TITLE PAGE

Species Boundaries in Scleractinian Corals: A Case Study of the *Acropora humilis* Species Group

Thesis submitted by

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ABSTRACT

Accurate identification of species is critical for studies of biological systems, including biodiversity analyses, understanding evolutionary processes and ecological dynamics, and for effective conservation and management of the environment. However, defining species boundaries in scleractinian corals is impeded by the difficulties of distinguishing between ecological and evolutionary influences on the appearance of colony morphology. In this study, I used three criteria, i.e. reproductive, morphometric and molecular evidence to determine the extent to which intraspecific and interspecific morphological variation is indicative of evolutionary relationships in species of the *Acropora humilis* species group. Reproductive criteria, including relative timing of spawning and potential to interbreed in fertilization experiments, provided the greatest level of taxonomic resolution. Discriminant analysis of morphometric data provided a moderate level of resolution. Molecular phylogenetic analysis of two markers, the 28S rDNA unit (domains 1 and 2) and the mtDNA intergenic region, provided the lowest level of resolution of the three criteria.

Twenty-one morphs were recognized in field surveys, conducted in seven regions in the western and central Pacific, and these were used as sampling units throughout this study. The morphs were defined using morphological characters that are traditionally used to identify species of the genus *Acropora* and included the eight currently recognized species of the *A. humilis* group, seven intermediate morphs and six sub-morphs. The intermediate morphs were characterized by intermediate morphologies that prevented colonies from being confidently assigned to a single species, and the sub-morphs formed recognizable units within the range of morphological variation of one species. Differentiation between species and morphs greatly enhanced the interpretation of evolutionary relationships in this species group, with consistent patterns being found throughout the geographic scale of this project. Colonies identified as *A. humilis, A. samoensis, A. gemmifera, A. monticulosa* and *A. digitifera* were shown to be valid species on the basis of reproductive data. Although these species showed no potential to interbreed, it is possible that indirect introgression, through interbreeding between some members of these species and morphs may be retarding divergence of these species. Reproductive data were not obtained for *A. globiceps, A. retusa* and *A. multiacuta*. The taxonomic status of *A. globiceps* is

therefore unresolved, due to its lack of morphological and genetic differentiation from *A. humilis*. *Acropora retusa* and *A. multiacuta* appear to be valid species, on the basis of morphological and molecular differentiation. *Acropora humilis, A. samoensis, A. globiceps* and morphs of these species share the greatest evolutionary affinity, on the basis of morphological overlap and lack of genetic differentiation. The most closely related to these three species appears to be *A. gemmifera*, with this species and morphs common to these four species also being genetically undifferentiated. *Acropora digitifera* was morphologically and genetically distinct from all other species of the *A. humilis* group, although an intermediate morph between this species and *A. gemmifera* was genetically undifferentiated but reproductively isolated from *A. digitifera*. On the basis of morphological affinity, this morph is proposed as a possible hybrid between these species. *Acropora monticulosa* was morphologically distinct from all other species, although it appears to share evolutionary connections on two fronts. Firstly, low levels of genetic differentiation for the mitochondrial marker, between this species and *A. humilis, A. samoensis, A. globiceps* and *A. gemmifera*, suggest recent divergence from these species. Secondly, *A. monticulosa* also appears to share evolutionary affinities with *A. digitifera* on the basis of morphological similarities between morphs of each of these species, with one of these morphs grouping with *A. monticulosa* for the 28S marker and with *A. digitifera* for the mitochondrial marker.

This study demonstrates that examining intraspecific and interspecific patterns of polymorphism are valuable for interpreting evolutionary relationships in corals. Evidence derived from these criteria suggest that the morphs are at various stages of divergence from the species with which they share morphological characters and that the morphs may indicate possible zones of speciation and hybridization. Recognition of morphs also avoided the possibility of taxonomic error as a result of ‘forcing’ colonies into incorrect or inappropriate species categories and was therefore essential for accurate interpretation of evolutionary boundaries. Using multiple criteria and samples collected across a broad biogeographic scale facilitated the clarification of relationships within and between species.

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CHAPTER 1: GENERAL INTRODUCTION

1.1 SPECIES BOUNDARIES IN SCLERACTINIAN CORALS

Species are the basic units of measurement of biodiversity. Accurate definition of species is therefore critical for describing patterns of biodiversity, understanding evolutionary processes and ecological dynamics, and effective conservation and management of the environment. Yet, despite the importance of species in studies of biological systems, the formation of species represent one of the most elusive subjects in evolutionary biology (Palumbi 1994). Species boundaries form the interface at which groups of individuals diverge to form separate evolutionary lineages (Avise and Ball 1990; Brower et al. 1996; Avise and Wollenberg 1997). This process of divergence is ongoing, on an evolutionary time scale. Consequently, depending on time since divergence, boundaries between species may be difficult to define, due to different rates of divergence of the criteria used to delineate them (Avise and Ball 1990; Brower et al. 1996; Avise and Wollenberg 1997). It is also possible that species boundaries may only be partially formed, with gene flow still occurring between some individuals or in some locations, further complicating the delineation of boundaries between species (Wu 2001).

Defining species boundaries is particularly complex in scleractinian corals (Lang 1984; Knowlton 2001; Frank and Mokady 2002). Species of corals are traditionally defined using morphological characters (e.g. Wells 1956; Veron and Wallace 1984; Wallace 1999), with morphological discontinuities used to determine the boundaries between them (Lang 1984; Wallace and Willis 1994). However, this is problematical because of the high levels of morphological polymorphism within species, as they are currently defined (Lang 1984; Wallace and Willis 1994; Veron 1995). Intraspecific morphological variation is common, and in some cases, there appear to be discrete morphs within currently defined species, particularly for studies within a small geographic area (Veron and Pichon 1976). Morphological discontinuities between species are often blurred by the existence of individuals that share morphological characters (Lang 1984). It is also possible that cryptic species, which are evolutionarily but not morphologically distinct, are concealed within this morphological variability (Knowlton and Jackson 1994; Knowlton 2000; Stobart and Benzie 1994). This phenotypic variability raises the issue of the extent to which morphological boundaries

and currently defined species boundaries represent evolutionary boundaries in corals (Wallace and Willis 1994) and whether patterns of morphological variability are indicative of microevolutionary processes (Sattler and Rutishauser 1997; Wiens 1999). For example, it is possible that morphological similarity may indicate recent divergence, and shared or intermediate morphological characters may indicate merging of species through hybridization. Alternatively, morphology may be evolving independently of other criteria used to define evolutionary relationships in corals (van Oppen et al. 2001).

Morphological variability in species of corals is partly due to environmental influences (Veron and Pichon 1976; Lang 1984; Willis 1990) and may also be due to variable patterns of interbreeding between species (Babcock 1995). Transplant experiments have demonstrated that morphology of coral colonies can change in response to light and energy regimes as well as space availability (Foster 1979; Willis 1985; Bruno and Edmunds 1998; Muko et al. 2000). Synchronous mass spawning by many species of corals, in which gametes from these species are mixed in spawning slicks (Harrison et al. 1984; Babcock et al. 1986; Hayashibara et al. 1993; Babcock et al. 1994), raises the issue of whether interspecific hybridization has contributed to morphological variability in coral species (Wallace and Willis 1994; Babcock 1995; Willis et al. 1997). *In vitro* fertilization experiments demonstrate that some species of corals hybridize, while other species show little or no potential to interbreed under laboratory conditions (Stobart 1994; Knowlton et al. 1997; Miller and Babcock 1997; Szmant et al. 1997; Willis et al. 1997; Hatta et al. 1999; Fukami et al. 2003). However, the extent to which hybridization occurs in nature and its effects on morphological variability are yet to be determined. Timing of spawning varies by up to 3 hours between species spawning on the same night (Babcock et al. 1986; Hayashibara et al. 1993; Knowlton et al. 1997; Fukami et al. 2003), sufficient to form temporal reproductive barriers between species (Knowlton et al. 1997; van Oppen et al. 2002b; Fukami et al. 2003). Other species are clearly reproductively isolated from the mass spawning, reproducing weeks or months out of phase with this phenomenon (Willis et al. 1985; Babcock et al. 1986; Hayashibara et al. 1993; Hayashibara and Shimoike 2002).

Electrophoretic and molecular techniques have been used to explore whether morphological variation in corals can be explained on the basis of genetic criteria.

Several studies demonstrate near or complete concordance between morphological and genetic boundaries, providing support for the validity of the species examined (Ayre et al. 1991; Weil 1992; Garthwaite et al. 1994; Stobart and Benzie 1994; Márquez et al. 2002a; Márquez et al. 2002b; Maté 2003). In other studies, incomplete genetic divergence between species may be due to hybridization or incomplete lineage sorting. Most authors to date have favoured introgression through continued interbreeding or hybridization as the likely mechanism preventing or retarding divergence (Miller and Benzie 1997; Odorico and Miller 1997b; Hatta et al. 1999; Medina et al. 1999; Diekmann et al. 2001; van Oppen et al. 2001; van Oppen et al. 2002b). However, it is also argued that the contribution of hybridization has been overestimated and that incomplete lineage sorting, due partly to the slow rate of evolution of some molecular markers, is also a major reason for the lack of genetic distinction between many species of corals (Knowlton 2001; Vollmer and Palumbi 2002).

Species boundaries in corals have typically been examined on a restricted spatial scale, often limited to a single location. However, examination of morphological, genetic and reproductive criteria within and between species on a broad biogeographic scale is necessary to gain a greater understanding of the evolutionary relationships between species. Taxonomic assemblages vary in different locations, due to the variable geographic ranges of species (Wallace 1999; Veron 2000). Patterns in timing of spawning and other spawning characters also vary in different locations, as well as in different years in the same location, in response to varying environmental or physical factors (Baird et al. 2002). It is therefore likely that patterns of evolutionary divergence and potential for hybridization will vary between locations on a biogeographic scale. For example, the inconsistent hybridization and the variable occurrence of intermediate colonies reported for the *Montastraea annularis* species complex in different locations (Knowlton et al. 1997; Szmant et al. 1997), may indicate that the formation of evolutionary boundaries within this species complex are at different stages in different locations within the Caribbean. Studies of species boundaries in scleractinian corals must therefore be at a broad biogeographic scale, using multiple criteria, to determine whether local varieties are in fact distinct evolutionary units and to enable the evolutionary limits of species with broad distributions to be accurately defined.

1.2 SPECIES CONCEPTS IN SCLERACTINIAN CORALS

The definition of species remains one of the most controversial issues in biology. Ongoing debates continue to evaluate the merits and limitations of different species concepts for resolving the most appropriate framework to define evolutionary relationships between taxonomic groups, particularly at the level of species (e.g. Claridge et al. 1997; Wilson 1999). Three of the most influential species concepts are (1) the morphological species concept in which phenotypic discontinuities are assumed to indicate evolutionary boundaries; (2) the biological species concept in which ability to interbreed is assumed to provide the cohesive mechanism within a species and evolutionary separation from other species; and (3) the phylogenetic species concept in which groups species as the smallest biological entities that are monophyletic. Controversy stems from the fact that the most appropriate criteria for defining species appear to vary for different taxonomic groups. Factors influencing these criteria include mode of reproduction and likelihood of gamete mixing within and between species, geographic distribution and connectivity between populations and therefore potential of individuals to interbreed, and usefulness of phylogenetic characters for defining evolutionary lineages. The authors of many reviews also argue that a single concept is inadequate for defining species because of the enormous diversity of patterns of evolution (e.g. Dupré 1999).

In corals, the inconsistent alignment of morphological, reproductive and genetic boundaries between morphologically defined species, questions the applicability of the morphological species concept as the most appropriate basis for defining species of scleractinian corals (Willis 1990) and raises the issue of whether alternative or multiple species concepts may provide a more appropriate framework. Developing a more comprehensive understanding of species boundaries in corals and interpreting how currently defined species correspond with evolutionary groups of individuals, will contribute substantially to determining the most appropriate theoretical framework for defining species in scleractinian corals.

1.3 THE *ACROPORA HUMILIS* SPECIES GROUP

The genus *Acropora* is the largest genus of scleractinian corals. The review by Wallace (1999) describes 114 species while Veron (2000) describes 170 species within the genus *Acropora*. Veron and Wallace (1984) and Wallace (1999) use species groups to

subdivide this large genus for convenience of identification, stating that they do not imply taxonomic affinity (Veron and Wallace 1984). The *Acropora humilis* species group contains eight species, as defined by Wallace (1999). These species are *A. humilis* (Dana 1846), *A. gemmifera* (Brook 1892), *A. multiacuta* (Nemenzo 1967), *A. monticulosa* (Brüggemann 1879), *A. digitifera* (Dana 1846), *A. samoensis* (Brook 1891), *A. retusa* (Dana 1846) and *A. globiceps* (Dana 1846). In a systematic revision of the genus *Acropora*, based on morphological characters, Wallace (1999) found the species of the *A. humilis* species group formed a paraphyletic assemblage (Fig. 1.1). The species *A. humilis* and *A. gemmifera* formed a highly derived clade, independent of all other species in this species group and the genus *Acropora*. The species *A. multiacuta* and *A. monticulosa* each formed independent lineages. The species *A. digitifera*, *A. samoensis* and *A. retusa* grouped within a monophyletic clade, the DND clade ("*digitifera–nasuta-divaricata groups*") shared by the species of two other species groups i.e. the *Acropora nasuta* and *Acropora divaricata* species groups. The eighth species, *A. globiceps* was not recognized at the time of the analysis, although Wallace (1999) notes that this species is separated from *A. humilis* by one morphological character, and therefore is assumed to be a sister species of *A. humilis*. The species *A. humilis*, *A. gemmifera*, *A. monticulosa*, *A. digitifera* and *A. samoensis* have broad Indo-Pacific distributions. *Acropora globiceps* is only recorded from Pacific Ocean locations, *A. multiacuta* is only recorded from isolated locations in the central Indo-Pacific and *A. retusa* is recorded from western Indian Ocean and central Pacific locations (Wallace 1999).

The species of the *Acropora humilis* group have high levels of intraspecific morphological variation, while morphological boundaries between some species are unclear. This polymorphism contributes to the complex taxonomic history and difficulties in interpreting the evolutionary history of these species, on the basis of

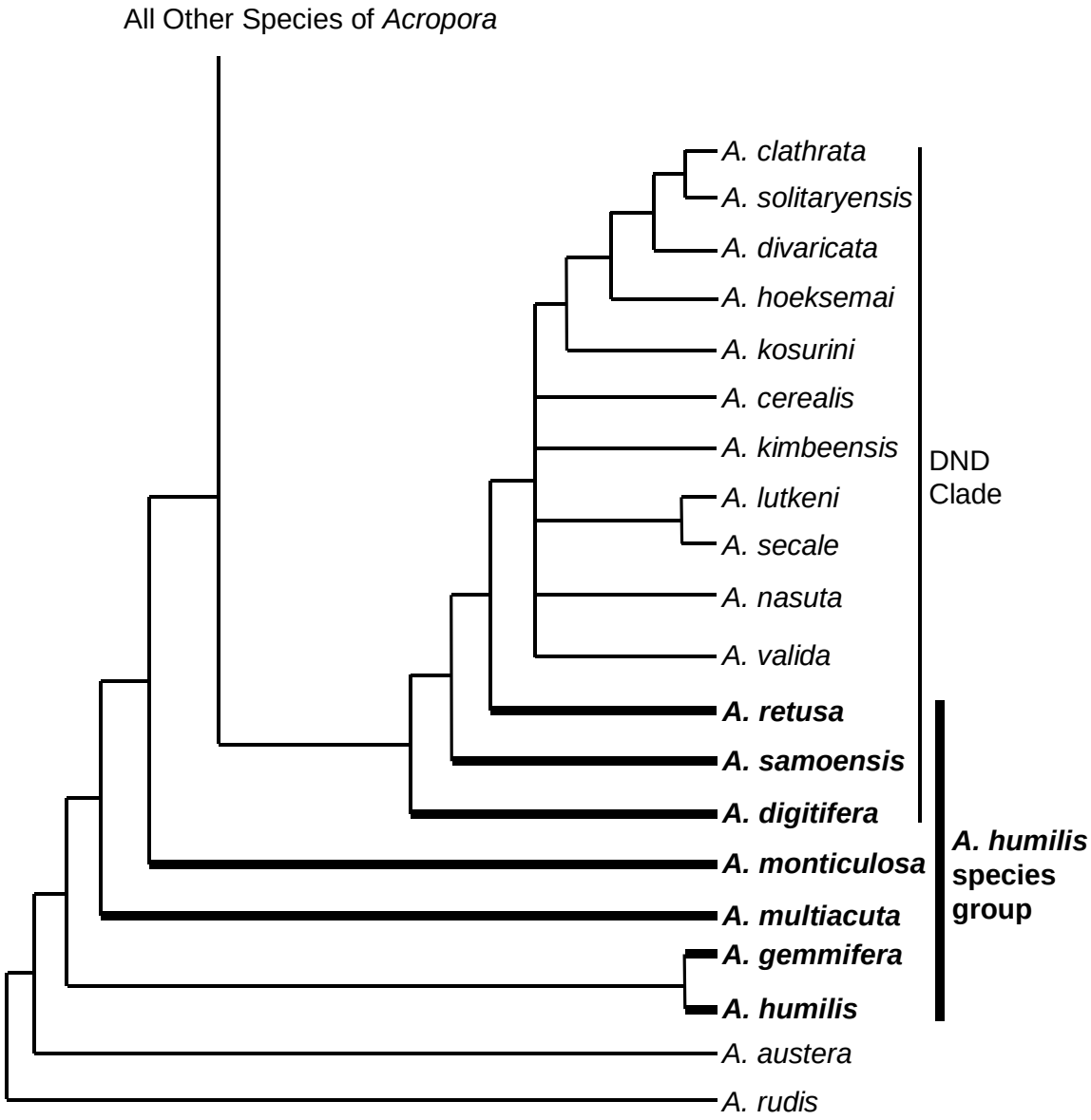

Fig 1.1 Basal branches of a strict consensus tree from the parsimony analysis of morphological characters of the genus *Acropora* by Wallace (1999), showing the relationship between species of the *A. humilis* species group and other species within the genus *Acropora* proposed in this phylogeny. Species of the *A. humilis* species group are highlighted in bold and with thickened lines. This figure is modified and redrawn from Fig. 44 in Wallace (1999).

morphological characters. In the first comprehensive review of the species now grouped within the *A. humilis* species group, Wells (1954) recognized only two species, *A. humilis* and *A. digitifera*. Within *A. humilis*, Wells (1954) synonymised the species *A. globiceps*, *A. gemmifera* and *A. samoensis* and thirteen other species, many of which continue to be considered as synonyms of currently recognized species in the *A. humilis* group. Wells (1954) recognized *A. digitifera* as a distinct species with no additional synonyms. In a major review of the genus *Acropora* for eastern Australia, Veron and Wallace (1984) recognized *A. humilis* and *A. digitifera*, as well as *A. gemmifera* and *A. samoensis* as distinct species, but did not discuss the status of *A. globiceps*. In the same monograph, Veron and Wallace (1984) recognized *A. monticulosa* and the recently described *A. multiacuta* as valid species. Veron and Wallace (1984) described close morphological affinities between the species *A. humilis*, *A. gemmifera* and *A. monticulosa* as well as *A. humilis* and *A. samoensis*, but note the distinction of *A. multiacuta* from all other species in the genus *Acropora*. In the most recent and only worldwide review of the genus *Acropora*, Wallace (1999) concurred with the designation of *A. humilis*, *A. digitifera*, *A. gemmifera*, *A. samoensis*, *A. monticulosa* and *A. multiacuta* as valid species, as well as reviving *A. globiceps* and *A. retusa* as valid species. In this review, Wallace (1999) states the need for further research to resolve boundaries between the species of the *A. humilis* group, particularly for the species *A. humilis* and *A. gemmifera*. Wallace (1999) notes apparent morphological affinities between *A. gemmifera* and *A. humilis*; *A. globiceps* with *A. samoensis* and *A. humilis*; and *A. monticulosa* with *A. humilis* and *A. gemmifera*. Based on the conclusions of these previous studies, it appears that the species *A. digitifera*, *A. multiacuta* and *A. retusa* are most clearly defined, while morphological boundaries between *A. humilis*, *A. globiceps*, *A. samoensis*, *A. gemmifera* and *A. monticulosa* are less clear. This differs from the results of the only two publications that have examined evolutionary relationships between species of the *A. humilis* group using non-morphological criteria, both of which present molecular phylogenies of the genus *Acropora* for a nuclear and mitochondrial marker (van Oppen et al. 2001; Márquez et al. 2002b). Van Oppen et al. (2001) found that *A. humilis* and *A. digitifera* share a close relationship which is distinct from *A. gemmifera*. This was supported by Márquez et al. (2002b) who used the same sequences of the previous study as well as additional sequences from other colonies of the same and different species of *Acropora*.

1.4 THESIS OUTLINE

The major aim of this thesis is to determine the extent to which morphology is indicative of evolutionary relationships within and between currently defined species of the *Acropora humilis* species group. To achieve this, I defined morphological sampling units, at multiple sites from the west to central Pacific, on the basis of morphological characters that are traditionally used to identify species of corals in the genus *Acropora* (Wallace 1999). I then examined evolutionary relationships between these sampling units using morphometric, molecular and reproductive criteria. The morphological sampling units included species as well as putative (intraspecific and interspecific) morphs. The species categories correspond with the known species of the *Acropora humilis* species group. The intraspecific morphs are termed sub-morphs and include colonies that appear to form morphologically discrete groups within the known species. The interspecific morphs are termed intermediate morphs and include colonies that appear to share morphological characters with more than one species. The sub-morphs and intermediate morphs are named after the species with which they appear to share greatest morphological affinity.

In chapter 2, I determine the most useful morphometric characters for comparing morphological relationships within and between the species and morphs of the *Acropora humilis* species group, using samples from American Samoa. Relationships between the species and morphs used in this analysis are examined using a nuclear molecular marker, domains 1 and 2 of the 28S rDNA unit.

In chapter 3, I survey the relative abundance of each species and morph of the *Acropora humilis* group at Lizard Island, Great Barrier Reef, Australia and examine the potential of these species and morphs to interbreed. The potential to interbreed is explored on two fronts. Firstly, timing of spawning is documented at the scales of month, day and hour to determine whether some species or morphs would be unable to interbreed due to temporal reproductive isolation. Secondly, I test the ability of colonies of the same and different species and morphs, which spawned on the same night, to form viable larvae in laboratory fertilization experiments. I use a mitochondrial molecular marker, the mtDNA intergenic region, to determine whether this marker indicates a genetic basis for the reproductive patterns that I found.

In chapter 4, I examine morphological variation and the biogeographic distribution of species and morphs of the *Acropora humilis* species group. I use the morphometric characters developed in chapter 2 to compare patterns of morphological variation within and between species and morphs of the *A. humilis* species group. Patterns of variation are documented across a broad geographic range, from the west to the central Pacific. Locations sampled include American Samoa, using the same samples analysed in chapter 2, in addition to six other locations: Taiwan, Indonesia, Australia (Lizard Island, Great Barrier Reef), Papua New Guinea, Solomon Islands and French Polynesia. I define morphological species categories in a discriminant analysis and then calculate the probability of classification of each colony of each morph into these species categories. To document the biogeographic distribution of each species and morph recognized in this study, I supplement the biogeographic data collected during my field work, by identifying all specimens of the *A. humilis* species group in the collections of the Museum of Tropical Queensland using the morphological categories (species and morphs) defined in this chapter. These identifications are used to map the worldwide distribution of each species and morph. The museum collections are also examined to determine if additional morphs, to those that I recorded in my field surveys, are evident.

In chapter 5, I explore genetic relationships between the species and morphs examined in chapter 4, using the mtDNA intergenic region. This analysis used the same sequences presented in chapter 3 for samples from Lizard Island, Great Barrier Reef, Australia as well as sequences from the other geographic locations i.e. Taiwan, Indonesia, Papua New Guinea, Solomon Islands, American Samoa and French Polynesia. Phylogenetic relationships determined for this marker are compared with the results of the molecular analysis of the 28S rDNA marker (chapter 2) and the morphological phylogeny presented by Wallace (1999), for species of the *Acropora humilis* species group and closely related species (Fig. 1.1). I also examine patterns of intra-individual variation for the mtDNA intergenic region, to determine whether these patterns provide additional evidence of evolutionary relationships between the species and morphs.

In chapter 6, I consolidate the findings of each chapter, summarizing the relationships between the species and morphs of the *Acropora humilis* species group, based on the

combined morphological, molecular and reproductive evidence. I then interpret these findings within an evolutionary framework and discuss the implications of my results for understanding the systematics of the genus *Acropora* and scleractinian corals.

CHAPTER 2: SPECIES BOUNDARIES WITHIN THE *ACROPORA HUMILIS* SPECIES GROUP (CNIDARIA; SCLERACTINIA): A MORPHOLOGICAL AND MOLECULAR INTERPRETATION OF EVOLUTION[*]

2.1 ABSTRACT

Species boundaries remain unresolved in many scleractinian corals. In this study, we examine evolutionary boundaries of species in the *Acropora humilis* species group. Five morphologically discrete units are recognized using principal components and hierarchical cluster analyses of quantitative and qualitative characters respectively. Maximum parsimony and likelihood analyses of partial 28S rDNA sequences suggest that these morphological units diverged to form two evolutionarily distinct lineages, with *A. humilis* and *A. gemmifera* in one lineage and *A. digitifera* and two morphological types of *A. monticulosa* in the other. Low levels of sequence divergence but distinct morphologies of *A. humilis* and *A. gemmifera* within the former lineage suggest recent divergence or ongoing hybridization between these species. Substantially higher levels of divergence within and between *A. digitifera* and *A. monticulosa* suggest a more ancient divergence between these species, with sequence types being shared through occasional introgression without disrupting morphological boundaries. These results suggest that morphology has evolved more rapidly than the 28S rDNA marker, and demonstrate the utility of using morphological and molecular characters as complementary tools for interpreting species boundaries in corals.

2.2 INTRODUCTION

Species are the basic units of measurement of biodiversity and therefore their accurate definition is critical to understanding evolutionary processes and ecological dynamics. Yet, despite the importance of species in studies of living systems, their definition and formation have long represented one of the most elusive subjects in evolutionary biology (Palumbi 1994). In scleractinian corals, a number of issues impede our understanding of the extent to which currently defined species represent evolutionary entities. Species of corals are traditionally described using morphological characters

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Wolstenholme, J. K., C. C. Wallace, C.A. Chen (2003) Species boundaries within the *Acropora humilis* species group (Cnidaria; Scleractinia): a morphological and molecular interpretation of evolution. *Coral Reefs* **22**(2): 155-166.

(e.g. Wells 1956; Veron and Wallace 1984; Wallace 1999), with morphological discontinuities being used to determine the boundaries between species (Wallace and Willis 1994). However, morphological discontinuities between currently defined species of corals are often not clear. An inherent factor contributing to this lack of resolution is morphological plasticity (Lang 1984), due to environmental influences such as light and energy regimes as well as space availability (e.g. Veron and Pichon 1976; Willis 1985; Budd et al. 1994; Muko et al. 2000). Therefore, distinguishing between morphological plasticity and genetic variation, including the recognition of possible sibling species, is essential for accurate definition of species of corals (Knowlton and Jackson 1994).

Molecular techniques greatly enhance our understanding of the evolutionary relationships between morphologically defined species. Indeed, during the past decade, electrophoretic and DNA sequence data have already provided substantial insight into these issues. Species boundaries within the genus *Porites* from the Atlantic and eastern Pacific, were unable to be resolved using morphological characters (Brakel 1977; Jameson 1997) but were resolved using electrophoretic data (Weil 1992). Two species of *Montipora*, previously synonymized as a single species, were distinguished on the basis of morphological and breeding criteria and have also been shown to be electrophoretically distinct (Stobart and Benzie 1994; Stobart 2000). Substantial morphological variability exists within the genus *Montastraea* (e.g. Foster 1985; Weil and Knowlton 1994). However, whether this variation represents separate species or morphotypes within a single polymorphic species continues to be debated (Lopez et al. 1999; Medina et al. 1999). Near or complete concordance of morphological and genetic characters has been demonstrated between species within the genera *Porites*, *Goniastrea* and between two species of *Acropora* (*A. palifera* and *A. cuneata*) (Ayre et al. 1991; Budd et al. 1994; Garthwaite et al. 1994; Babcock and Miller 1997; Hunter et al. 1997). In contrast, genetic exchange appears to be ongoing between morphological species of *Platygyra* (Miller and Benzie 1997). Genetic overlap has also been demonstrated between some species within the genera *Acropora* and *Madracis*, while other species within these genera are genetically distinct for the same molecular marker (van Oppen et al. 2000; Diekmann et al. 2001).

In corals, hybridization during multi-species mass spawning events has been proposed as the means by which common gene pools are maintained between species of corals (Miller and Benzie 1997; Hatta et al. 1999; Diekmann et al. 2001; van Oppen et al. 2001), and has been demonstrated to be possible under laboratory conditions (e.g. Willis et al. 1997). Based on this evidence, with additional support from karyotypic data, a reticulate evolutionary hypothesis has been proposed for scleractinian corals (Veron 1995; Kenyon 1997). Conversely, genetic overlap between species may merely be due to incomplete lineage sorting of ancestral genotypes, due to slow rates of molecular evolution in corals (Knowlton 2001).

Tracing the evolutionary history of scleractinian corals is clearly a very complex task but one fundamental to defining species boundaries within the Scleractinia. This is particularly true for the genus *Acropora*, the largest extant genus of scleractinian corals (Veron and Wallace 1984; Wallace 1999), with recently proposed phylogenies based on morphological characters (Wallace 1999) and molecular sequence data (van Oppen et al. 2001) suggesting conflicting patterns of evolution. Fossil records indicate that the high diversity of this genus appears to be the result of relatively recent and rapid speciation in the Indo-Pacific during and since the Miocene (Wallace 1999). Consequently, unresolved morphological and genetic boundaries between currently described species, and the ability of some species to interbreed under laboratory conditions (Wallace and Willis 1994; Willis et al. 1997), could indicate that many species of *Acropora* are still in the process of diverging.

In this paper, we examine the evolutionary relationships between species within the *A. humilis* species group in American Samoa, using morphological and molecular data. The purpose of the morphological analyses is to define morphological groupings of corals, with the aim of determining whether morphological entities can be recognized within currently described species, or alternatively whether currently described species merge to form larger overlapping morphological entities. The morphological entities defined in this study were then analyzed using partial sequences of the 28S ribosomal DNA unit. Finally, we propose evolutionary relationships for the morphological entities, based on the combined results of the morphological and molecular data.

2.3 METHODS

2.3.1 Sampling

Field work was carried out in American Samoa in January 1999. Samples were collected from seven sites on the islands of Tutuila, Ofu and Olosega (Fig. 2.1). Putative morphs, distinguished using field-recognizable and gross skeletal characters, were used as the sampling units in this study. Seven morphs were recognized from the *A. humilis* species group in American Samoa. Five colonies of each putative morph were sampled, except two rare forms for which only two and four colonies were sampled. All sites were exposed to very exposed. Each site was searched for morphs of the *A. humilis* species group, from a depth of approximately 20 m up to the reef flat.

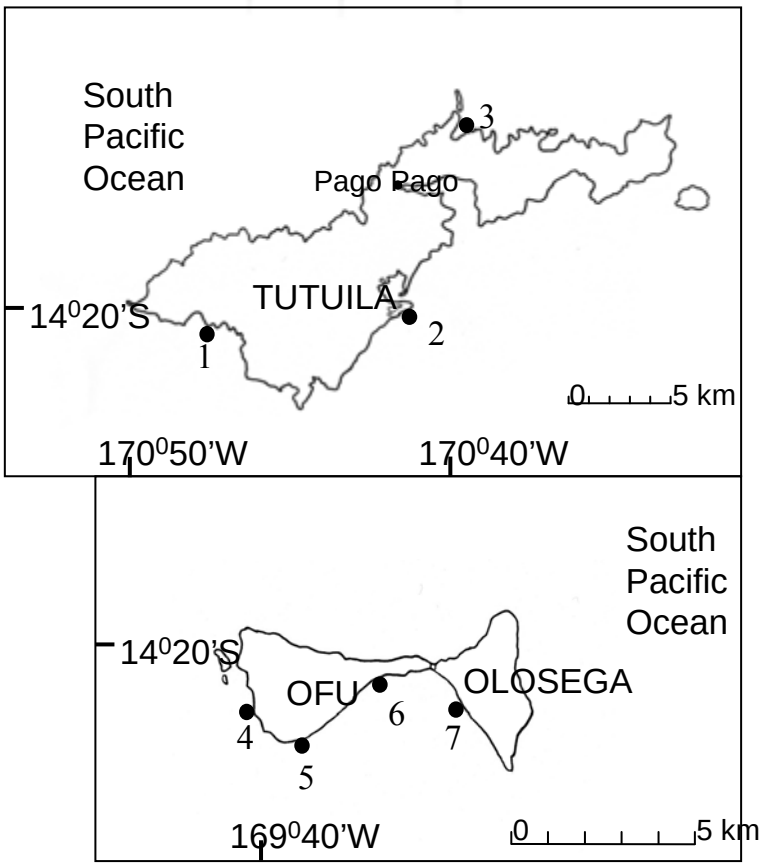

Fig. 2.1 Maps of Tutuila and Ofu-Olosega, American Samoa: indicated sampling sites and numbers correspond with Table 3. The islands of Ofu and Olosega are approximately 100 km east of Tutuila.

Samples for morphological and molecular analyses were collected from each colony sampled. All samples were used in the morphological analyses and representative samples for each putative morph were used in the molecular analysis. Samples were collected using the following protocol. First, the colony was photographed in profile to record colony appearance and the distance between branches for morphological analysis (see below). Colour of colonies and polyps, whether polyps were extended, overall colony appearance and any other distinguishing features were recorded. Five branches (the largest branches in the colony that did not have additional secondary branches developed) were collected for morphological analysis. Lastly, branch samples were collected for molecular analysis. Molecular samples for each colony were preserved in 95% (v/w) high-grade ethanol. The morphological branch samples were secured within labeled nylon bags and bleached in a sodium hypochlorite solution to remove all tissue, then rinsed in fresh water and dried. All morphological samples and corresponding molecular samples used in this study are deposited at the Museum of Tropical Queensland, Townsville, Australia (registration numbers G55587–G55617).

2.3.2 MORPHOLOGICAL ANALYSES

Analyses of morphometric and descriptive characters were used as complementary techniques to define morphological units within and between the morphs recognized in the field surveys. The morphometric analysis quantified characters as continuous variables and was therefore less subjective than the descriptive analysis. In contrast, the descriptive analysis allowed characters to be included which could not readily be quantified, particularly colony growth form, radial corallite shape, and coenosteal structure.

2.3.2.1 MORPHOMETRIC CHARACTERS

Characters used for the morphometric analysis (Table 2.1 and Fig. 2.2) were adapted from a previous study (Wallace et al. 1991). Character 1 (distance between branches) was measured from photos of live colonies, using Image Tool 2.00 (Wilcox et al. 1995-96). Characters 2–14 were measured directly from skeletal branch samples, using Vernier calipers for branch dimensions (characters 2–5) and a microscope and ocular graticule for corallite dimensions (characters 8–14). Characters 6 and 7 were measured by counting the number of corallites intersecting a 3 cm transect around the branch. Diameters and lengths of radial corallites were measured from mature corallites,

Table 2.1 Morphometric characters measured in this study

No.	Character	Code	Description
1	Branch spacing	brdist	Distance to the five nearest branches
2	Basal branch diameter	diambase	Diameter at base of branch
3	Mid branch diameter	diammid	Diameter at mid-point of branch length
4	Branch tip diameter	diamtip	Diameter 5 mm from tip of branch
5	Branch length	brlength	Distance from tip to base of branch
6	Radial crowding	radcor	Average number of regular radial corallites / 3 transects
7	No. of subimmersed radials	subimm	Average number of subimmersed radial corallites / 3 transects
8	Diameter of axial calice	axcal	Average distance between inner walls of axial corallite, measured as perpendicular diameters
9	Axial wall thickness	axwall	Width of axial wall
10	Septal length	axsepta	Average length of primary septa (usually 6) in axial corallite
11	Profile length	rcprolen	Maximum distance from base to outer edge of corallite
12	Corallite diameter	rcordiam	Maximum diameter of corallite from inner to outer wall
13	Calice diameter	rcaldiam	Maximum diameter of calice from inner to outer wall
14	Outer wall thickness	rcwall	Thickness of outer wall of radial corallite

defined as the largest radial corallites on the branch that did not have smaller corallites budding from their surface.

Morphometric characters were analyzed using principal components analysis (PCA). PCA is an exploratory tool, in which no a priori assumptions are made. PCA was therefore used to explore morphological distance, both within and between morphs. Characters 2–10 were measured from five branches. Characters 11–14 were measured for five radial corallites on each of five branches. The average value for each character for each coral colony was used in the analysis. The data matrix was standardized as a correlation matrix, to equally weight the branch and corallite measurements. Analysis was carried out in SPSS 9.0, using the factor analysis option.

2.3.2.2 DESCRIPTIVE CHARACTERS

Characters used for the descriptive analysis are listed in Table 2.2 and were adapted from a previous study (Wallace 1999). The same colonies and branch samples used in the morphometric analysis were used in this analysis. Characters 1 and 2 were coded from photos and field notes. Characters 3–20 were coded directly from the skeletal branch samples. The descriptive characters were analyzed using hierarchical cluster

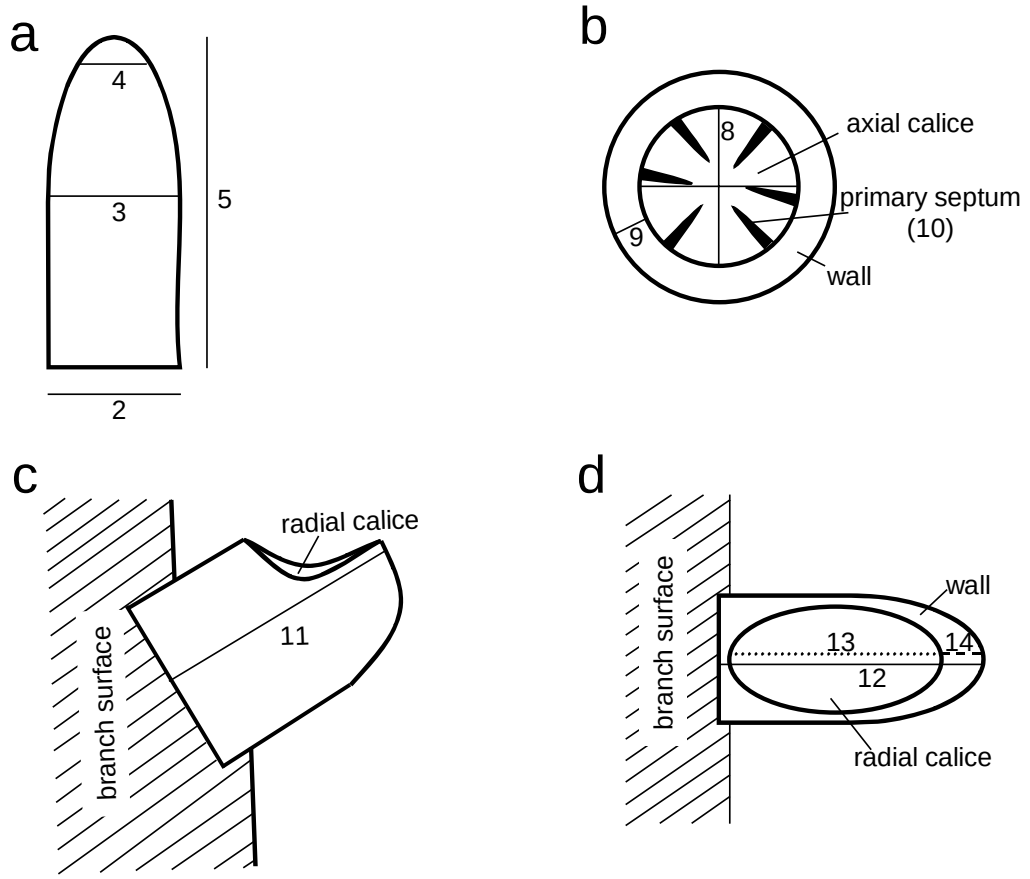

Fig. 2.2 Diagrammatic branch and corallite dimensions measured in the morphometric analysis: **a** single branch; **b** upper view of axial corallite; **c** profile view of radial corallite; and **d** upper view of radial corallite. *Numbers* correspond with characters 2–5 and 8–14 listed in Table 1. Characters 1, 6 and 7 are described in Table 1 and in the 'Methods' section.

analysis. As in the morphometric analysis, no prior assumptions were made about the relationships between colonies. Analysis was carried out using NTSYSpc 2.10d (Rohlf 1986-2000), using the sequential agglomerative hierarchical nested (SAHN) cluster analysis option. The clustering method used was the Unweighted Pair-Group Method using Arithmetic Averages (UPGMA).

Table 2.2 Descriptive morphological characters used in this study

No.	Character	Code	States	Coding
1	Colony outline	determ	Determinate from a focused origin	0
			Indeterminate	1
2	Predominant outline	growth	Arborescent/divergent	0
			Corymbose	1
			Digitate	2
3	Branch structure	axvsrad	Axial dominated	0
			Axials ≅ radials	1
4	Coenosteum	coentype	Same on and between radial corallites	0
			Different on and between radial corallites	1
5	Coenosteum on radial corallites	radcoen	Costate or reticulo-costate	0
			Open spinules	1
6	Coenosteum between radial corallites	axcoen	Reticulo-costate	0
			Reticulate	1
			Open spinules	2
7	Spinule shape	spinules	Single pointed, fine	0
			Blunt, irregular, sturdy pointed	1
			Elaborate	2
8	Radial corallite sizes	rcsize	One size or graded, with occasional, scattered small radials	0
			Two distinct sizes	1
			Variable	2
9	Radial corallite inner wall	rcinwall	Developed	0
			Not developed	1
			Reduced	2
10	Radial corallite shape	rcshape	Tubo-nariform	0
			Dimidiate	1
			Lipped	2
			Tubular	3
11	Radial corallite openings	rcopen	Oval	0
			Rounded	1
12	Axial corallite diameter	axdiam	Large, > 3.0 mm	0
			Medium, 2.8-3.0 mm	1
			Small, < 2.8 mm	2
13	Radial corallites	relsize	Large	0
			Medium	1
			Small	2
14	Maximum branch thickness	brthick	> 25 mm	0
			20-25 mm	1
			15-20 mm	2
			< 15 mm	3
15	Branch taper (tip=3 mm below branch tip)	taper	Broad conical (base > twice tip)	0
			Conical (base broader than tip)	1
			Terete (no to slight taper)	2
16	Maximum branch length	brlength	≥ 80 mm	0
			≥ 70 mm	1
			≥ 60 mm	2
			≥ 50 mm	3
			≥ 40 mm	4
			≥ 30 mm	5
17	Radial crowding	crowding	Radials do not touch	0
			Some radials touch	1
			Radials crowded, touching	2
18	No. axial corallite synapticular rings	axrings	2	0
			3–4	1
			> 4	2
19	Skeletal porosity	porosity	Radial walls porous	0
			Radial walls not porous	1
20	No. radial corallite synapticular rings	rcrings	2–3	0
			> 3	1

2.3.3 MOLECULAR ANALYSIS

The 28S nuclear large subunit rDNA (domains 1 and 2) was used for the molecular analysis. DNA was extracted from branch fragments of approximately 3–4 g wet weight, based on protocols described by Chen et al. (2000) and Chen and Yu (2000). Branch fragments were ground to a fine powder in liquid nitrogen and mixed with an equal volume of DNA extraction buffer (5M NaCl, 0.5M EDTA, pH 8.0, 2%SDS), to which 100 µg/ml of proteinase K was added. The solution was incubated overnight in a water bath at 50 °C. DNA was extracted using phenol/chloroform and precipitated in absolute ethanol. Following precipitation, the genomic DNA was dried, resuspended in TE buffer and stored at –20 °C. The target segments, domains 1 and 2 from 28S rDNA, were amplified using the primers 5S: 5'-GCCGACCCGCTGAATTCAAGCATAT-3' and B35: 5'-CCAGAGTTTCCTCTGGCTTCACCCTATT-3' (developed by Chen et al. 2000). The amplification reaction used 100–200 ng of DNA template and BRL *Taq* polymerase in a 50 µl reaction, in the presence of the buffer supplied with the enzyme (as per manufacturer's instructions). PCR was performed in a PC-960G gradient thermal cycler using the following thermal cycles: 1 cycle at 95 °C (4 min); 30 cycles at 94 °C (30 sec), 50 °C (1 min), 72 °C (2 min); 1 cycle at 72 °C (10 min); 1 cycle at 25 °C (30 sec). PCR products were electrophoresed in a 0.8% agarose (FMC Bioproduct) gel in 1x TAE buffer to assess the yield. PCR products were cloned using the ligation kit, pGEM T easy (Promega) and DH5α competent cells (BRL), under the conditions recommended by the manufacturers. Bacterial colonies containing the vector were picked with a sterile toothpick and cultured for 6–12 hours in a 4 ml LB nutrient solution and purified using a plasmid DNA mini-prep kit (Viogene). Nucleotide sequences were generated for pairs of complementary strands on an ABI 377 Genetic Analyzer using the ABI Big-dye Ready Reaction kit following standard cycle sequencing protocol. The sequences were submitted to GenBank under accession numbers AY139650–AY139681.

