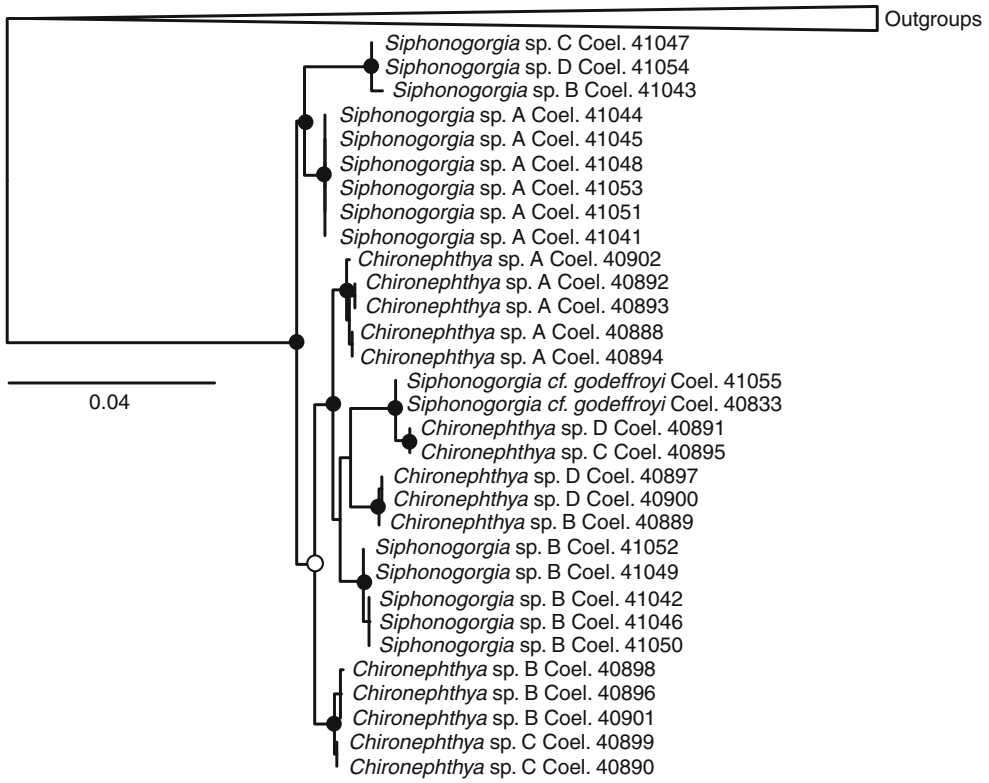

Fig. 2 Maximum likelihood phylogenetic reconstruction of species of *Chironephthya* and *Siphonogorgia* from Palau [partitioned analysis of concatenated mt + 28S barcode alignment with 100 bootstrap replicates run using Garli 2.0 (Zwickl 2006)]. *Solid circles* on nodes: bootstrap support >90 %; *open circles* bootstrap support >70 %. Outgroup includes genera *Nidalia*, *Sinularia*, *Sarcophyton* and *Lobophytum*

different loci included in a barcode, however, making a single-locus barcode a more economical option if relatively little is to be gained by sequencing additional loci. In our Palau biodiversity survey results, species richness accuracy (the agreement between numbers of species estimated using MOTUs vs. morphospecies concepts) varied considerably (from 0.576 to 1.085) depending on the barcode and genetic distance threshold employed, but was generally higher when 28S was used, either alone or in combination with the mt barcode. Concordance between MOTUs and morphospecies identifications, however, varied relatively little among barcodes (71–75 % at 0.1 and 0.3 % thresholds), and the highest concordance was obtained using a single-locus barcode (*mtMutS*). A high percentage of *Sinularia* species could also be identified to species correctly using just *mtMutS* sequences and gross colony morphology. Until we gain a better understanding of species boundaries in particular groups of octocorals, a single-locus barcode is likely to be adequate for comparative biodiversity studies that encompass a wide range of taxa. Among the three barcode loci examined here, *mtMutS* has been the most widely sequenced to date and has the most comparative data available across a wide range of octocoral taxa (McFadden et al. 2010).

