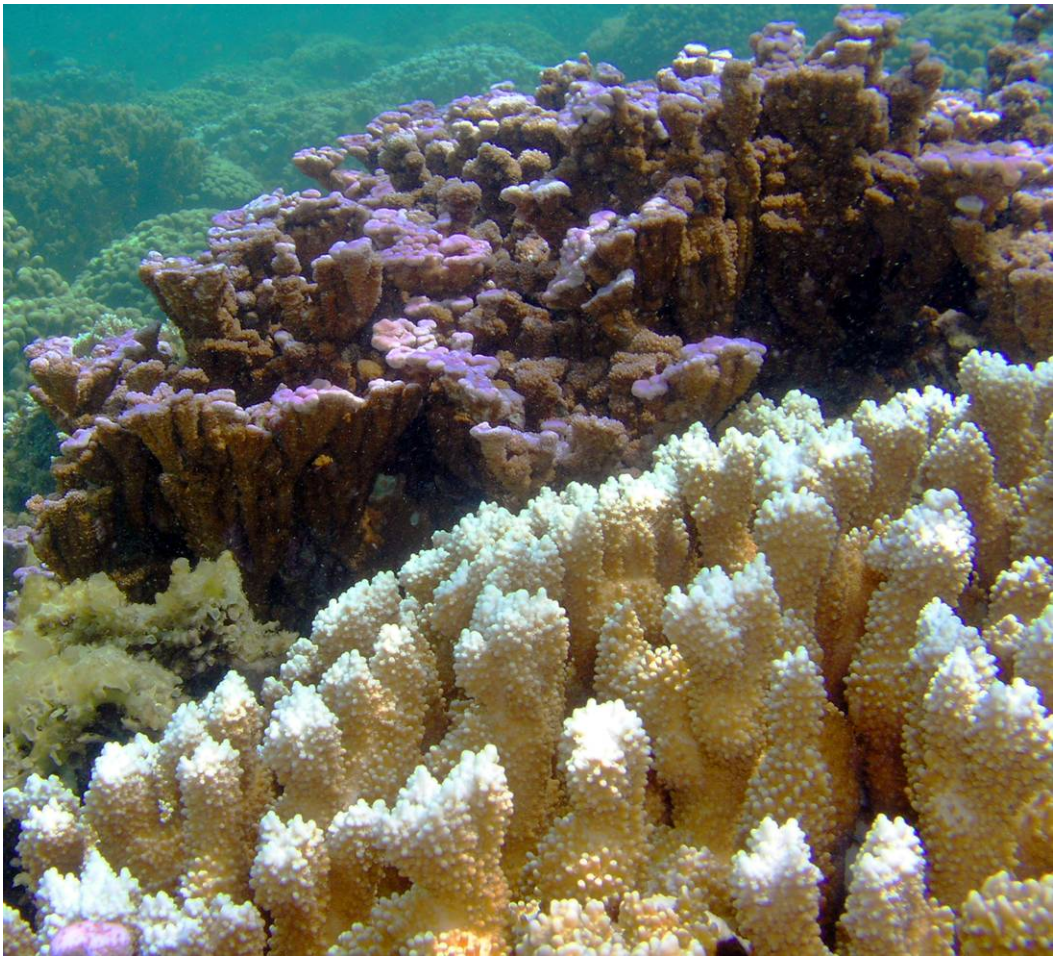
Is *Montipora dilatata* an endangered coral species or an ecotype? Genes and skeletal microstructure lump seven Hawaiian species into four groups



Z.H.Forsman[1], G.T.Concepcion[1], R.D.Haverkort[1], R.W.Shaw[3], J.E.Maragos[2], and R.J.Toonen[1]

[1] *Hawaii Institute of Marine Biology*
P.O. Box 1346, Kaneohe, HI 96744

[2] *Pacific/Remote Islands National Wildlife Refuge Complex*
U.S. Fish and Wildlife Service
300 Ala Moana Blvd., Rm 5-231, Box 50167
Honolulu, HI 96850

[3]*Grant MacEwan University*
P.O.Box 1796, Edmonton, AB T5J2P2 Canada

Image by J. E. Maragos. Foreground: M. capitata; above: M. dilatata; above, lower left: invasive algae (Kappaphycus/Eucheuma spp.)

Executive summary

Montipora dilatata is considered to be one of the rarest corals known. Thought to be endemic to Hawaii, only a few colonies have ever been found despite extensive surveys. Endangered species status would have major conservation implications; however, coral species boundaries are poorly understood. In order to examine genetic and morphological variation in Hawaiian *Montipora*, a suite of molecular markers (mitochondrial: COI, CR, Cyt-B, 16S, ATP6; nuclear: ATPsβ, ITS), in addition to a suite of measurements on skeletal microstructure, were examined. The ITS region and mitochondrial markers revealed four distinct clades: I) *M. patula/M. verilli*, II) *M. incrassata*, III) *M. capitata, IV) M. dilatata/M. flabellata/M. turgescens*. The nuclear ATPsβ intron tree had several exceptions that are generally interpreted as resulting from recent hybridization between clades or incomplete lineage sorting. Since the multicopy nuclear ITS region was concordant with the mitochondrial data, incomplete lineage sorting of the ATPsβ intron is a more likely explanation. Principal components analysis (PCA) of microstructure measurements agreed with the genetic clades rather than the nominal taxa. These species groups therefore either represent recent or insipient (CA <1MYA) species or morphological variants of the same biological species. These clades are likely to occur outside of Hawaii according to mitochondrial control region haplotypes from previous studies. Common garden experiments were conducted on distinct morphotypes of *M. capitata* to test the hypothesis that micro-skeletal traits can be phenotypically plastic in this genus. Although the experiment suffered high mortality from parasitic flatworms, verrucae (rice-grain sized bumps) were documented to form on formerly smooth colonies, indicating plasticity. This study contributes towards understanding the relationship between genetic and morphological variation in this taxonomically challenging group, which is essential for effective conservation and the key to understanding the evolution and biodiversity of reef building corals.

Management implications

This study identified two closely related species complexes (*M. dilatata/M. flabellata/M. turgescens* and *M. patula/M. verilli*) that may occur outside of Hawaii. We found no significant genetic or micro-skeletal differences between nominal taxa within these species complexes; however, this study may not be capable of detecting recent or insipient species (CA <1MYA). Now that these species complexes have been identified, population genetic and/or reproductive studies are necessary to determine if the nominal species within each complex are actively interbreeding. The findings of rapid evolution and plasticity of traits used for species identification challenges the reliability of traditional taxonomy, especially regarding gross colony-level skeletal morphology.

Introduction

Montipora dilatata is thought to be one of the rarest corals known. It has only been found in Kāneʻohe Bay, Oʻahu, and tentatively on Maro reef (*M. c.f. dilatata*) in the Northwestern Hawaiian Islands (NOAA 2007; Fenner 2005). In 2000, extensive surveys identified only three colonies of *M. dilatata* in Kāneʻohe Bay, where previously it was more abundant (NOAA 2007). The decline of this species in Kāneʻohe Bay has been attributed to high sensitivity to coral bleaching, in addition to other general threats (freshwater kills, sedimentation/habitat degradation, overgrowth by alien algae, and anchor/boat damage) that may impact a small population with a limited geographic distribution (NOAA 2007). *Montipora*, like many coral genera, has a high degree of morphological variation that can make confident identification problematic. There are some morphotypes of *M. dilatata* that are clearly distinct from other congeners; however, there are also intermediate morphotypes with some similarities to *M. capitata* (Jim Maragos pers.comm) or *M. flabellata* (Cynthia Hunter pers.comm).

Montipora taxonomy (as with all reef building coral) is based on skeletal morphology, which recent studies have shown can be remarkably variable, with surprising examples of convergent evolution (Fukami *et al.* 2004; Fukami *et al.* 2008), rapid evolution and homoplasy (Forsman *et al.* 2009), and phenotypic plasticity (Bruno & Edmunds 1997; Todd *et al.* 2008). Adding to taxonomic confusion, the conceptual nature of the coral species is a subject of debate, particularly regarding the permeability of reproductive boundaries between morphospecies, and the evolutionary significance and prevalence of interspecific hybridization (e.g., Veron 1995, 2000; Miller & van Oppen 2003; van Oppen *et al*. 2004; Volmer & Palumbi 2002). *Montipora* species are classified by arrangement and size of protrusions between corallites (e.g., "papillae" are smaller than corallites while "verrucae" are larger) and by colony form (laminar, encrusting, massive, and branching) which can often vary within an individual colony. Because colony form, size, and arrangement of microskeletal characters are rarely discrete, taxonomists frequently disagree about the number and names of *Montipora* species that occur in a geographic region, with all authors disagreeing on names and ranges of some Hawaiian species (Maragos 1977; Veron 2000; Fenner 2005).