Sequences were initially aligned using ClustalX (Thompson et al. 1997) and then optimised manually within variable regions. The distance matrix comparing the pair wise differences was calculated in PAUP* 4.0b10 (Swofford 2002), as were the maximum parsimony and maximum likelihood analyses. Maximum parsimony was run using the heuristic search option, with 10 random additions of sequences to search for

the most parsimonious trees. Bootstrapping with 1,000 pseudoreplicates determined the robustness of clades, with branches supported by <50% collapsed. Analyses were run with gaps excluded from the analysis, as well as treating gaps as a fifth character. The most appropriate evolutionary model for the maximum likelihood analysis was selected using Modeltest (Posada and Crandall 1998). Maximum likelihood analysis was run using the heuristic search option, with 10 random additions of sequences. Bootstrapping with 500 pseudoreplicates determined the robustness of clades, with branches supported by <50% collapsed.

Acropora palifera, of the subgenus *Isopora*, was used as the outgroup. This species was selected as an appropriate outgroup taxon because the two subgenera (*Isopora* and *Acropora*) are thought to have diverged early in the history of the genus, and the *A. humilis* species group occupies a basal position within the morphological phylogeny of the genus *Acropora* (Wallace 1999). The *A. palifera* sample used was collected by C. C. Wallace in September 1999 in the Togian Islands, central Sulawesi, Indonesia (Museum of Tropical Queensland registration number G55715).

2.4 RESULTS

2.4.1 MORPHOLOGICAL ANALYSES

The seven morphs recognized during field surveys (Fig. 2.3) clustered as five morphological units (Figs. 2.4 and 2.5). These morphological units correspond with the species *A. humilis* (Dana 1846), *A. gemmifera* (Brook 1892), A. *digitifera* (Dana 1846), and two forms (branching and digitate) of *A. monticulosa* (Brüggemann 1879). Characters describing each morph are summarized in Table 2.3. In both the quantitative and qualitative morphological analyses, all morphs except *A. humilis* formed discrete, non-overlapping units corresponding with the putative groupings (Figs. 2.4 and 2.5). The *A. humilis* morph comprises the remaining three undifferentiated putative groupings (*A. humilis* 1, *A. humilis* 2 and *A. humilis* 3). All morphs, except the two forms of *A. monticulosa*, were generally common at each of the seven sites surveyed. All colonies of the branching form of *A. monticulosa* were sampled at site 1 and both colonies of the digitate form of *A. monticulosa* at site 7 (Fig. 2.1). Data matrices used in the morphometric and descriptive analyses are available as electronic supplementary material (appendices I and II respectively).

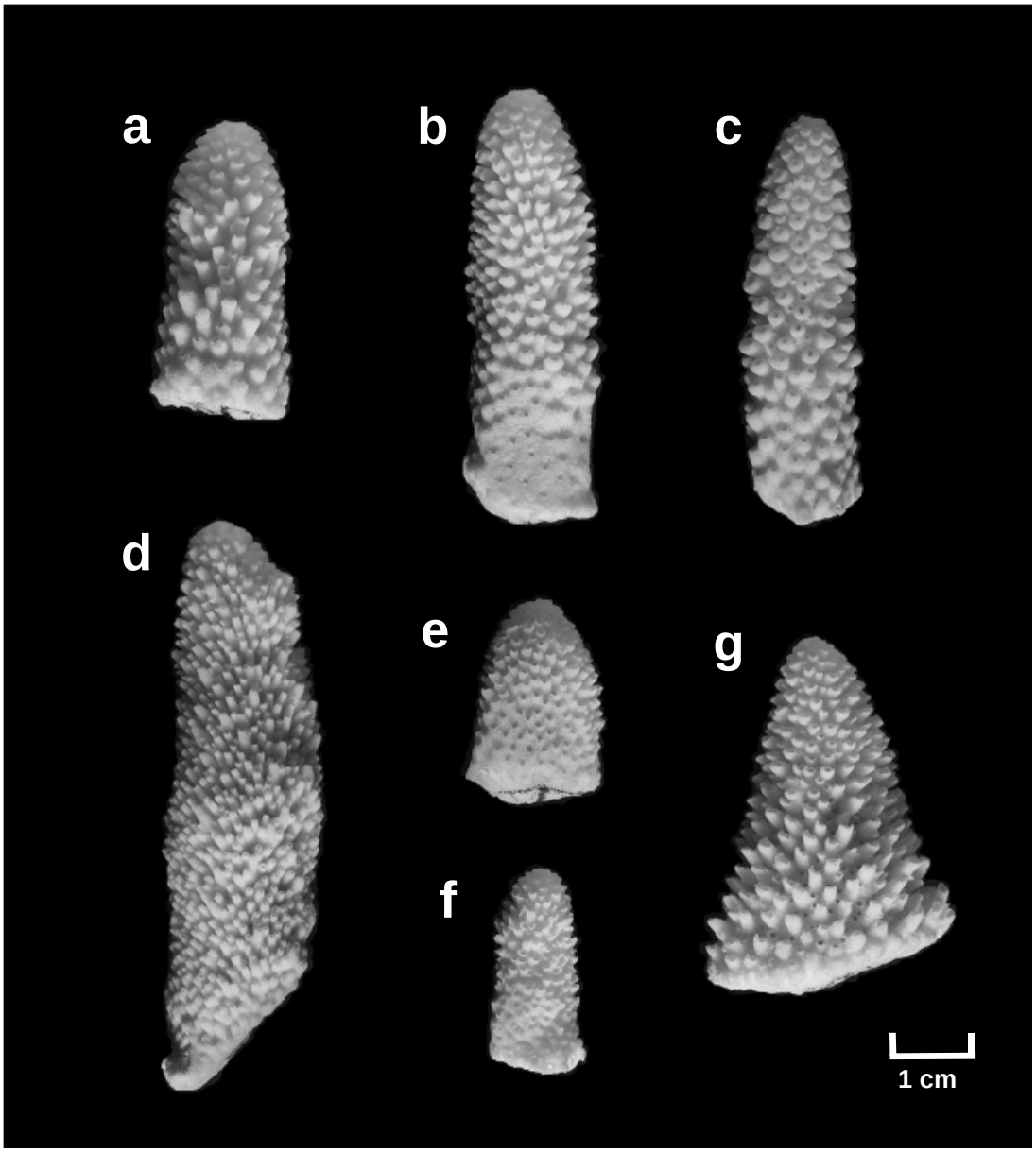

Fig. 2.3 Branch skeletons of each putative morph. Museum of Tropical Queensland registration numbers are listed in brackets after the name of each morph: **a** "*A. humilis* 1" (G55591); **b** "*A. humilis* 2" (G55593); **c** "*A. humilis* 3" (G55599); **d** "branching *A. monticulosa*" (G55617); **e** "digitate *A. monticulosa*" (G55612); **f** *A. digitifera* (G55602); **g** *A. gemmifera* (G55607).

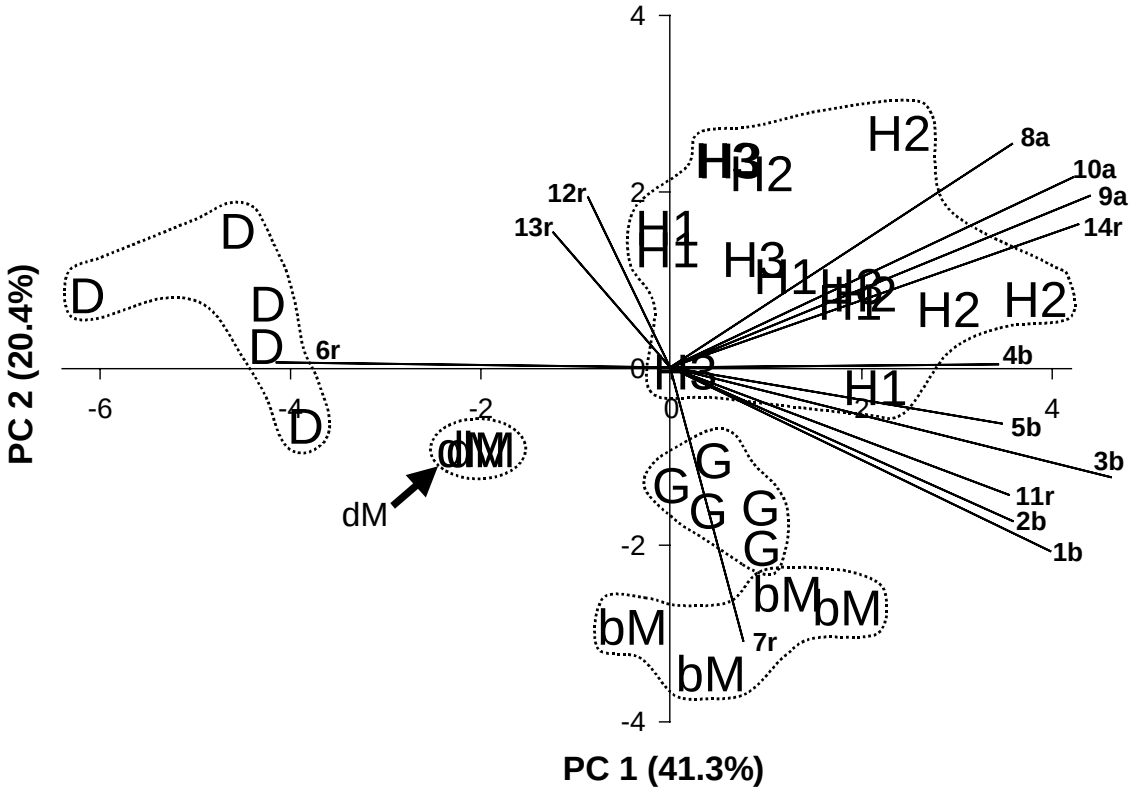

Fig. 2.4 PCA scatterplot of morphometric characters for principal components (PC) 1 and 2. Each data point in the PCA plot represents a single colony. Codes for each data point are indicated by codes for each morph as follows: *H1* "*A. humilis* 1"; *H2* "*A. humilis* 2"; *H3* "*A. humilis* 3"; *bM* "branching *A. monticulosa*"; *dM* "digitate *A. monticulosa*"; *D A. digitifera*; *G A. gemmifera*. *Envelopes* highlighting the clusters of each morph were drawn by eye. Length and direction of vectors indicate the relative effect of each character on distribution of morphs within the plot. *Numbers* for each vector correspond with character codes in Table 1 and *letters* indicate vector type: *b* branch character; *a* axial character; *r* radial character. Two colonies of H3 and two colonies of dM almost overlay each other; one colony of H3 lies almost at the origin.

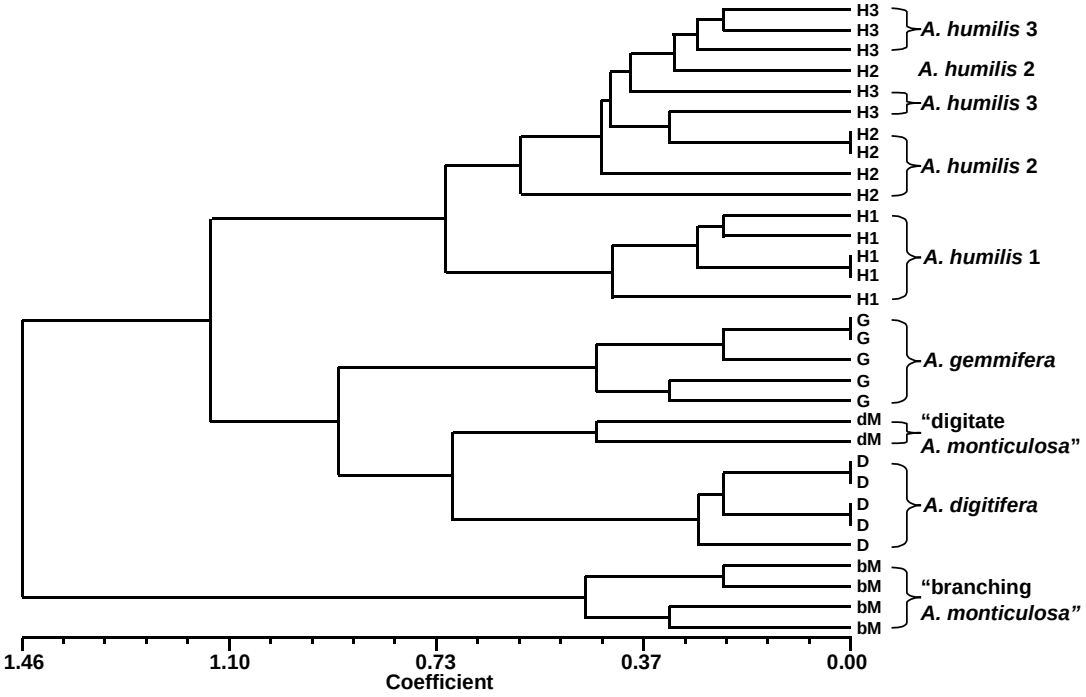

Fig. 2.5 Hierarchical cluster analysis (UPGMA) of descriptive morphological characters. Each branch of the dendrogram represents a single colony. Codes for each morph are the same as listed for Fig. 4.

2.4.1.1 MORPHOMETRIC CHARACTERS

The relationships between the five morphs revealed by PCA of the morphometric characters are presented in Fig. 2.4, with each data point representing a single colony. Colonies of each of the five morphs (*A. humilis*, *A. gemmifera*, A. *digitifera*, "branching *A. monticulosa* and "digitate *A. monticulosa*") formed discrete clusters. The morphometric characters quantified branch, axial corallite, and radial corallite dimensions. Characters quantifying radial corallites (size and spacing) were most useful for separating the five morphs. The three axial corallite characters were highly correlated, as were the five characters that quantified branch dimensions. The analysis therefore indicates that *A. digitifera* colonies were characterized by relatively crowded, small radial corallites and thin, short branches. The morph "branching *A. monticulosa*" was characterized by a high proportion of subimmersed radial corallites, which were oval rather than elongate in cross section. The three putative morphs of *A. humilis* were all strongly characterized by the axial characters and radial corallite wall thickness and width, with the radials tending to be widely spaced. The morphs "digitate *A. monticulosa*" and *A. gemmifera* were not strongly influenced by any particular characters. The short branches and relatively small, thin walled axials were the most

Table 2.3 Descriptions of morphs recognized from the *Acropora humilis* species group in American Samoa in this study. Characters are described relative to the other morphs in this table. Characters that are most important for distinguishing morphs are highlighted in *bold*. The distinctive features of the three putative morphs of *A. humilis* are noted. Sites sampled correspond with sites in Fig. 2.1.

	Habitat	Growth Form	Branches	Axial Corallites	Radial Corallites	Colony Colour	Sites Sampled
A. humilis	Exposed slopes, just subtidal to >20m *A. humilis* 1: shallower more exposed slopes	Digitate to corymbose *A. humilis* 3: approaching caespito-corymbose due to more secondary branching	Terete and long *A. humilis* 1: shorter branches *A. humilis* 2: longer branches	**Large to very large**	One size, large, nariform to tubo-nariform, not crowded	Brown with pale brown, white or green branch tips, polyps are white, green or sometimes pale brown and may be partly extended during the day	*A. humilis* 1: 4, 6, 7 *A. humilis* 2: 4, 6 *A. humilis* 3: 1, 4, 5
A. gemmifera	Exposed slopes, just subtidal to >10m	Digitate to corymbose	Conical and medium length	Medium	**Two sizes**, large: tubular with **dimidiate openings**; small: subimmersed, crowded	Brown with paler to white or sometimes blue branch tips, polyps brown or white and may be partly extended during the day	2, 3, 4
"branching *A. monticulosa*"	Shallow, semi-exposed reef tops to ~4m	**Divergent arborescent branching, proximal parts of branches dead**	Tapering at branch tips, variable length	Small	Mixed sizes, small, **elongate, cylindrical tubes interspersed with scattered subimmersed corallites, crowded**	Brown with a yellow or green tinge all over, sometimes slightly paler at branch tips, polyps are the same colour as the surrounding corallites	1
"digitate *A. monticulosa*"	Shallow, wave exposed crests to ~4m	Digitate	Terete, short, evenly sized, regularly spaced	Small	One size, small, **nariform**, crowded	Pale to dark brown sometimes with paler blue or paler brown branch tips, polyps dark brown and maybe partly extended during the day	7
A. digitifera	shallow, wave exposed crests to ~4m	Digitate	Terete, short, evenly sized, regularly spaced	Small	Mixed sizes, small, interspersed with scattered subimmersed corallites, **lipped**, crowded	Pale to dark brown sometimes with paler blue or paler brown branch tips, polyps dark brown and maybe partly extended during the day	1, 3

distinctive characters for the "digitate *A. monticulosa*" colonies and the high proportion of subimmersed radial corallites was the most distinctive character for colonies of *A. gemmifera*.

2.4.1.2 DESCRIPTIVE CHARACTERS

Qualitative analysis of the morphological characters showed the same separation of morphs as the morphometric analysis, with colonies of each of the five morphs (*A. humilis*, *A. gemmifera*, A. *digitifera*, "branching *A. monticulosa*" and "digitate *A. monticulosa*") clustering as distinct groups. The relationships within and between the morphs, based on UPGMA analysis of the descriptive characters, are shown in Fig. 2.5. A high cophenetic correlation of 0.96 calculated for this dendrogram indicates that the pattern of clustering is a true representation of the original data set. Analysis of the descriptive characters, using both single and complete linkage methods (calculated separately and as a strict consensus tree) grouped colonies in a similar pattern to the UPGMA analysis, differing only in branch lengths and the ordering of colonies within the *A. humilis* cluster.

As also demonstrated in the morphometric analysis, the two morphs of *A. monticulosa* clearly have very distinct morphologies. Colonies of "branching *A. monticulosa*" showed the greatest dissimilarity to all other morphs within the *A. humilis* species group, while colonies of "digitate *A. monticulosa*" shared most morphological characters with *A. digitifera* and *A. gemmifera*. Within the *A. humilis* cluster, "*A. humilis* 1" colonies formed a subcluster, while "*A. humilis* 2" and "*A. humilis* 3" colonies were not differentiated. Colonies of the putative morph "*A. humilis* 1" all had short branches and a different coenosteal structure compared with "*A. humilis* 2" and "*A. humilis* 3", while all other characters were shared between these three putative morphs.

2.4.2 MOLECULAR ANALYSIS

The major findings in this analysis, based on the sequences examined, were that the morphs *A. digitifera*, "branching *A. monticulosa*", and probably "digitate *A. monticulosa*" were distinct from the morphs *A. humilis* and *A. gemmifera*. Variability between sequences of the former three morphs was substantially greater than between the latter two morphs. Cloned sequences from domains 1 and 2 of the 5' end of 28S

rDNA were obtained from colonies of the seven putative morphs. In total, 32 clones from 15 coral colonies were sequenced. Sequence divergence ranged from 0–14.8% between morphs of the *A. humilis* species group, compared with 22.3–30.8% when compared with the *A. palifera* outgroup sequences. Nucleotide composition was similar for all clones, with an average GC (Guanine-Cytosine) content of 62.57%. GC content was slightly lower in clones isolated from morphs of the *A. humilis* species group (61.15–63.53%) compared with the two *A. palifera* outgroup sequences (64.98%).

The aligned sequences consisted of 907 positions, with individual sequences ranging in length from 782–851 bp. Within the aligned sequences, 635 positions were constant, 37 variable characters were not parsimony informative, and 235 (25.9%) were parsimony informative.

Maximum parsimony (MP) and maximum likelihood (ML) analyses grouped the sequences into four strongly supported clades. Sequences from *A. digitifera* and the two morphs of *A. monticulosa* were substantially more divergent than those from *A. humilis* or *A. gemmifera*. The phylogenetic tree from the MP analysis (Fig. 2.6, 50% majority-rule consensus tree based on 885,920 trees) formed two branches grouping clades I, II and III separately from clade IV. There were only low levels of divergence, indicating high levels of similarity between sequences within each of the four clades. Clade I grouped all but one sequence from *A. digitifera* (seven sequences from two colonies) with the other sequence from a third colony grouping with "branching *A. monticulosa*" in clade III. Sequences from "digitate *A. monticulosa*" grouped in clades I and II, indicating that this morph shares sequence types with both *A. digitifera* and "branching *A. monticulosa*". Sequences from two colonies of "branching *A. monticulosa*" grouped in clades II and III, indicating that there were two distinct types present within each colony of this morph. The remaining clade IV contained all sequences from the *A. humilis* and *A. gemmifera* morphs in addition to one sequence from the digitate morph of *A. monticulosa*. This latter sequence from "digitate *A. monticulosa*" appears to be an

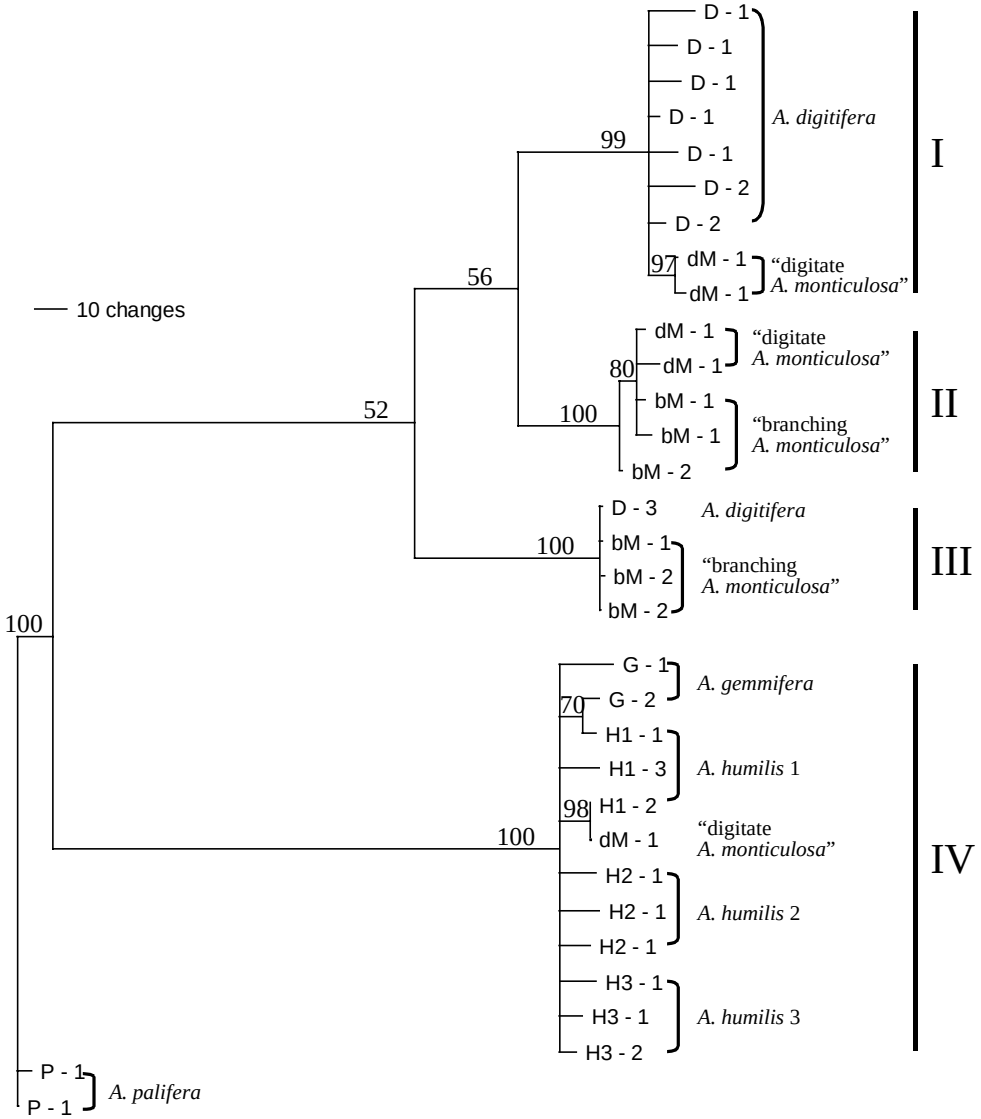

Fig. 2.6 Maximum parsimony consensus tree (50% majority-rule) of the partial 28S sequences (domains 1 and 2). *Numbers* above branches indicate percent bootstrap support; branches with <50% support have been collapsed. Tree length: 481; CI: 0.696; RI: 0.850; HI: 0.304. *Vertical bars at right of tree* indicate the 4 major clades (I–IV). Codes for each morph are the same as listed in Fig. 4. *Numbers after the hyphen* identify different coral colonies within each putative morph. *Scale bar* indicates number of nucleotide substitutions along branches.

erroneous sequence for two reasons. First, this sequence is almost identical to one of the *A. humilis* sequences. Second, the sequences cloned from the A. *humilis* and *A. gemmifera* morphs have very low levels of divergence and are otherwise very distinct from all sequences cloned from the other morphs (Fig. 2.6). Negative controls were consistently clear in all PCR reactions and so the source of error is most likely to have occurred during cloning. It is possible that this sequence is a cloning artifact, although contamination may also be the source.

The four clades were identical in composition when gaps were excluded from the analysis or treated as a fifth character in the MP analysis. Treating gaps as a fifth character produced a tree with two differences to that in Fig. 2.6. A shorter branch connected the ingroup and outgroup sequences and bootstrap support increased for the branch grouping clades I and II from 56 to 91%. Analysis of the sequences using ML also produced four clades identical in composition to the MP analysis, with 70, 96, 99 and 100% bootstrap support for clades I–IV, respectively. The structure of the ML tree differed in that the four clades formed a polytomy (compared with the grouping of clades I–III as a single branch in the MP analysis) with a longer branch length separating the ingroup from the outgroup sequences. The GTR + G + I model (G = 0.5612 and I = 0.3768) was selected for the maximum likelihood analysis.

2.5 DISCUSSION

2.5.1 SYNTHESIS OF MORPHOLOGICAL AND MOLECULAR FINDINGS

The seven putative morphs recognized in this study, within the *A. humilis* species group in American Samoa, clustered as five morphological units (Figs. 2.4 and 2.5). These morphological units correspond with the species *A. humilis*, *A. gemmifera*, *A. digitifera*, and 2 forms (digitate and branching) of *A. monticulosa*. Cloned sequences of the 28S nuclear rDNA unit from each of these morphs formed four strongly supported clades (Fig. 2.6). All sequences from *A. humilis* and *A. gemmifera* grouped in a single clade with little further differentiation. Sequences from *A. digitifera* and the two morphs of *A. monticulosa* grouped in the other three clades with sequences from pairs of each of these three morphs in each clade, indicating high levels of sequence variation within and between these three morphs. Based on the partial 28S rDNA sequences cloned in this study, we propose that the morphs *A. humilis* and *A. gemmifera* are evolutionarily distinct from *A. digitifera* and *A. monticulosa*. The distinct morphologies but low levels

of sequence divergence between *A. humilis* and *A. gemmifera* suggest recent divergence between these morphs. In contrast, sequence types appear to be shared through occasional introgression while maintaining the distinct morphologies of the three morphs, *A. digitifera*, "digitate *A. monticulosa*", and "branching *A. monticulosa*".

2.5.1.1 DIVERGENCE BETWEEN CLADES

All sequences cloned from colonies of the morphs *A. humilis* and *A. gemmifera* grouped in a single clade with complete bootstrap support (Fig. 2.6, clade IV) in both maximum parsimony and likelihood analyses. Based on these analyses, these morphs appear to be an evolutionary lineage that is distinct from *A. digitifera* and *A. monticulosa*. To confirm this proposal, additional sequences from colonies of "digitate *A. monticulosa*" are necessary to verify that the anomalous sequence for this morph in clade IV is erroneous. Alternatively, additional sequences will reveal the mechanisms of introgression operating between this and the other morphs examined in this study. The other three clades (Fig. 2.6, clades I, II and III), comprising sequences from *A. digitifera* and *A. monticulosa*, form a single branch with weak bootstrap support in the parsimony analysis and a polytomy with clade IV in the likelihood analysis. This indicates that each of these clades are also distinct, but probably share greater affinity with each other than with clade IV.

2.5.1.2 DIVERGENCE WITHIN CLADES

The extremely low level of sequence divergence between *A. humilis* and *A. gemmifera* in clade IV (Fig. 2.6) can be interpreted by the fact that either these morphs diverged too recently for these lineages to be assorted or they have not diverged and interbreeding between these two morphs is ongoing. The species *A. humilis* and *A. gemmifera* are often very similar morphologically at many locations within their distribution range, to the extent that the two species may be difficult to distinguish (Wallace 1999). However, in American Samoa the morphs representing these two species were morphologically distinct, with no overlap or merging of morphological characters (Table 2.3, Figs. 2.4 and 2.5), suggesting that recent divergence seems most plausible. Analysis of a maternally inherited mitochondrial marker is now underway, which will provide additional evidence on whether or not the low level of sequence divergence reported in this study represents recent common ancestry between these

now morphologically distinct morphs. Breeding trials are also being conducted to test the potential for these species to interbreed.

The partial 28S sequences cloned from *A. digitifera* and both forms of *A. monticulosa* were highly divergent, due to distinct sequence types being found within and between these morphs. The high to complete bootstrap support for clades I, II and III indicate that distinct sequence types are present in different colonies of *A. digitifera*, the *A. monticulosa* morphs and even in single colonies of each of the *A. monticulosa* morphs (Fig. 2.6). The high levels of sequence variation within and shared sequence types between these morphs were surprising because the three morphs were morphologically distinct, and colonies within each of these morphs were indistinguishable as live colonies and in skeletal samples (personal observation; Figs. 2.4 and 2.5). It therefore appears that these three morphs share a common ancestral lineage, in which lineage sorting is incomplete, and that gene flow may still be occurring between the three morphs through occasional hybridization and backcrossing. The high levels of divergence in these morphs may also be due to common ancestry with species not examined in this study, possibly those that share the monophyletic clade with *A. digitifera* in the phylogeny proposed by Wallace (1999) for the genus *Acropora*. More sequences are necessary from the same colonies and additional colonies of these morphs in order to confirm these proposals.

The grouping of sequences from "branching *A. monticulosa*" and "digitate *A. monticulosa*" in different clades is significant, given that these two morphs fall within the limits of a single species according to the current taxonomic description of *A. monticulosa* (Wallace 1999). Although these two morphs belong to the same taxonomic species, they were distinct morphologically, having different growth forms, differently shaped radial corallites and coenosteal structure (Figs. 2.4 and 2.5), as well as different colours of live colonies (Table 2.3). Despite the distinct morphological groupings of the *A. digitifera* and two *A. monticulosa* morphs (Figs. 2.4 and 2.5), the digitate form of *A. monticulosa* also shared apparent affinities with the *A. digitifera* colonies, having a similar growth form and colour of live colonies. Based on these morphological affinities and the shared sequence types, we tentatively propose that the morph "digitate *A. monticulosa*" may have been derived from *A. digitifera* and "branching *A. monticulosa*", and that the two *A. monticulosa* morphs represent sibling species.

2.5.2 EVOLUTIONARY IMPLICATIONS

The large subunit 28S nuclear rDNA gene is usually used in phylogenetic studies to examine the evolutionary relationships at higher taxonomic levels than species because of its relatively slow rate of evolution (Hillis and Dixon 1991). For example, it has been used to examine phylogenetic relationships between taxa within the Phylum Cnidaria (Odorico and Miller 1997a), Class Anthozoa (Chen et al. 1995), and Order Scleractinia (Veron et al. 1996; Romano and Cairns 2000). In contrast, more rapidly evolving markers, such as ITS-1, ITS-2, and the 5.8S gene of the nuclear ribosomal DNA unit, the mtDNA putative control region, *Pax-C* and the mini-collagen gene have been used to study species boundaries within the genus *Acropora* (Odorico and Miller 1997b; Hatta et al. 1999; van Oppen et al. 2000; van Oppen et al. 2001). These studies all conclude that the lack of resolution found between many species of *Acropora* using these markers indicates that this genus is evolving in a reticulate rather than a divergent pattern. It seems likely, however, based on the molecular evidence in these papers and the morphological and molecular evidence in this paper, that complex evolutionary relationships exist between species within the genus *Acropora*, with boundaries between species currently at various stages of formation.

In this study, we demonstrate that domains 1 and 2 of the 28S nuclear rDNA unit contain important information for interpreting the evolutionary relationships between *A. digitifera*, the *A. monticulosa* morphs and the *A. humilis–A. gemmifera* lineage, while more rapidly evolving markers are likely to be most useful for interpreting evolutionary relationships between more recently diverged species such as *A. humilis* and *A. gemmifera*. Additional sequences are necessary to fully utilise the potential of this 28S marker and provide a more comprehensive analysis of the evolutionary relationships between species examined in this study as well as their relationship with other species in the genus *Acropora*. To achieve this, additional sequences are needed from the individuals and morphs examined in this project, particularly the *A. digitifera* and *A. monticulosa* morphs, as well as other species of the genus *Acropora* occuring in American Samoa. These additional sequences will reveal the number of sequence types within individual colonies and individual morphs as well as the levels of divergence and evolutionary affinities between morphs. The 28S marker is also likely to be useful

for examining evolutionary relationships between these species over a broader biogeographic area because of its slow rate of evolution.

The existence of recognizably discrete groups of organisms, as seen in this study, "argues against the idea that species are simply peaks in a continuum of variation" (Vogler 2001).This view is reiterated for corals by Wallace and Willis (1994), who state that the apparent morphological consistency of many species across a broad geographic range supports the validity of species as real taxonomic entities. The presence of the five discrete morphs of the *A. humilis* species group in American Samoa, based on morphological characters, supports this view. Shared DNA sequences between currently described species, previously interpreted as evidence for reticulate evolution in corals (Odorico and Miller 1997b; Hatta et al. 1999; van Oppen et al. 2000; Diekmann et al. 2001; van Oppen et al. 2001), are equally likely to represent common ancestry and be evidence of either recent or ancient divergence. Substantial genetic overlap may exist between sister or other closely related species because only one or a small number of genes may cause speciation, with large regions of the genome remaining unchanged until the process of speciation is complete (Mayr 1963; Wu 2001).

Hybridization has been proposed by advocates of reticulate evolution as the mechanism preventing species of corals from diverging as discrete evolutionary lineages (Veron 1995; Odorico and Miller 1997b; Hatta et al. 1999; van Oppen et al. 2000; Diekmann et al. 2001; van Oppen et al. 2001). The opportunity for hybridization in corals appears to be considerable because fertilization in many species occurs externally during interspecific mass spawning events, in which gametes are mixed as they are released and aggregate at the water surface. However, it is equally plausible that hybridization in corals may retard but not prevent the final stages of divergence. If the latter is true, a major implication is that the time since speciation is likely to be underestimated in molecular phylogenetic analyses because genetic characters may have been shared through occasional interbreeding. Hybridization, leading to polyploidy, has also been proposed as a direct mechanism of rapid, sympatric speciation in the genus *Acropora* (Kenyon 1997) as is well known in angiosperms [reviewed by Arnold (1997)]. Therefore, as well as maintaining shared gene pools between recently diverged species, hybridization may also be a sudden means of divergent evolution. Further research is

necessary to determine the extent to which hybridization occurs under natural circumstances and its role in the evolutionary history of corals.

The results presented in this paper demonstrate the importance of interpreting morphological and molecular characters in complementary analyses to resolve species boundaries in corals (Willis 1990; Stobart 2000), with analysis of morphological characters providing additional information not revealed in the phylogeny of the sequence data. This conflicts with the findings of van Oppen (2001) that morphology has little predictive value in defining distinct evolutionary units. Concordance of the morphological and molecular data for *A. humilis*, grouping the three putative morphs as a single unit, strengthens support for colonies of these putative groupings belonging to a single species, despite the morphological variation recognized in field surveys. The recognition of two morphs within *A. monticulosa*, which may represent two sibling species, also demonstrates the utility of examining corals at the intraspecific level for tracing evolutionary relationships between species.

To enable further resolution of the evolutionary relationships of morphologically defined species of corals, it will be necessary to examine fossil and extant morphs across broad biogeographic ranges as well as trace their ancestry both directly in fossil lineages and indirectly using increasingly sophisticated molecular tools. Augmenting morphological and molecular studies with interspecific breeding experiments will also provide valuable insights into the current reproductive potential of individual morphs. Clearly, as demonstrated in this study, to resolve species boundaries and the evolutionary relationships of species in corals, it is important to work at the intraspecific level. This will enable possible sibling and intermediate species, as well as evolutionarily discrete species, to be recognized.

CHAPTER 3: TEMPORAL REPRODUCTIVE ISOLATION AND GAMETIC COMPATIBILITY ARE EVOLUTIONARY MECHANISMS IN THE *ACROPORA HUMILIS* SPECIES GROUP (CNIDARIA; SCLERACTINIA)[*]

3.1 ABSTRACT

Patterns of interbreeding between individuals are fundamental to the structure and maintenance of evolutionary boundaries between species. In corals, both hybridization and reproductive isolation appear to be important evolutionary mechanisms. In this study, I examine evolutionary boundaries using morphological, molecular and reproductive criteria within the *Acropora humilis* species group at Lizard Island on the Great Barrier Reef, Australia. Five species and seven morphs are recognized on the basis of morphological appearance of features traditionally used to identify corals of the genus *Acropora*. In a molecular phylogenetic analysis, I examine relationships for the mtDNA putative control region, using maximum parsimony and maximum likelihood methods. The reproductive criteria explore whether species and morphs are reproductively isolated on the basis of temporal or fertilization barriers. Timing of gamete maturity is surveyed for each species and morph, from the month prior to and three months after the mass spawning. Time of spawning is documented at the levels of night and hour of spawning, and time taken for egg-sperm bundles to separate. Laboratory fertilization experiments tested the potential of species and morphs to interbreed. High levels of intraspecific and extremely low or zero fertilization levels between the five species indicated that they are valid species. Based on the combined assessment of morphological, molecular and reproductive criteria, *A. humilis* and *A. gemmifera* appear to be the most closely related species, which are most closely related to the remaining species in the following order: *A. samoensis*, *A. monticulosa* and *A. digitifera*. Evidence derived from one or more of these criteria suggest that the morphs (i) are at various stages of divergence from the species with which they share morphological characters, and (ii) may indicate possible zones of speciation and hybridization. Identification of morphs avoided the possibility of taxonomic error and was essential for accurate interpretation of evolutionary boundaries. Confirmation of

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morphology as an informative character of evolutionary boundaries is of great significance because most coral research projects rely on morphology as the primary tool for identification of species.

3.2 INTRODUCTION

Hybridization is an important mechanism of speciation in many groups of plants and animals (reviewed in Arnold 1997; Rieseberg 1997). Hybridization may also promote speciation in scleractinian corals (Willis et al. 1997) and may contribute to the taxonomic difficulties of defining boundaries between species (Wallace and Willis 1994; Babcock 1995; Veron 1995). However the extent to which hybridization occurs in nature, its evolutionary role and its phenotypic effect in corals is unknown.

Many corals reproduce during synchronous multi-specific spawning events (Harrison et al. 1984; Babcock et al. 1986; Hayashibara et al. 1993; Babcock et al. 1994), potentially providing opportunities for interspecific hybridization (Babcock 1995; Willis et al. 1997). Gametes are viable for 6-8 hours after spawning (Willis et al. 1997) and those released from species which spawn synchronously aggregate and mix at the water surface, providing the potential for fertilization between different species. Interspecific fertilization occurs under laboratory conditions (Knowlton et al. 1997; Miller and Babcock 1997; Szmant et al. 1997; Willis et al. 1997; Hatta et al. 1999; Fukami et al. 2003) and some molecular studies conclude that common DNA sequence types in different species of corals are evidence of interspecific hybridization (Odorico and Miller 1997b; Hatta et al. 1999; van Oppen et al. 2000; Diekmann et al. 2001; van Oppen et al. 2001; van Oppen et al. 2002b; Fukami et al. 2003). A study of chromosome numbers concluded that hybridization contributes to the development of polyploidy and rapid speciation in the genus *Acropora* (Kenyon 1997).

Although there is potential for hybridization in corals, prezygotic mechanisms also appear to be important in limiting interspecific breeding in corals. The most apparent prezygotic mechanisms in corals include temporal reproductive isolation and gametic incompatibility. Temporal isolation has been proposed for some species on the scale of hours within mass spawning periods (Knowlton et al. 1997; Szmant et al. 1997; van Oppen et al. 2001; Fukami et al. 2003). Many additional species spawn up to 1-3 hours apart (Babcock et al. 1986; Wallace 1999) and may also be reproductively isolated.

Other species spawn weeks or months out of phase with the mass spawning (Willis et al. 1985; Babcock et al. 1986; Hayashibara et al. 1993; Wallace 1999; Hayashibara and Shimoike 2002) and may be partially or completely reproductively isolated by temporal barriers. Gametes of many species also appear to be incompatible. Within synchronously spawning species of *Acropora*, rates of intraspecific fertilization in experimental crosses are high (often > 90%) for many species compared with rates of fertilization in many interspecific crosses (Fig 3a and 4a in Willis et al. 1997; Table 1 in Hatta et al. 1999). The likelihood of interspecific fertilization in coral spawning slicks also appears to be reduced by the presence of sperm attractants in eggs, which enhance conspecific fertilization and reduce interspecific fertilization, as demonstrated for species of *Montipora* (Coll et al. 1994).

Detailed examination of morphologically similar species and intraspecific morphs, using molecular and reproductive criteria, suggest that evolutionary boundaries within and between coral taxa are at various stages of formation. In one study, congruent patterns of shared DNA sequences and high levels of fertilization between morphologically divergent species of *Acropora* provide strong evidence of interspecific hybridization (Hatta et al. 1999). In the same study, Hatta and colleagues also recognized three morphs in *A. nasuta* and two in *A. muricata* (synonym of *A. formosa*), which showed high levels of intramorph fertilization and (in all but one cross) extremely low levels (≤1.6%) of intermorph fertilization. This suggests that breeding boundaries have formed between the morphs within each of these species. Márquez and colleagues demonstrate that the species *Acropora hyacinthus* and *A. cytherea* are closely related but have now evolved to form statistically distinct lineages which hybridize infrequently in nature, despite having high levels of hybridization in laboratory experiments (Márquez et al. 2002a; Márquez et al. 2002b). Two studies have concluded that *Acropora palmata* and *A. cervicornis* are evolutionarily distinct species and that *A. prolifera* is a hybrid, derived from these species (van Oppen et al. 2000; Vollmer and Palumbi 2002). The latter study also demonstrated that colonies of *A. prolifera* are first generation hybrids with limited potential to interbreed, concluding that the effect of hybridization has been the generation of new morphologies without speciation. Three species of *Montastraea*, initially described as separate species and then synonymised within *Montastraea annularis*, are now recognized on the basis of

morphological, molecular and behavioral differences, as well as timing of spawning and fertilization potential (Knowlton et al. 1997; Szmant et al. 1997). However, no single character has been found which separates the three species. This and the variable occurrence of morphologically intermediate colonies suggest that boundaries may be at different stages of formation in different locations within the Caribbean (Knowlton et al. 1997; Szmant et al. 1997). Similarly, the species pairs *Acropora millepora* and *A. spathulata* and *Montipora digitata* and *M. tortuosa* were each previously regarded as single morphologically variable species, but are now recognized as distinct species on the basis of morphological and breeding criteria (Wallace 1999; Stobart 2000), and fixed genetic differences for the *Montipora* species (Stobart and Benzie 1994). In the genus *Platygyra*, seven morphospecies from the Great Barrier Reef have been defined in multivariate analyses, although no single morphological, reproductive or genetic character has been found which separates them (Miller and Babcock 1997; Miller and Benzie 1997). As suggested by Willis et al. (1997), based on the small number of species in the genus *Platygyra* compared with the genus *Acropora*, the effect of hybridization may have been to merge species or retard speciation in the former genus, but promote speciation in the latter genus.