Tests with *Alcyonium* have indicated that character-based methods of species discrimination yield higher rates of species identification than distance-based methods (Table 1). For example, using the combined mt + 28S barcode, character-based methods discriminated 9 MOTUs among the 10 species of *Alcyonium*; only one pair of species could not be distinguished. In contrast, distance-based methods only discriminated 6 MOTUs, lumping three species each into two MOTUs and two into another. Character-based (diagnostic) methods have been shown to outperform distance-based methods when a barcoding gap between intraspecific and interspecific divergence is absent (Kelly et al. 2007; Reid et al. 2011; van Velzen et al. 2012). The application of such methods, however, requires a priori knowledge of intraspecific sequence variation that can only be assessed by sequencing multiple individuals that have been assigned with confidence to the same species. At this time, we have not adequately sampled intraspecific variation in enough species to be able to apply this approach to octocorals and are instead constrained to define MOTUs based on genetic distance thresholds. Once a sufficient database of vouchered barcode sequences exists for octocorals, however, it may be possible to use character-based methods to

assign unknown specimens to known morphospecies, with the attendant prospect of increased identification rates.

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References

Alderslade P (1986) An unusual leaf-like gorgonian (Coelenterata: Octocorallia) from the Great Barrier Reef, Australia. Beagle 3:81–93

Amaral-Zettler L, Artigas LF, Baross J, Bharathi L, Boetius A, Chandromohan D, Herndl G, Kogure K, Neal P, Pedrós-Alió C, Ramette A, Schouten S, Stal L, Thessen A, de Leeuw J, Sogin M (2010) A global census of marine microbes. In: McIntyre AD (ed) Life in the world's oceans: Diversity, distribution and abundance. Blackwell Publishing, Oxford, pp 223–245

Bayer FM (1981) Status of knowledge of octocorals of world seas. Seminarios de Biologia Marinha, Academia Brasileira de Ciencias Rio de Janeiro 1981:3–11

Benayahu Y, Loya Y (1981) Competition for space among coral reef sessile organisms at Eilat, Red Sea. Bull Mar Sci 31:514–522

Benayahu Y, van Ofwegen LP, Alderslade P (1998) A case study of variation in two nominal species of *Sinularia* ((Coelenterata:octocorallia), *S. brassica* (May, 1898), and *S. dura* (Pratt, 1903), with a proposal for their synonymy. Zool Verh (Leiden) 323:1–30

Bilewitch JP, Coates KA, Currie DC, Trapido-Rosenthal HG (2010) Molecular and morphological variation supports monotypy of the octocoral *Briareum* Blainville, 1830 (Octocorallia, Alcyonacea) in the Western Atlantic. Proc Biol Soc Wash 123:93–112

Bouchet P (2006) The magnitude of marine biodiversity. In: Duarte CM (ed) The exploration of marine biodiversity: scientific and technological challenges. Fundacion BBVA, Bilbao, Spain, pp 31–64

Bridge TCL, Fabricius KE, Bongaerts P, Wallace CC, Muir PR, Done TJ, Webster JM (2012) Diversity of Scleractinia and Octocorallia in the mesophotic zone of the Great Barrier Reef, Australia. Coral Reefs 31:179–189

Bruno JF, Siddon CE, Witman JD, Colin PL, Toscano MA (2001) El Nino related coral bleaching in Palau, Western Caroline Islands. Coral Reefs 20:127–136

Buhl-Mortensen L, Vanreusel A, Gooday AJ, Levin LA, Priede IG, Buhl-Mortensen P, Gheerardyn H, King NJ, Raes M (2010) Biological structures as a source of habitat heterogeneity and biodiversity on the deep ocean margins. Mar Ecol 31:21–50

Cairns S (2007) Deep-water corals: an overview with special reference to diversity and distribution of deep-water scleractinian corals. Bull Mar Sci 81:311–322

Chanmethakul T, Chansang H, Watanasit S (2010) Soft coral (Cnidaria: Alcyonacea) distribution patterns in Thai waters. Zool Stud 49:72–84