The confusing range of morphological variation observed in coral is widely thought to be primarily due to widespread interspecific hybridization, with some clear examples based on molecular and reproductive studies (e.g., Willis et al. 1997; Wallace and Willis 1994; Miller and Babcock 1997; Szmant et al. 1997; Hatta *et al*. 1999; Volmer & Palumbi 2002). *Montipora* from Indonesia and the Great Barrier Reef were examined by van Oppen *et al*. (2004) with the putative mitochondrial control region (hereafter referred to as CR), and the Pax-C intron. Although the mitochondrial genes resolved clear clades, several morphological species shared identical haplotypes and could not be separated, while some morphological species were clearly not monophyletic. The Pax-C intron data was generally congruent with the mitochondrial trees, with some exceptions which were interpreted to be evidence of past introgression of nuclear genes from hybridization; however, contrasting rates of lineage sorting could not be ruled out. Recent studies from other coral families provide examples of alternative interpretations of disagreement between morphology and genetics and discordance between genes. For example, Forsman *et al*. 2009 also observed discordance between genes and morphology in *Porites* (some morphological species shared identical haplotypes, while others were not monophyletic); however, because of strong congruence between mitochondrial (COI, CR) and nuclear (ITS) markers, there was an alternative explanation: rapid evolution and possible intraspecific variation (phenotypic polymorphism) of gross colony-level skeletal morphology. Likewise, Flot *et al.* 2008 examined two mitochondrial and four nuclear markers on five morphospecies of Hawaiian *Pocillopora*, and each gene showed varying levels of concordance with classification based on morphology: mitochondrial genes resolved four morphospecies, ITS-2 resolved two, while single copy nuclear genes (calmodulin, EF-1α, ATPs β) had divergent allelic copies that

failed to resolve any groups. This pattern was more consistent with variable rates of lineage sorting among markers, than hybridization between mitochondrial lineages followed by introgression by nuclear genes.

There are many clear examples of hybridization in reef building corals, but it is not yet clear how much hybridization accounts for the observed patterns of molecular or morphological variation. Furthermore, it is technically and conceptually difficult to distinguish hybridization from intraspecific population-level variation, especially if there are more than three species involved. The majority of studies on closely related coral taxa have had difficulty resolving closely related taxa, in part because mitochondrial markers evolve unusually slowly in Anthozoa (van Oppen *et al.* 1999; Shearer *et al.* 2002; Hellberg 2006), and in part because the scale of morphological and molecular genetic variation that corresponds to species as opposed to populations is not understood.

This study examines genetic and morphological variation in the genus *Montipora* in Hawaii, with a focus on *Montipora dilatata*. The specific goals were: 1) to develop a suite of molecular markers for genetic analysis of *Montipora*, 2) to identify the closest relatives of *M. dilatata*, and 3) compare morphological variation of microskeletal features with patterns of genetic variation in Hawaiian congeners. This study is an example of how knowledge of species boundaries in corals is not only necessary for understanding patterns of biodiversity and evolution, but is essential for conservation.

Methods

A set of 71 samples were examined for this study, representing 7 *Montipora* species from the Main and Northwestern Hawaiian Islands (Appendix 1). To ensure consistency, a leading coral taxonomist in the region (Dr. Jim Maragos) confirmed the identification of the samples to species level (Figure 1). DNA extractions were accomplished following coral DNA extraction protocols developed previously in our laboratory (Concepcion *et al.* 2006). Briefly, DNA was extracted from small pieces of coral tissue (5 mm^3) by digestion for 2–3 h in 200µL of DNAB (0.4m NaCl, 50 mm Na_2 EDTA pH 8.0) + 1% SDS+ 10µL proteinase K (10µg/mL) on a shaker at 55°C. An equal volume of 2X CTAB (cetyltrimethyl ammonium bromide) + 10µL/mL β -mercaptoethanol was then added, and the tube was vortexed before being incubated at 65°C for an additional 30–60 min. An equal volume of chilled chloroform was added prior to vortexing. The samples were then left on a rotating platform for 2–3 h. Finally, the supernatant was precipitated with 95% EtOH, pelleted by centrifugation, and subsequently washed with 70% EtOH. DNA was resuspended in 50µL deionized (dI) water before making 50 fold dilutions (approximate final concentration of ~5 ng/µL) in dI water for subsequent use as template for subsequent Polymerase Chain Reaction (PCR).

In order to examine genealogical concordance, multiple loci were examined. PCR primers were based on conserved portions of aligned sequences from the National Center for Biological Information's (NCBI) GenBank database, and designed with the aid of Primer 3 v 0.4.0 (Rozen & Skaletsky 2000), or based on previously published primers (Table 1). PCR reactions were performed on a Bio-Rad MyCycler thermal cycler. Each 25µL PCR contained 1µL of DNA template, 2.5µL of 10X ImmoBuffer, 0.1µL IMMOLASE DNA polymerase (Bioline), 3 mm MgCl2, 10 mm total dNTPs, 13 pmol of each primer, and dI water to volume. Hot-start PCR amplification conditions varied slightly depending on the primer set used, but was generally: 95°C for 10 min (1 cycle), 95°C for 30s, annealing temperature (2 degrees less than primer melting temperature, ranging between 50 and 60°C) for 30s, and 72°C for 60s (35 cycles) followed by a final extension at 72°C for 10 min (1 cycle). PCR products were visualized using 1.0% agarose gels (1X TAE) stained with Gelstar. For the ITS region, PCR products were ligated into the PgemT-EZ cloning vector (Promega Inc.) and transformed into JM109 competent cells following manufacturer's recommendations. After blue/white colony selection, colonies were screened for the correct sized insert by PCR with the M13 vector

primers and direct sequenced. PCR products for direct sequencing were treated with 2 U of exonuclease I and 2 U of shrimp alkaline phosphatase (Exo:SAP) using the following thermocycler profile: 37°C for 60 min, 80°C for 10 min. Treated PCR products were then cycle-sequenced using BigDye Terminators (PerkinElmer) run on an ABI-3130XL automated sequencer at the EPSCoR core genetics facility at HIMB. Resulting sequences were inspected and aligned using Geneious Pro 4.8.5 (Drummond *et al.* 2009) to implement either ClustalW (Tompson *et al.* 1994) or Muscle (Edgar 2004).

The majority of samples were examined with the mt CR and ATPs β; however, 17 samples were examined in greater detail with a suite of additional markers (Appendix 1). Some ATPs β sequences were heterozygous at a few nucleotide positions as indicated by clear double peaks. Phase (Stephens *et al.* 2001) as implemented in DNAsp 5.10.01 (Librado *et al*. 2009) was used to estimate the haplotypes for each heterozygous individual. Any samples for which mtDNA and nDNA disagreed were re-extracted, re-sequenced and/or cloned and sequenced. For all ITS and CR alignments, gaps were coded as present or absent using the "simple" gap coding method employed in GapCoder v1.0 (Young & Healy 2003). The nucleotide substitution model was selected in Modeltest V.3.7 (Posada & Crandall 1998), selected by the Akaike information criterion. All phylogenetic analyses were performed with Bayesian Inference (BI) and Maximum Likelihood (ML). BI trees were generated with Mr.Bayes 3.1.2 (Hulsenbeck & Ronquist 2001), with 1,100,000 generations and a burnin of 110,000 generations, and ML trees were generated from RaxML (Stamatakis *et al*. 2008). Skeletal fragments (2-3cm diameter) were mounted on stubs and sputter-coated with gold particles for imaging with a Hitachi S-800 Field Emission Scanning Electron Microscope operated at 15KV with a minimum resolution of 20 mm. Digital images were calibrated and measured in ImageJ V.1.40 (available at http://rsb.info.nih.gov/ij; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD). Nineteen morphological characters were measured from 130 images of 47 specimens. Thirty-one images (including all *M. incrassata* images) were excluded from the final analysis because they did not include measurable papillae, verrucae, bumps or ridges within the 20 mm field of view. Principal components analysis (PCA) and discriminant function analysis were performed in R (2.10.1) with the candisc package for canonical discriminate analysis and MANOVA.