These studies clearly indicate that it is necessary to use morphs, rather than species, as sampling units to accurately interpret evolutionary boundaries in corals. This will provide the greatest opportunity of elucidating evolutionary relationships in corals, separating genetic versus environmental influences on morphological variability, and avoiding the potential of confused phylogenies due to taxonomic error. In this study, I examine evolutionary relationships between morphs of the *Acropora humilis* species group at Lizard Island (Great Barrier Reef), defined on the basis of morphological appearance. The major aim of this project is to determine the extent to which morphology is indicative of evolutionary relationships within and between currently defined species in this group of corals. To do this, I use molecular and breeding criteria. The *A. humilis* species group is of particular interest because species within this group have a high level of intraspecific morphological variability, with boundaries between species appearing indistinct (Wallace 1999). In addition, based on previous surveys at Lizard Island (Wallace 1999 and B. Kojis pers. comm.), it appears that temporal reproductive isolation may occur within this species group.

3.3 METHODS

3.3.1 SPECIES AND MORPHS

The sampling units used in this study were putative morphs, distinguished using the morphological characters that are traditionally used to identify species of the genus *Acropora* (Wallace 1999; Wolstenholme et al. 2003). These putative morphs were defined during pilot searches and include known species, and morphs within and between these species. Morphs are named using abbreviations from the species with which they share most morphological similarity. Characters used to distinguish the species and morphs are summarized in Table 3.1.

3.3.2 RELATIVE ABUNDANCE

Surveys were carried out to assess the relative abundance of each putative morph in the *Acropora humilis* species group at Lizard Island ($14^0$40'S $145^0$28'E). Surveys were conducted in the five habitats in which colonies of the *A. humilis* species group commonly occur at Lizard Island. These habitats were exposed reef flats, exposed crests, exposed slopes, lagoonal margins of the reef flat and lagoonal patch reefs. Surveys were conducted at two locations for each habitat. The two locations of the exposed habitats were approximately 6 km apart, facing north-easterly and southerly directions. Lagoonal reef flat locations were approximately 1 km apart at the south-east of the Lizard Island lagoon, and lagoonal patch reefs were approximately 0.5 km apart at the north-west of the lagoon. All habitats except the slope were shallow, ranging in depth from about 0-2m. The slope habitat ranged in depth from about 4m, at the edge of the reef wall to about 15-20m where corals were extremely sparse or absent.

In each survey, each of the first hundred colonies encountered from the *Acropora humilis* species group were identified as one of the putative morphs. Surveys were conducted over a distance of approximately 50-200 metres in the crest, flat and patch reef habitats. Colonies of the *A. humilis* species group were more sparsely distributed in the slope habitats and therefore it was necessary to search distances of 1-2 km. Five replicate surveys were made for each habitat, except the lagoon patch reefs for which only 3 replicate surveys were possible due to the small size of the reefs. Colonies that were too small to be confidently identified (usually <5cm) were not included. Due to the very high abundance of colonies of the *A. humilis* group on the reef crests at Lizard Island, surveys were conducted along a 1metre belt transect to ensure less conspicuous

colonies were included. Surveys in the other habitats were conducted by haphazard swimming, with all colonies encountered being identified.

3.3.3 MOLECULAR TECHNIQUES AND ANALYSIS

Molecular samples were collected to analyze genetic relationships between the putative morphs. One or two colonies of all but two of the rarer morphs ("mont-hum" and "mont-gem") and three colonies of *Acropora austera*, the outgroup taxon for this study, were sampled. The species *A. austera* was selected as the most appropriate outgroup because it is directly ancestral to the species examined in this study, based on a morphological phylogeny of the genus *Acropora* (Wallace 1999). For each colony sampled, skeletal branch samples were collected and photographs taken, providing a reference of the appearance of the colony. Molecular samples were collected following the protocol described by Wolstenholme et al. (2003).

DNA was extracted from a volume of approximately 200 µl of tissue and skeleton, using a Viogene blood and tissue genomic DNA extraction kit. The tissue and skeleton were ground to a fine powder in liquid nitrogen, mixed with an equal volume of lysis buffer and 20 µl of proteinase E. Samples were incubated overnight in a 60^0C waterbath. DNA was extracted as per the manufacturer's instructions and precipitated in isopropanol. Following precipitation, the genomic DNA was dried, resuspended in ddH_20 and stored at -20^0C. The marker used for the molecular analysis was the mtDNA putative control region (referred to as the mtDNA intergenic region for the remainder of this paper, as in van Oppen et al. (2001)). Target segments were amplified using the primers AcrdloopF: 5'-TGTTAGTACAAATCGCCCGTCGCC-3', AcrdloopInt: 5'-CGTGAGCAGGACGCTTCAG-3' and AcrdloopR: 5'-CATCCATATCATTTGGTTGAGCCTTCT-3', designed by van Oppen et al. (1999a). The amplification reaction used 100-200ng of DNA template and BRL Taq polymerase in a 50µl reaction, in the presence of the buffer supplied with the enzyme (as per manufacturer's instructions). PCR was performed in a PC-960G Gradient Thermal Cycler using the following steps: incubation period of 4 min at 95^0C; 5 cycles of 30 sec at 94^0C, 1 min at 50^0C and 2 min at 72^0C; followed by 30 cycles of 30 sec at 94^0C, 1 min at 55^0C and 2 min at 72^0C; ending with a 10 min extension at 72^0C. PCR products were electrophoresed in a 0.8% agarose (FMC Bioproduct) gel in 1X TAE buffer to assess the yield.

Sequences were initially obtained by direct sequencing but these could not be interpreted, apparently due to the variable occurrence of repeat sequence blocks within individuals. All sequences analysed in this study were therefore obtained by cloning. Multiple clones were sequenced from most individuals (Table 3.2) enabling variation within and between individuals to be compared. PCR products were cloned using the ligation kit, pGEM® T easy (Promega) and transformed into DH5α^{TM} competent cells (BRL), under conditions recommended by the manufacturers. Bacterial colonies containing the vector were picked with a sterile toothpick and cultured for 6-12 hours in a 4ml LB nutrient solution and purified using a Viogene plasmid DNA mini-prep kit. Nucleotide sequences were generated for both strands on an ABI 377 Genetic Analyzer using the ABI Big-dye Ready Reaction kit following standard cycle sequencing protocol.

Sequences were aligned manually in Seqapp 1.99 (Gilbert 1994). Calculation of the pairwise sequence distance matrix and the maximum parsimony and likelihood phylogenetic analyses were performed in PAUP* 4.0b10 (Swofford 2002). Phylogenetic analyses used the heuristic search option. Bootstrapping with 1000 pseudoreplicates determined the robustness of clades, with branches supported by <50% being collapsed as polytomies. The maximum parsimony analysis was run with gaps excluded from the analysis, as well as treating gaps as a fifth character. The best-fit model of sequence evolution (TrN+I) was determined using Modeltest 3.06 (Posada and Crandall 1998) and the Akaike Information Criterion (AIC) method, for the maximum likelihood analysis.

Sequences have been submitted to GenBank under accession numbers AY364090 to AY364162 and the morphological reference samples are deposited at the Museum of Tropical Queensland (MTQ), Townsville, Australia (registration numbers G56366-G56383). GenBank accession numbers and MTQ registration numbers are cross-referenced with the reciprocal institutions.

3.3.4 BREEDING POTENTIAL

3.3.4.1 TIMING OF GAMETE MATURITY

Surveys of the six most abundant morphs were carried out from November 1998 to December 2001, to determine the proportion of colonies containing mature eggs and an indication of the timing of spawning of each morph. Surveys were conducted 1-3 days after the full moons during the spawning season, which extended from one month before and three months after the mass spawning event (months –1, 0, 1, 2, 3 with month 0 being the mass spawning month). Up to 30 colonies of *Acropora humilis*, *A. gemmifera*, *A. samoensis*, *A. digitifera*, *A. monticulosa* and the morph "dig-gem" were surveyed each month, with fewer colonies surveyed during the earlier spawning seasons. Surveys were conducted around Lizard Island for all morphs. Colonies of *A. digitifera* are rare at Lizard Island (Fig. 3.1) but common at a nearby island, North Direction Island (5km SE of the Lizard Island group). Surveys of 30 colonies were therefore conducted at North Direction Island for *A. digitifera*, with fewer colonies sampled around Lizard Island during the earlier phases of the project. Based on the results of these surveys, no difference in timing of spawning was evident between Lizard and North Direction Islands.

The surveys were conducted following the protocol described in Baird et al. (2002). Eggs develop over approximately a nine month period in species of *Acropora*, becoming pigmented about three weeks prior to spawning (Wallace 1985). Testes are visible microscopically four to six weeks prior to spawning (Wallace 1985). To determine timing of gamete maturity and month of spawning, branches were broken from colonies and scored as follows: colonies in which eggs were not visible or only just visible microscopically were scored as containing 'no eggs'; colonies with visible but white eggs were recorded as 'immature'; and colonies with pigmented eggs were recorded as 'mature' and ready to spawn. Up to five branches were broken from each colony. If any of these branches contained mature eggs, then the colony was scored as mature. If reproductive status could not be confidently assessed in the field (i.e. 'no eggs' vs. 'immature'), branch samples were collected, preserved in 5% formalin, decalcified in 3% hydrochloric acid and examined under a dissecting microscope.

3.3.4.2 SPAWNING AND FERTILIZATION TRIALS

Laboratory-based fertilization experiments were carried out, following the procedure in Willis et al. (1997). Fertilization trials were only conducted between colonies spawning during the same period of gametic viability (i.e. the same night), to test the breeding potential within and between species and morphs that could feasibly interbreed in nature. Potential for colonies to interbreed was tested between pairs of colonies of the same morph, between pairs of colonies of different morphs and gametes from a single colony (selfs) for all morphs, except the rare morph "mont-gem".

Colonies used in the fertilization experiments were collected 1-5 days after the full moon. Each colony was placed in a separate aquarium. Once colonies had released a substantial amount of spawn, egg-sperm bundles were collected and poured into a cup with a plankton mesh base, in a bowl of seawater. The cup was then gently agitated to separate egg-sperm bundles, with sperm being strained through the plankton mesh and eggs retained within the cup. Plankton mesh with a pore size of 210μm was used in the main spawning month. However, in the first spawning season, I found that this mesh was too coarse to retain eggs released from colonies that spawned in the later months (months 2 and 3). Plankton mesh with a pore size of 62μm was therefore used to separate eggs and sperm in the later months. Sperm diluted to approximately 10^6 per ml and approximately 100 eggs were then combined in 25ml glass vials. Controls were set up for each colony used in the experiment, to ensure that extraneous sperm had not contaminated any of the crosses. All crosses and controls were replicated three times. Vials were suspended at the surface in a tank of aerated water, simulating the conditions in which eggs and sperm are mixed in a spawning slick. The proportions of regular embryos were counted after 6-10 hours. Fertilization levels in all self crosses were <10% and usually <3%, indicating that selfing is possible but most likely a consequence of the artificial conditions. Fertilization levels in all controls were <3% and usually 0%. Recorded fertilization levels may therefore include occasional embryos due to selfing or sperm contamination. Crosses that showed low levels of fertilization were therefore interpreted cautiously. Time of spawning (nights after the full moon and time of night) and time taken for gametes to separate were also recorded, providing additional indications of breeding potential within and between morphs.

3.4 RESULTS

3.4.1 SPECIES AND MORPHS

Twelve morphs were recognized within the *Acropora humilis* species group. Five of the morphs corresponded with currently recognized valid species in this group and are referred to as species for the rest of this paper, distinguishing them from the other seven morphs. The species are *A. humilis* (Dana 1846), *A. gemmifera* (Brook 1892), *A. samoensis* (Brook 1891), *A. monticulosa* (Brüggemann 1879) and *A. digitifera* (Dana 1846). Six of the remaining seven morphs were morphologically intermediate between these species and are named after the species with which they appear to share greatest morphological affinity (Table 3.1). The seventh morph, "terete mont" appeared to be most closely associated with *A. monticulosa*, and is named for its apparent affinity with this species and its less conical (terete) branches. Four of the morphs, "dig-gem", "mont-hum", "mont-gem" and "terete mont" were distinct. The "hum-gem" morph was morphologically variable, forming a continuum between the species *A. humilis* and *A. gemmifera*, with some colonies appearing most similar to one of these species and other colonies clearly sharing characters with both species. Colonies of the morphs "sam-hum" and "sam-gem" each appeared to share closest morphological affinity with *A. samoensis*.

3.4.2 RELATIVE ABUNDANCE

The most abundant species or morph was the morph "dig-gem", dominating the crest and flat habitats, common on the lagoon patch reefs but absent on the slopes (Fig. 3.1). *Acropora humilis*, *A. gemmifera* and the morph "hum-gem" were moderately common in all habitats. Colonies of *A. monticulosa* and morphs sharing characters with this species were mainly found on the reef crest, but were not abundant in any habitat and were absent on the lagoon patch reefs and slopes. *Acropora digitifera* was present but rare at all shallow sites and absent on the slopes. *Acropora samoensis* was the most abundant species or morph of the *A. humilis* species group in the deeper slope habitat, with "sam-hum" being the next most abundant. Both of these morphs were extremely rare or absent in all exposed habitats, while a small number of colonies were present on the lagoon patch reefs. The morph "sam-gem" was always rare, with only a few colonies being recorded on the slope and one lagoon patch reef.

Table 3.1 Primary characters used to identify species and morphs in the *A. humilis* species group.

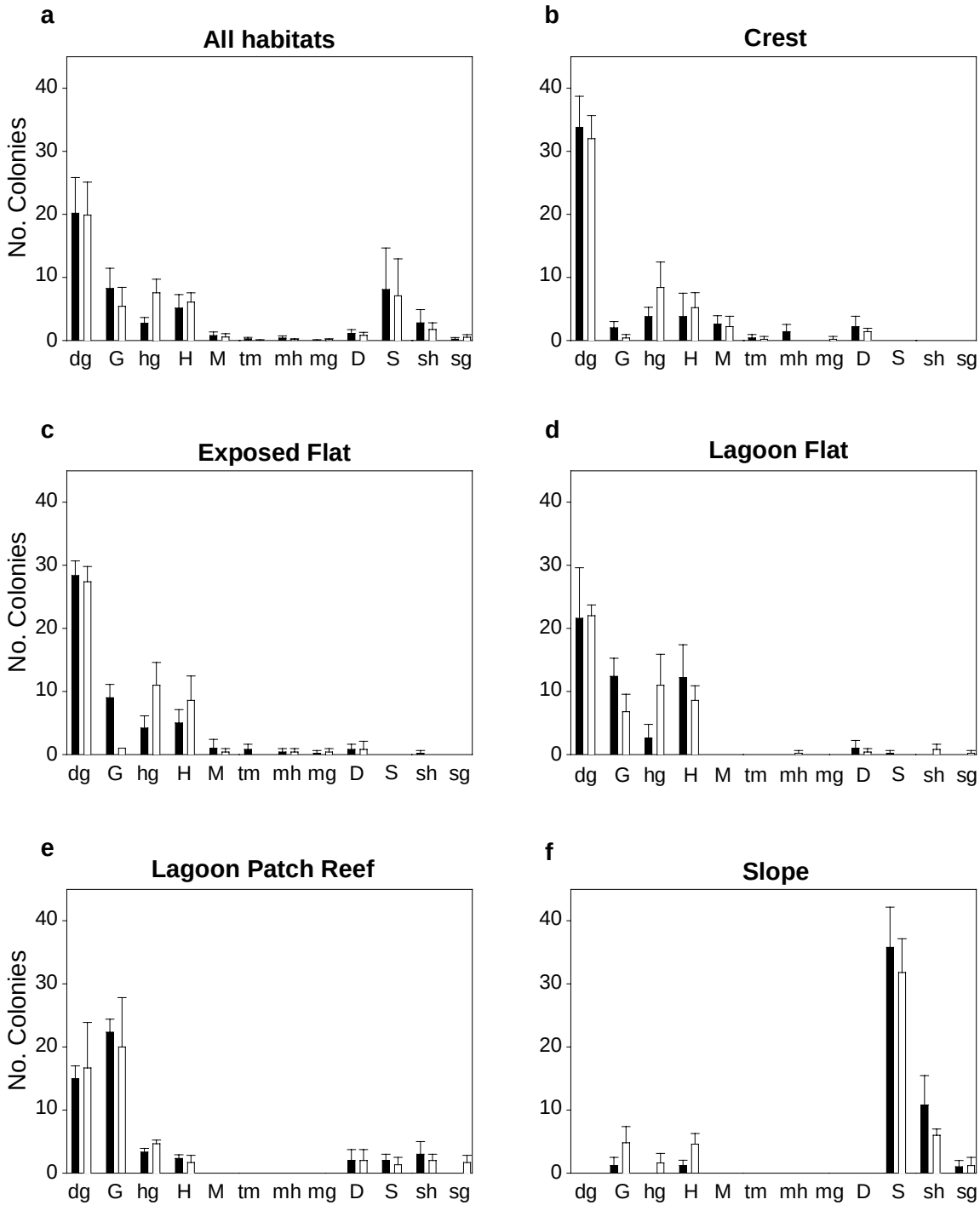
Species	Growth Form	Branch Shape	Axial Corallites	Radial Corallites[a]	Colony Colour
A. samoensis	**caespito-corymbose**	thin, terete	large	one size, large, tubular to tubo-nariform, **not crowded**	brown with edges of radial corallites, calice of axial corallites and ring around axial corallites pale green-yellow, pale brown, white or pale blue; polyps green-yellow (in colonies with this colour), white or pale brown
A. humilis	corymbose	thick, terete	**large to very large**	**one size**, large, **nariform to tubo-nariform,** crowded	often brightly coloured; brown, green, blue, purple or combinations of these colours; often paler or white around axial corallites; polyps yellow, green, white, pale blue or pale brown
A. gemmifera	corymbose	thick, conical	large	**two sizes**, large, **flaring dimidiate to tubo-nariform**, crowded	brown or pale brown, often with an orange, blue or yellow tinge; branch tips, edges of radial corallites and edges of colony often paler or white; polyps white or pale brown
A. monticulosa	**digitate**	very thick, **very conical**	small	one size, small, **nariform**, crowded	colonies usually the same colour along length of all branches; brown with pale or strong green-yellow or purple-blue tinge; polyps green-yellow (in colonies with this colour), white or pale brown
A. digitifera	corymbose	very thin, terete	small	mixed sizes, large, **flaring dimidiate to flaring lips**, crowded	pale brown to brown; sometimes with blue or cream branch tips; polyps pale or dark brown

Table 3.1 continued

Morphs[b]					
"sam-hum"	corymbose to caespito-corymbose	thin, terete	large	**one size**, large, **tubo-nariform**, usually crowded	usually as described for *A. samoensis*, but sometimes a single colour within the range of that seen in *A. humilis*
"sam-gem"	corymbose to caespito-corymbose	thin, terete	large	**one size**, large, **tubo-nariform to dimidiate**, usually crowded	same as *A. samoensis*
"hum-gem"	corymbose	thick, terete	large	**one to two sizes**, large, **tubo-nariform to dimidiate**, crowded	colonies similar in colour to *A. humilis* but not usually as brightly coloured; polyps white or pale brown
"mont-hum"	corymbose	thick, terete to conical	**large to very large**	**one size**, large, **nariform**, crowded	colonies recorded in this study were usually brown sometimes with a pink or yellow tinge; often calices of radial and axial corallites and ring around axial corallite were paler or white; polyps white
"mont-gem"	digitate	very thick, **very conical**	**small**	**two sizes**, small, **dimidiate**, crowded	same as *A. gemmifera*
"terete mont"	digitate	very thick, **sometimes conical**	large	mixed sizes, small, dimidiate, crowded	brown with a green-yellow or purple-blue tinge, calices of radial and axial corallites paler in colour, polyps white
"dig-gem"	corymbose	**thick, conical**	small	mixed sizes, large, **flaring dimidiate to flaring lips**, crowded	same as *A. digitifera*

[a] The first size category in the description of radial corallites, refers to the size range of radial corallites within that morph and the second size category refers to the size of radial corallites relative to other morphs. [b] Morphs are named according to the species with which they share most morphological characters. Characters that are most important for distinguishing each species and morph are highlighted in bold.

Fig. 3.1 Relative abundance of species and morphs in the *Acropora humilis* species group at Lizard Island in five habitats. **a** Overall abundance for all habitats and **b-f** abundance in each habitat. Columns represent average number of colonies for five replicate surveys for crest, flat and slope habitats and three replicate surveys for lagoon patch reefs. Black and white bars distinguish the two sites surveyed for each habitat. Error bars indicate 1 SD. Species and morphs are ordered along the horizontal axis by overall abundance (most to least abundant) in non-slope and then slope habitats. Upper case letters represent species and lower case letters represent morphs as follows: *dg* "dig-gem"; *G A. gemmifera*; *hg* "hum-gem"; *H A. humilis*; *M A. monticulosa*; *tm* "terete mont"; *mh* "mont-hum"; *mg* "mont-gem"; *D A. digitifera*; *S A. samoensis*; *sh* "sam-hum"; *sg* "sam-gem".


a
All habitats
No. Colonies
0
10
20
30
40
dg G hg H M tm mh mg D S sh sg
b
Crest
dg G hg H M tm mh mg D S sh sg
c
Exposed Flat
dg G hg H M tm mh mg D S sh sg
d
Lagoon Flat
dg G hg H M tm mh mg D S sh sg
e
Lagoon Patch Reef
dg G hg H M tm mh mg D S sh sg
f
Slope
dg G hg H M tm mh mg D S sh sg

3.4.3 MOLECULAR PHYLOGENETIC ANALYSIS

Phylogenetic analysis of the mtDNA intergenic region, using both maximum parsimony and maximum likelihood methods, divided the species and morphs into two clades. *Acropora digitifera* and "dig-gem" formed clade I and all other species and morphs formed clade II (Fig. 3.2). Complete (100%) bootstrap support for clade I indicates that *A. digitifera* and "dig-gem" are distinct from the other species and morphs of the *A. humilis* species group for this marker. Within clade I, sequences from the colonies of *A. digitifera* and "dig-gem" were indistinguishable. Within clade II, divergence is also apparent for *A. monticulosa* (clade IIa) from *A. humilis*, *A. samoensis*, *A. gemmifera* and intermediate morphs of these three species (clade IIb), with each of these subclades having strong (95%) bootstrap support. There was little additional resolution in clade IIb, suggesting a close evolutionary relationship between these species and morphs.

Repeat sequence blocks were present in some but not all cloned sequences from single colonies of *Acropora gemmifera*, *A. monticulosa*, "terete mont" and all cloned sequences from the three colonies of *A. austera* (Table 3.2), indicating that these repeat sequences do not represent a consistent phylogenetic signal. The repeat sequence blocks were therefore deleted prior to the phylogenetic analysis. In addition to the repeat sequence blocks, there were low levels of variation between sequences cloned from each individual. The number of base differences between cloned sequences from an individual coral ranged from 0 to 10 (Table 3.2). This variation may indicate polymorphic sites within an individual or PCR error. Single consensus sequences for each individual were therefore used in the analyses. At nucleotide sites that varied within an individual, the most commonly occurring base at that site was used in the consensus sequence. If different bases occurred with equal frequency at a particular site, that site was recoded using the IUPAC (International Union of Pure and Applied Chemistry) ambiguity code.

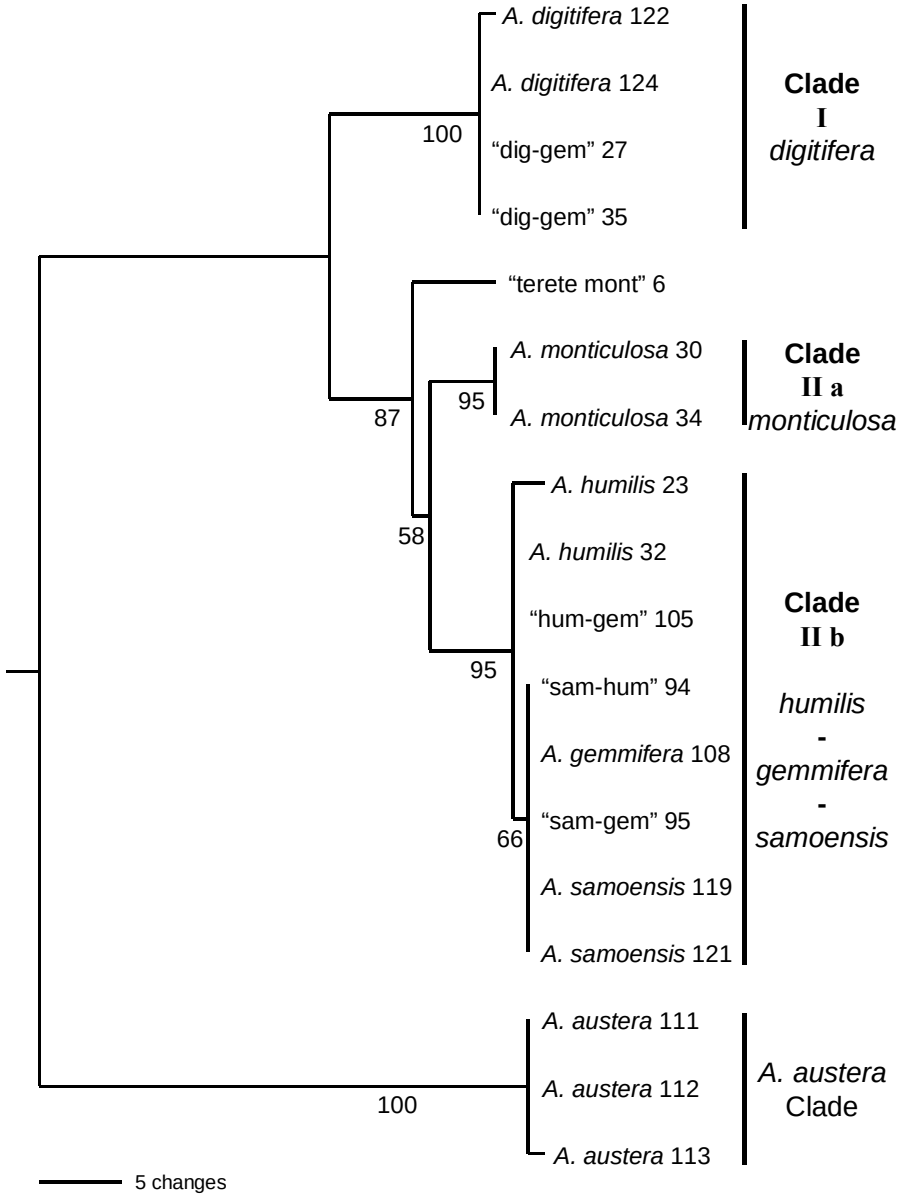


Fig. 3.2 Maximum parsimony consensus tree (50% majority rule) with mid-point rooting, produced in the analysis of the mtDNA intergenic region for species and morphs of the *Acropora humilis* species group. Gaps were treated as missing characters in this analysis. Numbers below branches indicate bootstrap values (1000 replicates) for branches with >50% support. Species names and sample codes are given for each individual. Sample codes are as listed in Table 3.2.

Table 3.2 Number of sequences obtained from individuals of each species and morph of the *Acropora humilis* species group in the molecular analysis. GenBank Accession numbers and corresponding Museum of Tropical Queensland (MTQ) registration numbers for morphological reference samples are listed for each colony.

Species or Morph	Sample Code	# Sequences / Individual	# base differences between sequences within an individual	GenBank Accession Numbers [a]	MTQ Registration Number
A. samoensis	119	5	1–7	AY364090–4	G56366
	121	5	2–4	AY364095–9	G56367
A. humilis	23	2	2	AY364100–1	G56368
	32	3	2–7	AY364102–4	G56369
A. gemmifera	108	4	0–10	AY364105–8 [a]	G56370
A. monticulosa	30	8	0–9	AY364109–16 [a]	G56371
	34	4	3–6	AY364117–20	G56372
A. digitifera	122	1		AY364121	G56373
	124	5	0–3	AY364122–6	G56374
“sam-hum”	94	5	1–3	AY364127–31	G56375
“sam-gem”	95	3	0–2	AY364132–4	G56376
“hum-gem”	105	5	1–7	AY364135–9	G56377
“terete mont”	6	1		AY364155[a]	G56378
“dig-gem”	27	9	1–6	AY364140–8	G56379
	35	6	2–10	AY364149–54	G56380
A. austera	111	3	2–6	AY364156–8[a]	G56381
	112	2	5	AY364159–60[a]	G56382
	113	2	9	AY364161–2[a]	G56383

[a] Sequences with the accession numbers AY364106 (*A. gemmifera*); AY364111, AY364112 and AY364116 (*A. monticulosa*); AY364155 (“terete mont”) and all sequences from colonies of *A. austera* did not contain the repeat sequence block, as described in the text.

Base composition was homogeneous between sequences from morphs of the *Acropora humilis* species group and *A. austera* (Table 3.3). The level of divergence between sequences obtained in this study was low (Table 3.4), as also reported in Márquez et al. (2002b) and van Oppen et al. (2001) for species of the genus *Acropora*. Maximum sequence divergence between species and morphs of the *A. humilis* species group and *A. austera* was 6.4% (Table 3.4). This was similar to the level of divergence for a broad range of species of *Acropora* (6.9%) reported in

Table 3.3 Mean base compositions (%) for species and morphs of the *Acropora humilis* species group and *Acropora austera* [a].

A	C	G	T
24.7 (0.002)	17.1 (0.002)	26.4 (0.002)	31.7 (0.004)

[a] Standard deviations are given in parentheses.

Table 3.4 Average Kimura 2-parameter pairwise sequence distances (%) within and between species and morphs of the *Acropora humilis* species group and *Acropora austera* [a].

	Within Morphs	Between Morphs	*A. austera*
A. humilis species group	0.7 (1.3)	1.4 (3.1)	5.6 (6.4)
A. austera			0.5 (0.8)

[a] Maximum values are given in parentheses.

van Oppen et al. (2001) and double the level of divergence between sequences from species and morphs within the *A. humilis* species group. Despite this distinction, sequences from colonies of *A. austera* were not sufficiently different to form a natural outgroup. Trees produced in the phylogenetic analyses were therefore constructed using the midpoint rooting option. The aligned consensus sequences consisted of 1075 positions with repeat sequence blocks deleted. Prior to deletion of repeat sequence blocks, individual sequences ranged in length from 1094 to 1233 bp. Within the aligned sequences, 999 positions were constant, 7 variable characters were not parsimony informative and 69 were parsimony informative.

Maximum parsimony (MP) and maximum likelihood (ML) analyses produced trees with similar topologies and levels of bootstrap support, differing in that the single "terete mont" sequence formed a third branch with weak bootstrap support in clade II in the MP analysis (Fig. 3.2) and a polytomy within this clade in the ML analysis. Additional sequences are needed to clarify the phylogenetic position of this morph, but based on the analyses in this study, it appears to be distinct from the species *Acropora monticulosa*. Treating gaps as a fifth character in the MP analysis did not change the tree topology, as also reported by van Oppen et al. (2001). MP analysis with gaps treated as missing characters produced 607 most parsimonious trees of 81 steps. A consistency index of 0.938, homoplasy index of 0.062 and a retention index of 0.973 indicate a strong phylogenetic signal in the sequence data.

3.4.4 BREEDING POTENTIAL

The results of this component of the study demonstrate that prezygotic mechanisms restrict the interbreeding potential between species in the *Acropora humilis* species group. Temporal reproductive barriers were present at two levels, i.e. month of gamete maturity and hour of spawning, while isolation of species or morphs was not evident on the basis of day of spawning. Fertilization barriers also existed between species that spawned synchronously (i.e. on the same night, within a 4 hour period).

3.4.4.1 TIMING OF GAMETE MATURITY

Surveys of the timing of egg maturity in colonies of the *Acropora humilis* species group at Lizard Island provided a direct indication of timing of spawning for each morph, for each month of the spawning season (Fig. 3.3). The development of eggs through the three stages of maturation is evident for each morph (Fig. 3.3) and timing of spawning was validated by field and laboratory observations. No spawning was recorded in the days prior to collection of this survey data. Spawning was only recorded by morphs containing mature eggs, and colonies recorded with mature eggs in the surveys contained no eggs in the days following spawning (Table 3.5).

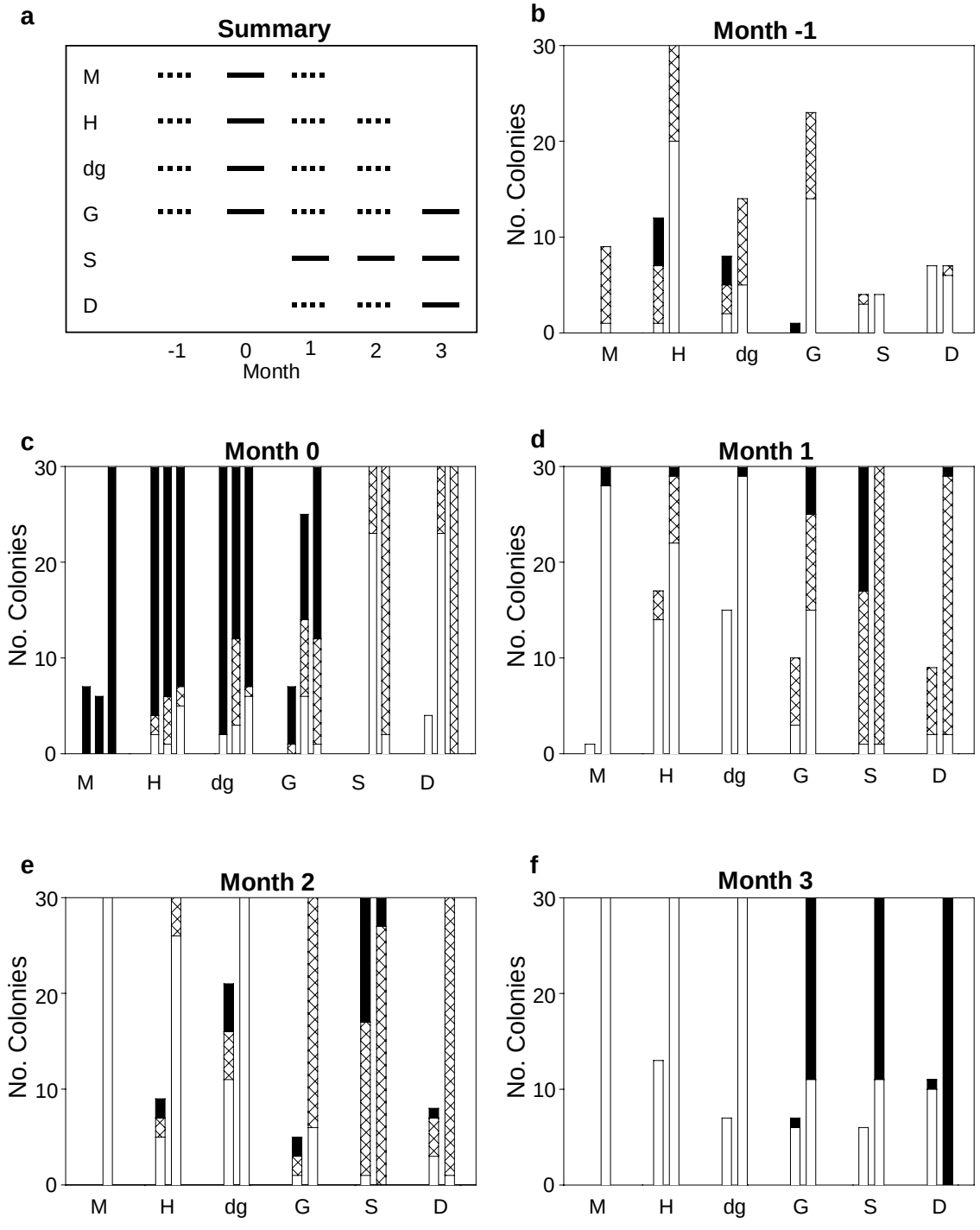
Table 3.5 Observations of spawning in laboratory aquaria for species and morphs of the *Acropora humilis* species group recorded during three spawning seasons (1999–2000, 2000–2001 and 2001–2002).

	# Colonies spawning[a]	# Colonies not spawning[b]	Month[c]	Night[d]	Time of Spawning (hrs after sunset)[e]	Separation of Bundles[f]
A. samoensis	9	1	2, 3	3, 4	1.5 – 2	5 – 30 mins
A. humilis	*13*	*1*	0	*5, 6, 7, 8*	2.5 – 3.5	*1 – 4 hrs*
A. gemmifera	9	0	0, 1,3	6, 7, 8	2.5 – 3.5	5 – 30 mins
A. monticulosa	*9*	*1*	0	*6, 7, 8*	*5 – 6.25*	*30 – 60 mins*
A. digitifera	4	0	3	7	2.75	5 – 15 mins
"sam-hum"	2	3	2	4, 6, 7	2 – 2.5	30 – 60 mins
"sam-gem"	2	0	2	4, 6	1.5 – 2	< 5 mins
"hum-gem"	*4*	*1*	0, 2	*6, 7, 9*	2.5 – 3	*30 – 90 mins*
"mont-hum"	3	1	0	7, 8	5 – 6.25	30 – 60 mins
"terete mont"	2	0	0	7	2.5 – 3	1 – 2 hrs
"dig-gem"	14	1	0	6, 7, 8	2.5 – 3.5	< 5 mins

[a] # Colonies spawning indicates the number of colonies spawning in aquaria, on which the observations in this table are based; [b] # colonies not spawning indicates the number of colonies in aquaria which did not spawn; [c] Month = month of spawning relative to the mass spawning (month 0), codes for the month of spawning are the same as those in Fig. 3; [d] Night = the number of nights after the full moon (night 0) on which spawning was recorded; [e] spawning times ranged from 8.30pm to 1.15am; [f] separation of bundles indicates the time interval taken for egg-sperm bundles to be separated.

The most striking finding in the pattern of timing of gamete maturity was that a second substantial spawning event occurred three months after the mass spawning, with all but one species spawning only during one of these events (Fig. 3.3a). Colonies of *Acropora monticulosa* had the greatest level of synchronicity, with almost all colonies (96%) containing mature eggs during the mass spawning month (month 0) and 4% during the month following the mass spawning (month 1). Similarly, most colonies of *A. humilis* and "dig-gem" contained mature eggs during the mass spawning month (90% and 89% respectively). In contrast, *A. samoensis* never contained mature gametes during the mass spawning, with all colonies of this species spawning during months 1, 2 or 3. The absence of mature gametes in colonies of *A. digitifera* during month 0 indicates that it also is not a mass spawning species. The predominant time of spawning for this species appears to be month 3, based on the data available, with almost all colonies (94%) containing mature eggs in this month. *Acropora gemmifera* showed the greatest variability in timing of spawning, with high proportions of colonies containing mature

Fig. 3.3 Timing of egg maturity for the five species and the morph “dig-gem” of the *Acropora humilis* species group during the spawning season at Lizard Island. Months were standardized in relation to the month of mass spawning (month 0) as follows: month –1: November 1998, October 1999; Month 0: November 1999, November 2000, December 2001; Month 1: December 1999, December 2000; Month 2: January 2000, January 2001; Month 3 March 1999, February 2001. Horizontal axis is ordered by predominant spawning time, using the same codes for species and “dig-gem” as in Fig. 3.1. **a** Summary of timing of egg maturity for species and “dig-gem”. No line indicates months when no colonies contained mature eggs; dotted lines indicate <40% of colonies contained mature eggs; solid lines indicate >40% of colonies contained mature eggs. **b-f** Stages of egg maturity for each month in the spawning season: white indicates no or extremely small eggs were present, cross-hatching indicates immature eggs were present, black indicates the presence of mature eggs. Multiple bars for each taxon represent data recorded in different spawning seasons (years).


a
Summary
M
H
dg
G
S
D
-1
0
1
2
3
Month
b
Month -1
No. Colonies
30
20
10
0
M
H
dg
G
S
D
c
Month 0
No. Colonies
30
20
10
0
M
H
dg
G
S
D
d
Month 1
No. Colonies
30
20
10
0
M
H
dg
G
S
D
e
Month 2
No. Colonies
30
20
10
0
M
H
dg
G
S
D
f
Month 3
No. Colonies
30
20
10
0
M
H
dg
G
S
D

eggs during the mass spawning and three months afterwards (55 and 32% of all colonies respectively), with ≤8% of all colonies spawning in each of the other months of the spawning season.

Colonies of the rarer morphs were also scored when encountered during the survey period. Colonies of “mont-hum”, “mont-gem” and “terete mont” were only recorded with mature eggs in month 0. The majority of colonies of “hum-gem” also contained mature eggs during month 0 (83%), with ≤7% of colonies containing mature eggs in each of the other months surveyed. Colonies of “sam-hum” and “sam-gem” appear to follow the timing of spawning recorded for *Acropora samoensis*, with sampled colonies containing mature eggs only during months 2 and 3.