Chen I-P, Tang C-Y, Chiou C-Y, Hsa J-H, Wei NW, Wallace CC, Muir P, Wu H, Chen CA (2009) Comparative analyses of coding and non-coding DNA regions indicate that *Acropora* (Anthozoa: Scleractinia) possesses a similar evolutionary tempo of nuclear vs. mitochondrial genomes as in plants. J Mar Biotech 11:141–152

Cornish AS, DiDonato EM (2004) Resurvey of a reef flat in American Samoa after 85 years reveals devastation to a soft coral (Alcyonacea) community. Mar Pollut Bull 48:768–777

Costello MJ, Wilson S, Houlding B (2012) Predicting total global species richness using rates of species description and estimates of taxonomic effort. Syst Biol 61:871–883

Daly M, Brugler MR, Cartwright P, Collins AG, Dawson MN, Fautin DG, France SC, McFadden CS, Opresko DM, Rodriguez E, Romano S, Stake J (2007) The phylum Cnidaria: a review of phylogenetic patterns and diversity 300 years after Linnaeus. In: Zhang Z-Q, Shear WA (eds) Linnaeus Tercentenary: progress in invertebrate taxonomy. Zootaxa 1668: 1–766

Dinesen ZD (1983) Patterns in the distribution of soft corals across the central Great Barrier Reef. Coral Reefs 1:229–236

Fabricius KE (1995) Slow population turnover in the soft coral genera *Sinularia* and *Sarcophyton* on mid- and outer-shelf reefs of the Great Barrier Reef. Mar Ecol Prog Ser 126:145–152

Fabricius KE (1997) Soft coral abundance on the central Great Barrier Reef: effects of *Acanthaster planci*, space availability, and aspects of the environment. Coral Reefs 16:159–167

Fabricius KE (1998) Reef invasions by soft corals: which taxa and which habitats? In: Greenwood JG, Hall NJ (eds) Proceedings of the Australian coral reef society 75th anniversary conference, Heron Island October 1997. The University of Queensland, Brisbane, School of Marine Science, pp 77–90

Fabricius KE (1999) Tissue loss and mortality in soft corals following mass-bleaching. Coral Reefs 18:54

Fabricius K, Alderslade P (2001) Soft corals and sea fans: a comprehensive guide to the tropical shallow-water genera of the Central West-Pacific, the Indian Ocean and the Red Sea. Australian Institute of Marine Science, Townsville

Fabricius KE, De'ath G (2008) Photosynthetic symbionts and energy supply determine octocoral biodiversity in coral reefs. Ecology 89:3163–3173

Fabricius KE, Alderslade P, Williams GC, Colin PL, Golbuu Y (2007) Octocorallia in Palau, Micronesia: effects of biogeography and coastal influences on local and regional biodiversity. In: Kayanne H, Omori M, Fabricius K, Verheij E, Colin P, Golbuu Y, Yurihira H (eds) Coral reefs of Palau. Palau International Coral Reef Centre, Palau, pp 79–92

Felsenstein J (2005) PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle

Floyd R, Abebe E, Papert A, Blaxter M (2002) Molecular barcodes for soil nematode identification. Mol Ecol 11:839–850

Fox HE, Pet JS, Dahuri R, Caldwell RL (2003) Recovery in rubble fields: Long-term impacts of blast fishing. Mar Pollut Bull 46:1024–1031

Grasshoff M (1999) The shallow water gorgonians of New Caledonia and adjacent islands. Senckenb Biol 78:1–121

Gutiérrez-Rodríguez C, Barbeitos MS, Sánchez JA, Lasker HR (2009) Phylogeography and morphological variation of the branching octocoral *Pseudopterogorgia elisabethae*. Mol Phylogenet Evol 50:1–15

Hellberg ME (2006) No variation and low synonymous substitution rates in coral mtDNA despite high nuclear variation. BMC Evol Biol 6:24

Huang D, Meier R, Todd PA, Chou LM (2008) Slow mitochondrial COI sequence evolution at the base of the metazoan tree and its implications for DNA barcoding. J Mol Evol 66:167–174

Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Briefings in Bioinformatics 9:286–298