Results & Discussion

Comparisons with the NCBI GenBank database confirmed that the correct gene region was amplified, with the highest sequence similarity to other *Montipora* sequences in the database. The Hawaiian CR sequences clustered into four strongly supported shallow clades, each separated from each other by three to seven fixed nucleotide differences (Figure 2). There were no differences in haplotypes within the clades, with the exception of M059, which differed by one base pair from other *M. capitata* samples. There were no differences between *M. dilatata*, *M. flabellata*, and *M. turgescens* (Figure 2, clade IV), or between *M. patula* and *M. verilli* (Figure 2, clade I). When compared to CR data from van Oppen (2004), clade I (*M. verrilli* and *M. patula*) shared identical haplotypes with species outside of Hawaii that also have papillae (*M. altasepta*, *M. hispida*, *M. peltiformis*, and *M. aequituberculata*) (Figure 3). Clade III (*M. capitata*) shared identical haplotypes with species from outside of Hawaii that also have large verrucae (*M. capitata*, *M. verrucosa*, and *M. danae*). Clade IV (*M. dilatata*/*flabellata*/*turgescens*) shared identical haplotypes with *M. turtlensis*, which tends to have irregular ridges (Figure 3). Although mitochondrial genes in corals evolve very slowly and may not be able to resolve species-level differences, it is interesting to note that these closely related genetic groups appear to have similar micro-morphology. It is also interesting to note that colony level morphology and color can be extraordinarily variable, for example clade III (*M. capitata*) contains colonies with thick branches, thin branches, plates, whirls, and hues of brown, red, orange and yellow.

Additional mitochondrial genes (ATP-6, COI, CYT-B, 16S) revealed no fixed differences corresponding to species within the clades (Figure 4). The tree resolved an additional clade nested within clade IV: clade IV' differs only by a single nucleotide position in ATP-6, which is not fixed between species (Figure 4). Unlike the CR tree, the concatenated mitochondrial data can be aligned and therefore rooted with an outgroup (*Acropora*) and can provide estimates for the divergence time between clades. Since Anthozoan mitochondrial genes evolve slowly, we have limited power to detect differences in recently diverged species. If we assume that mutations occur in a somewhat regular clock-like fashion, and that *Montipora* and *Acropora* diverged approximately 54 mya in accordance with the fossil record (Wells 1956), then the rate of mitochondrial evolution is approximately $0.0005 bp/10^6$ years (which also corresponds to the rate estimated for Anthozoan mitochondrial genes in Helberg 2006). Since approximately 3232 bp of mitochondrial DNA were surveyed, we would expect to find only 1.6 mutations for species that have been separated by one million years. Therefore, if *M. dilatata* is indeed a separate species, it has evolved fairly recently (e.g., within the last million years).

The ITS region tree (Figure 5) resolved the same four groups as the CR tree, and an additional clade (IV') similar to the ATP-6/COI/CYT-B/16S tree in Figure 4. The ITS region is a multi-copy marker with thousands of copies within a typical genome. Intragenomic variation was limited to occur within the mitochondrial clades (as indicated by colored lines in Figure 5). *M. dilatata* (M080a) and *M. flabellata* (M060e) share the same ITS sequence, which could be due to either hybridization or incomplete lineage sorting, but in either case it is not likely these species have been reproductively isolated over long evolutionary time scales. The ATPs β tree (Figure 6), however, contrasted sharply with the mitochondrial and ITS trees. Although the same clades are discernable, there are some individuals that occur in unexpected clades, and some heterozygous individuals that bridge across several clades (Figure 6). Since the ITS region tree is very similar to the mitochondrial trees it is unlikely that the discordance in the ATPs β is due solely to hybridization between mitochondrial lineages followed by nuclear introgression. Incomplete lineage sorting is a more likely explanation, especially when the following morphological results are taken into account.

A PCA analysis was conducted on the 19 measurements of micro-skeletal features (Table 2, Figure 7). Thirty-one images were excluded from the analysis (including all *M. incrassata* samples) because they did not contain measurable papillae, verrucae, or ridges (SEM resolution was too high to capture these features in all images). The morphological traits were highly variable (PC1 only accounted for 20.1 percent of the variation, and 12 principal components were necessary to encompass 90 percent of the variation). The size, shape, and density of papillae, verrucae, or ridges were important in distinguishing the genetic groups (Figure 7B). The morphological measurements strongly agreed with the genetic groups (MANOVA; Wilks test statistic; $p < 0.001$), but all of the species within the genetic groups showed a high degree of overlap and could not be distinguished (MANOVA; Willks test statistic; $p = 0.1912$; Table 3). In other words, according to these measurements *M. dilatata* could not be distinguished from M. *flabellata* or M. *turgescens*, and *M. patula* could not be distinguished from *M. verilli*. The micro-skeletal morphology agrees with the mitochondrial and ITS region dataset, which strengthens the case for incomplete lineage sorting as an explanation for discordance and incongruence with the ATPs β dataset (although hybridization can not be entirely ruled out). This interpretation is similar to Flot *et al*.'s (2008) conclusion that mitochondrial genes correspond well to morphological groups, followed by the ITS region, with little correspondence with single copy nuclear genes, which had considerable allelic diversity.

Although the size, shape and density of papillae, verrucae, or ridges are important in distinguishing the genetic groups, these traits were not always present to measure in some samples. In an effort to determine if these structures are phenotypically plastic, we conducted a common garden experiment to examine the effects of light and water motion on corals from

high and low flow environments. Unfortunately this experiment suffered from very high mortality from an infestation by a parasitic flatworm, and was inconclusive. Nevertheless, phenotypic plasticy of microskeletal traits was observed to occur on leftover fragments in a holding tank that were photographed through time (Figure 8). The parent colony was completely smooth, with no micro-skeletal features necessary for identification (Figure 8A). CR sequences indicated the colony was in clade III (*M. capitata*). The colony was collected from a turbid and shaded environment (leeward mangroves), and after five months in a holding tank it began to grow clear verrucae characteristic of *M. capitata* (Figure 8C). This observation indicates that verrucae can be phenotypically plastic (in this case present or absent depending on environmental conditions). It remains to be determined which environmental cue might initiate growth of verrucae; perhaps they form in response to water motion (creating small eddies in high water motion allowing greater feeding efficiency, whereas smooth colonies may shed sediment more easily in turbid environments). Micro skeletal traits are very important for identifying species in this genus, and this observation indicates that the absence of these traits alone is not a reliable diagnostic feature. Colony-level morphology is not evolutionarily conserved and varies wildly within the genetic groups. On the whole, these findings challenge the reliability of traditional taxonomy in this group, especially with regards to gross colony-level skeletal morphology.

Conclusion

Until very recently, the study of evolutionary relationships among coral species relied solely on morphological characters; however, recent genetic evidence has called the validity of taxonomy by gross colony-level morphology into question (Fukami *et al.* 2004; Fukami *et al.* 2008; Forsman *et al.* 2009). In addition to genetics, studies on phenotypic plasticity in corals have revealed that fragments taken from the same colony can exhibit strikingly different growth forms in different environments (Bruno & Edmunds 1997; Todd 2008). The extent of both genetic and morphological intraspecific variation in corals is poorly understood, as there are still relatively few evolutionary studies. This is clearly problematic, because morphological taxonomy is the current basis for estimating species distributions, abundance, and extinction risk. These factors complicate the understanding and management of potentially threatened coral species such as *M. dilatata.* This study identified no fixed genetic or micro-morphological differences between *M. flabellata*, *M. turgescens*, and *M. dilatata*, or between *M. patula* and *M. verilli*. These species complexes are either closely related (e.g., within one million years), or actively interbreeding. According to the CR data, the geographic ranges of these species complexes are likely to extend beyond Hawaii into the central Pacific. Now that these species complexes have been identified, future work (population genetic and/or reproductive studies) is necessary to determine if the nominal species within each complex successfully interbreed.