3.4.4.2 SPAWNING AND FERTILIZATION TRIALS

Time (hour) of spawning and time taken for egg-sperm bundles to break apart were consistent for each species and morph, between different nights, months and years. Most colonies of species and morphs spawning during the same month, spawned over the same range of nights (Table 3.5). This suggests that the former two factors, hour of spawning and time taken for egg-sperm bundles to separate, play an important role in determining which morphs have the greatest potential to interbreed while ‘night of spawning’ does not. Colonies of *Acropora humilis*, *A. gemmifera*, “hum-gem”, “dig-gem” and “terete mont” spawned at similar times (2.5-3.5 hours after sunset), while colonies of *A. monticulosa* and “mont-hum” spawned around 2-3 hours later (Table 3.5). Following spawning, time taken for egg-sperm bundles to break apart varied substantially (Table 3.5), despite similar levels of agitation for spawn collected from each colony. Bundles released from colonies of “dig-gem” and some colonies of *A. gemmifera* broke apart almost instantaneously upon reaching the water surface. Bundles released from colonies of “hum-gem”, “terete mont”, *A. monticulosa*, “mont-hum” and some colonies of *A. gemmifera* broke up over a 0.5-2 hour period. In contrast, time taken for bundles to separate from colonies of *A. humilis* ranged from 1-4 hours. It therefore seems that all species and morphs which spawned 2.5-3.5 hours after sunset, except *A. humilis*, would be unlikely to breed with the later spawning colonies of *A. monticulosa* and “mont-hum”. The longer time taken for bundles to separate for some colonies of *A. humilis* suggest a greater potential for this species to interbreed

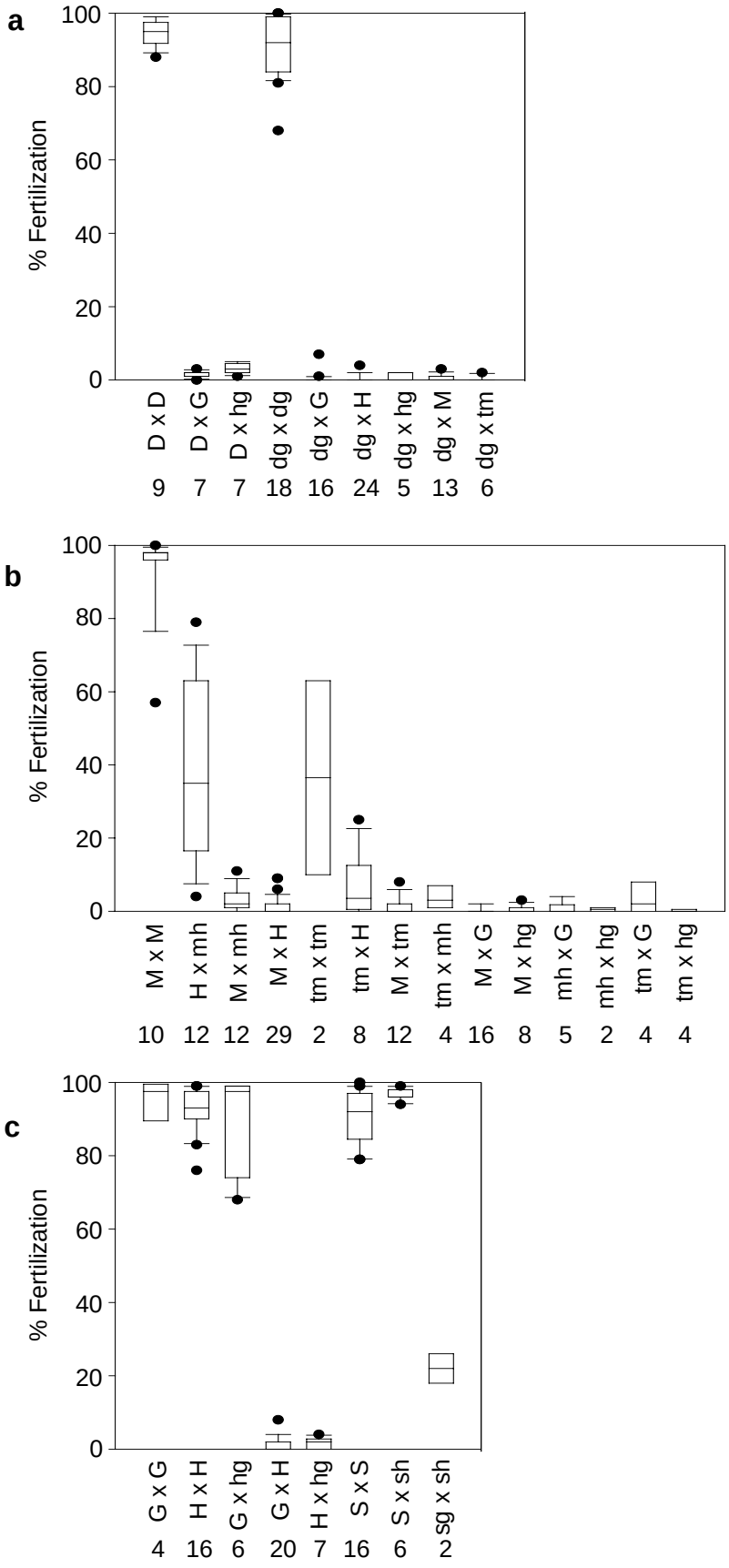
with *A. monticulosa* and “mont-hum”, both of which released egg-sperm bundles that separated relatively quickly.

Although timing of spawning and time taken for egg-sperm bundles to break apart did not indicate opportunities for reproductive isolation between species and morphs which spawned after the mass spawning event, timing of these traits do provide evidence of possible evolutionary connections for these taxa. Colonies of *Acropora samoensis*, “sam-hum”, and “sam-gem” spawned 1.5-2.5 hours after sunset and *A. digitifera* about 3 hours after sunset (Table 3.5). Relative to colonies of *A. samoensis*, colonies of “sam-hum” spawned later and egg-sperm bundles took longer to break apart, while the bundles released from colonies of “sam-gem” separated more rapidly. These observations suggest a distinction between colonies of *A. samoensis* and the intermediate morphs “sam-hum” and “sam-gem”, and a possible link with the species *A. humilis* and *A. gemmifera* respectively. Similarly, egg-sperm bundles released from colonies of *A. digitifera* separated rapidly, as seen for colonies of “dig-gem” (Table 3.5).

Preliminary observations in this study suggest that egg size may be related to timing of spawning, with smaller eggs being released from colonies in the second and third months after the mass spawning compared with eggs spawned during the mass spawning month. This proposal is based on the finer plankton mesh needed to separate eggs and sperm from colonies that spawned in months 2 and 3. Measurement of spawned eggs from each species and morph, for each month that they spawn, is necessary to confirm these observations.

Levels of fertilization between colonies of the same species were high to very high (usually >90%) and negligible between colonies of different species (Fig. 3.4). This suggests that strong pre-zygotic fertilization barriers exist between species of the *Acropora humilis* species group that spawn on the same night. Levels of fertilization between colonies of “dig-gem” were also high to very high and negligible between colonies of this morph and all other species and morphs, suggesting that fertilization barriers are well developed between this and other synchronously spawning species and

Fig. 3.4 Boxplots indicating the range of fertilization levels (average/3 replicates) for each cross that was tested. Interspecific and intermorph crosses are only presented in one figure: **a** "dig-gem" and *A. digitifera* **b** *A. monticulosa* and intermediate morphs of this species **c** *A. gemmifera, A. humilis, A. samoensis* and intermediate morphs of these species. The top and bottom of the boxes represent the upper and lower quartiles (i.e. 50% of the data) with the horizontal line within the box indicating the median value; whiskers at either end of the boxes indicate values within 1.5 inter-quartile ranges and dots indicate extreme values beyond the whiskers. Codes for species and/or morphs for each pair of colonies used in each cross are indicated on the horizontal axis and are the same as those in Fig. 3.1. Numbers below the codes for each morph indicate the number of crosses tested.


a
% Fertilization
100
80
60
40
20
0
D x D
D x G
D x hg
dg x dg
dg x G
dg x H
dg x hg
dg x M
dg x tm
9
7
7
18
16
24
5
13
6
b
% Fertilization
M x M
H x mh
M x mh
M x H
tm x tm
tm x H
M x tm
tm x mh
M x G
M x hg
mh x G
mh x hg
tm x G
tm x hg
10
12
12
29
2
8
12
4
16
8
5
2
4
4
c
% Fertilization
G x G
H x H
G x hg
G x H
H x hg
S x S
S x sh
sg x sh
4
16
6
20
7
16
6
2

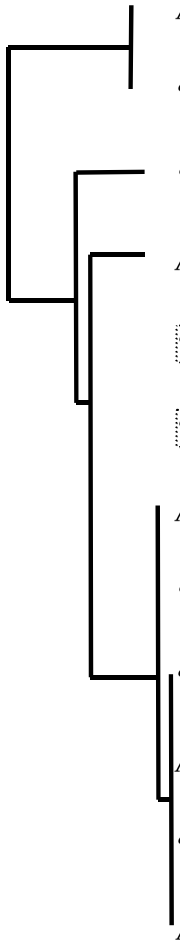
morphs. “Terete mont” was the only other morph that was tested for interbreeding potential because no other pairs of colonies of the same morph spawned on the same night. Although only two colonies were tested, the low to moderate levels of fertilization between colonies of “terete mont” contrasts strongly with the high levels of fertilization for all intraspecific and “dig-gem” crosses, suggesting that this morph may be a recent hybrid. Further crosses are necessary to explore this proposal. Extremely low levels of fertilization were recorded between colonies of *A. humilis* and *A. monticulosa*, while moderate but never high levels of fertilization were recorded between colonies of *A. humilis* and the morphs “mont-hum” and “terete mont”, suggesting an evolutionary link between these species and morphs. Negligible levels of fertilization between colonies of *A. monticulosa*, “mont-hum” and “terete mont” with colonies of *A. gemmifera* and “hum-gem” suggest strong reproductive barriers exist between these species and morphs. High levels of fertilization between colonies of “hum-gem” and *A. gemmifera* and low levels with *A. humilis* and all other species and morphs with which colonies of “hum-gem” were crossed, suggest that this morph shares greatest affinity with *A. gemmifera*. Likewise, high levels of fertilization between colonies of *A. samoensis* and “sam-hum” suggest a strong evolutionary affinity. Moderate fertilization levels between “sam-hum” and “sam-gem” reinforce the distinction between these morphs and *A. samoensis*, as is also evident in the timing of spawning and separation of bundles.

3.5 DISCUSSION

3.5.1 EVOLUTIONARY RELATIONSHIPS WITHIN THE *ACROPORA HUMILIS* SPECIES GROUP

The morphological, molecular and reproductive data presented in this study all contribute to the interpretation of evolutionary relationships between species and morphs in the *Acropora humilis* species group, with the five species and seven morphs at different stages of speciation. Predominant trends for each morph are summarized in Fig. 3.5. The molecular phylogenetic analysis (Fig. 3.2) provided the least resolution, indicating two levels of divergence for this marker. Divergence was largest between *A. digitifera* and other species and morphs of the *A. humilis* species group, while a more recent divergence of *A. monticulosa* was evident from the species *A. samoensis*, *A.*

Fig. 3.5 Summary of predominant patterns for each species and morph of the *Acropora humilis* species group, for molecular, ecological (habitat depth) and reproductive criteria. The figure or table from which each column is summarized is indicated under the heading of each column. The tree at left and first column of species and morph names represent a reduced version of the tree produced from the phylogenetic analysis of the mtDNA intergenic region. Each row in the remainder of the figure corresponds with the species and morphs listed within the tree. Molecular data were not obtained for "mont-hum" or "mont-gem": these morphs are placed in dashed boxes to separate them from the molecular tree. Depth is coded as *shallow* or *deep*, indicating the relative depth of the habitat in which species and morphs most commonly occurred; egg maturity is coded as after mass spawning: *After MS* or mass spawning: *MS*; spawning time is coded as *regular* or *late*, relative to the hour of spawning of other species and morphs; bundle separation is coded as *fast*, *moderate* or *slow*, relative to time taken for bundles to break apart for colonies of each species and morph; fertilization potential indicates which species and morphs showed potential to interbreed, based on the fertilization data, and are coded as *low*, *moderate* or *high*; the morph "terete mont" showed moderate intra-morph levels of fertilization while all other species or morphs had high levels of intraspecific or intra-morph levels of fertilization; *A. digitifera*, "dig-gem" and *A. monticulosa* showed no or extremely low potential to interbreed with other species or morphs; no reproductive observations were recorded for "mont-gem".



Molecular (Fig. 3.2)	Depth (Fig. 3.1)	Egg Maturity (Fig. 3.3)	Spawning Time (Table 3.5)	Bundle Separation (Table 3.5)	Fertilization Potential (Fig. 3.4)
A. digitifera	Shallow	After MS	Regular	Fast	----------
“dig-gem”	Shallow	MS	Regular	Fast	----------
“terete mont”	Shallow	MS	Regular	Moderate	Intra: moderate
A. monticulosa	Shallow	MS	Late	Moderate	----------
“mont-hum”	Shallow	MS	Late	Moderate	*A. humilis*: low
“mont-gem”	Shallow				
A. humilis	Shallow	MS	Regular	Slow - moderate	“mont-hum”: low
“hum-gem”	Shallow	MS	Regular	Moderate	*A. gemmifera*: high
“sam-hum”	Deep	After MS	Regular	Moderate	*A. samoensis*: high “sam-gem”: moderate
A. gemmifera	Shallow	MS & After MS	Regular	Moderate	“hum-gem”: high
“sam-gem”	Deep	After MS	Regular	Fast	“sam-hum”: moderate
A. samoensis	Deep	After MS	Regular	Moderate	“sam-hum”: high

humilis, A. gemmifera and morphs of these species. The reproductive data provided greater resolution, indicating that temporal and prezygotic reproductive barriers are important mechanisms, maintaining and possibly structuring species boundaries in the *A. humilis* species group. Temporal reproductive isolation is apparent at the scale of months over the summer spawning season, based on timing of gamete maturity (Fig. 3.3) and hours within the mass spawning (Table 3.5). Evidence of reproductive isolation based on night of spawning was not apparent, with most colonies of species and morphs spawning during the same month, also spawning over the same range of nights (Table 3.5). High levels of intraspecific and negligible levels of interspecific fertilization potential confirmed that the species of the *A. humilis* species group are valid species, while fertilization potential between species and morphs corresponded with apparent evolutionary affinity based on morphological appearance.

Phylogenetic analysis of the mtDNA intergenic region resolved two distinct clades within the *Acropora humilis* species group, with sequences from colonies of *A. digitifera* and "dig-gem" forming clade I and sequences from all other species and morphs forming clade II (Fig. 3.2). This distinction between the two clades was corroborated by the results of the breeding experiments, which demonstrated that the potential for colonies of *A. digitifera* or "dig-gem" to interbreed with the species or morphs in clade II against which they were tested were negligible (Fig. 3.4a).

Within clade I, sequences from the colonies of *Acropora digitifera* and "dig-gem" were indistinguishable. Morphologically colonies of this species and morph are very similar, differing in that colonies of *A. digitifera* have thin branches compared with the thicker and more conical shaped branches of "dig-gem" (Table 3.1). Live colonies of this species and morph also share identical patterns of colour variation (Table 3.1). Considering the molecular and morphological similarities, it was surprising to discover that an almost complete temporal reproductive barrier separates this species and morph, with "dig-gem" predominantly spawning during the mass spawning month and *A. digitifera* spawning three months after the mass spawning. These differences in timing of spawning indicate that *A. digitifera* and "dig-gem" are unlikely to interbreed at Lizard Island, although fertilization experiments are necessary to test whether they could interbreed if colonies spawned at the same time. The other species with which the morph "dig-gem" shares morphological characters is *A. gemmifera*, which also has

conical shaped branches and radial corallites whose shape merges with that of "dig-gem" (Table 3.1). No temporal reproductive barrier was evident between "dig-gem" and *A. gemmifera*, with both spawning simultaneously during the mass spawning. However, inter-fertilization potential was negligible (Fig. 3.4a), indicating that a pre-zygotic barrier reproductively isolates them. Based on the combined evidence of the morphological, molecular and reproductive results, three hypotheses could explain the origin of "dig-gem". Firstly, this morph may have evolved from within the species *A. digitifera* due to or reinforced by a differential timing of spawning. A second hypothesis is that *A. digitifera* evolved from "dig-gem", also as a result of or reinforced by a differential timing of spawning. A third hypothesis is that "dig-gem" may be derived from *A. digitifera* and *A. gemmifera*, through hybridization. Hybridization between *A. digitifera* and *A. gemmifera* would be most likely to occur in the third month after the mass spawning when most colonies of *A. digitifera* and many colonies of *A. gemmifera* spawned. Irrespective of the evolutionary origin of "dig-gem", it now comprises a discrete evolutionary unit, which is more abundant than any other species or morph of the *A. humilis* species group at Lizard Island (Fig. 3.1). Analysis of molecules involved in gamete recognition, e.g. bindin or lysin (Palumbi 1994), would be most useful for testing each of the three hypotheses and resolving the evolutionary origin of "dig-gem".

Within clade II of the molecular phylogenetic analysis, sequences from colonies of *Acropora monticulosa* grouped in a subclade (clade IIa) with high bootstrap support (95%), suggesting that this species has also diverged from other species within the *A. humilis* species group (Fig. 3.2). This apparent divergence of *A. monticulosa* is congruent with the extremely low potential of this species to interbreed with other species in clade II (Fig. 3.4), as well as the late spawning time of this species (Table 3.5) forming a temporal reproductive barrier from other mass spawning species. Colonies of *A. monticulosa* were the most synchronized in timing of spawning compared with all other species within the *A. humilis* species group, spawning almost exclusively in the mass spawning month during a time interval of just over one hour. This species consistently spawned 5-6.25 hours after sunset, while most species of *Acropora* spawn 2-3.5 hours after sunset, as recorded in this study (Table 3.5) and by Babcock et al. (1986), with the latest previous recorded spawning time for any species of *Acropora* being 3.8 hours after sunset (Babcock et al. 1986). The separation in

timing of spawning of *A. monticulosa* from other species monitored in this study by 2-3 hours, and 1-2 hours after the latest time recorded for any other congeneric species provides convincing evidence that this species is reproductively isolated by temporal barriers. In comparison, temporal reproductive isolation has also been proposed for other species of *Acropora* (van Oppen et al. 2001; Fukami et al. 2003) with separation times ranging from 0.5-3 hours (Babcock et al. 1986; Hayashibara et al. 1993; Fukami et al. 2003). Temporal reproductive isolation has also been suggested in the *Montastraea annularis* species complex, with *M. franksi* spawning 1-2 hours earlier than *M. annularis* and *M. faveolata* (Knowlton et al. 1997; Szmant et al. 1997).

The species *Acropora monticulosa* appears to share greatest evolutionary affinity with *A. humilis*, with several factors supporting this proposal. It is feasible that the prolonged period of separation for egg-sperm bundles released from colonies of *A. humilis*, and the later spawning time of *A. monticulosa* (Table 3.5) has provided or maintained an opportunity for these species to continue to interbreed, that does not exist for *A. monticulosa* and other species of the *A. humilis* species group. Colonies of the morph "mont-hum" were the only other colonies to spawn at the later time recorded for *A. monticulosa*, while "mont-hum" egg-sperm bundles also separated over a 30-60 min interval (Table 3.5). Levels of fertilization between *A. monticulosa* and colonies of *A. humilis* and morphs of these species were extremely low, but slightly higher than levels with any other species or morphs, supporting the proposed divergence of *A. monticulosa* but closest evolutionary affinity with *A. humilis*. Meanwhile moderate levels of fertilization were recorded between colonies of *A. humilis* and the morphs "mont-hum" and "terete mont". Based on these fertilization records and the morphological affinities of these morphs, it is possible that they may be of hybrid origin, derived from *A. humilis* and *A. monticulosa* and able to backcross with colonies of *A. humilis* but rarely with colonies of *A. monticulosa*. The low to moderate levels of fertilization between two colonies of "terete mont", in contrast to the high levels of fertilization for all other intra-species and intra-morph crosses provides additional evidence that this morph may be a hybrid, in which sterility barriers partially reduce the potential for colonies of this morph to inter-breed. Further support for the hybrid origin of the morph "terete mont" is indicated in the molecular phylogeny, with the sequence for this morph having low bootstrap support and grouping neither within clade IIa or IIb.

Sequences from colonies of *Acropora humilis, A. gemmifera, A. samoensis* and morphs of these species formed a second subclade (IIb) also with high bootstrap support (95%), in the molecular phylogenetic analysis (Fig. 3.2). There was little further differentiation between sequences within this subclade, suggesting a close evolutionary relationship between these species relative to *A. digitifera* and *A. monticulosa*. The existence of intermediate morphs between *A. humilis, A. gemmifera* and *A. samoensis* also suggests a close relationship between these species, with the morphological continuum between *A. humilis* and *A. gemmifera* (this study and Wallace (1999)) and molecular evidence (Wolstenholme et al. 2003) suggesting these two species are closely related. The consistent late maturation of gametes in colonies of *A. samoensis* also suggests this is the most distinct of the three species. Greatest evolutionary affinity of "sam-hum" and "sam-gem" with *A. samoensis* is suggested by morphological similarity and supported by the same late maturation of gametes in the second and third months after the mass spawning. High levels of fertilization between *A. samoensis* and "sam-hum" suggest reproductive barriers have not formed between this species and morph. Meanwhile, substantially lower levels of fertilization between "sam-hum" and "sam-gem" suggests that their morphological differences reflect the reduced breeding compatibility and a possible hybrid status for these morphs.

Further investigation of egg size may contribute to understanding factors determining timing of spawning. Preliminary evidence from this study indicates that the relatively small eggs in the species *Acropora samoensis* and *A. digitifera* may be related to timing of spawning. These species do not appear to be closely related, based on the molecular data and morphological appearance. They also differ in the habitats that they occupy, with *A. samoensis* mostly occurring in slope and lagoonal habitats and *A. digitifera* on reef flats. Therefore, the smaller eggs in these species do not appear to be attributable to environmental conditions. Measurement of eggs released from colonies of each species and morph, particularly those that spawned in different months, would clarify whether there is a correlation between egg size and species/morph or timing of spawning. Such patterns may have been overlooked in previous studies, which only measured egg size in preserved branch samples, irrespective of timing of egg maturity and spawning.

3.5.2 TAXONOMIC IMPLICATIONS

Species of corals are well known to be morphologically variable, with boundaries between many species remaining unclear (e.g. Lang 1984). This study demonstrates the value of working at the morph level for clarifying evolutionary boundaries in corals. Recognition of morphs within or between species reduces taxonomic error as a result of 'forcing' colonies into incorrect or inappropriate species categories. The most outstanding case in this study is the morph "dig-gem", which was substantially more abundant than any other species or morph at Lizard Island. According to the current taxonomy, this morph could have been identified as either *Acropora digitifera* or *A. gemmifera* (Table 3.1), but it is distinct from both of these species. Identification of this morph as either of these species would conceal important evolutionary distinctions between these species. If colonies of "dig-gem" were identified as *A. digitifera,* it would appear that this species spawns from months –1 to month 3, with no temporal reproductive barrier isolating it from other species and morphs of the *A. humilis* species group. Conversely, if colonies of "dig-gem" were identified as *A. gemmifera,* DNA sequences for the marker examined in this study would be present in both clades I and II for this species and fertilization levels would range from 0 to 100%. In the case of the other species examined in this study, the most serious consequences of not distinguishing the species and morphs would be the interpretation of substantially broader levels of fertilization for each species, which in many cases would range from 0-100%.

Accurate identification of morphological species and morphs provided the foundation for interpreting relationships between the species and morphs examined in this study. This is in contrast to the conclusions of van Oppen et al. (2001), who state that "skeletal morphology may have been effectively uncoupled from the genotype in the case of *Acropora* evolution" and in the case of *A. humilis*, "morphology may have arisen several times independently". The current study demonstrates that close examination of morphological boundaries, using molecular and breeding criteria, is a powerful technique for resolving evolutionary boundaries in corals, as proposed by Willis (1990), Stobart (2000) and Wolstenholme et al. (2003). In addition, the morphs provided a valuable tool for testing possible evolutionary links between species, while the absence of intermediate morphs between other species, e.g. between *A. monticulosa*, *A. samoensis* and *A. digitifera* corresponds with the low potential of these

species to interbreed. This is the first study to assess the potential of species of the *A. humilis* group to interbreed, and therefore comparison with other studies that have tested fertilization potential under laboratory conditions is not possible for these species. Sperm competition experiments would provide a further test of prezygotic barriers and therefore evolutionary boundaries (Márquez et al. 2002a), between species and morphs. This could be done for pairs of species and morphs, which showed no potential to interbreed, to confirm the existence of prezygotic barriers. In cases where there was potential for interbreeding, for example between *A. gemmifera* and "hum-gem", sperm competition experiments would establish whether fertilization potential varied between the same and different morphological groups.

Intermediate morphologies and breeding potential are not conclusive evidence of hybrid status. For example, many hybrids exhibit extreme (positive or negative) phenotypic characters relative to parent species (reviewed in Rieseberg et al. 1999). To confirm the evolutionary affinities of morphs recognized in this study, it will be necessary to examine the species and morphs using a combination of nuclear and mitochondrial molecular markers. This has recently been demonstrated for *Acropora prolifera*. At least two morphs of *A. prolifera*, both of which are morphologically intermediate between the species *A. cervicornis* and *A. palmata*, are now known to be of hybrid origin and derived from these species (Vollmer and Palumbi 2002). Examination of chromosome number is also likely to contribute to understanding evolutionary relationships between the species and morphs examined in this study. Kenyon (1997) concluded that polyploidy, resulting from the combination of sets of chromosomes from different species during hybridization events is a likely source of gametic incompatibility between species in the genus *Acropora*. Evaluation of chromosome numbers in species as well as morphs may therefore also provide an important tool for tracing evolutionary relationships within the genus *Acropora* and possibly other groups of corals.

The variation between sequences within individual colonies was surprising, given that sequences from mitochondrial markers are not expected to vary at this level (Avise 2000). Two patterns contributed to this variation. Firstly, although repetitive sequences are a typical feature of control regions (van Oppen et al. 2002a and references within), the occurrence of repeat sequence blocks in this study was not consistent within

individuals. Repeat sequence blocks have been reported for other species of *Acropora*, but no other cnidarians for which data are available (van Oppen et al. 2002a), while patterns of intra-individual variation have not been examined in other studies of cnidarians. Further research is necessary to understand the evolutionary significance of these repeat regions (van Oppen et al. 2002a) and whether they have the potential to contribute to the interpretation of evolutionary relationships in the genus *Acropora* (MS in prep., Wolstenholme et al.). Secondly, differences in sequences (excluding the repeat regions) from single individuals, varied by up to 10 base pairs (Table 3.2). Although some of this variation is likely to be due to PCR error (Saiki et al. 1985), sites which varied as a result of PCR error and those that are due to intra-individual polymorphisms cannot be distinguished. Further interpretation of this variation, within an evolutionary context, is therefore not possible.

This study was restricted to the *Acropora humilis* species group at Lizard Island on the Great Barrier Reef, Australia. Species within this group and other species groups in the genus *Acropora* were arbitrarily assigned based on apparent morphological similarity (Veron and Wallace 1984; Wallace 1999). It is possible that the species and morphs in this species group also share evolutionary affinities with other species in the genus *Acropora*. This particularly applies to *A. digitifera*, given the clear distinction between this species and other species of the *A. humilis* species group in this study. Phylogenetic analysis of morphological characters of the genus *Acropora* suggests that *A. digitifera* may be most closely related to species of the *Acropora nasuta* and *A. divaricata* species groups (Wallace 1999). Broader analyses, which examine additional species within the genus *Acropora* using complementary techniques as in this study, are necessary to explore this possibility. It is likely that the status of evolutionary relationships between species and morphs will vary in different geographic locations (e.g. Hayashibara and Shimoike 2002). Therefore, such projects must also be conducted at a broad geographic scale before the taxonomic status of the morphs and the boundaries of current species can be fully resolved.

This study demonstrates that morphology is a valuable tool for interpreting evolutionary relationships in the *Acropora humilis* species group. This is likely to also be true for other species of *Acropora* and other coral taxa. In particular, morphs may indicate active zones of speciation (e.g. between *A. humilis* and *A. gemmifera*) or

hybridization (e.g. between *A. digitifera* and *A. gemmifera*), which can then be tested using genetic and reproductive criteria. Confirmation of morphology as an informative character of evolutionary boundaries is of great significance because most coral research projects rely on morphology as the primary tool for recognizing species.

CHAPTER 4: MORPHOLOGICAL VARIATION AND BIOGEOGRAPHY OF SPECIES AND MORPHS OF THE *ACROPORA HUMILIS* SPECIES GROUP

4.1 INTRODUCTION

Morphological appearance or external form is the primary and most easily accessible means for defining and recognizing species (Stuessy 1990; Maddison 1996). Virtually all species of plants and animals are defined on the basis of morphological differentiation and once species have been defined, they are most readily recognized in other fields of research (e.g. genetics, biogeography, biology, ecology, physiology) on the basis of their morphological appearance. Recently, species have also been proposed on the basis of non-morphological criteria e.g. using genetic differentiation. In some cases, subsequent examination has revealed that these taxa can also be distinguished morphologically, while others appear to have not diverged morphologically. Irrespective of the level of morphological differentiation, it is essential that species are defined accurately according to the current state of knowledge (Knowlton and Jackson 1994). This is necessary so that advances in the interpretation of evolutionary relationships can be incorporated into the current taxonomic framework, enabling them to be assessed in future systematic studies and examined in other fields of research. To ensure results of past studies can be verified and compared in future research projects, voucher specimens need to be deposited in museum collections (Paulay 1997; Wheeler 2001).

In scleractinian corals, skeletal characters are most important for defining and recognizing species. The taxonomy of scleractinian corals is based almost entirely on skeletal characters (e.g. Wells 1956; Veron and Wallace 1984; Wallace 1999) and corresponding type and voucher specimens are dried skeletons, for which until recently, tissue samples were not preserved and live appearance was not recorded. The skeleton also forms the structure of all colonies, directly reflecting the morphological appearance of living corals. In addition, although the fossil record of scleractinian corals is extensive, it only provides evidence of the skeletal appearance of fossil taxa. Skeletal characters are therefore fundamental to the recognition and study of extant and fossil corals, connecting all fields of research on scleractinian coral species.

Despite the fundamental importance of skeletal characters in the taxonomy of scleractinian corals, defining species on the basis of these characters has been problematic, due to high levels of polymorphism in many taxa (reviewed in chapters 1-3). Intraspecific polymorphism has typically been attributed to environmental influences (e.g. Veron and Pichon 1976), but may also be an important indicator of recent or ongoing microevolutionary processes within or between species (Sattler and Rutishauser 1997; Wiens 1999). It is possible therefore, that unresolved boundaries between morphologically similar species of scleractinian corals may be highly informative, with shared or intermediate morphological characters indicating incomplete speciation or evidence of hybridization. Similarly, discrete morphs within currently defined species may be evidence of recent divergence (chapter 3).

Morphometric analyses have been used in numerous studies to examine morphological variation in scleractinian corals. These investigations have mostly focused on the Faviidae (*Montastraea* and *Platygyra*) and Poritidae (*Porites*), with few or no studies being conducted in other families. Morphological variation has been extensively examined within the genus *Montastraea*. Colonies previously assigned to one polymorphic species, *M. annularis*, are now recognized as this and two previously synonymised species, *M. faveolata* and *M. franksi*, on the basis of colony, corallite and septal characters (Weil and Knowlton 1994), as well as life-history, ecological and genetic differences (Knowlton et al. 1992; Knowlton et al. 1997). Morphometric analysis of these three extant species and fossil colonies of the genus *Montastraea* demonstrates that the extant species have been morphologically distinct for at least 2-4 million years, while evolutionary relationships of additional fossil species remain unresolved, pending further analysis (Budd and Klaus 2001). Within the genus *Platygyra*, morphometric analysis of corallite and septal characters confirm five described species and two additional morphospecies on the Great Barrier Reef, Australia are morphologically distinct, and that their appearance is independent of environmental influences (Miller 1994). However, these species do not appear to be separated by reproductive or genetic boundaries, indicating that evolutionary barriers have not formed between them (Miller and Babcock 1997; Miller and Benzie 1997). Defining morphological species boundaries in the genus *Porites* has been difficult because of polymorphic variation as well as the small size of taxonomic skeletal characters (Veron and Pichon 1982). Use of morphometric colony and corallite

characters has been useful in resolving boundaries within the genus *Porites* with complete resolution in one study (Weil 1992) and 90% agreement between morphological and genetic boundaries in another (Budd et al. 1994). Morphometric differences, supported by electrophoretic and reproductive criteria, confirmed the validity of three species in the genus *Pavona* (Maté 2003) and two in the genus *Montipora* (Stobart 2000). Despite the widespread abundance and large number of species of *Acropora*, only one preliminary study has examined species boundaries in this genus using morphological characters. In this study, species of the *A. selago* group were differentiated morphologically, using branch and axial corallite characters (Wallace et al. 1991). However, this study did not examine variability between colonies within each species.

Previous studies that have examined species boundaries in corals, have been on a restricted biogeographic scale, and often limited to a single location. However, to gain a greater understanding of the evolutionary relationships between species, it is necessary to examine patterns at a broad biogeographic scale (Hughes et al. 2002). Analysis of broad-scale biogeographic patterns of morphological variation in corals, within and between species, will provide a foundation upon which non-morphological criteria can be used to test the extent to which morphological variation is indicative of microevolutionary processes (Wiens 1999) and whether the same evolutionary processes are occurring between the same combinations of species in different locations. This will enable the delineation of boundaries between species, across their entire distributional range, to be more accurately defined. It will also enable the evolutionary significance of morphological variants within currently defined species to be resolved.

In this chapter, I assess patterns of morphological variation in the *Acropora humilis* species group, for several west and central Pacific locations. Qualitative characters are used to identify species as well as interspecific and intraspecific morphs. Morphological variation for each species is defined in a discriminant analysis of morphometric characters. The results of the discriminant analysis are then used to determine morphological affinities of colonies of the interspecific and intraspecific morphs, by calculating the classification probabilities of each colony. Finally, museum collections of samples from the entire Indo-Pacific region are examined to determine

the geographic distribution of each species and morph, and whether additional morphs of the *A. humilis* species group not recorded in the field surveys of this study could be recognized.

4.2 METHODS

4.2.1 COLLECTION OF SAMPLES

Samples of the *Acropora humilis* species group were collected in seven regions from the western to central Pacific, in Taiwan, Indonesia, Australia, Papua New Guinea, Solomon Islands, American Samoa and French Polynesia. Sampling locations within each region are listed in Table 4.1. The sampling units used in this study were putative morphs, distinguished using morphological characters, as described in chapters 2 and 3.

Table 4.1 Regions and locations where corals were sampled.

Region[a]		Location	Latitude	Longitude
Taiwan (Tai)	1	Taiwan Strait, Penghu Islands	23^{0}38'N	119^{0}33'E
	2	Southern Taiwan, Wanlitung	22^{0}04'N	120^{0}43'E
	3	Southern Taiwan, Nan-Wan Bay	22^{0}00'N	120^{0}48'E
	4	Northern coast	25^{0}12'N	121^{0}37'E
	5	Lanyu	22^{0}10'N	121^{0}31'E
Indonesia (Ind)	1	Central Sulawesi, Togian Islands	00^{0}20'S	121^{0}49'E
	2	North Sulawesi, Malibagu	00^{0}21'N	124^{0}03'E
	3	North Sulawesi, Bunaken National Park	01^{0}38'N	124^{0}45'E
	4	North Sulawesi, Lembeh Strait	01^{0}29'N	125^{0}14'E
Australia (Aus)	1	GBR, Lizard Island	14^{0}40’S	145^{0}28’E
Papua New Guinea (PNG)	1	New Britain, Kimbe Bay	05^{0}25'S	150^{0}05'E
Solomon Islands (Sol)	1	New Georgia Islands, Ghizo Island	08^{0}06'S	156^{0}53'E
American Samoa (Sam)	1	Tutuila	14^{0}20'S	170^{0}48'W
	2	Ofu	14^{0}11'S	169^{0}41'W
	3	Olosega	14^{0}11'S	169^{0}37'W
French Polynesia (Pol)	1	Society Islands, Moorea	17^{0}35'S	149^{0}50'W

[a] Abbreviations for each region (used in Tables 2, 4 and 5) are given in parentheses

The colonies analysed in chapters 2 and 3 (from American Samoa and Lizard Island, respectively) are included in this chapter. The putative morphs include known species,

intermediate morphs and sub-morphs. The characters used to define the species are based on the descriptions for each of these species (Wallace 1999). The intermediate morphs are characterized by intermediate morphologies that prevented colonies from being confidently assigned to a particular species, and are named using abbreviations from the species with which they appear to share most morphological characters. The sub-morphs formed recognizable units within the range of morphological variation of single species and are named using an abbreviation of this species name and a morphological feature or the location from which it was collected. Habitats and locations in which species and morphs were recorded, and characters used to distinguish them are summarized in Tables 4.2 and 4.3 respectively. Colonies were sampled following the protocol described in chapter 2.

4.2.2 MORPHOMETRIC ANALYSIS

Five colonies of each morph, from each region in which it occurred, were usually used in the analyses. In cases where fewer than five colonies were recorded from a region, all colonies were used in the analyses (Table 4.2). A total of 226 samples were examined in this analysis. All morphological samples used in this study are deposited at the Museum of Tropical Queensland, Townsville, Australia. Morphometric characters used in this study are the same as those used in chapter 2 (Table 2.1). Analyses were performed using the statistical package SPSS 10.0.5.

Variation within and between all species and morphs was compared for each morphometric character (Fig. 4.3). Morphological relationships between the species *A. samoensis, A. humilis, A. globiceps, A. gemmifera, A. monticulosa* and *A. digitifera* were also assessed in a Bayesian linear discriminant analysis of the morphometric variables. Variables were selected in the discriminant analysis using the stepwise method. The species *A. retusa* and *A. multiacuta* were not included in this analysis because they were clearly morphologically distinct from all other species and morphs examined in this study (Table 4.3a and Fig. 4.3). Morphological affinities between the species in the discriminant analysis and the intermediate and sub-morphs were tested by determining the probability of classification of each morph colony into the categories

Table 4.2a Habitats and locations in which species were recorded.

Species	Habitat	Recorded Location[a]	n [b]
A. globiceps	exposed reef slopes, just subtidal to ~15m	Pol: **1**	5
A. humilis	exposed reef slopes and lagoons, just subtidal to >20m but usually <10m	Tai: 2, **3**, 4, 5	5
		Ind: **3**, **4**	5
		Aus: **1**	5
		PNG: **1**	5
		Sol: **1**	5
		Sam: 1, **2**, 3	5
A. samoensis	reef slopes and lagoons, subtidal to > 20m, usually in calm or protected habitats	Ind: **1**	3
		Aus: **1**	5
		PNG: **1**	5
		Sol: **1**	5
A. gemmifera	exposed reef slopes and lagoons, just subtidal to ~10m	Ind: **1**, **2**, **3**, 4	5
		Aus: **1**	5
		PNG: **1**	5
		Sol: **1**	5
A. monticulosa	shallow, wave exposed reef crests to ~4m	Tai: **5**	2
		Aus: **1**	5
		Sol: **1**	3
		Sam: **1**	4
A. digitifera	shallow, exposed reef crests and flats to ~4m	Tai: **2**, **3**, **5**	3
		Ind: **1**, **2**, 3	5
		Aus: **1**	5
		PNG: **1**	5
		Sol: **1**	5
A. retusa	exposed reef slopes and lagoons, just subtidal to ~15m	Pol: **1**	5
A. multiacuta	protected reef slopes and lagoons, just subtidal to ~20m	Ind: **1**	5

[a] Recorded locations in bold indicate colonies from these locations were used in the analyses. Location numbers are listed in Table 4.1; [b] n is the number of colonies used in the analyses.

Table 4.2b Habitats and locations in which morphs were recorded.

Morph	Habitat	Recorded Location [a]	n [b]
"hum-gem"	exposed reef slopes and lagoons, just subtidal to >20m but usually < 10m	Ind: **1**	3
		Aus: **1**	5
		PNG: **1**	5
"hum-gem plate"	exposed reef slopes to ~10m	Tai: 1, **2**, **3**	5
"sam-hum"	as for *A. samoensis*	Ind: **1**	4
		Aus: **1**	4
		PNG: **1**	3
		Sol: **1**	3
"sam-gem"	as for *A. samoensis*	Ind: **1**	4
		Aus: **1**	4
		Sol: **1**	1
"mont-hum"	shallow, exposed reef crests and flats to ~4m	Aus: **1**	5
		Sol: **1**	2
"mont-gem"	as for *A. monticulosa*	Tai: **2**, **3**, **5**	5
		Ind: **2**, **3**, **4**	5
		Aus: **1**	3
		PNG: **1**	5
		Sol: **1**	5
		Sam: **1**, **2**	5
"dig-gem"	as for *A. digitifera*	Aus: **1**	5
"Penghu hum"	turbid reef slopes, subtidal to ~10m	Tai: **1**	5
"fine sam"	protected reef slopes and lagoons, subtidal to ~10m	Ind: **1**	5
"terete mont"	as for *A. monticulosa*	Aus: **1**	3
		Sol: **1**	1
"digitate mont"	as for *A. monticulosa*	Tai: **5**	2
		Sol: **1**	1
		Sam: **3**	2
"Samoan dig"	shallow, wave exposed crests to ~4m	Sam: **1**	5
"encrusting dig"	as for *A. digitifera*	Tai: **5**	1

[a] Recorded locations in bold indicate colonies from these locations were used in the analyses. Location numbers are listed in Table 1; [b] n is the number of colonies used in the analyses.

defined for the six species in the discriminant analysis. The classification coefficients derived from the discriminant analysis were used to calculate the classification probabilities for individual colonies, using the equation:

$$P\left(\Pi_j \mid \overrightarrow{x_o}\right) = \frac{P\left(\Pi_j\right) P\left(\overrightarrow{x_o} \middle| \Pi_j\right)}{\sum_{j=1}^{g} P\left(\Pi_j\right) P\left(\overrightarrow{x_o} \middle| \Pi_j\right)}$$

where π_j is the species category, $\overrightarrow{x_o}$ is the classification vector (morphometric values for each colony), g is the maximum number of categories (i.e. 6) and j ranges from 1 to the maximum number of categories. The classification probability was not calculated for the sub-morph "encrusting dig" because morphological characters of this morph were not directly comparable with the other species and morphs in this study, due to its peculiar growth form (Table 4.3c).

4.2.3 INDO-PACIFIC MORPHOLOGICAL VARIATION AND DISTRIBUTION

Collections from the entire Indo-Pacific region, at the Museum of Tropical Queensland, of each of the eight species of the *Acropora humilis* species group were examined to compare morphological variation and map the geographic distribution of the morphs recognized in this study. These collections were also examined to determine if additional morphs not recorded in this study were evident. Specimens in the collections (1267 specimens) were assigned to each species or morph, using the same skeletal morphological characters that were used to identify colonies in this study (Table 4.3).

4.3 RESULTS

4.3.1 SPECIES AND MORPHS

In field surveys of this project, 21 putative morphs were recognized within the *Acropora humilis* species group (Table 4.3). Eight of the putative morphs correspond with the known species in this group. These are *A. samoensis* (Brook 1891), *A. humilis* (Dana 1846), *A. globiceps* (Dana 1846), *A. gemmifera* (Brook 1892), *A. monticulosa* (Brüggemann 1879), *A. digitifera* (Dana 1846), *A. retusa* (Dana 1846) and *A. multiacuta* (Nemenzo 1967). The remaining putative morphs comprised seven

Table 4.3a Species in the *Acropora humilis* species group and characters used to identify them [a].

Species	Growth Form	Branch Shape	Axial Corallites	Radial Corallites [b]	Colony Colour
A. globiceps	corymbose	thick, terete	**large**, wall thin, funnel-shaped calice	one size, large (radial corallites around axial corallite are quite large in comparison with *A. humilis*), **nariform to tubo-nariform**, crowded	brown, edges of corallites paler in colour, polyps white
A. humilis	corymbose	thin to thick, terete	**large to very large**	**one size**, large, **nariform to tubo-nariform**, crowded to not crowded	brown, green, blue, yellow or combinations of these colours, corallites may be paler at edges, polyps are white, green or pale brown
A. samoensis	**caespito-corymbose** to corymbose	thin, terete	large	one size, size variable, tubular to tubo-nariform, **not crowded**	brown usually with a blue, orange or yellow tinge, edges of corallites often paler in colour, polyps white, yellow or pale brown
A. gemmifera	corymbose	thick, conical	large	**two sizes**, large, **dimidiate (sometimes flaring) to tubo-nariform**, crowded	brown usually with a blue or orange tinge, corallites may be paler at edges, polyps white or pale brown
A. monticulosa	**digitate or irregular digitate to arborescent**	very thick, **very conical**	small	one to mixed sizes, small, **nariform to tubular**, crowded	brown with a blue, yellow or green tinge all over, polyps pale brown
A. digitifera	corymbose	thin, terete	small	mixed sizes, small or large, **flaring dimidiate to flaring lips**, crowded	pale to dark brown sometimes with paler blue or paler brown branch tips, polyps dark or pale brown
A. retusa	corymbose	terete, **spiky**, long and exsert radial corallites	small	mixed sizes, large, **elongate tubes**, crowded	brown sometimes with a blue tinge, edges of corallites may be paler in colour, polyps not seen
A. multiacuta	irregular corymbose	thin, tapering	**very elongate but small diameter**	mixed sizes, small, tubular or tubular appressed, **widely and unevenly scattered, most at base of branches**	brown with a blue, pink or cream tinge, polyps not seen

[a] Characters are described relative to other species and morphs, with those that are most important for distinguishing each species highlighted in bold. [b] Two size categories of radial corallites are given. The first category refers to the size range of radial corallites within that species: corals with two sizes have subimmersed and regular sized corallites; corals with mixed sizes have corallites that range in size from subimmersed to regular. The second category refers to the size of radial corallites relative to other species and morphs.

Table 4.3b Intermediate morphs in the *Acropora humilis* species group and characters used to identify them [a].