Kelly RP, Sarkar IN, Eernisse DJ, DeSalle R (2007) DNA barcoding using chitons (genus *Mopalia*). Mol Ecol Notes 7:177–183

Keshavmurthy S, Yang S-Y, Alamaru A, Chuang Y–Y, Pichon M, Obura D, Fontana S, De Palmas S, Stefani F, Benzoni F, MacDonald A, Noreen AME, Chen C, Wallace CC, Moothein Pillay R, Denis V, Yang Amri A, Reimer JD, Mezaki T, Sheppard C, Loya Y, Abelson A, Suleiman Mohammed M, Baker AC, Ghavam Mostafavi P, Suharsono BA, Chen CA (2013) DNA barcoding reveals the coral "laboratory-rat", *Stylophora pistillata* encompasses multiple identities. Sci Rep 3: 1520 [doi:10.1038/srep01520]

Kim E, Lasker HR, Coffroth MA, Kim K (2004) Morphological and genetic variation across reef habitats in a broadcast-spawning octocoral. Hydrobiologia 530(531):423–432

Knowlton N, Brainard RE, Fisher R, Moews M, Plaisance L, Caley MJ (2010) Coral reef biodiversity. In: McIntyre AD (ed) Life in the world's oceans: Diversity, distribution and abundance. Blackwell Publishing, Oxford, pp 65–77

Krieger KJ, Wing BL (2002) Megafauna associations with deepwater corals (*Primnoa* spp.) in the Gulf of Alaska. Hydrobiologia 471:83–90

Loya Y, Sakai K, Yamazato K, Nakano Y, Sambali H, van Woesik R (2001) Coral bleaching: the winners and the losers. Ecol Lett 4:122–131

McFadden CS (1999) Genetic and taxonomic relationships among Northeastern Atlantic and Mediterranean populations of the soft coral *Alcyonium coralloides*. Mar Biol 133:171–184

McFadden CS, Hutchinson MB (2004) Molecular evidence for the hybrid origin of species in the soft coral genus *Alcyonium* (Cnidaria: Anthozoa: Octocorallia). Mol Ecol 13:1495–1505

McFadden CS, van Ofwegen LP (2013) A second, cryptic species of the soft coral genus *Incrustatus* (Anthozoa: Octocorallia: Clavulariidae) from Tierra del Fuego, Argentina revealed by DNA barcoding. Helgol Mar Res 67:137–147

McFadden CS, Sánchez JA, France SC (2010) Molecular phylogenetic insights into the evolution of Octocorallia: a review. Integr Comp Biol 50:389–410

McFadden CS, Donahue RM, Hadland BK, Weston R (2001) A molecular phylogenetic analysis of reproductive trait evolution in the soft coral genus *Alcyonium*. Evolution 55:29–42

McFadden CS, Alderslade P, van Ofwegen LP, Johnsen H, Rusmevichientong A (2006) Phylogenetic relationships within the tropical soft coral genera *Sarcophyton* and *Lobophytum* (Anthozoa, Octocorallia). Invertebr Biol 125:288–305

McFadden CS, van Ofwegen LP, Beckman EJ, Benayahu Y, Alderslade P (2009) Molecular systematics of the speciose Indo-Pacific soft coral genus, *Sinularia* (Anthozoa: Octocorallia). Invertebr Biol 128:303–323

McFadden CS, Benayahu Y, Pante E, Thoma JN, Nevarez PA, France SC (2011) Limitations of mitochondrial gene barcoding in Octocorallia. Mol Ecol Res 11:19–31

Meyer CP, Paulay G (2005) DNA barcoding: error rates based on comprehensive sampling. PLoS Biol 3(12): e422

Mora C, Tittensor DP, Adl S, Simpson AGB, Worm B (2011) How many species are there on earth and in the ocean? PLoS Biol 9(8):e1001127

Plaisance L, Knowlton N, Paulay G, Meyer C (2009) Reef-associated crustacean fauna: biodiversity estimates using semi-quantitative sampling and DNA barcoding. Coral Reefs 28:977–986

Plaisance L, Caley MJ, Brainard RE, Knowlton N (2011) The diversity of coral reefs: what are we missing? PLoS One 6:e25026