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Tables

Table 1. List of primers used for this study: mt = mitochondrial, n = nuclear**.**

Gene		Sequence	Aprox. size	Reference
mt16S	Z16Sf	CCTCGGCTGTTTACCAAAAA	790bp	This study
	Z16Sr	AACATCGAGGTCGCAAACAT		This study
mtATP-6	ZATP6F	GGCTCTGATCGCCTTGACTA	534bp	This study
	ZATP6R	GGCCCACTTGCAACTAACAT		This study
mtCyt-B	ZMcytbf	GGGACAGATGTTGTGCAATG	649bp	This study
	Zmcytbr	CCCCCAACAAAGGGATTAGT		This study
mtCox-1	ZCOIF	TCAACTAATCATAAAGATATTGGTACG	609bp	Forsman et al. 2009
	ZCOIR	TAAACCTCTGGATGCCCAAA		Forsman et al. 2009
mtCR	Ms FP2	TAGACAGGGCCAAGGAGAAG	650bp	van Oppen et al. 2004
	MON RP2	GATAGGGGCTTTTCATTTGTTTG		van Oppen et al. 2004
nITS	ZITS1	TAAAAGTCGTAACAAGGTTTCCGTA	750bp	Forsman et al. 2009
	ZITS2	CCTCCGCTTATTGATATGCTTAAA T		Forsman et al. 2009
nATPsβ	atpsbF2	CGTGAGGGAAATGATTTCTACCATGAGATGAT	280bp	This study
	atpsbR2	CGGGCACGGGCGCCGGGGGGTTCGTTCAT		
	atpsbF3	TGATTGTGTCTGGTGTAATCAGC		

Table 2. List and definitions of morphological characters used.

BC	Distance from center of corallite to nearest verrucae, papillae or ridge
NC	Number of corallites
NTP	Number of verrucae, papillae or ridges
MXDvSL	Maximum corallite diameter (2N) divided by septal length (3N)
LBp	Proportion of maximum to minimum diameter of largest verrucae, papillae or ridge
SBp	Proportion of maximum to minimum diameter of smallest verrucae, papillae or ridge
LPp	Proportion of maximum to minimum diameter of largest pore
SPp	Proportion of maximum to minimum diameter of smallest pore
MXD	Maximum corallite diameter (average of 2 measurements)
Dist	Distance between corallites
SL	Maximum septa length (average of 3 measurements)
LBmx	Maximum diameter of largest verrucae, papillae or ridge
LBmn	Minimum diameter of largest verrucae, papillae or ridge
SBmx	Maximum diameter of smallest verrucae, papillae or ridge
SBmn	Minimum diameter of smallest verrucae, papillae or ridge
LPmx	Maximum diameter of largest pore
LPmn	Minimum diameter of largest pore
SPmx	Maximum diameter of smallest pore
Spmn	Minimum diameter of smallest pore

Table 3. Type II MANOVA of morphological measurements by group. Tests: Wilks test statistic 0 '***' 0.001 '**' 0.01 '*' 0.05

	Df	Test stat	Approx F	Df (groups)	Df (obs)	Pr(>F)
Species	1	0.92654	1.4498	7	128	0.1912
Genetic group	1	0.62219	11.1034	7	128	6.39E-11***

Figures

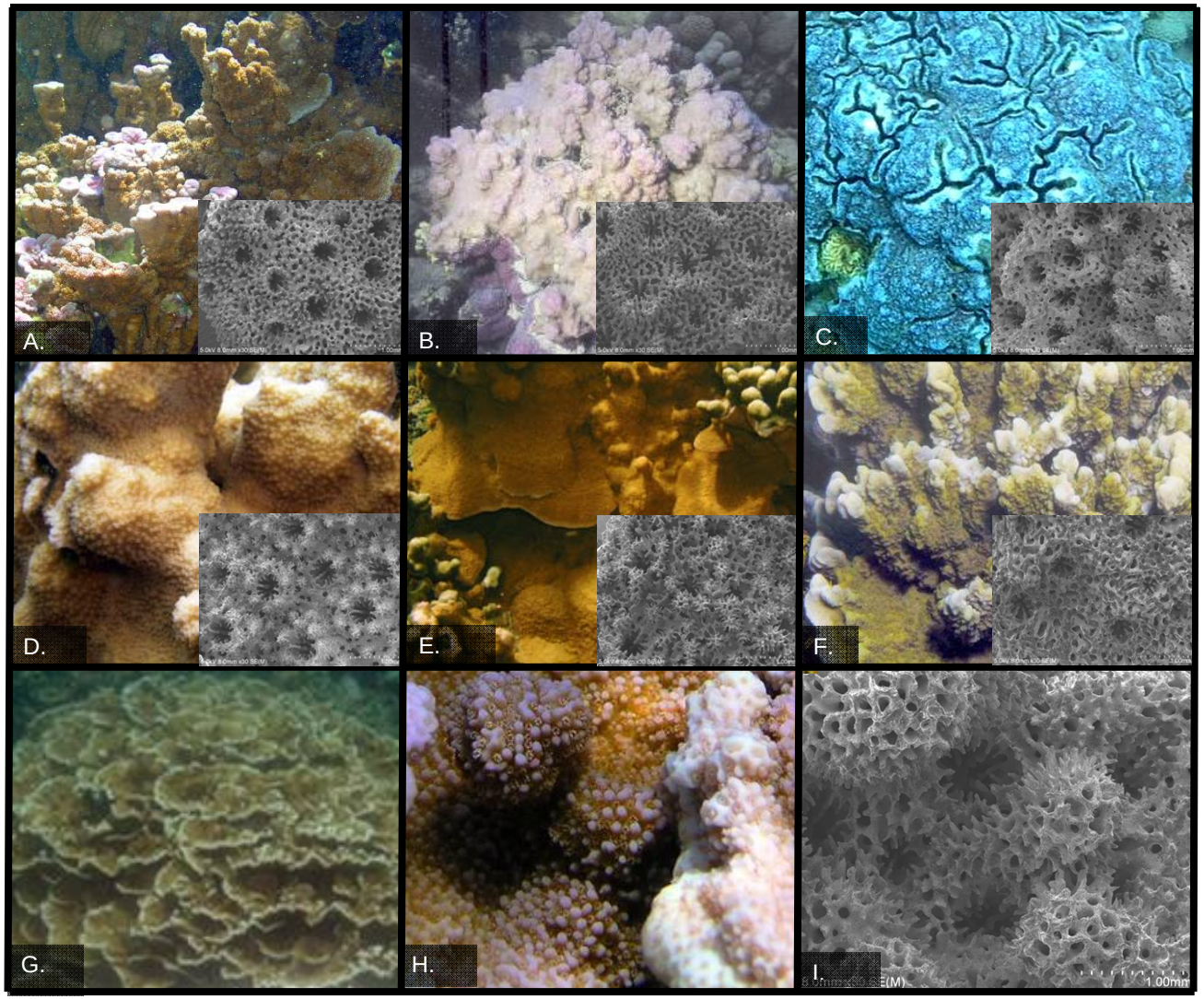

Figure 1. Hawaiian *Montipora* species and micro-morphology: **(A)** *M. dilatata*; **(B)** *M. turgescens*; **(C)** *M. flabellata*; **(D)** *M. patula*; **(E)** *M. verrilli*; **(F)** *M. incrassata*; **(G)** *M. capitata* (plating morph); **(H)** *M. capitata* (close up of branching morph); **(I)** *M. capitata* (SEM image).