Intermediate Morph	Growth Form	Branch Shape	Axial Corallites	Radial Corallites [b]	Colony Colour
"hum-gem"	corymbose	thick, terete	large	**one to two sizes**, large, **tubo-nariform to dimidiate**, crowded	as for *A. humilis* but usually less brightly coloured
"hum-gem plate"	corymbose plate	thick, short, conical	small	one to two sizes, nariform to dimidiate, crowded	brown with blue, orange or yellow tinge, polyps brown
"sam-hum"	corymbose to caespito-corymbose	thin to thick, terete	large	**one size**, large, **tubo-nariform**, usually crowded	as for *A. samoensis*
"sam-gem"	corymbose to caespito-corymbose	thin to thick, terete	large	**one or two sizes**, large, **tubo-nariform to dimidiate**, usually crowded	as for *A. samoensis*
"mont-hum"	corymbose	thick, terete to conical	**large to very large**	**one size**, large, **nariform**, crowded	brown or yellow-brown, paler at edges of corallites, polyps white or pale brown
"mont-gem"	digitate	very thick, **very conical**	**small**	**two sizes**, small, **dimidiate**, crowded	brown with paler to white or sometimes blue branch tips, polyps brown or white
"dig-gem"	corymbose	thick, conical	small	mixed sizes, large, **flaring dimidiate to flaring lips**, crowded	as for *A. digitifera*

[a] and [b] notes as for Table 4.3a

Table 4.3c Sub-morphs in the *Acropora humilis* species group and characters used to identify them [a].

Sub-Morph	Growth Form	Branch Shape	Axial Corallites	Radial Corallites [a]	Colony Colour
"Penghu hum"	corymbose	thin terete	**small**	**one size**, large, **nariform to tubo-nariform**, crowded	brown, sometimes with blue, orange or cream tinge, paler to white axials, polyps green or brown
"fine sam"	**caespitose**	thin terete	small	mixed sizes, large, **tubular**, **not crowded**	pale brown with blue or yellow tinge, polyps bright yellow
"terete mont"	digitate	very thick, **sometimes conical**	large	mixed sizes, small, dimidiate, crowded	brown with blue or green corallites, polyps white
"digitate mont"	digitate	terete, short, evenly sized	small	mixed sizes, small, **nariform to tubo-nariform**, crowded	as for *A. digitifera*
"Samoan dig"	digitate	thin, terete	small	mixed sizes, small, **lipped**, crowded	as for *A. digitifera*
"encrusting dig"	digitate	barely developed	small	immature	as for *A. digitifera*

[a] and [b] notes as for Table 4.3a

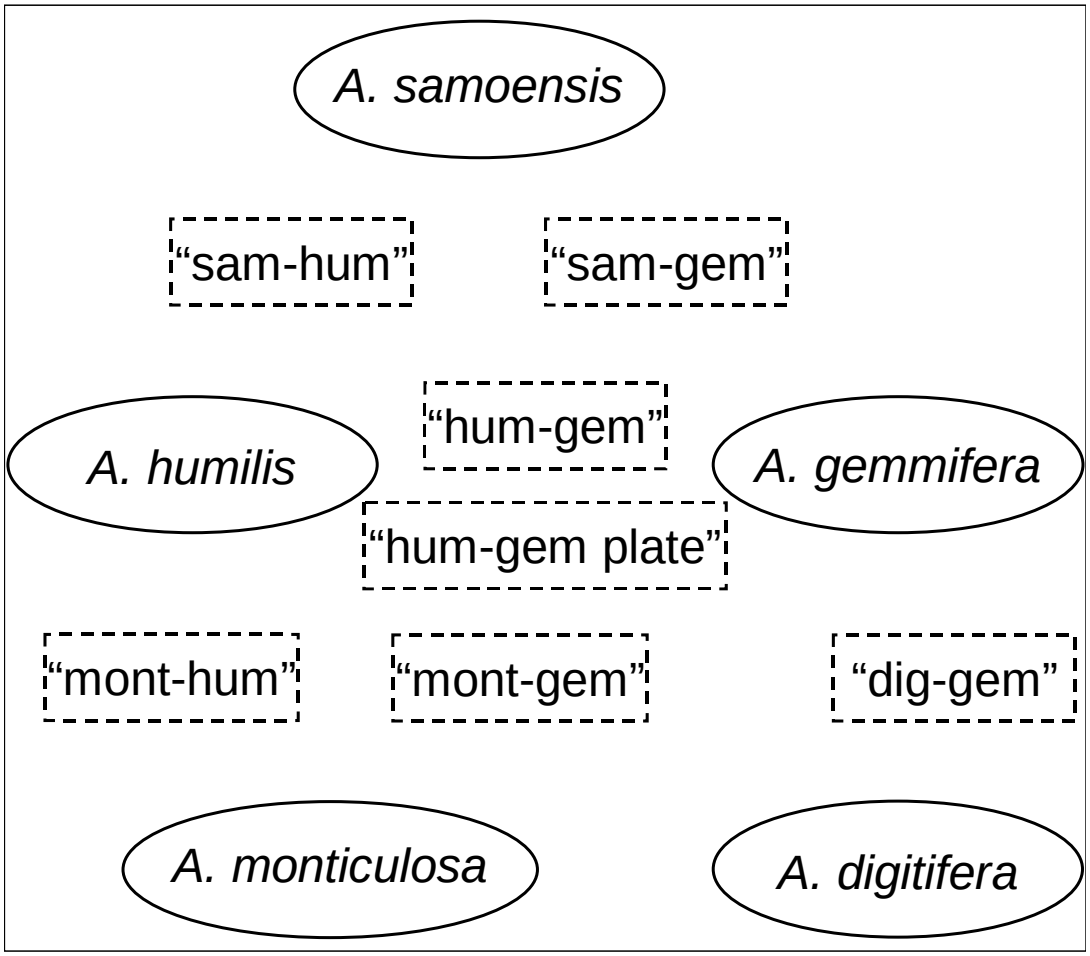


Fig. 4.1 Diagrammatic representation of the intermediate morphs in relation to the species after which they were named.

intermediate morphs (Fig. 4.1) and six sub-morphs (Fig. 4.2). No intermediate or sub-morphs were recorded for the species *A. globiceps, A. retusa* and *A. multiacuta* and no intermediate morphs were recorded between the species *A. samoensis*, *A. monticulosa* and *A. digitifera*. Intermediate morphs were recorded between *A. gemmifera* and *A. samoensis, A. humilis*, *A. monticulosa* and *A. digitifera*; and between *A. humilis* and *A. samoensis* and *A. monticulosa* but not *A. digitifera*. The intermediate morphs were "sam-hum", "sam-gem", "hum-gem", "hum-gem plate", "mont-hum", "mont-gem" and "dig-gem". Colonies of *A. gemmifera* from American Samoa were reclassified as "mont-gem" after comparison with all samples in the broader geographical context of this study. Of the six sub-morphs, none were recorded for the species *A. gemmifera*, one each for *A. samoensis* and *A. humilis* and two each for *A. monticulosa* and *A.*

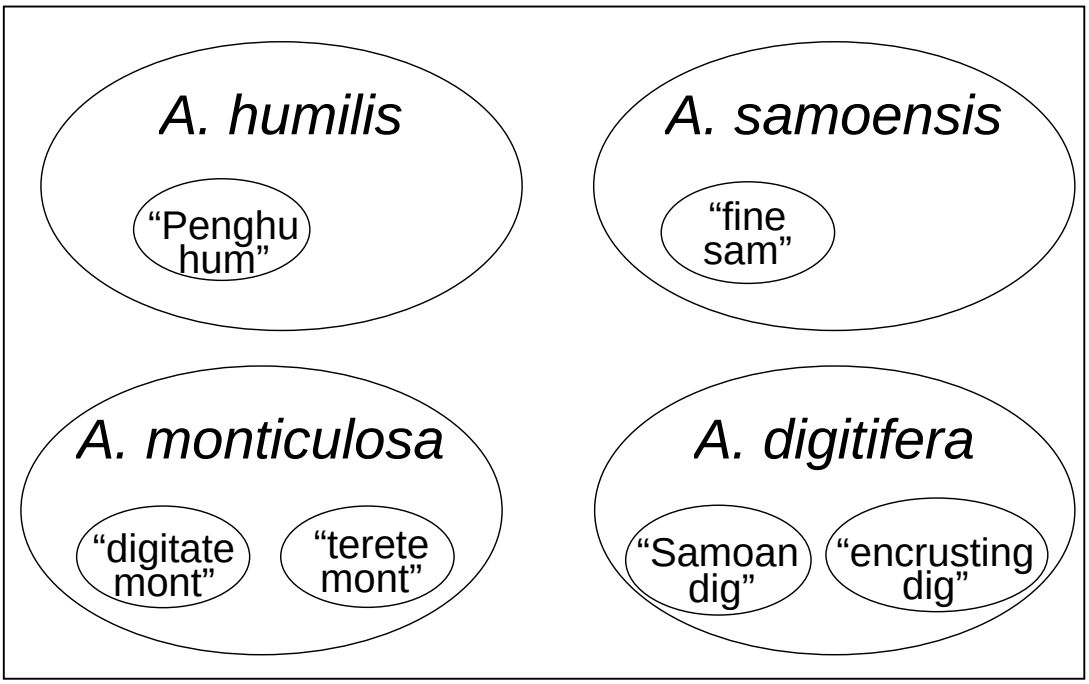


Fig. 4.2 Diagrammatic representation of the sub-morphs in relation to the species after which they were named.

digitifera. These were “fine sam”, “Penghu hum”, “terete mont”, “digitate mont”, “Samoan dig” and “encrusting dig”. Two morphs of *A. monticulosa* were recognized in American Samoa in chapter 2 (“branching *A. monticulosa*” and “digitate *A. monticulosa*”). The digitate morph remained a distinct morph when compared with other samples collected in this study and in the museum collections and is referred to as “digitate mont” in this chapter. The only distinguishing character of the morph “branching *A. monticulosa*”, when compared with other samples, was the arborescent branching growth form. Samples of this latter morph were therefore reclassified as the species *A. monticulosa*. Similarly, “Samoan dig” was identified as *A. digitifera* in chapter 2, but was clearly distinct from all other colonies of this species in this study and was therefore renamed as this sub-morph in this chapter.

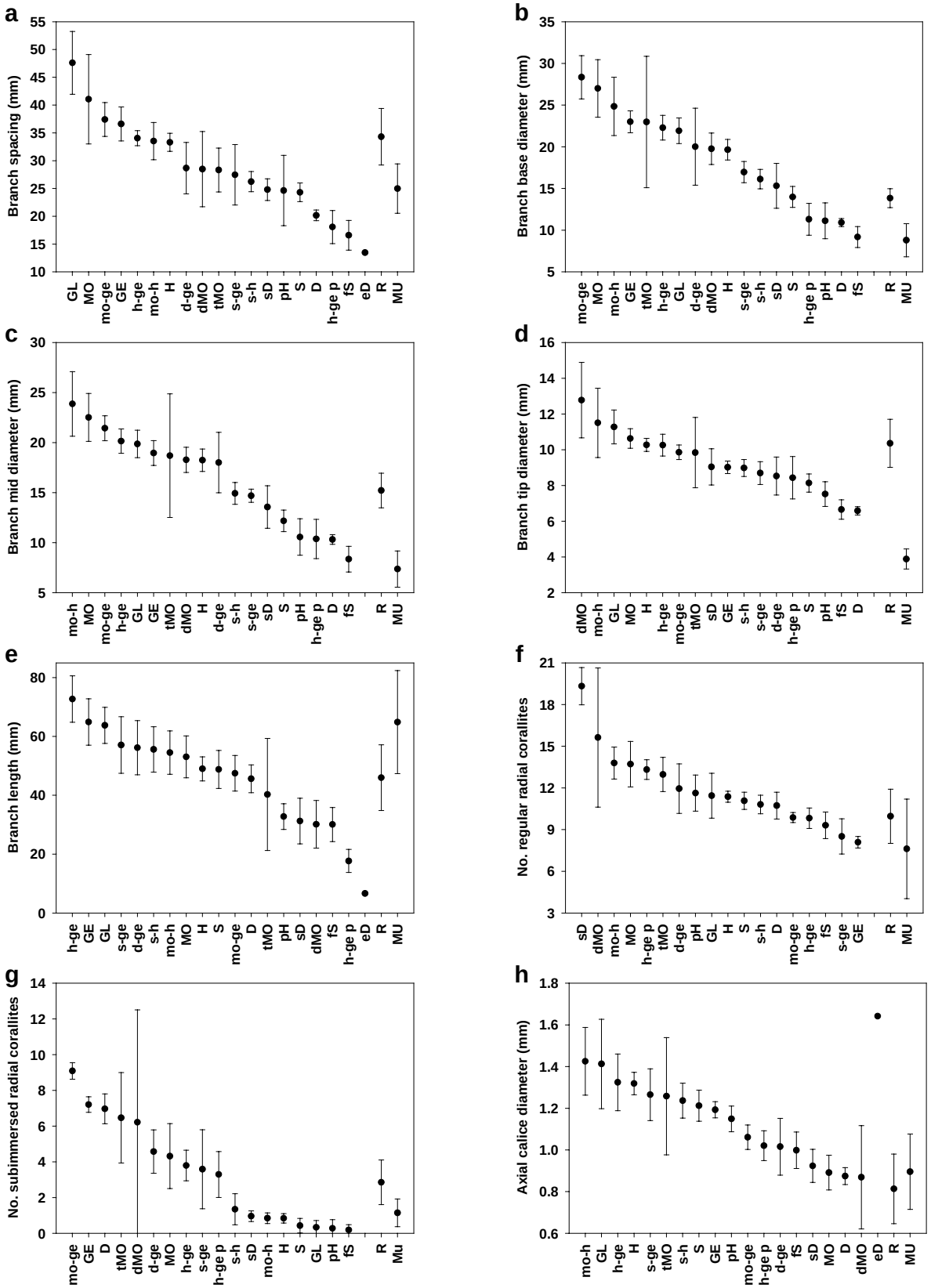
In addition to the species and morphs recognized in the field surveys, an intermediate morph and two sub-morphs were recognized in the museum collections, all of which appeared to share morphological affinities with the species *A. digitifera*. The intermediate morph was “dig-gem 1”. The sub-morphs were *Acropora* sp 1 aff. *digitifera*, which has been previously recognized and described (Hayashibara and Shimoike 2002) and “Indonesian dig”.

4.3.2 MORPHOLOGICAL VARIATION BETWEEN SPECIES AND MORPHS

There was no single morphological character that consistently differentiated the species and morphs in the *Acropora humilis* species group. This was the case for both qualitative (Table 4.3) and morphometric characters, with most morphometric characters varying continuously between most species and morphs (Fig. 4.3). *Acropora samoensis, A. humilis* and *A. globiceps* were morphologically similar in that they had terete branches and tubo-nariform radial corallites. These three species differed in that dimensions for branch and radial corallite characters and axial calice diameter were smallest in *A. samoensis*, intermediate in *A. humilis* and largest in *A. globiceps*. Width of the axial walls was similar in *A. samoensis* and *A. humilis* and about one-third thinner in *A. globiceps*. Of the remaining species, *A. gemmifera* is morphologically most similar to these three species (particularly *A. humilis*), but differs in having conical branches and radial corallites that varied from tubo-nariform to dimidiate in shape. The most distinctive character of *A. gemmifera* is the large number of subimmersed corallites compared to the small number of regular radial corallites. In *A. monticulosa*, the very conical branches were the most distinctive feature, being of intermediate length and having large basal diameters that taper to small axial corallites. Radial corallite shape in *A. digitifera* is most similar to but less tubular, more flaring and smaller compared to *A. gemmifera*. *Acropora gemmifera* and *A. digitifera* were also similar in having a high number of subimmersed radial corallites, but are distinguished by the thin, non-tapering branches and small axial corallite dimensions in the latter species. *Acropora retusa* and *A. multiacuta* were the most morphologically distinct of all species and morphs. The most distinguishing feature of *A. retusa* was the elongate tubular radial corallites (4.8mm mean length), which were approximately twice the length, but of similar diameter, to those in other species and morphs (Fig. 4.3 k-n). *Acropora multiacuta* was easily separated from all other species by its long thin branches (64.9mm mean length; Fig. 4.3e) with few radial corallites (7.6 regular and 1.1 subimmersed radial corallites/3cm; Fig. 4.3 f and g).

The intermediate morphs are recognized on the basis of intermediate or shared morphological traits with the species after which they are named (Table 4.3b). Morphometric dimensions of the morphs “sam-hum”, “sam-gem” and “hum-gem” were

Fig. 4.3 Mean values for each morphometric character for each species and morph examined in this study. Morphometric characters are indicated on the vertical axes. Error bars indicate 95% confidence intervals. Abbreviations for each species and morph are: *S A. samoensis*; *H A. humilis*; *GL A. globiceps*; *GE A. gemmifera*; *MO A. monticulosa*; *D A. digitifera*; *R A. retusa*; *MU A. multiacuta*; *s-h* "sam-hum"; *s-ge* "sam-gem"; *h-ge* "hum-gem"; *h-ge p* "hum-gem plate"; *mo-h* "mont-hum"; *mo-ge* "mont-gem"; *d-ge* "dig-gem"; *fS* "fine sam"; *pH* "Penghu hum"; *tMO* "terete mont"; *dMO* "digitate mont"; *sD* "Samoan dig"; *eD* "encrusting dig". Species and morphs are ordered on the horizontal axes from maximum to minimum mean values, with the exception of "encrusting dig", *A. retusa* and *A. multiacuta*. This morph and these species are placed in the same order at the right hand side of each plot. Branch diameters and radial corallite counts were not applicable for "encrusting dig" due to the absence of developed branches.


a
Branch spacing (mm)
GL MO mo-ge GE h-ge mo-h H d-ge dMO tMO s-ge s-h sD pH S D h-ge p fS eD R MU
b
Branch base diameter (mm)
mo-ge MO mo-h GE tMO h-ge GL d-ge dMO H s-ge s-h sD S h-ge p pH D fS R MU
c
Branch mid diameter (mm)
mo-h MO mo-ge h-ge GL GE tMO dMO H d-ge s-h s-ge sD S pH h-ge p D fS R MU
d
Branch tip diameter (mm)
dMO mo-h GL MO H h-ge mo-ge tMO sD GE s-h s-ge d-ge h-ge p S pH fS D R MU
e
Branch length (mm)
h-ge GE GL s-ge d-ge s-h mo-h MO H S mo-ge D tMO pH sD dMO fS h-ge p eD R MU
f
No. regular radial corallites
sD dMO mo-h MO h-ge p tMO d-ge pH GL H S s-h D mo-ge h-ge fS s-ge GE R MU
g
No. subimmersed radial corallites
mo-ge GE D tMO dMO d-ge MO h-ge s-ge h-ge p s-h sD mo-h H S GL pH fS R Mu
h
Axial calice diameter (mm)
mo-h GL h-ge H s-ge tMO s-h S GE pH mo-ge h-ge p d-ge fS sD MO D dMO eD R MU

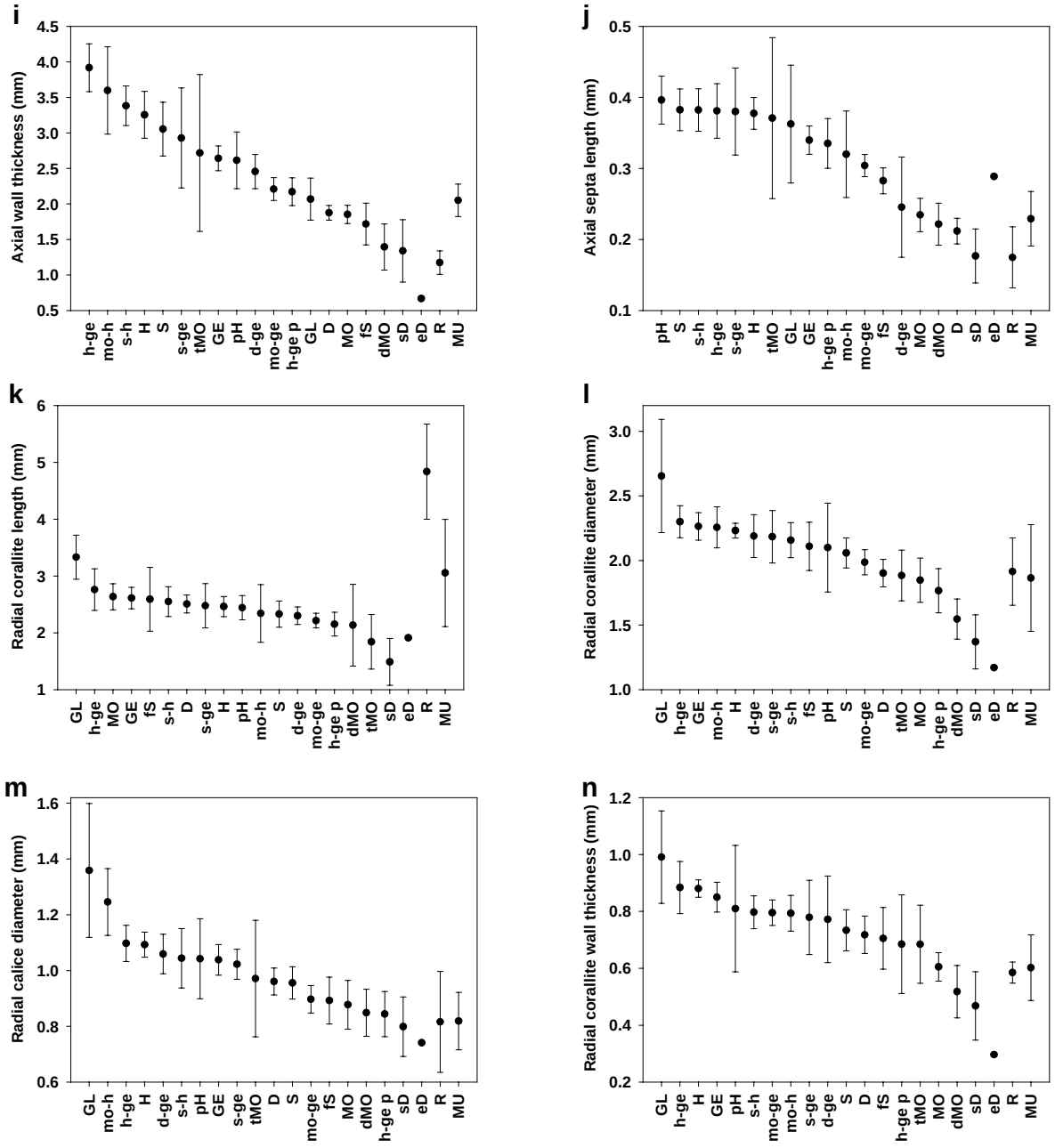


Fig. 4.3 continued

similar to or intermediate between dimensions for the species *A. samoensis*, *A. humilis* and *A. gemmifera*. There was little variation between the morphs "sam-hum" and "sam-gem" for most characters, except the former had more regular and fewer subimmersed radial corallites and thicker axial walls, consistent with the dimensions recorded for *A. humilis* and *A. gemmifera* respectively. Branch spacing and diameters of the morph "hum-gem" were intermediate to *A. humilis* and *A. gemmifera* but slightly larger than branch length, and axial and radial corallite dimensions in these species. Morphological affinity of the intermediate morph "hum-gem plate" was not clear. The shape of radial corallites in this morph suggested that this morph is probably most similar to *A. gemmifera* or "hum-gem". The growth form of corymbose plates in this morph seemed to be common for a range of species of *Acropora* in the Penghu Islands and southern Taiwan (pers. obs.), and so was not considered a diagnostic character for identifying this morph. In comparison to *A. monticulosa* and *A. humilis*, branches of the morph "mont-hum" were most similar to but less conical than those in the former species while axial and corallite dimensions were most similar to the latter species. The morph "mont-gem" was intermediate between *A. monticulosa* and *A. gemmifera* for most characters except branch diameters, which were more similar to the former species. Morphometric dimensions of the morph "dig-gem" were intermediate between *A. gemmifera* and *A. digitifera* but closest to *A. gemmifera* for almost all characters.

Morphological dimensions of the sub-morphs were generally smaller than those of the corresponding species after which they were named (Table 4.3c and Fig. 4.3). All colonies of the sub-morph "fine sam" were identical in colour to some colonies of the species *A. samoensis*, with this morph differing in that it had thinner, more compact branches and smaller axial corallites. The sub-morph "Penghu hum" differed from *A. humilis* in having smaller branch dimensions, smaller axial corallites but similarly long axial septa, and similar but variably sized radial corallites. Most skeletal characters of the sub-morph "terete mont" were highly variable, despite the consistent appearance of live colonies. Compared to *A. monticulosa*, this sub-morph had smaller and less conical branches, larger axial and similar radial dimensions. The sub-morph "digitate mont" was distinct from *A. monticulosa* and "terete mont", distinguished by short, thin, terete branches. Colonies of "digitate mont" had most resemblance to the morph "Samoan dig", which had similarly shaped but smaller branches, similarly small corallites and the same colour in live colonies. In comparison to *A. digitifera*, colonies of "Samoan

dig" had thicker, shorter branches with smaller axial corallites and smaller, more crowded radial corallites. The single colony of the morph "encrusting dig" was named because of its similar colony colour to that commonly seen in *A. digitifera*, although the peculiar growth form clearly distinguished this morph from *A. digitifera* and all other species and morphs examined in this study.

Examination of museum specimens of the *Acropora humilis* species group revealed increased morphological variation for most species and morphs, when compared across their Indo-Pacific distributions. This variation was within the range described for each species and morph (Table 4.3), for all species except *A. digitifera*. Within this species, many colonies from eastern Indian Ocean locations (Gulf of Thailand, Riau/Lingga Archipelago SE of Singapore, and Western Australia) had densely arranged radial corallites that approached a nariform shape. However, these colonies were not recognized as a distinct morph, because variation in these characters merged with those described in Table 4.3a for *A. digitifera*. The additional variation within the species *A. digitifera* was also due to the presence of another intermediate and two sub-morphs. The intermediate morph "dig-gem 1" appeared to have most affinity with *A. digitifera* and *A. gemmifera*, being distinguished by a combination of growth form and radial corallite characters. Colonies of "dig-gem 1" formed caespito-corymbose sub-branches which developed from a few larger horizontal branches. Radial corallites were most similar to those of *A. gemmifera*, being of mixed sizes ranging from subimmersed to large, with larger radials having a dimidiate shape. The sub-morph *Acropora* sp 1 aff. *digitifera* is distinguished from colonies of *A. digitifera* by its short, conical branches arising from a solid base (Hayashibara and Shimoike 2002), while "Indonesian dig", was distinguished from *A. digitifera* by the large and strongly flaring shape of the radial corallites.

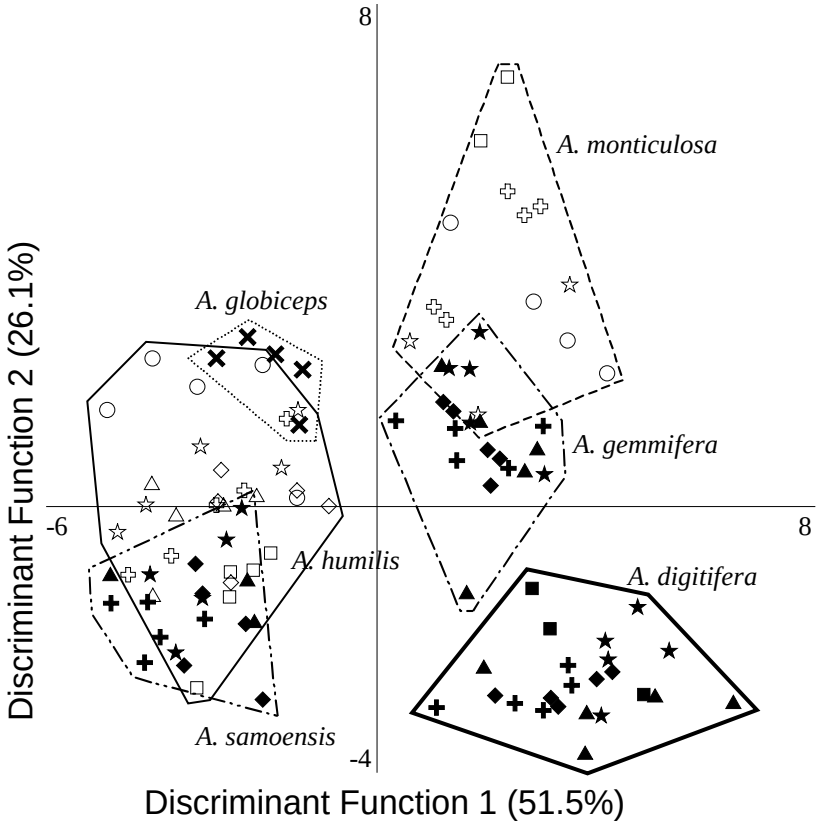
4.3.3 MORPHOMETRIC ANALYSIS

4.3.3.1 DEFINING SPECIES CATEGORIES

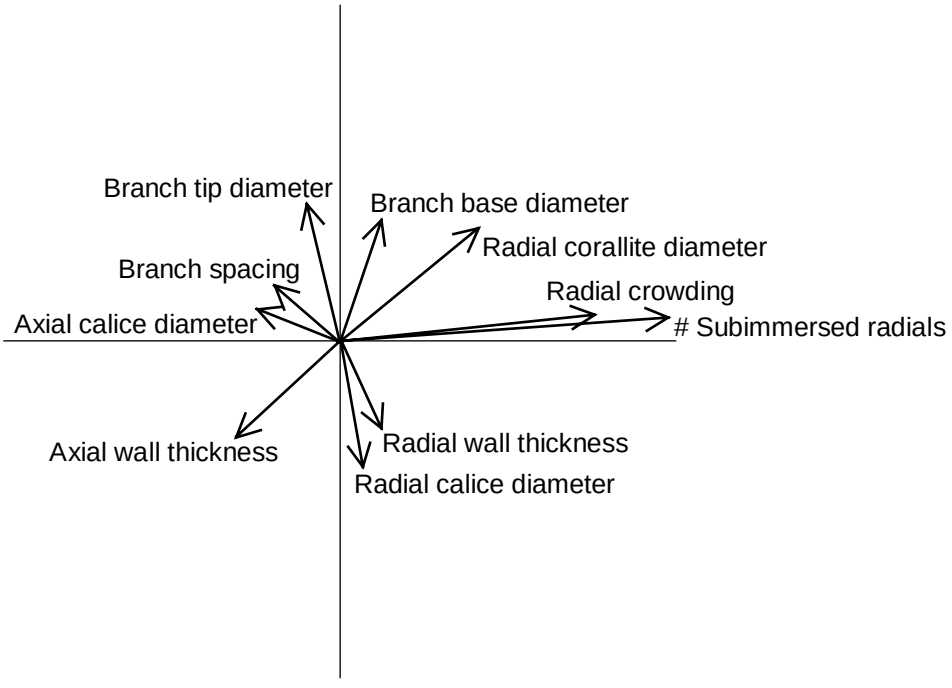
Discriminant analysis of the species *A. samoensis*, *A. humilis*, *A. globiceps*, *A. gemmifera*, *A. monticulosa* and *A. digitifera* indicated that 77.6% of the morphometric variation between these species was explained by the first two discriminant functions (DF1 and DF2) (Fig. 4.4a). The stepwise model used 10 characters to explain the variation between the species (Fig. 4.4b) and removed the characters branch mid-

Fig. 4.4 a Plot of the first two discriminant functions for the discriminant analysis of the species *A. samoensis*, *A. humilis*, *A. globiceps*, *A. gemmifera*, *A. monticulosa* and *A. digitifera*. Each data point represents a single colony. Polygons are drawn around colonies for each species with line styles as follows: *A. samoensis* dash with two dots; *A. humilis* thin continuous; *A. globiceps* dotted; *A. digitifera* thick continuous; *A. gemmifera* dash with one dot; *A. monticulosa* dashed. Symbols for each data point indicate the region in which colony was sampled: Taiwan: ■, Indonesia: ▲, Australia: ✚, PNG ◆, Solomon Islands ★, American Samoa ●, French Polynesia ✖. To differentiate between colonies of species in overlapping polygons, solid symbols are used for colonies of the species *A. globiceps*, *A. samoensis*, *A. gemmifera* and *A. digitifera* and empty symbols for colonies of the species *A. humilis* and *A. monticulosa*. **b** Vector plot showing the effect of each morphometric character on the distribution of colonies of each species.

a


8
A. monticulosa
A. globiceps
A. gemmifera
-6
8
A. humilis
A. digitifera
A. samoensis
-4
Discriminant Function 2 (26.1%)
Discriminant Function 1 (51.5%)

b


Branch tip diameter
Branch base diameter
Radial corallite diameter
Branch spacing
Radial crowding
Axial calice diameter
Subimmersed radials
Axial wall thickness
Radial wall thickness
Radial calice diameter

diameter, branch length, length of axial septa and length of radial corallites (characters 3, 5, 10 and 11, respectively; Table 2.1) from the analysis because they contributed little additional discrimination. The rate of correct classification of colonies for each species was extremely high (97.3%), indicating that the morphometric characters used in the analysis were successful in defining the species categories. All colonies of *A. globiceps*, *A. gemmifera*, *A. monticulosa* and *A. digitifera* were correctly classified. Only colonies of *A. samoensis* and *A. humilis* were misclassified, with one colony of *A. samoensis* being classified as *A. humilis* and two colonies of *A. humilis* as *A. samoensis*. Of the correctly classified individuals, most variation in the predicted allocation of colonies of each species was also between *A. humilis* and *A. samoensis*, emphasizing the morphological similarity of these species (Table 4.4). Most (13 of 18) colonies of *A. samoensis* had a small (<3%) potential of being classified as *A. humilis*, 11 of 30 colonies of *A. humilis* had a 0.1 – 15% likelihood of being classified as *A. samoensis* and 5 colonies of these species had a moderate likelihood (20.8 – 79.2%) of being allocated to either species.

The first discriminant function separated *A. samoensis*, *A. humilis* and *A. globiceps* from *A. gemmifera*, *A. monticulosa* and *A. digitifera*, with separation being mainly due to the characters 'number of subimmersed radial corallites' and 'radial crowding' (Fig. 4.3). The former three species had fewer subimmersed radials and less crowded radial corallites compared with the latter three species. The second discriminant function separated the species *A. samoensis* and *A. globiceps* but did not separate either of these species from *A. humilis*, with morphological variability between colonies of *A. humilis* extending to the extremes of each of the other two species. The multivariate spread of colonies in these three species was mostly due to the thicker axial walls and relatively small radial corallites of *A. samoensis* and some colonies of *A. humilis* compared to the larger branch dimensions, larger axial calices, thinner walls and smaller calices of radial corallites of *A. globiceps* and other colonies of *A. humilis*. The second discriminant function also separated *A. gemmifera* and *A. monticulosa* from *A. digitifera*, but did not separate *A. gemmifera* and *A. monticulosa*. Colonies of *A. digitifera* had larger radial calices and thicker radial walls compared to the other two species, which both had thicker branches and larger overall size of radial corallites. *Acropora gemmifera* and *A. monticulosa* both had relatively thin axial walls, while the character providing most separation between these two species was the relatively larger

Table 4.4 Post-hoc classification rates (%) for colonies of each species of the *Acropora humilis* species group into the species categories predicted by the discriminant analysis [a].

Species	Region	S	H	GL	GE	MO	D
S	Aus	**51.6***	**48.4**				
		99.7*	0.3				
		97.1*	2.9				
		100*	0.0				
		99.9*	0.1				
	PNG	**100***	0.0				
		99.8*	0.2				
		98.6*	1.4				
		98.7*	1.3				
		99.5*	0.5				
	Sol	**99.7***	0.3				
		99.6*	0.4				
●●●●●		11.3	**88.7***				
		79.2*	**20.8**				
		97.1*	2.9				
	Ind	**98.5***	1.5				
		99.2*	0.8				
		98.0*	2.0				
H	Sam		**100***				
			100*				
			100*				
			100*				
			100*				
	Aus		**100***				
			100*				
			100*				
		12.1	**87.9***				
			100*				
	PNG	6.9	**93.1***				
			100*				
			100*				
		4.4	**95.6***				
		2.1	**97.9***				
	Sol	0.3	**99.7***				
			100*				
			100*				
		0.1	**99.9***				
		0.1	**99.9***				
	Tai	1.1	**98.9***				
●●●●●		**93.3***	6.7				
		48.5	**51.5***				
		1.1	**98.9***				
●●●●●		**92.6***	7.4				
	Ind	0.7	**99.3***				
			100*				
		30.5	**69.5***				
		14.2	**85.8***				
		24.0	**76.0***				
GL	Pol			**100***			
				100*			
				100*			
				100*			
				100*			

Species	Region	S	H	GL	GE	MO	D
GE	Aus				**100***		
					100*		
					100*		
					100*		
					100*		
	PNG				**91.7***	8.3	
					100*		
					100*		
					100*		
					100*		
	Sol				**100***		
					100*		
					100*		
					100*		
					100*		
	Ind				**100***		
					99.9*	0.1	
					100*		
			0.2		**99.8***		
					100*		
MO	Aus					**100***	
						100*	
						100*	
						100*	
						100*	
	Sol					**100***	
						100*	
	Sam					**100***	
						100*	
						100*	
						100*	
	Sol					**100***	
	Tai					**100***	
						100*	
D	Aus						**100***
							100*
							100*
							100*
							100*
	PNG						**100***
							100*
							100*
							100*
							100*
	Sol						**100***
							100*
							100*
							100*
	Tai					0.1	**99.9***
							100*
							100*
							100*
	Ind						**100***
							100*
							100*
							100*
							100*

[a] Abbreviations for species categories are S: *A. samoensis*, H: *A. humilis*, GL: *A. globiceps*, GE: *A. gemmifera*, MO*: A. monticulosa*, D: *A. digitifera*. ●●●●● in the species column indicates misclassified individuals. The highest value for each colony is highlighted with an * and values >20% are in bold. Values of 0% are not shown.

basal branch diameters in *A. monticulosa*.

4.3.3.1.1 REGIONAL MORPHOLOGICAL VARIATION WITHIN SPECIES

Morphological variation between colonies for each region within each species of the *Acropora humilis* species group was generally overlapping (Fig. 4.4a). This suggests that no other morphs (in addition to those already recognized in this study) were evident within the regions. Within this overall pattern, some minor trends were also evident. The large variation between colonies of *A. humilis* for the second discriminant function, was mostly due to colonies from Taiwan showing greater morphological similarity to colonies of *A. samoensis*, colonies from American Samoa showing greater similarity to *A. globiceps* and colonies from the other regions mostly being distributed between these extremes. Within the species *A. samoensis*, colonies from Australia tended to have the thickest axial corallite walls, while variation between colonies from the other regions was overlapping. The amount of regional variation between colonies within the species *A. gemmifera* and *A. digitifera* was similar, although one colony of *A. gemmifera* from Indonesia was quite distinct, showing greatest affinity with *A. digitifera*. Morphological variability between colonies of *A. monticulosa* was mostly due to the variation in branch diameter, with colonies from Taiwan being largest and colonies from the Solomon Islands being smallest.

4.3.3.2 CLASSIFICATION OF MORPHS WITHIN SPECIES CATEGORIES

The classification probabilities of the intermediate morphs confirmed that morphological affinity of most colonies was closest to the pair of species after which they were named. No geographical patterns, for example of colonies from one region being classified as a different species to other colonies of the same morph, were evident (Table 4.5a). Some colonies showed they were most likely to be classified as one or other of the pair of species after which they were named, while others had a similar chance of being classified as either species. The morph “sam-hum” had the largest number of colonies with a moderate chance of being classified as both of the species, *A. samoensis* and *A. humilis*. Of the 14 “sam-hum” colonies, probabilities of being classified as either *A. samoensis* or *A. humilis* ranged from 39.5 – 56.3% for 4 colonies, 15.7 – 84.3% for a further 4 colonies, while the remaining 6 colonies had a 88.4 – 97.8% chance of being classified as *A. samoensis*. Colonies of “sam-gem” had an 83.9 – 100% chance of being classified as *A. samoensis* or *A. gemmifera*. Most colonies of

"hum-gem" were likely to be classified as the species *A. humilis* (82.0 – 99.6% for 7 colonies) or *A. gemmifera* (98.2 – 100% for 3 colonies), while one colony had a 77.5% and 22.3% chance of being classified as *A. humilis* and *A. gemmifera* respectively. All but one colony of "mont-hum" were most likely to be classified as *A. humilis*. Within the 28 colonies of "mont-gem", 17 had a 96 – 100% chance of being classified as *A. gemmifera*, five as *A. monticulosa* and one as *A. digitifera*. Three of the remaining colonies had a moderate chance of being classified as *A. gemmifera* or *A. monticulosa* (15.1 – 84.9%), while two were most likely to be classified as *A. gemmifera* or *A. digitifera* (31.0 – 67.7%). Classification probability of the colonies of "dig-gem" were variable, with single colonies each having a 99.9 – 100% chance of being classified as *A. digitifera, A. gemmifera* or *A. monticulosa*, while the other two colonies had a 57.7% and 62.9% chances of being classified as *A. digitifera*. The apparent morphological affinity was not consistent with classification probabilities for the morph "hum-gem plate", with four colonies most likely to be classified as *A. digitifera* (94.0 – 100%) and one colony as *A. monticulosa* (78.9%). This was due to the plating growth form of this species and therefore smaller branch, axial and radial dimensions compared to colonies of *A. humilis* and *A. gemmifera*.

On the basis of the classification probabilities, only "fine sam" and "digitate mont" of the six sub-morphs were consistently assigned to the species with which they appeared to share most morphological affinity (Table 4.5b). These two sub-morphs had a 100% probability of being classified as the species *A. samoensis* and *A. monticulosa* respectively. Three colonies of the morph "Penghu hum" had moderate chances of being classified as the species *A. humilis* or *A. samoensis* (34.9 – 65.1%) while two colonies had a high chance (84.3 – 99.9%) of being classified as *A. samoensis*. The sub-morphs "terete mont" and "Samoan dig" were both more likely to be classified as different species than the species after which they were named. Three colonies of "terete mont" had a 94.5 – 99.7% chance of being classified as *A. gemmifera* while one colony had a 99.8% chance as *A. monticulosa*. Colonies of the morph "Samoan dig" were most likely to be classified as *A. monticulosa*, with 4 colonies having a 97.4 – 100% chance of being classified as this species, and only one colony was most likely (89.1%) to be classified as the species *A. digitifera*.

Table 4.5a Classification probability (%) for colonies of each intermediate morph into the species categories defined in the discriminant analysis [a].

Morph	Region	S	H	GL	GE	MO	D
s-h	Ind	**94.5***	5.5				
		56.3*	**43.7**				
		29.3	**70.6***		0.1		
		15.7	**84.3***				
	Aus	**88.4***	11.6				
		19.9	**80.1***				
		49.1	**50.9***				
		39.5	**53.4***		7.0		
	PNG	**91.1***	8.9				
		51.7*	**48.3**				
		97.8*	2.2				
	Sol	**90.0***	10.0				
		93.9*	6.1				
		29.2	**70.8***				
s-ge	Ind	**99.8***	0.2				
		83.9*	16.1				
					100*		
		0.9	0.1		**99.0***		
	Aus				**100***		
		97.3*	2.7				
		1.0	9.9		**88.6***		0.4
		99.9*	0.1				
	Sol	**93.0***	7.0				
h-ge	Ind	17.9	**82.0***		0.1		
		3.8	**39.7**		**56.5***		
		0.8	1.0		**98.2***		
	Aus	0.2	**77.5***		**22.3**		
					100*		
		0.6	**99.4***				
		0.9	**99.1***				
			0.4		**99.4***	0.1	
	PNG	0.4	**99.6***		0.1		
		60.7*	**39.3**				
		0.2	**93.0***		6.8		
		1.2	**98.8***				
		2.1	**97.3***		0.6		
h-ge p	Tai	4.0	0.7				**95.3***
		0.7	1.4				**97.8***
		1.0	3.2		3.7	**78.9***	13.2
		0.6	5.3				**94.0***
							100*

Morph	Region	S	H	GL	GE	MO	D
mo-ge	Tai				3.3	0.2	**96.4***
						100*	
					100*		
					98.6*		1.3
					31.0	1.2	**67.7***
	Ind				**97.5***		2.5
					0.5	**99.5***	
					64.1*	0.1	**35.8**
					100*		
					99.6*	0.4	
	Aus				**100***		
					99.7*	0.2	0.1
					100*		
	PNG				0.3	**99.7***	
					0.6	**99.4***	
					98.5*	1.5	
					15.1	**84.9***	
					99.5*	0.5	
	Sol				**99.8***	0.2	
					100*		
					100*		
					96.0*	4.0	
						100*	
	Sam				**99.9***	0.1	
					56.6*	**43.4**	
					100*		
					100*		
					28.9	**71.1***	
mo-h	Aus		**100***				
			100*				
			100*				
		0.1	**72.6***	3.6	9.7	14.0	
		0.2	**93.2***	6.6			
	Sol	0.1	**36.2**	0.1	**63.4***	0.3	
		0.7	**99.3***				
d-ge	Aus					**100***	
					0.1		**99.9***
					100*		
					16.3	**26.0**	**57.7***
					37.1		**62.9***

[a] Abbreviations for morphs are the same as in Fig. 4.1. Abbreviations for species categories are the same as in Table 4.4. The highest probability for each colony is highlighted with an * and probabilities > 20% are in bold. Probabilities of 0% are not shown.