Prada C, Schizas NV, Yoshioka PM (2008) Phenotypic plasticity or speciation? A case from a clonal marine organism. BMC Evol Biol 8:47

Prada C, Weil E, Yoshioka PM (2010) Octocoral bleaching during unusual thermal stress. Coral Reefs 29:41–45

Rach J, DeSalle R, Sarkar IN, Schierwater B, Hadrys H (2008) Character-based DNA barcoding allows discrimination of genera, species and populations in Odonata. Proc R Soc Biol Sci Ser B 275:237–247

Reaka-Kundla M (1997) The global biodiversity of coral reefs: a comparison with rain forests. In: Reaka-Kudla M, Wilson DE, Wilson EO (eds) Biodiversity II: Understanding and protecting our biological resources. Joseph Henry Press, Washington DC, pp 83–108

Reid BN, Le M, McCord WP, Iverson JB, Georges A, Bergmann T, Amato G, DeSalle R, Naro-Maciel E (2011) Comparing and combining distance-based and character-based approaches for barcoding turtles. Mol Ecol Res 11:956–967

Ruzicka R, Colella M, Semon K, Brinkhuis V, Morrison J, Kidney J, Porter J, Meyers M, Christman M, Colee J. (2010) CREMP 2009 Final Report. Fish & Wildlife Research Institute/Florida Fish & Wildlife Conservation Commission, Saint Petersburg, FL, p 110

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF (2009) Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol 75:7537–7541

Shearer TL, Coffroth MA (2008) Barcoding corals: limited by interspecific divergence not intraspecific variation. Mol Ecol Res 8:247–255

Shearer TL, Van Oppen MJH, Romano SL, Wörheide G (2002) Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Mol Ecol 11:2475–2487

Skoufas G (2006) Comparative biometry of *Eunicella singularis* (Gorgonian) sclerites at East Mediterranean Sea (North Aegean Sea, Greece). Mar Biol 149:1365–1370

Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR, Arrieta JM, Herndl GJ (2006) Microbial diversity in the deep sea and the underexplored "rare biosphere". Proc Natl Acad Sci USA 103:12115–12120

Stobart B, Teleki K, Buckley R, Downing N, Callow M (2005) Coral recovery at Aldabra Atoll, Seychelles: five years after the 1998 bleaching event. Philos Trans R Soc A 363:251–255

Stone RP (2006) Coral habitat in the Aleutian Islands of Alaska: depth distribution, fine-scale species associations, and fisheries interactions. Coral Reefs 25:229–238

Tilot V, Leujak W, Ormond RFG, Ashworth JA, Mabrouk A (2008) Monitoring of South Sinai coral reefs: influence of natural and anthropogenic factors. Aquatic Conserv: Mar Freshw Ecosyst 18:1109–1126

Tursch B, Tursch A (1982) The soft coral community on a sheltered reef quadrat at Laing Island (Papua New Guinea). Mar Biol 68:321–332

van Ofwegen LP (2008) The genus *Sinularia* (Octocorallia: Alcyonacea) at Palau, Micronesia. Zool Med (Leiden) 82:631–735

Van Oppen MJH, Mieog JC, Sanchez CA, Fabricius KE (2005) Diversity of algal symbionts (zooxanthellae) in octocorals: the roles of geography and host relationships. Mol Ecol 14: 2403–2417

van Velzen R, Weitschek E, Felici G, Bakker FT (2012) DNA barcoding of recently diverged species: relative performance of matching methods. PLoS One 7:e300490

West JM (1997) Plasticity in the sclerites of a gorgonian coral: tests of water motion, light level, and damage cues. Biol Bull 192: 279–289

West JM, Harvell CD, Walls A-M (1993) Morphological plasticity in a gorgonian coral (*Briareum asbestinum*) over a depth cline. Mar Ecol Prog Ser 94:61–69

Williams GC, Cairns SC (2013) Systematic list of valid octocoral genera. Octocoral Research Center, California Academy of Science. http://research.calacademy.org/redirect?url=http://researcharchive.calacademy.org/research/izg/orc_home.html

Zwickl DJ (2006) Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. thesis, University of Texas, Austin