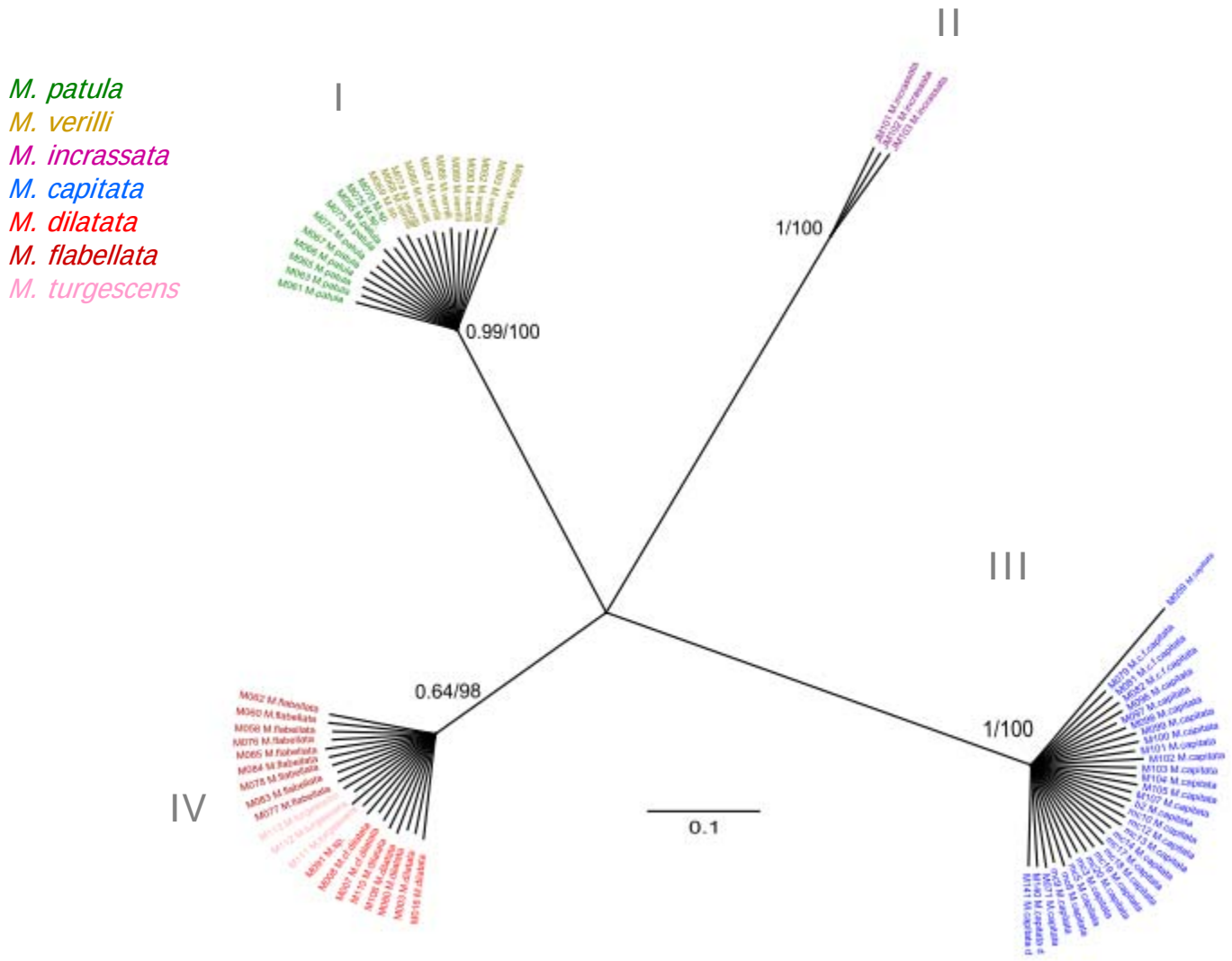

Figure 2. Bayesian inference tree of the mitochondrial control region for Hawaiian specimens. BI and ML confidence values are shown.

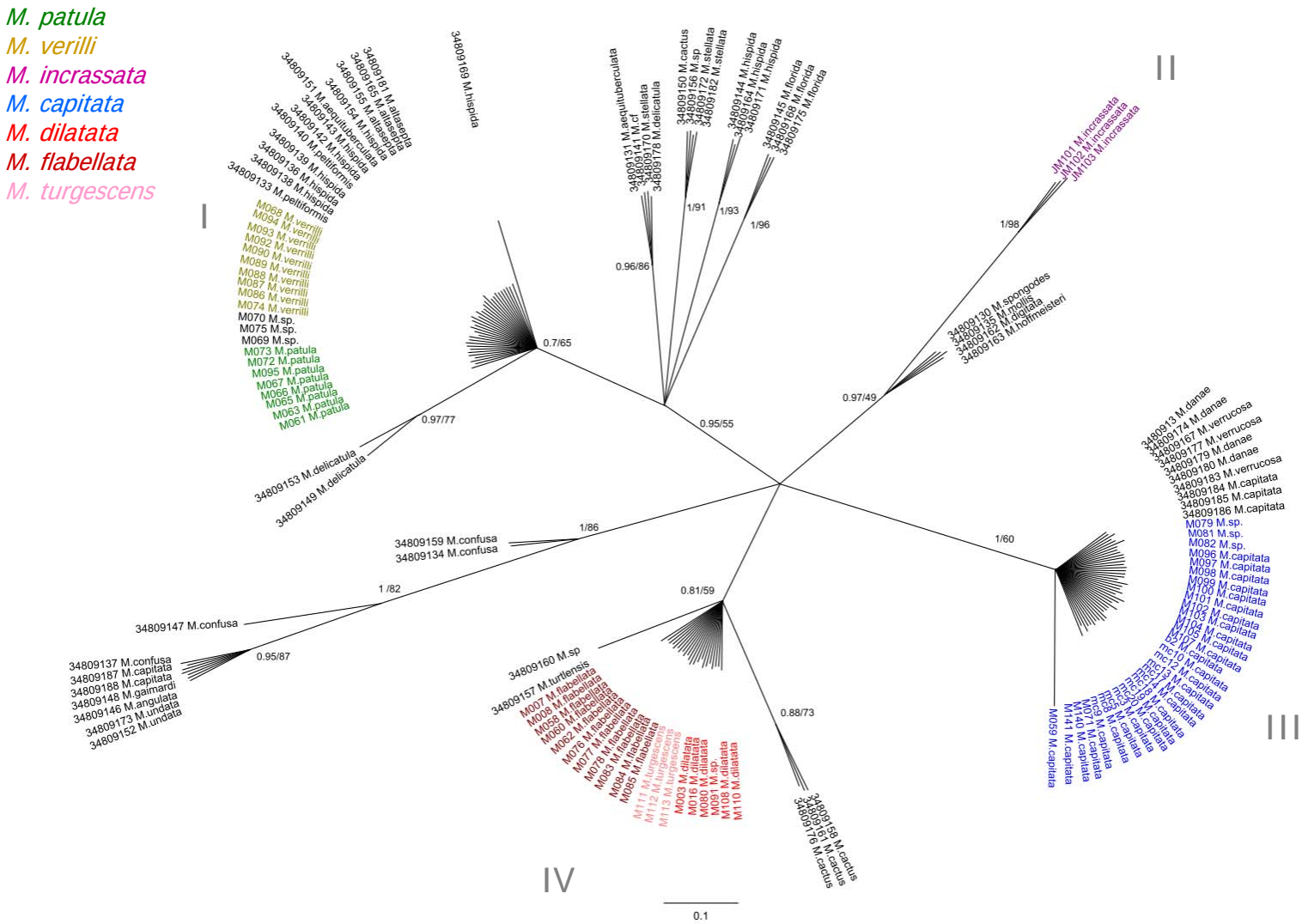

Figure 3. Bayesian inference tree of the mitochondrial control region for Hawaiian specimens and data from van Oppen *et. al.* (2004). BI/ML confidence values are shown.