Table 4.5b Classification probability (%) for colonies of each sub-morph into the species categories defined in the discriminant analysis [a].

Morph	Region	S	H	GL	GE	MO	D
pH	Tai	**84.3***	15.7				
		37.1	**62.8***	0.1			
		62.4*	**37.6**				
		65.1*	**34.9**				
		99.9*	0.1				
fS	Ind	**100***					
		100*					
		100*					
		100*					
		100*					
tMO	Aus				**94.5***	5.5	
					99.7*		0.3
					0.1	**99.8***	0.1
	Sol				**99.7***	0.2	0.1
dMO	Tai					**100***	
						100*	
	Sol					**100***	
	Sam					**100***	
						100*	
sD	Sam					**100***	
						97.4*	2.6
						100*	
						10.9	**89.1***
						100*	

[a] Abbreviations and codes are the same as in Table 4.5a.

4.3.4 DISTRIBUTION OF SPECIES AND MORPHS

Ranges of the intermediate morphs were generally broad and similar to the species after which they were named (Figs 4.5 and 4.6). Specimens of the morphs “sam-hum”, “sam-gem” and “hum-gem” were collected from the greatest number of locations, ranging from the Red Sea to the central pacific (Tubuai Islands, Cook Islands and Pitcairn Island respectively), with the Pacific distributions of the former two morphs extending beyond that of *A. samoensis*. The intermediate morphs “mont-hum” and “mont-gem” were commonly recorded but mostly restricted to the central Indo-Pacific. Specimens of “dig-gem” were recognized from relatively few locations but had a broad distribution from the Red Sea to the Cook Islands. Two of the intermediate morphs

“hum-gem plate” and “dig-gem 1” were only recorded over small areas i.e. Taiwan – southern Japan and central Indonesia, respectively.

In contrast to the generally broad distributions of the intermediate morphs, the sub-morphs had more restricted distributions, which were within the distribution of the species after which they were named (Fig. 4.7). There were few colonies of each of the sub-morphs in the museum collections (less than 10 colonies of each sub-morph), suggesting that these morphs are also rare within their relatively small range (Fig. 4.7). Specimens of “Penghu hum”, “encrusting dig” and *A.* sp 1 aff. *digitifera* were only recorded from the western Pacific with the former two morphs only being found at single locations in Taiwan in field surveys during this study, while colonies of the latter morph were only recorded from southern Japan in the museum collections. The sub-morph “Samoan dig” was only recorded in American Samoa and the Cook Islands, suggesting that it is restricted to the central Pacific. Colonies of “fine sam”, “terete mont” and “Indonesian dig” were commonly recorded, but only within narrow distribution ranges, within the central Indo-Pacific region. The sub-morph “digitate mont” was also rare, but found in relatively distant locations of Taiwan, Solomon Islands, American Samoa and the Cook Islands.

4.4 DISCUSSION

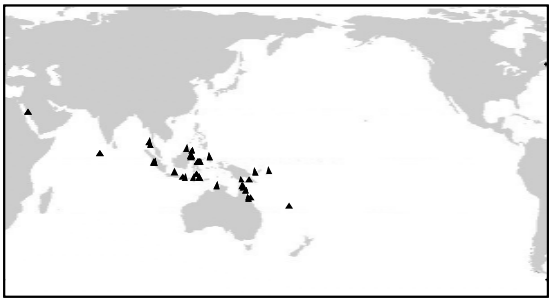
4.4.1 MORPHOLOGICAL COMPARISON OF SPECIES AND MORPHS

This study demonstrates that the taxonomic difficulties of defining species in the *Acropora humilis* species group are due to two levels of morphological variation. Firstly, morphological boundaries between species are blurred by colonies with intermediate morphologies, which share morphological affinities with more than one species. Secondly, there are morphs with distinct morphologies within the morphological boundaries of species as they are currently described. In this study, 21 morphs in the *A. humilis* species group were recognized from the western to central Pacific on the basis of qualitative characters (Table 4.3). These morphs comprise the eight described species of the group, seven intermediate morphs and six sub-morphs. Morphometric classification of intermediate morphs was generally within either of the species after which they were named, or in some cases, colonies had moderate chances of being classified as either of these species. In contrast, four of the six sub-morphs

Fig. 4.5 Distribution of species of the *Acropora humilis* species group, based on records from this study and specimens in the collections at the Museum of Tropical Queensland: **a** *A. samoensis*; **b** *A. humilis*; **c** *A. globiceps*; **d** *A. gemmifera*; **e** *A. monticulosa*; **f** *A. digitifera*; **g** *A. retusa*; **h** *A. multiacuta*.

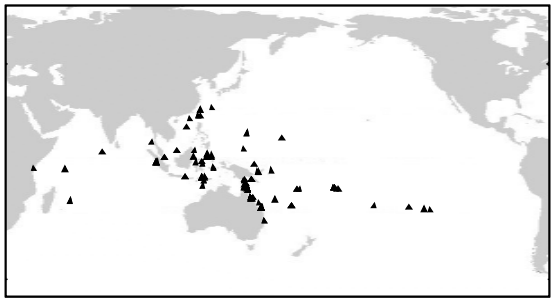
a

A. samoensis



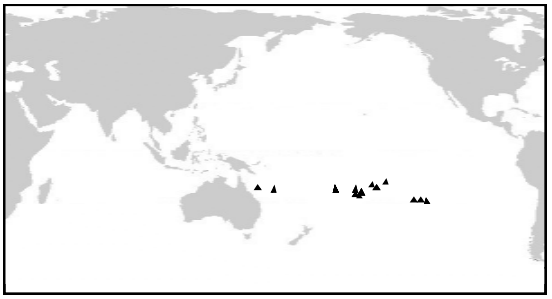
b

A. humilis



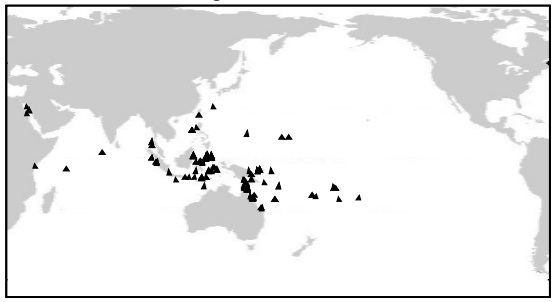
c

A. globiceps



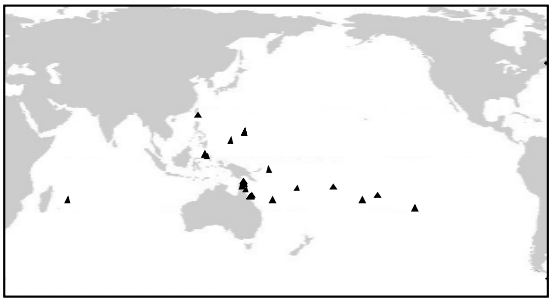
d

A. gemmifera



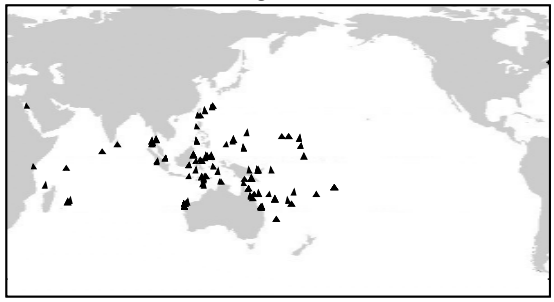
e

A. monticulosa



f

A. digitifera



g

A. retusa

h

A. multiacuta

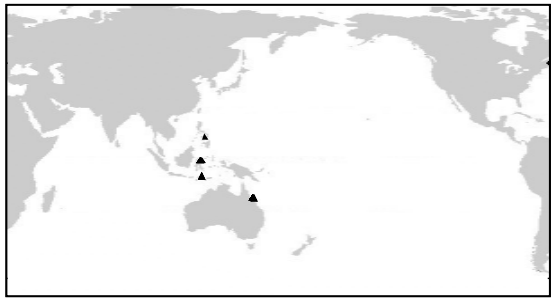


Fig. 4.6 Distribution of intermediate morphs of the *Acropora humilis* species group, based on records from this study and specimens in the collections at the Museum of Tropical Queensland: **a** “sam-hum”; **b** “sam-gem”; **c** “hum-gem”; **d** “hum-gem plate”; **e** “mont-hum”; **f** “mont-gem”; **g** “dig-gem”; **h** “dig-gem 1”. Polygons indicate the extreme locations that the species, after which each morph was named, was recorded. Polygons are drawn by eye for each species from Fig. 4.5. The solid polygon indicates the distribution of the first and the dashed polygon indicates the distribution of the second species in the name of the morph.

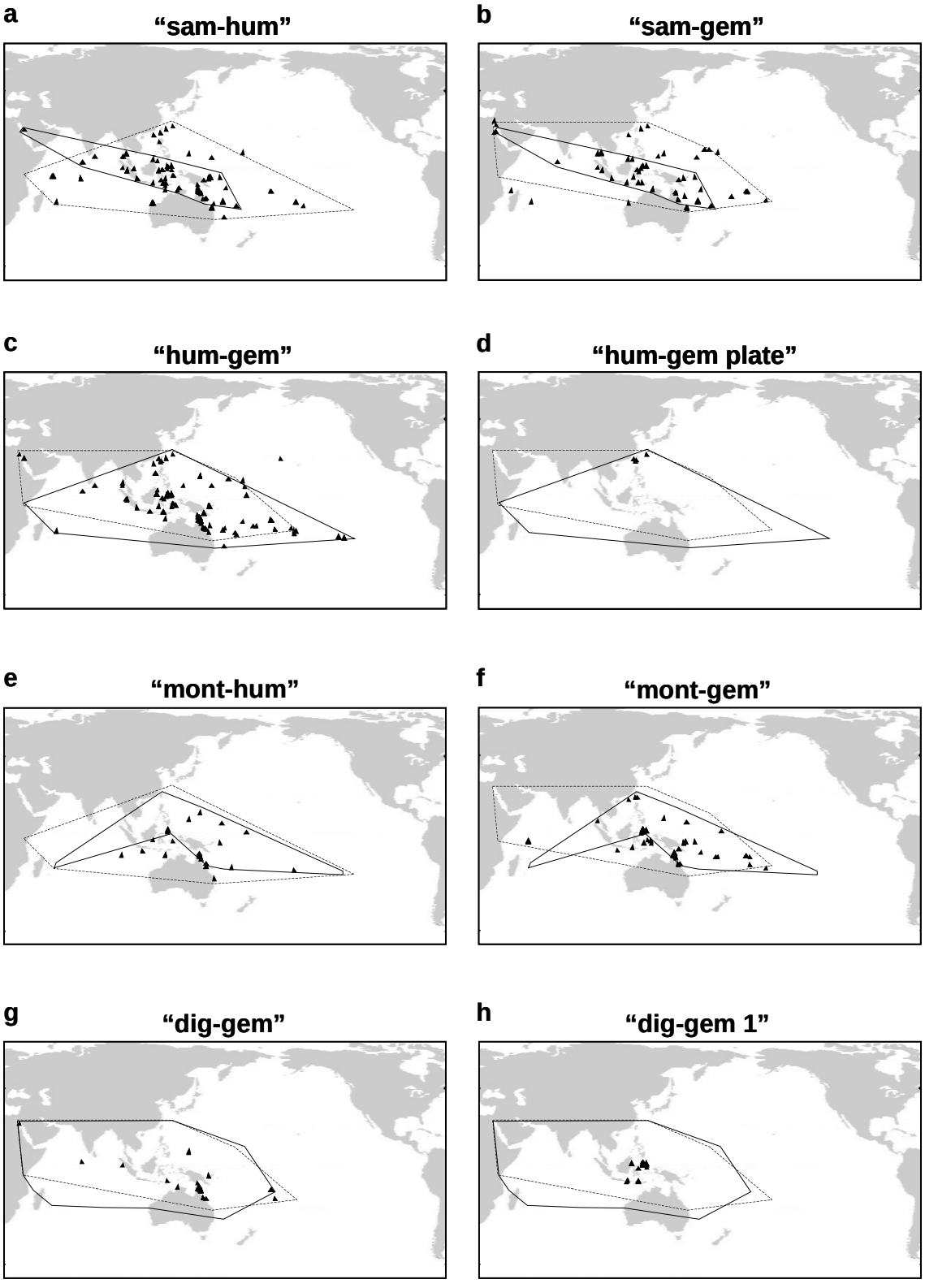
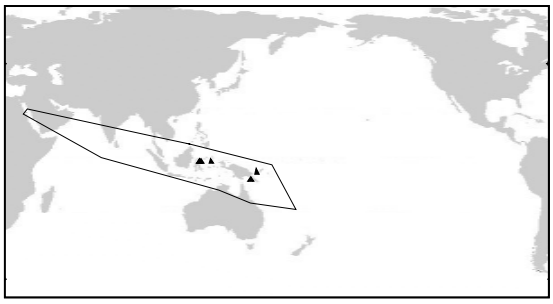

a
“sam-hum”
b
“sam-gem”
c
“hum-gem”
d
“hum-gem plate”
e
“mont-hum”
f
“mont-gem”
g
“dig-gem”
h
“dig-gem 1”

Fig. 4.7 Distribution of sub-morphs of the *Acropora humilis* species group, based on records from this study and specimens in the collections at the Museum of Tropical Queensland: **a** “fine sam”; **b** “Penghu hum”; **c** “terete mont”; **d** “digitate mont”; **e** “Samoan dig”; **f** “encrusting dig”; **g** “*A.* sp 1 aff. *digitifera*”; **h** “Indonesian dig”. Polygons indicate the extreme locations that the species, after which each morph was named, was recorded. Polygons are drawn by eye for each species from Fig. 4.5.

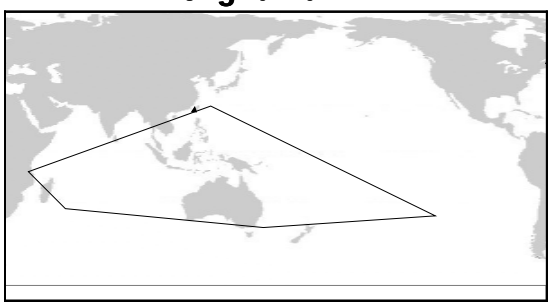
a

“fine sam”



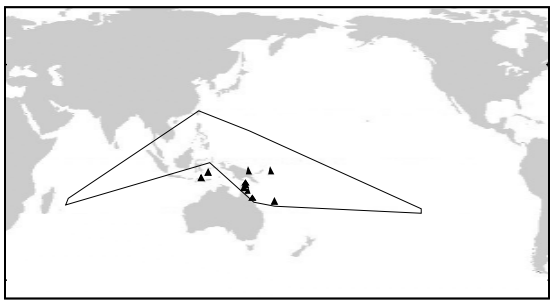
b

“Penghu hum”



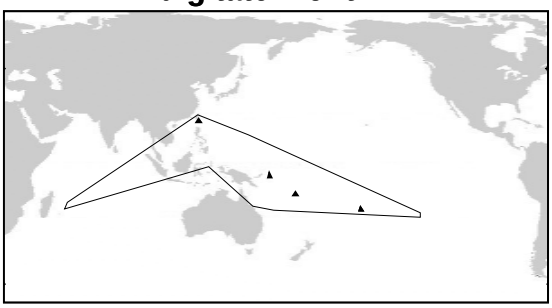
c

“terete mont”



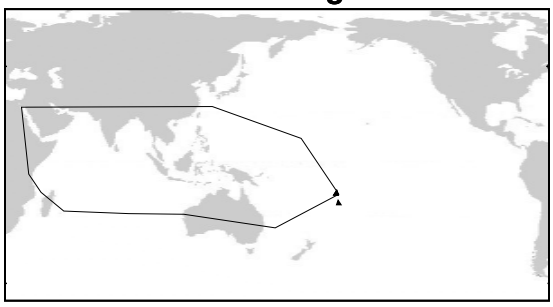
d

“digitate mont”



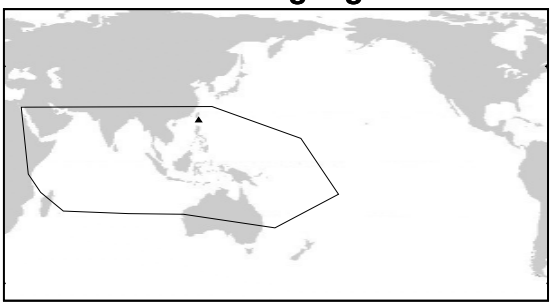
e

“Samoan dig”



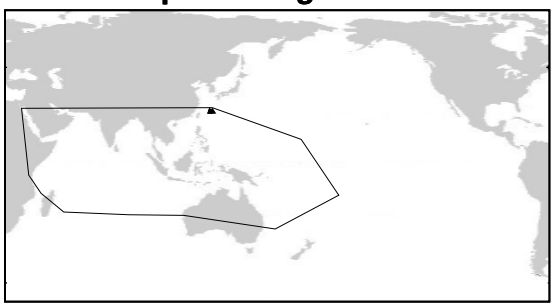
f

“encrusting dig”



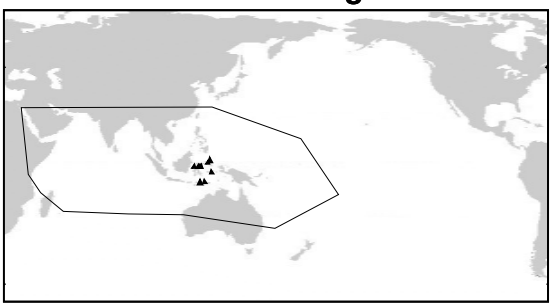
g

A.* sp 1 aff. *digitifera



h

“Indonesian dig”



were not classified within the species after which they were named, confirming the distinction of these morphs from the species with which they appeared to have most morphological affinity. Apparent affinities between the species and morphs, based on a synthesis of the evidence from this chapter and chapters 2 and 3, are proposed in this section.

Discriminant analysis of the morphometric characters indicated that the most morphologically similar species were *Acropora samoensis*, A. *humilis* and *A. globiceps*, with the greatest overlap being between the former two species (Fig. 4.4a). The classification probabilities of the intermediate morph "sam-hum" also demonstrated the close morphological similarities between the species *A. samoensis* and *A. humilis*, with all colonies of this morph having some chance, and one-third of the colonies having a moderate chance of being classified as either of these species (Table 4.5a). Despite the morphological and genetic similarity of these species and this morph (chapters 3), the predominant time of spawning of *A. samoensis* and "sam-hum" was out of phase with *A. humilis* and the mass spawning event at Lizard Island (chapter 3). Documenting timing of spawning for these species and this morph, both in locations where they do and do not co-occur, would contribute to understanding the evolutionary significance of the different time of spawning of *A. samoensis* and whether the boundary between *A. samoensis* and *A. humilis* can be more accurately defined. Reproductive studies may also contribute to further understanding evolutionary relationships for "Penghu hum", which classified as *A. samoensis* and *A. humilis*, and "fine sam" which consistently classified as *A. samoensis* in the morphometric analysis (Table 4.5b). Morphological similarity between *A. globiceps* and the species *A. samoensis* and *A. humilis* is indicated in the qualitative descriptions of each of these species (Table 4.3a) and confirmed in the discriminant analysis (Fig. 4.4a). This similarity is also recognized by Wallace (1999), who describes branch and radial corallite shape as most similar to *A. samoensis* and branch thickness and growth form as most similar to *A. humilis*.

The species with greatest morphological similarity to *A. samoensis*, *A. humilis* and *A. globiceps* was *A. gemmifera* (Table 4.3a). *Acropora gemmifera* was separated from the other three species by the first but not the second discriminant function (Fig. 4.4a). Although *A. gemmifera* was well defined in the discriminant analysis (Table 4.4),

boundaries are less distinct when the morphs of this species, particularly “hum-gem”, are considered (Table 4.3). The difficulty of distinguishing the species *A. gemmifera* from *A. humilis* is also noted by Wallace (1999). Morphometric analysis of colonies of this morph generally resolved the apparent gradation between these species with most colonies having a high probability of being classified as one or other of these species, although two colonies had moderate chances of being classified as either species (Table 4.5a). Morphological affinities of the morph “sam-gem” were resolved in the morphometric analysis, with all colonies being classified as either the species *A. gemmifera* or *A. samoensis* (Table 4.5a).

The species *Acropora monticulosa* formed a clearly defined group in the discriminant analysis (Table 4.4). However, in shallow wave exposed habitats such as the reef crest, other species of *Acropora* can also have digitate, conical branches (pers. obs.) and may therefore be misidentified as *A. monticulosa*. *Acropora gemmifera* appears to be the species most likely to be confused with *A. monticulosa*, based on the morphological overlap between these species in the discriminant analysis (Fig. 4.4a) and the morphological characters of the intermediate morph “mont-gem” (Table 4.3b). Although I was unable to confidently identify colonies of this morph as either of these species, the classification probabilities indicated that colonies of “mont-gem” were most likely to be classified as *A. gemmifera* (Table 4.5a). Similarly, colonies of “mont-hum” could not confidently be assigned to the species *A. monticulosa* or *A. humilis*, although classification probabilities suggested that these colonies were usually most similar to the latter species (Table 4.5a).

Colonies of the sub-morphs “digitate mont” and “terete mont” were also found in the same habitat as *A. monticulosa* and shared morphological affinities with this species. However, reproductive and genetic evidence for these morphs (chapters 2 and 3, respectively) suggest that each is evolving independently of this species. The morph, “digitate mont” was consistently classified as *A. monticulosa* (Table 4.5b), although evolutionary affinities were also proposed with “Samoan dig”, on the basis of morphological and molecular evidence (chapter 2). The morph “terete mont” also appeared to share greatest morphological affinity with *A. monticulosa*, but could not be confirmed due to the morphological variability and small number of colonies assessed for this morph. The large axial corallites of this morph resembled *A. humilis* and the

dimidiate radial corallites resembled *A. gemmifera* (Table 4.3). Based on the combined assessment of morphological appearance, the broad range of fertilization potential between colonies and timing of reproduction for this morph, it was proposed as a possible hybrid between the species *A. monticulosa* and *A. humilis* (chapter 3).

The species *Acropora digitifera* was the most clearly defined of all species in the discriminant analysis, being the only species to form a separate group (Fig. 4.4a), although this species appeared to have affinities with several morphs. Characters of this species were most similar to those in the species *A. gemmifera*, with the intermediate morph of these species, "dig-gem" sharing characters with both species (Table 4.3), although classification probabilities were inconclusive due to the morphological variability and small number of colonies examined of this morph. The intermediate morph "dig-gem 1" also appeared to share greatest morphological affinity with the same species, although was most similar to the species *A. gemmifera*, having radial corallites most similar in size and shape to this species. This morph was only recorded in the museum collections, in central Indonesia (Fig. 4.6h). Two sub-morphs were recognized within the species *A. digitifera* in the field surveys. The sub-morph "Samoan dig" appeared to have greatest affinity with the species *A. digitifera* (Table 4.3). However, classification probabilities for colonies of this morph suggested greatest affinity was with *A. monticulosa* (Table 4.5b), supporting the proposed evolutionary affinities between the morphs "Samoan dig" and "digitate mont" (chapter 2). Only a single colony of the sub-morph of "encrusting dig" was recorded in this study. Affinity of this morph is tentatively proposed, primarily on the basis of the colours of this colony matching those commonly seen in *A. digitifera*, but could not be tested with the morphometric characters used in this study due to the absence of branch and fully developed radial corallite characters. Two other sub-morphs of the species *A. digitifera* were recognized in the museum collections but not field surveys. One of these sub-morphs has been described from southern Japan as a cryptic species of *A. digitifera*, being distinguished by its encrusting growth form (Hayashibara and Shimoike 2002). The other sub-morph recorded in the museum collections was morphologically similar to *A. digitifera*, being distinguished by large and strongly flaring radial corallites and was only found in central Indonesia (Fig. 4.7f).

The species *Acropora multiacuta* and *A. retusa* were morphologically distinct from each other and all other species in the *A. humilis* species group. Wallace (1999) concluded that *A. multiacuta* appears to be the most misplaced species in this species group, noting the reduced number of radial corallites as being an unusual feature of this species. This feature and the long, thin branches of this species distinguished it from all other species and morphs of the *A. humilis* species group (Table 4.3 and Fig. 4.3). The species *A. retusa* was also morphologically distinct from other species and morphs in the *A. humilis* species group. The most distinguishing feature of this species was the elongate tubular radial corallites (Table 4.3 and Fig. 4.3k). Because of the morphological distinction of the species *A. multiacuta* and *A. retusa* from other species and morphs in the *A. humilis* species group, more extensive analyses which incorporate other species from the genus *Acropora* are necessary to determine the species with which they have greatest morphological affinities.

4.4.2 MORPHOLOGICAL VARIATION CONTRIBUTES TO THE INTERPRETATION OF EVOLUTIONARY RELATIONSHIPS

The morphometric analysis greatly enhanced this investigation of morphological variation within and between the species and morphs, throughout their geographic distributions. The analysis compared patterns of morphological variation of each species (Fig. 4.4), and provided a framework for assessing, on a case-by-case basis, the morphological affinity of colonies of each morph (Table 4.5). Given that morphological characters vary between species, they must also vary within species at some stage in their evolution, although such variation is not usually considered in systematic analyses (Wiens 1999). Rather than considering morphologically variable species as discrete evolutionary units, examination of patterns of intraspecific and interspecific variation may provide evidence of microevolutionary processes (Wiens 1999). Morphological affinities demonstrated in this study provide a foundation for testing this hypothesis with additional evidence, such as molecular and reproductive criteria. This will help to clarify whether colonies of morphs that had a high probability of being classified with only one species are correctly assigned, providing a more accurate delineation of the morphological boundaries between species. Non-morphological criteria can also be used to test whether there is evidence of evolutionary divergence between colonies that have not diverged morphologically across broad geographic scales, if intermediate

morphs indicate lineages that are diverging or hybridizing, and if sub-morphs are cryptic or recently diverged species.

Within the genus *Acropora*, congruence between morphological and evolutionary boundaries has been questioned (Wallace and Willis 1994; Willis et al. 1997; van Oppen et al. 2001), although few studies have examined these boundaries using detailed morphological analyses. The genus *Acropora* encompasses a large number of species in which there is "bewildering within-species variability coupled with a high degree of between-species similarity" (Wallace and Willis 1994). Furthermore, morphological characters at all structural levels, i.e. colony, branch, axial corallite, radial corallite and septa, are phenotypically variable, even within a single colony. It is clear therefore, that detailed morphometric analyses are necessary to synthesize patterns of polymorphism within and between species of *Acropora* and provide a sound framework for using non-morphological criteria to interpret evolutionary boundaries in this genus. Prior to the current study, only one study had used detailed morphological analyses to examine evolutionary boundaries in the genus *Acropora*. In that investigation, two morphs of the species *A. millepora* were found to be morphologically distinct and reproductively incompatible (Wallace and Willis 1994). On this basis, these morphs are now tentatively recognized as the species *A. millepora* and *A. spathulata* (Wallace 1999), with the former having thinner branches and smaller radial corallites than the latter species (Radford 1998). In the current study, using putative morphs as the sampling units has greatly enhanced the interpretation of reproductive and genetic relationships within the *A. humilis* species group (chapter 3). For example, recognition of the intermediate morph "dig-gem" was critical for exploring evolutionary relationships between the species *A. digitifera* and *A. gemmifera*. This morph was most similar in appearance to *A. digitifera* when compared as live colonies (Table 4.3), most similar to and usually identified as *A. gemmifera* when skeletal samples are compared (pers. obs. in museum collections), but evolutionarily distinct from each of these species on the basis of reproductive and genetic criteria respectively. In contrast, the species *A. humilis*, *A. samoensis* and *A. gemmifera* from Lizard Island were reproductively but not genetically distinct (chapter 3). Intermediate morphs of these species are also genetically undifferentiated from these species, although timing of spawning and potential to interbreed suggests that morphological appearance of these species and morphs is indicative of patterns of

recent divergence within these taxa (chapter 3). Interpretation of evolutionary relationships within the *A. humilis* species group is discussed further in chapter 5.

The morphometric characters used in this study are based on linear measurements, providing an indication of size but not shape of the dimensions measured. These linear measurements reflect taxonomic differences in branch and axial corallite characters, although comparison of radial corallite size only partially reflects differences between the species and morphs. More detailed analyses of radial corallites may refine the classification probability of intermediate morphs and sub-morphs. In the current study, incorporating shape into the morphometric analyses would be particularly useful for more detailed examination of radial corallites of *A. humilis*, *A. gemmifera* and intermediate morphs of these species, clarifying whether changes in shape between these species and morphs form a continuous gradient, or alternatively, marking where discontinuities occur. This is also likely to be particularly informative for the morphs, "hum-gem plate", "Penghu hum", "digitate mont" and "Samoan dig", because the relatively small dimensions of these morphs may have overshadowed differences in radial corallite shape. Use of landmark techniques may be useful for comparing the shape of radial corallites in the genus *Acropora*, as demonstrated in the genus *Porites* (Budd et al. 1994; Johnson and Budd 1996). Landmark techniques involve the measurement of distances between homologous points, documenting shape in two or three dimensions (Bookstein 1990). The number of homologous points, which can be defined in radial corallites of different shapes, may however restrict the application of this technique in the genus *Acropora*. Further investigation is therefore necessary to explore the potential of using landmark characters for increasing taxonomic resolution in the genus *Acropora*.

Morphological variation within the *Acropora humilis* species group was analysed using hierarchical cluster analysis for a single location with moderate diversity (American Samoa: chapter 2). Characters in this previous study were coded for qualitative character states, including traditional categories for radial corallite shape and coenosteal structure (skeletal matrix). This procedure was also attempted in the current study. However, the substantial levels of polymorphism within and between colonies of the species and morphs over the broader geographic range precluded meaningful classification, with many colonies possessing character states for more than one

category. In addition, classification clusters were unstable, especially for colonies of *A. globiceps*, *A. humilis*, *A. samoensis*, *A. gemmifera* and morphs of these species. These problems demonstrate the difficulties of assessing morphological variability of polymorphic individuals, and emphasize the value of using morphometric characters, ordination techniques and the potential of incorporating new morphometric characters not assessed in the present study, such as radial corallite shape and possibly coenosteal structure.

4.4.3 BIOGEOGRAPHY OF SPECIES AND MORPHS OF THE *ACROPORA HUMILIS* SPECIES GROUP

Mapping distributions of samples of species and morphs from the field surveys of this study and museum collections of the *Acropora humilis* species group provided additional information for interpreting evolutionary relationships between these taxa. The intermediate morphs tended to have broad geographic distributions that were similar to the species with which they appeared to share most morphological affinity (Fig. 4.6). Records from field surveys and museum collections indicated that the sub-morphs tended to be rarer and have relatively restricted distributions, within the distributions of the species after which they were named (Fig. 4.7).

The species *A. humilis*, *A. samoensis*, *A. gemmifera* and intermediate morphs of these species have broad Indo-Pacific distributions (Fig. 4.5 b, c, d and Fig. 4.6 a, c, d). Combined genetic and reproductive evidence suggests that these species and morphs have recently or incompletely diverged (chapters 2 and 3). If the intermediate morphs are indicative of recent divergence between these species, the broad and overlapping distributions for these species and intermediate morphs suggests that this apparent recent divergence may be the case throughout their range. Distributions of the intermediate morphs of these species extend further than the range of some of the species. The morphs “sam-hum” and “sam-gem” had broader distributions than *A. samoensis,* and similar distributions to *A. humilis* and *A. gemmifera*, although *A. humilis* was not recorded in the Red Sea (Fig. 4.6c and d). The distribution recorded for the morph “hum-gem” was also broad and similar to *A. humilis* and *A. gemmifera*, with two notable exceptions being that *A. humilis* was not recorded in the Red Sea and neither of these species were recorded from Hawaii (Fig. 4.6a). The colonies identified as “hum-gem” from Hawaii (French Frigate Shoals) shared characters with *A. humilis*

and *A. gemmifera* according to the categories defined in this study (Table 4.3), although colonies from this location are usually identified as *A. humilis* (e.g. Wallace 1999; Veron 2000). Further analyses of morphological and non-morphological criteria will clarify whether some colonies identified as intermediate morphs should be identified as one of the three species. For example, fertilization experiments demonstrated that colonies of "hum-gem" were able to interbreed with *A. gemmifera* but not *A. humilis* (chapter 3), suggesting that this morph may include colonies of *A. gemmifera*.

Potts (1983) proposed that the high levels of intraspecific variability and broad distributions of many shallow-water coral species may be due to frequent changes in sea level which cyclically separated and reconnected populations. He proposed that during periods of isolation, levels of intraspecific variability increased, although these time intervals were insufficient for the process of speciation to be completed. Based on available evidence, such factors could be retarding the evolutionary divergence of *A. humilis, A. samoensis* and *A. gemmifera*. *Acropora digitifera* also has a broad Indo-Pacific distribution (Fig. 4.5f), although intermediate colonies were only recorded with *A. gemmifera*. The morph "dig-gem" was recorded across a similar range but in relatively few locations beyond the Great Barrier Reef (Fig. 4.6g). Reproductive and genetic studies are necessary to confirm whether the evolutionary distinction of this morph from each of these species, demonstrated in chapter 3, is consistent for the other locations in which colonies of this morph were identified in the museum collections. Similarly, the intermediate morph "mont-hum" had a more restricted distribution than the species after which it was named, and on the basis of reproductive criteria, may also be a hybrid morph of these species (chapter 3). Discriminant analysis of colonies of "mont-gem" demonstrates that many colonies of this morph are likely to be *A. gemmifera*, with their growth form altered by environmental conditions. The distribution recorded for this morph is therefore likely to include some colonies that are *A. gemmifera*, but were identified in the museum collections as *A. monticulosa*. Colonies of other intermediate morphs and sub-morphs had relatively restricted distributions, with the majority only being recorded within the high diversity region of the central Indo-Pacific (sensu Wilson and Rosen 1998), or just north of this region in Taiwan and southern Japan (Figs 4.6 and 4.7). Similarly, the species *A. multiacuta* was recorded within and just south of this region on the Great Barrier Reef, Australia (Fig. 4.5h). In contrast, colonies of the species *A. globiceps*, *A. retusa* and "Samoan dig"

were only recorded outside this region (Fig. 4.5a and g and Fig. 4.7g), while the morph "digitate mont" was recorded rarely from distant locations in the western to central Pacific (Fig. 4.7d). These more restricted distribution patterns may indicate that recent divergence has occurred within and beyond the region of high diversity, but more frequently within this region for taxa of the *A. humilis* species group.

4.4.4 CONCLUSIONS

This study demonstrates that patterns of morphological variability within the genus *Acropora* may indicate recent or ongoing evolutionary processes. In contrast, previous studies of this genus have treated species as discrete taxa and therefore not revealed such connections. Comprehensive documentation of morphological variability throughout the distribution of the *A. humilis* species group was essential for exploring such patterns, as well as ensuring that the sampling units are clearly defined for further analysis of evolutionary relationships. Calculation of classification probabilities of colonies of intermediate and sub-morphs provided a valuable technique for assessing apparent morphological affinities. This analysis of intraspecific and interspecific affinities provides a framework for testing evolutionary relationships and resolving the nature of morphological boundaries between taxa, using alternative criteria e.g. genetic and reproductive criteria (chapters 2, 3 and 5). Techniques used in this study will be useful for resolving evolutionary relationships between other extant species complexes in the genus *Acropora* and other groups of scleractinian corals. They will also be useful for exploring whether species and morphs examined in this study share greater evolutionary affinities with taxa in other species groups in the genus *Acropora*. Classification probabilities may also be a valuable tool for assessing patterns of morphological variability and affinities of fossil specimens, in relation to other morphologically similar fossils and extant corals (Hillis 1987). Increased taxonomic resolution of fossil specimens will greatly enhance our understanding of the evolutionary history of scleractinian corals, providing evidence of patterns of morphological variability through time and more accurate means of calibrating molecular data.

CHAPTER 5: PHYLOGENY OF SPECIES AND MORPHS OF THE *ACROPORA HUMILIS* SPECIES GROUP BASED ON A MITOCHONDRIAL MOLECULAR MARKER

5.1 INTRODUCTION

Molecular markers provide alternative tools to traditional morphological characters for assessing boundaries and evolutionary relationships between species. They provide additional evidence, which is independent of morphological characters, for resolving evolutionary relationships and boundaries between species (Avise 1994). This is useful because the morphological appearance of a species constitutes a multitude of characters, many of which may have an environmental or other non-genetic component. Furthermore, molecular markers offer a direct means of tracing the evolutionary history of species, by mapping genetic lineages.

Despite the advantages of using molecular markers to explore evolutionary relationships of species, molecular phylogenies may not reflect species phylogenies. Primary sources of incongruence between gene trees and species trees are incomplete lineage sorting and introgression (e.g. Avise and Wollenberg 1997; Doyle 1997; Maddison 1997). Incomplete lineage sorting refers to the situation in which a genetic sequence, which existed prior to a speciation event, persists without or only partially diverging within new evolutionary lineages following speciation and therefore provides no indication of divergence. Introgression refers to the exchange of genes through hybridization between species that have previously diverged. Ongoing introgression may lead to stable hybrid populations, which may or may not evolve to form new species (Arnold 1997). Alternatively, introgression may merge species, or lead to extinction of one of the parent species through continued backcrossing between the other parent species and the hybrid offspring (Arnold 1997).

Resolution between the contribution of incomplete lineage sorting and introgression in evolutionary lineages is controversial, and for many taxa it is possible to argue that either is the source of incongruence based on the data that is currently available (Brower et al. 1996). This controversy is partly due to the difficulty of defining whether species have actually diverged to form separate evolutionary lineages (Avise and Ball 1990; Brower et al. 1996; Avise and Wollenberg 1997) and therefore whether it is valid

to argue that shared genetic characters are due to merging of lineages through hybridization. In addition, existing molecular evidence for many taxa is still preliminary and therefore conclusions based on this data are likely to change, as more data becomes available (Doyle 1997).

In studies that have examined species boundaries in scleractinian corals, both incomplete lineage sorting and introgression are commonly considered as possible reasons for discordance between molecular and morphological boundaries. However, in most studies, one mechanism is usually favoured over the other. For example, in the genus *Acropora*, introgression has been proposed as the most likely reason for discordance of both nuclear and mitochondrial markers with morphological species, based on the ability of species of this genus to hybridize under laboratory conditions (Odorico and Miller 1997b; Hatta et al. 1999; van Oppen et al. 2000; van Oppen et al. 2001; van Oppen et al. 2002b). In the morphologically similar species *Acropora cytherea* and *A. hyacinthus*, introgression through hybridization seemed to be infrequent, with assortative mating and disruptive selection apparently operating to maintain these species as statistically distinguishable lineages (Márquez et al. 2002a; Márquez et al. 2002b). Similarly, Vollmer and Palumbi (2002) demonstrate that hybridization occurs rarely between *Acropora cervicornis* and *A. palmata*, resulting in the hybrid *A. prolifera*. Low levels of introgression were apparent for one mitochondrial marker, while there was no evidence of introgression for two others. Shared alleles for one of the nuclear markers are due to incomplete lineage sorting, while the other has diverged completely between the parent species. In the genus *Madracis*, two species were completely resolved, while introgressive hybridization is the most likely mechanism maintaining shared polymorphisms between three other species (Diekmann et al. 2001). In the family Faviidae, the lack of electrophoretic differentiation between morphologically distinct species in the genus *Platygyra* (Miller and Benzie 1997) is consistent with the ability of these species to interbreed (Miller and Babcock 1997). Conflicting results have been reported for the three morphologically distinct species of the *Montastraea* species complex. In one study, there was no evidence of divergence between these species for a nuclear and a mitochondrial marker (Medina et al. 1999). In contrast, one of these species is distinct based on electrophoretic data (Knowlton et al. 1992), AFLP (amplified fragment length polymorphism) patterns and possibly a microsatellite locus (Lopez et al. 1999). Lack of

differentiation between the other two species appears to be due to incomplete lineage sorting (Lopez et al. 1999). Consistent patterns occur between morphological and electrophoretic criteria between species of *Porites* (Weil 1992; Garthwaite et al. 1994), *Pavona* (Maté 2003) and *Montipora* (Stobart and Benzie 1994) suggesting divergence has occurred between the species examined.

The large number of species in the genus *Acropora* (113 species in Wallace 1999) may be due to relatively rapid and recent speciation. While the earliest fossils of the genus *Acropora* are from the Paleocene and Eocene (Wallace 1999), fossil records indicate that most speciation in this genus has occurred since the start of the Neogene, coinciding with the notable increase in shallow water carbonates and reef development in the south-east Asian region (Wilson and Rosen 1998). This is corroborated by the paucity of fossils of the genus *Acropora* in an early Miocene assemblage in southern Iran, with a marked radiation for the genus *Acropora* in the Indo-West Pacific dated in the late Miocene or Pliocene (McCall et al. 1994). Similarly, Fukami et al. (2000) conclude, on the basis of combined sequence divergence of the cytochrome *b* and ATP6 molecular markers, that radiation within the genus *Acropora* has occurred since the late Miocene. If this apparent rapid and recent divergence in the genus *Acropora* is correct, this would support the proposal of incomplete lineage sorting rather than introgression as an explanation of the lack of divergence reported for many species of this genus. Criteria used for interpreting evolutionary relationships between corals and other taxa, such as morphological, molecular and breeding characters are least likely to be congruent during divergence, with congruence between different criteria becoming more likely as the time since divergence increases and the process of speciation is completed (Avise and Ball 1990).

The high levels of divergence generally reported for mitochondrial genomes in many animals, which make mitochondrial markers useful for phylogenetic analyses, are inconsistent in anthozoans (reviewed in Shearer et al. 2002). This slower rate of evolution is likely to have contributed to the discordant patterns of molecular and morphological boundaries reported between species of corals, in studies that have used mitochondrial markers. In these studies, clear patterns of evolutionary divergence are only evident in relatively distantly related taxa. Sequences from the mitochondrial cytochrome *c* oxidase subunit I (COI) differed by 2.4-2.7 % between the three species

of the *Montastraea annularis* species complex and *M. cavernosa*, but were virtually identical for the three species within the species complex (Medina et al. 1999). Sequence divergence for the cytochrome *b* gene ranged from 0.0-0.8% for nine species of *Acropora*, compared to 4.8-5.5% for these nine species and a confamilial species *Montipora aequituberculata* (van Oppen et al. 1999b). Similarly, for the same marker, Fukami et al. (2000) reported differences of 0.0-0.95% for eight species of *Acropora* and up to 8.46% when these species were compared with seven additional species of different genera within the same family. Using a second molecular marker, ATP-6 and the same set of species, Fukami et al. (2000) also reported divergence levels of 0.0-0.46% between the eight species of *Acropora* and up to 6.13% for the additional seven species. The only other mitochondrial marker that has been used to examine species boundaries in scleractinian corals is the putative control region, identified and described by van Oppen and colleagues (van Oppen et al. 1999a; van Oppen et al. 2002a). The control region is thought to be the most rapidly evolving region of the mitochondrial genome (Shearer et al. 2002) and therefore is expected to have the greatest potential for resolving boundaries between closely related taxa. However, surprisingly low levels of sequence divergence (0.0-6.9%) have also been recorded for this marker, for a range of species within the subgenus *Acropora* (Chapter 3; van Oppen et al. 2001; Márquez et al. 2002b). Substantially higher levels were recorded between species of the subgenera, *Acropora* and *Isopora*, ranging from 15.11-18.84% (van Oppen et al. 2001). Despite the low levels of divergence of the putative control region, distinct clades were resolved for all studies that have used this marker to examine species boundaries in the genus *Acropora* (Chapter 3; van Oppen et al. 2001; Márquez et al. 2002b; Vollmer and Palumbi 2002).