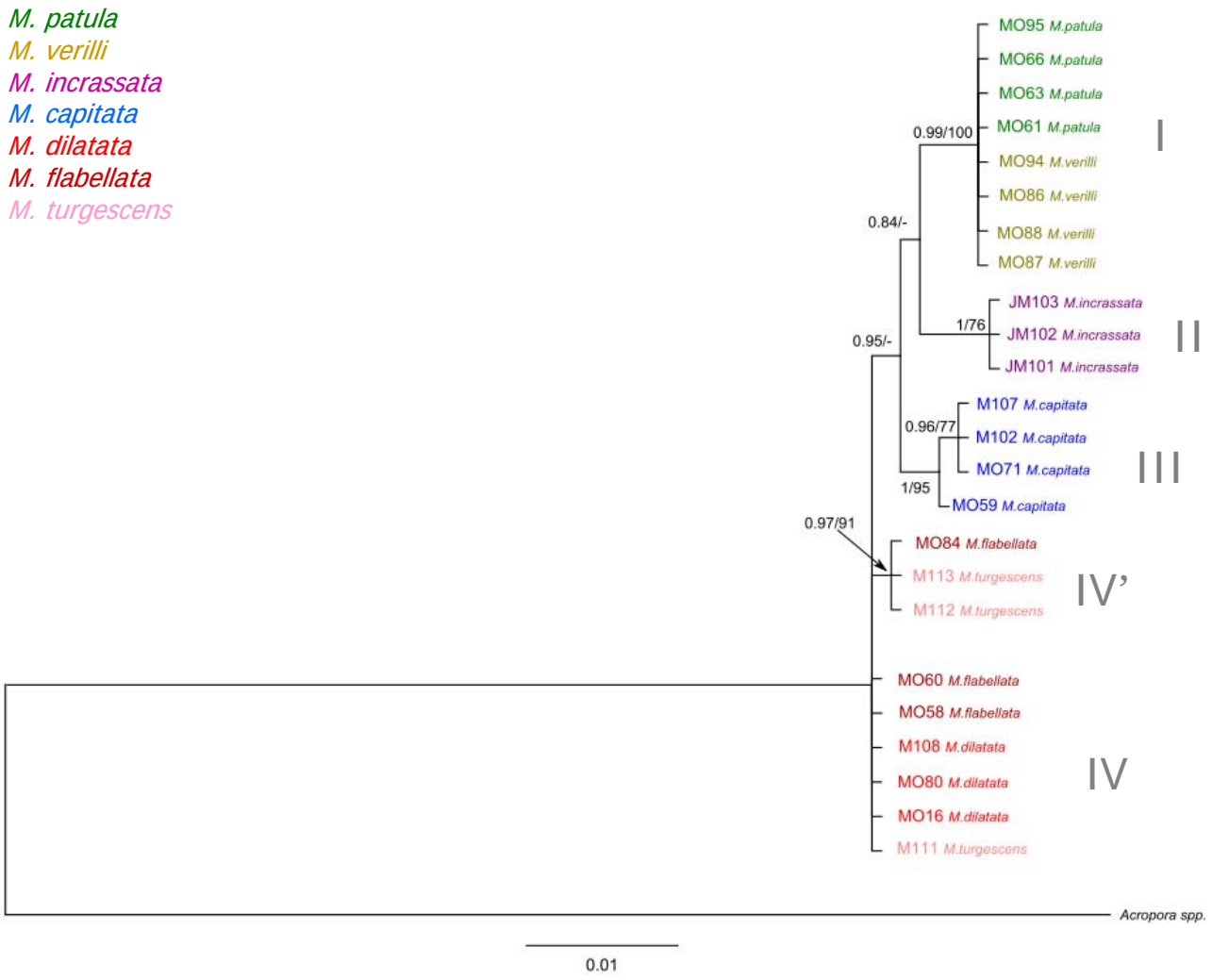

Figure 4. Bayesian inference tree of the concatenated mitochondrial dataset (ATP-6, COI, CYT-B, 16S) for Hawaiian specimens.

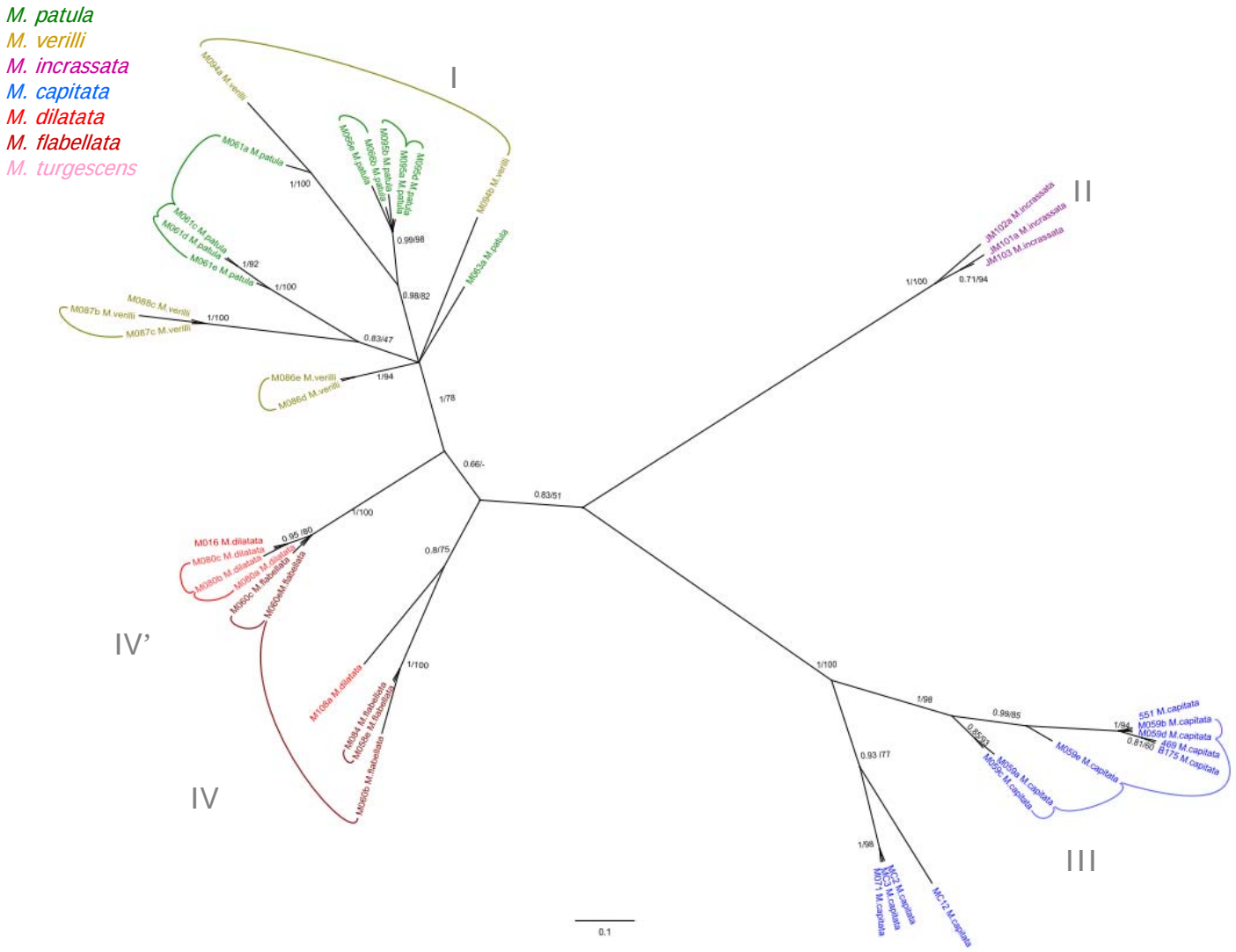

Figure 5. Bayesian inference tree of cloned ITS region sequences for Hawaiian *Montipora*. Lines represent individuals that contained multiple sequence types.

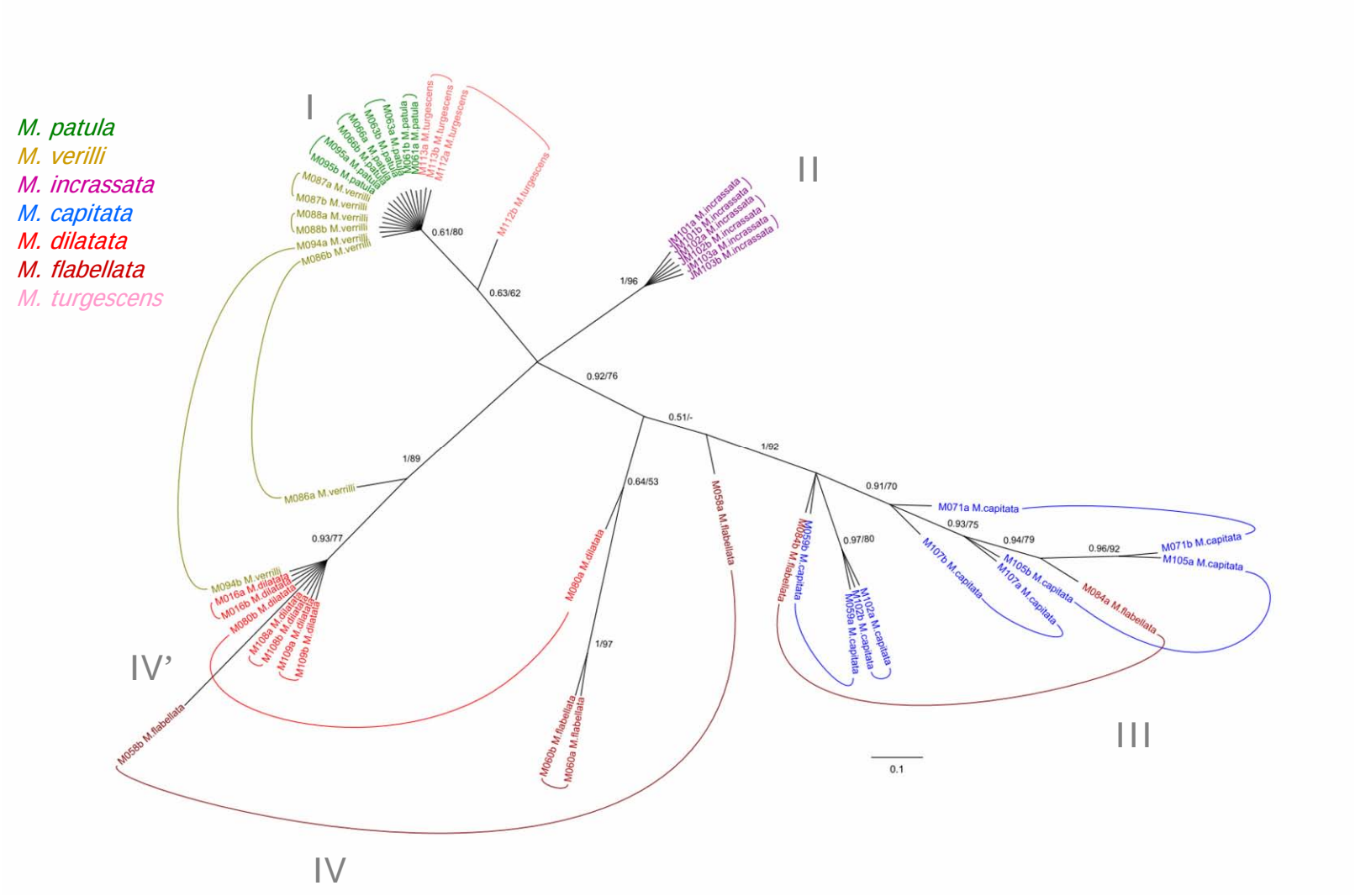

Figure 6. Bayesian inference tree of ATPs β haplotypes. Colored lines represent individuals that were inferred to be heterozygous.