Polymorphic sequence blocks within the mtDNA control region have been excluded from previous phylogenetic analyses of the genus *Acropora* (Chapter 3; van Oppen et al. 2001; Márquez et al. 2002b), despite their potential to contribute additional information to the interpretation of evolutionary relationships (Wiens 1999). These polymorphic regions have not been included in previous studies for at least two reasons. The mtDNA control region is generally considered to occur in single copies within an individual, and therefore single sequences are thought to be adequate for analyzing phylogenetic relationships using this marker (Avise 1994). On this premise, it has been necessary to exclude these polymorphic regions before analysis, because

they do not give a consistent phylogenetic signal for a single individual. In addition, the mechanisms by which these regions evolve were uncertain (van Oppen et al. 2001), although possible mechanisms and their evolutionary significance have recently been investigated (van Oppen et al. 2002a). However, patterns of variation within an individual have not been examined, even though the repeat sequence blocks commonly occur within the mtDNA control region, and therefore contribute to the variation within this marker (Avise 1994; van Oppen et al. 2002a), as demonstrated for colonies of the *Acropora humilis* species group (Chapter 3). To determine the evolutionary significance and mechanisms by which these regions may be evolving, it is necessary to first document patterns of variation within and between individuals.

In this study, I use molecular phylogenetic analyses of the putative control region to examine whether interpretation at the sub-species level can resolve evolutionary boundaries within the *Acropora humilis* species group and to examine how relationships vary within and between species across a broad biogeographic range, for this marker. To achieve these objectives, I use the sequences analysed in chapter 3 as well as additional sequences from samples collected across the west to central Pacific of the species and morphs defined in Chapter 4. To further explore evolutionary relationships of the species of the *A. humilis* group, I compare the phylogenetic position of these sequences with GenBank sequences of the same molecular marker (from van Oppen et al. 2001). In this comparison, the GenBank sequences are sequences from species of the *A. humilis* group and from species that appear to be closely related, based on a morphological phylogeny of the genus *Acropora* (Wallace 1999). I also compare the molecular phylogeny presented in this chapter with the morphological phylogeny of Wallace (1999), to test for congruence between these phylogenies. Finally, I compare patterns of variation within and between individuals for the putative control region, based on the occurrence of repeat sequence blocks, to examine whether they may provide additional phylogenetic information for interpreting evolutionary relationships between the species and morphs of the *A. humilis* species group.

5.2 METHODS

5.2.1 COLLECTION OF MOLECULAR SAMPLES

Samples for molecular analysis were collected using the protocol described in Chapter 2 and identified as species or morphs of the *Acropora humilis* species group. Colonies

used in this chapter are a subset, representing taxonomic and biogeographic components, of the colonies examined in the morphological analyses in Chapter 4. One to four colonies were examined for each species from each region, with two exceptions (Table 5.1). Firstly, six colonies of *A. humilis* were analysed from American Samoa, including two colonies of each of the putative morphs of this species, as described in Chapter 2. Secondly, colonies of *A. gemmifera* from the Solomon Islands were not used because the DNA was degraded in these samples. Intermediate and sub-morphs from most regions and locations in which they were recorded, with the exception of "mont-hum", were also examined in the molecular analysis (Table 5.1). *Acropora austera* was selected as the most appropriate outgroup because it is directly ancestral to the species examined in this study (Chapter 3), based on a morphological phylogeny of the genus *Acropora* (Wallace 1999) (Fig. 1.1).

5.2.2 MOLECULAR TECHNIQUES AND ANALYSIS

Extraction, amplification, cloning and sequencing of samples follow the same protocols used in Chapter 3. As described in Chapter 3, the molecular marker was the mtDNA putative control region, amplified using primers developed by van Oppen et al. (1999a). This marker is referred to as the mtDNA intergenic region for the remainder of this paper, as in van Oppen et al. (2001). Multiple cloned sequences were obtained for most individuals (as indicated in Table 5.1), to examine variation in the frequency of occurrence and position of repeat sequence blocks within and between individuals of the *Acropora humilis* species group, across the biogeographic range of this study.

Phylogenetic analysis of this marker was based on consensus sequences for each individual, with repeat sequence blocks deleted (as in Chapter 3). Sequences were aligned manually using Seqapp 1.99 (Gilbert 1994). Calculation of the pairwise sequence distance matrix and the maximum parsimony and likelihood phylogenetic analyses were performed in PAUP* 4.0b10 (Swofford 2002). Phylogenetic analyses used the heuristic search option. Bootstrapping with 1000 pseudoreplicates determined the robustness of clades, with branches supported by <50% being collapsed as polytomies. The maximum parsimony analysis was run with gaps excluded from the analysis, as well as treating gaps as a fifth character. The best-fit model of sequence

Table 5.1 Number of colonies sequenced and number of cloned sequences of the mtDNA intergenic region obtained from each species and morph of the *Acropora humilis* species group and *A. austera*.

Morph	Region: Location [a]	Sample Code	Sequences / Individual	Sequence Type	Base Differences [d]	GenBank Accession No. [b]	MTQ Registration No. [b]
A. samoensis	Indonesia: 1	62	2	I 2	5		G56648
		75	3	I 3	1-5		G56649
		76	1	I 1			G56650
	Australia: 1	119	5	I 5	1-7	AY364090–4	G56366
		121	5	I 5	2-4	AY364095–9	G56367
	PNG: 1	14	5	I 5	1-7		G56651
	Solomon Islands: 1	44	10	III 10	2-8		G56657
A. humilis	Taiwan: 3	7	4	I 4	1-4		G56665
		4	1	III 1			G56663
	Indonesia: 3	9	5	I 5	1-6		G56668
		13	4	I 4	0-2		G56669
	Australia: 1	23	2	I 2	2	AY364100–1	G56368
		32	3	I 3	2-7	AY364102–4	G56369
	PNG: 1	17	6	I 6	1-7		G56672
		33	7	I 7	3-9		G56674
	Solomon Islands: 1	26	4	I 4	2-11		G56677
		27	7	I 7	1-9		G56678
		29	1	I 1			G56679
"*A. humilis* 1" [c]	American Samoa: 2	15	1	I 1			G55587
		51	2	I 2	2		G55589
"*A. humilis* 2" [c]	American Samoa: 2	61	2	I 1	1		G55594
				II 1			
		79	5	I 3	1-8		G55595
				III 2			
"*A. humilis* 3" [c]	American Samoa: 2	66	5	I 3	2-6		G55599
				III 2			
		80	6	I 6	1-6		G55600
A. globiceps	French Polynesia: 1	9	2	I 2	2		G56682
		10	1	I 1			G56683
		12	5	I 5	3-5		G56684
A. gemmifera	Indonesia: 1	50	1	I 1			G56689
	Australia: 1	108	4	I 3	0-10	AY364105 AY364107-8	G56370
				III 1		AY364106	
	PNG: 1	10	7	I 7	1-8		G56692
		31	2	I 1	20		G56694
				III 1			
A. monticulosa	Taiwan: 5	76	7	I 6	2-19		G56701
				III 1			
		84	7	I 6	1-6		G56702
				III 1			
	Australia: 1	30	8	I 5	0-9	AY364109–10 AY364113–15	G56371
				III 3		AY364111–12 AY364116	
		34	4	I 4	3-6	AY364117–20	G56372
	Solomon Islands: 1	12	7	I 7	0-6		G56703
		32	9	III 9	1-4		G56704
	American Samoa: 1	125	4	I 4	0-5		G55616
		128	3	I 3	0-5		G55617
A. digitifera	Taiwan: 2	22	1	I 1			G56707
	Taiwan: 3	14	1	I 1			G56706
	Indonesia: 1	45	2	I 2	10		G56799
		46	8	I 8	2-7		G56800
	Indonesia: 2	39	5	I 4	3-6		G56801
				III 1			
		40	7	I 5	2-8		G56802
				III 2			
	Australia: 1	122	1	I 1		AY364121	G56373
		124	5	I 5	0-3	AY364122–6	G56374

Table 5.1 continued

	PNG: 1	1	8	I 8	1-10		G56714
		2	7	I 7	2-5		G56715
	Solomon Islands: 1	13	8	I 6	1-7		G56720
				III 2			
A. retusa	French Polynesia: 1	14	11	I 10	2-6		G56728
				III 1			
A. multiacuta	Indonesia: 1	65	3	III 3	2-6		G56730
		82	6	III 6	0-6		G56731
"sam-hum"	Australia: 1	94	5	I 5	1-3	AY364127–31	G56375
	Solomon Islands: 1	20	3	I 1	2-6		G56741
				III 2			
"sam-gem"	Australia: 1	95	3	I 3	0-2	AY364132–4	G56376
"hum-gem"	Australia: 1	105	5	I 5	1-7	AY364135–9	G56377
"hum-gem plate"	Taiwan: 3	11	1	I 1			G56758
"mont-gem"	Taiwan: 3	10	1	I 1			G56765
	Taiwan: 5	73	6	I 3	1-4		G56767
				III 3			
	Indonesia: 3	3	1	I 1			G56769
	Indonesia: 4	31	3	III 3	3-12		G56770
		32	4	I 4	1-7		G56771
	PNG: 1	3	5	I 3	1-4		G56774
				III 2			
		6	1	I 1			G56775
		19	1	I 1			G56777
	Solomon Islands: 1	17	8	I 8	2-6		G56780
		19	5	I 5	2-7		G56781
	American Samoa: 1	102	7	I 5	1-17		G55610
				III 2			
	American Samoa: 2	64	5	I 5	0-7		G55607
"dig-gem"	Australia: 1	27	9	I 9	1-6	AY364140–8	G56379
		35	6	I 6	2-10	AY364149–54	G56380
"fine sam"	Indonesia: 1	89	4	I 4	2-3		G56787
"Penghu hum"	Taiwan: 1	39	1	I 1			G56789
	Taiwan: 4	68	6	III 6	0-5		G56793
"terete mont"	Australia: 1	6	1	III 1		AY364155	G56378
"digitate mont"	Taiwan: 5	71	4	IV 3	1-7		G56795
				V 1			
		72	1	I 1			G56796
	Solomon Islands: 1	18	5	I 1	1-15		G56797
				IV 4			
	American Samoa: 3	24	1	I 1			G55613
"Samoan dig"	American Samoa: 1	121	6	I 6	1-5		G55605
		124	3	I 3	0-9		G55606
"encrusting dig"	Taiwan: 5	75	4	I 4	2-6		G56798
A. austera	Australia: 1	111	3	VI 3	2-6	AY364156–8	G56381
		112	2	VI 2	5	AY364159–60	G56382
		113	2	VI 2	9	AY364161–2	G56383
	PNG: 1	45	4	VI 4	0-7		G56803
		46	3	VI 3	2-6		G56804
		49	7	VI 7	0-6		G56805

[a] Location codes within regions are given in Table 4.1; Sequences from Lizard Island, Australia are also presented in chapter 3. [b] GenBank Accession numbers and corresponding Museum of Tropical Queensland (MTQ) registration numbers for morphological reference samples are listed for each colony. [c] Putative morphs 1, 2 and 3 of *A. humilis* from American Samoa, as described in chapter 2. [d] Base differences is the number of base differences between sequences within an individual, excluding repeat sequence blocks.

evolution (HKY + G) was determined using Modeltest 3.06 (Posada and Crandall 1998) and the Akaike Information Criterion method, for the maximum likelihood analysis. Sequences from samples from Lizard Island, Australia have been submitted to GenBank (Table 5.1 and Chapter 3). Sequences from samples from other locations will be available from GenBank following publication of this chapter. Morphological samples are deposited at the Museum of Tropical Queensland (MTQ), Townsville, Australia (Accession numbers are listed in Table 5.1). GenBank accession numbers and MTQ registration numbers will be cross-referenced with the reciprocal institutions.

5.2.2.1 COMPARISON WITH GENBANK SEQUENCES

The phylogenetic relationship between colonies of the species and morphs examined in this study are compared with results of van Oppen et al. (2001) for the same molecular marker, for species of the *Acropora humilis* species group and other species within the DND clade ("*digitifera–nasuta-divaricata groups*") (Wallace 1999) (see Fig. 1.1). Sequences from the study of van Oppen et al. (2001) were downloaded from GenBank. Accession numbers for these sequences are as follows: *A. humilis*: AY26440, AY26441, AY26442; *A. gemmifera*: AY26437, AY26438, AY26439; *A. digitifera*: AY26431; *A. valida*: AY26460; *A. nasuta*: AY26450; *A. cerealis*: AY26424, AY26425, AY26426; *A. divaricata* AY26432, AY26433.

5.2.2.2 COMPARISON OF MOLECULAR AND MORPHOLOGICAL PHYLOGENIES

Comparison is also made between the molecular phylogeny in this study and the morphological phylogeny of Wallace (1999). Consensus sequences of the mtDNA intergenic region, for each species, were used in this comparison, with gaps treated as a fifth character. Consensus sequences for species of the *Acropora humilis* species group and *A. austera* were reduced from all sequences obtained in this study. GenBank sequences from the study of van Oppen et al. (2001) for the species *A. nasuta*, *A. valida*, a consensus sequence of the two *A. divaricata* sequences and the sequence for *A. cerealis* in clade I (Fig. 5.1) were also included in the comparison. No explanation was evident for the anomalous position of the two sequences of *A. cerealis* in clade IIc (Fig. 5.1), and therefore they were not included in this analysis. The molecular phylogeny was forced to match the morphological phylogeny of Wallace (1999), using the program MacClade 4.03 (Maddison and Maddison 2001). This forced topology was

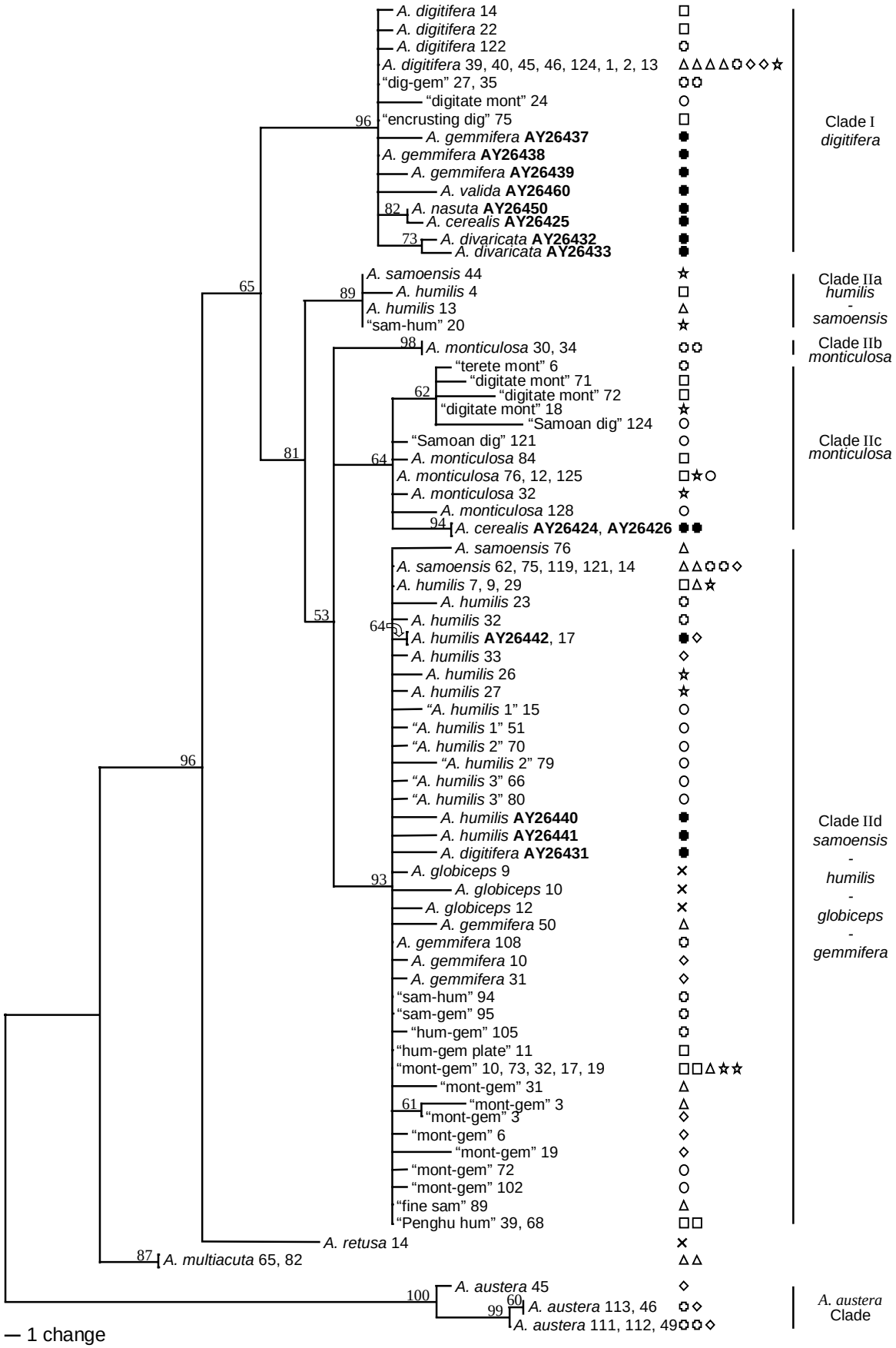
then compared to trees produced in an exhaustive search for the mtDNA intergenic region, in PAUP* 4.0b10 (Swofford 2002), to test whether the morphological phylogeny was significantly different from the molecular phylogeny, using the one-tailed Kishino-Hasegawa test and the two-tailed Templeton-Wilcoxon signed-ranks test.

5.3 RESULTS

5.3.1 PHYLOGENETIC ANALYSIS

Phylogenetic analysis of the sequences obtained in this study for the mtDNA intergenic region separated the species and morphs of the *Acropora humilis* species group into three major evolutionary lineages (Fig. 5.1). Within each lineage, there was a clear pattern of taxonomic structuring for all samples, across the biogeographic range of this study. Sequences from the species *A. digitifera* grouped in one lineage (clade I), sequences from *A. samoensis*, *A. humilis*, *A. globiceps*, *A. gemmifera* and *A. monticulosa* grouped in a second composite lineage (clade II) and sequences from *A. multiacuta* formed a third lineage distinct from all other species and morphs. The single sequence from *A. retusa* branched from clades I and II, with weak bootstrap support. Sequences from the morphs "dig-gem", "encrusting dig" and one colony of "digitate mont" grouped with the *A. digitifera* sequences in clade I. Four sub-clades were present within clade II. Clade IIa comprised one sequence from a colony of *A. samoensis*, two from colonies of *A. humilis* and one from a colony of "sam-hum". Clades IIb and IIc were both '*monticulosa*' clades with the two colonies of *A. monticulosa* from Australia forming clade IIb and the remaining sequences of this species from Taiwan, the Solomon Islands and American Samoa grouping with sequences from colonies of "terete mont", "digitate mont" and "Samoan dig" in clade IIc. Clade IId contained the remaining sequences from *A. samoensis* and *A. humilis* and all sequences from *A. globiceps* and *A. gemmifera*. Sequences from the three putative morphs of *A. humilis* from American Samoa, as defined in Chapter 2 were undifferentiated within this sub-clade, as were sequences from the intermediate morphs "sam-hum", "sam-gem", "hum-gem plate", "mont-gem" and the sub-morphs "fine sam" and "Penghu hum".

Fig. 5.1 Maximum parsimony consensus tree (50% majority rule) with mid-point rooting, produced in the analysis of the mtDNA intergenic region for species and morphs of the *Acropora humilis* species group, *A. austera* and GenBank sequences from the study by van Oppen et al. (2001) for the species *A. humilis, A. gemmifera, A. digitifera, A. cerealis, A. nasuta, A. valida* and *A. divaricata*. Gaps were treated as missing characters in this analysis. Numbers above branches indicate bootstrap values (1000 replicates) for branches with >50% support. Species names and sample codes are given for each individual. Sample codes are as listed in Table 5.1 for sequences from this study. GenBank accession numbers for the study by van Oppen et. al. (2001) are in bold font and contain 5 digit number prefixed by AY. Clade I and sub-clades of clade II are named after the species of the *Acropora humilis* species group within each clade. Symbols indicate the region in which colonies were sampled: Taiwan □ , Indonesia △, Australia ✪ , PNG ◇, Solomon Islands ☆, American Samoa ◯, French Polynesia ✖. Filled symbols highlight GenBank sequences from Australia.


A. digitifera 14
A. digitifera 22
A. digitifera 122
A. digitifera 39, 40, 45, 46, 124, 1, 2, 13
"dig-gem" 27, 35
"digitate mont" 24
"encrusting dig" 75
A. gemmifera AY26437
A. gemmifera AY26438
A. gemmifera AY26439
A. valida AY26460
A. nasuta AY26450
A. cerealis AY26425
A. divaricata AY26432
A. divaricata AY26433
A. samoensis 44
A. humilis 4
A. humilis 13
"sam-hum" 20
A. monticulosa 30, 34
"terete mont" 6
"digitate mont" 71
"digitate mont" 72
"digitate mont" 18
"Samoan dig" 124
"Samoan dig" 121
A. monticulosa 84
A. monticulosa 76, 12, 125
A. monticulosa 32
A. monticulosa 128
A. cerealis AY26424, AY26426
A. samoensis 76
A. samoensis 62, 75, 119, 121, 14
A. humilis 7, 9, 29
A. humilis 23
A. humilis 32
A. humilis AY26442, 17
A. humilis 33
A. humilis 26
A. humilis 27
"A. humilis 1" 15
"A. humilis 1" 51
"A. humilis 2" 70
"A. humilis 2" 79
"A. humilis 3" 66
"A. humilis 3" 80
A. humilis AY26440
A. humilis AY26441
A. digitifera AY26431
A. globiceps 9
A. globiceps 10
A. globiceps 12
A. gemmifera 50
A. gemmifera 108
A. gemmifera 10
A. gemmifera 31
"sam-hum" 94
"sam-gem" 95
"hum-gem" 105
"hum-gem plate" 11
"mont-gem" 10, 73, 32, 17, 19
"mont-gem" 31
"mont-gem" 3
"mont-gem" 3
"mont-gem" 6
"mont-gem" 19
"mont-gem" 72
"mont-gem" 102
"fine sam" 89
"Penghu hum" 39, 68
A. retusa 14
A. multiacuta 65, 82
A. austera 45
A. austera 113, 46
A. austera 111, 112, 49
96
82
73
65
89
98
62
81
64
94
53
64
96
93
61
87
100
60
99
Clade I
digitifera
Clade IIa
humilis
-
samoensis
Clade IIb
monticulosa
Clade IIc
monticulosa
Clade IId
samoensis
-
humilis
-
globiceps
-
gemmifera
A. austera
Clade
— 1 change

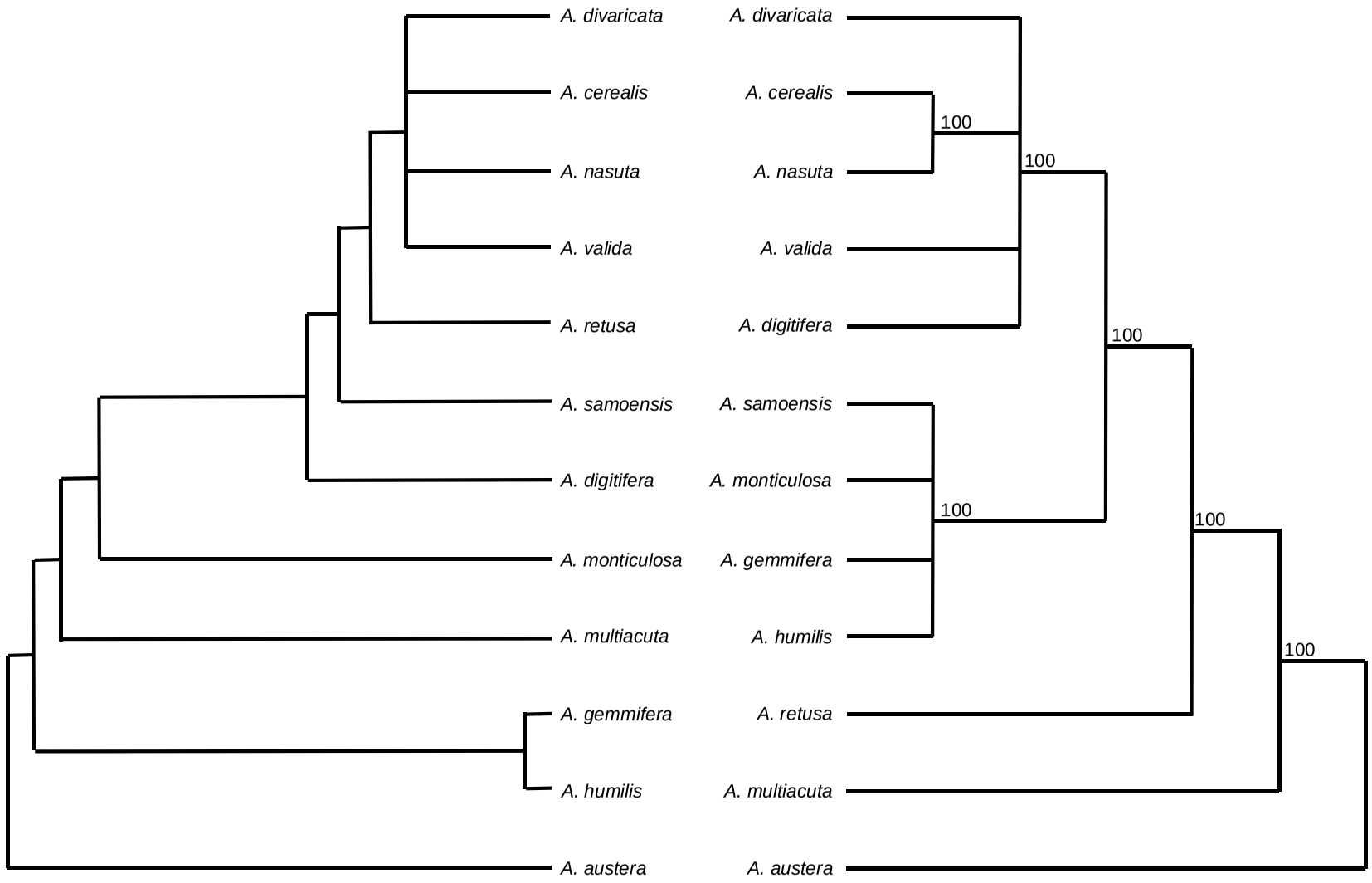
5.3.1.1 COMPARISON WITH GENBANK SEQUENCES

The phylogenetic position of GenBank sequences from a previous study by van Oppen et al. (2001), for colonies of the *Acropora humilis* species group, were consistent with those obtained in this study for *A. humilis* but not for *A. gemmifera* and *A. digitifera* (Fig. 5.1). Sequences from the three colonies of *A. humilis* grouped in clade IId, as did most sequences obtained in this study for this species. In contrast, the three GenBank sequences for *A. gemmifera* grouped within the '*digitifera*' clade (clade I) while the GenBank *A. digitifera* sequence grouped in the '*samoensis-humilis-globiceps-gemmifera*' clade (clade IId). Most sequences from species of the DND ('*digitifera-nasuta-divaricata*', Fig. 1.1) clade in the morphological phylogenetic analysis of Wallace (1999), grouped in the '*digitifera*' clade (clade I) (Fig. 5.1). One sequence of each of *A. valida, A. nasuta* and *A. cerealis* and sequences from two colonies of *A. divaricata* were in clade I, while sequences from two other colonies of *A. cerealis* were in the '*monticulosa*' clade (clade IIc).

5.3.1.2 COMPARISON OF MOLECULAR AND MORPHOLOGICAL PHYLOGENIES

Forcing the topology of the molecular phylogenetic analysis to follow that of the morphological tree of Wallace (1999) resulted in a tree that was significantly different ($p<0.0001$ for both the one and two tailed tests), with an additional 29 steps (Fig. 5.2). An exhaustive search of the consensus sequences of the mtDNA intergenic region produced two equally parsimonious trees of 127 steps, differing only in that *Acropora valida* and *A. divaricata* were sister taxa in one tree and a polytomy in the other. In comparison with these trees, the tree with the forced topology required 156 steps. Differences in the trees with and without the forced topology were due to the relative positions of species of the *A. humilis* species group, while the positions of *A. austera, A. divaricata, A. cerealis, A. nasuta* and *A. valida* were consistent. The greatest differences in the two trees were due to *A. retusa, A. samoensis* and *A. monticulosa*. In the morphological tree, *A. retusa* branched directly from the polytomy formed by A. *divaricata, A. cerealis, A. nasuta* and *A. valida*. In the molecular tree, however, *A. retusa* had a more basal position branching with strong bootstrap support from the polytomies formed by the species of clades I and II (Fig. 5.1). *Acropora samoensis* is the next most derived species, branching from *A. retusa* in the morphological tree, in

Fig. 5.2 Comparison of the morphological tree of Wallace (1999) and the molecular tree of the mtDNA intergenic region for the species of the *Acropora humilis* species group, *A. austera* and other species of the DND clade for which sequences were available: i.e. *A. cerealis*, *A. nasuta*, *A. valida* and *A. divaricata*. The molecular tree is a 50% majority rule consensus tree drawn from the two equally most parsimonious trees of an exhaustive search of single sequences for each species. Numbers above branches are bootstrap values (1000 replicates), for branches with >50% support. The bootstrap values of 100% indicated that the same species grouped together in both of the most parsimonious trees.



Morphological Phylogeny (redrawn from Wallace 1999)

Consensus Tree: mtDNA Intergenic Region

contrast to the molecular tree in which it groups within a polytomy with the other clade II species. *Acropora monticulosa* also groups within this clade II polytomy in the molecular tree, but forms its own lineage in the morphological tree. *Acropora digitifera* appears to be relatively closely related to *A. divaricata*, *A. cerealis*, *A. nasuta* and *A. valida* in both analyses. *Acropora humilis* and *A. gemmifera* form polytomies in each tree, emphasizing the close relationship of these species. *Acropora multiacuta* formed a separate lineage in both analyses, supporting the apparent distinction of this species.

Base composition of consensus sequences obtained in this study from species and morphs of the *Acropora humilis* group and *A. austera* was homogeneous (Table 5.2), and almost identical to that reported in Chapter 3 for a single geographic location. Level of divergence between sequences obtained in this study (Table 5.3) was low, as also reported in Márquez et al. (2002b) and van Oppen et al. (2001) and similar to that reported in Chapter 3. Maximum sequence divergence between the *A. humilis* species group and *A. austera* was 6.03% (Table 5.3), more than double the level of divergence between sequences from species and morphs within the *A. humilis* species group.

Table 5.2 Mean base compositions (%) for sequences obtained in this study from the mtDNA intergenic region for species and morphs of the Acropora humilis species group and Acropora austera [a].

A	C	G	T
24.8 (0.002)	17.2 (0.002)	26.4 (0.002)	31.6 (0.003)

[a] Standard deviations are given in parentheses.

Table 5.3 Average Kimura 2-parameter pairwise distances (%) within and between sequences obtained in this study from the mtDNA intergenic region for species and morphs of the *Acropora humilis* species group and *Acropora austera* [a].

	A. humilis species group	*A. austera*
A. humilis species group	0.96 (2.56)	5.31 (6.03)
A. austera		0.24 (0.66)

[a] Maximum values are given in parentheses.

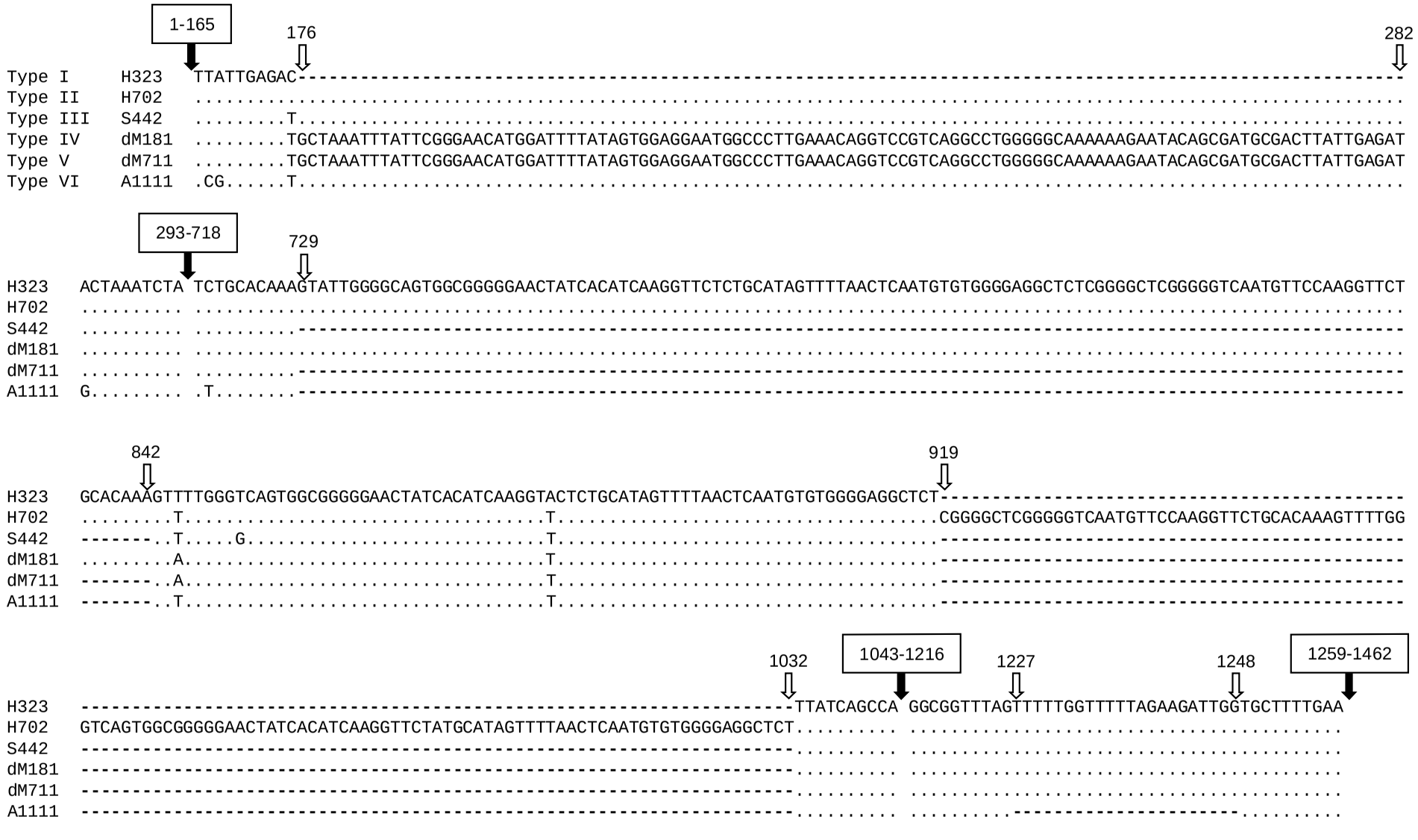
Nonetheless, sequences from colonies of *A. austera* were not sufficiently different to form a natural outgroup. Trees produced in the phylogenetic analyses were therefore drawn using the midpoint rooting option (as in Chapter 3). The aligned consensus sequences consisted of 1077 positions with repeat sequence blocks deleted. Prior to deletion of repeat sequence blocks, individual sequences ranged in length from 1094 to 1343 bp. Within the aligned sequences, 923 positions were constant, 69 variable characters were not parsimony informative and 85 were parsimony informative.

Maximum parsimony (MP) and maximum likelihood (ML) analyses of the single data set, comprising the consensus sequences obtained in this study and the GenBank sequences from van Oppen et al. (2001), produced trees with similar topologies and levels of bootstrap support and the same combination of sequences in each clade. The trees resulting from each analysis mainly differed in the structure of the sub-clades in clade II. In the MP analysis, clade IIa formed a separate branch with 81% bootstrap support, while the remaining three clades (IIb, IIc and IId) formed a polytomy (Fig. 5.1). In the ML analysis the four sub-clades formed a polytomy with 60% bootstrap support. The single sequence from *Acropora retusa* branched from clades I and II, with weak bootstrap support in both analyses (65% in the MP and 62% in the ML analysis). Treating gaps as a fifth character in the MP analysis did not change the tree topology, as also reported in Chapter 3 and by van Oppen et al. (2001). All *A. austera* sequences grouped as a distinct clade with strong (100%) bootstrap support in both MP analyses and the ML analysis. MP analysis with gaps treated as missing characters produced 29 most parsimonious trees of 183 steps. A consistency index of 0.863, a retention index of 0.971 and a homoplasy index of 0.137 indicate a strong phylogenetic signal in the sequence data.

5.3.2 VARIABILITY OF SEQUENCE REPEATS IN THE mtDNA INTERGENIC REGION

Alignment of all cloned sequences of the mtDNA intergenic region obtained in this study indicated the existence of six sequence types, defined according to the presence and position of repeat sequence blocks or deletions (Fig. 5.3). Type I sequences were the most common, against which all other sequence types are compared. Type II sequences differed from type I in having a unique repeat sequence block between positions 919 and 1032, repeating bases from positions 805-918. Sequence types IV

Fig. 5.3 Alignment of cloned sequences of the mtDNA intergenic region, showing examples of the six sequence types (Types I - VI) found in this study. Dots indicate the same bases as shown in the upper (Type I) sequence and dashes indicate alignment gaps. Positions of repeat sequence blocks are indicated with unfilled arrows. Location of bases from positions 1-165, 293-718, 1043-1216 and 1259-1462 are indicated with solid arrows, but not shown in the alignment. Codes for sequences are as follows: H323 *Acropora humilis* from Lizard Island, Australia; H702 "*A. humilis* 2" from Ofu, American Samoa; S442 *A. samoensis* from Ghizo Island, Solomon Islands; dM181 "digitate mont" from Ghizo Island, Solomon Islands; dm711 "digitate mont" from Lanyu, Taiwan; A1111 *A. austera* from Lizard Island, Australia. The last digit for each sequence code indicates the clone number, the digits preceding the clone numbers correspond with the sample code in Table 5.1.


1-165
176
282
Type I H323 TTATTGAGAC
Type II H702
Type III S442
Type IV dM181 TGCTAAATTTATTCGGGAACATGGATTTTATAGTGGAGGAATGGCCCTTGAAACAGGTCCGTCAGGCCTGGGGGCAAAAAAGAATACAGCGATGCGACTTATTGAGAT
Type V dM711 TGCTAAATTTATTCGGGAACATGGATTTTATAGTGGAGGAATGGCCCTTGAAACAGGTCCGTCAGGCCTGGGGGCAAAAAAGAATACAGCGATGCGACTTATTGAGAT
Type VI A1111 .CG.....T
293-718
729
H323 ACTAAATCTA TCTGCACAAAGTATTGGGGCAGTGGCGGGGGAACTATCACATCAAGGTTCTCTGCATAGTTTTAACTCAATGTGTGGGGAGGCTCTCGGGGCTCGGGGGTCAATGTTCCAAGGTTCT
H702
S442
dM181
dM711
A1111 G..... .T.....
842
919
H323 GCACAAAGTTTTGGGTCAGTGGCGGGGGAACTATCACATCAAGGTACTCTGCATAGTTTTAACTCAATGTGTGGGGAGGCTCT
H702 .....T.....T.....CGGGGCTCGGGGGTCAATGTTCCAAGGTTCTGCACAAAGTTTTGG
S442 --------T.....G.....T.....
dM181 .....A.....T.....
dM711 --------A.....T.....
A1111 --------T.....T.....
1032
1043-1216
1227
1248
1259-1462
H323 TTATCAGCCA GGCGGTTTAGTTTTTGGTTTTTAGAAGATTGGTGCTTTTGAA
H702 GTCAGTGGCGGGGGAACTATCACATCAAGGTTCTATGCATAGTTTTAACTCAATGTGTGGGGAGGCTCT
S442
dM181
dM711
A1111

and V contained a sequence block from positions 176 to 282, which was not found in any other sequence types. This sequence block repeated bases from positions 68-175. Type IV sequences were otherwise the same as type I sequences. Type V sequences also differed from type I sequences in that they contained an additional sequence block from positions 729-842. This additional sequence block was also present in type III and VI sequences, and repeated bases from positions 843-956. Type III sequences were otherwise the same as type I sequences, while type VI sequences had a unique deletion from positions 1227 to 1248.

Various combinations of pairs of sequence types were found in 18 of the 89 colonies sequenced, indicating that the mtDNA intergenic region can occur as more than one copy within individuals (Table 5.1) and comparison of this variability may provide additional information for revealing evolutionary relationships between species and morphs. No individuals were recorded with more than two sequence types. Sequence type I was present in at least some individuals of all species and morphs from all regions, with the exception of both colonies of *Acropora multiacuta* and the single colony of "terete mont" in which only type III was recorded. Type III sequences were the second most common type, and when present in an individual, usually co-occurred with sequence type I. Type I and III sequences were found in individuals from all regions, but not the same species and morphs in each region. Both I and II were present in colonies of *A. humilis* from American Samoa and Taiwan, *A. gemmifera* from Australia and PNG, *A. monticulosa* from Taiwan, Australia and the Solomon Islands, *A. digitifera* from Indonesia and the Solomon Islands, *A. retusa* from French Polynesia, "sam-hum" from the Solomon Islands, "mont-gem" from Taiwan, Indonesia, PNG and American Samoa and "Penghu hum" from Taiwan. The colony of *A. samoensis* from the Solomon Islands contained only sequence type III, while colonies of *A. globiceps*, "sam-gem", "hum-gem", "hum-gem plate", "dig-gem" or "fine sam" contained only type I sequences. Sequence type II was present in only one cloned sequence, from a single colony of "*A. humilis*" from American Samoa. Type IV sequences were found only in colonies of "digitate mont" from Taiwan and the Solomon Islands, while one of the Taiwan colonies of this morph also contained the only type V sequence. Type VI sequences were found in all colonies of *A. austera* but not in colonies of any other species or morph.

5.4 DISCUSSION

5.4.1 PHYLOGENY OF THE *ACROPORA HUMILIS* SPECIES GROUP

Three main evolutionary lineages were evident between species and morphs within the *Acropora humilis* group, based on the phylogenetic analysis of the sequences obtained in this study (Fig. 5.1). A clear pattern of taxonomic structure was evident between lineages, for all samples across the biogeographic range of this study. Clades I and II formed two of the lineages, separating *A. digitifera* in clade I from sequences of *A. monticulosa, A. samoensis, A. humilis, A. globiceps* and *A. gemmifera* in the composite clade II. The single sequence from *A. retusa* branched from clades I and II, with weak bootstrap support. Sequences from the species *A. multiacuta* formed a third lineage that was distinct from all other species and morphs.

Clade I comprised all sequences from the species *Acropora digitifera*, the intermediate morph "dig-gem" and the sub-morphs "encrusting dig" and "dig mont". The very high bootstrap support for this clade indicates a strong divergence of this species and these morphs from those in clade II. Clade I includes the sequences from colonies of *A. digitifera* and "dig-gem" from Lizard Island, Australia in clade I in Chapter 3, as well as additional sequences from colonies of *A. digitifera* from Taiwan, Indonesia, Papua New Guinea and the Solomon Islands. This finding demonstrates the strong similarity between colonies of *A. digitifera* across this broad biogeographic range and reinforces the lack of differentiation between this species and "dig-gem", for the mtDNA intergenic region. The grouping of the sub-morph "encrusting dig" within this clade supports the tentative identification of this morph, based on colony colour (Chapter 4).

Divergence between *Acropora samoensis, A. humilis, A. globiceps,* and *A. gemmifera* and morphs of these species, across the west to central Pacific, was not evident for the mtDNA intergenic region, suggesting recent or incomplete divergence between these taxa for this marker (Fig. 5.1). Lack of genetic differentiation was also demonstrated for the species *A. humilis* and *A. gemmifera* in the phylogenetic analysis of the nuclear 28S rDNA marker (Chapter 2, with colonies of "mont-gem" identified as *A. gemmifera*). Despite the lack of divergence of these molecular markers, reproductive barriers have been demonstrated between the species *A. samoensis, A. humilis* and *A. gemmifera* at Lizard Island, Australia (Chapter 3), suggesting recent rather than incomplete divergence for these species. The grouping of sequences from colonies of *A.*

globiceps in this clade is consistent with the close morphological similarity between this species and *A. humilis* (Chapter 4), although testing the potential of these species to interbreed would confirm whether evolutionary boundaries exist between these species. The grouping of sequences from all colonies of "mont-gem" within this clade is consistent with the strong morphological affinity between this morph and the species *A. gemmifera*, demonstrated by the likelihood of most colonies of this morph having a higher probability of being classified as *A. gemmifera* rather than *A monticulosa* (Chapter 4). Grouping of sequences from the morphs "hum-gem plate", "fine sam" and "Penghu hum" in clade IId is consistent with their apparent morphological affinities. Clarification of whether the morphological differentiation of these morphs, from the species after which they were named, reflected environmental polymorphism or cryptic species status was not possible due to the lack of further differentiation of the marker used in this study.

All sequences from the species *Acropora monticulosa* grouped in clades IIb or IIc (Fig. 5.1). The grouping of sequences from the two colonies of the putative morph "branching mont" from American Samoa with colonies of *A. monticulosa* from Taiwan and the Solomon Islands, supports my decision to classify this morph within this species (Chapter 4), despite its arborescent rather than the familiar digitate growth form of this species (Chapter 2). The grouping of sequences from *A. monticulosa* within clade II, but in separate sub-clades from *A. samoensis*, *A. humilis*, *A. globiceps* and *A. gemmifera* suggests that *A. monticulosa* has diverged from but shares greatest evolutionary affinity with these four species. Evolutionary affinity is however also evident between *A. monticulosa* and *A. digitifera*, based on relationships with the morphs "digitate mont" and "Samoan dig". The morph "digitate mont" is morphologically similar to *A. monticulosa* (Chapter 4), although sequences from different colonies of this morph grouped in both the '*digitifera*' clade I and the '*monticulosa*' clade IIc. Despite the morphological resemblance of the morph "Samoan dig" to *A. digitifera*, it also appears to have a strong evolutionary affinity with *A. monticulosa*. Four of five colonies of this morph were most likely to be classified as *A. monticulosa* in the morphometric analysis (Chapter 4) and sequences of the mtDNA intergenic region and the 28S rDNA region all grouped with *A. monticulosa* (Fig. 5.1, this chapter and Chapter 2 respectively). Evolutionary affinities of the morph "terete mont" also appears to be with two species, i.e. *A. monticulosa* and *A. humilis*. The

sequence from this morph grouped in the '*monticulosa*' clade IIc (Fig. 5.1), while it showed a greatest but only a moderate potential to interbreed with *A. humilis*. On this basis and its morphological appearance, this morph may be a hybrid, derived from the species *A. monticulosa* and *A. humilis*, as suggested in Chapter 3.

5.4.1.1 COMPARISON WITH GENBANK SEQUENCES

Phylogenetic relationships for sequences of the mtDNA intergenic region obtained in this study were consistent with van Oppen et al. (2001) for *Acropora humilis* but inconsistent for *A. gemmifera* and *A. digitifera* (Fig. 5.1). The three sequences from colonies of *A. humilis* grouped within clade IId, as did most sequences for this species in this study. However, the three sequences from colonies of *A. gemmifera* grouped within the '*digitifera*' clade (clade I), while the single sequence from *A. digitifera* grouped within clade IId. Based on the consistent grouping of colonies of *A. digitifera* and "dig-gem" in a well-supported clade (96% bootstrap support) that is distinct from the other species of the *A. humilis* group (Fig. 5.1) and the morphological affinities of the intermediate morph "dig-gem" with *A. digitifera* and *A. gemmifera* (Chapter 4), it seems likely that the colonies identified as *A. gemmifera* in the study by van Oppen et al. (2001), may in fact belong to the morph "dig-gem". Furthermore, the colony identified as *A. digitifera* by van Oppen et al. (2001), may be *A. humilis*, and hence explain the grouping of the sequence from this colony in clade IId. This proposal is supported by the collection details of this colony, which was collected from Magnetic Island on the Great Barrier Reef, Australia (Table 1: van Oppen et al. 2001). Specifically, *A. digitifera* does not occur at Magnetic Island and *A. humilis* is the only species of the *A. humilis* species group that does (Museum of Tropical Queensland collections).

5.4.1.2 COMPARISON OF MOLECULAR AND MORPHOLOGICAL PHYLOGENIES

Significantly different trees were drawn from the phylogeny of the mtDNA intergenic region and the morphological phylogeny of Wallace (1999), for the same set of species (Fig. 5.2). Differences were due to inconsistent relationships between species of the *Acropora humilis* species group. In contrast, *A. austera* occupied a basal position while *A. cerealis*, *A. nasuta*, *A. valida* and *A. divaricata* were closely related and occupied derived positions in both phylogenies. The close relationship of *A. digitifera* with the *A.*

nasuta and *A. divaricata* species groups was evident in both phylogenies, with *A. digitifera* grouping in monophyletic clades shared by the species of these groups in both analyses. The grouping of *A. retusa* and *A. samoensis* in the same monophyletic clade in the morphological phylogeny did not match the molecular phylogeny. In the molecular phylogeny, *A. retusa* branched from the polytomies formed by the species from clade I and clade II, with strong bootstrap support. *Acropora retusa* occupied the same position in the phylogeny of all sequences from all locations (Fig. 5.1), but with weak bootstrap support. It therefore appears to be distinct from other species of the *A. humilis* species group, although sequences from additional colonies of this species are needed to confirm this distinction. The position of *A. samoensis* was the most incongruent in the comparison of the morphological and molecular phylogenies. Although this species grouped with *A. digitifera* and *A. retusa* in the morphological phylogeny, it formed a polytomy with *A. humilis, A. gemmifera* and *A. monticulosa* in the molecular analysis. Based on the available evidence, I propose that the molecular phylogeny provides a more accurate indication of the evolutionary relationships of *A. samoensis*, consistent with its morphological similarity to *A. humilis* (Chapter 4). *Acropora samoensis* tends to occur deeper on the reef slope compared to other species of the *A. humilis* species group (Chapter 3; Wallace, 1999) and therefore it appears that the phylogenetic coding of morphological characters of this species reflect environmental rather than evolutionary features. *Acropora monticulosa* formed an independent lineage in the morphological phylogeny. Although this species shares a close relationship with *A. samoensis, A. humilis* and *A. gemmifera* in the molecular tree, the morphological distinction of this species is likely to be real, as also suggested by the grouping of sequences from this species in separate sub-clades in the analysis of colonies from all locations (Fig. 5.1). *Acropora multiacuta* formed independent lineages in both the morphological and molecular phylogenies, supporting the distinction of this species from other species of the *A. humilis* group. *Acropora humilis* and *A. gemmifera* formed a highly derived monophyletic clade in the morphological phylogeny and this close relationship was reflected in the molecular phylogeny. *Acropora globiceps* was not included in the morphological phylogeny of Wallace (1999), although she noted that it was separated from *A. humilis* by only one character and therefore was assumed to be a sister species. Therefore, although *A. globiceps* was not included in the statistical comparison of the morphological and molecular

phylogenies, this apparently close relationship was supported by the results of this study, with each of these species grouping within clade IId (Fig. 5.1).

5.4.2 VARIABILITY OF SEQUENCE REPEATS IN THE mtDNA INTERGENIC REGION

Variation in the distribution of repeat sequence blocks in the mtDNA intergenic region suggests this marker has the potential to provide additional support or information for revealing evolutionary relationships within the *Acropora humilis* species group. Type I sequences were most common, being found in colonies of all species and morphs of the *A. humilis* species group except *A. multiacuta* and the single sequence obtained from the sub-morph "terete mont". Type I sequences were also absent in *A. austera*, with all sequences of this species having type VI sequences, confirming the distinction of this species. All sequences from the two colonies of *A. multiacuta* were of type III, supporting the phylogenetic analysis, which found it to be the most distinctive species within the *A. humilis* species group. The reverse situation, i.e. the presence of type I sequences and the absence of type III sequences is less informative due to the wide occurrence of type I sequences. This reverse pattern was found in the colonies of *A. globiceps* and "dig-gem", with no other sequences types in this species and morph. The presence of sequence types I and III in all species other than *A. globiceps* within clade IId provides preliminary support for the distinction of *A. globiceps*. Similarly, the presence of type I sequences only in the colonies of "dig-gem", compared to both sequence types I and III in colonies of *A. digitifera* and *A. gemmifera*, also supports the distinction of this morph. Type IV sequences were recorded only in colonies of the morph "dig mont" from Taiwan and the Solomon Islands, supporting the apparent distinction of this morph from *A. monticulosa*, based on morphological appearance.

5.4.2.1 COMPARISON WITH OTHER *ACROPORA* SPECIES

The sequence types recorded in this study were different from all sequence types recorded for five other species of the genus *Acropora*: *A. aspera*, *A. cervicornis*, *A. loripes*, *A. tenuis* and *A. cuneata* (van Oppen et al. 2002a). The repeat sequence blocks recorded in this study from positions 176-282 (sequence types IV and V) and 919-1032 (sequence type II) (Fig. 5.3) were not present in any of the sequences in this previous study. The first of these sequence blocks (from sequence types IV and V) is located between positions 330 and 331, within the 'repeat 1, copy 3' block. The second

sequence block (from sequence type II) is located between positions 1065 and 1066, at the end of the ‘repeat 4, copy 2’ block and just prior to the conserved sequence block ‘f’ in the alignment by van Oppen et al. (2002a). The repeat sequence block recorded from positions 729-842 in this study (sequence types I, II and IV) aligned with positions 876 to 990, from within ‘repeat 3, copy 2’ to within ‘repeat 3, copy 3’in the alignment of the previous study. The deletion in all sequences from colonies of *A. austera*, from positions 1227 to 1248 was also present as a deletion in *A. tenuis*. Five segments in the aligned sequences in the study by van Oppen et al. (2002a), from positions 126-263, 715-764, 828-875, 1336-1422 and 1468-1603, were not present in the sequences of the current study.

It is clear, therefore, that patterns of variation exist between the repeat blocks in species and morphs of the *A. humilis* species group, and other species of the genus *Acropora*, which appear to be phylogenetically informative, although further research is necessary to understand the evolutionary significance of these repeat regions (van Oppen et al. 2002a). Development of phylogenetic analysis programs that can incorporate within-individual variation (e.g. Wiens 2001; Legendre and Makarenkov 2002) in the mtDNA intergenic region, the nuclear rDNA unit and other markers will assist in the interpretation of the evolutionary significance of this molecular polymorphism, and therefore relationships between species.

5.4.3 INCOMPLETE LINEAGE SORTING AND INTROGRESSION

Incomplete lineage sorting of the mtDNA intergenic region appears to be the primary explanation of the low levels of divergence between the species and morphs of the *Acropora humilis* species group, and is supported by the proposal of relatively rapid and recent speciation in the genus *Acropora* (Wilson and Rosen 1998; Fukami et al. 2000). The low levels or lack of divergence between *A. samoensis, A. humilis, A. globiceps, A. gemmifera, A. monticulosa* and morphs of these species, for all colonies across the broad biogeographic scale of this project, strongly suggests that lineage sorting is incomplete for this molecular marker. Although these species showed no potential to interbreed (Chapter 3), their rate of divergence may be retarded by indirect introgression through backcrossing via morphs of these species, leading to indirect mixing of genomes between what appear to be otherwise distinct species (Arnold 1997). Examples of species and morphs that showed moderate and high potentials to

interbreed include *A. humilis* with “terete mont” and *A. samoensis* with “sam-hum”, respectively (Chapter 3).

Although the close phylogenetic relationships between these species and most morphs of the *Acropora humilis* species group appear to be explained by incomplete lineage sorting of the mtDNA intergenic region, there was also some evidence for introgression through hybridization in two cases. Firstly, the morph “dig-gem” was the only intermediate morph that did not share the same clade as both of the species after which it was named. If the apparent morphological affinities of this morph are indicative of its ancestry, then the position of the putative parent species in the distinct clades, supports the tentative proposal that this morph may be derived from hybridization of *A. digitifera* and *A. gemmifera* (Chapter 3). Secondly, possible indications of hybridization between *A. monticulosa* and *A. digitifera* were also evident from morphological and molecular features of these species and the sub-morphs “digitate mont” and “Samoan dig”. These morphs were most like *A. monticulosa* in their morphological features, although their colony colour and diminutive, terete branches also resembled *A. digitifera* (Chapter 4). The molecular data also supports evolutionary affinities, possibly as hybrids of these species, for both morphs. “Digitate mont” grouped in both the ‘*monticulosa*’ clade (clade IIc) and the ‘*digitifera*’ clade (clade I) in the phylogenetic analysis of the mtDNA intergenic region (Fig. 5.1), and with *A. monticulosa* and “Samoan dig” for the 28S rDNA unit (Chapter 2). Meanwhile, the morph “Samoan dig” grouped within the ‘*monticulosa*’ clade (clade IIc) in the analysis of the mtDNA intergenic region.

5.4.4 SPECIATION IN THE GENUS *ACROPORA*

The ancestral history of species and morphs in the *Acropora humilis* species group is likely to be more complex than the results of this study indicate, with some species being more closely related to species outside the *A. humilis* group than to species within this group. Species from groups outside the *A. humilis* group may therefore have contributed to new lineages, recognized as species or morphs in this study. Furthermore, new lineages may have evolved from hybridization events that involved more than two species (Chapter 3). However, following the close examination of genetic relationships between species and morphs in this study, it appears that hybridization between species within this genus is more structured and not as

widespread as suggested previously (e.g. van Oppen et al. 2001). To explore this proposal further, it will be necessary to use molecular markers that are evolving under evolutionary constraints, because the low rate of divergence of neutral markers such as the mtDNA intergenic region are inadequate for resolving relationships between all species in corals and other anthozoans (Shearer et al. 2002). Proteins directly involved in preventing fertilization will be particularly useful for resolving evolutionary boundaries in corals (Palumbi 1994). In addition, reproductive proteins appear to evolve rapidly and may play a pivotal role in speciation (reviewed in Swanson and Vacquier 2002). Molecular markers developed from these proteins are likely to greatly enhance our understanding of the evolutionary relationships between apparently closely related species, such as *A. samoensis*, *A. humilis*, *A. globiceps* and *A. gemmifera* and of the ancestral history of possible hybrid morphs, such as "dig-gem", "digitate mont" and "Samoan dig".

Patterns of divergence between coral taxa have the potential to vary geographically. Different locations have different taxonomic assemblages and different patterns in the timing of spawning, influencing which species interbreed. Species composition varies considerably across the biogeographic range of the genus *Acropora*, most notably decreasing in diversity outwards from the Indo-West Pacific centre of biodiversity (Wallace 1999). Relative timing of spawning also varies between taxa in different geographic locations (e.g. Table 4 in Wallace 1999). Of particular relevance to the interpretation of the results of this study, timing of spawning of *A. digitifera* varies in relation to other species of the *A. humilis* species group and other mass spawning corals in different locations. The documented time of spawning of *A. digitifera* is February to March at Lizard Island, Australia (Chapter 3, Wallace 1999), while most other species of the genus *Acropora* spawn during the mass spawning months in November to December (Wallace 1999). Similarly, *A. digitifera* does not seem to spawn during the mass spawning in Guam (S. Romano, pers. comm.). However, in Papua New Guinea (April 2000) and the Solomon Islands (November 2000) this species contained mature gametes at the same time as other species of the *A. humilis* group and other mass spawning species (pers. obs.), and therefore appears to spawn at the same time as species from which it is temporally reproductively isolated in other locations. The three month separation in the predominant time of spawning between *A. digitifera* and the morph "dig-gem" at Lizard Island, Australia (Chapter 3) and between *A. digitifera* and

the cryptic species *A.* sp 1 aff. *digitifera* (also by three months) in southern Japan (Hayashibara and Shimoike 2002), provides clear evidence of the potential for geographically different patterns of divergence. This potential is supported by the abundance of colonies of "dig-gem" at Lizard Island, with this morph being substantially more common than any other species or morph of the *A. humilis* species group (Chapter 3). The morph "dig-gem" has only been recorded from a few other biogeographic locations (Chapter 4), while the cryptic species *A.* sp 1 aff. *digitifera* has not been recorded from other locations (Chapter 4; Hayashibara and Shimoike 2002). This apparent potential for different patterns of divergence in different geographic locations highlights the complexity of resolving evolutionary boundaries in the genus *Acropora*, and possibly other coral taxa. Different biogeographic patterns of divergence also supports incomplete lineage sorting as the predominant explanation of evolutionary relationships in the *A. humilis* species group, because the same patterns of hybridizing taxa over the broad biogeographic scale of this study are unlikely (and for many locations not possible) given the same combinations of taxa were not present in each location.

This study demonstrates the value of examining polymorphism of morphological and molecular characters, within and between species, for interpreting evolutionary boundaries in corals as suggested by Wiens (1999). Recognition of morphological polymorphism within species enabled intermediate morphs and sub-morphs to be defined and used as additional categories. Exploitation of this variability, rather than reducing it to previously defined species categories, assisted in interpreting evolutionary relationships as well as reducing the likelihood of taxonomic error. To exploit this variability in future studies and guard against taxonomic error, it is essential that voucher specimens be lodged in museums for permanent housing (Paulay 1997; Wheeler 2001). This is particularly important now that genetic data from previous studies are publicly available on databases such as GenBank. Lodgment of voucher specimens will enable the question of identity to be revisited as new techniques and evidence become available, and confirm or correct what is reported in the literature (Wheeler 2001). Examination of patterns of molecular variation can also contribute to interpreting evolutionary relationships in corals. The variable occurrence of the repeat sequence blocks, within and between individuals provided additional support for interpreting the relationships determined in the molecular phylogenetic analysis, given

the low levels of divergence for the mtDNA intergenic region. The repeat sequence blocks also highlighted the distinction of the morph “digitate mont” from *Acropora monticulosa* and other morphs of this species. Incorporating patterns of polymorphism into future analyses of are therefore recommended, to maximize the potential for resolving evolutionary relationships in corals.

CHAPTER 6: GENERAL DISCUSSION

6.1 EVOLUTIONARY RELATIONSHIPS IN THE *ACROPORA HUMILIS* SPECIES GROUP

In this thesis, the recognition of morphs within and between currently defined species greatly enhanced the interpretation of evolutionary relationships in the *Acropora humilis* species group. I demonstrate that, rather than considering species as discrete units, assessing their patterns of morphological variability provided valuable information, complementary to the molecular and reproductive criteria, for interpreting evolutionary affinities in this group of corals (Tables 6.1 and 6.2). Although, most studies that have examined evolutionary relationships in the genus *Acropora* have only recognized species as discrete units (e.g. Wallace 1999; van Oppen et al. 2001; Márquez et al. 2002b), recognition of morphs within the species *A. millepora*, *A. nasuta* and *A. prolifera* also helped to resolve evolutionary relationships for these species (Wallace and Willis 1994; Hatta et al. 1999; Vollmer and Palumbi 2002). In this thesis, all criteria contributed to the interpretation of evolutionary relationships. However, different levels of resolution for different combinations of species and morphs indicated variable patterns and rates of divergence for each of the criteria. Reproductive evidence revealed the greatest level of divergence, morphological characters revealed a moderate level of divergence and molecular criteria revealed the least divergence.

Acropora humilis, *A. samoensis* and *A. gemmifera* were shown to be valid biological species on the basis of the reproductive criteria, despite their morphological similarity and a lack of genetic differentiation between these species. The morphological and genetic affinities within and between these species appear to be due to recent divergence, with each of these species and their intermediate and sub-morphs sharing a close genetic relationship across the geographic range sampled. Although these species showed no potential to interbreed, it is possible that introgression through interbreeding between some of these morphs and species is retarding the rate of species divergence. Further research is necessary to confirm the taxonomic status of *A. globiceps*, as it was not distinguished from these three species based on the molecular data or from *A. humilis* for the morphological data.

Reproductive data indicates that *A. digitifera* is a valid biological species, and was distinct from all other species of the *A. humilis* species group for the mtDNA intergenic region across the geographic range of this project. This species may share a closer evolutionary relationship with species of the *A. nasuta* and *A. divaricata* species groups, as suggested by comparison of sequence data for this marker, and a morphological phylogeny of the genus *Acropora* (Wallace 1999). Recognition of the intermediate morph "dig-gem" was particularly important because it is evolutionarily distinct from both *A. digitifera* and *A. gemmifera*, on the basis of reproductive and molecular criteria respectively. Further research, incorporating reproductive and molecular criteria, is necessary to confirm that skeletal samples identified as "dig-gem"

Table 6.1a Summary of inferred affinities of the species of the *Acropora humilis* species group for each of the criteria examined in this study.

Species	Morphological Appearance	Morphometric Analysis	28S rDNA Unit (Domains 1 and 2)	mtDNA Intergenic Region	Reproduction
	All Chapters	Chapters 2 & 4	Chapter 2	Chapters 3 & 5	Chapters 3 & 5
A. samoensis	*A. humilis*	*A. humilis*		*A. humilis* *A. globiceps* *A. gemmifera*	None
A. humilis	*A. samoensis* *A. globiceps* *A. gemmifera*	*A. samoensis* *A. globiceps*	"mont-gem"	*A. samoensis* *A. globiceps* *A. gemmifera*	None
A. globiceps	*A. humilis*	*A. humilis*		*A. samoensis* *A. humilis* *A. gemmifera*	
A. gemmifera	*A. humilis* *A. digitifera*	*A. monticulosa* *A. digitifera*		*A. samoensis* *A. humilis* *A. globiceps*	None
A. monticulosa	*A. gemmifera*	*A. gemmifera*	"Samoan dig" "digitate mont"	*A. samoensis* *A. humilis* *A. globiceps* *A. gemmifera*	None
A. digitifera	*A. gemmifera*	A. gemmifera		None	None
A. retusa	None	None		None	
A. multiacuta	None	None		None	

Notes: Blank cells indicate criteria that were not tested for that species; *Acropora gemmifera*, "branching mont" and *A. digitifera* from chapter 2 are reclassified as "mont-gem", *A. monticulosa* and "Samoan dig", respectively in chapters 4 and 5; "digitate *A. monticulosa*" in chapter 2 is renamed "digitate mont" in chapters 4 and 5.

Table 6.1b Summary of inferred affinities of the intermediate and sub-morphs of the *Acropora humilis* species group for each of the criteria examined in this study.

Morph	Morphological Appearance All Chapters	Morphometric Analysis Chapters 2 & 4	28S rDNA Unit (Domains 1 and 2) Chapter 2	mtDNA Intergenic Region Chapters 3 & 5	Reproduction Chapters 3 & 5
Intermediate Morphs					
"sam-hum"	*A. samoensis* *A. humilis*	*A. samoensis* *A. humilis*		*A. samoensis* *A. humilis* *A. globiceps* *A. gemmifera*	*A. samoensis* * & **
"sam-gem"	*A. samoensis* *A. gemmifera*	*A. samoensis* *A. gemmifera*		*A. samoensis* *A. humilis* *A. globiceps* *A. gemmifera*	*A. samoensis* **
"hum-gem"	*A. humilis* *A. gemmifera*	*A. humilis* *A. gemmifera*		*A. samoensis* *A. humilis* *A. globiceps* *A. gemmifera*	*A. gemmifera* *
"hum-gem plate"	*A. humilis* *A. gemmifera*	*A. digitifera*		*A. samoensis* *A. humilis* *A. globiceps* *A. gemmifera*	
"mont-hum"	*A. monticulosa* *A. humilis*	*A. humilis*			*A. humilis* * *A. monticulosa* **
"mont-gem"	*A. monticulosa* *A. gemmifera*	*A. gemmifera*	*A. humilis*	*A. samoensis* *A. humilis* *A. globiceps* *A. gemmifera*	
"dig-gem"	*A. digitifera* *A. gemmifera*	*A. gemmifera* *A. monticulosa* *A. digitifera*		*A. digitifera*	*A. digitifera* ** *A. gemmifera* **
Sub-Morphs					
"fine sam"	*A. samoensis*	*A. samoensis*		*A. samoensis* *A. humilis* *A. globiceps* *A. gemmifera*	
"Penghu hum"	*A. humilis*	*A. samoensis* *A. humilis*		*A. samoensis* *A. humilis* *A. globiceps* *A. gemmifera*	
"terete mont"	*A. monticulosa* *A. gemmifera* *A. humilis*	*A. gemmifera* *A. monticulosa*		*A. monticulosa*	*A. humilis* *
"digitate mont"	*A. monticulosa* "Samoan dig"	*A. monticulosa*	*A. monticulosa* "Samoan dig"	*A. monticulosa*	
"Samoan dig"	*A. digitifera* "digitate mont"	A. monticulosa	*A. monticulosa* "digitate mont"	*A. monticulosa*	
"encrusting dig"	None			*A. digitifera*	

Notes: Blank cells indicate criteria that were not tested for that morph; Species and morphs were reclassified or renamed, as in Table 6.1a; For the reproductive criteria * indicates affinity is based on fertilization potential and ** indicates affinity is based on spawning details.

Table 6.2 Summary of inferred affinities of each morph, of the *Acropora humilis* species group based on the combined evidence for each of the criteria examined in this study.

	Species Affinities
Intermediate Morphs	
"sam-hum"	*A. samoensis*
"sam-gem"	*A. samoensis*
"hum-gem"	*A. humilis* *A. gemmifera*
"hum-gem plate"	*A. humilis* *A. gemmifera*
"mont-hum"	*A. humilis* *A. monticulosa*
"mont-gem"	*A. gemmifera*
"dig-gem"	*A. digitifera*
Sub-morphs	
"fine sam"	*A. samoensis*
"Penghu hum"	*A. humilis* *A. samoensis*
"terete mont"	*A. humilis* *A. monticulosa*
"digitate mont"	*A. monticulosa*
"Samoan dig"	*A. monticulosa*
"encrusting dig"	*A. digitifera*

in the museum collections from locations beyond the geographic range of this project (chapter 4) are also this morph, and therefore resolve the taxonomic status and biogeographic range of this morph.

The criteria examined in this project revealed a complex evolutionary relationship for *Acropora monticulosa*. It is a valid biological species based on reproductive criteria, while morphs of this species indicate that it has a range of evolutionary affinities with other species of the *A. humilis* species group. Phylogenetic analysis of the mtDNA intergenic region indicated that this species shares a close relationship with *A. humilis*, *A. samoensis*, *A. globiceps* and *A. gemmifera* for this marker. However, the grouping of

sequences from *A. monticulosa* in separate sub-clades for all regions suggests divergence from these four species. The grouping of sequences from all colonies of the morph "mont-gem" in the same sub-clade as *A. gemmifera* rather than *A. monticulosa* suggests that this morph is an ecological variant of *A. gemmifera*, with morphological appearance being determined by environmental rather than evolutionary factors. The low levels of fertilization between *A. monticulosa* and "mont-hum" and low to moderate levels between colonies of *A. humilis* and this morph, suggested that "mont-hum" was distinct from both species. Classification probabilities of colonies of "mont-hum" indicated that all but one colony of this morph were most morphologically similar to *A. humilis*. Based on the combined assessment of these criteria, overall appearance and the low abundance of this morph, "mont-hum" is tentatively proposed as a hybrid of these species. Similarly, the morph "terete mont" is proposed as a possible hybrid morph of the same pair of species, on the basis of morphological appearance, low abundance, lack of molecular distinction from *A. monticulosa*, a moderate within-morph level of fertilization and low potential to interbreed with either of these species. The morphs "digitate mont" and "Samoan dig" were morphologically similar but distinct from each other, with the shape of the radial corallites suggesting greatest affinity with *A. monticulosa* and *A. digitifera* respectively. However, based on the appearance of these morphs and evidence obtained in this study, they may each be derived from both of these species. Phylogenetic analysis of the 28S marker grouped sequences from the morph "digitate mont" with *A. monticulosa* and sequences from the mtDNA marker with *A. digitifera*, while all colonies of this morph consistently classified with the former species. In the case of "Samoan dig", although it appeared to most closely resemble *A. digitifera*, phylogenetic analysis of sequences from both markers grouped this morph with *A. monticulosa*, while four colonies were classified as *A. monticulosa* and one as *A. digitifera* in the morphometric analysis.

Acropora multiacuta and *A. retusa* were both distinct from all other species of the *A. humilis* species group, based on morphological characters and phylogenetic analysis of the mtDNA intergenic region. Analyses of the same criteria presented in this thesis, which incorporate other species of the genus *Acropora*, are therefore necessary to resolve evolutionary affinities of these species.

6.2 CONCLUSIONS

This study demonstrates the value of incorporating intraspecific and interspecific variation for clarifying evolutionary boundaries, as proposed by Wiens (1999), by revealing patterns that would not have been evident if only species categories were used. Recognition of morphs within and between species of the *Acropora humilis* group also reduced the chance of taxonomic error, by not 'forcing' colonies into incorrect or inappropriate species categories. In addition, the interspecific (intermediate) morphs provided a valuable means of exploring evolutionary links between species, while the absence of intermediate morphs between other species was consistent with apparent evolutionary distinction. Development of phylogenetic analysis programs that can incorporate this variability (e.g. Wiens 2001; Legendre and Makarenkov 2002) will greatly enhance the potential of accurately interpreting evolutionary relationships in scleractinian corals.

The broad biogeographic scale of this project also enhanced the interpretation of evolutionary relationships within and between species of the *Acropora humilis* group. Most importantly, I demonstrate that relationships are determined by taxonomic rather than geographic affinities, indicating that incomplete or recent divergence, rather than hybridization within populations, is the predominant explanation for the low or lack of divergence in this group of corals. If hybridization is the predominant process, genetic relationships between taxa would be expected to vary in different geographic locations, because of a variable potential of different combinations of species to interbreed. Factors influencing which species are able to interbreed in different regions include taxonomic composition, due to the different geographic ranges of species (Wallace 1999; Veron 2000), and patterns of spawning. The potential of species to interbreed may differ in different locations due to variable patterns in timing of spawning and fertilization (e.g. compare Knowlton et al. 1997; Szmant et al. 1997). However, as demonstrated in this project, species and morphs from the different regions show consistent molecular phylogenetic relationships, and species within one location (Lizard Island, chapter 3) showed no potential to interbreed, supporting the proposal that relationships are predominantly due to ancestral patterns of divergence.

In scleractinian corals, including the genus *Acropora*, the role of divergence appears to have been underemphasized and the significance of hybridization may have been

overemphasized as evolutionary processes (Knowlton 2001; Vollmer and Palumbi 2002), as demonstrated in this study. The combined assessment of the morphological, molecular and reproductive criteria indicates that lack of genetic distinction between the species *A. humilis*, *A. samoensis*, *A. globiceps*, *A. gemmifera* and morphs of these species appears to be due to incomplete or recent divergence, rather than hybridization. Furthermore, in situations where hybrids have formed, they may contribute little to the evolutionary history in the genus *Acropora* or other scleractinian corals. Two studies have shown that *A. prolifera* is a hybrid of *A. cervicornis* and *A. palmata* (van Oppen et al. 2000; Vollmer and Palumbi 2002). In this case, hybridization has produced new morphologies without speciation, due to the limited potential of *A. prolifera* to interbreed (Vollmer and Palumbi 2002). Although hybridization does not appear to be as widespread and unstructured as suggested in previous studies, (e.g. Veron 1995; van Oppen et al. 2001), the role of hybridization in the formation of new species (Arnold 1997) remains unresolved in scleractinian corals. Proposed examples of hybrids in this study include the morphs "dig-gem", "mont-hum", "terete mont", "digitate mont" and "Samoan dig". Continued detailed studies, which incorporate morphological, reproductive and molecular criteria, are necessary to resolve whether these morphs are indicative of incipient speciation. With respect to the molecular criteria, phylogenetic analysis of sequences from proteins directly involved in regulating fertilization are likely to be particularly informative (reviewed in Palumbi 1994; Swanson and Vacquier 2002), compared to mitochondrial markers which appear to be evolving unusually slowly and are therefore inadequate for resolving boundaries between species of anthozoans (reviewed in Shearer et al. 2002).

As demonstrated in this study, interpreting evolutionary relationships in scleractinian corals requires the integration of multiple criteria, including morphological, molecular and reproductive data (Willis 1990; Stobart 2000), and examination of patterns of intraspecific and interspecific polymorphism (Wiens 1999). Further research, particularly with increasingly sophisticated molecular analytical tools, is likely to reveal even more complex relationships in scleractinian corals than those recognized in this and previous studies. It is therefore essential that skeletal voucher samples be lodged in museums for permanent housing, enabling specimens from previous studies to be reexamined and possibly reinterpreted as more information becomes available (Paulay 1997; Wheeler 2001). This is particularly necessary with the public availability

of sequence and other molecular data from databases such as GenBank, so that species and morphs from which such data are derived can be revised in the light of new evidence.

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Appendix I Morphometric measurements. Character codes as listed in Table 2.1 and results presented in Fig. 2.4. Col. code = colony code used throughout the project; MTQ # = Museum of Tropical Queensland specimen registration number (submitted as electronic supplementary material to the journal *Coral Reefs*, with the publication of Chapter 2: DOI 10.1007/s00338-003-0299-0).

Identification	Colony Code in Fig. 2.6	MTQ #	Character Number and Code													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14
			brdist	diambase	diammid	diamtip	brlength	radcor	subimm	axcal	axwall	axsepta	rcprolen	rcordiam	rcaldiam	rcwall
A. humilis 1	1	G55587	33.6	19.8	19.6	10.6	28.4	11.0	0.13	1.42	1.18	0.33	1.89	1.10	0.94	0.69
A. humilis 1		G55588	40.2	25.8	25.0	12.6	36.6	12.9	0.14	1.42	1.26	0.34	1.61	0.95	0.68	0.76
A. humilis 1	3	G55589	33.2	22.0	20.8	11.4	38.6	12.2	0.04	1.38	1.25	0.33	1.90	0.97	0.82	0.94
A. humilis 1		G55590	33.2	18.4	17.2	10.0	36.4	11.9	0.05	1.40	1.07	0.28	2.02	1.06	0.77	0.98
A. humilis 1	2	G55591	36.2	20.2	20.4	11.6	40	10.4	0.03	1.47	1.13	0.40	2.43	0.90	0.83	0.90
A. humilis 2		G55592	36.0	17.4	16.6	9.8	42.0	9.7	0.04	1.40	1.24	0.43	2.07	1.06	0.99	0.90
A. humilis 2		G55593	37.3	18.4	17.6	10.0	65.0	10.0	0.03	1.43	1.20	0.31	1.97	1.05	0.84	0.85
A. humilis 2		G55594	32.8	17.4	16.6	9.0	47.6	9.7	0.09	1.36	1.30	0.43	2.13	1.10	0.89	0.97
A. humilis 2	1	G55595	38.1	22.2	19.4	10.8	51.0	11.7	0.14	1.53	1.14	0.46	2.29	0.99	0.82	0.81
A. humilis 2		G55596	33.0	19	18.8	9.8	46.8	10.4	0.13	1.15	1.10	0.28	2.32	0.97	0.77	0.79
A. humilis 3		G55597	40.6	20.6	21.2	10.6	53.6	11.1	0.13	1.26	1.33	0.44	2.00	1.02	0.77	0.93
A. humilis 3		G55598	36.1	19.4	19.6	10.8	55.4	10.7	0.03	1.34	1.31	0.39	2.10	1.22	1.01	0.73
A. humilis 3	2	G55599	37.3	20.2	20.0	12.0	59.4	11.3	0.05	1.54	1.49	0.43	2.26	1.21	0.98	0.87
A. humilis 3	1	G55600	41.5	23.4	21.2	11.2	63.0	9.7	0.26	1.46	1.18	0.43	2.29	1.04	0.83	0.98
A. humilis 3		G55601	39.7	28.4	25.0	11.0	58.2	9.7	0.06	1.42	1.36	0.44	2.21	1.05	0.78	1.11
A. digitifera	3	G55602	24.4	13.6	12.4	10.0	26.4	19.9	0.06	0.94	0.95	0.20	1.29	1.12	0.98	0.46
A. digitifera		G55603	25.0	12.8	11.4	8.4	24.0	19.7	0.05	0.90	0.46	0.15	1.26	1.16	0.93	0.36
A. digitifera		G55604	22.4	18.0	15.6	9.4	39.8	20.3	0.03	1.03	0.65	0.21	1.19	1.09	0.91	0.41
A. digitifera	1	G55605	25.9	15.4	13.6	8.0	32.6	19.0	0.05	0.88	0.66	0.19	1.86	1.01	0.93	0.62
A. digitifera	2	G55606	26.4	16.8	14.8	9.4	33.4	17.6	0.06	0.88	0.64	0.14	1.84	0.97	0.83	0.49
A. gemmifera	2	G55607	43.5	26.0	21.2	10.0	47.6	8.1	1.04	1.15	0.85	0.30	2.34	1.05	0.98	0.67
A. gemmifera		G55608	47.6	27.8	21.6	10.4	43.0	10.8	0.85	1.10	1.02	0.24	1.75	0.99	0.88	0.76
A. gemmifera		G55609	37.2	23.2	19.4	10.0	31.8	8.6	1.15	1.37	0.89	0.28	2.01	0.95	0.85	0.82
A. gemmifera	1	G55610	37.7	27.4	20.8	8.8	52.4	8.8	1.20	1.15	0.84	0.27	2.04	1.14	1.01	0.60
A. gemmifera		G55611	39.5	28.6	21.2	11.0	27.4	9.4	0.97	1.06	0.88	0.24	2.00	1.07	0.91	0.74
"digitate *A. monticulosa"*		G55612	31.0	21.4	18.4	10.6	29.4	18.4	0.01	1.13	0.57	0.26	1.65	0.91	0.64	0.57
"digitate *A. monticulosa"*	1	G55613	37.0	21.0	19.4	11.8	41.4	20.1	0.07	0.80	0.73	0.23	1.42	1.05	0.84	0.49
"branching *A. monticulosa"*		G55614	40.4	21.4	19.8	10.2	54.0	7.5	1.54	0.82	0.92	0.17	2.91	0.95	0.79	0.60
"branching *A. monticulosa"*		G55615	43.5	19.2	18.8	9.6	51.6	10.8	0.89	0.83	0.79	0.19	2.64	1.02	0.74	0.63
"branching *A. monticulosa"*	1	G55616	46.1	19.8	19.2	11.2	69.6	14.2	0.50	1.03	1.03	0.27	2.68	0.94	0.63	0.63
"branching *A. monticulosa"*	2	G55617	58.4	25.2	25.2	10.8	71.6	19.2	0.23	0.91	1.01	0.32	2.58	1.04	0.86	0.54

Appendix II Descriptive morphological characters. Codes as listed in Table 2.2 and results presented in Fig. 2.5. Col. code = colony code used during the study; MTQ # = Museum of Tropical Queensland specimen registration number (submitted as electronic supplementary material to the journal *Coral Reefs*, with the publication of Chapter 2: DOI 10.1007/s00338-003-0299-0).

Identification	Colony Code in Fig. 2.6	MTQ #	Character Number and Code																			
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
			determ	growth	axvsrad	coentype	radcoen	axcoen	spinules	rcsize	rcinwall	rcshape	rcopen	axdiam	relsize	brthick	taper	brlength	crowding	axrings	porosity	rcrings
A. humilis 1	1	G55587	0	1	1	1	0	1	1	0	2	0	0	0	0	2	1	5	0	1	1	1
A. humilis 1		G55588	0	1	1	1	0	1	1	0	2	0	0	0	0	0	0	4	0	1	1	1
A. humilis 1	3	G55589	0	1	1	1	0	1	1	0	2	0	0	0	0	1	1	4	0	1	1	1
A. humilis 1		G55590	0	1	1	1	0	1	1	0	2	0	0	0	0	2	1	4	0	1	1	1
A. humilis 1	2	G55591	0	1	1	1	0	1	1	0	2	0	0	0	0	1	1	4	0	1	1	1
A. humilis 2		G55592	0	1	1	1	0	0	1	0	2	0	0	0	0	2	2	3	0	2	1	1
A. humilis 2		G55593	0	1	1	1	0	0	1	0	2	0	0	0	0	2	2	1	0	2	1	1
A. humilis 2		G55594	0	1	1	1	0	0	1	0	2	0	0	0	0	2	2	2	0	2	1	1
A. humilis 2	1	G55595	0	1	1	1	0	0	1	0	2	0	0	0	0	1	1	2	0	2	1	1
A. humilis 2		G55596	0	1	1	1	0	0	1	0	2	0	0	0	0	2	2	3	1	2	1	1
A. humilis 3		G55597	0	1	1	1	0	1	1	0	2	0	0	0	0	1	2	2	1	1	1	1
A. humilis 3		G55598	0	1	1	1	0	0	1	0	2	0	0	0	0	2	2	2	0	1	1	1
A. humilis 3	2	G55599	0	1	1	1	0	0	1	0	2	0	0	0	0	1	2	1	0	1	1	1
A. humilis 3	1	G55600	0	1	1	1	0	0	1	0	2	0	0	0	0	1	2	1	0	1	1	1
A. humilis 3		G55601	0	1	0	1	0	0	1	0	2	0	0	0	0	0	1	1	0	1	1	1
A. digitifera	3	G55602	0	2	0	1	0	1	0	0	1	2	1	2	2	3	1	4	2	0	0	0
A. digitifera		G55603	0	2	0	1	0	1	0	0	1	2	1	2	2	3	1	5	2	0	0	0
A. digitifera		G55604	0	2	0	1	0	1	0	0	1	2	1	2	2	2	1	4	2	0	0	0
A. digitifera	1	G55605	0	2	0	1	0	1	0	0	1	2	1	2	2	3	1	4	2	0	0	0
A. digitifera	2	G55606	0	2	0	1	0	1	0	0	1	2	1	2	2	2	1	4	2	0	0	0
A. gemmifera	2	G55607	0	2	0	1	1	1	0	1	2	1	1	1	1	0	0	3	1	1	1	0
A. gemmifera		G55608	0	2	0	1	1	1	0	1	2	1	1	1	1	0	0	3	1	1	1	0
A. gemmifera		G55609	0	2	0	1	1	1	0	1	2	1	1	1	1	1	0	4	1	1	1	0
A. gemmifera	1	G55610	0	2	0	1	1	1	0	1	2	1	1	1	1	0	0	2	1	1	1	0
A. gemmifera		G55611	0	2	0	1	1	1	0	1	2	1	1	1	1	0	0	5	1	1	1	0
"digitate *A. monticulosa"*		G55612	0	2	0	1	0	0	0	0	2	0	1	2	2	1	1	5	2	0	0	0
"digitate *A. monticulosa"*	1	G55613	0	2	0	1	0	0	0	0	2	0	1	2	2	1	1	3	2	0	0	0
"branching *A. monticulosa"*		G55614	1	0	0	0	1	2	2	2	0	3	1	2	2	1	1	1	2	2	1	0
"branching *A. monticulosa"*		G55615	1	0	0	0	1	2	2	2	0	3	1	2	2	1	1	2	2	2	1	0
"branching *A. monticulosa"*	1	G55616	1	0	0	0	1	2	2	2	0	3	1	1	2	1	2	0	2	2	1	0
"branching *A. monticulosa"*	2	G55617	1	0	0	0	1	2	2	2	0	3	1	1	2	0	1	0	2	2	1	0