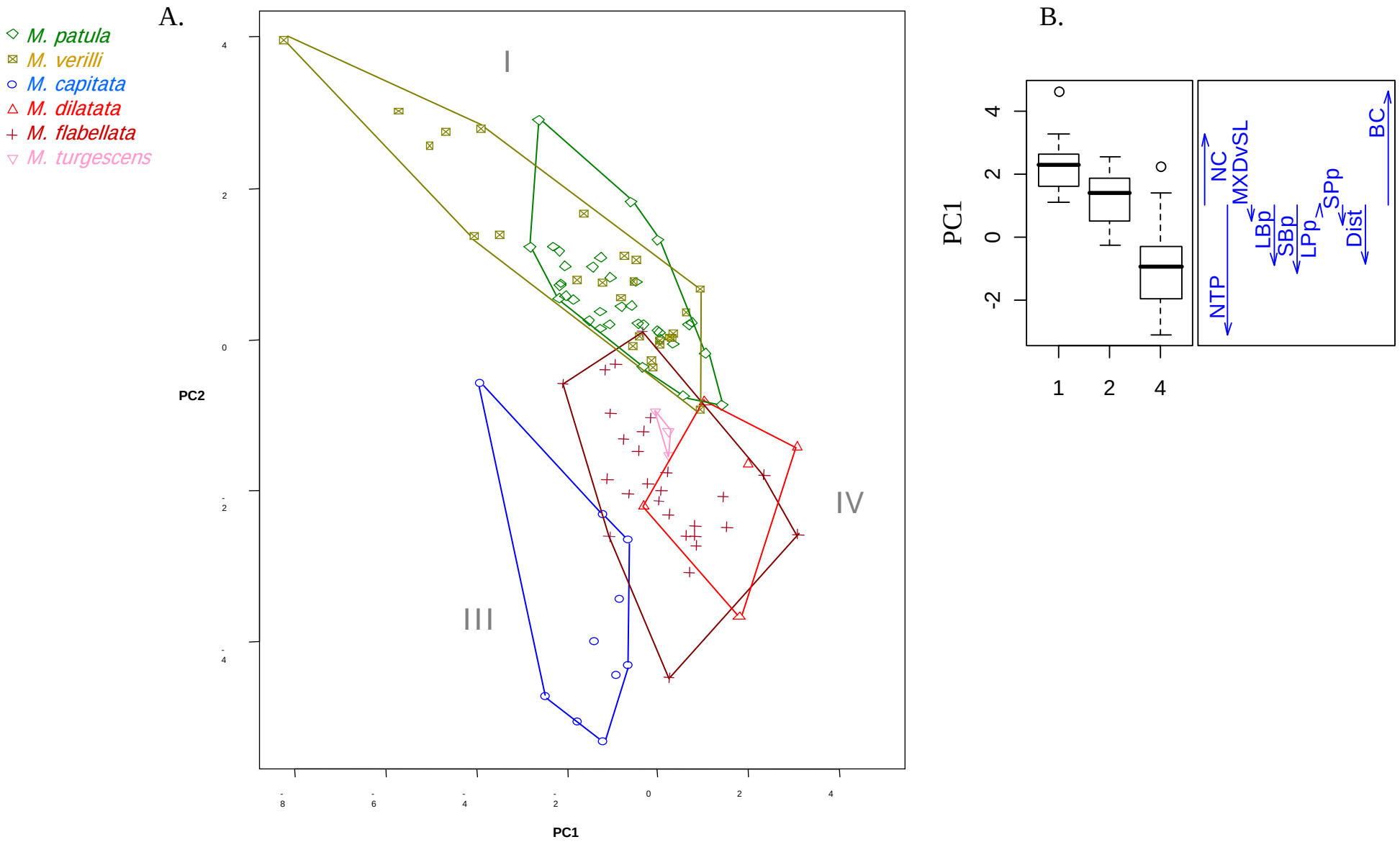

Figure 7. PCA analysis of micro-morphological traits. **(A)** A plot of the first two principal components color coded by species; **(B)** The first principle component plotted against mitochondrial genetic groups, and the relative influence of each morphological trait on separating the groups.

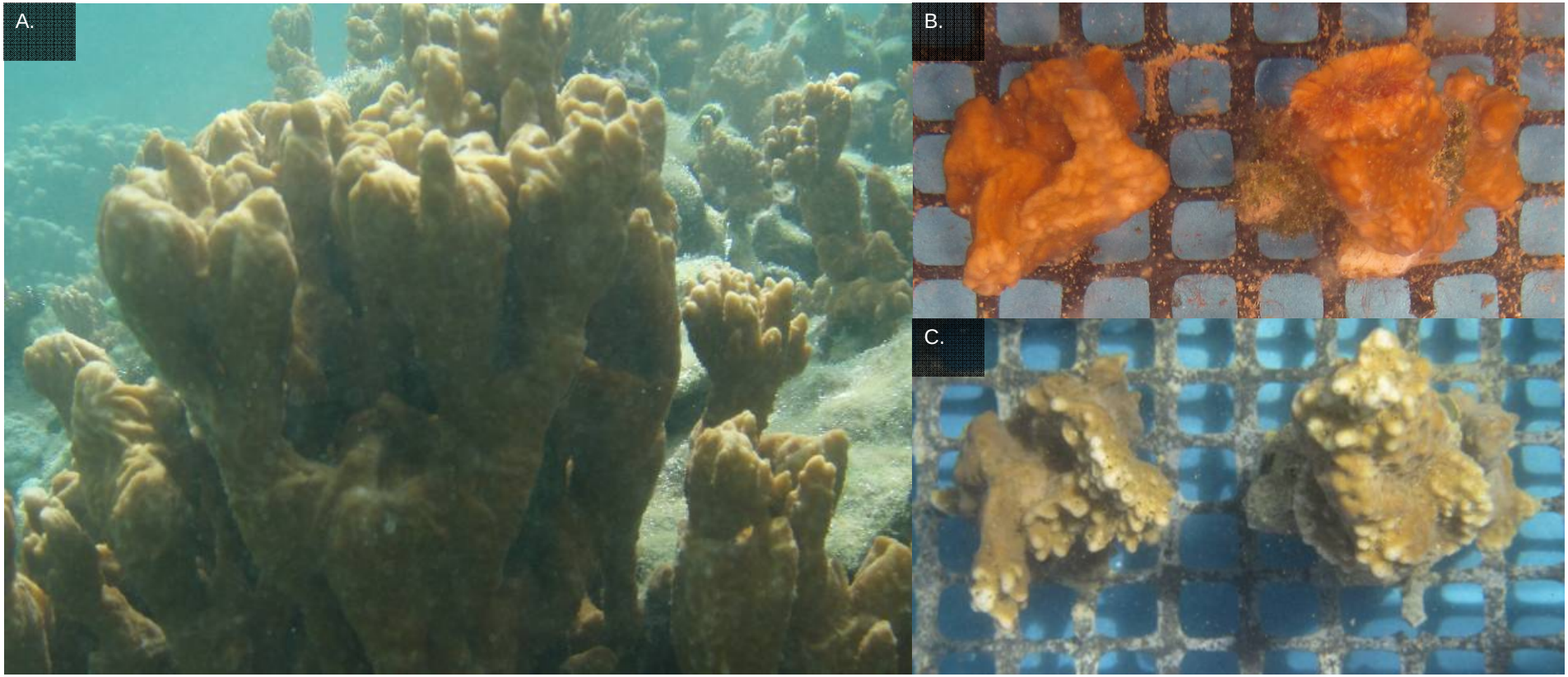

Figure 8. Observation of phenotypic plasticity of micro-skeletal traits: **(A)** Smooth colony with no verrucae photographed 10/1/2009 *in-situ*; **(B)** fragments from the same colony on 11/4/2009; **(C)** the same two fragments on 3/3/210 showing clear verrucae after 5 months growth in a holding tank.

Appendix 1. Table of sample collection and sequencing information. Check marks indicate successfully sequenced samples. Abbreviations: G.C.= Greg Concepcion, R.H.= Roxanne Haverkort, Z.F.= Zac Forsman, E.C.= Evelyn Cox, C.H. = Cynthia Hunter, M.T.= Molly Timmers, I.B. = Iliana Baums, G.A.= Greta Abey, B = branching morphology, P = plating morphology, O = orange color morph, R = red color morph.

Code	Species	Location	Collector	CR	ATPsβ	ITS	COI	ATP-6	Cyt-B	16S
B3	*M. capitata* B.	Oahu, HIMB	G.C.	✓						
MC3	*M. capitata* B.O.	Oahu, HIMB	G.A.	✓		✓				
MC5	*M. capitata* B.O.	Oahu, HIMB	G.A.	✓						
MC8	*M. capitata* B.R.	Oahu, HIMB	G.A.	✓						
MC9	*M. capitata* B.O.	Oahu, HIMB	G.A.	✓						
MC10	*M. capitata* B.O.	Oahu, HIMB	G.A.	✓						
MC12	*M. capitata* B.R.	Oahu, HIMB	G.A.	✓		✓				
MC13	*M. capitata* B.R.	Oahu, HIMB	G.A.	✓						
MC14	*M. capitata* B.R.	Oahu, HIMB	G.A.	✓						
MC17	*M. capitata* B.O.	Oahu, HIMB	G.A.	✓						
MC18	*M. capitata* B.O.	Oahu, HIMB	G.A.	✓						
MC19	*M. capitata* B.O.	Oahu, HIMB	G.A.	✓						
MC20	*M. capitata* B.R.	Oahu, HIMB	G.A.	✓						
M016	M. dilatata	Oahu, Kaneohe bay	C.H.	✓	✓	✓	✓	✓	✓	✓
M058	*M. flabellata*	Oahu, Magic Isl.	G.C.;R.H.	✓	✓	✓	✓	✓	✓	✓
M059	*M. capitata*	Oahu, Magic Isl.	G.C.;R.H.	✓	✓	✓	✓	✓	✓	✓
M060	*M. flabellata*	Oahu, Magic Isl.	G.C.;R.H.	✓	✓	✓	✓	✓	✓	✓
M061	*M. patula*	Oahu, Magic Isl.	G.C.;R.H.	✓	✓	✓	✓	✓	✓	✓
M062	*M. flabellata*	Oahu, Magic Isl.	G.C.;R.H.	✓	✓					
M063	*M. patula*	Oahu, Magic Isl.	G.C.;R.H.	✓	✓	✓	✓	✓	✓	✓
M065	*M. patula*	Oahu, Lanikai	G.C.;R.H.	✓	✓					
M066	*M. patula*	Oahu, Lanikai	G.C.;R.H.	✓	✓	✓	✓	✓	✓	✓
M067	*M. patula*	Oahu, Lanikai	G.C.;R.H.	✓	✓					
M068	*M. verrilli*	Oahu, Lanikai	G.C.;R.H.	✓	✓					
M069	*M. sp.*	Oahu, Lanikai	G.C.;R.H.	✓	✓					
M070	*M. sp.*	Oahu, Lanikai	G.C.;R.H.	✓	✓					
M071	*M. capitata*	Oahu, Lanikai	G.C.;R.H.	✓	✓	✓	✓	✓	✓	✓
M072	*M. patula*	Oahu, Lanikai	G.C.;R.H.	✓	✓					
M073	*M. patula*	Oahu, Lanikai	G.C.;R.H.	✓	✓					
M074	*M. verrilli*	Oahu, Lanikai	G.C.;R.H.	✓	✓					
M075	*M. sp.*	Oahu, Lanikai	G.C.;R.H.	✓	✓					
M076	*M. flabellata*	Oahu, Kaneohe bay	G.C.;R.H.	✓	✓					
M077	*M. flabellata*	Oahu, Kaneohe bay	G.C.;R.H.	✓	✓					
M078	*M. flabellata*	Oahu, Kaneohe bay	G.C.;R.H.	✓	✓					
M079	*M. sp.*	Oahu, Kaneohe bay	J.S.	✓	✓					
M080	*M. dilatata*	Oahu, Kaneohe bay	C.H.;Z.F.	✓	✓	✓	✓	✓	✓	✓
M081	*M. sp.*	Molokai, Pukoo	C.H.;Z.F.	✓						
M082	*M. sp.*	Molokai, Pukoo	C.H.;Z.F.	✓						
M083	*M. flabellata*	Oahu, Kaneohe bay	E.C.	✓	✓					
M084	*M. flabellata*	Oahu, Kaneohe bay	E.C.	✓	✓	✓	✓	✓	✓	✓
M085	*M. flabellata*	Oahu, Kaneohe bay	E.C.	✓	✓					
M086	*M. verrilli*	Oahu, Kaneohe bay	C.H.;Z.F.;R.H.	✓	✓	✓	✓	✓	✓	✓

Appendix 1 cont.

Code	Species	Location	Collector	CR	ATPsβ	ITS	COI	ATP-6	Cyt-B	16S
M087	*M. verrilli*	Oahu, Kaneohe bay	C.H.;Z.F.;R.H.	✓	✓	✓	✓	✓	✓	✓
M088	*M. verrilli*	Oahu, Kaneohe bay	C.H.;Z.F.;R.H.	✓	✓	✓	✓	✓	✓	✓
M089	*M. verrilli*	Oahu, Kaneohe bay	C.H.;Z.F.;R.H.	✓	✓					
M090	*M. verrilli*	Oahu, Kaneohe bay	C.H.;Z.F.;R.H.	✓	✓					
M091	*M. sp.*	Oahu, Kaneohe bay	C.H.;Z.F.;R.H.	✓	✓					
M092	*M. verrilli*	Oahu, Kaneohe bay	C.H.;Z.F.;R.H.	✓	✓					
M093	*M. verrilli*	Oahu, Kaneohe bay	C.H.;Z.F.;R.H.	✓	✓					
M094	*M. verrilli*	Oahu, Kaneohe bay	C.H.;Z.F.;R.H.	✓	✓					
M095	*M. patula*	Oahu, Kaneohe bay	C.H.;Z.F.;R.H.	✓	✓					
M096	*M. capitata*	Maro Reef, NWHI	I.B.	✓	✓					
M097	*M. capitata*	Mare Reef, NWHI	I.B.	✓	✓					
M098	*M. capitata*	Maro Reef, NWHI	I.B.	✓	✓					
M099	*M. capitata*	Pearl and Hermes, NWHI	G.C.	✓	✓					
M100	*M. capitata*	Kure Atoll, NWHI	G.C.	✓	✓					
M101	*M. capitata*	Lisianski, NWHI	G.C.	✓						
M102	*M. capitata*	Lisianski, NWHI	G.C.	✓	✓					
M103	*M. capitata*	Lisianski, NWHI	G.C.	✓	✓					
M104	*M. capitata*	Pearl and Hermes, NWHI	G.C.	✓	✓					
M105	*M. capitata*	Pearl and Hermes, NWHI	G.C.	✓	✓					
M106	*M. capitata*	Pearl and Hermes, NWHI	G.C.	✓						
M107	*M. capitata*	Kure Atoll, NWHI	G.C.	✓	✓					
M108	*M. dilatata*	Waikiki Aquarium	G.C.	✓	✓	✓	✓	✓	✓	✓
M109	*M. dilatata*	Waikiki Aquarium	G.C.	✓	✓					
M110	*M. dilatata*	Waikiki Aquarium	G.C.	✓	✓					
M111	*M. turgescens*	PHR-26	M.T.	✓			✓	✓	✓	✓
M112	*M. turgescens*	LIS-10	M.T.	✓	✓		✓	✓	✓	✓
M113	*M. turgescens*	PHR-31	M.T.	✓	✓		✓	✓	✓	✓
M140	*M. sp.* Deep water			✓						
M141	*M. sp.* Deep water			✓				✓	✓	